

# World Journal of *Hepatology*

*World J Hepatol* 2016 January 8; 8(1): 1-82





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 469 members, representing a team of worldwide experts in hepatology. They are from 53 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (1), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (98), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (19), Greece (11), Hungary (5), India (15), Indonesia (2), Iran (4), Israel (1), Italy (52), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (11), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (17), and United States (56).

### EDITORS-IN-CHIEF

Clara Balsano, Rome  
Wan-Long Chuang, Kaohsiung

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, Kaohsiung  
Tai-An Chiang, Tainan  
Chi-Tan Hu, Hualien  
Sen-Yung Hsieh, Taoyuan  
Wenya Huang, Tainan  
Liang-Yi Hung, Tainan  
Jih RU Hwu, Hsinchu  
Jing-Yi Lee, Taipei  
Mei-Hsuan Lee, Taipei  
Chih-Wen Lin, Kaohsiung  
Chun-Che Lin, Taichung  
Wan-Yu Lin, Taichung  
Tai-Long Pan, Tao-Yuan  
Suh-Ching Yang, Taipei  
Chun-Yan Yeung, Taipei

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, Batna



**Argentina**

Fernando O Bessone, Rosario  
Maria C Carrillo, Rosario  
Melisa M Dirchwolf, Buenos Aires  
Bernardo Frider, Buenos Aires

Jorge Quarleri, Buenos Aires  
Adriana M Torres, Rosario



**Armenia**

Narina Sargsyants, Yerevan



**Australia**

Mark D Gorrell, Sydney



**Austria**

Harald Hofer, Vienna  
Gustav Paumgartner, Vienna  
Matthias Pinter, Vienna  
Thomas Reiberger, Vienna



**Bangladesh**

Shahinul Alam, Dhaka  
Mamun Al Mahtab, Dhaka



**Belgium**

Nicolas Lanthier, Brussels  
Philip Meuleman, Ghent  
Luisa Vonghia, Antwerp



**Botswana**

Francesca Cainelli, Gaborone

Sandro Vento, Gaborone



**Brazil**

Edson Abdala, Sao Paulo  
Ilka FSF Boin, Campinas  
Niels OS Camara, Sao Paulo  
Ana Carolina FN Cardoso, Rio de Janeiro  
Roberto J Carvalho-Filho, Sao Paulo  
Julio CU Coelho, Curitiba  
Flavio Henrique Ferreira Galvao, São Paulo  
Janaina L Narciso-Schiavon, Florianopolis  
Sílvia HC Sales-Peres, Bauru  
Leonardo L Schiavon, Florianópolis  
Luciana D Silva, Belo Horizonte  
Vanessa Souza-Mello, Rio de Janeiro  
Jaques Waisberg, Santo André



**Bulgaria**

Mariana P Penkova-Radicheva, Stara Zagora  
Marieta Simonova, Sofia



**Canada**

Runjan Chetty, Toronto  
Michele Molinari, Halifax  
Giada Sebastiani, Montreal



**Chile**

Luis A Videla, Santiago





## China

Guang-Wen Cao, Shanghai  
 En-Qiang Chen, Chengdu  
 Gong-Ying Chen, Hangzhou  
 Jin-lian Chen, Shanghai  
 Jun Chen, Changsha  
 Alfred Cheng, Hong Kong  
 Chun-Ping Cui, Beijing  
 Shuang-Suo Dang, Xi'an  
 Ming-Xing Ding, Jinhua  
 Zhi-Jun Duang, Dalian  
 He-Bin Fan, Wuhan  
 Xiao-Ming Fan, Shanghai  
 James Yan Yue Fung, Hong Kong  
 Yi Gao, Guangzhou  
 Zuo-Jiong Gong, Wuhan  
 Zhi-Yong Guo, Guangzhou  
 Shao-Liang Han, Wenzhou  
 Tao Han, Tianjin  
 Jin-Yang He, Guangzhou  
 Ming-Liang He, Hong Kong  
 Can-Hua Huang, Chengdu  
 Bo Jin, Beijing  
 Shan Jin, Hohhot  
 Hui-Qing Jiang, Shijiazhuang  
 Wan-Yee Joseph Lau, Hong Kong  
 Guo-Lin Li, Changsha  
 Jin-Jun Li, Shanghai  
 Qiang Li, Jinan  
 Sheng Li, Jinan  
 Zong-Fang Li, Xi'an  
 Xu Li, Guangzhou  
 Xue-Song Liang, Shanghai  
 En-Qi Liu, Xi'an  
 Pei Liu, Shenyang  
 Zhong-Hui Liu, Changchun  
 Guang-Hua Luo, Changzhou  
 Yi Lv, Xi'an  
 Guang-Dong Pan, Liuzhou  
 Wen-Sheng Pan, Hangzhou  
 Jian-Min Qin, Shanghai  
 Wai-Kay Seto, Hong Kong  
 Hong Shen, Changsha  
 Xiao Su, Shanghai  
 Li-Ping Sun, Beijing  
 Wei-Hao Sun, Nanjing  
 Xue-Ying Sun, Harbin  
 Hua Tang, Tianjin  
 Ling Tian, Shanghai  
 Eric Tse, Hong Kong  
 Guo-Ying Wang, Changzhou  
 Yue Wang, Beijing  
 Shu-Qiang Wang, Chengdu  
 Mary MY Wayne, Hong Kong  
 Hong-Shan Wei, Beijing  
 Danny Ka-Ho Wong, Hong Kong  
 Grace Lai-Hung Wong, Hong Kong  
 Bang-Fu Wu, Dongguan  
 Feng Wu, Chongqing  
 Xiong-Zhi Wu, Tianjin  
 Chun-Fang Xu, Suzhou  
 Rui-An Xu, Quanzhou  
 Rui-Yun Xu, Guangzhou  
 Wei-Li Xu, Shijiazhuang  
 Shi-Ying Xuan, Qingdao  
 Ming-Xian Yan, Jinan  
 Lv-Nan Yan, Chengdu  
 Jin Yang, Hangzhou  
 Ji-Hong Yao, Dalian  
 Winnie Yeo, Hong Kong

Zheng Zeng, Beijing  
 Qi Zhang, Hangzhou  
 Shi-Jun Zhang, Guangzhou  
 Xiao-Lan Zhang, Shijiazhuang  
 Xiao-Yong Zhang, Guangzhou  
 Xin-Chen Zhang, Harbin  
 Yong Zhang, Xi'an  
 Hong-Chuan Zhao, Hefei  
 Ming-Hua Zheng, Wenzhou  
 Yu-Bao Zheng, Guangzhou  
 Ren-Qian Zhong, Shanghai  
 Fan Zhu, Wuhan  
 Xiao Zhu, Dongguan



## Czech Republic

Kamil Vysloulzil, Olomouc



## Denmark

Henning Gronbaek, Aarhus  
 Christian Mortensen, Hvidovre



## Egypt

Ihab T Abdel-Raheem, Damanhour  
 NGB G Bader EL Din, Cairo  
 Hatem Elalfy, Mansoura  
 Mahmoud M El-Bendary, Mansoura  
 Mona El SH El-Raziky, Cairo  
 Mohammad El-Sayed, Cairo  
 Yasser M Fouad, Minia  
 Mohamed AA Metwally, Benha  
 Hany Shehab, Cairo  
 Mostafa M Sira, Shebin El-koom  
 Ashraf Taye, Minia  
 MA Ali Wahab, Mansoura



## France

Laurent Alric, Toulouse  
 Sophie Conchon, Nantes  
 Daniel J Felmlee, Strasbourg  
 Herve Lerat, Creteil  
 Dominique Salmon, Paris  
 Jean-Pierre Vartanian, Paris



## Germany

Laura E Buitrago-Molina, Hannover  
 Enrico N De Toni, Munich  
 Oliver Ebert, Muenchen  
 Rolf Gebhardt, Leipzig  
 Janine V Hartl, Regensburg  
 Sebastian Hinz, Kiel  
 Benjamin Juntermanns, Essen  
 Roland Kaufmann, Jena  
 Viola Knop, Frankfurt  
 Veronika Lukacs-Kornek, Homburg  
 Benjamin Maasoumy, Hannover  
 Jochen Mattner, Erlangen  
 Nadja M Meindl-Beinker, Mannheim  
 Ulf P Neumann, Aachen  
 Margarete Odenthal, Cologne  
 Yoshiaki Sunami, Munich

Christoph Roderburg, Aachen  
 Frank Tacke, Aachen  
 Yuchen Xia, Munich



## Greece

Alex P Betrosian, Athens  
 George N Dalekos, Larissa  
 Ioanna K Delladetsima, Athens  
 Nikolaos K Gatselis, Larissa  
 Stavros Gourgiotis, Athens  
 Christos G Savopoulos, Thessaloniki  
 Tania Siahaniidou, Athens  
 Emmanouil Sinakos, Thessaloniki  
 Nikolaos G Symeonidi, Thessaloniki  
 Konstantinos C Thomopoulos, Larissa  
 Konstantinos Tziomalos, Thessaloniki



## Hungary

Gabor Banhegyi, Budapest  
 Peter L Lakatos, Budapest  
 Maria Papp, Debrecen  
 Ferenc Sipos, Budapest  
 Zsolt J Tulassay, Budapest



## India

Deepak N Amarapurkar, Mumbai  
 Girish M Bhopale, Pune  
 Sibnarayan Datta, Tezpur  
 Nutan D Desai, Mumbai  
 Sorabh Kapoor, Mumbai  
 Jaswinder S Maras, New Delhi  
 Nabeen C Nayak, New Delhi  
 C Ganesh Pai, Manipal  
 Amit Pal, Chandigarh  
 K Rajeshwari, New Delhi  
 Anup Ramachandran, Vellore  
 D Nageshwar Reddy, Hyderabad  
 Shivaram P Singh, Cuttack  
 Ajith TA, Thrissur  
 Balasubramaniyan Vairappan, Pondicherry



## Indonesia

Cosmas RA Lesmana, Jakarta  
 Neneng Ratnasari, Yogyakarta



## Iran

Seyed M Jazayeri, Tehran  
 Sedigheh Kafi-Abad, Tehran  
 Iradj Maleki, Sari  
 Fakhraddin Naghibalhossaini, Shiraz



## Israel

Stephen DH Malnick, Rehovot



## Italy

Francesco Angelico, Rome

Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Andrea Galli, *Florence*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Salvatore Gruttadauria, *Palermo*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*



**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*  
 Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*

Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhito Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*



**Jordan**

Kamal E Bani-Hani, *Zarqa*



**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*



**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*



**Moldova**

Angela Peltec, *Chishinev*



**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*



**Nigeria**

CA Asabamaka Onyekwere, *Lagos*



**Pakistan**

Bikha Ram Devrajani, *Jamshoro*



**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*



**Poland**

Jacek Zielinski, *Gdansk*



**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*



**Qatar**

Reem Al Olaby, *Doha*



**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*



**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*



**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*



**Singapore**

Ser Yee Lee, *Singapore*



**South Korea**

Young-Hwa Chung, *Seoul*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonju*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*



**Spain**

Ivan G Marina, *Madrid*  
 Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*

José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodríguez-Frias, *Córdoba*  
 Manuel L Rodríguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Traperó-Marugán, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpaboon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*  
 Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*

Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*



#### **United States**

Naim Alkhouri, *Cleveland*  
 Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*

Brian B Borg, *Jackson*  
 Ronald W Busuttill, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Grigoriy E Gurvits, *New York*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Kusum K Kharbanda, *Omaha*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**TOPIC HIGHLIGHT**

- 1** Metabonomic window into hepatitis B virus-related hepatic diseases  
*Hou Q, Duan ZJ*
- 9** Chaperones in hepatitis C virus infection  
*Khachatoorian R, French SW*
- 36** Vascular complications following liver transplantation: A literature review of advances in 2015  
*Piardi T, Lhuair M, Bruno O, Memeo R, Pessaux P, Kianmanesh R, Sommacale D*

**REVIEW**

- 58** Selection of patients with hepatocellular carcinoma for liver transplantation: Past and future  
*Soriano A, Varona A, Gianchandani R, Moneva ME, Arranz J, Gonzalez A, Barrera M*

**MINIREVIEWS**

- 69** Treatment strategies for chronic hepatitis C prior to and following liver transplantation  
*Perumpail RB, Hahambis TA, Aggarwal A, Younossi ZM, Ahmed A*

**ORIGINAL ARTICLE****Observational Study**

- 74** Adipokines, cytokines and body fat stores in hepatitis C virus liver steatosis  
*González-Reimers E, López-Prieto J, Quintero-Platt G, Pelazas-González R, Alemán-Valls MR, Pérez-Hernández O, de-la-Vega-Prieto MJ, Gómez-Rodríguez MA, Martín-González C, Santolaria-Fernández F*

## Contents

*World Journal of Hepatology*  
Volume 8 Number 1 January 8, 2016

### ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Henning Gronbaek, MD, PhD, Professor, Department of Hepatology and Gastroenterology, Aarhus University Hospital, 8000 Aarhus, Denmark

### AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed Central, PubMed, Digital Object Identifier, Directory of Open Access Journals, and Scopus.

### FLYLEAF

#### I-IV Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Xue-Mei Gong*  
Proofing Editorial Office Director: *Xin-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
January 8, 2016

#### COPYRIGHT

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

#### SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

#### INSTRUCTIONS TO AUTHORS

Full instructions are available online at [http://www.wjgnet.com/1948-5182/g\\_info\\_20100316080002.htm](http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm)

#### ONLINE SUBMISSION

<http://www.wjgnet.com/esps/>



2016 Advances in Hepatitis B Virus

## Metabonomic window into hepatitis B virus-related hepatic diseases

Qiang Hou, Zhi-Jun Duan

Qiang Hou, Zhi-Jun Duan, Department of Gastroenterology, the First Affiliated Hospital of Dalian Medical University, Dalian 116011, Liaoning Province, China

**Author contributions:** Hou Q searched the literature and drafted the review; Duan ZJ supervised the review.

**Supported by** Liaoning Natural Science Foundation of China, No. 2013B003.

**Conflict-of-interest statement:** The authors declare no conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Zhi-Jun Duan, Professor, Department of Gastroenterology, the First Affiliated Hospital of Dalian Medical University, Zhongshan Road, Xigang District, Dalian 116011, Liaoning Province, China. [cathydoctor@sina.com](mailto:cathydoctor@sina.com)  
 Telephone: +86-411-83635963

Received: April 29, 2015

Peer-review started: May 7, 2015

First decision: August 11, 2015

Revised: November 23, 2015

Accepted: December 17, 2015

Article in press: December 18, 2015

Published online: January 8, 2016

### Abstract

Metabonomics has recently been widely used to

discover the pathogenesis and find potential metabolic markers with high sensitivity and specificity. Furthermore, it develops new diagnosis and treatment methods, increases early phase diagnosis rates of certain diseases and provides a new basis for targeted therapy. This review mainly analyzes the research progress of the metabonomics of hepatitis B virus (HBV)-related hepatic diseases, hoping to discover some potential metabolic markers for identification of HBV-related hepatic diseases from other etiologies and for HBV-related hepatitis, liver cirrhosis and hepatocellular carcinoma. This can contribute to early discovery, diagnosis and treatment, eventually increasing the survival rate of HBV-related hepatic diseases.

**Key words:** Metabonomics; Hepatitis B virus-related hepatic diseases; Hepatitis B; Hepatitis B virus-related liver cirrhosis; Hepatitis B virus-related hepatocellular carcinoma

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This review mainly analyzes the research progress of the metabonomics of hepatitis B virus (HBV)-related hepatic diseases, hoping to discover some potential metabolic markers which can distinguish HBV-related hepatic diseases from other etiologies and discover potential metabolic markers of HBV-related hepatitis, liver cirrhosis and hepatocellular carcinoma, which can contribute to early discovery, diagnosis and treatment.

Hou Q, Duan ZJ. Metabonomic window into hepatitis B virus-related hepatic diseases. *World J Hepatol* 2016; 8(1): 1-8 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i1/1.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i1.1>

## METABONOMICS AND THE LIVER IN BRIEF

The main function of the liver is the synthesis and metabolism of various proteins, polysaccharides and fats and the detoxification of the body's normal metabolic wastes, such as uric acid, drugs and chemical products<sup>[1,2]</sup>. There are many hepatic diseases that threaten health. However, because of a lack of effective early diagnosis methods, a large number of the diseases are in the middle to late stages when detected, which seriously affects the prognosis. Therefore, it is important to find tumor markers with high sensitivity and specificity as well as to elucidate the pathogenesis.

Metabonomics, a branch of systematic biology, is a recent newly developing subject. It aims to explore biological systems, like the changes in metabolites of the cells, tissues and certain organisms in the environment of exogenous stimulations, especially studying metabolites weighing less than 1000. Metabonomics integrates gene regulation, post-transcriptional regulation and the interaction of the pathways together, which makes different metabolites manifest significant biological phenotypes through the stages of the cell directly. Compared to the vast information in genomics, transcriptomics and proteomics, there is more information about apparent learning<sup>[3]</sup>. Thus, metabonomics has recently been widely used to discover the pathogenesis, finding potential metabolic markers with high sensitivity and specificity and exploring new diagnosis and treatment methods in order to increase early phase diagnosis rates of certain diseases and provide a new basis for targeted therapy<sup>[4]</sup>.

The morbidity of hepatocellular carcinoma (HCC) ranks 5<sup>th</sup> and its mortality ranks 3<sup>rd</sup> as a malignancy worldwide<sup>[5]</sup>. The incidence in southeast Asia and Africa is especially high, about 20 per 100000 population<sup>[6]</sup>. HCC has many risks with HBC as the primary one, causing 780000 death yearly<sup>[7]</sup>. The evolutionary progress of chronic hepatic disease is from chronic hepatitis B (CHB), hepatitis B virus (HBV)-related cirrhosis to HBV-related HCC. Nowadays, liver biopsy is the golden criteria in differentiating hepatic fibrosis, liver cirrhosis (LC) and HCC but cannot be used universally because of the invasiveness. Abdominal ultrasound is still the first screening method for hepatic diseases. It is widely used clinically because it is noninvasive and cheap. However, its sensitivity is affected by the machine, operators and different states of the disease. The sensitivity of diagnosing early cirrhosis is especially low, only 32% to 65% in HCC<sup>[8,9]</sup>. However, as a widely used clinical serum biomarker for HCC, alpha fetoprotein shows no increase in 80% of small HCC and its overall sensitivity is just 70%<sup>[8-11]</sup>. Some liver fibrosis indexes, such as hyaluronic acid, procollagen type III, procollagen type IV and laminin, can indicate early hepatic cirrhosis by analyzing the proliferation and degeneration of hepatic fibrosis. However, its sensitivity and specificity remain

unknown<sup>[12]</sup>. As an essential metabolic organ, any organic disease of the liver will lead to changes in the whole body's metabolism, causing widespread concern for medical staff. Research on the relationship between hepatic diseases and metabonomics has been increasing yearly. This review mainly analyzes the research progress of the metabonomics of HBV-related hepatic diseases, hoping to discover some potential metabolic markers for identification of HBV-related hepatic diseases from other etiologies and for HBV-related hepatitis, LC and HCC. It can contribute to early discovery, diagnosis and treatment, eventually increasing the survival rate of HBV-related hepatic diseases.

## THE METABONOMIC WINDOW INTO HBV-RELATED HEPATIC DISEASES

### CHB

Chronic HBV infection is a global problem, mainly in developing countries and especially in southeast Asia and Africa. About 600000 people die of acute or chronic HBV infection each year<sup>[13]</sup>. Chronic HBV infection can result in hepatitis, hepatic fibrosis and even LC and HCC. Presently, the main treatment methods for chronic HBV infection are interferon treatment<sup>[14-16]</sup>, nucleotide analogue treatment<sup>[17-19]</sup>, immune treatment<sup>[20-22]</sup>, *etc.* Although they can reduce the transformation from CHB to LC and HCC, their cure rates still need to improve. In the meantime, the pathogenic pathway of chronic HBV infection is still unclear. In the metabonomic study of patients with chronic HBV infection, some metabolites with a significant difference were found, which may provide some basis for discovering a pathogenic pathway and ideas for new targeted therapy.

As shown in Table 1, there are 2 studies concerning CHB. Zhou *et al.*<sup>[23]</sup> analyzed the metabolites in serum from CHB patients and a control group by liquid chromatography-mass spectrometry (LC-MS) and discovered 12 metabolites with a difference that were involved in fatty acids, amino acids, bile acids and energy metabolism and other pathways<sup>[24]</sup>. To date, there are still few metabonomic studies about CHB so it is a research domain that needs to be expanded. Autoimmune hepatitis (AIH) is an inflammatory reaction of the liver caused by autoantibodies. Early diagnosis can result in successful treatment. However, due to the unknown pathogenesis, the diagnostic rate is low and the prognosis cannot be estimated. Wang *et al.*<sup>[25]</sup> studied metabonomic characteristics of AIH by nuclear magnetic resonance (NMR) for the first time, providing a basis for researching the pathogenesis of AIH and discovering potential metabolic markers further. About 11% of patients with nonalcoholic steatohepatitis (NASH) will develop LC after 15 years and 7% will develop HCC through LC or directly after 6.5 years<sup>[26]</sup>. The metabolic changes of NASH refer to the metabolism of fatty acids, carbohydrates and bile acids<sup>[27-29]</sup>. The metabonomic research for chronic hepatitis C has discovered that the

**Table 1 Summary of metabolomic studies of chronic hepatitis B**

Ref.	Year	Species	Tissue	Platform	Up-regulated	Down-regulated
Zhou <i>et al</i> <sup>[23]</sup>	2012	Human CHB 30 N 30 CHB/N	Serum	LC-MS	Cortisol, GCA, GCDCA, LysoPC (15:0), LysoPE (22:6), C16:1-CN	Tryptophan, C10-CN, C10:1-CN, C8-CN, C6-CN
Soga <i>et al</i> <sup>[24]</sup>	2011	Human CHB 7 N53 CHB/N	Serum	CE-TOM LC-MS	$\gamma$ -Glu-Thr	

CHB: Chronic hepatitis B; LC-MS: Liquid chromatography-mass spectrometry; GCA: Glycocholic acid; GCDCA: Glycochenodeoxycholic acid; LysoPC: Lysophosphatidylcholine; LysoPE: Lysophosphatidylethanolamine; CN: C16:1-acylcarnitine.

**Table 2 Summary of metabolomic studies of hepatitis B virus-related liver cirrhosis**

Ref.	Year	Species	Tissue	Platform	Up-regulated	Down-regulated
Liu <i>et al</i> <sup>[42]</sup>	2013	Human LC 42 N 18 LC/N	Serum	NMR LC-MS	L-phenylalanine, C16 sphinganine, alpha- CEHC, LysoPC (18:1), linoelaidic acid, PC (18:4/20:1), bilirubin	L-carnitine, decanoyl-L-carnitine, phytosphingosine, 3 $\alpha$ , 6 $\alpha$ , 7 $\alpha$ , 12 $\alpha$ -tetrahydroxy-5 $\beta$ -cholan-24-oic acid PC (14:1/14:1), LysoPC (16:0)
Wang <i>et al</i> <sup>[39]</sup>	2012	Human LC 63 N 31 LC/N	Urine	GC-MS UPLC-TOFMS	Prolile, citrate, aconitate, 3,4-dihydroxyphenylacetate, taurohyocholate, glycocholate, glycoursodeoxycholate	Threonine, hippurate, 2-aminobutyrate, cis- aconitate, pyroglutamate, alpha-hydroxyisobutyrate, 3-hydroxyisovalerate, alpha-hydroxyhippurate, estrone
Zhou <i>et al</i> <sup>[23]</sup>	2012	Human CIR 30 N 30 CIR/N	Serum	LC-MS	GCA, GCDCA, CN	Tryptophan, LysoPC (20:5), LysoPC (0:0/14:0), LysoPC (22:6), LysoPC (14:0/0:0), LysoPE (20:4), C10-CN, C10:1-CN, C8-CN, C6-CN
Yin <i>et al</i> <sup>[41]</sup>	2009	Human LC25 N25 LC/N	Serum	RRLC	Taurocholic acid fragment, GCA, bilirubin, TCDCA fragment, GCDCA, oleic acid fragment, taurocholic acid fragment, carnitine fragment, L-acetylcarnitine	Hypoxanthine, lysoPC C18:2, LPC C18:3, LPC C16:1, LPC C18:0, Hypoxanthine fragment, inosine, taurine, 6-methylnicotinic acid
Xue <i>et al</i> <sup>[40]</sup>	2009	HBV infected human LC20 non-LC 20 LC/non-LC	Serum	GC-MS	Acetic acid, hexanoic acid, 1-naphthalenamine, butanoic acid	Sorbitol, D-Lactic acid, phosphoric acid, D-glucitol, glucose

HBV: Hepatitis B virus; LC-MS: Liquid chromatography-mass spectrometry; GCA: Glycocholic acid; TCDCA: Taurochenodeoxycholic acid; GCDCA: Glycochenodeoxycholic acid; LC: Liver cirrhosis; PC: Phosphatidylcholine; NMR: Nuclear magnetic resonance; Alpha-CEHC: 2,5,7,8-Tetramethyl-2-(2'-carboxyethyl)-6-hydroxychroman; LysoPC: Lysophosphatidylcholine; LysoPE: Lysophosphatidylethanolamine; UPLC-TOFMS: Ultra-high performance liquid chromatography-time of flight-mass spectrometer; CN: C16:1-acylcarnitine; GC-MS: Gas chromatography-mass spectrometer.

up-regulation of AKR1B10 expression in urine leads to abnormal glucose metabolism<sup>[30]</sup>. In studies about acute alcoholic hepatitis, Rachakonda *et al*<sup>[31]</sup> detected metabolites that were distinctly different from those in alcoholic LC that were involved in the metabolic process of fatty acids, bile acids, proteins and carbohydrates.

## LC

LC is the terminal stage of chronic liver diseases (CLD), with a high morbidity worldwide. Chronic HBV infection is an important pathogenic factor of LC<sup>[32]</sup> and the evolution of HBV-related LC is a gradual progress<sup>[33]</sup>. Due to a lack of specific diagnostic methods, the incidence rate of LC is 3.7 per 100 person-years in HBV carriers<sup>[34]</sup> and the 5 years survival rate of decompensated LC patients is only 14% to 35%<sup>[35,36]</sup>, while 70% to 90% of HBV-related HCC developed from decompensated LC<sup>[37,38]</sup>. To date, there are still few valuable markers for early diagnosis of HBV-related LC and it is especially important to detect potential biomarkers with a higher

sensitivity and specificity.

Table 2 shows 5 studies regarding the metabonomics of HBV-related LC, 4 of them based on serum and 1 based on urine. According to the Child-Pugh scores, all the LC patients were classified into three groups, A, B and C. Wang *et al*<sup>[39]</sup> carried out a urinary metabonomic study on the different stages of HBV-related LC and healthy controls using a gas chromatography-mass spectrometer (GC-MS) and ultra performance liquid chromatography time-of-flight mass spectrometry. They found metabolites with a significant difference in the three groups of LC, which may be potential metabolic markers in different stages of LC, providing a basis for the estimate of progress. Differently from the other three studies, Xue *et al*<sup>[40]</sup> chose patients with CHB as a control group and found nine metabolites with an obvious difference in total. The study also further verified the distinguishing ability by SAS software, showing that five out of twenty LC patients in Child-Pugh A were misdiagnosed as patients with CHB due to the small

sample size. Zhou *et al.*<sup>[23]</sup> and Yin *et al.*<sup>[41]</sup> analyzed the metabolites in the serum of a HBV-related LC group and healthy control group by LC-MS and NMR, with both methods discovering metabolites with differences<sup>[42]</sup>. Among these five articles, only one used hepatitis B patients as a control group and the others chose healthy volunteers. In these present studies, we still lack research that uses CHB patients as a control group. The identification sensitivity of potential metabolic markers in patients with early HBV-related LC and patients with CHB found in present studies should be further discussed.

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are two diseases relevant to metabolic disorders of bile acid. Due to the insidious onset and lack of effective diagnostic methods with high specificity, patients are usually in an advanced stage when diagnosed<sup>[43]</sup>. Trottier *et al.*<sup>[44]</sup> analyzed the metabolic changes of 17 bile acids in patients with these two diseases by LC-MS. Compared to healthy volunteers, the primary bile acid in serum in the two diseases increased significantly, which may be associated with impairment of the enterohepatic circulation. Compared with PBC, the levels of secondary bile acid in the PSC group decreased obviously. It suggests that PBC is only relevant to the impairment of the extrahepatic bile duct, while PSC involves both the intrahepatic and extrahepatic bile duct. Furthermore, Bell *et al.*<sup>[45]</sup> also drew similar conclusions by LC-MS. Acute-on-chronic liver failure (ACLF) is acute liver failure resulting from the acute deterioration of liver function on the basis of CLD, which can be accompanied by multiple organ failure at the same time. Due to its yearly increasing incidence and high mortality rate, ACLF is receiving more and more attention from the medical profession<sup>[46]</sup>. Amathieu *et al.*<sup>[47,48]</sup> studied the metabonomic characteristics of LC patients with and without ACLF and detected obvious differences in the metabolic features of the two groups. Nie *et al.*<sup>[49]</sup> discovered 17 potential markers by comparing HBV-related ACLF with HBV-related LC in Child-Pugh A and 11 of them had improved survival after treatment, which has implications for the early diagnosis and prognosis assessment of ACLF. Lian *et al.*<sup>[50]</sup> researched metabolic differences in alcoholic LC and HBV-related LC by LC-MS and found that oleamide and myristamide increased significantly in patients with alcoholic LC but decreased distinctly in patients with HBV-related LC, which indicated that they both could be used as specific metabolic markers to distinguish alcoholic LC from HBV-related LC. By GC-MS and LC-MS, Fitian AI, Soga *et al.*<sup>[24]</sup> and Fitian *et al.*<sup>[51]</sup> found that some bile acids and dicarboxylic acids increased in hepatitis C virus (HCV)-related LC. Also,  $\gamma$ -glutamyl dipeptides were mentioned in both studies and there was thought to be some expressed differences in different types of hepatic diseases. Therefore, it can be used as a potential metabolic marker to differentiate various hepatic diseases. So far, metabonomics of various hepatic diseases is still in the primary stages,

lacking the metabolomic difference analysis comparing the diverse types of hepatic diseases. Therefore, the field of metabonomics of hepatic diseases needs further research.

## HCC

In China, over 80% of HCC cases resulted from chronic HBV infection, an evolutionary progress from CHB to LC and eventually to HCC<sup>[32,33]</sup>. To improve the diagnostic rate for early HCC, potential biomarkers with high specificity which can be adopted to screen the HBV-related LC need to be explored. Some metabolites which are specifically expressed in HBV-related HCC may provide a new horizon for the targeted treatment of HCC in the future.

In Table 3, 4 studies from China exploring metabonomics of HBV-related HCC are shown, complying with the regional differences of HCC. The potential metabolic markers found in these studies involve the metabolism pathways of fatty, amino and bile acids, energy and so on. Liu *et al.*<sup>[52]</sup> researched the metabolomic characteristics of liver tissue in 10 patients with liver carcinoma by LC-MS. Based on the comparison of the central area of the tumor and distant tissue, 14 metabolites were found with obvious differences and 9 of them<sup>[53-55]</sup> have also been mentioned in other studies. However, betasitosterol, quinaldic acid, arachidyl carnitine, tetradecanal and oleamide have rarely been mentioned, possibly because the levels of these 5 metabolites are too low in serum to be detected. It indicates that although metabolic profiling of tissue cannot reflect the changes of systemic metabolism in the human body, it could actually reflect the changes of metabolic characteristics of certain tissues or organs. Li *et al.*<sup>[56]</sup> compared the metabolomic characteristics of HBV infected HCC host cells HepG2.2.15 with HCC host cell HepG2 by NMR and found that 11 metabolites were obviously different. N-acetyl glucosamine kinase had a significantly increased expression in HepG2.2.15 and was involved in the hexosamine biosynthesis pathway, which demonstrated that the hexosamine biosynthesis pathway was activated in HBV infected cells, providing a new thought for studying targeted therapy for HBV infection in the future. Zhou *et al.*<sup>[23]</sup> and Yin *et al.*<sup>[41]</sup> analyzed the metabolites of HBV-related HCC and normal bodies by LC-MS and found some potential biomarkers of metabolism involved in the metabolism of fatty acid, phosphoric acid, amino acid and glucose. Both studies found that the expression of glycochenodeoxycholic acid, lysophosphatidylcholine and glycocholic acid were significantly different in patients with HCC.

Besides the infection with HBV, infection with HCV, the addition of alcohol and steatohepatitis are also important pathogenic factors in HCC. We found 3 studies regarding HCV-related HCC<sup>[51,57,58]</sup> from the United States. Compared to the research of HBV-related HCC, other body fluid samples were added, as well as serum, containing metabolomic characteristics of HCV-related HCC and LC. Bowers *et al.*<sup>[57]</sup> analyzed the metabolomic



**Table 3** Summary of metabolomic studies of hepatitis B virus-related hepatocellular carcinoma

Ref.	Year	Species	Tissue	Platform	Up-regulated	Down-regulated
Li <i>et al.</i> <sup>[56]</sup>	2015	Human	Liver	NMR	Fructose-bisphosphatealdolase, glucose-6-phosphate isomerase, alpha-enolase, citrate synthase	4-hydroxyphenylpyruvate dioxygenase
		Hepatoblastoma cell line HepG2.2.15/HepG2	Host cell		Phosphoglycerate kinase 1 Triosephosphate isomerase Succinate dehydrogenase Malate dehydrogenase	Fumarylacetoacetase
Liu <i>et al.</i> <sup>[52]</sup>	2013	Human	Liver	UPLC-MS	Sitosterol-beta, L-phenylalanine, LysoPC [18:2 (9Z, 12Z)], quinaldic acid	Arachidyl carnitine
		HCC 10			glycerophosphocholine, LysoPC (18:0) LysoPE (18:0/0:0), chenodeoxycholic acid glycine conjugate	Tetradecanal
		Central/distant			LysoPE [18:3 (9Z, 12Z, 15Z)/0:0] LysoPC [22:6 (4Z, 7Z, 10Z, 13Z, 16Z, 19Z)] M	Oleamide
Zhou <i>et al.</i> <sup>[23]</sup>	2012	Human	Serum	LC-MS	LysoPC [20:4 (5Z, 8Z, 11Z, 14Z)] GCA, GCDCA, C16:1-CN	Tryptophan, C10:1-CN, C8-CN, C10-CN, C6-CN, LysoPC (20:5)
		HCC 30 N 30 HCC/N				LysoPC (0:0/14:0), LysoPC (20:3), LysoPC (14:0/0:0)
Yin <i>et al.</i> <sup>[41]</sup>	2009	Human	Serum	LC-MS	Taurocholic acid, GCA, bilirubin, TCDCA, GCDCA, oleic acid, taurocholic acid, carnitine, L-acetylcarnitine	Hypoxanthine, phytosphingosine, dihydrosphingosine, LPC C18:2, LPC C18:3, LPC C16:1, LPC C18:0 phytosphingosine, inosine, hypoxanthine, taurine, 6-methylnicotinic acid
		HCC 24 N 25 HCC/N				

LC-MS: Liquid chromatography-mass spectrometry; LysoPC: Lysophosphatidylcholine; LysoPE: Lysophosphatidylethanolamine; LPC: Lysophosphatidylcholine; GCA: Glycocholic acid; TCDCA: Taurochenodeoxycholic acid; GCDCA: Glycochenodeoxycholic acid; UPLC-TOFMS: Ultra-high performance liquid chromatography-time of flight-mass spectrometer; CN: C16:1-acylcarnitine; HCC: Hepatocellular carcinoma.

characteristics in serum and urine from HCV-related HCC and chronic hepatitis C patients by LC-MS. Fitian *et al.*<sup>[51]</sup> and Baniasadi *et al.*<sup>[58]</sup> also studied the diversity of serum metabolomics in patients with HCV-related HCC and LC, resulting in some potential metabolic markers with significant differences being detected. There are increasing numbers of people addicted to alcohol with the speeding pace of modern society and about 1/3 of HCC cases result from alcohol worldwide<sup>[59]</sup>. Nahon *et al.*<sup>[60]</sup> analyzed the metabolic changes of alcoholic LC and HCC by NMR and discovered that the metabolites in a group of alcoholic LC without HCC were apparently different from that of alcoholic LC with large HCC. Glutamine decreased greatly, while metabolites such as glutamate and glycoprotein increased sharply. It indicated that glutamine degradation and glycolysis might be the main metabolic pathway of energy in hepatoma cells. With the improvement of living standards and the changes in lifestyle, the incidence of non-alcoholic fatty liver disease is increasing yearly and is currently up to 30% in developed countries<sup>[61,62]</sup>. Excessive deposition of fat in the liver can cause NASH, liver fibrosis, LC and even HCC<sup>[63]</sup>. Beyoğlu *et al.*<sup>[64]</sup> specifically analyzed the research about non-alcoholic HCC in their review. Most of the research used healthy people as the control group, while a small part used patients with LC. The potential metabolic markers detected were involved in the metabolic processes of fatty acids, bile acids and so on. There are some differences between the metabolic markers found in this research and in the research on HBV-related HCC. More research is needed to find the

pathogenesis in order to provide the basis for targeted treatment of HCC of different etiologies in the future.

## PROSPECTS

Metabonomics is still in the beginning and developing stage but it has drawn wide attention from the medical community. There are some short comings in its analysis technology and data analysis methods which require further completion and improvement. At present, metabonomics is just applied to common diseases. In our review, there are some obvious metabonomic differences between HBV-related hepatic diseases and other liver diseases, which have some research value and may provide evidence for detecting specific markers and elucidating the pathogenesis of HBV-related hepatic diseases. With the continuous development of medical technology, the prospect of metabonomics is immeasurable. It is expected to develop and enhance clinical diagnosis and treatment in the future, with genomics, transcriptomics and proteomics.

## REFERENCES

- 1 Zámbo V, Simon-Szabó L, Szelényi P, Kereszturi E, Bánhegyi G, Csala M. Lipotoxicity in the liver. *World J Hepatol* 2013; **5**: 550-557 [PMID: 24179614 DOI: 10.4254/wjh.v5.i10.550]
- 2 Yang WS, Va P, Bray F, Gao S, Gao J, Li HL, Xiang YB. The role of pre-existing diabetes mellitus on hepatocellular carcinoma occurrence and prognosis: a meta-analysis of prospective cohort studies. *PLoS One* 2011; **6**: e27326 [PMID: 22205924 DOI: 10.1371/journal.pone.0027326]



- 3 **Fitzpatrick M**, Young SP. Metabolomics--a novel window into inflammatory disease. *Swiss Med Wkly* 2013; **143**: w13743 [PMID: 23348753 DOI: 10.4414/smww.2013.13743]
- 4 **Friedrich N**. Metabolomics in diabetes research. *J Endocrinol* 2012; **215**: 29-42 [PMID: 22718433 DOI: 10.1530/JOE-12-0120]
- 5 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855]
- 6 **Han YF**, Zhao J, Ma LY, Yin JH, Chang WJ, Zhang HW, Cao GW. Factors predicting occurrence and prognosis of hepatitis-B-virus-related hepatocellular carcinoma. *World J Gastroenterol* 2011; **17**: 4258-4270 [PMID: 22090781 DOI: 10.3748/wjg.v17.i38.4258]
- 7 **Churin Y**, Roderfeld M, Roeb E. Hepatitis B virus large surface protein: function and fame. *Hepatobiliary Surg Nutr* 2015; **4**: 1-10 [PMID: 25713800 DOI: 10.3978/j.issn.2304-3881.2014.12.08]
- 8 **Singal AG**, Conjeevaram HS, Volk ML, Fu S, Fontana RJ, Askari F, Su GL, Lok AS, Marrero JA. Effectiveness of hepatocellular carcinoma surveillance in patients with cirrhosis. *Cancer Epidemiol Biomarkers Prev* 2012; **21**: 793-799 [PMID: 22374994]
- 9 **Singal A**, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, Marrero JA. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. *Aliment Pharmacol Ther* 2009; **30**: 37-47 [PMID: 19392863]
- 10 **Soper R**, Himmelreich U, Painter D, Somorjai RL, Lean CL, Dolenko B, Mountford CE, Russell P. Pathology of hepatocellular carcinoma and its precursors using proton magnetic resonance spectroscopy and a statistical classification strategy. *Pathology* 2002; **34**: 417-422 [PMID: 12408339]
- 11 **Saffroy R**, Pham P, Reffas M, Takka M, Lemoine A, Debuire B. New perspectives and strategy research biomarkers for hepatocellular carcinoma. *Clin Chem Lab Med* 2007; **45**: 1169-1179 [PMID: 17635075]
- 12 **Zhu C**, Cao H, Zhou X, Dong C, Luo J, Zhang C, Liu J, Ling Y. Meta-analysis of the clinical value of danshen injection and huangqi injection in liver cirrhosis. *Evid Based Complement Alternat Med* 2013; **2013**: 842824 [PMID: 24069058 DOI: 10.1155/2013/842824]
- 13 **Yu R**, Fan R, Hou J. Chronic hepatitis B virus infection: epidemiology, prevention, and treatment in China. *Front Med* 2014; **8**: 135-144 [PMID: 24810645 DOI: 10.1007/s11684-014-0331-5]
- 14 **Sonneveld MJ**, Rijckborst V, Zeuzem S, Heathcote EJ, Simon K, Senturk H, Pas SD, Hansen BE, Janssen HL. Presence of precore and core promoter mutants limits the probability of response to peginterferon in hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2012; **56**: 67-75 [PMID: 22307831 DOI: 10.1002/hep.25636]
- 15 **Sonneveld MJ**, Hansen BE, Piratvisuth T, Jia JD, Zeuzem S, Gane E, Liaw YF, Xie Q, Heathcote EJ, Chan HL, Janssen HL. Response-guided peginterferon therapy in hepatitis B e antigen-positive chronic hepatitis B using serum hepatitis B surface antigen levels. *Hepatology* 2013; **58**: 872-880 [PMID: 23553752 DOI: 10.1002/hep.26436]
- 16 **Lampertico P**, Viganò M, Di Costanzo GG, Sagnelli E, Fasano M, Di Marco V, Boninsegna S, Farci P, Fargion S, Giuberti T, Iannaccone C, Regep L, Masetto B, Facchetti F, Colombo M. Randomised study comparing 48 and 96 weeks peginterferon  $\alpha$ -2a therapy in genotype D HBeAg-negative chronic hepatitis B. *Gut* 2013; **62**: 290-298 [PMID: 22859496 DOI: 10.1136/gutjnl-2011-301430]
- 17 **Kitrinos KM**, Corsa A, Liu Y, Flaherty J, Snow-Lampart A, Marcellin P, Borroto-Esoda K, Miller MD. No detectable resistance to tenofovir disoproxil fumarate after 6 years of therapy in patients with chronic hepatitis B. *Hepatology* 2014; **59**: 434-442 [PMID: 23939953]
- 18 **Marcellin P**, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, Washington MK, Germanidis G, Flaherty JF, Aguilar Schall R, Bornstein JD, Kitrinos KM, Subramanian GM, McHutchison JG, Heathcote EJ. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* 2013; **381**: 468-475 [PMID: 23234725 DOI: 10.1016/S0140-6736(12)61425-1]
- 19 **Tenney DJ**, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, Wichroski MJ, Xu D, Yang J, Wilber RB, Colonno RJ. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009; **49**: 1503-1514 [PMID: 19280622 DOI: 10.1002/hep.22841]
- 20 **Hakim MS**, Spaan M, Janssen HL, Boonstra A. Inhibitory receptor molecules in chronic hepatitis B and C infections: novel targets for immunotherapy? *Rev Med Virol* 2014; **24**: 125-138 [PMID: 24757728]
- 21 **Wang L**, Zou ZQ, Liu CX, Liu XZ. Immunotherapeutic interventions in chronic hepatitis B virus infection: a review. *J Immunol Methods* 2014; **407**: 1-8 [PMID: 24747918 DOI: 10.1016/j.jim.2014.04.004]
- 22 **Atanley E**, van Drunen Littel-van den Hurk S. Future considerations for dendritic cell immunotherapy against chronic viral infections. *Expert Rev Clin Immunol* 2014; **10**: 801-813 [PMID: 24734867 DOI: 10.1586/1744666X.2014.907742]
- 23 **Zhou L**, Wang Q, Yin P, Xing W, Wu Z, Chen S, Lu X, Zhang Y, Lin X, Xu G. Serum metabolomics reveals the deregulation of fatty acids metabolism in hepatocellular carcinoma and chronic liver diseases. *Anal Bioanal Chem* 2012; **403**: 203-213 [PMID: 22349331 DOI: 10.1007/s00216-012-5782-4]
- 24 **Soga T**, Sugimoto M, Honma M, Mori M, Igarashi K, Kashiura K, Ikeda S, Hirayama A, Yamamoto T, Yoshida H, Otsuka M, Tsuji S, Yatomi Y, Sakuragawa T, Watanabe H, Nihei K, Saito T, Kawata S, Suzuki H, Tomita M, Suematsu M. Serum metabolomics reveals  $\gamma$ -glutamyl dipeptides as biomarkers for discrimination among different forms of liver disease. *J Hepatol* 2011; **55**: 896-905 [PMID: 21334394 DOI: 10.1016/j.jhep.2011.01.031]
- 25 **Wang JB**, Pu SB, Sun Y, Li ZF, Niu M, Yan XZ, Zhao YL, Wang LF, Qin XM, Ma ZJ, Zhang YM, Li BS, Luo SQ, Gong M, Sun YQ, Zou ZS, Xiao XH. Metabolomic Profiling of Autoimmune Hepatitis: The Diagnostic Utility of Nuclear Magnetic Resonance Spectroscopy. *J Proteome Res* 2014; Epub ahead of print [PMID: 24940827]
- 26 **Torres DM**, Williams CD, Harrison SA. Features, diagnosis, and treatment of nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2012; **10**: 837-858 [PMID: 22446927 DOI: 10.1016/j.cgh.2012.03.011]
- 27 **Kalhan SC**, Guo L, Edmison J, Dasarthy S, McCullough AJ, Hanson RW, Milburn M. Plasma metabolomic profile in nonalcoholic fatty liver disease. *Metabolism* 2011; **60**: 404-413 [PMID: 20423748 DOI: 10.1016/j.metabol.2010.03.006]
- 28 **Barr J**, Vázquez-Chantada M, Alonso C, Pérez-Cormenzana M, Mayo R, Galán A, Caballería J, Martín-Duce A, Tran A, Wagner C, Luka Z, Lu SC, Castro A, Le Marchand-Brustel Y, Martínez-Chantar ML, Veyrie N, Clément K, Tordjman J, Gual P, Mato JM. Liquid chromatography-mass spectrometry-based parallel metabolic profiling of human and mouse model serum reveals putative biomarkers associated with the progression of nonalcoholic fatty liver disease. *J Proteome Res* 2010; **9**: 4501-4512 [PMID: 20684516 DOI: 10.1021/pr1002593]
- 29 **Li H**, Wang L, Yan X, Liu Q, Yu C, Wei H, Li Y, Zhang X, He F, Jiang Y. A proton nuclear magnetic resonance metabonomics approach for biomarker discovery in nonalcoholic fatty liver disease. *J Proteome Res* 2011; **10**: 2797-2806 [PMID: 21563774 DOI: 10.1021/pr200047c]
- 30 **Semmo N**, Weber T, Idle JR, Beyoğlu D. Metabolomics reveals that aldose reductase activity due to AKR1B10 is upregulated in hepatitis C virus infection. *J Viral Hepat* 2015; **22**: 617-624 [PMID: 25487531]
- 31 **Rachakonda V**, Gabbert C, Raina A, Bell LN, Cooper S, Malik S, Behari J. Serum metabolomic profiling in acute alcoholic hepatitis identifies multiple dysregulated pathways. *PLoS One* 2014; **9**: e113860 [PMID: 25461442 DOI: 10.1371/journal.pone.0113860]
- 32 **Patel M**, Shariff MI, Ladep NG, Thillainayagam AV, Thomas HC, Khan SA, Taylor-Robinson SD. Hepatocellular carcinoma:

- diagnostics and screening. *J Eval Clin Pract* 2012; **18**: 335-342 [PMID: 21114800 DOI: 10.1111/j.1365-2753.2010.01599.x]
- 33 **Ott JJ**, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine* 2012; **30**: 2212-2219 [PMID: 22273662 DOI: 10.1016/j.vaccine.2011.12.116]
- 34 **Qi SW**, Tu ZG, Peng WJ, Wang LX, Ou-Yang X, Cai AJ, Dai Y. <sup>1</sup>H NMR-based serum metabolic profiling in compensated and decompensated cirrhosis. *World J Gastroenterol* 2012; **18**: 285-290 [PMID: 22294833 DOI: 10.3748/wjg.v18.i3.285]
- 35 **Fattovich G**, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol* 2008; **48**: 335-352 [PMID: 18096267]
- 36 **Peng CY**, Chien RN, Liaw YF. Hepatitis B virus-related decompensated liver cirrhosis: benefits of antiviral therapy. *J Hepatol* 2012; **57**: 442-450 [PMID: 22504333 DOI: 10.1016/j.jhep.2012.02.033]
- 37 **Yang JD**, Kim WR, Coelho R, Mettler TA, Benson JT, Sanderson SO, Therneau TM, Kim B, Roberts LR. Cirrhosis is present in most patients with hepatitis B and hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2011; **9**: 64-70 [PMID: 20831903 DOI: 10.1016/j.cgh.2010.08.019]
- 38 **Mittal S**, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. *J Clin Gastroenterol* 2013; **47** Suppl: S2-S6 [PMID: 23632345 DOI: 10.1097/MCG.0b013e3182872f29]
- 39 **Wang X**, Wang X, Xie G, Zhou M, Yu H, Lin Y, Du G, Luo G, Jia W, Liu P. Urinary metabolite variation is associated with pathological progression of the post-hepatitis B cirrhosis patients. *J Proteome Res* 2012; **11**: 3838-3847 [PMID: 22624806 DOI: 10.1021/pr300337s]
- 40 **Xue R**, Dong L, Wu H, Liu T, Wang J, Shen X. Gas chromatography/mass spectrometry screening of serum metabolomic biomarkers in hepatitis B virus infected cirrhosis patients. *Clin Chem Lab Med* 2009; **47**: 305-310 [PMID: 19676142 DOI: 10.1515/CCLM.2009.083]
- 41 **Yin P**, Wan D, Zhao C, Chen J, Zhao X, Wang W, Lu X, Yang S, Gu J, Xu G. A metabonomic study of hepatitis B-induced liver cirrhosis and hepatocellular carcinoma by using RP-LC and HILIC coupled with mass spectrometry. *Mol Biosyst* 2009; **5**: 868-876 [PMID: 19603122 DOI: 10.1039/b820224a]
- 42 **Liu Y**, Hong Z, Tan G, Dong X, Yang G, Zhao L, Chen X, Zhu Z, Lou Z, Qian B, Zhang G, Chai Y. NMR and LC/MS-based global metabolomics to identify serum biomarkers differentiating hepatocellular carcinoma from liver cirrhosis. *Int J Cancer* 2014; **135**: 658-668 [PMID: 24382646 DOI: 10.1002/ijc.28706]
- 43 **Tabibian JH**, Talwalkar JA, Lindor KD. Role of the microbiota and antibiotics in primary sclerosing cholangitis. *Biomed Res Int* 2013; **2013**: 389537 [PMID: 24232746 DOI: 10.1155/2013/389537]
- 44 **Trottier J**, Bialek A, Caron P, Straka RJ, Heathcote J, Milkiewicz P, Barbier O. Metabolomic profiling of 17 bile acids in serum from patients with primary biliary cirrhosis and primary sclerosing cholangitis: a pilot study. *Dig Liver Dis* 2012; **44**: 303-310 [PMID: 22169272 DOI: 10.1016/j.dld.2011.10.025]
- 45 **Bell LN**, Wulff J, Comerford M, Vuppalanchi R, Chalasani N. Serum metabolic signatures of primary biliary cirrhosis and primary sclerosing cholangitis. *Liver Int* 2015; **35**: 263-274 [PMID: 25181933 DOI: 10.1111/liv.12680]
- 46 **Jalan R**, Gines P, Olson JC, Mookerjee RP, Moreau R, Garcia-Tsao G, Arroyo V, Kamath PS. Acute-on chronic liver failure. *J Hepatol* 2012; **57**: 1336-1348 [PMID: 22750750 DOI: 10.1016/j.jhep.2012.06.026]
- 47 **Amathieu R**, Nahon P, Triba M, Bouchemal N, Trinchet JC, Beaugrand M, Dhonneur G, Le Moyec L. Metabolomic approach by <sup>1</sup>H NMR spectroscopy of serum for the assessment of chronic liver failure in patients with cirrhosis. *J Proteome Res* 2011; **10**: 3239-3245 [PMID: 21568267 DOI: 10.1021/pr200265z]
- 48 **Amathieu R**, Triba MN, Nahon P, Bouchemal N, Kamoun W, Haouache H, Trinchet JC, Savarin P, Le Moyec L, Dhonneur G. Serum <sup>1</sup>H-NMR metabolomic fingerprints of acute-on-chronic liver failure in intensive care unit patients with alcoholic cirrhosis. *PLoS One* 2014; **9**: e89230 [PMID: 24586615 DOI: 10.1371/journal.pone.0089230]
- 49 **Nie CY**, Han T, Zhang L, Li Y, Liu H, Xiao SX, Li Y, Kang H, Liu SY. Cross-sectional and dynamic change of serum metabolite profiling for Hepatitis B-related acute-on-chronic liver failure by UPLC/MS. *J Viral Hepat* 2014; **21**: 53-63 [PMID: 24329857 DOI: 10.1111/jvh.12122]
- 50 **Lian JS**, Liu W, Hao SR, Guo YZ, Huang HJ, Chen DY, Xie Q, Pan XP, Xu W, Yuan WX, Li LJ, Huang JR. A serum metabonomic study on the difference between alcohol- and HBV-induced liver cirrhosis by ultraperformance liquid chromatography coupled to mass spectrometry plus quadrupole time-of-flight mass spectrometry. *Chin Med J (Engl)* 2011; **124**: 1367-1373 [PMID: 21740750]
- 51 **Fitian AI**, Nelson DR, Liu C, Xu Y, Ararat M, Cabrera R. Integrated metabolomic profiling of hepatocellular carcinoma in hepatitis C cirrhosis through GC/MS and UPLC/MS-MS. *Liver Int* 2014; **34**: 1428-1444 [PMID: 24661807 DOI: 10.1111/liv.12541]
- 52 **Liu SY**, Zhang RL, Kang H, Fan ZJ, Du Z. Human liver tissue metabolic profiling research on hepatitis B virus-related hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 3423-3432 [PMID: 23801834 DOI: 10.3748/wjg.v19.i22.3423]
- 53 **Tan Y**, Yin P, Tang L, Xing W, Huang Q, Cao D, Zhao X, Wang W, Lu X, Xu Z, Wang H, Xu G. Metabolomics study of stepwise hepatocarcinogenesis from the model rats to patients: potential biomarkers effective for small hepatocellular carcinoma diagnosis. *Mol Cell Proteomics* 2012; **11**: M111.010694 [PMID: 22084000 DOI: 10.1074/mcp.M111.010694]
- 54 **Chen F**, Xue J, Zhou L, Wu S, Chen Z. Identification of serum biomarkers of hepatocarcinoma through liquid chromatography/mass spectrometry-based metabonomic method. *Anal Bioanal Chem* 2011; **401**: 1899-1904 [PMID: 21833635 DOI: 10.1007/s00216-011-5245-3]
- 55 **Chen T**, Xie G, Wang X, Fan J, Qiu Y, Zheng X, Qi X, Cao Y, Su M, Wang X, Xu LX, Yen Y, Liu P, Jia W. Serum and urine metabolite profiling reveals potential biomarkers of human hepatocellular carcinoma. *Mol Cell Proteomics* 2011; **10**: M110.004945 [PMID: 21518826 DOI: 10.1074/mcp.M110.004945]
- 56 **Li H**, Zhu W, Zhang L, Lei H, Wu X, Guo L, Chen X, Wang Y, Tang H. The metabolic responses to hepatitis B virus infection shed new light on pathogenesis and targets for treatment. *Sci Rep* 2015; **5**: 8421 [PMID: 25672227 DOI: 10.1038/srep08421]
- 57 **Bowers J**, Hughes E, Skill N, Maluccio M, Raftery D. Detection of hepatocellular carcinoma in hepatitis C patients: biomarker discovery by LC-MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2014; **966**: 154-162 [PMID: 24666728 DOI: 10.1016/j.jchromb.2014.02.043]
- 58 **Baniasadi H**, Gowda GA, Gu H, Zeng A, Zhuang S, Skill N, Maluccio M, Raftery D. Targeted metabolic profiling of hepatocellular carcinoma and hepatitis C using LC-MS/MS. *Electrophoresis* 2013; **34**: 2910-2917 [PMID: 23856972 DOI: 10.1002/elps.201300029]
- 59 **French SW**. Epigenetic events in liver cancer resulting from alcoholic liver disease. *Alcohol Res* 2013; **35**: 57-67 [PMID: 24313165]
- 60 **Nahon P**, Amathieu R, Triba MN, Bouchemal N, Nault JC, Zioli M, Seror O, Dhonneur G, Trinchet JC, Beaugrand M, Le Moyec L. Identification of serum proton NMR metabolomic fingerprints associated with hepatocellular carcinoma in patients with alcoholic cirrhosis. *Clin Cancer Res* 2012; **18**: 6714-6722 [PMID: 23136190 DOI: 10.1158/1078-0432.CCR-12-1099]
- 61 **Vernon G**, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]
- 62 **Patel NS**, Peterson MR, Lin GY, Feldstein A, Schnabl B, Bettencourt R, Seki E, Sirlin CB, Loomba R. Insulin Resistance Increases MRI-Estimated Pancreatic Fat in Nonalcoholic Fatty Liver Disease and Normal Controls. *Gastroenterol Res Pract* 2013;

- 2013; 498296 [PMID: 24348536 DOI: 10.1155/2013/498296]
- 63 **Karandish M**, Tamimi M, Shayesteh AA, Haghighizadeh MH, Jalali MT. The effect of magnesium supplementation and weight loss on liver enzymes in patients with nonalcoholic fatty liver disease. *J Res Med Sci* 2013; **18**: 573-579 [PMID: 24516489]
- 64 **Beyoğlu D**, Idle JR. The metabolomic window into hepatobiliary disease. *J Hepatol* 2013; **59**: 842-858 [PMID: 23714158 DOI: 10.1016/j.jhep.2013.05.030]

**P- Reviewer:** Ito H **S- Editor:** Ji FF  
**L- Editor:** Roemmele A **E- Editor:** Liu SQ



## 2016 Advances in Hepatitis C Virus

# Chaperones in hepatitis C virus infection

Ronik Khachatoorian, Samuel W French

Ronik Khachatoorian, Samuel W French, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at University of California, Los Angeles, CA 90095, United States

Samuel W French, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at University of California, Los Angeles, CA 90095, United States

Samuel W French, UCLA AIDS Institute, David Geffen School of Medicine at University of California, Los Angeles, CA 90024, United States

**Author contributions:** Khachatoorian R conducted an extensive article search, identified all relevant articles, developed the outline of the review article, prepared all the drafts, revised the accepted manuscript, and approved the article to be published; French SW contributed to the design and writing of the manuscript, reviewed and edited the drafts, revised the accepted manuscript, and approved the article to be published.

Supported by NIH R01DK090794, SWF.

**Conflict-of-interest statement:** The authors declare no conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Samuel W French, MD, PhD, Associate Professor, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at University of California, 10833 Le Conte Avenue, Los Angeles, CA 90095, United States. [sfrench@mednet.ucla.edu](mailto:sfrench@mednet.ucla.edu)  
 Telephone: +1-310-2672795  
 Fax: +1-310-2672058

Received: April 29, 2015  
 Peer-review started: May 7, 2015

First decision: September 8, 2015

Revised: October 1, 2015

Accepted: December 17, 2015

Article in press: December 18, 2015

Published online: January 8, 2016

## Abstract

The hepatitis C virus (HCV) infects approximately 3% of the world population or more than 185 million people worldwide. Each year, an estimated 350000-500000 deaths occur worldwide due to HCV-associated diseases including cirrhosis and hepatocellular carcinoma. HCV is the most common indication for liver transplantation in patients with cirrhosis worldwide. HCV is an enveloped RNA virus classified in the genus *Hepacivirus* in the *Flaviviridae* family. The HCV viral life cycle in a cell can be divided into six phases: (1) binding and internalization; (2) cytoplasmic release and uncoating; (3) viral polyprotein translation and processing; (4) RNA genome replication; (5) encapsidation (packaging) and assembly; and (6) virus morphogenesis (maturation) and secretion. Many host factors are involved in the HCV life cycle. Chaperones are an important group of host cytoprotective molecules that coordinate numerous cellular processes including protein folding, multimeric protein assembly, protein trafficking, and protein degradation. All phases of the viral life cycle require chaperone activity and the interaction of viral proteins with chaperones. This review will present our current knowledge and understanding of the role of chaperones in the HCV life cycle. Analysis of chaperones in HCV infection will provide further insights into viral/host interactions and potential therapeutic targets for both HCV and other viruses.

**Key words:** Hepatitis C; Hepatitis C virus; Chaperones; Heat shock proteins; Viral life cycle

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.



**Core tip:** Interaction of viral proteins with host chaperones is critical for the hepatitis C viral (HCV) life cycle. Some of these chaperones, such as cyclophilins have been studied in detail recently and have led to the advent of new therapies for HCV infection with high success rates. Further investigation of the role of chaperones in the viral life cycle may allow for development of novel therapies both for HCV and related viruses.

Khachatoorian R, French SW. Chaperones in hepatitis C virus infection. *World J Hepatol* 2016; 8(1): 9-35 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i1/9.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i1.9>

## INTRODUCTION

The hepatitis C virus (HCV) infects approximately 3% of the world population or more than 185 million people worldwide<sup>[1,2]</sup>. While infection is less prevalent in developed countries including North America, other areas face prevalence rates as high as 3.5% or more<sup>[1]</sup>. Each year, an estimated 350000-500000 deaths occur worldwide due to HCV-associated diseases<sup>[1-3]</sup>. HCV is mainly responsible for liver transplantation in patients with cirrhosis worldwide<sup>[4-6]</sup>. Furthermore, HCV is the most common chronic bloodborne pathogen in the United States affecting 1.5% of the population and is the major etiologic factor responsible for the recent doubling of hepatocellular carcinoma<sup>[5,7-9]</sup>.

HCV is an enveloped RNA virus classified in the genus *Hepacivirus* in the *Flaviviridae* family. It possesses an approximately 9.6 kb positive-sense RNA genome that is translated as a single polypeptide approximately 3000 amino acids in length<sup>[10,11]</sup>. It is subsequently proteolytically cleaved into 10 viral proteins including the structural proteins core, E1, and E2 as well as the non-structural (NS) proteins p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B<sup>[12]</sup>. Core is the viral nucleocapsid protein that encapsidates the viral genome in the virion. E1 and E2 are glycoproteins on the viral envelope that are involved in receptor-mediated viral entry. p7 is an integral membrane ion channel also called viroporin that functions to protect virions from acidification during maturation by allowing protons to flow<sup>[13]</sup>. NS2, NS3, and NS4A are the viral proteases, while NS4B is a helicase. NS5A, a 56-59 kDa multifunctional phosphoprotein, lacks any known enzymatic activity, is a component of the viral replicase complex, and has been implicated in regulation of HCV genome replication, internal ribosomal entry site (IRES)-mediated viral protein translation, and infectious virion assembly<sup>[14-18]</sup>. NS5B is the viral RNA-dependent RNA polymerase. In addition to these originally identified 10 proteins, another viral protein called the HCV F protein was observed<sup>[19,20]</sup> and later identified<sup>[21-23]</sup> to be expressed as a result of a ribosomal

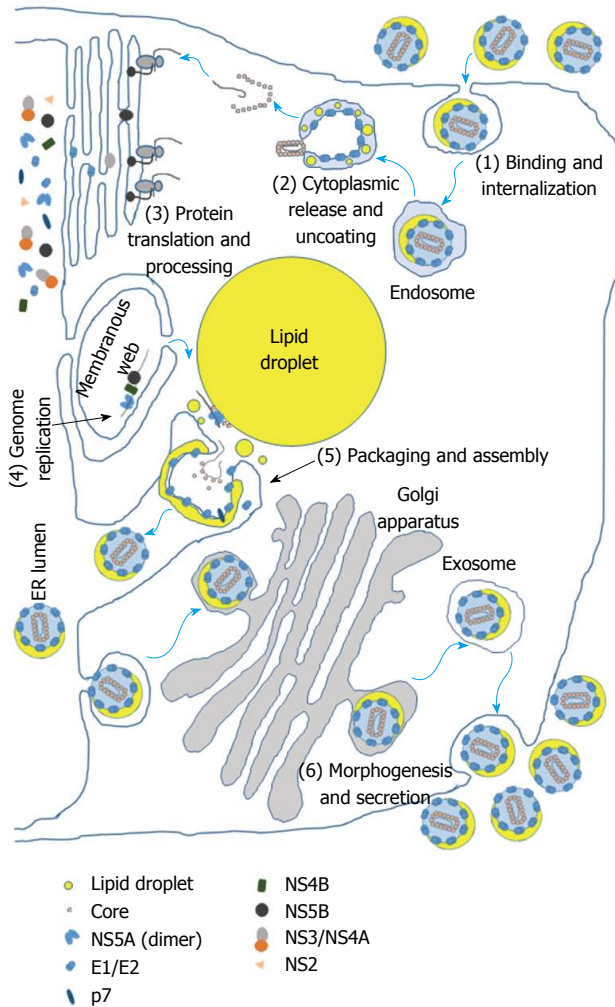
frameshift near the beginning of the core protein coding sequence. The F protein has been implicated in the regulation of protein degradation, inhibition of apoptosis, and immunoregulation<sup>[24-31]</sup>.

The 5' non-coding region (NCR) of the viral genome possesses an IRES, a cis-acting element found in some host RNA transcripts as well as in viruses that allows ribosomal translation initiation to occur internally within a transcript in lieu of 5' 7-methylguanylate cap-dependent translation<sup>[12,32]</sup>. The HCV viral life cycle in a cell can be divided into six phases: (1) binding and internalization; (2) cytoplasmic release and uncoating; (3) viral polyprotein translation and processing; (4) RNA genome replication; (5) encapsidation (packaging) and assembly; and (6) virus morphogenesis (maturation) and secretion<sup>[33]</sup> (Figure 1).

The viral life cycle begins with the attachment of the enveloped virion to the cell followed by entry, which is mediated by interaction of the E1 and E2 glycoproteins in the viral membrane with a number of hepatocyte cell surface receptors and proteins which include the low-density lipoprotein receptor (LDLR), glycosaminoglycans (GAGs), CD81, scavenger receptor B1 (SR-B1), claudin 1, occludin, and the cholesterol absorption receptor Niemann-Pick C1-like 1<sup>[34]</sup>. Subsequently, the viral particle is internalized through clathrin-mediated endocytosis or an alternative clathrin-independent pathway after which, the viral and cellular membranes fuse through acidification of the endosomal compartment, and the core-encapsidated viral genome is released into the cytosol, uncoated, and subsequently translated<sup>[35,36]</sup>. The resulting polyprotein is cleaved with the help of the cellular proteases signalase and signal peptide peptidase and the viral proteases NS2-NS3 and NS3-NS4A<sup>[37]</sup>. Viral genome replication is carried out by NS5B utilizing a negative-sense viral genome intermediate<sup>[38]</sup>. New virions are assembled at the sites of cytosolic lipid droplets in the vicinity of endoplasmic reticulum (ER) membrane where core protein encapsidates the viral genome followed by budding of the nascent virion into the lumen of ER<sup>[39]</sup>. The virions follow the Golgi-dependent secretory pathway during which they undergo maturation by addition of lipid components significantly decreasing their buoyant density<sup>[40,41]</sup>. Finally the mature virions are secreted through exocytosis<sup>[42]</sup>.

In order to establish successful infection, HCV depends on numerous host factors during its entire life cycle. In addition to performing virus-specific functions such as viral genome replication and virion assembly, HCV proteins alter cellular metabolism, critical signaling pathways, and organellar morphology and function to establish persistent infection and to escape the immune responses. Accumulation of misfolded viral proteins in the ER leads to ER stress and the unfolded protein response (UPR) which is a cellular program to help restore ER protein homeostasis by shutting down cellular protein synthesis, properly folding the misfolded proteins, targeting them to ER-associated degradation (ERAD) if folding is unsuccessful, and inducing apoptosis if the





**Figure 1 A schematic of the hepatitis C virus life cycle.** The six steps of the viral life cycle are indicated in colored boxes with numbers. (1) Binding and internalization. HSC70 is part of the viral particle and may play a role in viral entry. Also HCV internalization occurs at least in part through clathrin-mediated endocytosis which involves HSC70; (2) Cytoplasmic release and uncoating. The chaperone activity of E1 and E2 may be involved in membrane fusion that releases the core-encapsulated viral genome into the cytosol; (3) Protein translation and processing. HSP70, together with the DNAJA2 member of HSP40 co-chaperones, is the main chaperone involved in IRES-mediated translation of the viral genome, while HSP90 may play some role as well. Calnexin, calreticulin, and CypA are also involved; (4) Genome replication. HSP90, some members of HSP40 co-chaperones, TRiC/CCT, FKBP38, SigR1, and some Cyps are involved in viral genome replication. Core and NS3 may play some roles in genome replication as well; (5) Packaging and assembly. HSC70, PDI, and MTTP are the principal chaperones involved in infectious virion assembly, and Cyps also play important roles; and (6) Morphogenesis and secretion. MTTP which is involved in the VLDL pathway also plays important roles in viral particle maturation and secretion. Cyps are also involved. Cyp: Cyclophilin; ER: Endoplasmic reticulum; FKBP: FK506-binding protein; HCV: Hepatitis C virus; HSC70: Heat shock cognate protein 70; HSP: Heat shock protein; MTTP: Microsomal triglyceride transfer protein; NS: Non-structural; PDI: Protein disulfide isomerase; SigR1: Sigma non-opioid intracellular receptor 1; TRiC/CCT: TCP-1 ring complex/chaperonin-containing TCP-1; VLDL: Very low-density lipoprotein.

cell cannot cope with the ER stress<sup>[43]</sup>. HCV suppresses ERAD and apoptosis thereby maintaining cells under ER stress in order to persistently produce its own proteins. However, HCV maintains a balance between ER stress and the UPR and virus production through

different mechanisms some of which are presented in this review<sup>[44-46]</sup>. Additionally, HCV replication in cells disrupts mitochondrial homeostasis leading to formation of irregular mitochondrial morphology, overproduction of reactive oxygen species (ROS), and oxidative stress<sup>[47]</sup>. Oxidative stress leads to activation of antioxidant programs to cope with the stress, and if unsuccessful, apoptosis is triggered. As is the case with ER stress, HCV not only induces oxidative stress, but also activates antioxidant programs and suppresses mitochondria-induced apoptosis<sup>[44,47,48]</sup>. Again, this leads to persistent infection and benefits virus production<sup>[44]</sup>. Thus, while HCV infection and some viral proteins may be capable of inducing apoptosis<sup>[49-51]</sup>, it is generally agreed that apoptosis is effectively suppressed during infection. A few mechanisms that HCV utilizes to suppress apoptosis are also discussed in this review.

Virus infection of hepatocytes leads to rearrangements of ER membranes to generate double-membrane vesicles (DMVs) and to a lesser extent multi-membrane vesicles that are collectively referred to as the membranous web<sup>[52]</sup>. Viral genome replication occurs within the membranous web in replication complexes (RCs). Infection by all positive-strand RNA viruses results in the formation of membranous web. It is thought that the membranous web benefits viral replication by: (1) protecting viral RNA and proteins from degradation and intracellular antiviral defense; (2) increasing the local concentration of the factors involved in RNA replication; and (3) ensuring spatial proximity of viral RNA translation, viral genome replication, and virion assembly for efficient progression through the viral life cycle<sup>[39]</sup>.

HCV also hijacks the hepatocyte very low-density lipoprotein (VLDL) pathway for the maturation and secretion of infectious viral particles<sup>[53]</sup>. Lipid secretion is reduced during infection, and maturing viral particles acquire VLDL characteristics, while secreted viral particles are bound to VLDL particles<sup>[40,54]</sup>.

An important group of host factors intimately involved in essentially all steps of the HCV life cycle are molecular chaperones. The term chaperone reflects the significant role of these cytoprotective proteins in: (1) assisting client proteins to achieve native/functional conformation that is required for their function; (2) assembling/disassembling protein subunits; (3) preventing newly synthesized proteins or assembled protein subunits from forming nonfunctional aggregates and molecular crowding; (4) transporting proteins to particular subcellular compartments which is referred to as intracellular protein trafficking; and (5) targeting proteins to degradation if attempts to (re)fold or (re)assemble are not successful<sup>[55-57]</sup>. Newly synthesized proteins are assisted to fold properly by chaperones. Under stress conditions such as heat shock or viral infection, proteins can become misfolded, and chaperones attempt to refold such proteins. If folding is not successful, the protein gets targeted for proteasome-mediated degradation.

A large number of molecular chaperones belong

to the family of heat shock proteins (HSPs) originally identified as proteins that helped refolding proteins that were denatured as a result of heat stress<sup>[58]</sup>. HSPs are a highly evolutionarily conserved family of proteins that are typically classified into four different systems based on their molecular weight: HSP70, HSP90, HSP60, and small HSPs<sup>[57]</sup>. The HSP70, HSP90, and HSP60 systems consist of the adenosine triphosphate (ATP)-dependent main chaperones that utilize their enzymatic activity to induce conformational changes in the client polypeptide by hydrolyzing ATP to adenosine diphosphate (ADP). In addition, a number of co-chaperones may assist and regulate the activity of the main chaperones. Small HSPs, on the other hand, do not possess enzymatic activity, and instead, perform their chaperone function by functioning as holdases, *i.e.*, binding to client polypeptides, preventing their aggregation, and directing them to one of the ATP-dependent HSPs.

HCV has evolved a remarkable ability to interact with numerous chaperones to coordinate the diverse molecular systems and pathways that it requires for its propagation in hepatocytes (Table 1). This review presents our current knowledge and understanding of the chaperones that are involved in the HCV life cycle. First, HSPs are presented covering all four HSP systems HSP70, HSP90, HSP60, and small HSPs. Next, a diverse group of other molecular chaperones are discussed including BAG3, FK506-binding proteins (FKBPs), p23, prefoldin, apolipoprotein J [apoJ or clusterin (CLU)], protein disulfide isomerases (PDIs), microsomal triglyceride transfer protein (MTTP), calnexin (CANX), calreticulin (CALR), "endoplasmic reticulum degradation enhancer, mannosidase alpha-like 1" (EDEM1), EDEM3, sigma non-opioid intracellular receptor 1 (SigR1), prohibitin (PHB), and cyclophilins (CyPs). Finally the chaperone activity of the HCV proteins core, E1, E2, NS3, and NS4A are described. The gene names for the chaperones are also included in parentheses.

## HSP70/HSP40 SYSTEM

The HSP70 family of chaperones consists of a large number of proteins that are ubiquitously expressed throughout the cell. They play important roles in proper protein folding, protection of proteins from stress-induced damage, recovery/renaturing of damaged/aggregated proteins, protein degradation, protein translocation, and disassembly of protein complexes such as the DNA replication machinery<sup>[59,60]</sup>. This family of HSPs typically functions as a group of three proteins where the main HSP70 chaperone interacts with the client polypeptide through its substrate-binding domain (SBD), while the nucleotide-binding domain (NBD) binds to an ATP hydrolyzing it to ADP to induce conformational changes in SBD for its chaperone function. The hydrolysis is stimulated by substrate binding the chaperone resulting in a closed state where it tightly binds the substrate and helps with (re)folding it. Cofactor HSPs also known as co-chaperones, such as HSP40, typically interact with the

NBD to modulate chaperone activity and to determine the clients of HSP70s *via* their specificity in binding particular target proteins. A nucleotide exchange factor (NEF) assists with the removal of hydrolyzed ADP which causes the chaperone to revert to its open conformation releasing the substrate.

### HSP70 (HSPA1A)

HSP70 is an inducible chaperone that is expressed in conditions of stress such as heat shock and viral infection. HSP70 has been identified as one of the numerous host factors important for HCV production<sup>[61-64]</sup>. Knockdown of HSP70 led to decreased virus production<sup>[61,63]</sup> or replication in subgenomic replicon (SGR) systems<sup>[62,63]</sup>. Both HSP70 overexpression and autoantibodies against HSP70 in the sera of HCV-infected patients have also been reported<sup>[65]</sup>. Huh7 cells harboring an HCV SGR demonstrated upregulation of HSP70<sup>[66]</sup>. It was also found that expression of NS5A alone in huh7 cells was sufficient for upregulation of HSP70<sup>[67]</sup>. This upregulation was the result of NS5A-induced increased levels of nuclear factor of activated T cells 5 (NFAT5), one of the transcription factors responsible for HSP70 expression. The increased NFAT5 levels itself is mediated by NS5A-driven ROS production.

Our laboratory has shown NS5A to colocalize with HSP70 and HSP40 as well<sup>[63]</sup>. We further showed that knockdown of HSP70 inhibited NS5A-augmented IRES-mediated translation. The HSP synthesis inhibitor quercetin, a bioflavonoid, also suppressed the NS5A-augmented IRES-mediated translation<sup>[63,68]</sup>. In addition, we demonstrated that the NS5A/HSP70 interaction is direct and identified the site of NS5A/HSP70 interaction on NS5A to be a hairpin moiety at the C terminus of NS5A domain I<sup>[17]</sup>. Treatment of cells with a synthetic peptide corresponding to this hairpin moiety, which we termed the HSP-binding domain<sup>[69]</sup>, disrupted the NS5A/HSP70 interaction and suppressed NS5A-augmented IRES-mediated translation and virus production<sup>[17]</sup>. Others have shown that overexpression of HSP70 leads to increased viral RNA and protein levels, while knockdown of HSP70 has the opposite effect<sup>[64]</sup>. HSP70 was found to interact with NS3-NS4A protein and NS5B as well. HSP70 increases RC formation by interacting with viral proteins in RCs, increasing the stability of viral proteins, and enhancing NS5A-driven viral IRES-mediated translation. Further, HSP70 was found to interact with the 3' NCR of the viral genome<sup>[70]</sup>.

### Heat shock cognate protein 70 (HSPA8)

Heat shock cognate protein 70 (HSC70) is a constitutively-expressed housekeeping gene with diverse cellular functions including protein folding, signal transduction, apoptosis, autophagy, and many others<sup>[71]</sup>. Viral entry occurs at least in part through the HSC70-dependent clathrin-mediated endocytosis<sup>[35]</sup>. HSC70 activity was found to be significantly increased in an HCV SGR system<sup>[72]</sup>, and HSC70 levels were increased in a proteomic analysis of RCs<sup>[73]</sup>. HSC70 was also identified

**Table 1** Chaperones and their roles in the hepatitis C virus viral life cycle

Chaperone	Subcellular localization	Function in HCV infection/stage of viral life cycle
HSP70 family		
GRP75 (HSPA9)	Mitochondrial	Varied expression/activity <sup>[66,72]</sup> Interacts with NS5A <sup>[105]</sup>
GRP78 (HSPA5)	ER	Regulation of viral protein homeostasis and maintaining a balance between viral and cellular translation to prevent viral protein overload (involves induction of ER stress and the UPR) <sup>[43,85-96]</sup> Increased expression and activity <sup>[72,85,88,93,95]</sup>
HSC70 (HSPA8)	Cytosolic	Associated with the viral genome <sup>[70,76]</sup> Infectious virion assembly <sup>[118,74]</sup> Potentially contributes to stability of virion structure and viral entry through clathrin-mediated endocytosis <sup>[35,74]</sup> Associated with the viral genome <sup>[70,76]</sup> Increased expression and activity <sup>[72,73]</sup>
HSP70 (HSPA1A)	Cytosolic	Knockdown decreases lipid droplet size and virion assembly <sup>[118,74]</sup> IRES-mediated translation of viral genome <sup>[117,63,64,68,69]</sup> Increased expression <sup>[65-67]</sup>
HSP70B' (HSPA6)	Cytosolic	Knockdown decreases IRES activity and virus production <sup>[61,63]</sup> Associated with 3' NCR of HCV genome <sup>[70]</sup>
HSP40 family		
DNAJA1	Cytosolic	Co-immunoprecipitates with NS3-NS4A <sup>[105]</sup>
DNAJA2	Cytosolic	IRES-mediated translation of viral genome <sup>[63]</sup>
DNAJA3	Mitochondrial	Potentially HCV-induced mitochondrial dysfunction <sup>[61,127]</sup>
DNAJB1	Cytosolic	Potentially regulates apoptosis <sup>[61,117]</sup> Knockdown decreases virus production <sup>[61]</sup>
DNAJB6	Cytosolic	Potentially viral RNA replication <sup>[105]</sup> Interacts with NS5B <sup>[105]</sup> Potentially overexpressed <sup>[108]</sup> knockdown decreases viral RNA replication <sup>[105]</sup>
DNAJB9	ER	Potentially regulates apoptosis <sup>[124]</sup> Varied expression <sup>[108]</sup>
DNAJC1	ER	Interacts with E1 and E2 <sup>[107]</sup>
DNAJC7	Cytosolic	Potentially regulates apoptosis <sup>[118]</sup> Co-immunoprecipitates with NS3-NS4A <sup>[105]</sup>
DNAJC8	Cytosolic	Upregulated <sup>[119]</sup>
DNAJC10	ER	ER protein homeostasis likely benefiting virus production <sup>[126]</sup> Proper folding of LDLR (viral entry) <sup>[126]</sup> Likely overexpressed <sup>[125]</sup>
DNAJC14	ER	Viral RNA replication <sup>[62,121,122]</sup>
HSP110 family		
HSP105 (HSPH1)	Cytosolic	Overexpressed <sup>[129]</sup>
HSP70RY (HSPA4)	Cytosolic	Overexpressed <sup>[66,130]</sup> Knockdown decreases viral RNA replication <sup>[130]</sup>
Hip (HSPBP1)	Cytosolic	Knockdown decreases virus production <sup>[62,134]</sup>
HSP90 family		
GRP94 (HSP90B1)	ER	Regulation of viral protein homeostasis and maintaining a balance between viral and cellular translation to prevent viral protein overload (involves induction of ER stress and the UPR) <sup>[95,97,101]</sup> Suppression of HCV-induced apoptosis <sup>[50]</sup> Potentially HCV-induced liver fibrosis and autoimmune disease <sup>[155]</sup> Overexpressed <sup>[95,101,130]</sup>
HSP90 (HSP90AA1/HSP90AB1)	Cytosolic	Knockdown decreases viral RNA replication <sup>[130]</sup> HCV RNA replication <sup>[138,139,148,149]</sup> Maturation and stability of HCV proteins <sup>[140-143]</sup> IRES-mediated translation of viral genome <sup>[144]</sup> Circumventing IFN $\beta$ response in peripheral B cells <sup>[151]</sup> Potentially regulates miRNA levels in conjunction with GW182 <sup>[145]</sup> Interacts with NS5A and NS5B <sup>[105,107,143]</sup> Overexpressed <sup>[130,152]</sup> Knockdown decreases RNA replication <sup>[138]</sup>
HSP60 family (chaperonins)		
HSP60 (HSPD1/HSPD1)	Mitochondrial	Regulates ROS production and apoptosis <sup>[159]</sup> Interacts with core, NS3-NS4A, and viral genome <sup>[76,105,107,159]</sup> Varied expression <sup>[66,130]</sup>
TRiC/CCT (TCP1/CCT2-8)	Cytosolic	Viral RNA replication by assisting in RC assembly <sup>[73]</sup> Increased activity <sup>[129,130]</sup> Increased TCP1, CCT2, and CCT5 expression <sup>[130]</sup> Decreased CCT4 expression <sup>[129]</sup> CCT4 co-immunoprecipitates with NS3-NS4A <sup>[105]</sup>

		Knockdown of CCT5 decreases viral RNA replication <sup>[73]</sup>
Small HSPs		
HSP22 (HSPB8)	Cytosolic	Potentially blocks apoptosis <sup>[166]</sup> Overexpressed <sup>[119]</sup>
HSP27 (HSPB1)	Cytosolic	Potentially decreases apoptosis <sup>[164]</sup> Binds NS5A <sup>[164]</sup> Overexpressed <sup>[66]</sup>
Other chaperones		
ApoJ (clusterin) (CLU)	Cytosolic	Binds to and stabilizes core and NS5A <sup>[190]</sup> Overexpressed <sup>[190]</sup>
BAG3 (BAG3)	Cytosolic	Co-chaperone of HSP90 family Likely blocks ER-stress-induced apoptosis <sup>[104]</sup>
Calnexin (CANX)	ER	E1/E2 folding and glycosylation <sup>[98,107,219,220,223-225]</sup> HCV-induced ER stress and viral protein homeostasis <sup>[98]</sup> Knockdown decreases virus production <sup>[62]</sup>
Calreticulin (CALR)	ER	E1/E2 glycosylation <sup>[98,107]</sup> HCV-induced ER stress and viral protein homeostasis <sup>[98,101]</sup> Overexpressed <sup>[1101,130,226]</sup>
Cyp40 (PPID)	Cytosolic	Knockdown decreases virus production <sup>[62]</sup>
CypA (PPIA)	Cytosolic	Lipid trafficking and virion secretion <sup>[303]</sup> RC formation and viral RNA replication <sup>[263,270]</sup> NS5A and NS5B activation <sup>[276,280]</sup> Viral polyprotein cleavage <sup>[283,301]</sup> Regulates IFN response <sup>[304]</sup>
CypB (PPIB)	Cytosolic	Lipid trafficking and virion assembly and secretion <sup>[291,303]</sup> RC formation and viral RNA replication <sup>[271,272]</sup> NS5A and NS5B activation <sup>[271,272,274,276]</sup>
CypD (PPIF)	Mitochondrial	Inhibits mitochondrial function leading to ROS production <sup>[308]</sup>
EDEM1 (EDEM1)	ER	Downregulated <sup>[103,231]</sup> Binds E1 and E2 <sup>[230]</sup> HCV-induced ER stress <sup>[230]</sup>
EDEM3 (EDEM3)	ER	Targets misfolded glycoproteins to ERAD (viral protein homeostasis) <sup>[227,228]</sup> Binds E1 and E2 <sup>[230]</sup> HCV-induced ER stress <sup>[230]</sup> Targets misfolded glycoproteins to ERAD (viral protein homeostasis) <sup>[227,228]</sup>
Erp72 (PDIA4)	Cytosolic	Increased activity <sup>[72]</sup>
FKBP38 (FKBP8)	Cytosolic	Co-chaperone of HSP90 family <sup>[137]</sup> HCV RNA replication <sup>[137]</sup> Blocks apoptosis <sup>[177]</sup> Potentially regulates Ca <sup>2+</sup> homeostasis by interacting with S100 proteins <sup>[175]</sup> Interacts with NS5A <sup>[105,169]</sup>
FKBP54 (FKBP5)	Cytosolic	Knockdown decreases HCV RNA replication <sup>[137]</sup>
GRP58 (PDIA3)	Cytosolic	Interacts with NS5B <sup>[105]</sup> Overexpressed <sup>[125,130]</sup>
MTTP (MTTP)	Cytosolic	Knockdown decreases viral RNA replication <sup>[130]</sup> Part of the PDI/MTTP heterodimer involved in VLDL biogenesis <sup>[193]</sup> Potentially causes HCV-induced liver steatosis <sup>[193,198]</sup> Viral maturation and secretion <sup>[210,211]</sup> Decreased expression and activity <sup>[193,198-200]</sup>
p23 (PTGES3)	Cytosolic	Co-chaperone of HSP90 family <sup>[179]</sup> Potentially regulates telomerase activity <sup>[180,181]</sup>
PDI (P4HB)	ER	Folding and transfer of MTTP to ER as a PDI/MTTP heterodimer involved in VLDL biogenesis <sup>[193]</sup> Increased activity <sup>[129]</sup>
PDIR (PDIA5)	Cytosolic	Increased activity <sup>[72]</sup>
Prefoldin (PFDN1-2/VBP1/PFDN4-6)	Cytosolic	Co-chaperone of TRiC/CCT <sup>[182]</sup> Binds F protein <sup>[183]</sup> Regulates cytoskeleton likely to balance virus production in hepatocytes <sup>[183]</sup>
Prohibitin (PHB/PHB2)	Mitochondrial	Inhibits mitochondrial respiratory function leading to ROS production <sup>[237-240]</sup> Binds core <sup>[238]</sup> Overexpressed <sup>[236,237]</sup>
SigR1 (SIGMAR1)	Cytosolic	Viral RNA replication immediately after entry <sup>[44,234]</sup> Interorganellar communication between ER and mitochondria <sup>[44]</sup>
HCV chaperones		
Core		Viral RNA stabilization, dimerization, and structural rearrangement <sup>[311-315]</sup> Folding of E1 <sup>[316]</sup>



E1	Proper folding of E2 <sup>[224,318-320]</sup>
E2	Proper folding of E1 <sup>[317]</sup>
NS3	Interconversion of viral RNA species <sup>[322]</sup>
NS4A	Directs NS3 to ER <sup>[323]</sup> Increases NS3 stability <sup>[323]</sup>

Apo: Apolipoprotein; BAG: BCL2-associated athanogene; Cyp: Cyclophilin; EDEM: Endoplasmic reticulum degradation enhancer, mannosidase alpha-like; ER: Endoplasmic reticulum; ERAD: ER-associated degradation; FKBP: FK506-binding protein; GRP: Glucose-regulated protein; GW: Glycine-tryptophan; HCV: Hepatitis C virus; Hip: HSP70-interacting protein; HSC70: Heat shock cognate protein 70; HSP: Heat shock protein; IFN $\beta$ : Interferon beta; IRES: Internal ribosomal entry site; LDLR: Low-density lipoprotein receptor; MTTP: Microsomal triglyceride transfer protein; NCR: Non-coding region; NS: Non-structural; ROS: Reactive oxygen species; PDI: Protein disulfide isomerase; RC: Replication complex; SigR1: Sigma non-opioid intracellular receptor 1; TRiC/CCT: TCP-1 ring complex/chaperonin-containing TCP-1; UPR: Unfolded protein response; VLDL: Very low-density lipoprotein.

to be part of the HCV viral particles, and the viral E2 protein was found to contain the HSC70-interacting histidine-proline-aspartic acid (HPD) motif<sup>[74]</sup> which is required for the interaction of the HSP40 co-chaperones with HSP70 family of chaperones<sup>[75]</sup>. Pretreatment of the virus with HSC70 antibody significantly diminished infectivity suggesting that HSC70 is a part of the viral particle<sup>[74]</sup>. In addition, HSC70, core, and E2 were found to colocalize around lipid droplets, the site of virion assembly. RNAi-mediated knockdown of HSC70 significantly decreased the volume of lipid droplets and viral secretion, but not viral RNA replication levels. These results suggest that HSC70 plays an important role during virion assembly and may play a structural role for the virion as well. It has been observed that HSC70 associates with positive-strand subgenomic viral RNA (corresponding to domains III and IV of the 5' NCR and 36 nucleotides of core) and the 3' NCR of the viral genome as well<sup>[70,76]</sup>.

A number of compounds including IMB-DM122, N-substituted benzyl matrinic acid derivatives, and (+)-lycoridine were shown to downregulate HSC70 mRNA expression leading to decreased virus production<sup>[77-79]</sup>. Our lab demonstrated that HSC70 directly binds to NS5A *in vitro* and colocalizes with NS5A in infected cells<sup>[18]</sup>. We further showed that knockdown of HSC70 significantly impacted intracellular infectious virion assembly thereby establishing distinct functions of HSC70 and HSP70 in the HCV life cycle. This is further supported by the fact that HSC70 and HSP70 do not interact with each other. Based on the available evidence, therefore, it seems that HSC70 is important for virion assembly.

### HSP70B' (HSPA6)

HSP70B' is another member of the HSP70 family which is highly similar to HSPA1A in terms of sequence homology (82%) and function<sup>[80]</sup>. Both chaperones are stress inducible and work in conjunction to protect cells from stress. However, HSP70B' is the secondary responder to stress after HSPA1A, and proteasome inhibition is a potent inducer of HSP70B' expression<sup>[81]</sup>. HSP70B' was found to be associated with the 3' NCR of the HCV genome<sup>[70]</sup>.

### Glucose-regulated protein 78 (HSPA5)

Glucose-regulated protein 78 (GRP78), also known as

the binding immunoglobulin protein (BiP), is another member of the HSP70 family and is the major molecular chaperone in the ER<sup>[82]</sup>. The ER is involved in vital cellular processes including protein folding, protein transport, the UPR, and calcium homeostasis. The UPR is an adaptive signaling program that is activated in response to accumulation of unfolded or misfolded proteins in the ER, referred to as ER stress. Proteins that are not successfully folded are either sent for refolding or tagged for degradation through the ERAD pathway<sup>[83]</sup>. If the UPR program is unable to successfully relieve cells from ER stress, it initiates mitochondria-mediated apoptosis<sup>[84]</sup>. Under certain conditions such as heat stress and pathogen infection, unfolded or misfolded proteins can accumulate in the ER leading to ER stress and activation of UPR. Stimulation of GRP78 transcription is an indication of ER stress and induction of UPR, which occurs in HCV infection likely to repress cellular protein translation in order to utilize cellular resources for the IRES-mediated translation of viral proteins and to suppress innate immunity in order to establish persistent infection<sup>[43,85-96]</sup>. GRP78 activity was also found to be significantly increased in an HCV SGR system<sup>[72]</sup>.

UPR signaling can be initiated by three factors: Activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and double-stranded RNA-activated protein kinase R-like ER kinase (PERK)<sup>[43,92]</sup>. These three factors act as ER stress sensors and lead to induction of expression of GRP78, which is itself a negative regulator of the three ER stress sensors. ER stress may lead to the proteolytic cleavage of ATF6, an ER membrane-associated transmembrane protein. The 90 kDa ATF6 precursor, also known as pATF6 $\alpha$ (P), is cleaved to form an approximately 50 kDa N-terminal fragment pATF6 $\alpha$ (N) which translocates to the nucleus and activates transcription of ER chaperone genes such as GRP78 involved in the UPR. ER stress also leads to phosphorylation of IRE1 which results in the splicing of unspliced X-box-binding protein 1 to spliced XPB1 (sXBP1), a transcription factor that can induce expression of GRP78 and other genes involved in the UPR. Upon initiation of ER stress, PERK can also get activated and phosphorylate the eukaryotic initiation factor 2 alpha (eIF2 $\alpha$ ). Phosphorylated eIF2 $\alpha$  (peIF2 $\alpha$ ) results in global inhibition of cellular protein synthesis and enhanced ATF4 expression which leads to induction of UPR genes. HCV can activate all three ER stress



sensors.

It was found that the viral glycoprotein E2, and not E1, can induce transcription of GRP78 and that only E2 bound to GRP78<sup>[97]</sup>. Another group reported that both E1 and E2 bind GRP78<sup>[98]</sup>. However, it seems that GRP78 tends to bind to E1/E2 aggregates rather than monomeric glycoproteins. Expression of both E1 and E2 was also shown to lead to the UPR<sup>[99,100]</sup>. The HCV core protein has also been reported to induce expression of GRP78<sup>[101]</sup>. Induction of core, E1, E2, and p7 in mice liver led to ER stress and overexpression of GRP78<sup>[95]</sup>. Expression of HCV NS genes led to upregulation of GRP78<sup>[102]</sup>. The NS2 alone also induces ER stress and leads to upregulation of GRP78 protein levels<sup>[46]</sup>. NS4B alone can also induce ER stress and the UPR and upregulate GRP78 expression<sup>[87,103]</sup>. NS5A weakly binds GRP78, enhances GRP78 expression, and protects hepatocytes from ER stress-induced apoptosis leading to persistent infection<sup>[104,105]</sup>. It was also shown that HCV bearing certain mutations in NS5A and NS5B proteins (C2441S, P2938S or R2985P) displayed higher levels of GRP78 expression<sup>[94]</sup>. However, it was not clear whether NS5A alone can induce ER stress in these studies. Another group reported that NS5A does not lead to ER stress and the UPR<sup>[89,106]</sup>. An SGR system expressing all the NS proteins led to the UPR as well<sup>[106]</sup>. Thus, it is not clear whether the NS5 proteins alone can cause ER stress and the UPR. GRP78 was also shown to benefit virus production in a genome-wide expression analysis of multiple huh7-derived cell lines where interactions with core, E1, E2, p7, NS3, NS4B and NS5A were implicated<sup>[107]</sup>. Furthermore, GRP78 is a target of miR-30a, miR-30c, and miR-30e that were found to be downregulated in acute HCV infection potentially leading to GRP78 overexpression<sup>[108]</sup>.

In addition to the ER-targeted E1 and E2 proteins, cytosol-targeted E1 and E2 proteins have also been described with opposing functions in the context of ER stress<sup>[109-112]</sup>. In the cytosol, E1 binds to the cytoplasmic domain of PERK. Furthermore, cytosolic E1 leads to downregulation of GRP78. Similarly, E2 binds to PERK as well, inhibits its kinase activity, reverses PERK-mediated global translation repression, and confers resistance to ER stress. In addition, NS2 leads to phosphorylation of eIF2 $\alpha$  and decreased protein synthesis as well as reduction of IRES-mediated translation suggesting that NS2 can also provide a negative feedback regulation of ER stress by decreasing viral protein translation that is responsible for inducing ER stress<sup>[46]</sup>.

Thus, it seems that GRP78, as well as other ER-resident chaperones, play an important role in regulating and maintaining viral protein homeostasis to ensure the availability of sufficient viral proteins to establish a persistent infection while minimizing cellular protein expression and preventing viral protein overload. GRP78 was also found to be associated with positive-strand subgenomic viral RNA (corresponding to domains III and IV of the 5' NCR and 36 nucleotides of core) and the 3' NCR of the viral genome<sup>[70,76]</sup>.

A recent study reported that there was no significant difference in the mRNA levels of GRP74 and a number of other genes involved in ER stress and UPR between infected patients and healthy controls<sup>[113]</sup>. No difference in GRP78 protein levels were observed either. This may be attributed to the fact that typically HCV infects a small percentage of hepatocytes, and therefore, changes may not be detected.

### GRP75 (HSPA9)

GRP75 also known as mtHSP70 or mortalin is the mitochondria-resident HSP70 family member. It plays a number of critical roles in the cells including anti-apoptosis, protein transport into mitochondria which may involve HSP60 as well, protection of cells from ROS, and mitochondrial biogenesis<sup>[114]</sup>. It has also been implicated in membrane trafficking and human immunodeficiency virus (HIV) virion release<sup>[115]</sup>. In the context of HCV, it has been reported that GRP75 activity was significantly increased in one HCV SGR system<sup>[72]</sup>, while GRP75 protein was significantly downregulated in another SGR system<sup>[66]</sup>. These different results may reflect the HCV-mediated modulation of GRP75 activity/expression to accommodate its needs during the viral life cycle. Furthermore, NS5A was shown to co-immunoprecipitate with GRP75<sup>[105]</sup>.

### HSP40 family

The HSP40 family are co-chaperones of HSP70 proteins that regulate the activity of HSP70s and determine their client range by binding specific target proteins<sup>[60,116]</sup>. This large family of proteins are homologous with the bacterial DnaJ chaperone, and the term DNAJ is utilized in the gene nomenclature of the isoforms of this family. DNAJA1 and DNAJA2 are the most abundant cytosolic HSP40 co-chaperones<sup>[116]</sup>. DNAJA1 was reported to co-immunoprecipitate with the NS3-NS4A protein<sup>[105]</sup>. We have shown that DNAJA2 participates together with HSP70 in regulating the NS5A-augmented IRES-mediated translation of the viral genome<sup>[63]</sup>. The interaction of viral proteins with these co-chaperones may, therefore, modulate chaperone activity to benefit the viral life cycle. A genome-wide siRNA screening identified DNAJB1 to be important for HCV production<sup>[61]</sup>. DNAJB1 plays important roles in regulating apoptosis and cell proliferation<sup>[117]</sup>. DNAJC7 co-immunoprecipitates with NS3-NS4A protein<sup>[105]</sup>. DNAJC7 also regulates apoptosis by binding to the pro-apoptotic p53 protein and increasing its activity and stability<sup>[118]</sup>. Thus, it can be speculated that binding of NS3-NS4A may prevent the pro-apoptotic function of DNAJC7/p53 thereby suppressing apoptosis and contributing to persistent HCV infection. DNAJC8 was reported to be upregulated in quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) as well as microarray analyses of host gene expression in infected huh7 cells<sup>[119]</sup>. DNAJC8 has been shown to play an important role in regulating pre-mRNA splicing by the spliceosome<sup>[120]</sup>. This is achieved by the binding of DNAJC8 with "serine/arginine-rich splicing

factor protein kinase 1". DNAJB6 interacts with NS5B, and shRNA-mediated knockdown of DNAJB6 led to a significant decrease in viral RNA replication<sup>[105]</sup>. DNAJB6 may, therefore, be required for the stability or activity of NS5B for viral RNA replication. In addition, miR-17, miR-106a, and miR-106b with DNAJB6 as their target were found to be downregulated in acute HCV infection<sup>[108]</sup>.

There are seven ER-resident HSP40 co-chaperones: DNAJB9, DNAJB11, DNAJC1, DNAJC3, DNAJC10, DNAJC23 and DNAJC25. DNAJC14 was found as a host factor involved in HCV replication in an siRNA screen where knockdown of DNAJC14 led to increased viral replication<sup>[62]</sup>. Further, DNAJC14 has been reported to be involved in RNA replication of yellow fever virus (YFV) and other flaviviruses including HCV<sup>[121]</sup> and has been shown to be important for RC assembly in YFV<sup>[122]</sup>. Overexpression of DNAJC14 blocked viral RNA replication in all flaviviruses tested including HCV, while NS2/3 cleavage was not inhibited. siRNA-mediated knockdown of DNAJC14 also demonstrated similar results indicating that both elevated and reduced levels of DNAJC14 interferes with viral RNA replication. Also DNAJC14 is recruited to YFV RCs consistent with the normal cellular function of DNAJC14 as an ER-localized co-chaperone involved in protein transport<sup>[121,123]</sup>. DNAJB9 was identified in a microarray analysis as one of the host genes with most consistently modified expression as a result of acute HCV infection<sup>[108]</sup>. Further, miR-17, miR-106a, and miR-106b that target DNAJB9 were found to be downregulated. DNAJB9 has been shown to be involved in regulation of apoptosis<sup>[124]</sup>. DNAJC10 expression was found to be increased in HeLa cells expressing HCV polyprotein<sup>[125]</sup>. DNAJC10 is also a member of the PDI family of chaperones (discussed below) which is responsible for removing non-native disulfide bonds in conjunction with BiP and targeting misfolded proteins for degradation<sup>[126]</sup>. Interaction of DNAJC10 with EDEM1, an ER chaperone (discussed below), is required for disulfide bond reduction. Interestingly, DNAJC10 is also required for the correct folding of LDLR, one of the cell surface receptors utilized by HCV for entry. DNAJC1 was identified as an antiviral protein in a genome-wide expression analysis of multiple huh7-derived cell lines where interactions with E1 and E2 were implicated<sup>[107]</sup>.

Five members of the HSP40 family have been identified in mitochondria: DNAJA3, DNAJC11, DNAJC15, DNAJC19, and DNAJC20. DNAJA3 was identified as an HCV-interacting protein<sup>[61]</sup>. DNAJA3 is normally involved in maintaining mitochondrial morphology, and altering DNAJA3 levels leads to mitochondrial fragmentation and reduced cell viability<sup>[127]</sup>. HCV infection leads to mitochondrial dysfunction, and DNAJA3 may play a role in this process.

### NEFs

NEFs play an important role in normal chaperone functioning by facilitating replacement of the hydrolyzed ADP with an ATP<sup>[128]</sup>. Three families of NEFs have been identified for the HSP70 chaperones: (1) HSP110/

GRP170; (2) HSP70-interacting protein (Hip) (HSPBP1)/BiP-associated protein (SIL1); and (3) the BCL2-associated athanogene (BAG) family of proteins. The HSP110/GRP170 family consists of three cytosolic members HSP105 (HSPH1), HSP70RY (Apg-2) (HSPA4), and OSP94 (Apg-1) (HSPA4L), and one mitochondrial member GRP170 (HYOU1).

It was found that HSP105 and HSP70RY expression levels increase in HCV SGR systems<sup>[66,129,130]</sup>. Also knockdown of HSP70RY in an SGR system decreased viral RNA replication levels<sup>[130]</sup>. This is expected as the levels and activity of HSP70 family members increase during HCV infection which may require more NEFs for their function. Furthermore, HSP110 levels increase in stressed cells likely to assist in coping with stress, and in the context of HCV infection, increased HSP110 levels may help cells with HCV-induced ER stress. Similar effects of overexpression of HSP110 has been reported in cancer and gastric ulcer where targeting HSP110 had beneficial effects<sup>[131-133]</sup>. In siRNA screens, it was found that knockdown of Hip led to a significant decrease in virus production levels<sup>[62,134]</sup>. The role of BAG3 in HCV infection is discussed below.

## HSP90 SYSTEM

The HSP90 proteins are highly conserved evolutionarily and are involved in the folding of proteins especially those involved in signal transduction<sup>[135]</sup>. Thus, HSP90 possesses a more discrete range of clients compared with the HSP70 system. Like HSP70, HSP90 also undergoes conformational changes to assist with the folding of client proteins, a process which is driven by ATP hydrolysis, and co-chaperones also assist in regulating HSP90 function. HSP90 has been shown to be important for a large group of viruses including HCV<sup>[136]</sup>. The HSP90 family consists of the inducible cytosolic isoform HSP90 $\alpha$  (HSP90AA1), the constitutively expressed cytosolic isoform HSP90 $\beta$  (HSP90AB1), the inducible ER isoform GRP94 (HSP90B1), and the mitochondrial isoform "tumor necrosis factor (TNF) receptor-associated protein 1" (TRAP1) (HSP90L).

### HSP90 (HSP90AA1 and/or HSP90AB1)

HSP90 has been shown to be important for virus production<sup>[137]</sup>. siRNA-mediated knockdown of HSP90 as well as HSP90 inhibitors geldanamycin, "17-dimethylaminoethylamino-17-demethoxygeldanamycin" (17-DMAG), herbimycin A, and radicicol resulted in dose-dependent suppression of HCV in a replicon system<sup>[138]</sup>. Further, viral levels in chimeric mice with a humanized liver treated with 17-DMAG were significantly reduced. Other derivatives of geldanamycin as HSP90 inhibitors have also been reported to block HCV RNA replication<sup>[139]</sup>.

HSP90 is required for the maturation of the viral polyprotein complex specially to generate functional NS2/3 protease<sup>[140]</sup>. HSP90 inhibitors were shown to block NS2/3 cleavage. Expression of HCV core in *Saccharomyces cerevisiae* impaired the growth of yeast cells, and it was found that HSC82, the yeast homolog

of HSP90, is required for the stability of core protein<sup>[141]</sup>. Treatment of yeast cells with the HSP90 inhibitors geldanamycin, radicicol, herbimycin A, and herbimycin C suppressed core-induced growth impairment. HSP90 directly binds to NS3 through the NS3 helicase region and is required for NS3 stabilization<sup>[142,143]</sup>. In an SGR system, the HSP90 inhibitor “17-N-allylamino-17-demethoxygeldanamycin” (17-AAG) resulted in NS3 degradation specifically<sup>[142]</sup>. In the same SGR system, 17-AAG also suppressed HCV RNA replication in a dose-dependent manner. However, it was not clear if replication was affected directly or through decreased IRES translation. A subsequent study demonstrated the indirect interaction of HSP90 with the subunit C of eIF3c which involves and is dependent on the viral IRES RNA<sup>[144]</sup>. This interaction prevents the ubiquitination and the subsequent proteasome-dependent degradation of eIF3c which is required for IRES-mediated translation of the viral genome. Therefore, treatment with HSP90 inhibitors may prevent the chaperoning of eIF3c by HSP90 which leads to its degradation. Knockdown of eIF3c inhibited IRES-mediated translation, but not cellular 5' 7-methylguanylate cap-dependent translation.

HSP90 was found to colocalize and co-immunoprecipitate with glycine-tryptophan (GW) 182, an important component of GW bodies which are involved in mRNA degradation and translational repression *via* miRNAs<sup>[145]</sup>. Both HSP90 and GW182 also colocalized with NS3, core, and NS5A. Knockdown of GW182 significantly decreased HCV RNA levels in infected cells, while overexpression of GW182 resulted in a significant increase in viral RNA levels. The HSP90 inhibitor 17-DMAG and knockdown of HSP90 significantly decreased GW182 and miR-122 levels leading to decreased HCV RNA levels. Ethanol was shown to upregulate both GW182 and HSP90 thereby facilitating HCV RNA replication. Interestingly, the same group discovered infectious exosomes from sera of HCV-infected patients or supernatants of infected huh7.5 cells that contained negative-strand viral RNA in association with Argonaute 2 [a component of the RNA-induced silencing complex (RISC)], HSP90, and miR-122<sup>[146]</sup>. These exosomes are capable of transmitting HCV infection in a CD81, SR-B1, and apolipoprotein E (apoE) receptor-independent manner, which was blocked by miR-122 and HSP90 inhibitors. An interaction between NS5A and HSP90 was also implicated in a genome-wide expression analysis of multiple huh7-derived cell lines<sup>[107]</sup>. Thus, viral proteins may modulate GW182 activity in an HSP90-dependent manner in order to regulate viral RNA replication and miRNA levels. A number of miRNAs have been shown to be modulated by HCV infection<sup>[108]</sup>.

Treatment with the HSP90 inhibitor 17-DMAG was shown to destabilize phosphoinositide-dependent kinase 1 (PDK1), an upstream kinase of protein kinase C-related kinase 2 (PRK2)<sup>[147]</sup>. The PDK1-PRK2 signaling pathway leads to phosphorylation of NS5B, which is required for HCV RNA replication<sup>[148,149]</sup>. 17-DMAG-driven destabilization and degradation of PDK1 diminished NS5B

phosphorylation levels leading to suppression of viral RNA replication<sup>[147]</sup>. An interaction between NS5B and HSP90 has also been reported in a yeast two-hybrid system<sup>[143]</sup>. NS5B co-immunoprecipitates with both isoforms of HSP90 as well<sup>[105]</sup>.

Peripheral B cells have been proposed to serve as reservoirs for persistent HCV infection<sup>[150,151]</sup>. It was found that peripheral B cells in patients with chronic HCV infection circumvent the interferon beta (IFN $\beta$ )-mediated antiviral response in part by downregulating HSP90 which acts as a stabilizer of TANK-binding kinase 1 involved in phosphorylation of the interferon-regulatory factor 3 (IRF3) transcription factor that induces IFN expression<sup>[151]</sup>. Thus, by using this HSP90-mediated strategy, HCV in B cells evades detection by the immune system contributing to recurring infection even after liver transplant.

The constitutively expressed isoform of HSP90, HSP90AB1, was found to be significantly overexpressed in the mononuclear cells of HCV-infected patients<sup>[152]</sup>. Co-infection with HIV decreased the overexpression of HSP90AB1 in the same study. HSP90AB1 was also reproducibly enriched in the detergent-resistant membrane fraction of an SGR system<sup>[130]</sup>.

HSP90 also plays an important role in HCV RNA replication in conjunction with FKBP38, a co-chaperone of HSP90 family, which is a member of the immunophilin family of proteins. The role of FKBP38 and its interaction with HSP90 is discussed in detail in the FKBP38 section below. Another HSP90 co-chaperone p23 is also involved in the HCV life cycle and is discussed below as well.

### GRP94 (HSP90B1)

GRP94 is the ER-resident HSP90 isoform which is involved in folding of secreted proteins, ER stress, and the UPR<sup>[153]</sup>. It was found that the viral glycoprotein E2, and not E1, can lead to the ER stress response and induce transcription of GRP94<sup>[97]</sup>. This leads to activation of nuclear factor kappa B and induction of anti-apoptotic proteins<sup>[50]</sup>. In addition, knockdown of GRP94 abolished the anti-apoptotic activity of E2 suggesting that E2 inhibits apoptosis induced by HCV infection and leads to persistent viral infection in hepatocytes. The HCV core protein also contributes to ER stress by inducing the expression of GRP94<sup>[101]</sup>. Increased expression of GRP94 was also observed in the liver of mice conditionally expressing HCV structural proteins core, E1, E2 and p7<sup>[95]</sup>. No binding of GRP94 to either E1 or E2 glycoproteins was observed<sup>[98]</sup>. GRP94 was reproducibly enriched in the detergent-resistant membrane fraction of SGR cells<sup>[130]</sup>. HCV utilizes GRP94 as well as other ER-resident chaperones especially GRP78 to maintain viral protein homeostasis in the ER in order to establish persistent infection and suppress cellular protein translation. GRP94 was also shown to be beneficial for virus production in a genome-wide expression analysis of multiple huh7-derived cell lines where interactions with core, E2, NS3, and NS4B were implicated<sup>[107]</sup>. Knockdown of GRP94 in an SGR system led to a significant decrease

in viral RNA replication levels as well<sup>[130]</sup>.

GRP94 is prevented from translocating to the cell surface by "aminoacyl tRNA synthetase complex-interacting multifunctional protein 1" (AIMp1)/p43<sup>[154]</sup>, which is a cofactor of aminoacyl tRNA synthetase complex and is involved in regulating transforming growth factor beta (TGF- $\beta$ ) signaling. Translocation of GRP94 to the cell surface leads to activation of dendritic cells and leads to autoimmune diseases. The HCV E2 protein has been reported to directly bind AIMp1/p43 and lead to its degradation through ubiquitination and the proteasome pathway<sup>[155]</sup>. In addition, E2 interferes with the AIMp1/p43-GRP78 interaction leading to lower cellular AIMp1/p43 levels. Decreased AIMp1/p43 levels in cells leads to elevated TGF- $\beta$  signaling and cell surface expression of GRP94. Therefore, these mechanisms may be responsible for HCV-induced liver fibrosis and autoimmune diseases.

## HSP60 SYSTEM

HSP60 chaperones also known as chaperonins are an important family of HSPs involved in protein folding and macromolecular assembly<sup>[156]</sup>. The HSP60 family consists of mitochondrial and cytosolic proteins. The mitochondrial HSP60 (encoded by *HSPD1* and *HSPE1* genes), also known as mtHSP60, is thought to have originated in the bacterial ancestors that were engulfed by early eukaryotic cells giving rise to the mitochondrial organelle. HSPD1 (the homolog of bacterial GroEL) forms tetradecamers, composed of two stacked heptameric rings with a central cavity that accommodates the target protein. HSPE1 (the homolog of bacterial GroES) forms one heptameric ring that serves as a cap for the HSPD1 structure. The HSPD1/HSPE1 complex functions in protein folding in an ATP-dependent manner. The eukaryotic/cytosolic chaperonin, also known as "TCP-1 ring complex/chaperonin-containing TCP-1" (TRiC/CCT), is homologous to the Archean thermosome complexes forming hexadecamers consisting of two octameric rings to assist in oligomeric protein assembly<sup>[157]</sup> and folding of approximately 10% of the proteome<sup>[158]</sup>. TRiC/CCT is composed of eight paralogous subunits encoded by *TCP1* and *CCT2-8* genes. The TRiC/CCT complex lacks a GroES-like homolog and instead uses a built-in cap system. Typically, the term HSP60 is used to refer to the mitochondrial chaperonin, whereas the eukaryotic cytosolic homolog is referred to as TRiC/CCT.

### HSP60 (HSPD1/HSPE1)

Proteomic analyses of huh7 cells harboring an HCV SGR demonstrated downregulation of HSP60<sup>[66]</sup>, while it was shown to be reproducibly enriched in the detergent-resistant membrane fraction of another SGR system<sup>[130]</sup>. However, these studies did not validate HSP60 levels by Western analysis or in the context of viral infection. HSP60 has been shown to interact with core<sup>[107,159]</sup>. This interaction led to production of ROS and sensitization of cells to TNF $\alpha$ -induced apoptosis<sup>[159]</sup>.

Further, overexpression of HSP60 decreased ROS production and prevented apoptosis in core-expressing cells. Thus, binding of core to HSP60 seems to impair the function of HSP60 in regulating ROS production and apoptosis as a possible pro-oncogenic process. However, significant research is still required to elucidate the function of the HSP60 system in the context of HCV infection. Nevertheless, HSP60 has been shown to be important for Dengue virus production (also a positive-stranded RNA virus) although the exact function has not been elucidated<sup>[160]</sup>. Further, HSP60 is overexpressed in HBV and HIV infection<sup>[156,161]</sup>. Autoantibodies against HSP60 have been detected in sera of chronic HCV infected patients<sup>[162]</sup>. HSP60 has also been shown to co-immunoprecipitate with the NS3-NS4A protein<sup>[105]</sup> and associate with positive-strand subgenomic viral RNA (corresponding to domains III and IV of the 5' NCR and 36 nucleotides of core)<sup>[76]</sup>.

### TRiC/CCT (TCP1/CCT2-8)

The activity of TRiC/CCT, the cytosolic chaperonin, was reported to be increased in an SGR system<sup>[129]</sup>. Also TCP1, CCT2, and CCT5 were reproducibly enriched in the detergent-resistant membrane fraction of an SGR system<sup>[130]</sup>. TRiC/CCT also plays an important role in the assembly of RCs which mediate HCV RNA replication<sup>[73]</sup>. This may be facilitated by an interaction between the subunit CCT5 of TRiC/CCT and NS5B. siRNA-mediated knockdown of CCT5 suppressed viral RNA replication. Treatment with an antibody against CCT5 also suppressed HCV RNA synthesis in an *in vitro* cell-free assay. These observations suggest that NS5B may recruit TRiC/CCT to the RCs to assemble components of RCs in order to facilitate HCV RNA replication. It was also reported that that CCT4 can co-immunoprecipitate with the NS3-NS4A protein<sup>[105]</sup>. CCT4 activity was decreased in an SGR system<sup>[129]</sup>.

TRiC/CCT is regulated by a number of co-chaperones including prefoldin. The role of prefoldin in the HCV life cycle is discussed below.

## SMALL HSPS

Small HSPs constitute a family of ten proteins with molecular mass in the range of 12-43 kDa with diverse functions including protein folding, development, and eye lens tissue formation to name a few<sup>[163]</sup>. They lack enzymatic activity and work as holdases in conjunction with the ATP-dependent chaperones to carry out their functions<sup>[57]</sup>.

### HSP27 (HSPB1)

Proteomic analyses of huh7 cells harboring an HCV SGR have demonstrated upregulation of HSP27<sup>[66]</sup>. HSP27 was found to bind NS5A (and not NS5B) in co-immunoprecipitation studies and colocalize by immunofluorescence under heat shock conditions<sup>[164]</sup>. The N-terminal regions of both proteins were found to be involved in the interaction (amino acids 1-122 of



HSP27 and 1-181 of NS5A). While the function of this interaction is not known, it has been speculated that it may decrease infection-induced apoptosis. This is likely as HCV is known to modulate apoptosis in order to establish persistent infection. In fact, HSP27 is overexpressed and has anti-apoptotic roles in several cancers as well<sup>[165]</sup>.

### **HSP22 (HSPB8)**

HSP22 is a multifunctional chaperone involved in regulation of protein folding, macroautophagy, carcinogenesis, and apoptosis<sup>[166]</sup>. HSP22 was reported to be significantly overexpressed in infected huh7 cells as determined by qRT-PCR as well as microarray analyses of host gene expression<sup>[119]</sup>. HSP22 is an anti-apoptotic protein, and its upregulation by HCV may be one of the mechanisms that HCV utilizes to block apoptosis in hepatocytes.

## **OTHER CHAPERONES**

In addition to HSPs, cells possess a number of other molecular chaperones and co-chaperones that play critical roles in numerous cellular functions by assisting with protein folding and stability in their respective pathways.

### **BAG3 (BAG3)**

BAG3 is one of the BAG family of proteins and serves as a NEF for the HSP70 family of chaperones. BAG3 is the only heat stress-inducible BAG isoform and plays important roles in cell proliferation, apoptosis, adhesion, and migration<sup>[167]</sup>. It acts as an anti-apoptotic protein in different cancers. In the context of HCV infection, it was found that overexpression of NS5A in HepG2 cells upregulated a number of anti-apoptotic genes including BAG3 when the cells were treated with thapsigargin, an inducer of ER stress<sup>[104]</sup>. GRP78 was also overexpressed.

### **FKBP38 (FKBP8) and FKBP54 (FKBP5)**

FKBP38 is a co-chaperone of the HSP90 family and a member of the immunophilin family of chaperone proteins which possess peptidylprolyl isomerase (PPIase) activity and also serve as receptors for the immunosuppressive drug FK506<sup>[168]</sup>. FKBP38 was identified as an NS5A interacting protein in a fetal liver cDNA library screen, and both NS5A and FKBP38 colocalize to mitochondria and the ER<sup>[169]</sup>. NS5A and FKBP38 were also shown to co-immunoprecipitate<sup>[105]</sup>. FKBP38 interacts with HSP90 and plays an important role in HCV RNA replication. FKBP38 forms a complex with HSP90 and NS5A where FKBP38 binds to both HSP90 and NS5A through different sites in its tetratricopeptide repeat domain<sup>[137]</sup>. Both knockdown of FKBP38 and treatment with geldanamycin suppresses HCV RNA replication in a replicon system indicating that the HSP90/NS5A/FKBP38 complex is important for the regulation of HCV RNA replication. In fact, the FKBP38/NS5A interaction is so critical for the virus that a single amino acid mutation in NS5A that disrupts its binding with FKBP38 impairs virus

production<sup>[170]</sup>. The same group found that HSP90 binds to human butyrate-induced transcript 1 (hB-ind1)<sup>[171]</sup>, which is a member of the Rho family of GTPases and a component of the Ras-related C3 botulinum toxin substrate 1 (Rac1) signaling pathway<sup>[172,173]</sup>. hB-ind1 was found to bind to NS5A and is involved in viral RNA replication through its interaction with HSP90. Thus, by interacting with NS5A, hB-ind1 recruits HSP90 and FKBP38 to the RCs. In addition, through immunofluorescence analyses, it was found that hB-ind1 colocalizes with NS5A, FKBP38, and double-stranded viral RNA at the site of the membranous web<sup>[174]</sup>. These results further support the role of HSP90 in viral RNA replication. Moreover, treatment with an HSP90 inhibitor decreased the HCV-induced UPR which points to a potential involvement of HSP90 in an hB-ind1-mediated protein folding mechanism in the membranous web in order to circumvent the virus-induced UPR.

It was also found that a few members of the S100 family of proteins, S100A1, S100A2, S100A6, S100B and S100P directly bind FKBP38 in cell-free *in vitro* assays in a Ca<sup>2+</sup>-dependent manner<sup>[175]</sup>. The S100 proteins are a family of 24 Ca<sup>2+</sup> binding proteins which are involved in regulating inflammation, cell proliferation and differentiation, apoptosis, cell migration and invasion, and Ca<sup>2+</sup> homeostasis<sup>[176]</sup>. The S100/FKBP38 interactions blocked both NS5A/FKBP38 and HSP90/FKBP38 interactions<sup>[175]</sup>. Furthermore, overexpression of S100A1, S100A2 and S100A6 suppressed HCV RNA replication. S100P was identified as one of the proteins with most consistently modified expression in acute HCV infection<sup>[108]</sup>.

FKBP38 has also been reported to be involved in HCV suppression of apoptosis<sup>[177]</sup>. NS5A plays an important role in HCV pathogenesis by activating the mammalian target of rapamycin (mTOR) pathway. This leads to suppression of apoptosis and hepatocyte cell survival which is required for persistent infection. NS5A exerts its anti-apoptotic activity by blocking the interaction between FKBP38 and mTOR.

FKBP54 (p54), another FKBP family member, was reported to co-immunoprecipitate with NS5B<sup>[105]</sup>. FKBP54 is an important co-chaperone involved in regulating a number of signaling pathways, steroid hormone receptors, and autophagy<sup>[178]</sup>.

### **p23 (PTGES3)**

p23 (prostaglandin E synthase 3) is another HSP90 co-chaperone and an inhibitor of HSP90 ATP turnover<sup>[179]</sup>. In addition, p23 together with HSP90 are essential telomerase components, and telomerase activity as well as expression of multiple telomerase components were reported to be significantly induced in HCV infection of huh7.5 cell<sup>[180]</sup>. The same group also showed that expression of the La protein (Sjogren syndrome antigen B), a regulator of HCV IRES-mediated translation<sup>[181]</sup>, significantly correlated with the expression of telomerase components including telomerase RNA, p23 and HSP90 in HCV-infected patient tissues. Thus, HCV may regulate



telomerase activity in an HSP90-dependent manner which may potentially be linked to HCV-induced hepatocarcinogenesis.

### **Prefoldin (PFDN1-2/VBP1/PFDN4-6)**

Prefoldin is the co-chaperone of the cytosolic chaperonin TRiC/CCT. It is a hexameric protein complex consisting of the six subunits encoded by the PFDN1-2, VBP1 (PFDN3), and PFDN4-6 genes<sup>[182]</sup>. Newly synthesized proteins at ribosomes bind to prefoldin which in cooperation with HSP70/HSP40 transports them to TRiC/CCT for proper folding and preventing protein aggregation. Prefoldin also plays an important role in clearing aggregated proteins as a result of ER stress or proteasome inhibitor treatment.

The HCV F protein, a 17 kDa product of ribosomal frameshift at the beginning of the core protein coding sequence, was found to bind prefoldin 2<sup>[183]</sup>. Prefoldin is involved in the proper folding of actin and tubulin subunits and plays an important role in the formation of the cytoskeleton. It was found that overexpression of the HCV F protein interfered with the prefoldin 1 and 2 interaction and resulted in an aberrant tubulin cytoskeleton. It was speculated that since an intact cytoskeleton is needed for HCV production in infected cells<sup>[184-187]</sup>, the HCV F protein may modulate and decrease virus production in order to establish a persistent chronic infection<sup>[183]</sup>.

### **ApoJ/clusterin (CLU)**

ApoJ, also known as clusterin, is another chaperone with both intracellular and extracellular functions including protein folding and extracellular protein degradation and is involved in a number of age-related diseases including cardiovascular and neurodegenerative diseases and cancer likely by interacting with HSP60<sup>[188,189]</sup>. HCV infection led to increased clusterin expression both in cell culture and serum of infected patients<sup>[190]</sup>. siRNA-mediated silencing of clusterin led to decreased virus production without affecting viral RNA replication levels suggesting a subsequent step such as translation, assembly, or secretion is affected. It was found that clusterin binds to and stabilizes core and NS5A.

### **PDI (PDI family) and MTTP (MTTP)**

The PDI family of proteins are ER chaperones that are responsible for disulfide bond formation<sup>[191]</sup>. The term PDI typically refers to the beta subunit of the prolyl 4-hydroxylase (P4H) enzyme, PDIA1 (P4HB), which is the first characterized member of the PDI family<sup>[192]</sup>. P4HB is involved in the folding and transfer of MTTP, a chaperone itself, from the cytosol into the lumen of ER<sup>[193,194]</sup>. P4HB and MTTP subsequently form a heterodimer, and MTTP then lipidates and stabilizes apolipoprotein B (apoB), a component of the VLDL produced by hepatocytes. ApoB associates with triglyceride containing particles generating VLDLs, and MTTP is involved in VLDL secretion as well<sup>[194,195]</sup>.

It has been shown that core expression leads to

decreased MTTP activity, in an HCV genotype 3-dependent manner<sup>[196]</sup> thereby reducing VLDL formation and secretion, which leads to accumulation of lipids in HCV-infected hepatocytes and subsequently liver steatosis<sup>[193,197,198]</sup>. Viral NS proteins have also been shown to decrease MTTP expression and activity and implicated in inhibition of VLDL secretion likely due to interaction of NS5A and apoB<sup>[199]</sup>. NS5A overexpression was also shown to decrease the expression of MTTP and increase lipid droplet size<sup>[200]</sup>. Furthermore, MTTP gene polymorphisms contribute to the accumulation of lipids in hepatocytes and may predict sustained virological response (SVR) to antiviral therapy in patients infected with genotype 4<sup>[201-204]</sup>. Thus, HCV infection is highly dependent on modulation of lipid metabolism, possibly in a genotype-specific manner<sup>[205-207]</sup>, through interactions with MTTP<sup>[208]</sup>. During maturation, the newly assembled virions acquire low-density configuration prior to being secreted, a process that requires MTTP, and the secreted viral particles are bound to VLDL<sup>[54,209,210]</sup>. Secretion of viral particles depends on the apoB-positive lipoprotein particles in an MTTP-dependent manner, while virion assembly (and infectivity through LDLR and GAGs) requires apoE and is not MTTP and VLDL dependent<sup>[34,211-216]</sup>.

P4HB activity was found to be increased in an HCV SGR system<sup>[129]</sup>. GRP58 (PDIA3), an important ER chaperone<sup>[191,217]</sup>, was found to be overexpressed in HeLa cells expressing HCV polyprotein<sup>[125]</sup>. Further, GRP58 was reproducibly enriched in the detergent-resistant membrane fraction of an SGR system, and knockdown of GRP58 led to a significant decrease in viral RNA replication<sup>[130]</sup>. The activity of two other PDI family members ERp72 (PDIA4) and PDIR (PDIA5) were also significantly increased in an HCV SGR system<sup>[72,191]</sup>. ERp5 (PDIA6) activity was reduced in an SGR system. It should be noted that SGR systems do not produce infectious virus, and the activity/expression of PDIs may, therefore, not correspond with the context of viral infection.

The PDI family also includes DNAJC10, an HSP40 family member, which is discussed in the HSP40 section above.

### **Calnexin (CANX) and calreticulin (CALR)**

Protein glycosylation among other post-translational modifications is carried out in the ER/Golgi apparatus. Calnexin and calreticulin are ER-resident chaperones that play a crucial role in the proper folding and glycosylation of glycoproteins. Both chaperones are part of a quality control mechanism in the ER that occurs in a cyclical manner<sup>[218]</sup>. Both HCV E1 and E2 being glycoproteins undergo the same cycles of quality control until they achieve the proper folding conformations required for the assembly of virions<sup>[98]</sup>. siRNA-mediated knockdown of calnexin and calreticulin decreased virus production<sup>[62]</sup>.

Both E1 and E2 rapidly associate with calnexin immediately after synthesis in the ER, but dissociate slowly<sup>[61,98,107,219]</sup>. While E2 folding occurs rapidly and is complete upon cleavage of the E2-NS2 precursor

polyprotein, folding of E1 is slow. Their association with calnexin parallels this timing suggesting that calnexin plays a role in proper folding of the E1/E2 glycoprotein complexes<sup>[220]</sup>. Calreticulin binds to E1 and E2 glycoproteins as well<sup>[98,107]</sup>. Whereas calnexin preferentially binds to monomeric glycoproteins, calreticulin seems to bind to E1/E2 aggregates. The N-linked oligosaccharides on these glycoproteins are important for the formation of E1/E2 complexes and for their interactions with some chaperones as treatment with tunicamycin, a glycosylation inhibitor, blocked the interaction of E1/E2 complexes with calnexin and calreticulin preventing their maturation and suppressing virus production<sup>[98,221,222]</sup>. Virus infectivity may also be impaired due to incorporation of immature glycoproteins in some virions<sup>[222]</sup>. Rather than being secreted, the E1/E2 complexes seem to remain in the ER and do not migrate past the cis-Golgi apparatus and are subsequently utilized in assembly of virions after undergoing proper folding and complex formation. Properly folded E1/E2 heterodimers no longer interact with calnexin<sup>[223-225]</sup>.

NS2 was reported to co-immunoprecipitate with CANX in infected cells<sup>[105]</sup>. All viral NS proteins were found to colocalize with the newly synthesized HCV RNA and calnexin at RCs which are ER-derived perinuclear structures<sup>[52]</sup>. In agreement with this observation, calnexin was reported to be associated with positive-strand subgenomic viral RNA (corresponding to domains III and IV of the 5' NCR and 36 nucleotides of core)<sup>[76]</sup>. Calnexin is also a target of miR-130a, miR-130b and miR-310 that were shown to be downregulated in acute HCV infection<sup>[108]</sup>. HCV core protein causes ER stress thereby inducing the expression of calreticulin<sup>[101]</sup>. Calreticulin was reproducibly enriched in the detergent-resistant membrane fraction of an SGR system<sup>[130]</sup>. HCV infection was also found to increase calreticulin expression<sup>[226]</sup>.

### **EDEM1 (EDEM1) and EDEM3 (EDEM3)**

EDEMs that consist of three proteins EDEM1, EDEM2, and EDEM3 are lectin chaperones and regulators of ERAD that are involved in targeting misfolded glycoproteins to the ERAD pathway<sup>[227,228]</sup>. EDEMs binds to the target glycoproteins that are destined for degradation<sup>[229]</sup>. EDEMs also bind GRP78 and appear to provide the signal for degradation of the target glycoprotein<sup>[227]</sup>. EDEM1 and EDEM3, but not EDEM2, directly bind HCV glycoproteins and increase their ubiquitination<sup>[230]</sup>. Knockdown of EDEM1 and EDEM3 as well as treatment with kifunensine, an ERAD inhibitor, increased the half-life of E1 and E2 and virus production, and overexpression of the two EDEMs decreased virus production.

As mentioned above, misfolded proteins in the ER are targeted to the ERAD pathway if attempts to properly fold these proteins are unsuccessful<sup>[83]</sup>. While HCV production in cells leads to ER stress and the UPR, the virus has evolved strategies to prevent its proteins from being degraded through the ERAD pathway<sup>[44]</sup>. The ERAD pathway is activated downstream of the

IRE1 pathway, and the IRE1 pathway is activated in response to HCV-induced ER stress and activation of the UPR<sup>[92]</sup>. However, despite activation of the IRE1 pathway, activation of the ERAD pathway is inhibited in HCV infection<sup>[231]</sup>. Thus, although sXBP1 is produced indicating activation of the IRE1 pathway, expression of EDEM1 is suppressed. This seems to be unique for HCV as other flaviviruses do not suppress EDEM expression in presence of sXBP1 production<sup>[83,231]</sup>. HCV NS4B similarly leads to production of sXBP1, but suppresses EDEM expression<sup>[103]</sup>. The lack of EDEM induction may also lead to increased IRES-mediated translation of viral proteins<sup>[231]</sup>. These results suggest that EDEMs may play a crucial role in regulating viral protein homeostasis and maintaining a balance in viral protein production to establish persistent infection.

### **SigR1 (SIGMAR1)**

SigR1 is a cholesterol-binding chaperone in lipid-rich areas of ER and mitochondrion-associated ER membranes (MAMs)<sup>[232]</sup>. MAMs play an important role in pathogenesis of HCV by serving as interorganellar communication sites between ER and mitochondria both of which are crucial for HCV production<sup>[44]</sup>. SigR1 is normally involved in crucial processes including cellular response to stress, lipid and protein trafficking, cell survival, and neuroprotection<sup>[232,233]</sup>. SigR1 has been reported to play an important role for viral RNA replication immediately after virion entry, but not afterwards during persistent infection<sup>[44,234]</sup>. siRNA-mediated knockdown of SigR1 reduced viral RNA replication only in early stages of infection.

### **Prohibitin (PHB) and prohibitin 2 (PHB2)**

The mitochondrial chaperone prohibitin is involved in a variety of processes including mitochondrial protein folding and membrane potential, cell cycle, and apoptosis<sup>[235]</sup>. It forms a ring structure composed of two subunits encoded by the *PHB* and *PHB2* genes. The HCV core protein as well as viral infection lead to overexpression of prohibitin<sup>[236,237]</sup>, which is a target of the HCV core protein<sup>[238]</sup>. Core binds to prohibitin and impairs its chaperone function thereby preventing the proper function of mitochondrial respiratory chain leading to overproduction of ROS which may result in hepatocarcinogenesis<sup>[237,238]</sup>. This is likely caused by the core-mediated suppression of the interaction between prohibitin and subunit I and IV of cytochrome C oxidase<sup>[239,240]</sup>.

### **Cyps (PPI family)**

Cyps are an important family of molecular chaperones most of which possess PPIase activity and are involved in diverse cellular processes including protein folding, scaffolding, protein trafficking, and apoptosis<sup>[241]</sup>. The genes that encode Cyps are referred to as PPIs. Cyps have been reported to be important for replication of HCV as well as other flaviviruses<sup>[242]</sup>, and Cyp inhibitors such as cyclosporine A (CsA) have been shown to effectively block virus production when used alone

or in combination with other antiviral agents such as IFN<sup>[243-262]</sup>. CyPs have been suggested to play important roles in the HCV life cycle including viral RNA replication, membranous web formation, viral polyprotein cleavage, lipid trafficking, virion assembly, suppression of IFN-based antiviral response, and induction of mitochondrial dysfunction.

It has been suggested that NS5B is recruited to the RCs in the membranous web by cyclophilin A (CypA) (PPIA) likely to ensure NS5B retains its proper conformation for viral RNA replication<sup>[263]</sup>. In fact, both NS5B and CypA share a common binding site on NS5A<sup>[264]</sup> suggesting that CypA delivers NS5B to the RCs at which point NS5B binds NS5A. This function of CypA is supported by the finding that treatment of cells with CsA reduces the levels of NS5B in RCs, but not NS5A or NS3<sup>[263]</sup>. In addition, mutant NS5B from CsA-resistant replicons retained their RC incorporation in presence of CsA. Other published Cyp inhibitor-selected mutations in NS5B have been reported to increase its RNA binding capacity<sup>[265-267]</sup>. Also the observed CsA resistance of the JFH1 strain (genotype 2a) is NS5B dependent<sup>[268]</sup>. PPIase mutant CypA maintained its NS5B binding<sup>[263]</sup>. However, the mutant CypA was unable to rescue HCV replication in CypA knockdown cells implicating its PPIase activity is important for HCV replication. Another study reported that CypA does not recruit NS5B or NS5A to RCs as CsA treatment did not affect the RC association of NS5B and NS5A, concluding the possibility of a CypA-independent recruitment of NS5B and NS5A to RCs<sup>[269]</sup>. A recent report seems to resolve this discrepancy<sup>[270]</sup>. It was found that Cyp inhibitor treatment did not affect the replicase activity of RCs after active RCs are established. This suggests that Cyp inhibitors exert their antiviral activity prior to formation of active RCs supporting the originally proposed CypA-mediated NS5B recruitment model.

In addition, NS5B binds to CypB (PPIB) which is required to stimulate the RNA-binding activity of NS5B and RNA synthesis<sup>[271-273]</sup>. Both CypA and CypB activate NS5B replicase function, particularly RNA binding, *in vitro* where CypB demonstrates viral genotype 1b specificity<sup>[274]</sup>. It was shown that the lack of PPIase activity in mutant CypA and CypB had some effect on NS5B activation, but the PPIase mutant CypA and CypB were still capable of activating NS5B to a significant extent suggesting that the PPIase activity is dispensable for NS5B activation. However, these experiments were performed in a cell free system, whereas the previous experiments showing the importance of PPIase activity in HCV replication were performed in a replicon system. Others have shown NS5B/CypB interaction to be mediated by CsA-associated helicase-like protein in GST pulldown assays<sup>[275]</sup>.

Significant evidence also points to a role of CyPs in viral RNA replication through their PPIase activity likely inducing conformational changes in viral and/or host proteins for optimal functioning. NS5A is a substrate for the PPIase activity of CypA and CypB through many

proline residues in NS5A domain II and the linker region between NS5A domains II and III (known as the low-complexity sequence II or LCS-II)<sup>[276-278]</sup>. A three amino acid structural motif, a proline-tryptophan turn, is essential for HCV RNA replication and proper interaction with CypA and influences the PPIase activity of CypA on NS5A domain II<sup>[279]</sup>. CypA also binds NS5A domain III and has PPIase activity towards some peptidylprolyl bonds in NS5A domain III<sup>[280]</sup>. The NS5A/CypA interaction and the PPIase activity of CypA, which are both disrupted by Cyp inhibitors, have been shown to be critical for HCV production<sup>[280-289]</sup>, and the PPIase activity of CypA is required for the NS5A/CypA interaction<sup>[281]</sup>. Further, wild-type CypA rescued viral RNA replication under CypA knockdown, but a PPIase mutant did not<sup>[284]</sup>. Indeed, it was found that CypA interacts with NS5A and stimulates RNA binding of NS5A domain II in a PPIase-dependent manner<sup>[290,291]</sup>. Furthermore, some SNP mutations in the PPIase domain of CypA render hepatocytes resistant to HCV replication likely by decreasing the intracellular stability of CypA<sup>[292]</sup>. Mutant NS5A from Cyp inhibitor resistant virus still binds to CypA as wild-type NS5A *in vitro*<sup>[281,282,286]</sup>, whereas in cell culture the interaction appears much stronger than with wild-type NS5A implying other cellular proteins are important for this interaction<sup>[170]</sup>. NS5B was found to further strengthen this interaction as well. Others have provided an alternative mechanism for resistance through NMR analyses showing that the resistant NS5A exhibited a trans to cis conformational shift possibly rendering NS5A less dependent on the PPIase activity of CypA for isomerization<sup>[285]</sup>. Importantly, the Cyp inhibitor-induced NS5A mutation can rescue viral replication under CypA knockdown conditions<sup>[282]</sup> although it still requires CypA at lower levels<sup>[293]</sup>. Thus, most of the evidence to date suggests that CypA is the most important Cyp in the context of HCV replication and that CypA and NS5A are the main targets of Cyp inhibitor-mediated antiviral activity as knockdown of CypB, CypC (PPIC), and CypD (PPIF) failed to suppress viral replication, and NS5A mutations have the major role in Cyp inhibitor resistance compared with NS5B and other viral proteins<sup>[263,265,283,284,293-298]</sup>.

Cyp inhibitor treatment also prevents formation of DMVs that are required for RNA replication at RCs suggesting that CyPs are involved in formation of RCs as well<sup>[270]</sup>. While the NS3-NS5B polyprotein and even NS5A alone suffices for formation of DMVs, knockdown of CypA prevents DMV formation suggesting that CyPs and, in particular, CypA is required for DMV formation. In addition, the PPIase activity of CypA was found to be required for DMV formation indicating that both NS5A and CypA are crucial for formation of DMVs.

The JFH1 SGR (lacking NS2) is not very sensitive to CsA or NIM811 (another Cyp inhibitor)<sup>[299]</sup>, and it was shown that full-length JFH1 was inhibited much more efficiently by CsA implicating NS2 to be important for CsA-mediated viral inhibition in a CypA-dependent manner<sup>[283,300,301]</sup>. Subsequently, it was found that NS2

itself is not a target of CsA, but that the rate-limiting NS2-NS3 cleavage determines sensitivity to CsA<sup>[301]</sup>. It has been suggested that NS3 also binds Cyps and that mutations in NS3 may also lead to CsA resistance<sup>[297,302]</sup>. Also it was found that the CypA dependence of HCV replication correlates with the NS5A-NS5B cleavage kinetics as demonstrated by substitution mutants at this cleavage site<sup>[283]</sup>. These findings indicate that viral polyprotein cleavage may at least in part be dependent on Cyps especially CypA.

CsA has also been shown to affect hepatocyte lipids pointing to an additional role of Cyps in lipid trafficking and in HCV pathogenesis<sup>[303]</sup>. Cyp inhibitor treatment disrupts the VLDL pathway of virus maturation described above resulting in increased lipid droplet size, accumulation of apoB on lipid droplets, removal of NS5A from lipid droplets, and inhibition of infectious virion assembly<sup>[291,303]</sup>. The Cyps involved were found to be CypA and Cyp40 (PPID).

Yet another role of CypA in viral infection has been suggested in the context of the IFN pathway<sup>[304]</sup>. It was found that CypA and IRF9, a component of the JAK/STAT pathway, directly bind each other *via* the PPIase domain of CypA and the newly-identified CypA binding site in the IRF-association domain of IRF9. Cyp inhibitors prevent this complex formation. Interestingly, NS5A and IRF9 compete for binding to CypA, and CypA inhibition led to increased IFN-induced transcriptional activity through interferon-sensitive response elements (ISREs). Thus, it seems that HCV utilizes NS5A to dampen the IFN response by replacing IRF9 in the CypA/IRF9 complex, in order to establish persistent infection in hepatocytes. Furthermore, it was observed that Cyp inhibitor treatment blocks phosphorylation of protein kinase R (PKR) and its target eIF2 $\alpha$  which inhibits translation of interferon-stimulated genes<sup>[305,306]</sup>. Cyp inhibitors also blocked stress granule formation. CypA binds PKR, and this interaction was disrupted by Cyp inhibitor treatment as well<sup>[305]</sup>. However, it was reported that Cyp inhibitor-mediated inhibition of PKR phosphorylation is due to suppression/clearing of viral infection rather than being a direct effect<sup>[306]</sup>. Thus, the significance of the CypA/PKR interaction and its disruption by Cyp inhibitors is not clear.

It is also reported that CsA treatment of uninfected huh7 cells induces the UPR and upregulation of GRP78<sup>[307]</sup>. Further, treatment of cells with UPR-inducing agents suppressed HCV replication. This may suggest that CsA may also exert its antiviral activity by inducing UPR which likely leads to improper viral glycoprotein/protein folding, their aggregation, and subsequent degradation.

The Cyp inhibitor alisporivir has also been found to prevent and to some extent reverse the negative impacts of HCV infection on mitochondrial function revealing another potential role for Cyps in the context of viral infection<sup>[308]</sup>. In particular, alisporivir prevents HCV-mediated collapse of the mitochondrial membrane potential, overproduction of ROS, and mitochondrial

calcium overload through inhibition of CypD-mediated opening of the mitochondrial permeability transition pore<sup>[308-310]</sup>.

## HCV PROTEINS AS CHAPERONES

Remarkably, some HCV proteins possess chaperone functions that are critical for virus production. For example, core, in particular the N-terminal domain I, has been shown to play important chaperone roles for viral RNA stabilization, dimerization, and structural rearrangements<sup>[311-315]</sup>. Also core appears to be involved in folding of the E1 glycoprotein<sup>[316]</sup>. Both viral glycoproteins E1 and E2 have been reported to possess chaperone functions. E2 has been reported to be required for proper E1 folding<sup>[317]</sup>. The disulfide bonds in E1 have been shown to be required for the proper function of E2 during viral assembly and entry<sup>[318]</sup>, and E2 does not seem to be able to reach a native structure in the absence of E1<sup>[319]</sup>. Further, a monoclonal antibody was reported to recognize properly folded E2 only when complexed with E1<sup>[224]</sup>. Also the ectodomain of E2 was shown to fold only in presence of E1<sup>[320]</sup>. CANX may be important for the chaperone activities of HCV glycoproteins<sup>[220]</sup>. This is in agreement with the observation that E2, unlike E1, did not associate with cellular chaperones such as CANX in an infection-free system<sup>[319]</sup>. In many class II enveloped viruses, of which HCV is a member, one viral glycoprotein acts as a chaperone for the folding of the other one which carries out the membrane fusion after viral entry in order to release viral genome in the cytosol<sup>[321]</sup>. However, for HCV, the mechanism of membrane fusion and the role of glycoproteins is not fully understood. The NS3 protein which possesses a helicase domain has been reported to mediate functions beyond the known helicase activity as it is involved in "intermolecular annealing, resolves three-stranded RNA duplexes, and assists dsRNA and ssRNA inter-conversions to establish a steady state among RNA structures"<sup>[322]</sup>. NS4A directs NS3 to ER and increases the intracellular stability of NS3<sup>[323]</sup>.

## CONCLUSION

Chaperones play crucial roles in HCV infection, and essentially all phases of the viral life cycle depend on chaperone functions and the interaction of viral proteins with chaperones (Table 1). The critical roles of Cyps and HSP90 in HCV RNA replication among others, HSP70 in viral protein translation, HSC70 in virion assembly, and the ER chaperones GRP78 and GRP94 in viral protein stability and persistent infection are important examples. Better understanding of the role of chaperones in the viral life cycle will provide further insights into the mechanism of virus production and suppression of immune response. Recently, significant advancements have been achieved in HCV therapy, and IFN-free therapies utilizing combinations of direct-acting antivirals (DAAs) with or without ribavirin (RBV) are being used successfully to achieve SVR in the majority of cases.



Besides very high costs associated with some therapies, other issues include variability in activity across different genotypes, such as genotype 3 that can result in failure to achieve SVR<sup>[324]</sup>. If RBV is required, significant side effects can occur such as hemolytic anemia<sup>[325]</sup>. Treatment with DAAs can also result in resistant virus as targeting viral proteins puts direct selective pressure for resistant mutants. Furthermore, a small percentage of patients are infected with intergenotypic recombinant strains of HCV which may not respond optimally to the current standard treatments<sup>[326,327]</sup>. Analysis of the role of chaperones in the viral life cycle may allow for development of novel strategies to target HCV infection. Targeting host factors may reduce selective pressure on the virus to generate resistant mutants. Furthermore, insights obtained by studying chaperones in HCV infection may allow for development of therapies for other viruses especially flaviviruses.

## REFERENCES

- Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]
- World Health Organization. Hepatitis C, Fact Sheet N°164. 2014. [accessed 2015 Sept 30]. Available from: URL: <http://www.who.int/mediacentre/factsheets/fs164/en/>
- Gravitz L. Introduction: a smouldering public-health crisis. *Nature* 2011; **474**: S2-S4 [PMID: 21666731 DOI: 10.1038/474S2a]
- Freeman RB, Steffick DE, Guidinger MK, Farmer DG, Berg CL, Merion RM. Liver and intestine transplantation in the United States, 1997-2006. *Am J Transplant* 2008; **8**: 958-976 [PMID: 18336699 DOI: 10.1111/j.1600-6143.2008.02174.x]
- Biggins SW, Bambha KM, Terrault NA, Inadomi J, Shiboski S, Dodge JL, Gralla J, Rosen HR, Roberts JP. Projected future increase in aging hepatitis C virus-infected liver transplant candidates: a potential effect of hepatocellular carcinoma. *Liver Transpl* 2012; **18**: 1471-1478 [PMID: 23008049 DOI: 10.1002/lt.23551]
- Younossi ZM, Kanwal F, Saab S, Brown KA, El-Serag HB, Kim WR, Ahmed A, Kugelmas M, Gordon SC. The impact of hepatitis C burden: an evidence-based approach. *Aliment Pharmacol Ther* 2014; **39**: 518-531 [PMID: 24461160 DOI: 10.1111/apt.12625]
- Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; **5**: 558-567 [PMID: 16122679 DOI: 10.1016/S1473-3099(05)70216-4]
- Rich JD, Taylor LE. The beginning of a new era in understanding hepatitis C virus prevention. *J Infect Dis* 2010; **202**: 981-983 [PMID: 20726769 DOI: 10.1086/656213]
- El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004; **127**: S27-S34 [PMID: 15508094 DOI: 10.1053/j.gastro.2004.09.013]
- Baron S. Medical microbiology. 4th ed. Galveston, Tex.: University of Texas Medical Branch at Galveston, 1996
- Lindenbach BD, Rice CM. Unravelling hepatitis C virus replication from genome to function. *Nature* 2005; **436**: 933-938 [PMID: 16107832 DOI: 10.1038/nature04077]
- Wang C, Sarnow P, Siddiqui A. Translation of human hepatitis C virus RNA in cultured cells is mediated by an internal ribosome-binding mechanism. *J Virol* 1993; **67**: 3338-3344 [PMID: 8388503]
- Wozniak AL, Griffin S, Rowlands D, Harris M, Yi M, Lemon SM, Weinman SA. Intracellular proton conductance of the hepatitis C virus p7 protein and its contribution to infectious virus production. *PLoS Pathog* 2010; **6**: e1001087 [PMID: 20824094 DOI: 10.1371/journal.ppat.1001087]
- He Y, Yan W, Coito C, Li Y, Gale M, Katze MG. The regulation of hepatitis C virus (HCV) internal ribosome-entry site-mediated translation by HCV replicons and nonstructural proteins. *J Gen Virol* 2003; **84**: 535-543 [PMID: 12604803 DOI: 10.1099/vir.0.18658-0]
- Tellinghuisen TL, Foss KL, Treadaway JC, Rice CM. Identification of residues required for RNA replication in domains II and III of the hepatitis C virus NS5A protein. *J Virol* 2008; **82**: 1073-1083 [PMID: 18032500 DOI: 10.1128/JVI.00328-07]
- Hughes M, Griffin S, Harris M. Domain III of NS5A contributes to both RNA replication and assembly of hepatitis C virus particles. *J Gen Virol* 2009; **90**: 1329-1334 [PMID: 19264615 DOI: 10.1099/vir.0.009332-0]
- Khachatoorian R, Arumugaswami V, Ruchala P, Raychaudhuri S, Maloney EM, Miao E, Dasgupta A, French SW. A cell-permeable hairpin peptide inhibits hepatitis C viral nonstructural protein 5A-mediated translation and virus production. *Hepatology* 2012; **55**: 1662-1672 [PMID: 22183951 DOI: 10.1002/hep.25533]
- Khachatoorian R, Ganapathy E, Ahmadieh Y, Wheatley N, Sundberg C, Jung CL, Arumugaswami V, Raychaudhuri S, Dasgupta A, French SW. The NS5A-binding heat shock proteins HSC70 and HSP70 play distinct roles in the hepatitis C viral life cycle. *Virology* 2014; **454-455**: 118-127 [PMID: 24725938 DOI: 10.1016/j.virol.2014.02.016]
- Lo SY, Selby M, Tong M, Ou JH. Comparative studies of the core gene products of two different hepatitis C virus isolates: two alternative forms determined by a single amino acid substitution. *Virology* 1994; **199**: 124-131 [PMID: 8116235 DOI: 10.1006/viro.1994.1104]
- Ray RB, Lagging LM, Meyer K, Ray R. Hepatitis C virus core protein cooperates with ras and transforms primary rat embryo fibroblasts to tumorigenic phenotype. *J Virol* 1996; **70**: 4438-4443 [PMID: 8676467]
- Xu Z, Choi J, Yen TS, Lu W, Strohecker A, Govindarajan S, Chien D, Selby MJ, Ou J. Synthesis of a novel hepatitis C virus protein by ribosomal frameshift. *EMBO J* 2001; **20**: 3840-3848 [PMID: 11447125 DOI: 10.1093/emboj/20.14.3840]
- Walewski JL, Keller TR, Stump DD, Branch AD. Evidence for a new hepatitis C virus antigen encoded in an overlapping reading frame. *RNA* 2001; **7**: 710-721 [PMID: 11350035]
- Varaklioti A, Vassilaki N, Georgopoulou U, Mavromara P. Alternate translation occurs within the core coding region of the hepatitis C viral genome. *J Biol Chem* 2002; **277**: 17713-17721 [PMID: 11884417 DOI: 10.1074/jbc.M201722200]
- Fiorucci M, Boulant S, Fournillier A, Abraham JD, Lavergne JP, Paranhos-Baccala G, Inchauspé G, Bain C. Expression of the alternative reading frame protein of Hepatitis C virus induces cytokines involved in hepatic injuries. *J Gen Virol* 2007; **88**: 1149-1162 [PMID: 17374758 DOI: 10.1099/vir.0.82575-0]
- Shao SW, Wu WB, Bian ZQ, Yu JG, Zhao P, Zhao LJ, Zhu SY, Qi ZT. Hepatitis C virus F protein inhibits cell apoptosis by activation of intracellular NF-kappaB pathway. *Hepatol Res* 2009; **39**: 282-289 [PMID: 19054148 DOI: 10.1111/j.1872-034X.2008.00452.x]
- Yuksekk K, Chen WL, Chien D, Ou JH. Ubiquitin-independent degradation of hepatitis C virus F protein. *J Virol* 2009; **83**: 612-621 [PMID: 18971267 DOI: 10.1128/JVI.00832-08]
- Hu WT, Li HC, Lee SK, Ma HC, Yang CH, Chen HL, Lo SY. Both core and F proteins of hepatitis C virus could enhance cell proliferation in transgenic mice. *Biochem Biophys Res Commun* 2013; **435**: 147-152 [PMID: 23628415 DOI: 10.1016/j.bbrc.2013.04.059]
- Yue M, Deng X, Zhai X, Xu K, Kong J, Zhang J, Zhou Z, Yu X, Xu X, Liu Y, Zhu D, Zhang Y. Th1 and Th2 cytokine profiles induced by hepatitis C virus F protein in peripheral blood mononuclear cells from chronic hepatitis C patients. *Immunol Lett* 2013; **152**: 89-95 [PMID: 23680070 DOI: 10.1016/j.imlet.2013.05.002]
- Xu X, Yu X, Deng X, Yue M, Zhang J, Zhu D, Zhou Z, Zhai X, Xu K, Zhang Y. Hepatitis C virus alternate reading frame protein decreases interferon- $\alpha$  secretion in peripheral blood mononuclear cells. *Mol Med Rep* 2014; **9**: 730-736 [PMID: 24270940 DOI: 10.3892/mmr.2014.2427]

- 10.3892/mmr.2013.1816]
- 30 **Xiao W**, Jiang LF, Deng XZ, Zhu DY, Pei JP, Xu ML, Li BJ, Wang CJ, Zhang JH, Zhang Q, Zhou ZX, Ding WL, Xu XD, Yue M. PD-1/PD-L1 signal pathway participates in HCV F protein-induced T cell dysfunction in chronic HCV infection. *Immunol Res* 2015; Epub ahead of print [PMID: 26286967 DOI: 10.1007/s12026-015-8680-y]
- 31 **Zhu DY**, Deng XZ, Jiang LF, Xiao W, Pei JP, Li BJ, Wang CJ, Zhang JH, Zhang Q, Zhou ZX, Ding WL, Xu XD, Yue M. Potential Role of Hepatitis C Virus Alternate Reading Frame Protein in Negative Regulation of T-Bet Gene Expression. *Inflammation* 2015; **38**: 1823-1834 [PMID: 25894282 DOI: 10.1007/s10753-015-0160-y]
- 32 **Pacheco A**, Martinez-Salas E. Insights into the biology of IRES elements through riboproteomic approaches. *J Biomed Biotechnol* 2010; **2010**: 458927 [PMID: 20150968 DOI: 10.1155/2010/458927]
- 33 **Moradpour D**, Penin F, Rice CM. Replication of hepatitis C virus. *Nat Rev Microbiol* 2007; **5**: 453-463 [PMID: 17487147 DOI: 10.1038/nrmicro1645]
- 34 **Lindenbach BD**, Rice CM. The ins and outs of hepatitis C virus entry and assembly. *Nat Rev Microbiol* 2013; **11**: 688-700 [PMID: 24018384 DOI: 10.1038/nrmicro3098]
- 35 **Blanchard E**, Belouzard S, Goueslain L, Wakita T, Dubuisson J, Wychowski C, Rouillé Y. Hepatitis C virus entry depends on clathrin-mediated endocytosis. *J Virol* 2006; **80**: 6964-6972 [PMID: 16809302 DOI: 10.1128/JVI.00024-06]
- 36 **Matsuda M**, Suzuki R, Kataoka C, Watashi K, Aizaki H, Kato N, Matsuura Y, Suzuki T, Wakita T. Alternative endocytosis pathway for productive entry of hepatitis C virus. *J Gen Virol* 2014; **95**: 2658-2667 [PMID: 25096815 DOI: 10.1099/vir.0.068528-0]
- 37 **Scheel TK**, Rice CM. Understanding the hepatitis C virus life cycle paves the way for highly effective therapies. *Nat Med* 2013; **19**: 837-849 [PMID: 23836234 DOI: 10.1038/nm.3248]
- 38 **Behrens SE**, Tomei L, De Francesco R. Identification and properties of the RNA-dependent RNA polymerase of hepatitis C virus. *EMBO J* 1996; **15**: 12-22 [PMID: 8598194]
- 39 **Paul D**, Madan V, Bartenschlager R. Hepatitis C virus RNA replication and assembly: living on the fat of the land. *Cell Host Microbe* 2014; **16**: 569-579 [PMID: 25525790 DOI: 10.1016/j.chom.2014.10.008]
- 40 **Suzuki T**. Morphogenesis of infectious hepatitis C virus particles. *Front Microbiol* 2012; **3**: 38 [PMID: 22347224 DOI: 10.3389/fmicb.2012.00038]
- 41 **Popescu CI**, Riva L, Vlaicu O, Farhat R, Rouillé Y, Dubuisson J. Hepatitis C virus life cycle and lipid metabolism. *Biology (Basel)* 2014; **3**: 892-921 [PMID: 25517881 DOI: 10.3390/biology3040892]
- 42 **Ye J**. Hepatitis C virus: a new class of virus associated with particles derived from very low-density lipoproteins. *Arterioscler Thromb Vasc Biol* 2012; **32**: 1099-1103 [PMID: 22517369 DOI: 10.1161/ATVBAHA.111.241448]
- 43 **Merquiol E**, Uzi D, Mueller T, Goldenberg D, Nahmias Y, Xavier RJ, Tirosh B, Shibolet O. HCV causes chronic endoplasmic reticulum stress leading to adaptation and interference with the unfolded protein response. *PLoS One* 2011; **6**: e24660 [PMID: 21949742 DOI: 10.1371/journal.pone.0024660]
- 44 **Vasallo C**, Gastaminza P. Cellular stress responses in hepatitis C virus infection: Mastering a two-edged sword. *Virus Res* 2015; **209**: 100-117 [PMID: 25836277 DOI: 10.1016/j.virusres.2015.03.013]
- 45 **Shinohara Y**, Imajo K, Yoneda M, Tomeno W, Ogawa Y, Kirikoshi H, Funakoshi K, Ikeda M, Kato N, Nakajima A, Saito S. Unfolded protein response pathways regulate Hepatitis C virus replication via modulation of autophagy. *Biochem Biophys Res Commun* 2013; **432**: 326-332 [PMID: 23395875 DOI: 10.1016/j.bbrc.2013.01.103]
- 46 **von dem Bussche A**, Machida R, Li K, Loevinsohn G, Khander A, Wang J, Wakita T, Wands JR, Li J. Hepatitis C virus NS2 protein triggers endoplasmic reticulum stress and suppresses its own viral replication. *J Hepatol* 2010; **53**: 797-804 [PMID: 20801537 DOI: 10.1016/j.jhep.2010.05.022]
- 47 **Braut C**, Levy PL, Bartosch B. Hepatitis C virus-induced mitochondrial dysfunctions. *Viruses* 2013; **5**: 954-980 [PMID: 23518579 DOI: 10.3390/v5030954]
- 48 **Ivanov AV**, Bartosch B, Smirnova OA, Isagulians MG, Kochetkov SN. HCV and oxidative stress in the liver. *Viruses* 2013; **5**: 439-469 [PMID: 23358390 DOI: 10.3390/v5020439]
- 49 **Deng L**, Adachi T, Kitayama K, Bungyoku Y, Kitazawa S, Ishido S, Shoji I, Hotta H. Hepatitis C virus infection induces apoptosis through a Bax-triggered, mitochondrion-mediated, caspase 3-dependent pathway. *J Virol* 2008; **82**: 10375-10385 [PMID: 18768989 DOI: 10.1128/JVI.00395-08]
- 50 **Lee SH**, Song R, Lee MN, Kim CS, Lee H, Kong YY, Kim H, Jang SK. A molecular chaperone glucose-regulated protein 94 blocks apoptosis induced by virus infection. *Hepatology* 2008; **47**: 854-866 [PMID: 18273841 DOI: 10.1002/hep.22107]
- 51 **Zhao P**, Han T, Guo JJ, Zhu SL, Wang J, Ao F, Jing MZ, She YL, Wu ZH, Ye LB. HCV NS4B induces apoptosis through the mitochondrial death pathway. *Virus Res* 2012; **169**: 1-7 [PMID: 22542667 DOI: 10.1016/j.virusres.2012.04.006]
- 52 **El-Hage N**, Luo G. Replication of hepatitis C virus RNA occurs in a membrane-bound replication complex containing nonstructural viral proteins and RNA. *J Gen Virol* 2003; **84**: 2761-2769 [PMID: 13679611 DOI: 10.1099/vir.0.19305-0]
- 53 **Huang H**, Sun F, Owen DM, Li W, Chen Y, Gale M, Ye J. Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low-density lipoproteins. *Proc Natl Acad Sci USA* 2007; **104**: 5848-5853 [PMID: 17376867 DOI: 10.1073/pnas.0700760104]
- 54 **Nahmias Y**, Goldwasser J, Casali M, van Poll D, Wakita T, Chung RT, Yarmush ML. Apolipoprotein B-dependent hepatitis C virus secretion is inhibited by the grapefruit flavonoid naringenin. *Hepatology* 2008; **47**: 1437-1445 [PMID: 18393287 DOI: 10.1002/hep.22197]
- 55 **Ellis RJ**. Molecular chaperones: assisting assembly in addition to folding. *Trends Biochem Sci* 2006; **31**: 395-401 [PMID: 16716593 DOI: 10.1016/j.tibs.2006.05.001]
- 56 **Höfheld J**, Cyr DM, Patterson C. From the cradle to the grave: molecular chaperones that may choose between folding and degradation. *EMBO Rep* 2001; **2**: 885-890 [PMID: 11600451 DOI: 10.1093/embo-reports/kve206]
- 57 **Hartl FU**, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. *Nature* 2011; **475**: 324-332 [PMID: 21776078 DOI: 10.1038/nature10317]
- 58 **Kim YK**, Jang SK. Continuous heat shock enhances translational initiation directed by internal ribosomal entry site. *Biochem Biophys Res Commun* 2002; **297**: 224-231 [PMID: 12237106 DOI: 10.1016/S0006-291X(02)02154-X]
- 59 **Clerico EM**, Tilitsky JM, Meng W, Gierasch LM. How hsp70 molecular machines interact with their substrates to mediate diverse physiological functions. *J Mol Biol* 2015; **427**: 1575-1588 [PMID: 25683596 DOI: 10.1016/j.jmb.2015.02.004]
- 60 **Cyr DM**, Ramos CH. Specification of Hsp70 function by Type I and Type II Hsp40. *Subcell Biochem* 2015; **78**: 91-102 [PMID: 25487017 DOI: 10.1007/978-3-319-11731-7\_4]
- 61 **Li Q**, Brass AL, Ng A, Hu Z, Xavier RJ, Liang TJ, Elledge SJ. A genome-wide genetic screen for host factors required for hepatitis C virus propagation. *Proc Natl Acad Sci USA* 2009; **106**: 16410-16415 [PMID: 19717417 DOI: 10.1073/pnas.0907439106]
- 62 **Randall G**, Panis M, Cooper JD, Tellinghuisen TL, Sukhodolets KE, Pfeffer S, Landthaler M, Landgraf P, Kan S, Lindenbach BD, Chien M, Weir DB, Russo JJ, Ju J, Brownstein MJ, Sheridan R, Sander C, Zavolan M, Tuschl T, Rice CM. Cellular cofactors affecting hepatitis C virus infection and replication. *Proc Natl Acad Sci USA* 2007; **104**: 12884-12889 [PMID: 17616579 DOI: 10.1073/pnas.0704894104]
- 63 **Gonzalez O**, Fontanes V, Raychaudhuri S, Loo R, Loo J, Arumugaswami V, Sun R, Dasgupta A, French SW. The heat shock protein inhibitor Quercetin attenuates hepatitis C virus production. *Hepatology* 2009; **50**: 1756-1764 [PMID: 19839005 DOI: 10.1002/hep.23232]
- 64 **Chen YJ**, Chen YH, Chow LP, Tsai YH, Chen PH, Huang CY, Chen WT, Hwang LH. Heat shock protein 72 is associated

- with the hepatitis C virus replicase complex and enhances viral RNA replication. *J Biol Chem* 2010; **285**: 28183-28190 [PMID: 20601427 DOI: 10.1074/jbc.M110.118323]
- 65 **Chumpitazi BF**, Bouillet L, Drouet MT, Kuhn L, Garin J, Zarski JP, Drouet C. Biological autoimmunity screening in hepatitis C patients by anti-HepG2 lysate and anti-heat shock protein 70.1 autoantibodies. *Eur J Clin Microbiol Infect Dis* 2009; **28**: 137-146 [PMID: 18696130 DOI: 10.1007/s10096-008-0599-y]
  - 66 **Fang C**, Yi Z, Liu F, Lan S, Wang J, Lu H, Yang P, Yuan Z. Proteome analysis of human liver carcinoma Huh7 cells harboring hepatitis C virus subgenomic replicon. *Proteomics* 2006; **6**: 519-527 [PMID: 16317778 DOI: 10.1002/pmic.200500233]
  - 67 **Lim YS**, Shin KS, Oh SH, Kang SM, Won SJ, Hwang SB. Nonstructural 5A protein of hepatitis C virus regulates heat shock protein 72 for its own propagation. *J Viral Hepat* 2012; **19**: 353-363 [PMID: 22497815 DOI: 10.1111/j.1365-2893.2011.01556.x]
  - 68 **Khachatoorian R**, Arumugaswami V, Raychaudhuri S, Yeh GK, Maloney EM, Wang J, Dasgupta A, French SW. Divergent antiviral effects of bioflavonoids on the hepatitis C virus life cycle. *Virology* 2012; **433**: 346-355 [PMID: 22975673 DOI: 10.1016/j.virol.2012.08.029]
  - 69 **Khachatoorian R**, Ruchala P, Waring A, Jung CL, Ganapathy E, Wheatley N, Sundberg C, Arumugaswami V, Dasgupta A, French SW. Structural characterization of the HSP70 interaction domain of the hepatitis C viral protein NS5A. *Virology* 2015; **475**: 46-55 [PMID: 25462345 DOI: 10.1016/j.virol.2014.10.011]
  - 70 **Harris D**, Zhang Z, Chaubey B, Pandey VN. Identification of cellular factors associated with the 3'-nontranslated region of the hepatitis C virus genome. *Mol Cell Proteomics* 2006; **5**: 1006-1018 [PMID: 16500930 DOI: 10.1074/mcp.M500429-MCP200]
  - 71 **Liu T**, Daniels CK, Cao S. Comprehensive review on the HSC70 functions, interactions with related molecules and involvement in clinical diseases and therapeutic potential. *Pharmacol Ther* 2012; **136**: 354-374 [PMID: 22960394 DOI: 10.1016/j.pharmthera.2012.08.014]
  - 72 **Singaravelu R**, Blais DR, McKay CS, Pezacki JP. Activity-based protein profiling of the hepatitis C virus replication in Huh-7 hepatoma cells using a non-directed active site probe. *Proteome Sci* 2010; **8**: 5 [PMID: 20181094 DOI: 10.1186/1477-5956-8-5]
  - 73 **Inoue Y**, Aizaki H, Hara H, Matsuda M, Ando T, Shimoji T, Murakami K, Masaki T, Shoji I, Homma S, Matsuura Y, Miyamura T, Wakita T, Suzuki T. Chaperonin TRiC/CCT participates in replication of hepatitis C virus genome via interaction with the viral NS5B protein. *Virology* 2011; **410**: 38-47 [PMID: 21093005 DOI: 10.1016/j.virol.2010.10.026]
  - 74 **Parent R**, Qu X, Petit MA, Beretta L. The heat shock cognate protein 70 is associated with hepatitis C virus particles and modulates virus infectivity. *Hepatology* 2009; **49**: 1798-1809 [PMID: 19434724 DOI: 10.1002/hep.22852]
  - 75 **Walsh P**, Bursac D, Law YC, Cyr D, Lithgow T. The J-protein family: modulating protein assembly, disassembly and translocation. *EMBO Rep* 2004; **5**: 567-571 [PMID: 15170475 DOI: 10.1038/sj.embor.7400172]
  - 76 **Upadhyay A**, Dixit U, Manvar D, Chaturvedi N, Pandey VN. Affinity capture and identification of host cell factors associated with hepatitis C virus (+) strand subgenomic RNA. *Mol Cell Proteomics* 2013; **12**: 1539-1552 [PMID: 23429521 DOI: 10.1074/mcp.M112.017020]
  - 77 **Peng ZG**, Fan B, Du NN, Wang YP, Gao LM, Li YH, Li YH, Liu F, You XF, Han YX, Zhao ZY, Cen S, Li JR, Song DQ, Jiang JD. Small molecular compounds that inhibit hepatitis C virus replication through destabilizing heat shock cognate 70 messenger RNA. *Hepatology* 2010; **52**: 845-853 [PMID: 20593456 DOI: 10.1002/hep.23766]
  - 78 **Chen DZ**, Jiang JD, Zhang KQ, He HP, Di YT, Zhang Y, Cai JY, Wang L, Li SL, Yi P, Peng ZG, Hao XJ. Evaluation of anti-HCV activity and SAR study of (+)-lycoridine through targeting of host heat-stress cognate 70 (Hsc70). *Bioorg Med Chem Lett* 2013; **23**: 2679-2682 [PMID: 23511018 DOI: 10.1016/j.bmcl.2013.02.089]
  - 79 **Du NN**, Peng ZG, Bi CW, Tang S, Li YH, Li JR, Zhu YP, Zhang JP, Wang YX, Jiang JD, Song DQ. N-substituted benzyl matrinic acid derivatives inhibit hepatitis C virus (HCV) replication through down-regulating host heat-stress cognate 70 (Hsc70) expression. *PLoS One* 2013; **8**: e58675 [PMID: 23516533 DOI: 10.1371/journal.pone.0058675]
  - 80 **Stricher F**, Macri C, Ruff M, Muller S. HSPA8/HSC70 chaperone protein: structure, function, and chemical targeting. *Autophagy* 2013; **9**: 1937-1954 [PMID: 24121476 DOI: 10.4161/autophagy.26448]
  - 81 **Noonan EJ**, Place RF, Giardina C, Hightower LE. Hsp70B' regulation and function. *Cell Stress Chaperones* 2007; **12**: 393-402 [PMID: 18229458 DOI: 10.1379/CSC-278e.1]
  - 82 **Dudek J**, Benedix J, Cappel S, Greiner M, Jalal C, Müller L, Zimmermann R. Functions and pathologies of BiP and its interaction partners. *Cell Mol Life Sci* 2009; **66**: 1556-1569 [PMID: 19151922 DOI: 10.1007/s00018-009-8745-y]
  - 83 **Yu CY**, Hsu YW, Liao CL, Lin YL. Flavivirus infection activates the XBP1 pathway of the unfolded protein response to cope with endoplasmic reticulum stress. *J Virol* 2006; **80**: 11868-11880 [PMID: 16987981 DOI: 10.1128/JVI.00879-06]
  - 84 **Chakrabarti A**, Chen AW, Varner JD. A review of the mammalian unfolded protein response. *Biotechnol Bioeng* 2011; **108**: 2777-2793 [PMID: 21809331 DOI: 10.1002/bit.23282]
  - 85 **Ciccaglione AR**, Marcantonio C, Tritarelli E, Equestre M, Vendittelli F, Costantino A, Geraci A, Rapicetta M. Activation of the ER stress gene gadd153 by hepatitis C virus sensitizes cells to oxidant injury. *Virus Res* 2007; **126**: 128-138 [PMID: 17368854 DOI: 10.1016/j.virusres.2007.02.006]
  - 86 **Sekine-Osajima Y**, Sakamoto N, Mishima K, Nakagawa M, Itsui Y, Tasaka M, Nishimura-Sakurai Y, Chen CH, Kanai T, Tsuchiya K, Wakita T, Enomoto N, Watanabe M. Development of plaque assays for hepatitis C virus-JFH1 strain and isolation of mutants with enhanced cytopathogenicity and replication capacity. *Virology* 2008; **371**: 71-85 [PMID: 17949770 DOI: 10.1016/j.virol.2007.09.019]
  - 87 **Li S**, Ye L, Yu X, Xu B, Li K, Zhu X, Liu H, Wu X, Kong L. Hepatitis C virus NS4B induces unfolded protein response and endoplasmic reticulum overload response-dependent NF-kappaB activation. *Virology* 2009; **391**: 257-264 [PMID: 19628242 DOI: 10.1016/j.virol.2009.06.039]
  - 88 **Funaoka Y**, Sakamoto N, Suda G, Itsui Y, Nakagawa M, Kakinuma S, Watanabe T, Mishima K, Ueyama M, Onozuka I, Nitta S, Kitazume A, Kiyohashi K, Murakawa M, Azuma S, Tsuchiya K, Watanabe M. Analysis of interferon signaling by infectious hepatitis C virus clones with substitutions of core amino acids 70 and 91. *J Virol* 2011; **85**: 5986-5994 [PMID: 21490101 DOI: 10.1128/JVI.02583-10]
  - 89 **Waris G**, Tardif KD, Siddiqui A. Endoplasmic reticulum (ER) stress: hepatitis C virus induces an ER-nucleus signal transduction pathway and activates NF-kappaB and STAT-3. *Biochem Pharmacol* 2002; **64**: 1425-1430 [PMID: 12417255 DOI: 10.1016/S0006-2952(02)01300-X]
  - 90 **Ke PY**, Chen SS. Activation of the unfolded protein response and autophagy after hepatitis C virus infection suppresses innate antiviral immunity in vitro. *J Clin Invest* 2011; **121**: 37-56 [PMID: 21135505 DOI: 10.1172/JCI41474]
  - 91 **Mohl BP**, Tedbury PR, Griffin S, Harris M. Hepatitis C virus-induced autophagy is independent of the unfolded protein response. *J Virol* 2012; **86**: 10724-10732 [PMID: 22837205 DOI: 10.1128/JVI.01667-12]
  - 92 **Chan SW**. Unfolded protein response in hepatitis C virus infection. *Front Microbiol* 2014; **5**: 233 [PMID: 24904547 DOI: 10.3389/fmicb.2014.00233]
  - 93 **Joyce MA**, Walters KA, Lamb SE, Yeh MM, Zhu LF, Kneteman N, Doyle JS, Katze MG, Tyrrell DL. HCV induces oxidative and ER stress, and sensitizes infected cells to apoptosis in SCID/Alb-uPA mice. *PLoS Pathog* 2009; **5**: e1000291 [PMID: 19242562 DOI: 10.1371/journal.ppat.1000291]
  - 94 **Mishima K**, Sakamoto N, Sekine-Osajima Y, Nakagawa M, Itsui Y, Azuma S, Kakinuma S, Kiyohashi K, Kitazume A, Tsuchiya K, Imamura M, Hiraga N, Chayama K, Wakita T, Watanabe M.



- Cell culture and in vivo analyses of cytopathic hepatitis C virus mutants. *Virology* 2010; **405**: 361-369 [PMID: 20609455 DOI: 10.1016/j.virol.2010.06.020]
- 95 **Tumurbaatar B**, Sun Y, Chan T, Sun J. Cre-estrogen receptor-mediated hepatitis C virus structural protein expression in mice. *J Virol Methods* 2007; **146**: 5-13 [PMID: 17628708 DOI: 10.1016/j.jviromet.2007.05.025]
  - 96 **Chandra PK**, Gunduz F, Hazari S, Kurt R, Panigrahi R, Poat B, Bruce D, Cohen AJ, Bohorquez HE, Carmody I, Loss G, Balart LA, Wu T, Dash S. Impaired expression of type I and type II interferon receptors in HCV-associated chronic liver disease and liver cirrhosis. *PLoS One* 2014; **9**: e108616 [PMID: 25265476 DOI: 10.1371/journal.pone.0108616]
  - 97 **Liberman E**, Fong YL, Selby MJ, Choo QL, Cousens L, Houghton M, Yen TS. Activation of the grp78 and grp94 promoters by hepatitis C virus E2 envelope protein. *J Virol* 1999; **73**: 3718-3722 [PMID: 10196264]
  - 98 **Choukhi A**, Ung S, Wychowski C, Dubuisson J. Involvement of endoplasmic reticulum chaperones in the folding of hepatitis C virus glycoproteins. *J Virol* 1998; **72**: 3851-3858 [PMID: 9557669]
  - 99 **Chan SW**, Egan PA. Hepatitis C virus envelope proteins regulate CHOP via induction of the unfolded protein response. *FASEB J* 2005; **19**: 1510-1512 [PMID: 16006626 DOI: 10.1096/fj.04-3455fje]
  - 100 **Chan SW**, Egan PA. Effects of hepatitis C virus envelope glycoprotein unfolded protein response activation on translation and transcription. *Arch Virol* 2009; **154**: 1631-1640 [PMID: 19763778 DOI: 10.1007/s00705-009-0495-5]
  - 101 **Benali-Furet NL**, Chami M, Houel L, De Giorgi F, Vernejoul F, Lagorce D, Buscail L, Bartenschlager R, Icha S, Rizzuto R, Paterlini-Bréchet P. Hepatitis C virus core triggers apoptosis in liver cells by inducing ER stress and ER calcium depletion. *Oncogene* 2005; **24**: 4921-4933 [PMID: 15897896 DOI: 10.1038/sj.onc.1208673]
  - 102 **Ciccaglione AR**, Costantino A, Tritarelli E, Marcantonio C, Equestre M, Marziliano N, Rapicetta M. Activation of endoplasmic reticulum stress response by hepatitis C virus proteins. *Arch Virol* 2005; **150**: 1339-1356 [PMID: 15770357 DOI: 10.1007/s00705-004-0487-4]
  - 103 **Zheng Y**, Gao B, Ye L, Kong L, Jing W, Yang X, Wu Z, Ye L. Hepatitis C virus non-structural protein NS4B can modulate an unfolded protein response. *J Microbiol* 2005; **43**: 529-536 [PMID: 16410770]
  - 104 **Jiang X**, Kanda T, Wu S, Nakamoto S, Wakita T, Shirasawa H, Yokosuka O. Hepatitis C virus nonstructural protein 5A inhibits thapsigargin-induced apoptosis. *PLoS One* 2014; **9**: e113499 [PMID: 25409163 DOI: 10.1371/journal.pone.0113499]
  - 105 **Germain MA**, Chatel-Chaix L, Gagné B, Bonnell E, Thibault P, Pradezynski F, de Chasse B, Meyniel-Schicklin L, Lotteau V, Baril M, Lamarre D. Elucidating novel hepatitis C virus-host interactions using combined mass spectrometry and functional genomics approaches. *Mol Cell Proteomics* 2014; **13**: 184-203 [PMID: 24169621 DOI: 10.1074/mcp.M113.030155]
  - 106 **Tardif KD**, Mori K, Siddiqui A. Hepatitis C virus subgenomic replicons induce endoplasmic reticulum stress activating an intracellular signaling pathway. *J Virol* 2002; **76**: 7453-7459 [PMID: 12097557 DOI: 10.1128/JVI.76.15.7453-7459.2002]
  - 107 **MacPherson JI**, Sidders B, Wieland S, Zhong J, Targett-Adams P, Lohmann V, Backes P, Delpuech-Adams O, Chisari F, Lewis M, Parkinson T, Robertson DL. An integrated transcriptomic and meta-analysis of hepatoma cells reveals factors that influence susceptibility to HCV infection. *PLoS One* 2011; **6**: e25584 [PMID: 22046242 DOI: 10.1371/journal.pone.0025584]
  - 108 **Liu X**, Wang T, Wakita T, Yang W. Systematic identification of microRNA and messenger RNA profiles in hepatitis C virus-infected human hepatoma cells. *Virology* 2010; **398**: 57-67 [PMID: 20006370 DOI: 10.1016/j.virol.2009.11.036]
  - 109 **Pavio N**, Romano PR, Graczyk TM, Feinstone SM, Taylor DR. Protein synthesis and endoplasmic reticulum stress can be modulated by the hepatitis C virus envelope protein E2 through the eukaryotic initiation factor 2alpha kinase PERK. *J Virol* 2003; **77**: 3578-3585 [PMID: 12610133 DOI: 10.1128/JVI.77.6.3578-3585.2003]
  - 110 **Egan PA**, Sobkowiak M, Chan SW. Hepatitis C Virus Envelope Protein E1 Binds PERK and Represses the Unfolded Protein Response. *Open Virol J* 2013; **7**: 37-40 [PMID: 23667408 DOI: 10.2174/1874357901307010037]
  - 111 **Selby M**, Erickson A, Dong C, Cooper S, Parham P, Houghton M, Walker CM. Hepatitis C virus envelope glycoprotein E1 originates in the endoplasmic reticulum and requires cytoplasmic processing for presentation by class I MHC molecules. *J Immunol* 1999; **162**: 669-676 [PMID: 9916684]
  - 112 **Pavio N**, Taylor DR, Lai MM. Detection of a novel unglycosylated form of hepatitis C virus E2 envelope protein that is located in the cytosol and interacts with PKR. *J Virol* 2002; **76**: 1265-1272 [PMID: 11773402 DOI: 10.1128/JVI.76.3.1265-1272.2002]
  - 113 **McPherson S**, Powell EE, Barrie HD, Clouston AD, McGuckin M, Jonsson JR. No evidence of the unfolded protein response in patients with chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 2011; **26**: 319-327 [PMID: 21261722 DOI: 10.1111/j.1440-1746.2010.06368.x]
  - 114 **Dores-Silva PR**, Barbosa LR, Ramos CH, Borges JC. Human mitochondrial Hsp70 (mortalin): shedding light on ATPase activity, interaction with adenosine nucleotides, solution structure and domain organization. *PLoS One* 2015; **10**: e0117170 [PMID: 25615450 DOI: 10.1371/journal.pone.0117170]
  - 115 **Flachbartová Z**, Kovacech B. Mortalin - a multipotent chaperone regulating cellular processes ranging from viral infection to neurodegeneration. *Acta Virol* 2013; **57**: 3-15 [PMID: 23530819 DOI: 10.4149/av\_2013\_01\_3]
  - 116 **Baaklini I**, Wong MJ, Hantouche C, Patel Y, Shrier A, Young JC. The DNAJA2 substrate release mechanism is essential for chaperone-mediated folding. *J Biol Chem* 2012; **287**: 41939-41954 [PMID: 23091061 DOI: 10.1074/jbc.M112.413278]
  - 117 **Park SY**, Choi HK, Seo JS, Yoo JY, Jeong JW, Choi Y, Choi KC, Yoon HG. DNAJB1 negatively regulates MIG6 to promote epidermal growth factor receptor signaling. *Biochim Biophys Acta* 2015; **1853**: 2722-2730 [PMID: 26239118 DOI: 10.1016/j.bbamer.2015.07.024]
  - 118 **Kubo N**, Wu D, Yoshihara Y, Sang M, Nakagawara A, Ozaki T. Co-chaperon DnaJC7/TPR2 enhances p53 stability and activity through blocking the complex formation between p53 and MDM2. *Biochem Biophys Res Commun* 2013; **430**: 1034-1039 [PMID: 23261415 DOI: 10.1016/j.bbrc.2012.11.121]
  - 119 **Blackham S**, Baillie A, Al-Hababi F, Remlinger K, You S, Hamatake R, McGarvey MJ. Gene expression profiling indicates the roles of host oxidative stress, apoptosis, lipid metabolism, and intracellular transport genes in the replication of hepatitis C virus. *J Virol* 2010; **84**: 5404-5414 [PMID: 20200238 DOI: 10.1128/JVI.02529-09]
  - 120 **Zhong XY**, Ding JH, Adams JA, Ghosh G, Fu XD. Regulation of SR protein phosphorylation and alternative splicing by modulating kinetic interactions of SRPK1 with molecular chaperones. *Genes Dev* 2009; **23**: 482-495 [PMID: 19240134 DOI: 10.1101/gad.1752109]
  - 121 **Yi Z**, Sperzel L, Nürnberger C, Bredenbeek PJ, Lubick KJ, Best SM, Stoyanov CT, Law LM, Yuan Z, Rice CM, MacDonald MR. Identification and characterization of the host protein DNAJC14 as a broadly active flavivirus replication modulator. *PLoS Pathog* 2011; **7**: e1001255 [PMID: 21249176 DOI: 10.1371/journal.ppat.1001255]
  - 122 **Yi Z**, Yuan Z, Rice CM, MacDonald MR. Flavivirus replication complex assembly revealed by DNAJC14 functional mapping. *J Virol* 2012; **86**: 11815-11832 [PMID: 22915803 DOI: 10.1128/JVI.01022-12]
  - 123 **Qiu XB**, Shao YM, Miao S, Wang L. The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. *Cell Mol Life Sci* 2006; **63**: 2560-2570 [PMID: 16952052 DOI: 10.1007/s00018-006-6192-6]
  - 124 **Lee HJ**, Kim JM, Kim KH, Heo JI, Kwak SJ, Han JA. Genotoxic



- stress/p53-induced DNAJB9 inhibits the pro-apoptotic function of p53. *Cell Death Differ* 2015; **22**: 86-95 [PMID: 25146923 DOI: 10.1038/cdd.2014.116]
- 125 **Vandermeeren AM**, Gómez CE, Patiño C, Domingo-Gil E, Guerra S, González JM, Esteban M. Subcellular forms and biochemical events triggered in human cells by HCV polyprotein expression from a viral vector. *Virology* 2008; **5**: 102 [PMID: 18793431 DOI: 10.1186/1743-422X-5-102]
  - 126 **Oka OB**, Pringle MA, Schopp IM, Braakman I, Bulleid NJ. ERdj5 is the ER reductase that catalyzes the removal of non-native disulfides and correct folding of the LDL receptor. *Mol Cell* 2013; **50**: 793-804 [PMID: 23769672 DOI: 10.1016/j.molcel.2013.05.014]
  - 127 **Elwi AN**, Lee B, Meijndert HC, Braun JE, Kim SW. Mitochondrial chaperone DnaJA3 induces Drp1-dependent mitochondrial fragmentation. *Int J Biochem Cell Biol* 2012; **44**: 1366-1376 [PMID: 22595283 DOI: 10.1016/j.biocel.2012.05.004]
  - 128 **Bracher A**, Verghese J, GrpE, Hsp110/Grp170, HspBPI/Sil1 and BAG domain proteins: nucleotide exchange factors for Hsp70 molecular chaperones. *Subcell Biochem* 2015; **78**: 1-33 [PMID: 25487014 DOI: 10.1007/978-3-319-11731-7\_1]
  - 129 **Blais DR**, Brûlotte M, Qian Y, Bélanger S, Yao SQ, Pezacki JP. Activity-based proteome profiling of hepatoma cells during hepatitis C virus replication using protease substrate probes. *J Proteome Res* 2010; **9**: 912-923 [PMID: 19954226 DOI: 10.1021/pr900788a]
  - 130 **Hara H**, Aizaki H, Matsuda M, Shinkai-Ouchi F, Inoue Y, Murakami K, Shoji I, Kawakami H, Matsuura Y, Lai MM, Miyamura T, Wakita T, Suzuki T. Involvement of creatine kinase B in hepatitis C virus genome replication through interaction with the viral NS4A protein. *J Virol* 2009; **83**: 5137-5147 [PMID: 19264780 DOI: 10.1128/JVI.02179-08]
  - 131 **Park JM**, Kim JW, Hahm KB. HSPA4, the „Evil Chaperone” of the HSP Family, Delays Gastric Ulcer Healing. *Dig Dis Sci* 2015; **60**: 824-826 [PMID: 25732714 DOI: 10.1007/s10620-015-3597-9]
  - 132 **Yang Z**, Zhuang L, Szatmary P, Wen L, Sun H, Lu Y, Xu Q, Chen X. Upregulation of heat shock proteins (HSPA12A, HSP90B1, HSPA4, HSPA5 and HSPA6) in tumour tissues is associated with poor outcomes from HBV-related early-stage hepatocellular carcinoma. *Int J Med Sci* 2015; **12**: 256-263 [PMID: 25798051 DOI: 10.7150/ijms.10735]
  - 133 **Saito Y**, Yamagishi N, Hatayama T. Nuclear localization mechanism of Hsp105beta and its possible function in mammalian cells. *J Biochem* 2009; **145**: 185-191 [PMID: 19028714 DOI: 10.1093/jb/mvn155]
  - 134 **Tai AW**, Benita Y, Peng LF, Kim SS, Sakamoto N, Xavier RJ, Chung RT. A functional genomic screen identifies cellular cofactors of hepatitis C virus replication. *Cell Host Microbe* 2009; **5**: 298-307 [PMID: 19286138 DOI: 10.1016/j.chom.2009.02.001]
  - 135 **Eckl JM**, Richter K. Functions of the Hsp90 chaperone system: lifting client proteins to new heights. *Int J Biochem Mol Biol* 2013; **4**: 157-165 [PMID: 24380020]
  - 136 **Geller R**, Tagawa S, Frydman J. Broad action of Hsp90 as a host chaperone required for viral replication. *Biochim Biophys Acta* 2012; **1823**: 698-706 [PMID: 22154817 DOI: 10.1016/j.bbamcr.2011.11.007]
  - 137 **Okamoto T**, Nishimura Y, Ichimura T, Suzuki K, Miyamura T, Suzuki T, Moriishi K, Matsuura Y. Hepatitis C virus RNA replication is regulated by FKBP8 and Hsp90. *EMBO J* 2006; **25**: 5015-5025 [PMID: 17024179 DOI: 10.1038/sj.emboj.7601367]
  - 138 **Nakagawa S**, Umehara T, Matsuda C, Kuge S, Sudoh M, Kohara M. Hsp90 inhibitors suppress HCV replication in replicon cells and humanized liver mice. *Biochem Biophys Res Commun* 2007; **353**: 882-888 [PMID: 17196931 DOI: 10.1016/j.bbrc.2006.12.117]
  - 139 **Shan GZ**, Peng ZG, Li YH, Li D, Li YP, Meng S, Gao LY, Jiang JD, Li ZR. A novel class of geldanamycin derivatives as HCV replication inhibitors targeting on Hsp90: synthesis, structure-activity relationships and anti-HCV activity in GS4.3 replicon cells. *J Antibiot (Tokyo)* 2011; **64**: 177-182 [PMID: 21179047 DOI: 10.1038/ja.2010.161]
  - 140 **Waxman L**, Whitney M, Pollok BA, Kuo LC, Darke PL. Host cell factor requirement for hepatitis C virus enzyme maturation. *Proc Natl Acad Sci USA* 2001; **98**: 13931-13935 [PMID: 11707594 DOI: 10.1073/pnas.241510898]
  - 141 **Kubota N**, Inayoshi Y, Satoh N, Fukuda T, Iwai K, Tomoda H, Kohara M, Kataoka K, Shimamoto A, Furuichi Y, Nomoto A, Naganuma A, Kuge S. HSC90 is required for nascent hepatitis C virus core protein stability in yeast cells. *FEBS Lett* 2012; **586**: 2318-2325 [PMID: 22659183 DOI: 10.1016/j.febslet.2012.05.023]
  - 142 **Ujino S**, Yamaguchi S, Shimotohno K, Takaku H. Heat-shock protein 90 is essential for stabilization of the hepatitis C virus nonstructural protein NS3. *J Biol Chem* 2009; **284**: 6841-6846 [PMID: 19150985 DOI: 10.1074/jbc.M806452200]
  - 143 **Dolan PT**, Zhang C, Khadka S, Arumugaswami V, Vangeloff AD, Heaton NS, Sahasrabudhe S, Randall G, Sun R, LaCount DJ. Identification and comparative analysis of hepatitis C virus-host cell protein interactions. *Mol Biosyst* 2013; **9**: 3199-3209 [PMID: 24136289 DOI: 10.1039/c3mb70343f]
  - 144 **Ujino S**, Nishitsuji H, Sugiyama R, Suzuki H, Hishiki T, Sugiyama K, Shimotohno K, Takaku H. The interaction between human initiation factor eIF3 subunit c and heat-shock protein 90: a necessary factor for translation mediated by the hepatitis C virus internal ribosome entry site. *Virus Res* 2012; **163**: 390-395 [PMID: 22016036 DOI: 10.1016/j.virusres.2011.10.003]
  - 145 **Bukong TN**, Hou W, Kodys K, Szabo G. Ethanol facilitates hepatitis C virus replication via up-regulation of GW182 and heat shock protein 90 in human hepatoma cells. *Hepatology* 2013; **57**: 70-80 [PMID: 22898980 DOI: 10.1002/hep.26010]
  - 146 **Bukong TN**, Momen-Heravi F, Kodys K, Bala S, Szabo G. Exosomes from hepatitis C infected patients transmit HCV infection and contain replication competent viral RNA in complex with Ago2-miR122-HSP90. *PLoS Pathog* 2014; **10**: e1004424 [PMID: 25275643 DOI: 10.1371/journal.ppat.1004424]
  - 147 **Kim MG**, Moon JS, Kim EJ, Lee SH, Oh JW. Destabilization of PDK1 by Hsp90 inactivation suppresses hepatitis C virus replication through inhibition of PRK2-mediated viral RNA polymerase phosphorylation. *Biochem Biophys Res Commun* 2012; **421**: 112-118 [PMID: 22490666 DOI: 10.1016/j.bbrc.2012.03.126]
  - 148 **Kim SJ**, Kim JH, Sun JM, Kim MG, Oh JW. Suppression of hepatitis C virus replication by protein kinase C-related kinase 2 inhibitors that block phosphorylation of viral RNA polymerase. *J Viral Hepat* 2009; **16**: 697-704 [PMID: 19243496 DOI: 10.1111/j.1365-2893.2009.01108.x]
  - 149 **Kim SJ**, Kim JH, Kim YG, Lim HS, Oh JW. Protein kinase C-related kinase 2 regulates hepatitis C virus RNA polymerase function by phosphorylation. *J Biol Chem* 2004; **279**: 50031-50041 [PMID: 15364941 DOI: 10.1074/jbc.M408617200]
  - 150 **Ito M**, Murakami K, Suzuki T, Mochida K, Suzuki M, Ikebuchi K, Yamaguchi K, Mizuochi T. Enhanced expression of lymphomagenesis-related genes in peripheral blood B cells of chronic hepatitis C patients. *Clin Immunol* 2010; **135**: 459-465 [PMID: 20189883 DOI: 10.1016/j.clim.2010.02.002]
  - 151 **Ito M**, Masumi A, Mochida K, Kukihiro H, Moriishi K, Matsuura Y, Yamaguchi K, Mizuochi T. Peripheral B cells may serve as a reservoir for persistent hepatitis C virus infection. *J Innate Immun* 2010; **2**: 607-617 [PMID: 20714117 DOI: 10.1159/000317690]
  - 152 **Boukli NM**, Shetty V, Cubano L, Ricaurte M, Coelho-Dos-Reis J, Nickens Z, Shah P, Talal AH, Philip R, Jain P. Unique and differential protein signatures within the mononuclear cells of HIV-1 and HCV mono-infected and co-infected patients. *Clin Proteomics* 2012; **9**: 11 [PMID: 22958358 DOI: 10.1186/1559-0275-9-11]
  - 153 **Marzec M**, Eletto D, Argon Y. GRP94: An HSP90-like protein specialized for protein folding and quality control in the endoplasmic reticulum. *Biochim Biophys Acta* 2012; **1823**: 774-787 [PMID: 22079671 DOI: 10.1016/j.bbamcr.2011.10.013]
  - 154 **Han JM**, Park SG, Liu B, Park BJ, Kim JY, Jin CH, Song YW, Li Z, Kim S. Aminoacyl-tRNA synthetase-interacting multifunctional protein 1/p43 controls endoplasmic reticulum retention of heat shock protein gp96: its pathological implications in lupus-like autoimmune diseases. *Am J Pathol* 2007; **170**: 2042-2054 [PMID: 17024179 DOI: 10.1038/sj.emboj.7601367]

- 17525271 DOI: 10.2353/ajpath.2007.061266]
- 155 **Kim MS**, Kim S, Myung H. Degradation of AIMP1/p43 induced by hepatitis C virus E2 leads to upregulation of TGF- $\beta$  signaling and increase in surface expression of gp96. *PLoS One* 2014; **9**: e96302 [PMID: 24816397 DOI: 10.1371/journal.pone.0096302]
- 156 **Nakamura H**, Minegishi H. HSP60 as a drug target. *Curr Pharm Des* 2013; **19**: 441-451 [PMID: 22920899 DOI: 10.2174/1381612811306030441]
- 157 **Hemmingsen SM**, Woolford C, van der Vies SM, Tilly K, Dennis DT, Georgopoulos CP, Hendrix RW, Ellis RJ. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. *Nature* 1988; **333**: 330-334 [PMID: 2897629 DOI: 10.1038/333330a0]
- 158 **Leitner A**, Joachimiak LA, Bracher A, Mönkemeyer L, Walzthoeni T, Chen B, Pechmann S, Holmes S, Cong Y, Ma B, Ludtke S, Chiu W, Hartl FU, Aebersold R, Frydman J. The molecular architecture of the eukaryotic chaperonin TRiC/CCT. *Structure* 2012; **20**: 814-825 [PMID: 22503819 DOI: 10.1016/j.str.2012.03.007]
- 159 **Kang SM**, Kim SJ, Kim JH, Lee W, Kim GW, Lee KH, Choi KY, Oh JW. Interaction of hepatitis C virus core protein with Hsp60 triggers the production of reactive oxygen species and enhances TNF- $\alpha$ -mediated apoptosis. *Cancer Lett* 2009; **279**: 230-237 [PMID: 19264393 DOI: 10.1016/j.canlet.2009.02.003]
- 160 **Padwad YS**, Mishra KP, Jain M, Chanda S, Karan D, Ganju L. RNA interference mediated silencing of Hsp60 gene in human monocytic myeloma cell line U937 revealed decreased dengue virus multiplication. *Immunobiology* 2009; **214**: 422-429 [PMID: 19261350 DOI: 10.1016/j.imbio.2008.11.010]
- 161 **Cappello F**, Marino Gammazza A, Palumbo Piccionello A, Campanella C, Pace A, Conway de Macario E, Macario AJ. Hsp60 chaperonopathies and chaperonotherapy: targets and agents. *Expert Opin Ther Targets* 2014; **18**: 185-208 [PMID: 24286280 DOI: 10.1517/14728222.2014.856417]
- 162 **Fukuda Y**, Yotsuyanagi H, Ooka S, Sekine T, Koike J, Takano T, Suzuki M, Itoh F, Nishioka K, Kato T. Identification of a new autoantibody in patients with chronic hepatitis. *Hum Immunol* 2004; **65**: 1530-1538 [PMID: 15603881 DOI: 10.1016/j.hum-imm.2004.08.186]
- 163 **Bakthisaran R**, Tangirala R, Rao ChM. Small heat shock proteins: Role in cellular functions and pathology. *Biochim Biophys Acta* 2015; **1854**: 291-319 [PMID: 25556000 DOI: 10.1016/j.bbapap.2014.12.019]
- 164 **Choi YW**, Tan YJ, Lim SG, Hong W, Goh PY. Proteomic approach identifies HSP27 as an interacting partner of the hepatitis C virus NS5A protein. *Biochem Biophys Res Commun* 2004; **318**: 514-519 [PMID: 15120631 DOI: 10.1016/j.bbrc.2004.04.052]
- 165 **Wang X**, Chen M, Zhou J, Zhang X. HSP27, 70 and 90, anti-apoptotic proteins, in clinical cancer therapy (Review). *Int J Oncol* 2014; **45**: 18-30 [PMID: 24789222 DOI: 10.3892/ijo.2014.2399]
- 166 **Acunzo J**, Katsogiannou M, Rocchi P. Small heat shock proteins HSP27 (HspB1),  $\alpha$ B-crystallin (HspB5) and HSP22 (HspB8) as regulators of cell death. *Int J Biochem Cell Biol* 2012; **44**: 1622-1631 [PMID: 22521623 DOI: 10.1016/j.biocel.2012.04.002]
- 167 **Zhu H**, Liu P, Li J. BAG3: a new therapeutic target of human cancers? *Histol Histopathol* 2012; **27**: 257-261 [PMID: 22237703]
- 168 **Edlich F**, Lücke C. From cell death to viral replication: the diverse functions of the membrane-associated FKBP38. *Curr Opin Pharmacol* 2011; **11**: 348-353 [PMID: 21514222 DOI: 10.1016/j.coph.2011.03.011]
- 169 **Wang J**, Tong W, Zhang X, Chen L, Yi Z, Pan T, Hu Y, Xiang L, Yuan Z. Hepatitis C virus non-structural protein NS5A interacts with FKBP38 and inhibits apoptosis in Huh7 hepatoma cells. *FEBS Lett* 2006; **580**: 4392-4400 [PMID: 16844119 DOI: 10.1016/j.febslet.2006.07.002]
- 170 **Okamoto T**, Omori H, Kaname Y, Abe T, Nishimura Y, Suzuki T, Miyamura T, Yoshimori T, Moriishi K, Matsuura Y. A single-amino-acid mutation in hepatitis C virus NS5A disrupting FKBP8 interaction impairs viral replication. *J Virol* 2008; **82**: 3480-3489 [PMID: 18216108 DOI: 10.1128/JVI.02253-07]
- 171 **Taguwa S**, Okamoto T, Abe T, Mori Y, Suzuki T, Moriishi K, Matsuura Y. Human butyrate-induced transcript 1 interacts with hepatitis C virus NS5A and regulates viral replication. *J Virol* 2008; **82**: 2631-2641 [PMID: 18160438 DOI: 10.1128/JVI.02153-07]
- 172 **Courilleau D**, Chastre E, Sabbah M, Redeuilh G, Atfi A, Mester J. B-ind1, a novel mediator of Rac1 signaling cloned from sodium butyrate-treated fibroblasts. *J Biol Chem* 2000; **275**: 17344-17348 [PMID: 10747961 DOI: 10.1074/jbc.M000887200]
- 173 **Bosco EE**, Mulloy JC, Zheng Y. Rac1 GTPase: a "Rac" of all trades. *Cell Mol Life Sci* 2009; **66**: 370-374 [PMID: 19151919 DOI: 10.1007/s00018-008-8552-x]
- 174 **Taguwa S**, Kambara H, Omori H, Tani H, Abe T, Mori Y, Suzuki T, Yoshimori T, Moriishi K, Matsuura Y. Cochaperone activity of human butyrate-induced transcript 1 facilitates hepatitis C virus replication through an Hsp90-dependent pathway. *J Virol* 2009; **83**: 10427-10436 [PMID: 19656872 DOI: 10.1128/JVI.01035-09]
- 175 **Tani J**, Shimamoto S, Mori K, Kato N, Moriishi K, Matsuura Y, Tokumitsu H, Tsuchiya M, Fujimoto T, Kato K, Miyoshi H, Masaki T, Kobayashi R. Ca(2+)/S100 proteins regulate HCV virus NS5A-FKBP8/FKBP38 interaction and HCV virus RNA replication. *Liver Int* 2013; **33**: 1008-1018 [PMID: 23522085 DOI: 10.1111/liv.12151]
- 176 **Donato R**, Cannon BR, Sorci G, Riuzzi F, Hsu K, Weber DJ, Geczy CL. Functions of S100 proteins. *Curr Mol Med* 2013; **13**: 24-57 [PMID: 22834835]
- 177 **Peng L**, Liang D, Tong W, Li J, Yuan Z. Hepatitis C virus NS5A activates the mammalian target of rapamycin (mTOR) pathway, contributing to cell survival by disrupting the interaction between FK506-binding protein 38 (FKBP38) and mTOR. *J Biol Chem* 2010; **285**: 20870-20881 [PMID: 20439463 DOI: 10.1074/jbc.M110.112045]
- 178 **Zannas AS**, Wiechmann T, Gassen NC, Binder EB. Gene-Stress-Epigenetic Regulation of FKBP5: Clinical and Translational Implications. *Neuropsychopharmacology* 2016; **41**: 261-274 [PMID: 26250598 DOI: 10.1038/npp.2015.235]
- 179 **Rehn AB**, Buchner J. p23 and Aha1. *Subcell Biochem* 2015; **78**: 113-131 [PMID: 25487019 DOI: 10.1007/978-3-319-11731-7\_6]
- 180 **Shirasaki T**, Honda M, Mizuno H, Shimakami T, Okada H, Sakai Y, Murakami S, Wakita T, Kaneko S. La protein required for internal ribosome entry site-directed translation is a potential therapeutic target for hepatitis C virus replication. *J Infect Dis* 2010; **202**: 75-85 [PMID: 20497049 DOI: 10.1086/653081]
- 181 **Ali N**, Siddiqui A. The La antigen binds 5' noncoding region of the hepatitis C virus RNA in the context of the initiator AUG codon and stimulates internal ribosome entry site-mediated translation. *Proc Natl Acad Sci USA* 1997; **94**: 2249-2254 [PMID: 9122180]
- 182 **Abe A**, Takahashi-Niki K, Takekoshi Y, Shimizu T, Kitaura H, Maita H, Iguchi-Arigo SM, Ariga H. Prefoldin plays a role as a clearance factor in preventing proteasome inhibitor-induced protein aggregation. *J Biol Chem* 2013; **288**: 27764-27776 [PMID: 23946485 DOI: 10.1074/jbc.M113.476358]
- 183 **Tsao ML**, Chao CH, Yeh CT. Interaction of hepatitis C virus F protein with prefoldin 2 perturbs tubulin cytoskeleton organization. *Biochem Biophys Res Commun* 2006; **348**: 271-277 [PMID: 16876117 DOI: 10.1016/j.bbrc.2006.07.062]
- 184 **Lai CK**, Jeng KS, Machida K, Lai MM. Association of hepatitis C virus replication complexes with microtubules and actin filaments is dependent on the interaction of NS3 and NS5A. *J Virol* 2008; **82**: 8838-8848 [PMID: 18562541 DOI: 10.1128/JVI.00398-08]
- 185 **Roohvand F**, Maillard P, Laverne JP, Boulant S, Walic M, Andréo U, Goueslain L, Helle F, Mallet A, McLauchlan J, Budkowska A. Initiation of hepatitis C virus infection requires the dynamic microtubule network: role of the viral nucleocapsid protein. *J Biol Chem* 2009; **284**: 13778-13791 [PMID: 19269968 DOI: 10.1074/jbc.M807873200]
- 186 **Counihan NA**, Rawlinson SM, Lindenbach BD. Trafficking of hepatitis C virus core protein during virus particle assembly. *PLoS Pathog* 2011; **7**: e1002302 [PMID: 22028650 DOI: 10.1371/journal.ppat.1002302]
- 187 **Bost AG**, Venable D, Liu L, Heinz BA. Cytoskeletal requirements for hepatitis C virus (HCV) RNA synthesis in the HCV replicon cell

- culture system. *J Virol* 2003; **77**: 4401-4408 [PMID: 12634397]
- 188 **Trougakos IP**. The molecular chaperone apolipoprotein J/clusterin as a sensor of oxidative stress: implications in therapeutic approaches - a mini-review. *Gerontology* 2013; **59**: 514-523 [PMID: 23689375 DOI: 10.1159/000351207]
  - 189 **Chaiwatanasirikul KA**, Sala A. The tumour-suppressive function of CLU is explained by its localisation and interaction with HSP60. *Cell Death Dis* 2011; **2**: e219 [PMID: 22012253 DOI: 10.1038/cddis.2011.99]
  - 190 **Lin CC**, Tsai P, Sun HY, Hsu MC, Lee JC, Wu IC, Tsao CW, Chang TT, Young KC. Apolipoprotein J, a glucose-upregulated molecular chaperone, stabilizes core and NS5A to promote infectious hepatitis C virus virion production. *J Hepatol* 2014; **61**: 984-993 [PMID: 24996046 DOI: 10.1016/j.jhep.2014.06.026]
  - 191 **Galligan JJ**, Petersen DR. The human protein disulfide isomerase gene family. *Hum Genomics* 2012; **6**: 6 [PMID: 23245351 DOI: 10.1186/1479-7364-6-6]
  - 192 **Freedman RB**, Hirst TR, Tuite MF. Protein disulphide isomerase: building bridges in protein folding. *Trends Biochem Sci* 1994; **19**: 331-336 [PMID: 7940678]
  - 193 **Perlemuter G**, Sabile A, Letteron P, Vona G, Topilco A, Chrétien Y, Koike K, Pessayre D, Chapman J, Barba G, Bréchet C. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *FASEB J* 2002; **16**: 185-194 [PMID: 11818366 DOI: 10.1096/fj.01-0396com]
  - 194 **Gordon DA**. Recent advances in elucidating the role of the microsomal triglyceride transfer protein in apolipoprotein B lipoprotein assembly. *Curr Opin Lipidol* 1997; **8**: 131-137 [PMID: 9211060]
  - 195 **Burnett JR**, Barrett PH. Apolipoprotein B metabolism: tracer kinetics, models, and metabolic studies. *Crit Rev Clin Lab Sci* 2002; **39**: 89-137 [PMID: 12014529 DOI: 10.1080/10408360208951113]
  - 196 **Mirandola S**, Realdon S, Iqbal J, Gerotto M, Dal Pero F, Bortoletto G, Marcolongo M, Vario A, Datz C, Hussain MM, Alberti A. Liver microsomal triglyceride transfer protein is involved in hepatitis C liver steatosis. *Gastroenterology* 2006; **130**: 1661-1669 [PMID: 16697730 DOI: 10.1053/j.gastro.2006.02.035]
  - 197 **André P**, Perlemuter G, Budkowska A, Bréchet C, Lotteau V. Hepatitis C virus particles and lipoprotein metabolism. *Semin Liver Dis* 2005; **25**: 93-104 [PMID: 15732001 DOI: 10.1055/s-2005-864785]
  - 198 **Yamaguchi A**, Tazuma S, Nishioka T, Ohishi W, Hyogo H, Nomura S, Chayama K. Hepatitis C virus core protein modulates fatty acid metabolism and thereby causes lipid accumulation in the liver. *Dig Dis Sci* 2005; **50**: 1361-1371 [PMID: 16047488 DOI: 10.1007/s10620-005-2788-1]
  - 199 **Domitrovich AM**, Felmlee DJ, Siddiqui A. Hepatitis C virus nonstructural proteins inhibit apolipoprotein B100 secretion. *J Biol Chem* 2005; **280**: 39802-39808 [PMID: 16203724 DOI: 10.1074/jbc.M510391200]
  - 200 **Parvaiz F**, Manzoor S, Iqbal J, McRae S, Javed F, Ahmed QL, Waris G. Hepatitis C virus nonstructural protein 5A favors upregulation of gluconeogenic and lipogenic gene expression leading towards insulin resistance: a metabolic syndrome. *Arch Virol* 2014; **159**: 1017-1025 [PMID: 24240483 DOI: 10.1007/s00705-013-1892-3]
  - 201 **Zampino R**, Ingrosso D, Durante-Mangoni E, Capasso R, Tripodi MF, Restivo L, Zappia V, Ruggiero G, Adinolfi LE. Microsomal triglyceride transfer protein (MTP) -493G/T gene polymorphism contributes to fat liver accumulation in HCV genotype 3 infected patients. *J Viral Hepat* 2008; **15**: 740-746 [PMID: 18482281 DOI: 10.1111/j.1365-2893.2008.00994.x]
  - 202 **Mirandola S**, Osterreicher CH, Marcolongo M, Datz C, Aigner E, Schlabrakowski A, Realdon S, Gerotto M, Alberti A, Stickel F. Microsomal triglyceride transfer protein polymorphism (-493G/T) is associated with hepatic steatosis in patients with chronic hepatitis C. *Liver Int* 2009; **29**: 557-565 [PMID: 19018985 DOI: 10.1111/j.1478-3231.2008.01892.x]
  - 203 **Siqueira ER**, Oliveira CP, Correa-Giannella ML, Stefano JT, Cavaleiro AM, Fortes MA, Muniz MT, Silva FS, Pereira LM, Carrilho FJ. MTP -493G/T gene polymorphism is associated with steatosis in hepatitis C-infected patients. *Braz J Med Biol Res* 2012; **45**: 72-77 [PMID: 22147193 DOI: 10.1590/S0100-879X2011007500160]
  - 204 **Saad Y**, Shaker O, Nassar Y, Ahmad L, Said M, Esmat G. A polymorphism in the microsomal triglyceride transfer protein can predict the response to antiviral therapy in Egyptian patients with chronic hepatitis C virus genotype 4 infection. *Gut Liver* 2014; **8**: 655-661 [PMID: 25287167 DOI: 10.5009/gnl13374]
  - 205 **Ryan MC**, Desmond PV, Slavin JL, Congiu M. Expression of genes involved in lipogenesis is not increased in patients with HCV genotype 3 in human liver. *J Viral Hepat* 2011; **18**: 53-60 [PMID: 20196803 DOI: 10.1111/j.1365-2893.2010.01283.x]
  - 206 **Rojas Á**, del Campo JA, Maraver M, Aparcero R, García-Valdecasas M, Diago M, Carmona I, Andrade RJ, Solà R, Romero-Gómez M. Hepatitis C virus infection alters lipid metabolism depending on IL28B polymorphism and viral genotype and modulates gene expression in vivo and in vitro. *J Viral Hepat* 2014; **21**: 19-24 [PMID: 24188401 DOI: 10.1111/jvh.12209]
  - 207 **Bridge SH**, Sheridan DA, Felmlee DJ, Crossey MM, Fenwick FI, Lanyon CV, Dubuc G, Seidah NG, Davignon J, Thomas HC, Taylor-Robinson SD, Toms GL, Neely RD, Bassendine MF. PCSK9, apolipoprotein E and lipoviral particles in chronic hepatitis C genotype 3: evidence for genotype-specific regulation of lipoprotein metabolism. *J Hepatol* 2015; **62**: 763-770 [PMID: 25463543 DOI: 10.1016/j.jhep.2014.11.016]
  - 208 **Mirandola S**, Bowman D, Hussain MM, Alberti A. Hepatic steatosis in hepatitis C is a storage disease due to HCV interaction with microsomal triglyceride transfer protein (MTP). *Nutr Metab (Lond)* 2010; **7**: 13 [PMID: 20178560 DOI: 10.1186/1743-7075-7-13]
  - 209 **Gastaminza P**, Kapadia SB, Chisari FV. Differential biophysical properties of infectious intracellular and secreted hepatitis C virus particles. *J Virol* 2006; **80**: 11074-11081 [PMID: 16956946 DOI: 10.1128/JVI.01150-06]
  - 210 **Gastaminza P**, Cheng G, Wieland S, Zhong J, Liao W, Chisari FV. Cellular determinants of hepatitis C virus assembly, maturation, degradation, and secretion. *J Virol* 2008; **82**: 2120-2129 [PMID: 18077707 DOI: 10.1128/JVI.02053-07]
  - 211 **Icard V**, Diaz O, Scholtes C, Perrin-Cocon L, Ramière C, Bartenschlager R, Penin F, Lotteau V, André P. Secretion of hepatitis C virus envelope glycoproteins depends on assembly of apolipoprotein B positive lipoproteins. *PLoS One* 2009; **4**: e4233 [PMID: 19156195 DOI: 10.1371/journal.pone.0004233]
  - 212 **Owen DM**, Huang H, Ye J, Gale M. Apolipoprotein E on hepatitis C virion facilitates infection through interaction with low-density lipoprotein receptor. *Virology* 2009; **394**: 99-108 [PMID: 19751943 DOI: 10.1016/j.virol.2009.08.037]
  - 213 **Chang KS**, Jiang J, Cai Z, Luo G. Human apolipoprotein e is required for infectivity and production of hepatitis C virus in cell culture. *J Virol* 2007; **81**: 13783-13793 [PMID: 17913825 DOI: 10.1128/JVI.01091-07]
  - 214 **Benga WJ**, Krieger SE, Dimitrova M, Zeisel MB, Parnot M, Lupberger J, Hildt E, Luo G, McLauchlan J, Baumert TF, Schuster C. Apolipoprotein E interacts with hepatitis C virus nonstructural protein 5A and determines assembly of infectious particles. *Hepatology* 2010; **51**: 43-53 [PMID: 20014138 DOI: 10.1002/hep.23278]
  - 215 **Cun W**, Jiang J, Luo G. The C-terminal alpha-helix domain of apolipoprotein E is required for interaction with nonstructural protein 5A and assembly of hepatitis C virus. *J Virol* 2010; **84**: 11532-11541 [PMID: 20719944 DOI: 10.1128/JVI.01021-10]
  - 216 **Jiang J**, Luo G. Apolipoprotein E but not B is required for the formation of infectious hepatitis C virus particles. *J Virol* 2009; **83**: 12680-12691 [PMID: 19793818 DOI: 10.1128/JVI.01476-09]
  - 217 **Castillo V**, Oñate M, Woehlbiel U, Rozas P, Andreu C, Medinas D, Valdés P, Osorio F, Mercado G, Vidal RL, Kerr B, Court FA, Hetz C. Functional Role of the Disulfide Isomerase ERp57 in Axonal Regeneration. *PLoS One* 2015; **10**: e0136620 [PMID: 26361352]



- DOI: 10.1371/journal.pone.0136620]
- 218 **Caramelo JJ**, Parodi AJ. Getting in and out from calnexin/calreticulin cycles. *J Biol Chem* 2008; **283**: 10221-10225 [PMID: 18303019 DOI: 10.1074/jbc.R700048200]
  - 219 **Dubuisson J**, Rice CM. Hepatitis C virus glycoprotein folding: disulfide bond formation and association with calnexin. *J Virol* 1996; **70**: 778-786 [PMID: 8551615]
  - 220 **Dubuisson J**. Folding, assembly and subcellular localization of hepatitis C virus glycoproteins. *Curr Top Microbiol Immunol* 2000; **242**: 135-148 [PMID: 10592659]
  - 221 **Chapel C**, Garcia C, Roingard P, Zitzmann N, Dubuisson J, Dwek RA, Trépo C, Zoulim F, Durantel D. Antiviral effect of alpha-glucosidase inhibitors on viral morphogenesis and binding properties of hepatitis C virus-like particles. *J Gen Virol* 2006; **87**: 861-871 [PMID: 16528036 DOI: 10.1099/vir.0.81503-0]
  - 222 **Wohlfarth C**, Efferth T. Natural products as promising drug candidates for the treatment of hepatitis B and C. *Acta Pharmacol Sin* 2009; **30**: 25-30 [PMID: 19060918 DOI: 10.1038/aps.2008.5]
  - 223 **Deleersnyder V**, Pillel A, Wychowski C, Blight K, Xu J, Hahn YS, Rice CM, Dubuisson J. Formation of native hepatitis C virus glycoprotein complexes. *J Virol* 1997; **71**: 697-704 [PMID: 8985401]
  - 224 **Cocquerel L**, Quinn ER, Flint M, Hadlock KG, Fong SK, Levy S. Recognition of native hepatitis C virus E1E2 heterodimers by a human monoclonal antibody. *J Virol* 2003; **77**: 1604-1609 [PMID: 12502876 DOI: 10.1128/JVI.77.2.1604-1609.2003]
  - 225 **Meunier JC**, Fournillier A, Choukhi A, Cahour A, Cocquerel L, Dubuisson J, Wychowski C. Analysis of the glycosylation sites of hepatitis C virus (HCV) glycoprotein E1 and the influence of E1 glycans on the formation of the HCV glycoprotein complex. *J Gen Virol* 1999; **80** (Pt 4): 887-896 [PMID: 10211957]
  - 226 **Ahmed QL**, Manzoor S, Tariq M, Khalid M, Ashraf W, Parvaiz F, Imran M. Hepatitis C virus infection in vitro triggers endoplasmic reticulum stress and downregulates insulin receptor substrates 1 and 2 through upregulation of cytokine signaling suppressor 3. *Acta Virol* 2014; **58**: 238-244 [PMID: 25283858 DOI: 10.4149/av\_2014\_03\_238]
  - 227 **Määttä P**, Gehring K, Bergeron JJ, Thomas DY. Protein quality control in the ER: the recognition of misfolded proteins. *Semin Cell Dev Biol* 2010; **21**: 500-511 [PMID: 20347046 DOI: 10.1016/j.semcdb.2010.03.006]
  - 228 **Ni M**, Lee AS. ER chaperones in mammalian development and human diseases. *FEBS Lett* 2007; **581**: 3641-3651 [PMID: 17481612 DOI: 10.1016/j.febslet.2007.04.045]
  - 229 **Hosokawa N**, Wada I, Hasegawa K, Yoriyuzi T, Tremblay LO, Herscovics A, Nagata K. A novel ER alpha-mannosidase-like protein accelerates ER-associated degradation. *EMBO Rep* 2001; **2**: 415-422 [PMID: 11375934 DOI: 10.1093/embo-reports/kve084]
  - 230 **Saeed M**, Suzuki R, Watanabe N, Masaki T, Tomonaga M, Muhammad A, Kato T, Matsuura Y, Watanabe H, Wakita T, Suzuki T. Role of the endoplasmic reticulum-associated degradation (ERAD) pathway in degradation of hepatitis C virus envelope proteins and production of virus particles. *J Biol Chem* 2011; **286**: 37264-37273 [PMID: 21878646 DOI: 10.1074/jbc.M111.259085]
  - 231 **Tardif KD**, Mori K, Kaufman RJ, Siddiqui A. Hepatitis C virus suppresses the IRE1-XBP1 pathway of the unfolded protein response. *J Biol Chem* 2004; **279**: 17158-17164 [PMID: 14960590 DOI: 10.1074/jbc.M312144200]
  - 232 **Hayashi T**, Su TP. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. *Cell* 2007; **131**: 596-610 [PMID: 17981125 DOI: 10.1016/j.cell.2007.08.036]
  - 233 **Ruscher K**, Wieloch T. The involvement of the sigma-1 receptor in neurodegeneration and neurorestoration. *J Pharmacol Sci* 2015; **127**: 30-35 [PMID: 25704015 DOI: 10.1016/j.jphs.2014.11.011]
  - 234 **Friesland M**, Mingorance L, Chung J, Chisari FV, Gastaminza P. Sigma-1 receptor regulates early steps of viral RNA replication at the onset of hepatitis C virus infection. *J Virol* 2013; **87**: 6377-6390 [PMID: 23536676 DOI: 10.1128/JVI.03557-12]
  - 235 **Zhou TB**, Qin YH. Signaling pathways of prohibitin and its role in diseases. *J Recept Signal Transduct Res* 2013; **33**: 28-36 [PMID: 23327602 DOI: 10.3109/10799893.2012.752006]
  - 236 **Dang SS**, Sun MZ, Yang E, Xun M, Ma L, Jia ZS, Wang WJ, Jia XL. Prohibitin is overexpressed in Huh-7-HCV and Huh-7.5-HCV cells harboring in vitro transcribed full-length hepatitis C virus RNA. *Virol J* 2011; **8**: 424 [PMID: 21896168 DOI: 10.1186/1743-422X-8-424]
  - 237 **Tsutsumi T**, Matsuda M, Aizaki H, Moriya K, Miyoshi H, Fujie H, Shintani Y, Yotsuyanagi H, Miyamura T, Suzuki T, Koike K. Proteomics analysis of mitochondrial proteins reveals overexpression of a mitochondrial protein chaperon, prohibitin, in cells expressing hepatitis C virus core protein. *Hepatology* 2009; **50**: 378-386 [PMID: 19591124 DOI: 10.1002/hep.22998]
  - 238 **Fujinaga H**, Tsutsumi T, Yotsuyanagi H, Moriya K, Koike K. Hepatocarcinogenesis in hepatitis C: HCV shrewdly exacerbates oxidative stress by modulating both production and scavenging of reactive oxygen species. *Oncology* 2011; **81** Suppl 1: 11-17 [PMID: 22212930 DOI: 10.1159/000333253]
  - 239 **Nijtmans LG**, de Jong L, Artal Sanz M, Coates PJ, Berden JA, Back JW, Muijsers AO, van der Spek H, Grivell LA. Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. *EMBO J* 2000; **19**: 2444-2451 [PMID: 10835343 DOI: 10.1093/emboj/19.11.2444]
  - 240 **Koike K**. The oncogenic role of hepatitis C virus. *Recent Results Cancer Res* 2014; **193**: 97-111 [PMID: 24008295 DOI: 10.1007/978-3-642-38965-8\_6]
  - 241 **Kumari S**, Roy S, Singh P, Singla-Pareek SL, Pareek A. Cyclophilins: proteins in search of function. *Plant Signal Behav* 2013; **8**: e22734 [PMID: 23123451 DOI: 10.4161/psb.22734]
  - 242 **Qing M**, Yang F, Zhang B, Zou G, Robida JM, Yuan Z, Tang H, Shi PY. Cyclosporine inhibits flavivirus replication through blocking the interaction between host cyclophilins and viral NS5 protein. *Antimicrob Agents Chemother* 2009; **53**: 3226-3235 [PMID: 19451286 DOI: 10.1128/AAC.00189-09]
  - 243 **Wang P**, Heitman J. The cyclophilins. *Genome Biol* 2005; **6**: 226 [PMID: 15998457 DOI: 10.1186/gb-2005-6-7-226]
  - 244 **Watahi K**, Shimotohno K. Cyclophilin and viruses: cyclophilin as a cofactor for viral infection and possible anti-viral target. *Drug Target Insights* 2007; **2**: 9-18 [PMID: 21901058]
  - 245 **Gaither LA**, Borawski J, Anderson LJ, Balabanis KA, Devay P, Joberty G, Rau C, Schirle M, Bouwmeester T, Mickanin C, Zhao S, Vickers C, Lee L, Deng G, Baryza J, Fujimoto RA, Lin K, Compton T, Wiedmann B. Multiple cyclophilins involved in different cellular pathways mediate HCV replication. *Virology* 2010; **397**: 43-55 [PMID: 19932913 DOI: 10.1016/j.virol.2009.10.043]
  - 246 **Inoue K**, Sekiyama K, Yamada M, Watanabe T, Yasuda H, Yoshida M. Combined interferon alpha2b and cyclosporin A in the treatment of chronic hepatitis C: controlled trial. *J Gastroenterol* 2003; **38**: 567-572 [PMID: 12825133 DOI: 10.1007/s00535-002-1104-5]
  - 247 **Inoue K**, Yoshida M. Interferon combined with cyclosporine treatment as an effective countermeasure against hepatitis C virus recurrence in liver transplant patients with end-stage hepatitis C virus related disease. *Transplant Proc* 2005; **37**: 1233-1234 [PMID: 15848679 DOI: 10.1016/j.transproceed.2004.11.041]
  - 248 **Goto K**, Watahi K, Murata T, Hishiki T, Hijikata M, Shimotohno K. Evaluation of the anti-hepatitis C virus effects of cyclophilin inhibitors, cyclosporin A, and NIM811. *Biochem Biophys Res Commun* 2006; **343**: 879-884 [PMID: 16564500 DOI: 10.1016/j.bbrc.2006.03.059]
  - 249 **Watahi K**, Hijikata M, Hosaka M, Yamaji M, Shimotohno K. Cyclosporin A suppresses replication of hepatitis C virus genome in cultured hepatocytes. *Hepatology* 2003; **38**: 1282-1288 [PMID: 14578868 DOI: 10.1053/jhep.2003.50449]
  - 250 **Ma S**, Boerner JE, TiongYip C, Weidmann B, Ryder NS, Cooreman MP, Lin K. NIM811, a cyclophilin inhibitor, exhibits potent in vitro activity against hepatitis C virus alone or in combination with alpha interferon. *Antimicrob Agents Chemother* 2006; **50**: 2976-2982 [PMID: 16940091 DOI: 10.1128/AAC.00310-06]
  - 251 **Paeshuyse J**, Kaul A, De Clercq E, Rosenwirth B, Dumont JM, Scalfaro P, Bartenschlager R, Neyts J. The non-immunosuppressive



- cyclosporin DEBIO-025 is a potent inhibitor of hepatitis C virus replication in vitro. *Hepatology* 2006; **43**: 761-770 [PMID: 16557546 DOI: 10.1002/hep.21102]
- 252 **Mathy JE**, Ma S, Compton T, Lin K. Combinations of cyclophilin inhibitor NIM811 with hepatitis C Virus NS3-4A Protease or NS5B polymerase inhibitors enhance antiviral activity and suppress the emergence of resistance. *Antimicrob Agents Chemother* 2008; **52**: 3267-3275 [PMID: 18591281 DOI: 10.1128/AAC.00498-08]
  - 253 **Nakagawa M**, Sakamoto N, Enomoto N, Tanabe Y, Kanazawa N, Koyama T, Kurosaki M, Maekawa S, Yamashiro T, Chen CH, Itsui Y, Kakinuma S, Watanabe M. Specific inhibition of hepatitis C virus replication by cyclosporin A. *Biochem Biophys Res Commun* 2004; **313**: 42-47 [PMID: 14672695 DOI: 10.1016/j.bbrc.2003.11.080]
  - 254 **Teraoka S**, Mishihiro S, Ebihara K, Sanaka T, Yamaguchi Y, Nakajima I, Kawai T, Yagisawa T, Honda H, Fuchinoue S. Effect of cyclosporine on proliferation of non-A, non-B hepatitis virus. *Transplant Proc* 1988; **20**: 868-876 [PMID: 3133858]
  - 255 **Flisiak R**, Horban A, Galloway P, Bobardt M, Selvarajah S, Wiercinska-Drapalo A, Siwak E, Cielniak I, Higersberger J, Kierkus J, Aeschlimann C, Groscurin P, Nicolas-Métral V, Dumont JM, Porchet H, Crabbé R, Scalfaro P. The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfecting with hepatitis C and human immunodeficiency virus. *Hepatology* 2008; **47**: 817-826 [PMID: 18302285 DOI: 10.1002/hep.22131]
  - 256 **Coelmont L**, Kaptein S, Paeshuyse J, Vliegen I, Dumont JM, Vuagniaux G, Neyts J. Debio 025, a cyclophilin binding molecule, is highly efficient in clearing hepatitis C virus (HCV) replicon-containing cells when used alone or in combination with specifically targeted antiviral therapy for HCV (STAT-C) inhibitors. *Antimicrob Agents Chemother* 2009; **53**: 967-976 [PMID: 19104013 DOI: 10.1128/AAC.00939-08]
  - 257 **Flisiak R**, Feinman SV, Jablkowski M, Horban A, Kryczka W, Pawlowska M, Heathcote JE, Mazzella G, Vandelli C, Nicolas-Métral V, Groscurin P, Liz JS, Scalfaro P, Porchet H, Crabbé R. The cyclophilin inhibitor Debio 025 combined with PEG IFN $\alpha$ 2a significantly reduces viral load in treatment-naïve hepatitis C patients. *Hepatology* 2009; **49**: 1460-1468 [PMID: 19353740 DOI: 10.1002/hep.22835]
  - 258 **Inoue K**, Watanabe T, Yamada M, Yoshikumi H, Ogawa O, Yoshida M. Efficacy of interferon Beta combined with cyclosporine induction and intensified therapy for retreatment of chronic hepatitis C. *Transplant Proc* 2009; **41**: 246-249 [PMID: 19249526 DOI: 10.1016/j.transproceed.2008.10.056]
  - 259 **Hopkins S**, Scorneaux B, Huang Z, Murray MG, Wring S, Smitley C, Harris R, Erdmann F, Fischer G, Ribeill Y. SCY-635, a novel nonimmunosuppressive analog of cyclosporine that exhibits potent inhibition of hepatitis C virus RNA replication in vitro. *Antimicrob Agents Chemother* 2010; **54**: 660-672 [PMID: 19933795 DOI: 10.1128/AAC.00660-09]
  - 260 **Lawitz E**, Godofsky E, Rouzier R, Marbury T, Nguyen T, Ke J, Huang M, Praestgaard J, Serra D, Evans TG. Safety, pharmacokinetics, and antiviral activity of the cyclophilin inhibitor NIM811 alone or in combination with pegylated interferon in HCV-infected patients receiving 14 days of therapy. *Antiviral Res* 2011; **89**: 238-245 [PMID: 21255610 DOI: 10.1016/j.antiviral.2011.01.003]
  - 261 **Hopkins S**, DiMassimo B, Rusnak P, Heuman D, Lalezari J, Sluder A, Scorneaux B, Mosier S, Kowalczyk P, Ribeill Y, Baugh J, Galloway P. The cyclophilin inhibitor SCY-635 suppresses viral replication and induces endogenous interferons in patients with chronic HCV genotype 1 infection. *J Hepatol* 2012; **57**: 47-54 [PMID: 22425702 DOI: 10.1016/j.jhep.2012.02.024]
  - 262 **Chatterji U**, Garcia-Rivera JA, Baugh J, Gawlik K, Wong KA, Zhong W, Brass CA, Naoumov NV, Galloway PA. The combination of alisporivir plus an NS5A inhibitor provides additive to synergistic anti-hepatitis C virus activity without detectable cross-resistance. *Antimicrob Agents Chemother* 2014; **58**: 3327-3334 [PMID: 24687498 DOI: 10.1128/AAC.00016-14]
  - 263 **Liu Z**, Yang F, Robotham JM, Tang H. Critical role of cyclophilin A and its prolyl-peptidyl isomerase activity in the structure and function of the hepatitis C virus replication complex. *J Virol* 2009; **83**: 6554-6565 [PMID: 19386705 DOI: 10.1128/JVI.02550-08]
  - 264 **Rosnoblet C**, Fritzinger B, Legrand D, Launay H, Wieruszkeski JM, Lippens G, Hanoulle X. Hepatitis C virus NS5B and host cyclophilin A share a common binding site on NS5A. *J Biol Chem* 2012; **287**: 44249-44260 [PMID: 23152499 DOI: 10.1074/jbc.M112.392209]
  - 265 **Fernandes F**, Poole DS, Hoover S, Middleton R, Andrei AC, Gerstner J, Striker R. Sensitivity of hepatitis C virus to cyclosporine A depends on nonstructural proteins NS5A and NS5B. *Hepatology* 2007; **46**: 1026-1033 [PMID: 17600342 DOI: 10.1002/hep.21809]
  - 266 **Robida JM**, Nelson HB, Liu Z, Tang H. Characterization of hepatitis C virus subgenomic replicon resistance to cyclosporine in vitro. *J Virol* 2007; **81**: 5829-5840 [PMID: 17376913 DOI: 10.1128/JVI.02524-06]
  - 267 **Liu Z**, Robida JM, Chinnaswamy S, Yi G, Robotham JM, Nelson HB, Irsigler A, Kao CC, Tang H. Mutations in the hepatitis C virus polymerase that increase RNA binding can confer resistance to cyclosporine A. *Hepatology* 2009; **50**: 25-33 [PMID: 19489073 DOI: 10.1002/hep.22987]
  - 268 **Abe K**, Ikeda M, Ariumi Y, Dansako H, Wakita T, Kato N. HCV genotype 1b chimeric replicon with NS5B of JFH-1 exhibited resistance to cyclosporine A. *Arch Virol* 2009; **154**: 1671-1677 [PMID: 19779801 DOI: 10.1007/s00705-009-0502-x]
  - 269 **Chatterji U**, Bobardt MD, Lim P, Galloway PA. Cyclophilin A-independent recruitment of NS5A and NS5B into hepatitis C virus replication complexes. *J Gen Virol* 2010; **91**: 1189-1193 [PMID: 20107018 DOI: 10.1099/vir.0.018531-0]
  - 270 **Chatterji U**, Bobardt M, Tai A, Wood M, Galloway PA. Cyclophilin and NS5A inhibitors, but not other anti-hepatitis C virus (HCV) agents, preclude HCV-mediated formation of double-membrane-vesicle viral factories. *Antimicrob Agents Chemother* 2015; **59**: 2496-2507 [PMID: 25666154 DOI: 10.1128/AAC.04958-14]
  - 271 **Watahi K**, Ishii N, Hijikata M, Inoue D, Murata T, Miyazaki Y, Shimotohno K. Cyclophilin B is a functional regulator of hepatitis C virus RNA polymerase. *Mol Cell* 2005; **19**: 111-122 [PMID: 15989969 DOI: 10.1016/j.molcel.2005.05.014]
  - 272 **Heck JA**, Meng X, Frick DN. Cyclophilin B stimulates RNA synthesis by the HCV RNA dependent RNA polymerase. *Biochem Pharmacol* 2009; **77**: 1173-1180 [PMID: 19174155 DOI: 10.1016/j.bcp.2008.12.019]
  - 273 **Watahi K**, Shimotohno K. Chemical genetics approach to hepatitis C virus replication: cyclophilin as a target for anti-hepatitis C virus strategy. *Rev Med Virol* 2007; **17**: 245-252 [PMID: 17299803 DOI: 10.1002/rmv.534]
  - 274 **Weng L**, Tian X, Gao Y, Watahi K, Shimotohno K, Wakita T, Kohara M, Toyoda T. Different mechanisms of hepatitis C virus RNA polymerase activation by cyclophilin A and B in vitro. *Biochim Biophys Acta* 2012; **1820**: 1886-1892 [PMID: 22954804 DOI: 10.1016/j.bbagen.2012.08.017]
  - 275 **Morohashi K**, Sahara H, Watahi K, Iwabata K, Sunoki T, Kuramochi K, Takakusagi K, Miyashita H, Sato N, Tanabe A, Shimotohno K, Kobayashi S, Sakaguchi K, Sugawara F. Cyclosporin A associated helicase-like protein facilitates the association of hepatitis C virus RNA polymerase with its cellular cyclophilin B. *PLoS One* 2011; **6**: e18285 [PMID: 21559518 DOI: 10.1371/journal.pone.0018285]
  - 276 **Hanoulle X**, Badillo A, Wieruszkeski JM, Verdegem D, Landrieu I, Bartenschlager R, Penin F, Lippens G. Hepatitis C virus NS5A protein is a substrate for the peptidyl-prolyl cis/trans isomerase activity of cyclophilins A and B. *J Biol Chem* 2009; **284**: 13589-13601 [PMID: 19297321 DOI: 10.1074/jbc.M809244200]
  - 277 **Grisé H**, Frausto S, Logan T, Tang H. A conserved tandem cyclophilin-binding site in hepatitis C virus nonstructural protein 5A regulates Alisporivir susceptibility. *J Virol* 2012; **86**: 4811-4822 [PMID: 22345441 DOI: 10.1128/JVI.06641-11]
  - 278 **Yang F**, Robotham JM, Grise H, Frausto S, Madan V, Zayas M, Bartenschlager R, Robinson M, Greenstein AE, Nag A, Logan TM, Bienkiewicz E, Tang H. A major determinant of cyclophilin

- dependence and cyclosporine susceptibility of hepatitis C virus identified by a genetic approach. *PLoS Pathog* 2010; **6**: e1001118 [PMID: 20886100 DOI: 10.1371/journal.ppat.1001118]
- 279 **Dujardin M**, Madan V, Montserret R, Ahuja P, Huvent I, Launay H, Leroy A, Bartenschlager R, Penin F, Lippens G, Hanouille X. A Proline-Tryptophan Turn in the Intrinsically Disordered Domain 2 of NS5A Protein Is Essential for Hepatitis C Virus RNA Replication. *J Biol Chem* 2015; **290**: 19104-19120 [PMID: 26085105 DOI: 10.1074/jbc.M115.644419]
- 280 **Verdegem D**, Badillo A, Wieruszkeski JM, Landrieu I, Leroy A, Bartenschlager R, Penin F, Lippens G, Hanouille X. Domain 3 of NS5A protein from the hepatitis C virus has intrinsic alpha-helical propensity and is a substrate of cyclophilin A. *J Biol Chem* 2011; **286**: 20441-20454 [PMID: 21489988 DOI: 10.1074/jbc.M110.182436]
- 281 **Chatterji U**, Lim P, Bobardt MD, Wieland S, Cordek DG, Vuagniaux G, Chisari F, Cameron CE, Targett-Adams P, Parkinson T, Gallay PA. HCV resistance to cyclosporin A does not correlate with a resistance of the NS5A-cyclophilin A interaction to cyclophilin inhibitors. *J Hepatol* 2010; **53**: 50-56 [PMID: 20451281 DOI: 10.1016/j.jhep.2010.01.041]
- 282 **Hopkins S**, Bobardt M, Chatterji U, Garcia-Rivera JA, Lim P, Gallay PA. The cyclophilin inhibitor SCY-635 disrupts hepatitis C virus NS5A-cyclophilin A complexes. *Antimicrob Agents Chemother* 2012; **56**: 3888-3897 [PMID: 22585215 DOI: 10.1128/AAC.00693-12]
- 283 **Kaul A**, Stauffer S, Berger C, Pertel T, Schmitt J, Kallis S, Zayas M, Lohmann V, Luban J, Bartenschlager R. Essential role of cyclophilin A for hepatitis C virus replication and virus production and possible link to polyprotein cleavage kinetics. *PLoS Pathog* 2009; **5**: e1000546 [PMID: 19680534 DOI: 10.1371/journal.ppat.1000546]
- 284 **Chatterji U**, Bobardt M, Selvarajah S, Yang F, Tang H, Sakamoto N, Vuagniaux G, Parkinson T, Gallay P. The isomerase active site of cyclophilin A is critical for hepatitis C virus replication. *J Biol Chem* 2009; **284**: 16998-17005 [PMID: 19380579 DOI: 10.1074/jbc.M109.007625]
- 285 **Coelmont L**, Hanouille X, Chatterji U, Berger C, Snoeck J, Bobardt M, Lim P, Vlieghe I, Paeshuyse J, Vuagniaux G, Vandamme AM, Bartenschlager R, Gallay P, Lippens G, Neyts J. DEB025 (Alisporivir) inhibits hepatitis C virus replication by preventing a cyclophilin A induced cis-trans isomerisation in domain II of NS5A. *PLoS One* 2010; **5**: e13687 [PMID: 21060866 DOI: 10.1371/journal.pone.0013687]
- 286 **Fernandes F**, Ansari IU, Striker R. Cyclosporine inhibits a direct interaction between cyclophilins and hepatitis C NS5A. *PLoS One* 2010; **5**: e9815 [PMID: 20352119 DOI: 10.1371/journal.pone.0009815]
- 287 **Waller H**, Chatterji U, Gallay P, Parkinson T, Targett-Adams P. The use of AlphaLISA technology to detect interaction between hepatitis C virus-encoded NS5A and cyclophilin A. *J Virol Methods* 2010; **165**: 202-210 [PMID: 20132841 DOI: 10.1016/j.jviromet.2010.01.020]
- 288 **Gregory MA**, Bobardt M, Obeid S, Chatterji U, Coates NJ, Foster T, Gallay P, Leyssen P, Moss SJ, Neyts J, Nur-e-Alam M, Paeshuyse J, Pirae M, Suthar D, Warneck T, Zhang MQ, Wilkinson B. Preclinical characterization of naturally occurring polyketide cyclophilin inhibitors from the sanglifehrin family. *Antimicrob Agents Chemother* 2011; **55**: 1975-1981 [PMID: 21383094 DOI: 10.1128/AAC.01627-10]
- 289 **Gallay PA**, Bobardt MD, Chatterji U, Trepanier DJ, Ure D, Ordonez C, Foster R. The Novel Cyclophilin Inhibitor CPI-431-32 Concurrently Blocks HCV and HIV-1 Infections via a Similar Mechanism of Action. *PLoS One* 2015; **10**: e0134707 [PMID: 26263487 DOI: 10.1371/journal.pone.0134707]
- 290 **Foster TL**, Gallay P, Stonehouse NJ, Harris M. Cyclophilin A interacts with domain II of hepatitis C virus NS5A and stimulates RNA binding in an isomerase-dependent manner. *J Virol* 2011; **85**: 7460-7464 [PMID: 21593166 DOI: 10.1128/JVI.00393-11]
- 291 **Nag A**, Robotham JM, Tang H. Suppression of viral RNA binding and the assembly of infectious hepatitis C virus particles in vitro by cyclophilin inhibitors. *J Virol* 2012; **86**: 12616-12624 [PMID: 22973029 DOI: 10.1128/JVI.01351-12]
- 292 **von Hahn T**, Schiene-Fischer C, Van ND, Pfaender S, Karavul B, Steinmann E, Potthoff A, Strassburg C, Hamdi N, Abdelaziz AI, Sarrazin C, Müller T, Berg T, Trépo E, Wedemeyer H, Manns MP, Pietschmann T, Ciesek S. Hepatocytes that express variants of cyclophilin A are resistant to HCV infection and replication. *Gastroenterology* 2012; **143**: 439-447.e1 [PMID: 22580540 DOI: 10.1053/j.gastro.2012.04.053]
- 293 **Yang F**, Robotham JM, Nelson HB, Irsigler A, Kenworthy R, Tang H. Cyclophilin A is an essential cofactor for hepatitis C virus infection and the principal mediator of cyclosporine resistance in vitro. *J Virol* 2008; **82**: 5269-5278 [PMID: 18385230 DOI: 10.1128/JVI.02614-07]
- 294 **Goto K**, Watashi K, Inoue D, Hijikata M, Shimotohno K. Identification of cellular and viral factors related to anti-hepatitis C virus activity of cyclophilin inhibitor. *Cancer Sci* 2009; **100**: 1943-1950 [PMID: 19659609 DOI: 10.1111/j.1349-7006.2009.01263.x]
- 295 **Ansari IU**, Allen T, Berical A, Stock PG, Barin B, Striker R. Phenotypic analysis of NS5A variant from liver transplant patient with increased cyclosporine susceptibility. *Virology* 2013; **436**: 268-273 [PMID: 23290631 DOI: 10.1016/j.virol.2012.11.018]
- 296 **Garcia-Rivera JA**, Bobardt M, Chatterji U, Hopkins S, Gregory MA, Wilkinson B, Lin K, Gallay PA. Multiple mutations in hepatitis C virus NS5A domain II are required to confer a significant level of resistance to alisporivir. *Antimicrob Agents Chemother* 2012; **56**: 5113-5121 [PMID: 22802259 DOI: 10.1128/AAC.00919-12]
- 297 **Arai M**, Tsukiyama-Kohara K, Takagi A, Tobita Y, Inoue K, Kohara M. Resistance to cyclosporin A derives from mutations in hepatitis C virus nonstructural proteins. *Biochem Biophys Res Commun* 2014; **448**: 56-62 [PMID: 24751518 DOI: 10.1016/j.bbrc.2014.04.053]
- 298 **Ansari IU**, Striker R. Subtype specific differences in NS5A domain II reveals involvement of proline at position 310 in cyclosporine susceptibility of hepatitis C virus. *Viruses* 2012; **4**: 3303-3315 [PMID: 23342381 DOI: 10.3390/v4123303]
- 299 **Ishii N**, Watashi K, Hishiki T, Goto K, Inoue D, Hijikata M, Wakita T, Kato N, Shimotohno K. Diverse effects of cyclosporine on hepatitis C virus strain replication. *J Virol* 2006; **80**: 4510-4520 [PMID: 16611911 DOI: 10.1128/JVI.80.9.4510-4520.2006]
- 300 **Ciesek S**, Steinmann E, Wedemeyer H, Manns MP, Neyts J, Tautz N, Madan V, Bartenschlager R, von Hahn T, Pietschmann T. Cyclosporine A inhibits hepatitis C virus nonstructural protein 2 through cyclophilin A. *Hepatology* 2009; **50**: 1638-1645 [PMID: 19821520 DOI: 10.1002/hep.23281]
- 301 **Madan V**, Paul D, Lohmann V, Bartenschlager R. Inhibition of HCV replication by cyclophilin antagonists is linked to replication fitness and occurs by inhibition of membranous web formation. *Gastroenterology* 2014; **146**: 1361-1372.e1-9 [PMID: 24486951 DOI: 10.1053/j.gastro.2014.01.055]
- 302 **Puyang X**, Poulin DL, Mathy JE, Anderson LJ, Ma S, Fang Z, Zhu S, Lin K, Fujimoto R, Compton T, Wiedmann B. Mechanism of resistance of hepatitis C virus replicons to structurally distinct cyclophilin inhibitors. *Antimicrob Agents Chemother* 2010; **54**: 1981-1987 [PMID: 20176894 DOI: 10.1128/AAC.01236-09]
- 303 **Anderson LJ**, Lin K, Compton T, Wiedmann B. Inhibition of cyclophilins alters lipid trafficking and blocks hepatitis C virus secretion. *Virol J* 2011; **8**: 329 [PMID: 21711559 DOI: 10.1186/1743-422X-8-329]
- 304 **Bobardt M**, Hopkins S, Baugh J, Chatterji U, Hernandez F, Hiscott J, Sluder A, Lin K, Gallay PA. HCV NS5A and IRF9 compete for CypA binding. *J Hepatol* 2013; **58**: 16-23 [PMID: 22902549 DOI: 10.1016/j.jhep.2012.08.007]
- 305 **Daito T**, Watashi K, Sluder A, Ohashi H, Nakajima S, Borroto-Esoda K, Fujita T, Wakita T. Cyclophilin inhibitors reduce phosphorylation of RNA-dependent protein kinase to restore expression of IFN-stimulated genes in HCV-infected cells. *Gastroenterology* 2014; **147**: 463-472 [PMID: 24786893 DOI: 10.1053/j.gas-

- tro.2014.04.035]
- 306 **Bobardt M**, Chatterji U, Lim P, Gawlik K, Gallay P. Both Cyclophilin Inhibitors and Direct-Acting Antivirals Prevent PKR Activation in HCV-Infected Cells. *Open Virol J* 2014; **8**: 1-8 [PMID: 24799968 DOI: 10.2174/1874357901408010001]
  - 307 **Nakagawa M**, Sakamoto N, Tanabe Y, Koyama T, Itsui Y, Takeda Y, Chen CH, Kakinuma S, Oooka S, Maekawa S, Enomoto N, Watanabe M. Suppression of hepatitis C virus replication by cyclosporin a is mediated by blockade of cyclophilins. *Gastroenterology* 2005; **129**: 1031-1041 [PMID: 16143140 DOI: 10.1053/j.gastro.2005.06.031]
  - 308 **Quarato G**, D'Aprile A, Gavillet B, Vuagniaux G, Moradpour D, Capitanio N, Piccoli C. The cyclophilin inhibitor alisporivir prevents hepatitis C virus-mediated mitochondrial dysfunction. *Hepatology* 2012; **55**: 1333-1343 [PMID: 22135208 DOI: 10.1002/hep.25514]
  - 309 **Dionisio N**, Garcia-Mediavilla MV, Sanchez-Campos S, Majano PL, Benedicto I, Rosado JA, Salido GM, Gonzalez-Gallego J. Hepatitis C virus NS5A and core proteins induce oxidative stress-mediated calcium signalling alterations in hepatocytes. *J Hepatol* 2009; **50**: 872-882 [PMID: 19303156 DOI: 10.1016/j.jhep.2008.12.026]
  - 310 **Piccoli C**, Scrima R, Quarato G, D'Aprile A, Ripoli M, Lecce L, Boffoli D, Moradpour D, Capitanio N. Hepatitis C virus protein expression causes calcium-mediated mitochondrial bioenergetic dysfunction and nitro-oxidative stress. *Hepatology* 2007; **46**: 58-65 [PMID: 17567832 DOI: 10.1002/hep.21679]
  - 311 **Cristofari G**, Ivanyi-Nagy R, Gabus C, Boulant S, Lavergne JP, Penin F, Darlix JL. The hepatitis C virus Core protein is a potent nucleic acid chaperone that directs dimerization of the viral (+) strand RNA in vitro. *Nucleic Acids Res* 2004; **32**: 2623-2631 [PMID: 15141033 DOI: 10.1093/nar/gkh579]
  - 312 **Ivanyi-Nagy R**, Kanevsky I, Gabus C, Lavergne JP, Ficheux D, Penin F, Fossé P, Darlix JL. Analysis of hepatitis C virus RNA dimerization and core-RNA interactions. *Nucleic Acids Res* 2006; **34**: 2618-2633 [PMID: 16707664 DOI: 10.1093/nar/gkl240]
  - 313 **Ivanyi-Nagy R**, Lavergne JP, Gabus C, Ficheux D, Darlix JL. RNA chaperoning and intrinsic disorder in the core proteins of Flaviviridae. *Nucleic Acids Res* 2008; **36**: 712-725 [PMID: 18033802 DOI: 10.1093/nar/gkm1051]
  - 314 **Sharma Kk**, Didier P, Darlix JL, de Rocquigny H, Bensikaddour H, Lavergne JP, Pénin F, Lessinger JM, Mély Y. Kinetic analysis of the nucleic acid chaperone activity of the hepatitis C virus core protein. *Nucleic Acids Res* 2010; **38**: 3632-3642 [PMID: 20167640]
  - 315 **Sharma KK**, de Rocquigny H, Darlix JL, Lavergne JP, Pénin F, Lessinger JM, Mély Y. Analysis of the RNA chaperoning activity of the hepatitis C virus core protein on the conserved 3'X region of the viral genome. *Nucleic Acids Res* 2012; **40**: 2540-2553 [PMID: 22127859 DOI: 10.1093/nar/gkr1140]
  - 316 **Merola M**, Brazzoli M, Cocchiarella F, Heile JM, Helenius A, Weiner AJ, Houghton M, Abrignani S. Folding of hepatitis C virus E1 glycoprotein in a cell-free system. *J Virol* 2001; **75**: 11205-11217 [PMID: 11602760 DOI: 10.1128/JVI.75.22.11205-11217.2001]
  - 317 **Michalak JP**, Wychowski C, Choukhi A, Meunier JC, Ung S, Rice CM, Dubuisson J. Characterization of truncated forms of hepatitis C virus glycoproteins. *J Gen Virol* 1997; **78** (Pt 9): 2299-2306 [PMID: 9292018]
  - 318 **Wahid A**, Helle F, Descamps V, Duverlie G, Penin F, Dubuisson J. Disulfide bonds in hepatitis C virus glycoprotein E1 control the assembly and entry functions of E2 glycoprotein. *J Virol* 2013; **87**: 1605-1617 [PMID: 23175356 DOI: 10.1128/JVI.02659-12]
  - 319 **Brazzoli M**, Helenius A, Fong SK, Houghton M, Abrignani S, Merola M. Folding and dimerization of hepatitis C virus E1 and E2 glycoproteins in stably transfected CHO cells. *Virology* 2005; **332**: 438-453 [PMID: 15661174 DOI: 10.1016/j.virol.2004.11.034]
  - 320 **Ortega-Atienza S**, Lombana L, Gómez-Gutiérrez J, Yélamos B, Peterson DL, Gavilanes F. Production and characterization of the ectodomain of E2 envelope glycoprotein of hepatitis C virus folded in the presence of full-length E1 glycoprotein. *Protein Expr Purif* 2014; **104C**: 20-25 [PMID: 25255721 DOI: 10.1016/j.pep.2014.09.009]
  - 321 **Vaney MC**, Rey FA. Class II enveloped viruses. *Cell Microbiol* 2011; **13**: 1451-1459 [PMID: 21790946 DOI: 10.1111/j.1462-5822.2011.01653.x]
  - 322 **Huang ZS**, Wang CC, Wu HN. HCV NS3 protein helicase domain assists RNA structure conversion. *FEBS Lett* 2010; **584**: 2356-2362 [PMID: 20398661 DOI: 10.1016/j.febslet.2010.04.020]
  - 323 **Wölk B**, Sansonno D, Kräusslich HG, Dammacco F, Rice CM, Blum HE, Moradpour D. Subcellular localization, stability, and trans-cleavage competence of the hepatitis C virus NS3-NS4A complex expressed in tetracycline-regulated cell lines. *J Virol* 2000; **74**: 2293-2304 [PMID: 10666260 DOI: 10.1128/JVI.74.5.2293-2304.2000]
  - 324 **Donaldson EF**, Harrington PR, O'Rear JJ, Naeger LK. Clinical evidence and bioinformatics characterization of potential hepatitis C virus resistance pathways for sofosbuvir. *Hepatology* 2015; **61**: 56-65 [PMID: 25123381 DOI: 10.1002/hep.27375]
  - 325 **Shiffman ML**. What future for ribavirin? *Liver Int* 2009; **29** Suppl 1: 68-73 [PMID: 19207968 DOI: 10.1111/j.1478-3231.2008.01936.x]
  - 326 **Foster GR**. Mutant Ninja viruses. *Hepatology* 2015; **61**: 421-423 [PMID: 25266372 DOI: 10.1002/hep.27540]
  - 327 **Hedskog C**, Doehle B, Chodavarapu K, Gontcharova V, Crespo Garcia J, De Knecht R, Drenth JP, McHutchison JG, Brainard D, Stamm LM, Miller MD, Svarovskaia E, Mo H. Characterization of hepatitis C virus intergenotypic recombinant strains and associated virological response to sofosbuvir/ribavirin. *Hepatology* 2015; **61**: 471-480 [PMID: 25099344 DOI: 10.1002/hep.27361]

**P- Reviewer:** Bolhassani A, Tetsuya T **S- Editor:** Gong ZM

**L- Editor:** A **E- Editor:** Liu SQ



2016 Advances in Liver Transplantation

## Vascular complications following liver transplantation: A literature review of advances in 2015

Tullio Piardi, Martin Lhuire, Onorina Bruno, Riccardo Memeo, Patrick Pessaux, Reza Kianmanesh, Daniele Sommacale

Tullio Piardi, Martin Lhuire, Reza Kianmanesh, Daniele Sommacale, Department of General, Digestive and Endocrine Surgery, Hôpital Robert Debré, Centre Hospitalier Universitaire de Reims, Université de Reims Champagne-Ardenne, 51100 Reims, France

Onorina Bruno, Department of Radiology, Hôpital Beaujon, Assistance Publique des Hôpitaux de Paris, 92110 Clichy, France

Riccardo Memeo, Patrick Pessaux, Department of Hepato-Biliary and Pancreatic Surgery, Nouvel Hôpital Civil, 67000 Strasbourg, France

**Author contributions:** Piardi T and Lhuire M contributed equally to this work and wrote the paper; Piardi T, Lhuire M, Bruno O, Memeo R, Pessaux P, Kianmanesh R and Sommacale D contributed to literature research and bibliography analysis; Piardi T, Lhuire M, Pessaux P, Kianmanesh R and Sommacale D designed the manuscript.

**Conflict-of-interest statement:** Authors declare no conflict of interests for this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Daniele Sommacale, MD, Professor, Department of General, Digestive and Endocrine Surgery, Hôpital Robert Debré, Centre Hospitalier Universitaire de Reims, Université de Reims Champagne-Ardenne, Avenue du Général Koenig, 51100 Reims, France. [dsommacale@chu-reims.fr](mailto:dsommacale@chu-reims.fr)  
 Telephone: +33-3-26787095  
 Fax: +33-3-26788739

Received: June 3, 2015  
 Peer-review started: June 6, 2015

First decision: July 25, 2015  
 Revised: December 2, 2015  
 Accepted: December 18, 2015  
 Article in press: December 21, 2015  
 Published online: January 8, 2016

### Abstract

Although vascular complications (VCs) following orthotopic liver transplantation (OLT) seldom occur, they are the most feared complications with a high incidence of both graft loss and mortality, as they compromise the blood flow of the transplant (either inflow or out-flow). Diagnosis and therapeutic management of VCs constitute a major challenge in terms of increasing the success rate of liver transplantation. While surgical treatment used to be considered the first choice for management, advances in endovascular intervention have increased to make this a viable therapeutic option. Considering VC as a rare but a major and dreadful issue in OLT history, and in view of the continuing and rapid progress in recent years, an update on these uncommon conditions seemed necessary. In this sense, this review comprehensively discusses the important features (epidemiological, clinical, paraclinical, prognostic and therapeutic) of VCs following OLT.

**Key words:** Vascular complications; Orthotopic liver transplantation; Liver transplantation; Endovascular intervention

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Although vascular complications (VCs) following orthotopic liver transplantation (OLT) seldom occur, they are the most feared complications with a high incidence of both graft loss and mortality, as



they compromise the blood flow of the transplant (either inflow or outflow). Diagnosis and therapeutic management of VCs constitute a major challenge in terms of increasing the success rate of liver transplantation. This review comprehensively discusses the important features (epidemiological, clinical, paraclinical, prognostic and therapeutic) of VCs following OLT.

Piardi T, Lhuair M, Bruno O, Memeo R, Pessaux P, Kianmanesh R, Sommacale D. Vascular complications following liver transplantation: A literature review of advances in 2015. *World J Hepatol* 2016; 8(1): 36-57 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i1/36.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i1.36>

## INTRODUCTION

Although vascular complications (VCs) following orthotopic liver transplantation (OLT) are seldom, they are one of the most dreaded complications with a high incidence of both graft loss and mortality, as they compromise the blood flow of the transplant (either inflow or outflow). Khalaf<sup>[1]</sup>, in 2010, reported that patient who presented VCs had significantly inferior graft and patient survival rates. The overall incidence of VCs in adults varies widely among transplant centers worldwide, but remains around 7% in various series of deceased donor liver transplantation (DDLT), and around 13% involving living donor liver transplantation (LDLT)<sup>[1-5]</sup>. Bleeding, stenosis and thrombosis can arise at any of the vascular anastomoses, as well as aneurysms at the arterial anastomosis and exceptionally on the portal vein<sup>[6,7]</sup>, with an overall reported incidence of 7.2%-15% in adults (mainly arterial 5%-10%, following by portal 1%-3% and caval < 2%) (Table 1)<sup>[5,8-10]</sup>. In this sense, diagnosis and therapeutic management of VCs constitute a major challenge in terms of increasing the success rate of liver transplantation. This explains why, currently, many transplant teams perform close surveillance of all vascular anastomoses using Doppler ultrasonography, which allows prompt detection and treatment before ineluctable graft failure. All vascular problems must be treated aggressively, particularly in- or out-flows and sudden vascular occlusions (*i.e.*, thrombosis or kinking), such as hepatic artery thrombosis (HAT) and portal vein thrombosis (PVT), which are the most common, and more rarely hepatic veins or cavo-caval thrombosis. Indeed, they can suddenly interrupt hepatic blood supply with both high graft loss and retransplantation rates<sup>[1,5,10]</sup>. Usually, therapeutic options include surgical revascularization, percutaneous thrombolysis, percutaneous angioplasty, retransplantation and a conservative approach. Although surgical treatment used to be considered the first choice for management, advances in endovascular intervention have increased to make this a viable therapeutic option following OLT. In recent decades, huge advances in

the field of interventional radiology have radically changed the diagnostic and therapeutic approaches to VCs in liver transplant patients. For example, technical improvements made in the catheterization of hepatic vessels and computed imaging allow a specific and localized intervention on these pathological vessels, in a less invasive way<sup>[1,5,11-18]</sup>. As a matter of fact, percutaneous endovascular therapies (*i.e.*, catheter-based thrombolytic intervention, balloon angioplasty and stenting) provided by an experienced interventional radiologist are commonly employed and have supplanted surgery as the therapy of choice in almost all cases<sup>[18-20]</sup>.

Considering VCs as rare but as major and dreadful issues of OLT history, and in view of the continuing and rapid progresses in recent years, an update on these uncommon conditions seems necessary. In this sense, this review comprehensively presents the important features (either epidemiological, clinical, paraclinical, prognostic and therapeutic) of VCs following OLT. In this review, only VCs following adult OLT (DDLT or LDLT) are presented, excluding pediatric liver transplantation. Taking into account that biliary complications following OLT also constitute a major therapeutic challenge, and that they are intrinsically linked with hepatic arterial pathology, they are beyond the subject of this article and therefore will not be discussed herein.

## ARTERIAL COMPLICATIONS

Arterial complications are still a major source of morbidity and mortality after OLT. Normally, the liver allograft maintains a dual inflow blood supply: Portal and arterial. Hepatic artery (HA) plays a major physiological role, because it provides the blood supply for both the liver parenchyma and the biliary tree. Arterial reconstruction is a frequent therapeutic option after the ligation of different collaterals until, finally, the celiac trunk remains the only arterial vascular supply to the transplanted liver<sup>[21]</sup>. In patients with traumatic liver rupture with curative ligation of the hepatic artery, it has been reported that bile duct necrosis is not always associated<sup>[22]</sup>. On the contrary, the interruption or the reduction of arterial flow during liver transplant is frequently associated with biliary tree complications due to ischemic processes (*i.e.*, bile duct necrosis, liver abscesses and graft dysfunction)<sup>[23]</sup>. This discrepancy can be explained by the absence of collaterals in an OLT recipient<sup>[2,24]</sup>. In the native liver, HAT or even acute ligation, is usually well-tolerated due to the abundant arterial collateral sources which avoid ischemia of the liver parenchyma. In contrast, disruption of these collaterals inevitably occurs when performing total hepatectomy for OLT. Thus, the allograft may survive by portal and arterial inflows *via* portal and hepatic artery anastomoses. In cases of HA complications (HAC) perturbing the arterial inflow, the allograft may survive by portal inflow, but only if arterial collaterals exist<sup>[2,24,25]</sup>. These facts explain why recognition and prompt management of HAC is of great importance

**Table 1 Vascular complications following orthotopic liver transplantation**

Type	Delay (incidence)	Clinical presentation	Diagnosis	Treatment
Arterial complications HAT incidence: 3.5%	Early HAT (2.9%)	Abnormal transaminase	DUS	Emergent revascularization by endovascular intervention or surgical revascularization or rLT
		Fever	ce-MDCT	
		Biliary complications	Angiography	
		Graft failure		
		Coagulopathy		
	Late HAT (2.2%)	Asymptomatic		
		Fever		
		Abnormal transaminase		
		Bile leak		
		Hepatic abscess		
HAS incidence: 2%-13%	Early HAS	Cholangitis		Endovascular intervention or surgical revascularization
		Graft failure	DUS	
		Biliary complications	ce-MDCT	
	Late HAS	Asymptomatic	Angiography	Endovascular intervention or surgical revascularization
		Fever	DUS	
		Abnormal liver function	ce-MDCT	
HAP incidence: 2.5%		Asymptomatic	Angiography	Endovascular intervention or surgical resection and revascularization
		Abdominal pain	DUS	
HAR incidence: 0.64%		Fever	ce-MDCT	
		Gastrointestinal bleeding	Angiography	
		Massive bleeding through abdominal drains	None in emergency	
		Hemorrhagic shock		
Portal vein complications PVT incidence: < 3%	Early	Abnormal transaminase		rLT or surgical repair or endovascular interventions
		Graf dysfunction	DUS	
		Multi-organe failure	ce-MDCT	
		Variceal bleeding	(portal phase)	
			Portography	
	Late	Ascite	DUS	Curative anticoagulant therapy
		Portal vein hypertension	ce-MDCT	
		Splenomegaly	(portal phase)	
		Variceal bleeding	Portography	
			DUS	
	Early	Asymptomatic		Endovascular interventions
		Portal vein hypertension	ce-MDCT	
		Abnormal transaminase	(portal phase)	
			Portography	
			DUS	
	Late	Asymptomatic		Anticoagulant therapy and/or Endovascular interventions
		Ascite	ce-MDCT	
		Abnormal liver test function	(portal phase)	
			Portography	
Caval anastomosis complications Caval resection and end-to-end cavo-caval anastomosis	Early	Acute Budd-Chiari syndrome		Endovascular intervention or surgical repair or rLT
		Graf failure	DUS	
		Intestinal congestion	ce-MDCT	
		Renal dysfunction	Cavography	
		Lower limb edema		
	Late	Moderate Budd-Chiari syndrome		Endovascular intervention
		Ascite	DUS	
			ce-MDCT	
Piggy-back	Early	Acute Budd Chiari		Surgical repair or rLT
		Graf failure	DUS	
		Intestinal congestion	ce-MDCT	
		Renal dysfunction	Cavography	
		Lower extremity edema		
	Late	Moderate Budd-Chiari		Endovascular intervention
		Ascite	DUS	
		Lower extremity edema	ce-MDCT	
		Renal dysfunction	Cavography	
		Abdormal liver test function		

Clinical characteristics of arterial and caval complications. rLT: Re-liver transplantation; DUS: Doppler ultrasound; HAT: Hepatic artery thrombosis; HAS: Hepatic artery stenosis; HAP: Hepatic artery pseudoaneurysm; HAR: Hepatic artery rupture; PVT: Portal vein thrombosis; PVS: Portal vein stenosis; MDCT: Multi-detector computed tomography.

**Table 2** Hepatic artery thrombosis highlights**Summary of the clinical characteristics about HAT**

HA supplies exclusively the bile duct, so HAT is associated with a high frequency of biliary complications  
 HAT represents more than 50% of all arterial complications following OLT  
 The incidence of HAT following OLT is 3.5% with early and late HAT incidences of 2.9% and 2.2%, respectively  
 HAT carries an incidence of graft failure and mortality of more than 50% without prompt treatment  
 The median time to detection of early and late HAT was 6.9 d (range: 1-17.5 POD) and 6 mo (range: 1.8-79 mo), respectively  
 No differences in HAT incidences were observed between DDLT and LDLT  
 Clinical presentation spectrum: Mild elevation of serum transaminase and bilirubin levels (75%), biliary complications (15%), fever and sepsis (6%), graft dysfunction or failure (4%)  
 Risk factors of early HAT are mainly represented by technical problems, LDLT, cigarette smoking and hypercoagulability state, while late HAT is usually related to ischemic or immunologic injury: CMV positive donor, female donor and male recipient and hepatitis C seropositive recipient  
 Early diagnosis is achieved by assessing the serum transaminase level and performing Doppler ultrasound monitoring in the postoperative period and confirmed by contrast-enhanced abdominal CT scan and/or visceral angiography  
 Currently, the literature on the curative management of early HAT suggests the following procedures: First endovascular radiological intervention (IAT, PTA and stent placement), secondly open surgical revascularization, and finally retransplantation, which is associated with the best survival rate compared with revision or thrombolysis, but is a limited therapeutic option due to organ shortage

HA: Hepatic artery; HAT: Hepatic artery thrombosis; OLT: Orthotopic liver transplantation; DDLT: Deceased donor liver transplantation; LDLT: Living donor liver transplantation; CMV: Cytomegalovirus; IAT: Intra-arterial thrombolysis; PTA: Percutaneous transluminal angioplasty; CT: Computed tomography.

for graft and patient survival. The etiology underlying most HAC involves the anastomosis, including: (1) HAT: 1.9%-16.6% (the most frequent and pejorative); (2) anastomotic stricture [*i.e.*, hepatic artery stenosis (HAS)]: 0.8%-9.3%; (3) pseudoaneurysm formation [*i.e.*, hepatic artery pseudoaneurysm (HAP)]: 0%-3%; and (4) hepatic artery rupture (HAR): 0.64%<sup>[8,9,18,26]</sup>. These complications can be classified into two categories (Table 1): Early (< 1 mo) or late (delayed, *i.e.*, > 1 mo). Very particular attention should be focused on early complications, because they are associated with graft loss and a high mortality rate. In different studies, the definition of early and late complications continues to be discussed. Most of the authors have defined late complications as those occurring after 4 wk, and others after 6 mo<sup>[13,25,27,28]</sup>. In this review, we consider the recent consensus which defines early complication when it appears within the first month<sup>[10,13,18,27,28]</sup>.

**HAT**

HAT represent more than 50% of all arterial complications. It is the most frequent and severe vascular complication following OLT. Table 2 usually more frequent after pediatric liver transplantation<sup>[5,10,16,17,28-31]</sup>. It is the first cause of primary non-function of the liver transplant, which can lead to allotransplant loss and patient death in the early postoperative period. HAT is associated with a high incidence of liver transplant failure (more than 50%) and carries a mortality of more than 50% in the absence of revascularization or retransplantation. In recent years, early revascularization by means of endovascular catheter-based intervention has been a viable option for graft salvage before considering retransplantation. Indeed, the retransplantation rate is very high in untreated HAT (25%-83%) compared to graft revascularization treated patients (28%-35%)<sup>[3,10,13,16,17,30,32-40]</sup>.

**Definition:** HAT is defined as a thrombotic occlusion of the hepatic artery. It has been classified, as described above, into two types depending on the time of presentation following OLT: Early HAT [within the first 30 d of liver transplantation (LT)] and late HAT (after 30 d of LT)<sup>[13,17,28]</sup>. The hepatic artery supplies the biliary tree of the transplant, explaining the high frequency of biliary complications in HAT (*i.e.*, biliary ischemia, necrosis, stricture, sepsis) and eventually hepatic insufficiency and graft loss<sup>[31]</sup>.

**Incidence:** The true incidence of early HAT following OLT is unknown, but it varies widely from 0% to 12% in adults<sup>[5,9,24,25,27,30,38,41]</sup>. Bekker *et al*<sup>[28]</sup> (2009) reported in a systematic review comprising 21822 OLT cases an incidence of 843 cases (adults and children) of early HAT with an overall incidence of 4.4%. In adults, the incidence of HAT was 2.9%. They also showed that the incidence of early HAT had decreased over time since the first report in 1982 by Starzl (6.9% in 1996 vs 3.2% in 2006) with improvements in perioperative care. They reported that there were no differences in incidence among transplantation centers worldwide<sup>[2,28]</sup>. Median times to the occurrence detection of early and late HAT were respectively 6.9 postoperative days (range: 1-17.5) and 6 mo (range: 1.8-79 mo)<sup>[17]</sup>.

In literature, it does not confirm that HAT incidence in LDLT is significantly lower or higher compared to HAT incidence in DDLT. Many studies show contradictory results<sup>[1,9,17,28,41]</sup> but, a meta-analysis on HAT found no significant difference with an incidence of 3.1% and 4.6% in LDLT and DDLT, respectively<sup>[28]</sup>. Furthermore, it was reported that arterial anastomosis with operation microscope or loupe magnification did not show any difference in incidence HAT<sup>[9,17,28,41]</sup>.

Late HAT shows a lower incidence, ranging from 1% to 25%<sup>[38,42]</sup>. Torras *et al*<sup>[34]</sup> (1999) reported an

incidence of 7.5% (35/413) following OLT. Sixteen cases occurred during the first month (early HAT): Diagnosis made from 1 to 13 d after OLT (median: 2.5). Nineteen cases were late HAT (> 30 d, from 2 to 79 mo after OLT (median: 5 mo)<sup>[34]</sup>.

**Clinical presentation:** The clinical presentation of HAT range from a mild elevation in serum amino transferase (most frequently in patients with HAT) and bilirubin levels to fulminant hepatic necrosis. HAT is associated with elevated transaminases in 75%, biliary complications in 15%, fever and sepsis in 6% and graft dysfunction or failure in 4% of cases<sup>[5]</sup>. The clinical expression depends on the timing of the onset of HAT as well as on the existence of collaterals<sup>[5,25,27]</sup>. Usually, initial non-function or severe allograft dysfunction predominately occurs in patients with early HAT. This explains the importance of symptomatic expression, whereas biliary tract complications (*i.e.*, bile duct strictures or bile leaks sometimes leading to biliary hepatic abscesses) are more frequently, but not exclusively, associated with late HAT. Indeed, clinical expression depends on the existence of collaterals, which can develop as early as within two weeks<sup>[17,24,27]</sup>. Therefore, two main forms of HAT are recognized: (1) acute presentation (early HAT) characterized by a severe clinical course; and (2) delayed presentation (late HAT) generally associated with a milder clinical course<sup>[25]</sup>.

In every cases, early HAT clinically manifests with fever, increase leukocytosis and a important elevation in liver enzyme levels. The natural history of early HAT could be summarized as biliary tract necrosis followed by uncontrolled septic shock in the immunosuppressed population, and even by the patient's death<sup>[17,27,28,31,38]</sup>. The pathophysiological process of early HAT results in injury to the bile duct epithelium and to hepatocytes. This leads to massive necrosis in the allograft, partly due to the disruption of arterial inflows (*i.e.*, main flow by HA and accessory physiological collaterals), explaining the high incidence of biliary sepsis in early HAT<sup>[25,27,28]</sup>.

It is usually assumed that late HAT is due to ischemic or immunological damages with a more insidious onset. Up to 50% of patients with late HAT can be asymptomatic with elevated liver function tests only<sup>[10,19,27,36]</sup>. Symptomatic patients often present with biliary complications including recurrent cholangitis, bile duct stricture/stenosis, biliary leakage, biliary tract necrosis and abscess formation revealed by relapsing fever and bacteremia. The presentation may be insidious. Liver graft ischemia and liver failure are other classical insidious clinical outcomes revealing late HAT<sup>[17,27,28,36,38,42,43]</sup>.

**Risk factors:** Several reports studied the risk factors associated with HAT<sup>[5,10,17,19,25,27,28,34,44,45]</sup>. They can be divided into several categories. It is usually considered that technical problems are mainly associated with early HAT. Conversely, risk factors for late HAT are less well-defined. However, a donor positive CMV status and a

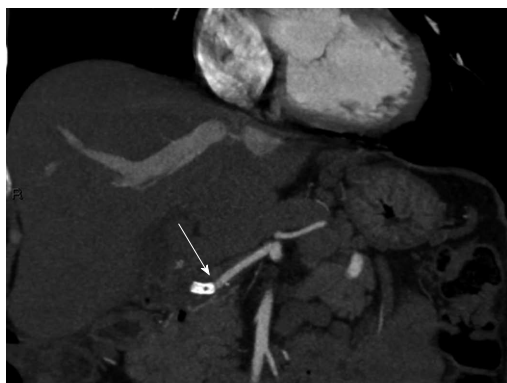
recipient negative cytomegalovirus (CMV) status have repeatedly been shown to be a possible risk factor for late HAT<sup>[27,45]</sup>. Moreover, specific factors of late HAT reported include the association of female donor and male recipient, hepatitis C virus positive recipients, episodes of rejection, tobacco consumption and retransplantation<sup>[10,17,27,45,46]</sup>. Besides, while some authors believe that HAS and hepatic artery kinking are the initiating factors, others suggest a perioperative hypercoagulable state as a possible underlying cause<sup>[5,10,17,28,29]</sup>.

Truly, the cause of early HAT is still under debate and remains unknown in most cases. Up to 20% of HAT cases are probably due to surgical causes (technical problems) in the arterial anastomosis, such as difficult anastomosis, technical imperfections with the anastomosis, kinking, stenotic anastomosis, small vessel size, reduction in a disparate diameters of the arteries, dissection of the hepatic arterial wall, celiac stenosis or compression by the median arcuate ligament, the presence of multiple arteries, aberrant or complex donor/recipient arterial anatomy or arterial abnormalities requiring complex arterial reconstructions, complex backtable arterial reconstruction of the allograft, poor quality donor and recipient vessels and high-resistance microvascular arterial outflow caused by rejection or severe ischemia-reperfusion injury. Those problems are more common among centers performing fewer than 30 OLT a year; the incidence of HAT diminishes with the surgical team's experience. Therefore, surgical causes probably do not represent the main risk factor for HAT<sup>[17,28,29,31,38]</sup>.

It has been reported that HAT can occurs within a few hours after LDLT, which indicates a population at higher risk of HAT. Indeed it has been shown that these patients displayed a higher rate of VCs explained by the complexity vascular reconstructions linked to smaller and shorter caliber of donor and recipient vessels<sup>[1,10,47]</sup>.

Regarding the non-surgical risk factors involved in the occurrence of HAT, donor age > 60 years, extended cold ischemia time, lack of ABO compatibility, cigarette smoking, hypercoagulability state, donor positive for CMV in a CMV-negative recipient, rejection, regrafts and transplant for primary sclerosing cholangitis have been shown to be statistically linked with the occurrence of HAT<sup>[17,28,38,46]</sup>. However, the literature review dealing with this issue displayed conflicting results. Indeed, some authors reported that some parameters like cold ischemic time, donor age and the presence of rejection were not found to be factors related to the development of HAT<sup>[34]</sup>. This emphasizes the difficulty in accurately determining the risk factors associated with early HAT. In a recent study, Panaro *et al.*<sup>[48]</sup> (2014) have shown a statistical association between TACE and the radiological and histological arterial wall injury, as in the past 25 years TACE has been widely used in the treatment of HCC. This procedure may potentially cause vascular lesions in the arterial wall (catheterization and drug infusion), suggesting that previous transarterial chemoembolization (TACE) could constitute a risk factor





**Figure 1** Contrast-enhanced-multidetector-row computed tomography-scan showing hepatic artery thrombosis after an endovascular intervention with stent placement. Thrombus (arrow).

of HAT when future OLT is performed<sup>[5,48]</sup>.

Some practices could prevent the occurrence of HAT, and the data reported by Duffy *et al.*<sup>[5]</sup> (2009) demonstrates that arterial reconstructions which restore the normal anatomy and gentle handling of vessels are of great importance in the accomplishment of hepatic arterial anastomosis. Some studies reported that recipients with multiple anastomoses for arterial reconstruction should receive aspirin and Doppler ultrasound (DUS) assessment to screen the patency of the reconstructed hepatic artery. Moreover, the use of aortic conduits for arterial reconstruction is a risk factor that warrants the initiation of prophylaxis in the post-transplant period<sup>[5,10,17,19,25,31,44]</sup>. For patients with inheritable thrombophilic diseases; given the devastating effects of HAT on graft outcomes, it should be necessary to identify these to prevent thrombotic complications. It is likely that patients who present both hematological and operative factors are most at risk, and routine anticoagulation in the post-OLT setting should be instituted. In sum, many studies recommend peritransplantation anticoagulation with heparin or an antiplatelet agent in patients with extraanatomic conduits, complex backtable reconstruction, or pre-OLT TACE. However, the best prophylactic approach is controversial, and this should be clarified by randomized, controlled trials<sup>[5,10,17,19,31,44,25]</sup>. An interesting report by Marín-Gómez *et al.*<sup>[40]</sup> (2012) demonstrates that intraoperative blood flow allows for a prediction of the occurrence of HAT when it is less than 100 mL/min with 84.5% sensitivity and a predictive positive value of 97.8%.

**Diagnosis:** Early diagnosis is mandatory to allow immediate treatment and to prevent graft loss. The detection of these patients includes biological (serum transaminase levels) and morphological (DUS) exams, while visceral angiography allows to confirm the diagnosis. DUS is a proven non-invasive technique and the gold standard investigation to assess hepatic artery patency. It detects the absence of hepatic artery flow, even in its intrahepatic branches. The DUS diagnosis comprises the lack of HA signal (Se = 92%) or an increased resistive index (RI)<sup>[25,17,38]</sup>. Even though the

screening protocol varies between liver transplant centers, a DUS surveillance protocol of the hepatic artery can detect reduced hepatic arterial flow and to allow for prompt revascularization management, which may result in transplant salvage<sup>[17]</sup>. In sum, in case of an abnormal elevation in liver enzymes and suggestive findings on DUS, abdominal computed tomography (CT) angiogram or angiography confirmed diagnosis and it can precisely shows an underlying anatomical defects (stenosis or kinking) with a high sensitivity and specificity specificity (Figure 1)<sup>[17]</sup>. Pareja *et al.*<sup>[38]</sup> (2010) established a screening protocol for early HAT, consisting of a first Doppler ultrasound within 48 h of OLT and in another Doppler ultrasound 7 d later. If the first examination is conclusive, they perform contrast ultrasound (microbubbles) or computed tomography. When HAT is confirmed, arteriography should be performed<sup>[38]</sup>. Intimal hyperplasia causing progressive HAS may precede late HAT and may be screen by regular (yearly) post-OLT DUS assessment. In some cases, HAS is likely to stimulate the development of arterial collaterals that protect the liver from ischemia at the time of HAT<sup>[25,48]</sup>.

**Therapeutic management:** Classically, we consider several treatment modalities for HAT: (1) revascularization (surgical or endovascular); (2) retransplantation; and (3) observation. Currently, the most effective treatment approach remains controversial and the choice of any of these treatments depends on the time of diagnosis. Early diagnosis, prompt revascularization and retransplantation have been considered the only solution to rescue patients with HAT. Historically, retransplantation is the treatment of choice for most groups, offering the best survival results<sup>[5,16]</sup>. However, this possibility is strongly conditioned by the shortage of donors and by the patient's condition<sup>[16,17,27,38,39]</sup>. Percutaneous endovascular treatments including intra-arterial thrombolysis (IAT), percutaneous transluminal angioplasty (PTA) and stent placement have shown hopeful outcomes in the literature. Finally, some patients survive without revascularization or retransplantation by developing collateral circulation distal to the thrombosis, but this occurs in rare cases<sup>[17,20,24,38,39]</sup>. Despite these encouraging results of endovascular interventions, the efficacy and risk of complications (mainly represented by hemorrhage risk) make this therapeutic option still controversial. Moreover, in some cases these are ineffective and surgical intervention (including anastomotic revision and retransplantation) must be applied. The complications of PTA include thrombosis, vascular dissection and rupture. Thus, urgent revascularization by means of endovascular interventions as a primary option offers could give a chance to avoid rLT, but only in asymptomatic patients<sup>[8,10,17,20]</sup>. Despite the proof of efficacy and safety of thrombolytic treatment with different products and regimens (urokinase, streptokinase, alteplase), the best protocol is not still known and there are currently no specific guidelines for

thrombolytic therapy application. Furthermore, several studies recommend low dose of heparin in association with thrombolytic despite increasing the risk of adverse bleeding. Indeed, hemorrhage is the most frequent adverse effect and concern about 20% of patients: Ranging from blood in the drainage to intra-abdominal hemorrhage, which could be fatal in some cases. This is mainly true in early postoperative period, but selective thrombolysis *via* the hepatic artery presents several advantages, such as a smaller thrombolytic dose, a highly localized concentration and little influence on systemic coagulation<sup>[17,20]</sup>. Endoluminal IAT with restoration of flow should be associated with underlying anatomic defects treatment if present, including reduction of kinking, treatment of an anastomotic stenosis and often requires balloon angioplasty and/or stent placement<sup>[16,20]</sup>. Association of IAT with PTA and/or stenting showed better efficacy and survival rates when compared to IAT alone. In summary, PTA and stent placement are currently tried first to resolve the problem in many centers<sup>[10,20]</sup>. Open surgical revascularization of thrombosed liver transplant is considered a viable option to save the transplant and to avoid retransplantation. Open surgical revascularization can be performed in various ways depending on the length and on the integrity of the recipient and on the graft arterial stumps. The procedure in its simplest form can be a Fogarty thrombectomy and a primary resuture of the end-to-end hepatic artery anastomosis<sup>[16]</sup>. Duffy *et al*<sup>[10]</sup> evaluated 4234 LT from 1984 to 2007: 203 (5%) developed HAT including 133 early and 70 late HAT; the occurrence of HAT was 3.9% in adults. Overall 90 patients were treated with surgical exploration, thrombectomy, or anastomotic revision. Nine patients were treated with catheter-based thrombolysis and 13 patients received anticoagulation. Of the patients with early HAT who underwent thrombectomy and anastomotic revision, only 9 (10.5%) had graft salvage, and the remaining patients needed re-transplantation. Overall, re-transplantation was necessary in 153 (75%) patients with HAT. Therefore, retransplantation after HAT has a better survival rate compared with revision or thrombolysis<sup>[5,10]</sup>.

In contrast, some patients with late HAT survive without revascularization or retransplantation by developing a collateral circulation distal to the thrombosis. The mean time between the diagnosis of HAT and the neovascularized liver is 4.1 mo (range: 3-5.5 mo). Four factors are associated with the development of a neovascularized liver: Late HAT, early HAS, site of thrombosis, and Roux-en-Y anastomosis<sup>[24,39]</sup>. These results confirm that a slow arterial obstruction process allows for the formation of arterial substitute pathways, but this striking neoangiogenesis capacity, only significant in cases of chronic ischemia, is insufficiently rapid in the case of early HAT. Given the improved outcome of the conservative treatment of liver transplant recipients, in whom late HAT develops without revascularization or retransplantation, revascularization in this condition is controversial. Based on two limitations (the relative

lack of utility of revascularization of late HAT and the contraindication to early postoperative thrombolysis), Saad *et al*<sup>[16]</sup> (2007) proposed that the clinical window of the applicability of transcatheter thrombolysis should be most likely from 1 to 3 wk to 1 to 3 mo post-transplantation, respecting contraindications to avoid fatal bleeding complications. However, successful and safe pharmaceutical thrombolysis was described by Figueras *et al*<sup>[11]</sup> (1995) 3 d after OLT. In the literature, the time interval between the transplant and thrombolysis procedures ranges from 2 to 120 d (mean, 53 d)<sup>[11,16,27,49-51]</sup>.

**Prognosis:** At the time of revascularization, survival rates is 40% in symptomatic vs 82% in asymptomatic patients<sup>[17]</sup>. The incidence of HAT has a significant impact on transplant and recipient survivals. Indeed, Silva *et al*<sup>[27]</sup> (2006) reported an overall mortality rate of 23% in those developing HAT post-OLT. In the meta-analysis reported by Bekker *et al*<sup>[28]</sup> (2009) HAT was a major cause of graft loss (53.1%) and mortality (33.3%) in the early postoperative period.

**Conclusion:** HAT is rare but it represents the most common vascular complication following LT. A definitive diagnosis is achieved by angiography, which may detect predisposing anatomical anomalies. Moreover, it allows prompt therapeutic management in the same time. IAT can be performed alone and an eventual anatomical anomaly may then be corrected by endovascular procedures such as balloon angioplasty and/or stent placement, or a surgical intervention. Currently, it seems reasonable to propose endovascular treatment first, mainly due to organ shortage and the high mortality related to retransplantation, considering the highly individualized outcome and depending of the competence of the transplant center. However, in the early post-transplant period, it is widely accepted that symptomatic patients with severe allotransplant dysfunction and symptoms related to arterial thrombosis need retransplantation.

### Hepatic artery stricture/HAS

**Definition:** HAS following OLT is defined as a narrowing of the transverse diameter of the HA, more or less extended, resulting in graft ischemia mainly revealed by elevated liver function tests<sup>[2,16,52-56]</sup>. Significant HAS is usually defined as a narrowing of the transverse diameter > 50% on angiogram associated with clinical suspicion and a RI < 0.5 (defined by peak systolic flow-end diastolic flow/peak systolic flow) and a peak systolic velocity > 400 cm/s detected by DUS<sup>[16,57,58]</sup>. HAS and HAT are the most common hepatic arterial complications, with high rates of morbidity and mortality<sup>[56,58]</sup> (Table 3).

**Incidence:** HAS occurs in 2% to 13% of transplants and has been suggested to progress to HAT implicating, at least in part, that HAS and HAT are two contiguous components of the broader allotransplant ischemic spectrum<sup>[2,16,30,52,53,55,56,58-60]</sup>. Wozney *et al*<sup>[2]</sup> (1986) reported three cases in which untreated anastomotic

**Table 3** Hepatic artery stenosis highlights**Summary of the clinical characteristics about HAS**

Significant HAS is defined as a narrowing of the transverse diameter > 50% on the angiogram associated with clinical suspicion, with a resistive index < 0.5 and a peak systolic velocity > 400 cm/s detected by DUS

HAS occurs in 2% to 13% of transplants, at the level of the anastomosis (59% of cases), graft HA (41%) or recipient HA (2.6%)

HAS has been speculated to progress to HAT in 65% of cases at 6 mo for untreated HAS

The median time to diagnosis is 100 (range: 1-1220) d following OLT

Most of patients with HAS are asymptomatic and most commonly present only with abnormal liver function tests and in rare cases with graft failure

Routine screening by DUS during the postoperative period is mandatory because of the insidious clinical presentation

The risk factors are not really known, but among these, technical and surgical factors (vascular injury such as clamp injury, intimal dissection, faulty placement of anastomotic sutures, excessive length with kinking and angulation, differences in the vessel caliber that require and oblique anastomosis, vasa vasorum disruption) or acute cellular rejection

DUS is a non-invasive method for the assessment of HA patency, but a contrast-enhanced CT scan and angiography are required to confirm the diagnosis

Radiological endovascular intervention by PTA with or without stent placement is often used to treat post-transplant HAS and are both efficacious, with 7% to 12% of complications including dissection and arterial rupture, restenosis or thrombosis (25%) and 12% failed attempts

Surgical revision and retransplant showed a high rate of success, but the overall mortality rate was as high as 20%. In some case, HAS may be an early sign of chronic rejection

DUS: Doppler ultrasound; HA: Hepatic artery; HAT: Hepatic artery thrombosis; HAS: Hepatic artery stenosis; OLT: Orthotopic liver transplantation; PTA: Percutaneous transluminal angioplasty; CT: Computed tomography.

strictures of the hepatic artery progressed to HAT. Saad *et al*<sup>[52]</sup> (2005) emphasized the correlative progression of untreated significant HAS to HAT with an incidence rate of 65% at six months for untreated HAS<sup>[2,16,52]</sup>. Abbasoglu *et al*<sup>[57]</sup> (1997) reported an incidence of 4.8% in a cohort of 857 consecutive OLT from 1988 to 1995. The median time to diagnosis was 100 d (range: 1-1220 d) following OLT, which was also reported by Denys *et al*<sup>[60]</sup> (2002) with a mean time to diagnosis at 94 d post-OLT<sup>[57,60]</sup>. Similar to HAT, HAS may be divided in two groups: HAS occurring within 30 d after OLT (early HAS), and HAS occurring more than 30 d after OLT (late HAS). Chen *et al*<sup>[61]</sup> (2009) reported an overall HAS incidence of 2.8%, with an early HAS incidence of 40% vs a late HAS incidence of 60% (mean time elapsed between transplantation to diagnosis: 91 d; range: 1-430 d). Abbasoglu *et al*<sup>[57]</sup> (1997) reported that stenosis occurred in 59% of cases at the level of the anastomosis with a median time of diagnosis at 75 d post-OLT, in 41% of cases at the level of the graft HA with a median time of diagnosis at 160 d post-OLT, and in 2.6% at the level of the recipient HA<sup>[57]</sup>. Saad *et al*<sup>[52]</sup> (2005) did not confirm these results. Indeed, the literature has established that the anastomotic stenosis is the most common place for the development of HAS within three months after LT<sup>[10,62]</sup>.

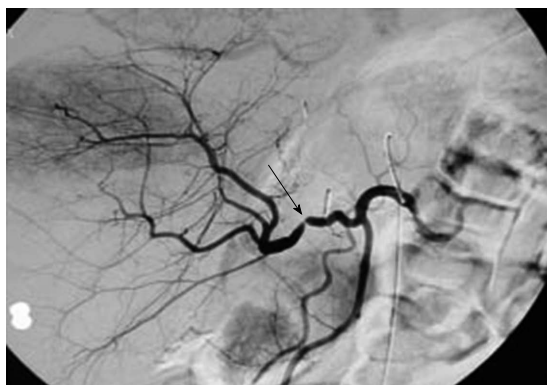
**Clinical presentation:** The clinical presentation of HAT range from normal liver function to transplant failure secondary to ischemia or necrosis. Moreover, HAS can lead to an insidious form of graft disorder, both in the early and later postoperative stages. Many patients with HAS are asymptomatic and most commonly present only with abnormal liver function tests (LFT)<sup>[16,52,57,58,60,63,64]</sup>. Indeed, Abbasoglu *et al*<sup>[57]</sup> (1997) reported that an elevation in LFT was the main clinical presentation. Most asymptomatic patients are detected during routine DUS screening. In fact, the non-specific and insidious clinical presentation of HAS mandates to perform routine

screening DUS at regular time intervals. In contrast, it is obvious that DUS screening should be highly required for OLT asymptomatic patients presenting elevated LFT.

Compared with HAT, the risks of developing biliary complications, including biliary strictures and bile leaks, are less frequent with HAS. Ideally, HAS should be diagnosed before the occurrence of biliary complications, because of the significant impact on both graft and patient survival<sup>[10,19,57]</sup>. Indeed, incidence of biliary complications is reported to be as high as 67% in liver transplant recipients with HAS<sup>[52,63,64]</sup>.

**Risk factors:** The risk factors of HAS are not really known and seem to have a multifactorial origin<sup>[60]</sup>. Many authors suggest perioperative factors (technical) of vascular injury (clamp injury, intimal dissection, faulty placement of anastomotic sutures), donor and recipient factors (excessive length with kinking and angulation, differences in vessel caliber that require oblique anastomosis), and others, such as extrinsic compression and microvascular injury, *i.e.*, vasa vasorum disruption or acute cellular rejection<sup>[52]</sup>. Abbasoglu *et al*<sup>[57]</sup> (1997) demonstrated that a low mean initial HA flow (less than 400 mL/min) after OLT is a risk factor for developing anastomotic HAS, but they did not identify a risk factor. Moreover, they showed that the presumed immunological bases, such as autoimmune hepatitis, primary biliary cirrhosis and primary sclerosing cholangitis for their OLT, were not risk factors for HAS<sup>[57]</sup>.

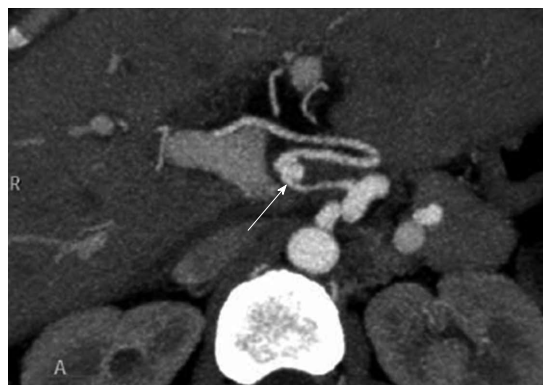
**Diagnosis:** DUS is a well-established non-invasive method for the assessment of HA patency, and its efficiency in the early diagnosis of HAS has been reported in several studies<sup>[52,57]</sup>. Abbasoglu *et al*<sup>[57]</sup> (1997) showed a DUS sensitivity of 85% in detecting HA stenosis. DUS showed a sensitivity of 100%, a specificity of 99.5%, a positive predictive value of 95% and a negative predictive value of 100%, and an overall accuracy of 99.5% in early HAS diagnosis<sup>[10,57,62,65]</sup>.



**Figure 2** Arteriography showing an anastomotic hepatic artery stenosis after orthotopic liver transplantation. Stenosis (arrow).

Many teams also use MDCTA and standard angiography to confirm the diagnosis, which is the gold standard for HAS diagnosis<sup>[10,62,65]</sup>.

**Therapeutic management:** The therapeutic management of HAS includes either surgical revision, retransplant or percutaneous endovascular interventions, such as PTA with or without stent placement<sup>[52,57,60,63,64,66]</sup> (Figures 2 and 3). Abbasoglu *et al*<sup>[57]</sup> (1997) reported 35 cases of surgical revision, including aortohepatic iliac artery graft (from banked donor vessels), autologous saphenous vein patch angioplasty and resection of the stenotic segment either with primary reanastomosis or with interposition of a banked iliac artery or saphenous vein graft. In this group, HA flow was reestablished successfully in all patients. At a mean follow-up of 25 mo, 67% of patients were asymptomatic with normal liver function. Six patients were treated with PTA. Five of them were found to be asymptomatic at a mean follow-up of 25 mo<sup>[57]</sup>. Indeed, balloon angioplasty can be an effective treatment option in these cases<sup>[10,19]</sup>. Similar to Abbasoglu *et al*<sup>[57]</sup> (1997), Saad *et al*<sup>[52]</sup> (2005) also reported 81% successful treatment of cases in a series of 42 cases of significant HAS treated by PTA, with an incidence of immediate complication of 7% including dissection and arterial rupture<sup>[52,57]</sup>. Delayed complications (*i.e.*, HAT) within 30 d of PTA occurred in 5% of cases, yielding a total complication rate of 12% and 12% total failed attempts without consequences. In this treatment modality, very different rates of restenosis have been reported from no restenosis to rates as high as 75%<sup>[60,63,64,67,68]</sup>. Denys *et al*<sup>[60]</sup> (2002) reported a low rate of HAT among 13 HAS patients treated by HA stent placement, which may be attributed to anticoagulation and/or antiplatelet regimens that were routinely given to their patients<sup>[52,60]</sup>. In their study, they also reported a post-HA stent placement HAT in one patient, and four patients with intra-stent restenosis in whom restenosis was dilated successfully. Other teams showed that primary stenting of the HA is feasible and offers a low complication rate with an acceptable one-year patency rate<sup>[60,69]</sup>. Ueno *et al*<sup>[69]</sup> (2006) reported an incidence of restenosis of 25% after stent placement, which is



**Figure 3** Contrast-enhanced-multidetector-row computed tomography-scan showing a hepatic artery pseudoaneurysm following orthotopic liver transplantation. Pseudoaneurysm (arrow).

significant, but Sommacale *et al*<sup>[56]</sup> (2013) demonstrated that repeated endovascular treatment of recurring HA stenosis carries a high rate of success<sup>[56,69]</sup>. However the best time for the earliest endovascular intervention after liver transplant is currently still discussed. Boyvat *et al*<sup>[66]</sup> (2008) reported endovascular intervention performed within seven days after transplant in nine patients, with a mean intervention time of 34.6 d (range: 6 h-210 d). They experienced extravasation or HAR in five patients and used graft-covered stents to solve this issue in all patients. They suggested that this technique should allow for safer endovascular intervention with no restriction time after surgery and with an acceptable benefit/risk ratio<sup>[66]</sup>. Finally, a recent published meta-analysis of case series has reported that interventional radiological procedures are often used to treat post-transplant HAS, and that PTA with balloon dilation alone or associated to stent placement are both efficacious and show similar complication rates and decrease the retransplantation rate<sup>[55]</sup>.

**Prognosis:** In the study by Abbasoglu *et al*<sup>[57]</sup> (1997) the overall mortality was 20%, mainly in the surgical revision group. Nineteen percent of patients with HAS had retransplantation with a median time of four months (range: 11 d-21 mo). It is interesting to note that among these, five had chronic rejection not diagnosed prior to HA revision, suggesting that HA stenosis should be an early sign of chronic rejection<sup>[57]</sup>. Therefore, Abbasoglu *et al*<sup>[57]</sup> (1997) recommended that every HAS patients should be screened for chronic rejection. The patient and graft survival rates at four years in the revised HA group were 65% and 56%, respectively; these rates were not significantly different from those of the control group<sup>[57]</sup>.

**Conclusion:** To conclude, HAS requiring revision is an uncommon condition after OLT. Early diagnosis by means of systematic DUS in the postoperative period and prompt revascularization procedures, with percutaneous endovascular methods with or without stent placement first, are usually successful with long-term graft and patient survival<sup>[56]</sup>. Individualized therapeutic regimens



**Table 4** Hepatic artery pseudoaneurysm highlights

Summary of the clinical characteristics about HAP
The reported incidence of HAP is ranging from 0.27% to 3% following OLT
In most cases, HAP is localized extra-hepatic and occurred in the early postoperative period around 1 mo post-OLT (69% within 20 d and 81% within 35 POD)
Clinical presentation varies from the asymptomatic state and incidental diagnosis to abdominal pain with fever and gastrointestinal bleeding (25% of cases, massive bleeding through the abdominal drain or acutely with hemorrhagic shock)
Risk factors include peritoneal infection, biliary leak, bilio-digestive anastomosis and digestive leak
Diagnosis of HAP is confirmed by DUS (with lower performance), contrast-enhanced CT scan, magnetic resonance angiography or angiography
Treatment of HAP includes reoperation (urgent laparotomy for HA ligation: Mortality rate 60%; HAP excision and immediate revascularization with a cryopreserved arterial allograft: Mortality rate 28%) or interventional radiology (HA embolization with a coil or HAP exclusion with a covered stent)
HAP has a worse prognosis with an overall mortality of more than 50% (ranging from 53% to 100%)
Early recognition of HAP in the population at high risk is mandatory and allows for a successful therapeutic outcome in 100% of cases

DUS: Doppler ultrasound; HA: Hepatic artery; HAP: Hepatic artery pseudoaneurysm; OLT: Orthotopic liver transplantation; CT: Computed tomography.

should be applied to treat HAS according to the technical platform available within transplant centers. When endovascular intervention fails to rescue arterial blood inflow, surgical revascularization should be attempted, especially if HAS is associated with biliary complications before considering retransplantation, which carries a higher mortality rate<sup>[70]</sup>. Finally, a meticulous arterial anastomosis suture with careful attention of a sufficient arterial flow into the liver transplant seems prevent this complication.

### HAP

**Definition:** HAP is defined as a dilated hepatic artery, which occurs after iatrogenic injury in most cases, causing blood to leak and pool outside the artery wall into surrounding tissue, with a persistent communication between the HA and the resultant adjacent cavity (Table 4). This is a very unusual event, with a reported incidence of 0.27%-3%<sup>[26,30,71-80]</sup>.

**Incidence:** In the retrospective cohort studied by Volpin *et al*<sup>[81]</sup> (2014) on 787 LT performed between January 1990 and 31 December 2005, a HAP incidence of 2.5% was reported, uniformly distributed over the 16-year period. The authors showed that this complication did not significantly affect any specific indication for liver transplantation. In the 16 patients that were concerned, the anatomical localization of HAP was extra-hepatic and occurred after the first liver transplant. In fact, most HAP occurred in the early postoperative period around one month post-OLT: 69% presented within 20 d and 81% within 35 d following LT. The median time of presentation of HAP was 13 d. This corresponds to the median time reported by many authors, varying from 13.4 to 29 d post-LT<sup>[26,30,78,80,81]</sup>.

**Clinical presentation:** The clinical presentation of HAP varies from the asymptomatic state and incidental diagnosis upon imaging to abdominal pain associated with fever, gastrointestinal bleeding (25% of cases), massive bleeding through the abdominal drain in the very early post-LT period (31% of cases) and acutely with hemorrhagic shock (81% of cases, the most frequent in

the series of Volpin *et al*<sup>[81]</sup>, 2014). These imply additional investigations, such as emergent abdominal imaging.

**Risk factors:** Several predisposing factors have been suggested, including peritoneal infections, technical difficulties during the completion of arterial anastomosis and biliary leak<sup>[26,30,71-83]</sup>. The rate of patients with extra-hepatic HAP and with bacterial or fungal organisms isolated from the peritoneal fluid or from the arterial wall is very high. In the series of Volpin *et al*<sup>[81]</sup> (2014), these patients accounted for 81% of the total (microorganisms cultured from the HAP wall: 50% of cases; cultured from the abdominal fluid: 31% of cases), and other authors report a rate varying from 66% to 100%<sup>[26,30,71,73-81,84,85]</sup>. Four patients of the Volpin series had a biliary leak discovered before or at the same time as HAP. Indeed, bile leak and bilio-digestive anastomosis were found to be risk factors for HAP, suggesting that enterotomy, bile and digestive leaks could be a source of peritoneal contamination, be considered very seriously and treated promptly because of the risk of HAP formation. In contrast, LDLT, reduced size, split, auxiliary LT and retransplantation were not risk factors for HAP.

**Diagnosis:** In the study by Volpin *et al*<sup>[81]</sup> (2014), the diagnosis of HAP was made by DUS, contrast-enhanced CT scan or angiography (Volpin *et al*<sup>[81]</sup>, 2014) (Figure 4).

**Therapeutic management:** Treatment of HAP can be achieved by reoperation or interventional radiology<sup>[26,75,78,81,86]</sup>. In the series of Volpin *et al*<sup>[81]</sup> (2014), five patients underwent urgent laparotomy for HA ligation; three of them died in the immediate postoperative course with a mortality rate of 60%. The two survivors had biliary complications<sup>[81]</sup>. Among patients treated by HA ligation, other authors confirmed this unfavorable outcome: 28% mortality in the series of Madariaga *et al*<sup>[73]</sup> (1992), 75% in the series of Marshall *et al*<sup>[78]</sup> (2001) and 85% in the series of Bonham *et al*<sup>[74]</sup> (1999). Moreover, this treatment exposes survivors to impaired liver function, graft loss and finally retransplantation<sup>[81,85]</sup>. Despite these poor outcomes, Boleslawski *et al*<sup>[26]</sup> (2013) reported that HA ligation without revascularization is



**Figure 4** Arteriography showing a hepatic artery stenosis due to a kinking following orthotopic liver transplantation. Kinking stenosis (arrow).

regarded as a reasonable option, with no early mortality in 10 patients with HAP rupture treated by ligation without revascularization. Six of them were still alive without retransplantation after a median follow-up of 70 mo<sup>[26]</sup>; seven patients underwent HAP excision and immediate revascularization. The arterial continuity was directly restored in five cases and cryopreserved arterial allograft conduits were interposed in two cases. In three cases, concomitant biliary complication was treated simultaneously by bilio-enteric anastomoses. Two patients died postoperatively (mortality rate of 28%). In this subgroup of treated patients, 66% of cases had an uneventful outcome, which seems to offer the best outcome in an emergency setting. Finally, two patients were treated by interventional radiology. One patient underwent embolization with a coil for deliberate HA occlusion; at 10.5 years of follow-up, this patient has good liver function without biliary complications. Another patient had HAP excision with a covered stent inserted into the HA; this patient has good liver function at 10 years of follow-up<sup>[81]</sup>.

**Prognosis:** Volpin *et al*<sup>[81]</sup> (2014) reported an overall mortality of 50%. Among patients who presented with HAP rupture, the mortality rate was 53%. The three patients treated before HAP rupture occurred are still alive after 10 years of follow-up<sup>[81]</sup>. In the literature, HAP is associated with a high mortality rate, ranging from 69% to 100%<sup>[26,30,71-81]</sup>.

**Conclusion:** To conclude, the early recognition of HAP in a high risk population (patient presenting with a documented peritoneal infection, bacteremia, bile and/or digestive leak, or bilio-digestive anastomosis) is crucial to expressly carry out diagnostic assessment and therapeutic management by percutaneous endovascular techniques first. Surgical intervention for HAP excision should be followed by immediate revascularization, even in an infected field, if endovascular management has failed. Recognition before rupture should allow a successful outcome in 100% of cases. Keeping in mind that HAP is usually asymptomatic before rupture, that

it occurs most often within the first five weeks post-LT and the poor performance of DUS<sup>[87]</sup>, Volpin *et al*<sup>[81]</sup> (2014) suggested that a contrast-enhanced CT scan or magnetic resonance angiography should be performed.

## HAR

**Definition, incidence and risk factors:** HAT is defined as a severe hemorrhage from the trunk or from a main branch of the HA. It is a very serious complication that results in the disruption of the arterial blood supply of the transplant. This is a very exceptional but a dramatic complication after OLT which carries very high incidence of liver transplant loss and high mortality rate. In most cases, this condition complicates a pseudoaneurysm of the HA, leading to major bleeding that requires emergency operation. Many reports reported the role of infectious pathogens as the cause in the development of pseudoaneurysms. Diagnosis of pseudoaneurysms is accessible with various radiological techniques, but in half of cases, HAP is not recognized before rupture, requiring immediate surgery<sup>[26]</sup> (Table 5).

In cases of acute bleeding, many therapeutic possibilities are available: endovascular intervention with embolization with or without stenting, surgical intervention for anastomotic revision, aorto-hepatic grafting, HA ligation or emergency/elective retransplantation. In case of HAR, mortality remains very high and currently there is no consensus on the indications for these procedures<sup>[26,73,78,80,88]</sup>. Boleslawski *et al*<sup>[26]</sup> (2013) published the largest series of ruptured post-transplant HAP; they highlighted the efficacy of primary HA ligation on both early and late survival. They reported an HAR incidence of 0.64% (17 patients out of 2649 OLTs from 1997 to 2007). The mean age was 47.9 years (range: 27-65 years; 13 men and 4 women). The median time between transplant and HAR occurrence was 29 d (range: 2-92 d), but the distribution of events was bimodal with only four late HA ruptures occurring after two months<sup>[26]</sup>.

**Clinical presentation and diagnosis:** In the study by Boleslawski *et al*<sup>[26]</sup> (2013), clinical presentation was always sudden hemorrhage: Hemoperitoneum in ten patients, gastrointestinal bleeding in five patients, hematoma in one patient and hemobilia in one patient. The presence of a fungal infection in the arterial wall was confirmed in six patients. Biliary leak was observed in seven patients<sup>[26]</sup>.

**Therapeutic management:** In the study by Boleslawski *et al*<sup>[26]</sup> (2013), immediate treatment included urgent laparotomy (15 patients) with definitive ligation of the HA (10 patients), anastomotic revision (3 patients) and aortohepatic grafting (2 patients). One patient had a percutaneous embolization and one patient died before treatment. Treatment of the associated biliary leak was performed either synchronously or after the first surgery in seven patients. In this series, the early mortality rate was 35% (0-80 d from HAR and 16-172 d from

**Table 5 Hepatic artery rupture highlights**

Summary of the clinical characteristics about HAR
HAT is defined as a severe hemorrhage from the trunk or from a main branch of the HA, resulting in disruption of graft arterial blood supply
This is a very rare (incidence of 0.64%) but a dramatic complication following OLT with a high mortality rate
In most cases, HAR complicates a pseudoaneurysm of the HA
The median time of HAR is 29 d (range: 2-92 d) following OLT
The clinical presentation is always a sudden hemorrhage: Hemoperitoneum, gastrointestinal bleeding, hematoma and hemobilia
Treatment comprises urgent laparotomy with definitive ligation of the HA, anastomotic revision and aortohepatic grafting or interventional radiology with percutaneous embolization

HA: Hepatic artery; HAT: Hepatic artery thrombosis; OLT: Orthotopic liver transplantation; HAR: Hepatic artery rupture.

transplantation) because of hemorrhagic relapse or sepsis<sup>[26]</sup>.

**Prognosis:** Boleslawski *et al*<sup>[26]</sup> (2013) also studied the effect of HA ligation on survival. They compared patients with ( $n = 10$ ) and without ( $n = 6$ ) HA ligation treatment. Of the 6 patients that received percutaneous embolization or revascularization, only 1 survived beyond 90 d (mortality rate: 83%). The 10 patients with HA ligation survived after postoperative day 90. Additionally, the one- and three-year graft survival rates for patients without HA ligation were 14% and 14%, respectively, vs 80% and 70%, respectively, in patients with HA ligation. The one- and three-year overall survival probabilities were 14% and 14%, respectively, in patients without HA ligation vs 100% and 80%, respectively, in patients with HA ligation<sup>[26]</sup>.

**Conclusion:** Finally, in this retrospective study, Boleslawski *et al*<sup>[26]</sup> (2013) recommended that HA revascularization should be avoided, especially when mycotic pseudoaneurysm is suspected (*i.e.*, if there was a gastrointestinal wound during liver procurement, documented systemic candidiasis prior to HAR, or if HAR occurred several weeks after transplant with associated lesions, such as biliary leak or gastroduodenal perforation). In contrast, HA ligation seems to be a reasonable life-saving option because it prevents hemorrhagic recurrence and should achieve a successful long-term outcome, with or even without retransplantation. Expected biliary complications, such as ischemic cholangitis, following HA ligation could be managed afterward by percutaneous and/or endoscopic interventions<sup>[26]</sup>.

## VENOUS COMPLICATIONS

Compared to arterial complications, venous complications are less frequent with an estimated overall incidence of less than 3%<sup>[4,5,8,9,62,89-91]</sup>. They can be potentially devastating and lead to graft failure, and therefore represent an important source of morbidity and mortality after OLT, especially if they occur in the early post-operative period<sup>[9,90,91]</sup>. Numerous literature reports have demonstrated that the incidence of venous complications in pediatric transplants is higher than in adult transplants<sup>[9,62,92,93]</sup>. Venous complications

following OLT include: Portal (1%-3%) and caval (< 2%) problems<sup>[5,8,9,91]</sup>. The etiology underlying most of these involves the anastomosis, including: (1) PVT: < 3% (the most pejorative), portal vein stenosis (PVS): 2%-3%; and (2) caval and hepatic veins with specific complications depending to the type of anastomosis either end to end caval anastomosis: Thrombosis, stenosis (< 2%); or piggyback: Thrombosis, stenosis, kinking < 2%<sup>[4,5,8,9,91,94,95]</sup>. In the same fashion as HACs, they can be classified into two categories (Table 1): Early (< 1 mo) or late (delayed, *i.e.*, > 1 mo). In the recent years, the literature has been in favor of endovascular intervention management of venous complications, with very good outcomes<sup>[8,9,10,62,91]</sup>.

### Portal vein complications

The incidence of portal vein complications (PVCs) following liver transplantation is relatively uncommon, occurring in 1% to 3% of patients<sup>[4,5,8,9,89-91,96]</sup>. These complications are associated with high morbidity and graft loss<sup>[8,9]</sup>. An another important fact to mention is that PVCs are more common with split liver and LDLT and also in pediatric transplantation<sup>[91,97]</sup>. Regarding PVCs, DUS, contrast enhanced ultrasound (CEUS) and contrast-enhanced CT are the usual tools for diagnosis; more recently, magnetic resonance venography using the gadofosveset trisodium agent has been proposed<sup>[8,9,98]</sup>. Therapeutic PVCs management ranges from thrombectomy and anastomosis revision to retransplantation depending to the delay of occurrence after OLT. Nowadays, except early PVT, endovascular procedures are now considered to be the first line treatment for post-transplant PVCs, and many studies have shown highly successful results<sup>[62,93,99,100]</sup>.

**PVT:** The incidence of PVT in OLT ranges from 0.3%-2.6%<sup>[1,90]</sup> (Table 6). From the UCLA experience, Duffy *et al*<sup>[5]</sup> (2009) reported a PVT incidence of 2% in more than 4200 patients. However, the incidence of PVT is close to 4% in adult LDLT due to technical difficulties in PV reconstructions, mainly related to a shorter vessel pedicle and limited vessel graft<sup>[101]</sup>. In LDLT, PVT occurs more frequently in the early period, defined as within 3 mo by Kyoden *et al*<sup>[101]</sup> (2008) (73% of cases from Kyoden's series; median, 58 d; range, 1-68 d).

The clinical presentation depends on the time the

**Table 6 Portal vein thrombosis highlights****Summary of the clinical characteristics about PVT**

The incidence of PVT is uncommon and ranges from < 3% following OLT  
 PVT incidence is higher in pediatric transplantation, LDLT and split liver transplantation  
 Early PVT is more frequent than late PVT with a median time to diagnosis of 5 d following OLT (range: 1 to 15 d)  
 The clinical presentation of early PVT ranges from portal hypertension manifestations (abdominal pain, ascites, gastrointestinal bleeding, splenomegaly) to severe allograft dysfunction and multiorgan failure  
 The most common causes leading to PVT are technical errors and anatomic complications such as venous redundancy, kinking and/or stenosis of the anastomosis  
 Risk factors are the presence of portal thrombosis prior OLT, small diameter of the portal vein, previous splenectomy, large portosystemic collaterals and the use of cryopreserved venous conduits for PV reconstruction  
 DUS, CEUS, contrast-enhanced CT, MRI and portography are imaging tools used for a positive diagnosis  
 PVT treatment includes systemic anticoagulation therapy, catheter-based thrombolytic therapy by percutaneous radiological intervention (transhepatic or transjugular access depending of the coagulation state) with or without stent placement to portosystemic shunting (TIPS) to retransplantation in highly unresolvable cases  
 PVT is associated with poor survival without treatment, but with prompt management, outcomes in terms of morbidity and mortality are satisfying

DUS: Doppler ultrasound; PVT: Portal vein thrombosis; OLT: Orthotopic liver transplantation; LDLT: Living donor liver transplantation; CEUS: Contrast enhanced ultrasound; MRI: Magnetic resonance imaging; CT: Computed tomography; TIPS: Transjugular intrahepatic portosystemic shunt.

thrombosis occurs. When it occurs early, severe acute liver insufficiency or graft failure predominates. If it occurs late, clinical symptoms depend of the portocaval collateral circulation existence. Portal hypertension manifestations including upper gastrointestinal bleeding due to esophagogastric varices and ascites are the most frequent clinical presentations. In contrast, liver failure is rare<sup>[30,90,96]</sup>. Langnas *et al.*<sup>[30]</sup> (1991) reported a mean diagnosis time of 5 d following OLT (range: 1 to 15 d), which was confirmed by Kyoden *et al.*<sup>[101]</sup> (2008), who reported that PVT occurred more frequently in the early period, *i.e.*, 8/11 cases (72%).

The most common causes of PVT are technical errors related to venous redundancy and kinking and/or stenosis of the anastomosis<sup>[90]</sup>. Other reported risk factors include prior surgery on the portal or splanchnic venous system or a pre-transplant portal thrombosis requiring thrombectomy during the operation, a small diameter of the portal vein (< 5 mm), previous splenectomy, hypoplastic portal vein, large portosystemic collaterals and the use of venous conduits for portal vein reconstruction<sup>[90,96]</sup>. Specific risk factors found in adult LDLT are: Small PV size, liver graft position and the type of venous conduits used to connect the PV of the donor to the recipient such as a cryo-preserved vein, the use of which is discouraged by Kyoden *et al.*<sup>[101]</sup> (2008)<sup>[30,90,96,102-105]</sup>.

DUS should be the first imaging tool used and is easily employed to evaluate vascular patency. It allows, in most cases, for an immediate non-invasive diagnosis and provides a rapid evaluation of vascular flow patency. DUS protocols vary widely worldwide among liver transplant centers, but most teams recommend performing DUS daily (some authors recommend twice daily) in the immediate post-operative period until POD 5 or in the presence of abnormalities of liver function tests or a clinical suspicion of the diagnosis<sup>[106-109]</sup>. Recently, other authors have proposed the use of CEUS to avoid frequent false-positive results after DUS<sup>[108,110]</sup>. CEUS may help in assessing the severity of portal insufficiency,

based on evidence of parenchymal perfusion status. It allows to show small thrombus in a peripheral portal branch<sup>[108,110]</sup>. In a retrospective evaluation of 23 patients, CEUS was used as an additional diagnostic method to DUS, CT and magnetic resonance imaging<sup>[110]</sup>. The authors reported new clinically relevant findings in 52% of cases, such as PVT confirmed during surgery or other radiological results.

Therapeutic options for PVT range from systemic anticoagulation to catheter-based thrombolytic therapy, to surgical revision until retransplantation. The three percutaneous options presented in the literature include transhepatic portal vein angioplasty (with or without stent placement), percutaneous thrombolytic therapy *via* transjugular intrahepatic portosystemic shunt (TIPS) creation and the transsplenic approach<sup>[111-114]</sup>. In practice, three different therapeutic situations that require specific care may be distinguished: (1) complete PVT within the first 48 h post-OLT; (2) PVT (complete or partial) at 48 h and not more than at 30 d (early PVT); and (3) after more than at 30 d (late PVT).

Early complete PVT within the first 72 h post-LT: In a patient who shows signs of multiorgan failure, surgical revision of the anastomosis is mandatory. In the presence of kinking or twisting that caused the thrombosis, anastomotic revision and systemic anticoagulation are sufficient to resolve this condition. If this procedure is unsuccessful in obtaining satisfactory portal transplant revascularization, emergent retransplantation should be indicated.

Early PVT (PVT > 72 h and < 30 d): Independently of PVT presentation (partial or complete), non-surgical treatment should be reasonably attempted. The most frequent procedure is percutaneous thrombolysis associated with stent placement<sup>[111,113,115-117]</sup>. Cherukuri *et al.*<sup>[113]</sup> (1998) reported the necessity that thrombolytic doses should be relatively low and maintained for only a few hours for efficacy and safety. Concerning the modality for stent placement, two different possibilities are described in the literature: The classical percutaneous



**Table 7 Portal vein stenosis highlights****Summary of the clinical characteristics about PVS**

The true incidence of PVS is not really known, but is thought to be < 3%  
 The major complication of PVS is the evolution to PVT if not treated  
 The majority of patients with PVS are asymptomatic and the diagnosis of stenosis is an incidental finding detected on routine DUS screening  
 Risk factors of PVS are almost exclusively represented by technical errors, particularly if a tapered anastomosis is required in the case of a vessel size mismatch between donor and recipient  
 Pre-OLT radiotherapy is another major predisposing factor of PVS  
 DUS with the finding of a stenosis ratio > 50% or a portal velocity ratio > 3:1 defines PVS. Contrast-enhanced CT and portography are used to confirm the diagnosis  
 If PVS is asymptomatic, no therapeutic intervention with close surveillance is possible, but anticoagulation therapy is recommended  
 In patients with clinical manifestations, percutaneous radiological intervention is the method of choice by transhepatic or transjugular access to perform angioplasty with or without stent placement; this prevents recurrence with a high rate of success and low rate of complications

PVT: Portal vein thrombosis; PVS: Portal vein stenosis; DUS: Doppler ultrasound; OLT: Orthotopic liver transplantation; CT: Computed tomography.

transhepatic approach and the transjugular approach. It is obvious that the latter should be preferred in patients with a coagulopathy or ascites, to minimize the risk of bleeding from transhepatic puncture<sup>[118-120]</sup>. This method has already been used in transplanted patients in the presence of decompensated cirrhosis, veno-occlusive disease or portal hypertension. The success rate with different endovascular methods ranges from 68%-100% and the mortality and morbidity rates are between 0% and 11%, respectively<sup>[121]</sup>.

Late PVT (PVT > 30 d): Two clinical presentations should be distinguished. Late PVT involving or not the superior mesenteric vein and normal liver function tests develop *de novo* hepato-portal collaterals and cavernoma formation. In these cases, observation may be justified, because of the appropriate venous inflow from the splenic circulation<sup>[19]</sup>; Late PVT with symptomatic manifestations such as acute gastroesophageal bleeding or ascites that should be treated with percutaneous or transjugular transhepatic procedures. Regarding the transjugular experience, Lodhia *et al.*<sup>[122]</sup> (2010) reported 3 cases of acute PVT occurring years following LT treated with an approach combining a TIPS and mechanical thrombectomy. To reduce the risk of periprocedural pulmonary emboli, the authors performed direct PV thrombolysis prior to placing the TIPS stent in order to allow time for clot dissolution<sup>[122]</sup>. Another possibility reported by Guckelberger *et al.*<sup>[123]</sup> (1999) was described for cases of late PVT with complete recanalization using a systemic low dose recombinant tissue plasminogen activator (rt-PA). The authors reported their experience with late PVT 45 mo after LT and justified the use of systemic low dose rt-PA lysis continuously for 10 d, along with 25000 IU heparin per day to adjust the partial thromboplastin time to favorable administration<sup>[123]</sup>. In fact, although, streptokinase (SK) and urokinase (UK) have been shown to be largely effective for thrombolytic therapies, both are characterized by limited thrombolytic potencies and major clinical disadvantages compared to rt-PA<sup>[124]</sup>. While streptokinase has a high antigenicity, both SK and UK, unlike rt-PA, lack fibrin-specific action which results in systemic consumption of plasminogen and decreased thrombolytic efficacy. Furthermore, it

may increase bleeding complications<sup>[124]</sup>.

PVT is associated with poor survival without treatment, but in cases of prompt diagnosis and adequate management, the literature shows good results in terms of morbidity and mortality.

To conclude, PVT is a rare but serious complication when it occurs in the early post-operative period. Diagnosis is mandatory as soon as possible by DUS screening protocols or with suspicious clinico-biological findings including abnormal abdominal pain and/or elevated liver enzymes and unexpected decrease PT. Surgical thrombectomy is traditionally required in the early post-operative period, but percutaneous radiological intervention has progressively become the best therapeutic option with good outcomes and safety.

**PVS**

The true incidence of PVS after LT is not really known, and the only data reported in the literature concerning the incidence of venous complications is < 3%<sup>[91]</sup> (Table 7).

When PVS occurs, it can be present with graft failure or the complication of portal hypertension<sup>[125]</sup>. In practice, the majority of patients with PVS are asymptomatic and the diagnosis of stenosis is an incidental finding detected on routine screening ultrasound. Conversely, when the patients are symptomatic, they may present with signs of portal hypertension, which include upper gastrointestinal tract bleeding from gastroesophageal varices, ascites and splenomegaly. Abnormal liver function tests are not constant, and are therefore not a reliable sign for PVS diagnosis<sup>[91]</sup>.

Regarding the risk factors of PVS, similar to PVT, it is well-established that the major concern is surgical technical errors<sup>[91]</sup>. Classically, the portal anastomosis is end-to-end and is usually simple in OLT, though a tapered anastomosis may be required when a significant size mismatch exists between the recipient and the donor, which constitutes a risk factor of stenosis. It explains in part why the pediatric population represents a population highly at risk to PVS<sup>[91]</sup>. In most cases, early PVS is the consequence of a surgical mistake due to technical difficulties in the anastomosis and could

evolve into an early thrombosis if not treated promptly. In contrast, it is assumed that late PVS is secondary to fibrosis or intimal hyperplasia<sup>[126]</sup>. Schneider *et al*<sup>[125]</sup> (2011) reported some cases of PVS after neoadjuvant radiotherapy for cholangiocarcinoma, and highlighted radiotherapy as a predisposing factor in venous complications; 21% of the patients who received a LT following the Mayo protocol for cholangiocarcinoma developed PVCs<sup>[125,127]</sup>.

Concerning a positive diagnosis, although DUS is the first screening morphological tool to use, its definition is still controversial because of the lack of definite and objective criteria. Moreover, DUS is sensitive for PVS but it is not specific. The PVS criteria for diagnosis include portal caliber size, velocities at the anastomotic site, as well as the preanastomotic and postanastomotic gradients. Recently Huang *et al*<sup>[107]</sup> (2010) reported a formula that can estimate the portal stenosis ratio in LDLT: They calculated the portal stenosis ratio (SR) = PRE-AS/PRE > 50% [anastomotic stenosis (AS); pre-stenotic stenosis (PRE)]; significant PVS was defined as a PVS with an SR > 50%. The portal velocity ratio (VR) was also calculated between AS and PRE, such that > 3:1 is defined as a significant VR value correlating with the SR evaluation. If these are confirmed, the patient should undergo contrast-enhanced CT to confirm the diagnosis<sup>[107]</sup>. Some authors consider the pressure gradient between the pre- and post-stenosis site. Wei *et al*<sup>[126]</sup> (2009) considered a gradient of > 5 mmHg to initiate treatment, while Shibata *et al*<sup>[128]</sup> (2005) used a significant gradient of > 3 mmHg. Other authors did not measure gradients if the stenosis was noted to be greater than 75% of the main portal vein diameter.

Surgical treatment, including anastomotic revision or retransplantation, is usually preferred for early portal inflow abnormalities following OLT<sup>[129]</sup>. In cases of asymptomatic patients with normal hepatic function test results, PVS may be solely observed with no therapeutic intervention<sup>[102]</sup>. In these particular cases, and in view of the possible evolution to PVT, it is reasonable to screen regularly by DUS to check for the patency of the PV. Moreover, in this condition, the use of anticoagulant therapy is still discussed and there is no international consensus or recommendation on this issue. In patients with clinical manifestations and radiological confirmation of significant stenosis, therapeutic intervention is mandatory to avoid graft loss, retransplantation and mortality. Interventional radiology has become widely recognized as the first choice for treatment for PVS after LT<sup>[103-105,111,125,126,128-132]</sup>. Regarding PVS management, it is possible to use the transhepatic access or transjugular access<sup>[133]</sup>, but most authors choose a transhepatic approach, usually from the right side. Shibata *et al*<sup>[128]</sup> (2005) reported that a single balloon dilatation was sufficient to maintain patency in 77.7% of patients, with a mean follow-up of 24.8 mo. In some series, stent placement associated with PTA was used to prevent recurrence. However, problems related to stent placement have been reported by Zajko *et al*<sup>[130]</sup> (1994),

*i.e.*, a thrombus that developed around the stent that could not be lysed, requiring retransplantation. However, Ko *et al*<sup>[129]</sup> (2007) reported on their experience in PVS management by percutaneous transhepatic primary stent placement after LDLT. In this series, technical and clinical success was obtained in 77.8% by using this method with a complication rate of 33% (including hemoperitoneum caused by blood oozing from the transhepatic tract and intrahepatic pseudoaneurysms)<sup>[129]</sup>. Finally, regarding the recurrence rate, this ranges between 0%-100%. Shibata *et al*<sup>[128]</sup> (2005) reported the most important series in the literature where the recurrence rate was 28.6%. Some authors recommend the use of anticoagulant therapy for the prevention of recurrent PVT<sup>[134]</sup>. Recently, Sanada *et al*<sup>[134]</sup> (2010) concluded that the use of three anticoagulant therapies, *i.e.*, low-molecular-weight heparin, warfarin and aspirin, significantly reduced the recurrence of thrombosis with a median follow-up of three months<sup>[134]</sup>. Additionally, some authors have coupled endovascular treatment with surgical PV access<sup>[106]</sup>.

To conclude, PVS represents an uncommon venous complication following OLT. This condition is more specific to pediatric LT and LDLT. As described earlier, a DUS screening protocol is an important diagnostic tool to help the clinician because the majority of asymptomatic cases can progress until PVT if not promptly treated, with negative effects on the prognosis of the graft and ultimately patient survival. Currently, it is obvious that percutaneous transhepatic radiological intervention with stent placement is the method of choice to address this complication with a high rate of success and a low rate of recurrence and/or complications.

### Caval vein complications

Currently, transplant outflow obstruction by kinking, stenosis or thrombosis of the inferior vena cava (IVC) or hepatic vein, especially in LDLT, are relatively uncommon complications following liver transplantation with an reported incidence of less than 3%<sup>[94,95]</sup> (Table 8 and Figure 5).

Clinical presentation ranges from lower limb edema, hepatomegaly, ascites, pleural effusions, Budd-Chiari syndrome, liver and renal failure to hypotension leading to allograft loss and multiorgan failure<sup>[4,89,135]</sup>.

The main risk factor leading to caval anastomosis complications (CACs) is represented by technical errors in the connection of caval anastomoses, which lead to kinking or thrombosis in the early post-operative course. In the late post-operative period, chronic stenosis in the anastomotic area is the result of fibrosis, hyperplasia and/or extrinsic compression from the enlarged liver graft<sup>[2,136,137]</sup>.

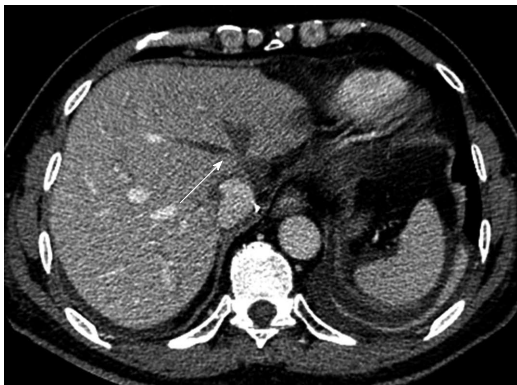
Diagnosis should be achieved by DUS, contrast-enhanced CT, and finally by cavography which allows for providing treatment.

Many techniques for caval anastomosis connection can avoid these complications, such as piggyback (PB) and subsequently modified-PB, first described by Starzl

**Table 8 Caval anastomosis complication highlights****Summary of the clinical characteristics about CAC**

The incidence of CAC is not known and is thought to be less than 3%  
 CAC is represented by stenosis, thrombosis and kinking depending on the type of caval anastomosis (cava resection or PB)  
 Clinical presentation of CAC ranges from lower limb edema, hepatomegaly, ascites, pleural effusions, Budd-Chiari syndrome, liver and renal failure, and hypotension, leading to allograft loss and even death  
 The main risk factor is a technical error in the creation of the anastomosis, which leads to kinking stenosis and thrombosis  
 Modified-PB with the three-hepatic vein seems to offer better outcomes because it has been demonstrated to be an efficient and safe method  
 Diagnosis tools include DUS, contrast-enhanced CT and cavography  
 Percutaneous radiological intervention is the method of choice *via* a transjugular approach or transhepatic approach if the anastomosis cannot be catheterized  
 It includes angioplasty by balloon dilatation and recurrences should be prevented by stent placement

CAC: Caval anastomosis complication; DUS: Doppler ultrasound; CT: Computed tomography; PB: Piggyback.



**Figure 5** Contrast-enhanced-multidetector-row computed tomography-scan showing median and left thromboses hepatic veins following orthotopic liver transplantation (arrow).

*et al.*<sup>[138]</sup> (1968). The method described by Starzl *et al.*<sup>[138]</sup> (1968) consists of a complete resection of the recipient IVC and interposition of the donor intrahepatic part of the vena cava with two end-to-end anastomoses<sup>[138-144]</sup>. The preservation of the recipient IVC with the PB technique has been associated with an increased risk of suprahepatic IVC thrombosis or stenosis, leading to acute or chronic Budd-Chiari syndrome in 0% to 1.6%, venous congestion of the liver allograft in 1%, and with an increased incidence of post-transplant ascites<sup>[89,135]</sup>. To avoid such complications, techniques for optimizing outflow with the piggyback technique have been described; the main of these in undoubtedly the width of the caval anastomosis, while other authors have reported methods using either the two-vein or the three-vein technique for anastomosis with a low rate of CACs<sup>[89,94,135,145-149]</sup>. Finally, several studies have demonstrated the superiority of modified-PB with the three-hepatic vein technique, which should be routinely used in OLT because it is safe and efficient and involves few surgical complications<sup>[89,94,143]</sup>. Hepatic venous stenosis is specific to LDLT with an incidence of 2% to 4%, because of the different techniques of donor graft outflow venoplasty, leading to Budd-Chiari syndrome or outflow block syndrome after LDLT<sup>[150]</sup>.

Therapeutic management of CACs depends on the time of the presentation and the delay following OLT. In

the case of severe allograft dysfunction or multiorgan failure, retransplantation is always indicated. Beyond this particular situation, percutaneous radiological intervention is the method of choice, where mortality after interventional transplant salvage procedure is 11.1% as compared with 41.6% mortality for those patients managed by retransplantation<sup>[121,137]</sup>. Treatment can be performed by transjugular approach, but percutaneous transhepatic access may be necessary when the anastomosis cannot be catheterized from the jugular access. Angioplasty by balloon dilatation can restore anastomotic patency in almost 100% of cases, but recidive of stenosis is frequent and repeat angioplasties may be applied<sup>[137]</sup>. PTA associated with stent placement may be the better solution with a high rate of success ranging from 73% to 100% in the literature; this technique is safe and apparently durable<sup>[121,130,136,137,151-157]</sup>.

To conclude, the incidence of CACs is very low, and particular attention should be paid to the caval anastomosis connection. Currently, modified-PB using the three-hepatic vein technique seems to show better outcomes. As with other VCs, prompt diagnosis and management are required if the patient is clinically symptomatic. The percutaneous endovascular method should be attempted to rescue the outflow patency, reserving surgical revision in unresolvable cases and ultimately retransplantation in patients presenting multiorgan failure.

## CONCLUSION

VCs continue to be a major problem following transplantation with a relatively frequent incidence (7%). They carry a high rate of morbidity and mortality, especially if they occur in the immediate post-operative period (first month) and if diagnosed late. The only solution to reduce their gravity is to prevent it by controlling risk factors and, if this is not possible, to diagnose them as early as can be, even in asymptomatic or paucisymptomatic patients. Many transplant teams worldwide advocate the routine use of complementary explorations such as DUS and, if in doubt, a contrast-enhanced CT scan or classical arteriography, which is

the reference. Currently, if recognized promptly, and if there is no graft or multiorgan failure, endovascular treatment should be attempted first if a technical plateau is available, because this has demonstrated efficacious and safe outcomes. Conversely, if there are severe liver repercussions, the most efficient therapeutic procedure is an emergency retransplant which shows better outcomes in terms of efficacy and survival, but the organ shortage dramatically limits this therapeutic option.

## ACKNOWLEDGMENTS

We thank Emmanuel Schaeffer and Pr. Jocelyne Wuibout for the proofreading and the correction of this manuscript.

## REFERENCES

- Khalaf H.** Vascular complications after deceased and living donor liver transplantation: a single-center experience. *Transplant Proc* 2010; **42**: 865-870 [PMID: 20430192 DOI: 10.1016/j.transproceed.2010.02.037]
- Wozney P, Zajko AB, Bron KM, Point S, Starzl TE.** Vascular complications after liver transplantation: a 5-year experience. *AJR Am J Roentgenol* 1986; **147**: 657-663 [PMID: 3529892]
- Karatzas T, Lykaki-Karatzas E, Webb M, Nery J, Tsaroucha A, Demirbas A, Khan F, Ciancio G, Montalvo B, Reddy R, Schiff E, Miller J, Tzakis AG.** Vascular complications, treatment, and outcome following orthotopic liver transplantation. *Transplant Proc* 1997; **29**: 2853-2855 [PMID: 9365590]
- Pawlak J, Grodzicki M, Leowska E, Malkowski P, Michałowicz B, Nyckowski P, Rowiński O, Pachó R, Zieniewicz K, Andrzejewska M, Ołdakowska U, Grzelak I, Patkowski W, Alsharabi A, Remiszewski P, Dudek K, Krawczyk M.** Vascular complications after liver transplantation. *Transplant Proc* 2003; **35**: 2313-2315 [PMID: 14529925]
- Duffy JP, Hong JC, Farmer DG, Ghobrial RM, Yersiz H, Hiatt JR, Busuttil RW.** Vascular complications of orthotopic liver transplantation: experience in more than 4,200 patients. *J Am Coll Surg* 2009; **208**: 896-903; discussion 903-905 [PMID: 19476857 DOI: 10.1016/j.jamcollsurg.2008.12.032]
- Bonnet S, Sauvagnet A, Bruno O, Sommacale D, Francoz C, Dondero F, Durand F, Belghiti J.** Long-term survival after portal vein arterialization for portal vein thrombosis in orthotopic liver transplantation. *Gastroenterol Clin Biol* 2010; **34**: 23-28 [PMID: 19643558 DOI: 10.1016/j.gcb.2009.05.013]
- Schwöpe RB, Margolis DJ, Raman SS, Kadell BM.** Portal vein aneurysms: a case series with literature review. *J Radiol Case Rep* 2010; **4**: 28-38 [PMID: 22470738 DOI: 10.3941/jrcr.v4i6.431]
- Pérez-Saborido B, Pacheco-Sánchez D, Barrera-Rebollo A, Asensio-Díaz E, Pinto-Fuentes P, Sarmentero-Prieto JC, Rodríguez-Vielba P, Martínez-Díaz R, Gonzalo-Martín M, Rodríguez M, Calero-Aguilar H, Pintado-Garrido R, García-Pajares F, Anta-Román A.** Incidence, management, and results of vascular complications after liver transplantation. *Transplant Proc* 2011; **43**: 749-750 [PMID: 21486590 DOI: 10.1016/j.transproceed.2011.01.104]
- Steinbrück K, Enne M, Fernandes R, Martinho JM, Balbi E, Agoglia L, Roma J, Pacheco-Moreira LF.** Vascular complications after living donor liver transplantation: a Brazilian, single-center experience. *Transplant Proc* 2011; **43**: 196-198 [PMID: 21335187 DOI: 10.1016/j.transproceed.2010.12.007]
- Hejazi Kenari SK, Zimmerman A, Eslami M, F Saidi R.** Current state of art management for vascular complications after liver transplantation. *Middle East J Dig Dis* 2014; **6**: 121-130 [PMID: 25093059]
- Figueras J, Busquets J, Dominguez J, Sancho C, Casanovas-Taltavull T, Rafecas A, Fabregat J, Torras J, Jaurrieta E.** Intra-arterial thrombolysis in the treatment of acute hepatic artery thrombosis after liver transplantation. *Transplantation* 1995; **59**: 1356-1357 [PMID: 7762074]
- Boyvat F, Aytekin C, Firat A, Harman A, Karakayali H, Haberal M.** Diagnostic and therapeutic management of hepatic artery thrombosis and stenosis after orthotopic and heterotopic liver transplantation. *Transplant Proc* 2003; **35**: 2791-2795 [PMID: 14612122]
- Stange BJ, Glanemann M, Nuessler NC, Settmacher U, Steinmüller T, Neuhaus P.** Hepatic artery thrombosis after adult liver transplantation. *Liver Transpl* 2003; **9**: 612-620 [PMID: 12783404]
- Zhou J, Fan J, Wang JH, Wu ZQ, Qiu SJ, Shen YH, Shi YH, Huang XW, Wang Z, Tang ZY, Wang YQ.** Continuous transcatheter arterial thrombolysis for early hepatic artery thrombosis after liver transplantation. *Transplant Proc* 2005; **37**: 4426-4429 [PMID: 16387137]
- Li ZW, Wang MQ, Zhou NX, Liu Z, Huang ZQ.** Interventional treatment of acute hepatic artery occlusion after liver transplantation. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 474-478 [PMID: 17897908]
- Saad WE, Davies MG, Saad NE, Westesson KE, Patel NC, Sahler LG, Lee DE, Kitanosono T, Sasson T, Waldman DL.** Catheter thrombolysis of thrombosed hepatic arteries in liver transplant recipients: predictors of success and role of thrombolysis. *Vasc Endovascular Surg* 2007; **41**: 19-26 [PMID: 17277239]
- Singhal A, Stokes K, Sebastian A, Wright HI, Kohli V.** Endovascular treatment of hepatic artery thrombosis following liver transplantation. *Transpl Int* 2010; **23**: 245-256 [PMID: 20030796 DOI: 10.1111/j.1432-2277.2009.01037.x]
- Chen J, Weinstein J, Black S, Spain J, Brady PS, Dowell JD.** Surgical and endovascular treatment of hepatic arterial complications following liver transplant. *Clin Transplant* 2014; **28**: 1305-1312 [PMID: 25091402 DOI: 10.1111/ctr.12431]
- Porrett PM, Hsu J, Shaked A.** Late surgical complications following liver transplantation. *Liver Transpl* 2009; **15** Suppl 2: S12-S18 [PMID: 19877292 DOI: 10.1002/lt.21893]
- Abdelaziz O, Hosny K, Amin A, Emadeldin S, Uemoto S, Mostafa M.** Endovascular management of early hepatic artery thrombosis after living donor liver transplantation. *Transpl Int* 2012; **25**: 847-856 [PMID: 22708507 DOI: 10.1111/j.1432-2277.2012.01509.x]
- Gordon SA, Carmody IC.** In: *Transplantation of the liver*. 2nd ed. Philadelphia, 2005: 953-961
- Moore FA, Moore EE, Seagraves A.** Nonresectional management of major hepatic trauma. An evolving concept. *Am J Surg* 1985; **150**: 725-729 [PMID: 3907382]
- Crossin JD, Muradali D, Wilson SR.** US of liver transplants: normal and abnormal. *Radiographics* 2003; **23**: 1093-1114 [PMID: 12975502]
- Panaro F, Gallix B, Bouyabrine H, Ramos J, Addeo P, Testa G, Carabalona JP, Pageaux G, Domergue J, Navarro F.** Liver transplantation and spontaneous neovascularization after arterial thrombosis: "the neovascularized liver". *Transpl Int* 2011; **24**: 949-957 [PMID: 21740470 DOI: 10.1111/j.1432-2277.2011.01293.x]
- Pastacaldi S, Teixeira R, Montalto P, Rolles K, Burroughs AK.** Hepatic artery thrombosis after orthotopic liver transplantation: a review of nonsurgical causes. *Liver Transpl* 2001; **7**: 75-81 [PMID: 11172388]
- Boleslawski E, Bouras AF, Truant S, Liddo G, Herrero A, Badic B, Audet M, Altieri M, Laurent A, Declerck N, Navarro F, Létoublon C, Wolf P, Chiche L, Cherqui D, Pruvot FR.** Hepatic artery ligation for arterial rupture following liver transplantation: a reasonable option. *Am J Transplant* 2013; **13**: 1055-1062 [PMID: 23398886 DOI: 10.1111/ajt.12135]
- Silva MA, Jambulingam PS, Gunson BK, Mayer D, Buckels JA, Mirza DF, Bramhall SR.** Hepatic artery thrombosis following orthotopic liver transplantation: a 10-year experience from a single centre in the United Kingdom. *Liver Transpl* 2006; **12**: 146-151 [PMID: 16382467]



- 28 **Bekker J**, Ploem S, de Jong KP. Early hepatic artery thrombosis after liver transplantation: a systematic review of the incidence, outcome and risk factors. *Am J Transplant* 2009; **9**: 746-757 [PMID: 19298450 DOI: 10.1111/j.1600-6143.2008.02541.x]
- 29 **Tzakis AG**, Gordon RD, Shaw BW, Iwatsuki S, Starzl TE. Clinical presentation of hepatic artery thrombosis after liver transplantation in the cyclosporine era. *Transplantation* 1985; **40**: 667-671 [PMID: 3907040]
- 30 **Langnas AN**, Marujo W, Stratta RJ, Wood RP, Shaw BW. Vascular complications after orthotopic liver transplantation. *Am J Surg* 1991; **161**: 76-82; discussion 82-83 [PMID: 1987861]
- 31 **Drazan K**, Shaked A, Olthoff KM, Imagawa D, Jurim O, Kiai K, Shackelton C, Busuttill R. Etiology and management of symptomatic adult hepatic artery thrombosis after orthotopic liver transplantation (OLT). *Am Surg* 1996; **62**: 237-240 [PMID: 8607585]
- 32 **Pinna AD**, Smith CV, Furukawa H, Starzl TE, Fung JJ. Urgent revascularization of liver allografts after early hepatic artery thrombosis. *Transplantation* 1996; **62**: 1584-1587 [PMID: 8970612]
- 33 **Sheiner PA**, Varma CV, Guarrera JV, Cooper J, Garatti M, Emre S, Guy SR, Schwartz ME, Miller CM. Selective revascularization of hepatic artery thromboses after liver transplantation improves patient and graft survival. *Transplantation* 1997; **64**: 1295-1299 [PMID: 9371671]
- 34 **Torras J**, Lladó L, Figueras J, Ramos E, Lama C, Fabregat J, Rafecas A, Escalante E, Dominguez J, Sancho C, Jaurieta E. Diagnostic and therapeutic management of hepatic artery thrombosis after liver transplantation. *Transplant Proc* 1999; **31**: 2405 [PMID: 10500642]
- 35 **Pawlak J**, Wróblewski T, Małkowski P, Nyckowski P, Zieniewicz K, Grzelak I, Alsharabi A, Michałowicz B, Krawczyk M, Karwowski A. Vascular complications related to liver transplantation. *Transplant Proc* 2000; **32**: 1426-1428 [PMID: 10996003]
- 36 **Bhattacharjya S**, Gunson BK, Mirza DF, Mayer DA, Buckels JA, McMaster P, Neuberger JM. Delayed hepatic artery thrombosis in adult orthotopic liver transplantation-a 12-year experience. *Transplantation* 2001; **71**: 1592-1596 [PMID: 11435970]
- 37 **Jain A**, Costa G, Marsh W, Fontes P, Devera M, Mazariegos G, Reyes J, Patel K, Mohanka R, Gadomski M, Fung J, Marcos A. Thrombotic and nonthrombotic hepatic artery complications in adults and children following primary liver transplantation with long-term follow-up in 1000 consecutive patients. *Transpl Int* 2006; **19**: 27-37 [PMID: 16359374]
- 38 **Pareja E**, Cortes M, Navarro R, Sanjuan F, López R, Mir J. Vascular complications after orthotopic liver transplantation: hepatic artery thrombosis. *Transplant Proc* 2010; **42**: 2970-2972 [PMID: 20970585 DOI: 10.1016/j.transproceed.2010.07.063]
- 39 **Fouzias I**, Sklavos A, Bismpa K, Paxiadakis I, Antoniadis N, Giakoustidis D, Katsiki E, Tatsou N, Mouloudi E, Karapanagiotou A, Tsitlakidis A, Karakatsanis A, Patsiaoura K, Petridis A, Gakis D, Imvrios G, Papanikolaou V. Hepatic artery thrombosis after orthotopic liver transplantation: 3 patients with collateral formation and conservative treatment. *Transplant Proc* 2012; **44**: 2741-2744 [PMID: 23146510 DOI: 10.1016/j.transproceed.2012.09.002]
- 40 **Marín-Gómez LM**, Bernal-Bellido C, Alamo-Martínez JM, Porras-López FM, Suárez-Artacho G, Serrano-Díaz-Canedo J, Padillo-Ruiz J, Gómez-Bravo MA. Intraoperative hepatic artery blood flow predicts early hepatic artery thrombosis after liver transplantation. *Transplant Proc* 2012; **44**: 2078-2081 [PMID: 22974916 DOI: 10.1016/j.transproceed.2012.07.077]
- 41 **Unal B**, Gonultas F, Aydin C, Otan E, Kayaalp C, Yilmaz S. Hepatic artery thrombosis-related risk factors after living donor liver transplantation: single-center experience from Turkey. *Transplant Proc* 2013; **45**: 974-977 [PMID: 23622602 DOI: 10.1016/j.transproceed.2013.02.070]
- 42 **Gunsar F**, Rolando N, Pastacaldi S, Patch D, Raimondo ML, Davidson B, Rolles K, Burroughs AK. Late hepatic artery thrombosis after orthotopic liver transplantation. *Liver Transpl* 2003; **9**: 605-611 [PMID: 12783403]
- 43 **Margarit C**, Hidalgo E, Lázaro JL, Murio E, Charco R, Balsells J. Biliary complications secondary to late hepatic artery thrombosis in adult liver transplant patients. *Transpl Int* 1998; **11** Suppl 1: S251-S254 [PMID: 9664990]
- 44 **Sakamoto Y**, Harihara Y, Nakatsuka T, Kawarasaki H, Takayama T, Kubota K, Kimura W, Kita Y, Tanaka H, Ito M, Hashizume K, Makuuchi M. Rescue of liver grafts from hepatic artery occlusion in living-related liver transplantation. *Br J Surg* 1999; **86**: 886-889 [PMID: 10417559]
- 45 **Oh CK**, Pelletier SJ, Sawyer RG, Dacus AR, McCullough CS, Pruett TL, Sanfey HA. Uni- and multi-variate analysis of risk factors for early and late hepatic artery thrombosis after liver transplantation. *Transplantation* 2001; **71**: 767-772 [PMID: 11330540]
- 46 **Pungpapong S**, Manzarbeitia C, Ortiz J, Reich DJ, Araya V, Rothstein KD, Muñoz SJ. Cigarette smoking is associated with an increased incidence of vascular complications after liver transplantation. *Liver Transpl* 2002; **8**: 582-587 [PMID: 12089709]
- 47 **Jiang XZ**, Yan LN, Li B, Zhao JC, Wang WT, Li FG, Wen TF, Ma YK, Zeng Y, Xu MQ, Yang JY, Li ZH. Arterial complications after living-related liver transplantation: single-center experience from West China. *Transplant Proc* 2008; **40**: 1525-1528 [PMID: 18589143 DOI: 10.1016/j.transproceed.2007.11.078]
- 48 **Panaro F**, Ramos J, Gallix B, Mercier G, Herrero A, Niampa H, Pageaux GP, Navarro F. Hepatic artery complications following liver transplantation. Does preoperative chemoembolization impact the postoperative course? *Clin Transplant* 2014; **28**: 598-605 [PMID: 24628275 DOI: 10.1111/ctr.12358]
- 49 **Vorwerk D**, Günther RW, Klever P, Riesener KP, Schumpelick V. Angioplasty and stent placement for treatment of hepatic artery thrombosis following liver transplantation. *J Vasc Interv Radiol* 1994; **5**: 309-311; discussion 312-314 [PMID: 8186600]
- 50 **Bjerkvik S**, Vatne K, Mathisen O, Søreide O. Percutaneous revascularization of postoperative hepatic artery thrombosis in a liver transplant. *Transplantation* 1995; **59**: 1746-1748 [PMID: 7604448]
- 51 **Cotroneo AR**, Di Stasi C, Cina A, De Gaetano AM, Evangelisti R, Paloni F, Marano G. Stent placement in four patients with hepatic artery stenosis or thrombosis after liver transplantation. *J Vasc Interv Radiol* 2002; **13**: 619-623 [PMID: 12050303]
- 52 **Saad WE**, Davies MG, Sahler L, Lee DE, Patel NC, Kitanoosono T, Sasson T, Waldman DL. Hepatic artery stenosis in liver transplant recipients: primary treatment with percutaneous transluminal angioplasty. *J Vasc Interv Radiol* 2005; **16**: 795-805 [PMID: 15947043]
- 53 **da Silva RF**, Raphe R, Felício HC, Rocha MF, Duca WJ, Arroyo PC, Palini GL, Vasquez AM, Miquelin DG, Reis LF, Silva AA, da Silva RC. Prevalence, treatment, and outcomes of the hepatic artery stenosis after liver transplantation. *Transplant Proc* 2008; **40**: 805-807 [PMID: 18455023 DOI: 10.1016/j.transproceed.2008.02.041]
- 54 **Hamby BA**, Ramirez DE, Loss GE, Bazan HA, Smith TA, Bluth E, Sternbergh WC. Endovascular treatment of hepatic artery stenosis after liver transplantation. *J Vasc Surg* 2013; **57**: 1067-1072 [PMID: 23332988 DOI: 10.1016/j.jvs.2012.10.086]
- 55 **Rostambeigi N**, Hunter D, Duval S, Chinnakotla S, Golzarian J. Stent placement versus angioplasty for hepatic artery stenosis after liver transplant: a meta-analysis of case series. *Eur Radiol* 2013; **23**: 1323-1334 [PMID: 23239061 DOI: 10.1007/s00330-012-2730-9]
- 56 **Sommacale D**, Aoyagi T, Dondero F, Sibert A, Bruno O, Fteriche S, Francoz C, Durand F, Belghiti J. Repeat endovascular treatment of recurring hepatic artery stenoses in orthotopic liver transplantation. *Transpl Int* 2013; **26**: 608-615 [PMID: 23551134 DOI: 10.1111/tri.12089]
- 57 **Abbasoglu O**, Levy MF, Vodapally MS, Goldstein RM, Husberg BS, Gonwa TA, Klintmalm GB. Hepatic artery stenosis after liver transplantation--incidence, presentation, treatment, and long term outcome. *Transplantation* 1997; **63**: 250-255 [PMID: 9020326]
- 58 **Sabri SS**, Saad WE, Schmitt TM, Turba UC, Kumer SC, Park AW, Matsumoto AH, Angle JF. Endovascular therapy for hepatic

- artery stenosis and thrombosis following liver transplantation. *Vasc Endovascular Surg* 2011; **45**: 447-452 [PMID: 21571780 DOI: 10.1177/1538574411407088]
- 59 **Blumhardt G**, Ringe B, Lauchart W, Burdelski M, Bechstein WO, Pichlmayr R. Vascular problems in liver transplantation. *Transplant Proc* 1987; **19**: 2412 [PMID: 3274527]
- 60 **Denys AL**, Qanadli SD, Durand F, Vilgrain V, Farges O, Belghiti J, Lacombe P, Menu Y. Feasibility and effectiveness of using coronary stents in the treatment of hepatic artery stenoses after orthotopic liver transplantation: preliminary report. *AJR Am J Roentgenol* 2002; **178**: 1175-1179 [PMID: 11959726]
- 61 **Chen GH**, Wang GY, Yang Y, Li H, Lu MQ, Cai CJ, Wang GS, Xu C, Yi SH, Zhang JF, Fu BS. Single-center experience of therapeutic management of hepatic artery stenosis after orthotopic liver transplantation. Report of 20 cases. *Eur Surg Res* 2009; **42**: 21-27 [PMID: 18971582 DOI: 10.1159/000166601]
- 62 **Uller W**, Knoppke B, Schreyer AG, Heiss P, Schlitt HJ, Melter M, Stroszczyński C, Zorger N, Wohlgemuth WA. Interventional radiological treatment of perihepatic vascular stenosis or occlusion in pediatric patients after liver transplantation. *Cardiovasc Intervent Radiol* 2013; **36**: 1562-1571 [PMID: 23572039 DOI: 10.1007/s00270-013-0595-1]
- 63 **Orons PD**, Sheng R, Zajko AB. Hepatic artery stenosis in liver transplant recipients: prevalence and cholangiographic appearance of associated biliary complications. *AJR Am J Roentgenol* 1995; **165**: 1145-1149 [PMID: 7572493]
- 64 **Orons PD**, Zajko AB, Bron KM, Trecha GT, Selby RR, Fung JJ. Hepatic artery angioplasty after liver transplantation: experience in 21 allografts. *J Vasc Interv Radiol* 1995; **6**: 523-529 [PMID: 7579858]
- 65 **Frangillo F**, Grossi U, Lirosi MC, Nure E, Sganga G, Avolio AW, Inchingolo R, Di Stasi C, Rinaldi P, Agnes S. Incidence, management, and results of hepatic artery stenosis after liver transplantation in the era of donor to recipient match. *Transplant Proc* 2013; **45**: 2722-2725 [PMID: 24034032 DOI: 10.1016/j.transproceed.2013.08.007]
- 66 **Boyvat F**, Aytekin C, Harman A, Sevmiş S, Karakayali H, Haberal M. Endovascular stent placement in patients with hepatic artery stenoses or thromboses after liver transplant. *Transplant Proc* 2008; **40**: 22-26 [PMID: 18261538 DOI: 10.1016/j.transproceed.2007.12.027]
- 67 **Abad J**, Hidalgo EG, Cantarero JM, Parga G, Fernandez R, Gomez M, Colina F, Moreno E. Hepatic artery anastomotic stenosis after transplantation: treatment with percutaneous transluminal angioplasty. *Radiology* 1989; **171**: 661-662 [PMID: 2524086]
- 68 **Mondragon RS**, Karani JB, Heaton ND, Thomas S, Wong PY, O'Grady JG, Tan KC, Williams R. The use of percutaneous transluminal angioplasty in hepatic artery stenosis after transplantation. *Transplantation* 1994; **57**: 228-231 [PMID: 8310513]
- 69 **Ueno T**, Jones G, Martin A, Ikegami T, Sanchez EQ, Chinnakotla S, Randall HB, Levy MF, Goldstein RM, Klintmalm GB. Clinical outcomes from hepatic artery stenting in liver transplantation. *Liver Transpl* 2006; **12**: 422-427 [PMID: 16498642]
- 70 **Sommacale D**, Rochas Dos Santos V, Dondero F, Francoz C, Durand F, Sibert A, Paugam-Burtz C, Sauvanet A, Belghiti J. Simultaneous surgical repair for combined biliary and arterial stenoses after liver transplantation. *Transplant Proc* 2011; **43**: 1765-1769 [PMID: 21693275 DOI: 10.1016/j.transproceed.2011.01.171]
- 71 **Houssin D**, Ortega D, Richardson A, Ozier Y, Stephan H, Soffer M, Chapuis Y. Mycotic aneurysm of the hepatic artery complicating human liver transplantation. *Transplantation* 1988; **46**: 469-472 [PMID: 3047941]
- 72 **Lerut J**, Gordon RD, Iwatsuki S, Starzl TE. Surgical complications in human orthotopic liver transplantation. *Acta Chir Belg* 1987; **87**: 193-204 [PMID: 3303776]
- 73 **Madariaga J**, Tzakis A, Zajko AB, Tzoracoleftherakis E, Tepetes K, Gordon R, Todo S, Starzl TE. Hepatic artery pseudoaneurysm ligation after orthotopic liver transplantation--a report of 7 cases. *Transplantation* 1992; **54**: 824-828 [PMID: 1440848]
- 74 **Bonham CA**, Kapur S, Geller D, Fung JJ, Pinna A. Excision and immediate revascularization for hepatic artery pseudoaneurysm following liver transplantation. *Transplant Proc* 1999; **31**: 443 [PMID: 10083180]
- 75 **Lowell JA**, Coopersmith CM, Shenoy S, Howard TK. Unusual presentations of nonmycotic hepatic artery pseudoaneurysms after liver transplantation. *Liver Transpl Surg* 1999; **5**: 200-203 [PMID: 10226110]
- 76 **Stange B**, Settmacher U, Glanemann M, Nuessler NC, Bechstein WO, Neuhaus P. Aneurysms of the hepatic artery after liver transplantation. *Transplant Proc* 2000; **32**: 533-534 [PMID: 10812100]
- 77 **Leonardi LS**, Soares C, Boin IF, Oliveira VC. Hemobilia after mycotic hepatic artery pseudoaneurysm after liver transplantation. *Transplant Proc* 2001; **33**: 2580-2582 [PMID: 11406253]
- 78 **Marshall MM**, Muiesan P, Srinivasan P, Kane PA, Rela M, Heaton ND, Karani JB, Sidhu PS. Hepatic artery pseudoaneurysms following liver transplantation: incidence, presenting features and management. *Clin Radiol* 2001; **56**: 579-587 [PMID: 11446757]
- 79 **Turrión VS**, Alvira LG, Jimenez M, Lucena JL, Ardaiz J. Incidence and results of arterial complications in liver transplantation: experience in a series of 400 transplants. *Transplant Proc* 2002; **34**: 292-293 [PMID: 11959290]
- 80 **Leelaudomlpi S**, Bramhall SR, Gunson BK, Candinas D, Buckels JA, McMaster P, Mirza DF, Mayer AD. Hepatic-artery aneurysm in adult liver transplantation. *Transpl Int* 2003; **16**: 257-261 [PMID: 12730806]
- 81 **Volpin E**, Pessaux P, Sauvanet A, Sibert A, Kianmanesh R, Durand F, Belghiti J, Sommacale D. Preservation of the arterial vascularisation after hepatic artery pseudoaneurysm following orthotopic liver transplantation: long-term results. *Ann Transplant* 2014; **19**: 346-352 [PMID: 25034853 DOI: 10.12659/AOT.890473]
- 82 **Jarzembowski TM**, Sankary HN, Bogetti D, Manzelli A, Ong E, Oberholzer J, Benedetti E, Testa G. Living donor liver graft salvage after rupture of hepatic artery pseudoaneurysm. *Int Surg* 2008; **93**: 300-303 [PMID: 19943434]
- 83 **Panaro F**, Miggino M, Bouyabrine H, Carabalona JP, Berthet JP, Canaud L, Nougaret S, Ramos J, Navarro F. Reversed saphenous bypass for hepatic artery pseudoaneurysm after liver transplantation. *Ann Vasc Surg* 2013; **27**: 1088-1097 [PMID: 23972638 DOI: 10.1016/j.avsg.2013.01.007]
- 84 **Sellers MT**, Haustein SV, McGuire BM, Jones C, Bynon JS, Diethelm AG, Eckhoff DE. Use of preserved vascular homografts in liver transplantation: hepatic artery aneurysms and other complications. *Am J Transplant* 2002; **2**: 471-475 [PMID: 12123215]
- 85 **Fistouris J**, Herlenius G, Bäckman L, Olausson M, Rizell M, Mjörnstedt L, Friman S. Pseudoaneurysm of the hepatic artery following liver transplantation. *Transplant Proc* 2006; **38**: 2679-2682 [PMID: 17098038]
- 86 **Patel JV**, Weston MJ, Kessel DO, Prasad R, Toogood GJ, Robertson I. Hepatic artery pseudoaneurysm after liver transplantation: treatment with percutaneous thrombin injection. *Transplantation* 2003; **75**: 1755-1757 [PMID: 12777870]
- 87 **Kim HJ**, Kim KW, Kim AY, Kim TK, Byun JH, Won HJ, Shin YM, Kim PN, Ha HK, Lee SG, Lee MG. Hepatic artery pseudoaneurysms in adult living-donor liver transplantation: efficacy of CT and Doppler sonography. *AJR Am J Roentgenol* 2005; **184**: 1549-1555 [PMID: 15855114]
- 88 **Golse N**, Spina A, Abdelaal A, Mennesson N, Feugier P, Dumortier J, Boillot O, Adham M. Extra-anatomical hepatic artery reconstruction following post-embolization iatrogenic dissection and arterial anastomotic rupture in two liver transplant recipients. *Gastroenterol Clin Biol* 2010; **34**: 111-114 [PMID: 20071115 DOI: 10.1016/j.gcb.2009.11.003]
- 89 **Parrilla P**, Sánchez-Bueno F, Figueras J, Jaurrieta E, Mir J, Margarit C, Lázaro J, Herrera L, Gómez-Fleitas M, Varo E, Vicente E, Robles R, Ramirez P. Analysis of the complications of the piggy-back technique in 1,112 liver transplants. *Transplantation* 1999; **67**: 1214-1217 [PMID: 10342311]

- 90 **Sánchez-Bueno F**, Hernández Q, Ramírez P, Robles R, Acosta F, Rodríguez JM, Parrilla P. Vascular complications in a series of 300 orthotopic liver transplants. *Transplant Proc* 1999; **31**: 2409-2410 [PMID: 10500645]
- 91 **Woo DH**, Laberge JM, Gordon RL, Wilson MW, Kerlan RK. Management of portal venous complications after liver transplantation. *Tech Vasc Interv Radiol* 2007; **10**: 233-239 [PMID: 18086428]
- 92 **Yilmaz A**, Arıkan C, Tuncgor G, Kilic M, Aydogdu S. Vascular complications in living-related and deceased donation pediatric liver transplantation: single center's experience from Turkey. *Pediatr Transplant* 2007; **11**: 160-164 [PMID: 17300495]
- 93 **Orlandini M**, Feier FH, Jaeger B, Kieling C, Vieira SG, Zanotelli ML. Frequency of and factors associated with vascular complications after pediatric liver transplantation. *J Pediatr (Rio J)* 2014; **90**: 169-175 [PMID: 24370174 DOI: 10.1016/j.jped.2013.08.010]
- 94 **Audet M**, Piardi T, Panaro F, Cag M, Habibeh H, Gheza F, Portolani N, Cinqualbre J, Jaecq D, Wolf P. Four hundred and twenty-three consecutive adults piggy-back liver transplantations with the three suprahepatic veins: was the portal systemic shunt required? *J Gastroenterol Hepatol* 2010; **25**: 591-596 [PMID: 19968745 DOI: 10.1111/j.1440-1746.2009.06084.x]
- 95 **Schmitz V**, Schoening W, Jelkmann I, Globke B, Pascher A, Bahra M, Neuhaus P, Puhl G. Different cava reconstruction techniques in liver transplantation: piggyback versus cava resection. *Hepatobiliary Pancreat Dis Int* 2014; **13**: 242-249 [PMID: 24919606]
- 96 **Lerut J**, Tzakis AG, Bron K, Gordon RD, Iwatsuki S, Esquivel CO, Makowka L, Todo S, Starzl TE. Complications of venous reconstruction in human orthotopic liver transplantation. *Ann Surg* 1987; **205**: 404-414 [PMID: 3551857]
- 97 **Buell JF**, Funaki B, Cronin DC, Yoshida A, Perlman MK, Lorenz J, Kelly S, Brady L, Leef JA, Millis JM. Long-term venous complications after full-size and segmental pediatric liver transplantation. *Ann Surg* 2002; **236**: 658-666 [PMID: 12409673]
- 98 **Strovski E**, Liu D, Scudamore C, Ho S, Yoshida E, Klass D. Magnetic resonance venography and liver transplant complications. *World J Gastroenterol* 2013; **19**: 6110-6113 [PMID: 24106414 DOI: 10.3748/wjg.v19.i36.6110]
- 99 **Goss JA**, Shackleton CR, McDiarmid SV, Maggard M, Swenson K, Seu P, Vargas J, Martin M, Ament M, Brill J, Harrison R, Busuttil RW. Long-term results of pediatric liver transplantation: an analysis of 569 transplants. *Ann Surg* 1998; **228**: 411-420 [PMID: 9742924]
- 100 **Charco R**, Fuster J, Fondevila C, Ferrer J, Mans E, García-Valdecasas JC. Portal vein thrombosis in liver transplantation. *Transplant Proc* 2005; **37**: 3904-3905 [PMID: 16386579]
- 101 **Kyoden Y**, Tamura S, Sugawara Y, Matsui Y, Togashi J, Kaneko J, Kokudo N, Makuuchi M. Portal vein complications after adult-to-adult living donor liver transplantation. *Transpl Int* 2008; **21**: 1136-1144 [PMID: 18764831 DOI: 10.1111/j.1432-2277.2008.00752.x]
- 102 **Kaneko J**, Sugawara Y, Ohkubo T, Matsui Y, Kokudo N, Makuuchi M. Successful conservative therapy for portal vein thrombosis after living donor liver transplantation. *Abdom Imaging* 2003; **28**: 58-59 [PMID: 12483385]
- 103 **Cheng YF**, Ou HY, Tsang LL, Yu CY, Huang TL, Chen TY, Concejero A, Wang CC, Wang SH, Lin TS, Liu YW, Yang CH, Yong CC, Chiu KW, Jawan B, Eng HL, Chen CL. Vascular stents in the management of portal venous complications in living donor liver transplantation. *Am J Transplant* 2010; **10**: 1276-1283 [PMID: 20353467 DOI: 10.1111/j.1600-6143.2010.03076.x]
- 104 **Azzam AZ**, Tanaka K. Management of vascular complications after living donor liver transplantation. *Hepatogastroenterology* 2012; **59**: 182-186 [PMID: 22251536 DOI: 10.5754/hge10453]
- 105 **Abdelaziz O**, Hosny K, Elmalt O, Emad-Eldin S, Hosny A. Intraoperative Ultrasound-guided Thrombectomy and Thrombolysis for Post-operative Portal Vein Thrombosis in Living Liver Donors. *Int J Organ Transplant Med* 2015; **6**: 33-40 [PMID: 25737775]
- 106 **Cheng YF**, Huang TL, Chen CL, Lee TY, Chen TY, Chen YS, Liu PP, Chiang YC, Eng HL, Wang CC, Cheung HK, Jawan B, Goto S. Intraoperative Doppler ultrasound in liver transplantation. *Clin Transplant* 1998; **12**: 292-299 [PMID: 9686322]
- 107 **Huang TL**, Cheng YF, Chen TY, Tsang LL, Ou HY, Yu CY, Wang CC, Wang SH, Lin CL, Cheung HK, Eng HL, Jawan B, Concejero AM, Chen CL. Doppler ultrasound evaluation of postoperative portal vein stenosis in adult living donor liver transplantation. *Transplant Proc* 2010; **42**: 879-881 [PMID: 20430195 DOI: 10.1016/j.transproceed.2010.02.036]
- 108 **Lee SJ**, Kim KW, Kim SY, Park YS, Lee J, Kim HJ, Lee JS, Song GW, Hwang S, Lee SG. Contrast-enhanced sonography for screening of vascular complication in recipients following living donor liver transplantation. *J Clin Ultrasound* 2013; **41**: 305-312 [PMID: 23553428 DOI: 10.1002/jcu.22044]
- 109 **Lee H**, Lim CW, Yoo SH, Koo CH, Kwon WI, Suh KS, Ryu HG. The effect of Doppler ultrasound on early vascular interventions and clinical outcomes after liver transplantation. *World J Surg* 2014; **38**: 3202-3209 [PMID: 25123179 DOI: 10.1007/s00268-014-2721-x]
- 110 **Reinert J**, Dornia C, Georgieva M, Roehrl S, Fellner C, Schleder S, Stroszczyński C, Jung EM. Identification of early complications following liver transplantation using contrast enhanced ultrasound (CEUS). First results. *J Gastrointest Liver Dis* 2012; **21**: 407-412 [PMID: 23256124]
- 111 **Olcott EW**, Ring EJ, Roberts JP, Ascher NL, Lake JR, Gordon RL. Percutaneous transhepatic portal vein angioplasty and stent placement after liver transplantation: early experience. *J Vasc Interv Radiol* 1990; **1**: 17-22 [PMID: 2151969]
- 112 **Durham JD**, LaBerge JM, Altman S, Kam I, Everson GT, Gordon RL, Kumpe DA. Portal vein thrombolysis and closure of competitive shunts following liver transplantation. *J Vasc Interv Radiol* 1994; **5**: 611-615; discussion 616-618 [PMID: 7949719]
- 113 **Cherukuri R**, Haskal ZJ, Naji A, Shaked A. Percutaneous thrombolysis and stent placement for the treatment of portal vein thrombosis after liver transplantation: long-term follow-up. *Transplantation* 1998; **65**: 1124-1126 [PMID: 9583875]
- 114 **Kensinger CD**, Sexton KW, Baron CM, Lipnik AJ, Meranze SG, Gorden DL. Management of portal vein thrombosis after liver transplantation with a combined open and endovascular approach. *Liver Transpl* 2015; **21**: 132-134 [PMID: 25262999 DOI: 10.1002/lt.24011]
- 115 **Haskal ZJ**, Naji A. Treatment of portal vein thrombosis after liver transplantation with percutaneous thrombolysis and stent placement. *J Vasc Interv Radiol* 1993; **4**: 789-792 [PMID: 8281002]
- 116 **Bhattacharjya T**, Olliff SP, Bhattacharjya S, Mirza DF, McMaster P. Percutaneous portal vein thrombolysis and endovascular stent for management of posttransplant portal venous conduit thrombosis. *Transplantation* 2000; **69**: 2195-2198 [PMID: 10852624]
- 117 **Baccarani U**, Gasparini D, Risaliti A, Vianello V, Adani GL, Sainz M, Sponza M, Bresadola F. Percutaneous mechanical fragmentation and stent placement for the treatment of early posttransplantation portal vein thrombosis. *Transplantation* 2001; **72**: 1572-1582 [PMID: 11707747]
- 118 **Lerut JP**, Goffette P, Molle G, Roggen FM, Puttemans T, Brenard R, Morelli MC, Wallemacq P, Van Beers B, Laterre PF. Transjugular intrahepatic portosystemic shunt after adult liver transplantation: experience in eight patients. *Transplantation* 1999; **68**: 379-384 [PMID: 10459541]
- 119 **Ciccarelli O**, Goffette P, Laterre PF, Danse E, Wittebolle X, Lerut J. Transjugular intrahepatic portosystemic shunt approach and local thrombolysis for treatment of early posttransplant portal vein thrombosis. *Transplantation* 2001; **72**: 159-161 [PMID: 11468552]
- 120 **López-Benítez R**, Barragán-Campos HM, Richter GM, Sauer P, Mehrabi A, Fonouni H, Golriz M, Schmidt J, Hallscheidt PJ. Interventional radiologic procedures in the treatment of complications after liver transplantation. *Clin Transplant* 2009; **23** Suppl 21: 92-101 [PMID: 19930322 DOI: 10.1111/j.1399-0012.2009.01115.x]
- 121 **Cavallari A**, Vivarelli M, Bellusci R, Jovine E, Mazziozzi A,



- Rossi C. Treatment of vascular complications following liver transplantation: multidisciplinary approach. *Hepatogastroenterology* 2001; **48**: 179-183 [PMID: 11268960]
- 122 **Lodhia N**, Salem R, Levitsky J. Transjugular intrahepatic portosystemic shunt with thrombectomy for the treatment of portal vein thrombosis after liver transplantation. *Dig Dis Sci* 2010; **55**: 529-534 [PMID: 19242796 DOI: 10.1007/s10620-009-0735-2]
  - 123 **Guckelberger O**, Bechstein WO, Langrehr JM, Kratschmer B, Loeffel J, Settmacher U, Neuhaus R, Lopez Haenninen E, Venz S, Vogl TJ, Neuhaus P. Successful recanalization of late portal vein thrombosis after liver transplantation using systemic low-dose recombinant tissue plasminogen activator. *Transpl Int* 1999; **12**: 273-277 [PMID: 10460873]
  - 124 **Gulba DC**, Bode C, Runge MS, Huber K. Thrombolytic agents-an overview. *Ann Hematol* 1996; **73** Suppl 1: S9-27 [PMID: 8853112]
  - 125 **Schneider N**, Scanga A, Stokes L, Perri R. Portal vein stenosis: a rare yet clinically important cause of delayed-onset ascites after adult deceased donor liver transplantation: two case reports. *Transplant Proc* 2011; **43**: 3829-3834 [PMID: 22172855 DOI: 10.1016/j.transproceed.2011.09.068]
  - 126 **Wei BJ**, Zhai RY, Wang JF, Dai DK, Yu P. Percutaneous portal venoplasty and stenting for anastomotic stenosis after liver transplantation. *World J Gastroenterol* 2009; **15**: 1880-1885 [PMID: 19370787]
  - 127 **Mantel HT**, Rosen CB, Heimbach JK, Nyberg SL, Ishitani MB, Andrews JC, McKusick MA, Haddock MG, Alberts SR, Gores GJ. Vascular complications after orthotopic liver transplantation after neoadjuvant therapy for hilar cholangiocarcinoma. *Liver Transpl* 2007; **13**: 1372-1381 [PMID: 17427173]
  - 128 **Shibata T**, Itoh K, Kubo T, Maetani Y, Shibata T, Togashi K, Tanaka K. Percutaneous transhepatic balloon dilation of portal venous stenosis in patients with living donor liver transplantation. *Radiology* 2005; **235**: 1078-1083 [PMID: 15845790]
  - 129 **Ko GY**, Sung KB, Yoon HK, Lee S. Early posttransplantation portal vein stenosis following living donor liver transplantation: percutaneous transhepatic primary stent placement. *Liver Transpl* 2007; **13**: 530-536 [PMID: 17394150]
  - 130 **Zajko AB**, Sheng R, Bron K, Reyes J, Nour B, Tzakis A. Percutaneous transluminal angioplasty of venous anastomotic stenoses complicating liver transplantation: intermediate-term results. *J Vasc Interv Radiol* 1994; **5**: 121-126 [PMID: 8136588]
  - 131 **Park KB**, Choo SW, Do YS, Shin SW, Cho SG, Choo IW. Percutaneous angioplasty of portal vein stenosis that complicates liver transplantation: the mid-term therapeutic results. *Korean J Radiol* 2005; **6**: 161-166 [PMID: 16145291]
  - 132 **Shiba H**, Sadaoka S, Wakiyama S, Ishida Y, Misawa T, Yanaga K. Successful treatment by balloon angioplasty under portography for late-onset stenosis of portal vein after cadaveric liver transplantation. *Int Surg* 2013; **98**: 466-468 [PMID: 24229043 DOI: 10.9738/INTSURG-D-12-00031.1]
  - 133 **Glanemann M**, Settmacher U, Langrehr JM, Kling N, Hidajat N, Stange B, Staffa G, Bechstein WO, Neuhaus P. Portal vein angioplasty using a transjugular, intrahepatic approach for treatment of extrahepatic portal vein stenosis after liver transplantation. *Transpl Int* 2001; **14**: 48-51 [PMID: 11263556]
  - 134 **Sanada Y**, Kawano Y, Mizuta K, Egami S, Hayashida M, Wakiya T, Fujiwara T, Sakuma Y, Hydo M, Nakata M, Yasuda Y, Kawarasaki H. Strategy to prevent recurrent portal vein stenosis following interventional radiology in pediatric liver transplantation. *Liver Transpl* 2010; **16**: 332-339 [PMID: 20209593 DOI: 10.1002/lt.21995]
  - 135 **Navarro F**, Le Moine MC, Fabre JM, Belghiti J, Cherqui D, Adam R, Pruvot FR, Letoublon C, Domergue J. Specific vascular complications of orthotopic liver transplantation with preservation of the retrohepatic vena cava: review of 1361 cases. *Transplantation* 1999; **68**: 646-650 [PMID: 10507483]
  - 136 **Weeks SM**, Gerber DA, Jaques PF, Sandhu J, Johnson MW, Fair JH, Mauro MA. Primary Gianturco stent placement for inferior vena cava abnormalities following liver transplantation. *J Vasc Interv Radiol* 2000; **11**: 177-187 [PMID: 10716387]
  - 137 **Darcy MD**. Management of venous outflow complications after liver transplantation. *Tech Vasc Interv Radiol* 2007; **10**: 240-245 [PMID: 18086429]
  - 138 **Starzl TE**, Groth CG, Brettschneider L, Penn I, Fulginiti VA, Moon JB, Blanchard H, Martin AJ, Porter KA. Orthotopic homotransplantation of the human liver. *Ann Surg* 1968; **168**: 392-415 [PMID: 4877589]
  - 139 **Calne RY**, Williams R. Liver transplantation in man. I. Observations on technique and organization in five cases. *Br Med J* 1968; **4**: 535-540 [PMID: 4881063]
  - 140 **Tzakis A**, Todo S, Starzl TE. Orthotopic liver transplantation with preservation of the inferior vena cava. *Ann Surg* 1989; **210**: 649-652 [PMID: 2818033]
  - 141 **Belghiti J**, Panis Y, Sauvanet A, Gayet B, Fékété F. A new technique of side to side caval anastomosis during orthotopic hepatic transplantation without inferior vena caval occlusion. *Surg Gynecol Obstet* 1992; **175**: 270-272 [PMID: 1514163]
  - 142 **Bismuth H**, Castaing D, Sherlock DJ. Liver transplantation by "face-à-face" venacavaplasty. *Surgery* 1992; **111**: 151-155 [PMID: 1736384]
  - 143 **Cherqui D**, Lauzet JY, Rotman N, Duvoux C, Dhumeaux D, Julien M, Fagniez PL. Orthotopic liver transplantation with preservation of the caval and portal flows. Technique and results in 62 cases. *Transplantation* 1994; **58**: 793-796 [PMID: 7940712]
  - 144 **Kishi Y**, Sugawara Y, Matsui Y, Akamatsu N, Makuuchi M. Late onset portal vein thrombosis and its risk factors. *Hepatogastroenterology* 2008; **55**: 1008-1009 [PMID: 18705318]
  - 145 **Lázaro JL**, Charco R, Revhaug A, Murio E, Balsells J, Hidalgo E, Mora A, Cortés C, Margarit C. Hemodynamics in human liver transplantation with inferior vena cava preservation. *Transplant Proc* 1997; **29**: 2851-2852 [PMID: 9365589]
  - 146 **Robles R**, Parrilla P, Acosta F, Bueno FS, Ramirez P, Lopez J, Lujan JA, Rodriguez JM, Fernandez JA, Picó F. Complications related to hepatic venous outflow in piggy-back liver transplantation: two- versus three-suprahepatic-vein anastomosis. *Transplant Proc* 1999; **31**: 2390-2391 [PMID: 10500634]
  - 147 **Wojcicki M**, Post M, Pakosz-Golanowska M, Zeair S, Lubikowski J, Jarosz K, Czuprynska M, Milkiewicz P. Vascular complications following adult piggyback liver transplantation with end-to-side cavo-cavostomy: a single-center experience. *Transplant Proc* 2009; **41**: 3131-3134 [PMID: 19857694 DOI: 10.1016/j.transproceed.2009.07.092]
  - 148 **Tayar C**, Kluger MD, Laurent A, Cherqui D. Optimizing outflow in piggyback liver transplantation without caval occlusion: the three-vein technique. *Liver Transpl* 2011; **17**: 88-92 [PMID: 21254349 DOI: 10.1002/lt.22201]
  - 149 **Nishida S**, Nakamura N, Vaidya A, Levi DM, Kato T, Nery JR, Madariaga JR, Molina E, Ruiz P, Gyamfi A, Tzakis AG. Piggyback technique in adult orthotopic liver transplantation: an analysis of 1067 liver transplants at a single center. *HPB (Oxford)* 2006; **8**: 182-188 [PMID: 18333273 DOI: 10.1080/13651820500542135]
  - 150 **Mizuno S**, Yokoi H, Yamagiwa K, Tabata M, Isaji S, Yamakado K, Takeda K, Uemoto S. Outflow block secondary to stenosis of the inferior vena cava following living-donor liver transplantation? *Clin Transplant* 2005; **19**: 215-219 [PMID: 15740557]
  - 151 **Yamagiwa K**, Yokoi H, Isaji S, Tabata M, Mizuno S, Hori T, Yamakado K, Uemoto S, Takeda K. Intrahepatic hepatic vein stenosis after living-related liver transplantation treated by insertion of an expandable metallic stent. *Am J Transplant* 2004; **4**: 1006-1009 [PMID: 15147437]
  - 152 **Wang SL**, Sze DY, Busque S, Razavi MK, Kee ST, Frisoli JK, Dake MD. Treatment of hepatic venous outflow obstruction after piggyback liver transplantation. *Radiology* 2005; **236**: 352-359 [PMID: 15955856]
  - 153 **Liu XL**, Li FQ, Li X, Li B, Yan LN, Wei YG. Treatment of hepatic venous outflow stenosis after living donor liver transplantation by insertion of an expandable metallic stent. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 424-427 [PMID: 19666414]
  - 154 **Ikeda O**, Tamura Y, Nakasone Y, Yamashita Y, Okajima H, Asonuma K, Inomata Y. Percutaneous transluminal venoplasty



- after venous pressure measurement in patients with hepatic venous outflow obstruction after living donor liver transplantation. *Jpn J Radiol* 2010; **28**: 520-526 [PMID: 20799017 DOI: 10.1007/s11604-010-0463-8]
- 155 **Lee JM**, Ko GY, Sung KB, Gwon DI, Yoon HK, Lee SG. Long-term efficacy of stent placement for treating inferior vena cava stenosis following liver transplantation. *Liver Transpl* 2010; **16**: 513-519 [PMID: 20213830 DOI: 10.1002/lt.22021]
- 156 **Ferro C**, Andorno E, Guastavino A, Rossi UG, Seitun S, Bovio G, Valente U. Endovascular treatment with primary stenting of inferior cava vein torsion following orthotopic liver transplantation with modified piggyback technique. *Radiol Med* 2014; **119**: 183-188 [PMID: 24356944 DOI: 10.1007/s11547-013-0325-4]
- 157 **Lorenz JM**, van Beek D, Funaki B, Van Ha TG, Zangan S, Navuluri R, Leef JA. Long-term outcomes of percutaneous venoplasty and Gianturco stent placement to treat obstruction of the inferior vena cava complicating liver transplantation. *Cardiovasc Intervent Radiol* 2014; **37**: 114-124 [PMID: 23665862]

**P- Reviewer:** Kambadakone A **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Liu SQ



## Selection of patients with hepatocellular carcinoma for liver transplantation: Past and future

Arturo Soriano, Aranzazu Varona, Rajesh Gianchandani, Modesto Enrique Moneva, Javier Arranz, Antonio Gonzalez, Manuel Barrera

Arturo Soriano, Aranzazu Varona, Rajesh Gianchandani, Modesto Enrique Moneva, Javier Arranz, Antonio Gonzalez, Manuel Barrera, Liver Transplantation Unit, University Hospital Nuestra Señora de Candelaria, Santa Cruz, 38010 Santa Cruz de Tenerife, Spain

**Author contributions:** Soriano A and Barrera M designed the research; Varona A, Moneva ME, Arranz J and Gonzalez A performed the research; Soriano A and Gianchandani R wrote the paper; Soriano A, Varona A, Gianchandani R, Moneva ME, Arranz J, Gonzalez A and Barrera M reviewed the article and provided final approval.

**Conflict-of-interest statement:** The authors declare no conflicts of interest for this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Manuel Barrera, MD, Chief, Liver Transplantation Unit, University Hospital Nuestra Señora de Candelaria, Santa Cruz, Ctra del Rosario n 145, 38010 Santa Cruz de Tenerife, Spain. [mbargom@yahoo.es](mailto:mbargom@yahoo.es)  
 Telephone: +34-92-2602075  
 Fax: +34-92-2602075

Received: April 29, 2015  
 Peer-review started: May 8, 2015  
 First decision: October 21, 2015  
 Revised: November 18, 2015  
 Accepted: December 8, 2015  
 Article in press: December 11, 2015  
 Published online: January 8, 2016

### Abstract

The aim of liver transplantation (LT) for hepatocellular carcinoma (HCC) is to ensure a rate of disease-free survival similar to that of patients transplanted due to benign disease. Therefore, we are forced to adopt strict criteria when selecting candidates for LT and prioritizing patients on the waiting list (WL), to have clarified indications for bridging therapy for groups at risk for progression or recurrence, and to establish certain limits for downstaging therapies. Although the Milan criteria (MC) remain the standard and most employed criteria for indication of HCC patients for LT by far, in the coming years, criteria will be consolidated that take into account not only data regarding the size/volume and number of tumors but also their biology. This criteria will mainly include the alpha fetoprotein (AFP) values and, in view of their wide variability, any of the published logarithmic models for the selection of candidates for LT. Bridging therapy is necessary for HCC patients on the WL who meet the MC and have the possibility of experiencing a delay for LT greater than 6 mo or any of the known risk factors for recurrence. It is difficult to define single AFP values that would indicate bridging therapy (200, 300 or 400 ng/mL); therefore, it is preferable to rely on the criteria of a French AFP model score > 2. Other single indications for bridging therapy include a tumor diameter greater than 3 cm, more than one tumor, and having an AFP slope greater than 15 ng/mL per month or > 50 ng/mL for three months during strict monitoring while on the WL. When considering the inclusion of patients on the WL who do not meet the MC, it is mandatory to determine their eligibility for downstaging therapy prior to inclusion. The upper limit for this therapy could be one lesion up to 8 cm, 2-3 lesions with a total tumor diameter up to 8 cm, or a total tumor volume of 115 cm<sup>3</sup>. Lastly, liver allocation and the prioritization of patients with HCC on

the WL should take into account the recently described HCC model for end-stage liver disease, which considers hepatic function, HCC size and the number and the log of AFP values. This formula has been calibrated with the survival data of non-HCC patients and produces a dynamic and more accurate assessment model.

**Key words:** Hepatocarcinoma; Liver transplantation; Alpha fetoprotein; Patient selection; Prioritization; Waiting list; Bridging therapy; Allocation; Downstaging

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This article aims to provide clinicians who treat patients with hepatocellular carcinoma, in whom liver transplantation may be indicated, with an actualized tool that considers a combination of morphological (size and number of tumors) and biological data (alpha fetoprotein value) and that facilitates the process of selecting candidates, predicts the indication of and response to neoadjuvant therapy prior to transplantation and also aids in the prioritization of patients once they are on the waiting list.

Soriano A, Varona A, Gianchandani R, Moneva ME, Arranz J, Gonzalez A, Barrera M. Selection of patients with hepatocellular carcinoma for liver transplantation: Past and future. *World J Hepatol* 2016; 8(1): 58-68 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i1/58.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i1.58>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is a major global health problem. It is the sixth most common cancer worldwide<sup>[1]</sup> and the third most common cause of cancer death<sup>[2]</sup>. Without treatment, the 5-year survival rate is 10%-12%<sup>[3,4]</sup>. In the early stages, curative treatment includes resection, radiofrequency ablation and liver transplantation (LT). The latter technique remains the most effective treatment method in cases of early HCC because it jointly eliminates the tumor and the underlying disease and shows 1- and 5-year survival rates of 85% and 70%, respectively<sup>[5]</sup>. However, LT does not completely eliminate the possibility of recurrence, which is still a serious problem; therefore, it is discussed in this review.

## DIAGNOSIS

In the last decade, great improvements in HCC diagnosis<sup>[6]</sup> have occurred, which are mainly based on imaging tests. In recent years, HCC has been diagnosed earlier<sup>[7]</sup>, and due to the improvements in imaging tests, a progressive decline in the use of alpha fetoprotein (AFP) levels for the surveillance of HCC in cirrhotic patients<sup>[6,8,9]</sup>

has occurred owing to their lack of appropriate sensitivity and specificity<sup>[8]</sup>.

For lesions less than 1 cm, ultrasonography is repeated every three months, and for lesions larger than 1 cm, a typical image (arterial hypervascularity and venous delayed phase wash out) can be used to confirm the diagnosis<sup>[8]</sup> because this method is 100% specific, with a very high predictive value<sup>[10]</sup>. When a surveillance test is positive, a more definitive noninvasive imaging exam is recommended. Recent guidelines endorse multiphasic computerized tomography (CT) and magnetic resonance imaging (MRI) with hepatobiliary agents as first-line modalities for this purpose. Both modalities provide excellent sensitivity for nodular HCCs larger than 2 cm, modest sensitivity for 1-2-cm HCCs, and poor sensitivity for HCCs smaller than 1 cm. However, MRI is emerging worldwide as a leading method for the diagnosis and staging of HCC, and it is the most sensitive method for the detection of small HCCs<sup>[11]</sup>. However, the combination of dual-phase CT-angiography in the arterial and portal phase with positron emission tomography (PET) imaging using (18)F-fluorodeoxyglucose [(18)FDG] appears to be a sensitive method for the detection of HCC with the alternative presence of hypervascularity or hyperaccumulation of (18)FDG<sup>[12]</sup>.

If the radiological pattern is not typical, the test should be repeated. If the result does not meet the criteria for HCC, a biopsy of the lesion can be performed while taking into account that a negative finding after a biopsy does not exclude HCC<sup>[1]</sup>, and the possible complications of a biopsy such as hemorrhage and needle track tumoral implant should be considered<sup>[13]</sup>. Although in a recent, long retrospective series the incidence of HCC was only 0.2%<sup>[14]</sup>, in a meta-analysis the incidence was 2.7% overall or 0.9% per year<sup>[15]</sup>.

## STAGING

The TNM classification, which is widely accepted for the staging of cancer, for HCC has a lower capacity to predict long-term survival<sup>[16]</sup>. For this reason, the Barcelona Clinic Liver Cancer (BCLC) staging and treatment strategy is most often used<sup>[9,17]</sup> because it includes information concerning the tumor, hepatic function and the general clinical status<sup>[18]</sup>. However, in spite of these facts, the TNM classification is used as the reference for pathological studies of surgical specimens.

## SELECTION OF CANDIDATES WITH HCC FOR LT

The aim of LT for HCC is to obtain a level of disease-free survival (DFS) similar to that of patients who are transplanted for benign disease; therefore, we are obliged to adopt strict selection criteria for candidates, with the intention of obtaining the maximum survival with the minimum possible recurrence.

**Table 1 Isolated biological criteria for the selection of candidates with hepatocellular carcinoma for liver transplantation**

Ref.	Pretransplant AFP levels (ng/mL)	Importance	
Figueras <i>et al</i> <sup>[19]</sup>	> 300	Factor for mortality	
Yao <i>et al</i> <sup>[16]</sup>	> 1,000	Reduced survival	
Bruix <sup>[20]</sup>	> 200	Significant worse outcomes	
Xu <i>et al</i> <sup>[21]</sup>	> 400	Higher tumor recurrence	
Mailey <i>et al</i> <sup>[22]</sup>	Low ( $\leq 20$ )	Medium and high: Higher mortality	
	Medium (20-399)		
	High ( $\geq 400$ )		
Muscari <i>et al</i> <sup>[23]</sup>		DFS	Recurrence
	Normal	71%	4%
	10-150	75%	10%
	150-500	57%	24%
	> 500	46%	62%
Chiao <i>et al</i> <sup>[24]</sup>	> 1,000	Reason for exclusion from the WL	
Hameed <i>et al</i> <sup>[25]</sup>			
Menon <i>et al</i> <sup>[26]</sup>	> 10,000	Reason for exclusion from the WL	

AFP: Alpha fetoprotein; DFS: Disease-free survival; WL: Waiting list.

### Isolated biological criteria for the selection and prognosis of patients with HCC for LT

More than a decade ago, several authors noted the importance of the isolated AFP value in predicting mortality and/or posttransplant recurrence. High AFP values may be a marker for vascular invasion or extra hepatic disease that has escaped detection by conventional imaging techniques. It has been observed that a pretransplant AFP level higher than 300 ng/mL is the only factor independently associated with mortality after LT<sup>[19]</sup>, and a level higher than 1000 ng/mL is a significant predictor of reduced survival<sup>[16]</sup>. In general, HCC patients on the waiting list (WL) with a baseline serum level of AFP > 200 ng/mL display significantly worse outcomes<sup>[20]</sup>; however, several detrimental cut-off values for AFP levels have been reported recently. Xu *et al*<sup>[21]</sup> found that pre-transplant AFP levels > 400 ng/mL were associated with higher tumor recurrence. Mailey *et al*<sup>[22]</sup> classified patients into low ( $\leq 20$  ng/mL), medium (20-399 ng/mL), or high ( $\geq 400$  ng/mL) AFP level groups. In a multivariate analysis, the medium and high AFP groups were associated with higher mortality. Another study<sup>[23]</sup> correlated the DFS and 5-year recurrence rate to the AFP level. Normal AFP values between 10-150 ng/mL, those from 150-500 ng/mL and those > 500 reduce DFS from 71% to 57%, 46% and 28%, respectively, and increase the recurrence rate from 4% to 10%, 24% and 62%, respectively. Recently, it was shown once again that an AFP level > 1000 ng/mL is a reason for exclusion from the WL<sup>[24,25]</sup>, confirming data reported in 2001<sup>[16]</sup>. However these data have not been taken into account by programs using expanded criteria that only consider an AFP level greater than 10000 ng/mL as a reason for exclusion<sup>[26]</sup>. This matter will be further examined when discussing the indications for downstaging of HCC prior to LT (Table 1).

In Japan, des-gamma carboxy prothrombin (DCP) is well established as a biomarker and is reported to

**Table 2 Selection criteria base on radiological/morphological tumor characteristics**

Ref.	Parameters	Importance
Bismuth <i>et al</i> <sup>[30]</sup>	Up to 3 nodules Each < 3 cm	Best results
Mazzaferro <i>et al</i> <sup>[31]</sup>	Single lesion < 5 cm	DFS > 75%
	< 3 lesions, each < 3 cm	Recurrence < 15%
	No macrovascular invasion	
	No extrahepatic disease	
Löhe <i>et al</i> <sup>[34]</sup>	Single tumor with size > 5 cm	Reduction in DFS
Yao <i>et al</i> <sup>[16]</sup>	Single lesion $\leq 6$ cm	DFS > 75%
	2-3 lesions each $\leq 4.5$ cm	Recurrence < 15%
	Total tumor diameter $\leq 8$ cm	
Mazzaferro <sup>[41]</sup>	Ordinates: <i>n</i> of tumors	Progressive reduction of 5 yr survival
	Abscissas: Tumor size	
Mazzaferro <i>et al</i> <sup>[42]</sup>	Up to 7, as the sum of: Largest tumor in centimeter and <i>n</i> of tumors	71.2% 5 yr survival
Jang <i>et al</i> <sup>[46]</sup>	10 as the sum of: Largest tumor in cm and <i>n</i> of tumors	If >: Decreased DFS

DFS: Disease-free survival.

correlate with post-LT recurrence of HCC<sup>[27,28]</sup>. We cannot predict whether new molecular markers of HCC such as PIVKA-II, a protein induced by the absence of Vit K, will have widespread use, but Japanese studies suggest that it is correlated with microvascular invasion<sup>[29]</sup>.

### Selection criteria based on radiological/morphologic tumor characteristics

Some criteria include the number and size of the tumors and the tumor volume.

**Criteria based on number and size:** In 1993, Bismuth *et al*<sup>[30]</sup> noted that patients transplanted for HCC with up to 3 nodules (each < 3 cm) exhibited the best results. In 1996, the Milan criteria (MC)<sup>[31]</sup> set clear limits on the selection of HCC patients for LT, consisting of a single lesion < 5 cm or fewer than three lesions, each < 3 cm and without macrovascular invasion or extrahepatic disease, which resulted in 5-year DFS > 75% and a recurrence rate < 15%<sup>[31]</sup>. Since that time, these standard selection criteria for LT due to HCC have been accepted worldwide<sup>[20,32,33]</sup>. Other authors have confirmed that a single tumor with a size > 5 cm causes a reduction in DFS<sup>[34]</sup>. The MC have received criticism because the radiological studies used for evaluations are not very accurate<sup>[35]</sup> and highly variable between centers. In addition, some authors have argued that these criteria are strict<sup>[20]</sup>, with tumor size and tumor number cut-offs that are somewhat arbitrary and too restrictive, and that they deprive patients of the possible benefit of LT<sup>[36]</sup> and therefore should be extended<sup>[16,37,38]</sup> (Table 2).

Thus, in 2001 the so-called expanded criteria of the University of San Francisco, California (UCSF) were proposed by Yao *et al*<sup>[16]</sup>, which set the limit for LT to a single lesion  $\leq 6.5$  cm in diameter or 2-3 lesions each  $\leq 4.5$  cm with a total maximum diameter  $\leq$



**Table 3 Selection criteria based on functional/radiological features of the tumor**

Ref.	Parameters	Importance
Hiraoka <i>et al</i> <sup>[56]</sup>	Hyperintensity on gadoteric acid-enhanced MRI	HCC with more malignant potential
Ferda <i>et al</i> <sup>[12]</sup>	Hipervascularity or hiperaccumulation of (18)FDG/PET/ with Dual-phase CT angiography (arterial/portal phase)	Distinguishing between welland Poorly differentiated HCC
Ochi <i>et al</i> <sup>[57]</sup>	High positivity in (18)FDG/PET/CT	Increase the risk of early recurrence
Kornberg <i>et al</i> <sup>[58]</sup>	mSUV	Reflects the existence of distant microsatellite
Kornberg <sup>[59]</sup>	Positivity in (18)FDG/PET/CT	Statistically significant lower survival post LT

CT: Computerized tomography; MRI: Magnetic resonance imaging; HCC: Hepatocellular carcinoma; PET: Positron emission tomography; LT: Liver transplantation; mSUV: Maximun standized uptake value; (18)FDG: (18)F-fluorodeoxyglucose.

8 cm, thus obtaining similar survival after LT to that obtained with the MC. These criteria were criticized because in this study, only 24% of the patients did not meet the MC<sup>[39]</sup>, and because it was a retrospective study based on the histology of explants<sup>[40]</sup>. By that time, Mazzaferro<sup>[41]</sup> had introduced the concept of the Metroticket calculator, a system of orderly Cartesian ordinates (number of tumors) and abscissa (tumor size) in which the progressive reduction of 5-year survival is graphically represented as these parameters increase, leading to the expression “the longer the trip, the higher the price”. In 2009, Mazzaferro *et al*<sup>[42]</sup> found that a total tumor diameter greater than 7 cm resulted in an increase in the percentage of recurrence and proposed a new MC (the so-called up-to-seven), using seven as the sum of the size of the largest tumor (in centimeter) and the number of tumors, which yielded 5-year overall survival of 71.2%. Many groups have validated these criteria<sup>[43,44]</sup>, but after 5 years, they have not been accepted as widely as the MC. Other authors have made similar suggestions<sup>[45]</sup>, however, others have placed this limit at 10 cm, which results in a decrease in DFS<sup>[46]</sup>. This value should be universally accepted as the upper limit<sup>[26]</sup>. The expanded criteria require further validation because recurrence could be less often reported, increasing the risk of vascular invasion, microsatellites and poorly differentiated tumors<sup>[35,47,48]</sup>.

**Morphological criteria based on the total tumor volume:** Toso *et al*<sup>[37]</sup> calculated the total tumor volume (TTV) as the sum of the volumes of all tumors using the formula  $(4/3) \pi r^3$ , where  $r$  is the maximum radius of each tumor. The radiological accuracy of this formula was greater, and based on the risk of recurrence, a threshold of 115 cm<sup>3</sup> was established, which allowed the selection of more patients for LT with results similar to those of the MC and UCSF criteria<sup>[37]</sup>. According to this mathematical formula, the largest tumor has the maximum importance. As a result, the possibility of correct staging increases because larger tumors are evaluated more accurately than smaller ones.

Expansion of the MC may be justified in regions with less organ shortage, but this will require demonstrating high survival rates for the newly eligible patients<sup>[49]</sup>. Regional variation in survival does not facilitate a national policy<sup>[50]</sup>, but it is undeniable that in the USA, 97% of patients transplanted for HCC meet the MC<sup>[51]</sup>,

and although this number has changed somewhat recently, the number of inclusions for patients for LT that do not meet the MC is still less than 5%<sup>[52]</sup>. It should be mentioned that until very recently, the criteria used in the United Kingdom for LT for HCC considered a maximum tumor diameter up to 15 cm (up to 5 tumors all  $\leq 3$  cm), which is well beyond the limit of the MC and UCSF criteria<sup>[26]</sup>.

### Selection criteria based on functional/radiological features of the tumor:

Dynamic MRI may constitute a non-invasive and promising method to assess the biology of HCC due to its greater avidity of contrast uptake, which implies a higher degree of microscopic vascular invasion and greater aggressiveness<sup>[53,54]</sup>. Tumors that are heterogeneously hyperintense in the hepatobiliary phase on gadoteric acid-enhanced MRI have more malignant potential than other types of HCC<sup>[55]</sup>. Other authors<sup>[56]</sup> have used 18F-fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) not only for detection<sup>[12]</sup> but also as a prognostic factor, which distinguishes between well and poorly differentiated HCC<sup>[12]</sup>. High positivity of HCC increases the risk of early recurrence after curative resection<sup>[56]</sup>, and the maximum standardized uptake value (mSUV) of 18F-FDG PET/CT reflects the existence of distant microsatellites; therefore, it can be a useful tool in the treatment protocol of HCC<sup>[57]</sup>. In a comparison of two groups of transplanted patients who did not meet the MC, other authors<sup>[58]</sup> found that patients with positive PET findings had significantly lower survival than PET negative patients (Table 3).

### Combined morphological and biological tumor parameters:

Adequate patient selection should be based on tumor biology assessed *via* serum or pathological parameters rather than on the macro morphology of HCC<sup>[59]</sup>. In fact, the aggressiveness of a tumor can be determined by a higher histological grade and greater microscopic vascular invasion, and a biopsy can be used to predict DFS. The Toronto criteria<sup>[60]</sup> select patients with HCC for LT who do not meet the MC by biopsy exclusion of poorly differentiated tumors, resulting in 5-year overall survival (OS) and DFS values of 70% and 66%, respectively, which are similar to those of the MC (72% and 70%, respectively). However, there is little correlation between the biopsy and

**Table 4 Combined morphological/biological selection criteria**

Ref.	Parameters	Importance
DuBay <i>et al</i> <sup>[60]</sup>	Liver tumor biopsy	Excluding poorly differentiated tumors
Toso <i>et al</i> <sup>[52]</sup>	TTV > 115 cm <sup>3</sup>	Reduced survival at 3 yr (< 50%)
	AFP > 400 ng/mL	Limit for indication for LT
Lai <i>et al</i> <sup>[62]</sup>	AFP > 400 ng/mL	Strongest predictor for recurrence
	Total tumor diameter > 8 cm	
Duvoux <i>et al</i> <sup>[63]</sup>	Model combining log10 AFP, tumor size and <i>n</i> of tumors: Score > or < 2	Score greater than 2 predict a marked increase in 5 yr risk of recurrence and decreased survival
Berry <i>et al</i> <sup>[66]</sup>	AFP < 15 or > 15 ng/mL	AFP levels predicts post-transplant survival independently of MC

TTV: Total tumor volume; AFP: Alpha fetoprotein; MC: Milan criteria; LT: Liver transplantation.

histology of an explant due to tumor heterogeneity and because, in multifocal disease, the dominant lesion is not always the most biologically representative. For these reasons, currently, the biopsy has a limited role in pre-LT staging<sup>[61]</sup> (Table 4).

In 2009, Toso *et al*<sup>[52]</sup> found that only the TTV and AFP levels predicted survival and established a composite score with a TTV > 115 cm<sup>3</sup> or AFP > 400 ng/mL as limits for indication for transplantation because patients with greater values for these parameters had 3-year survival rates < 50%.

Using a multivariate analysis, Lai *et al*<sup>[62]</sup> found that an AFP level > 400 ng/mL and a total tumor diameter > 8 cm were the strongest predictors for recurrence.

Recently, Duvoux *et al*<sup>[63]</sup> generated an improved prognostic model for predicting recurrence in LT candidates with HCC. A prognostic score was developed and validated prospectively. The AFP level independently predicted tumor recurrence and was correlated with vascular invasion and differentiation. A model combining the log10 value of the AFP, tumor size and number of tumors was highly predictive of tumor recurrence and death. Using a simplified version of the model with untransformed AFP values, a cut-off value of 2 was identified. In the validation cohort, a score greater than 2 predicted a marked increase in 5-year risk of recurrence and decreased survival. Among patients who exceeded the MC, a score of 2 or lower identified a subgroup of patients with AFP levels less than 100 ng/mL and a low 5-year risk of recurrence. In contrast, for patients who met the MC, a score greater than 2 identified a subgroup of patients with AFP levels greater than 1000 ng/mL and a high risk of recurrence. We will refer to this as the French model.

Our group<sup>[64]</sup>, based on our previous experience with LT for patients with HCC and cirrhosis, has performed an analysis of the risk factors for HCC relapse and applied the French AFP model to LT for HCC and cirrhosis patients who met the MC<sup>[65]</sup>. We were able to confirm the predictive value for tumor relapse of the French AFP model both pre- and postoperatively.

Berry *et al*<sup>[66]</sup> established that the AFP level, rather than the tumor burden, was most strongly associated with posttransplant survival. Thus, patients with HCC and AFP levels < 15 ng/mL at the time of transplantation

did not exhibit excess posttransplant mortality; increases in AFP (16-65 ng/mL; 66-320 ng/mL and > 320 ng/mL) result in progressively worse posttransplant mortality than similar increases in recipients without HCC. Patients who did not meet the MC showed excellent survival if their AFP level was < 15 ng/mL. In contrast, patients who met the MC exhibited poor survival if their serum AFP level was substantially elevated (serum AFP ≥ 66 ng/mL). AFP changes while on the WL closely corresponded to changes in posttransplant mortality. Not only the absolute serum AFP level but also changes in this level strongly predicted posttransplant survival independently of tumor burden.

These models, combining data related to the tumor (size and number of tumors) with preoperative levels of AFP, had previously been studied by Japanese authors<sup>[67]</sup> in living-donor liver transplant (LDLT) patients (Table 5). In these models, a value of 1 to 4 points (p) was assigned to each of the following parameters: tumor size: ≤ 3 cm (1 p), 3.1-5 cm (2 p), 5.1-6.5 cm (3 p), > 6.5 cm (4 p); number of tumors: 1 (1 p), 2-3 (2 p), 4-5 (3 p), > 5-6 nodules (4 p); AFP: ≤ 20 ng/mL (1 p), 20.1-200 ng/mL (2 p), 200.1-1000 ng/mL (3 p), and > 1000 ng/mL (4 p). Candidates with 3-6 total points were "transplantable" and those with 7-12 points were "non-transplantable". In Japan and other Asian countries, due to the severe organ shortage, LDLT comprises the majority of LT<sup>[68]</sup>. Each center has developed and proposed expanded selection criteria based on institutional and regional experience, which vary from the model of Tokyo University<sup>[68]</sup>, which only considers morphological tumor parameters, *i.e.*, up to 5 nodules with a maximum diameter ≤ 5 cm, without taking into account any biological markers. The Kyoto group<sup>[69]</sup> considers patients with less than 10 nodules, all less than 5 cm, with a DCP level < 400 mAU/mL, and the Kyushu group<sup>[70]</sup> also use extended criteria without limiting the number of nodules but require a maximum tumor diameter less than 5 cm and DCP levels under 300 mAU/mL.

## ORGAN ALLOCATION FOR LT

The allocation of organs for LT follows criteria of prioritization that have varied throughout the history

**Table 5** Japanese combined morphological/biological selection criteria for living-donor liver transplant

Ref.	Parameters					Importance: Limits for LDLT
	Value	1p	2p	3p	4p	
Yang <i>et al</i> <sup>[67]</sup>	T size (cm)	≤ 3	3.1-5	5.1-6.5	> 6.5	Patients with 3-6 points are transplantable Those with 7-12 points are not transplantable
	<i>n</i> of tumors	1	2-3	4-5	> 5 or 6	
	AFP (ng/mL)	< 20	20-200	200.1-1.000	< 1.000	
Akamatsu <i>et al</i> <sup>[68]</sup>	Up to 5 nodules					Upper limit for LDLT
Kaido <i>et al</i> <sup>[69]</sup>	Maximum diameter ≤ 5					Upper limit for LDLT
	Less that 10 nodules, all < 5 cm					
	DCP < 400 mAu/mL					
Shirabe <i>et al</i> <sup>[70]</sup>	<i>n</i> of nodules: No limit					Upper limit for LDLT
	Maximun diameter: < 5 cm					
	DCP < 300 mAu/mL					

AFP: Alpha fetoprotein; DCP: Des-gamma carboxy prothrombin; LDLT: Living-donor liver transplant.

of LT, from prioritization of the more serious patients based on the Child-Turcotte-Pugh score and the time of inclusion on the WL to the more recent model for end-stage liver disease (MELD) score. However, because this method does not consider the risk of neoplastic growth while on the WL, HCC patients are prioritized based on their exception points and the MELD exception, with the goal of obtaining similar WL mortality for neoplastic and non-neoplastic patients. Exception points are assigned every 3 mo<sup>[36]</sup> because progression of HCC can produce a 15% increase in mortality<sup>[71]</sup>. Paradoxically, several years later, it was found that the likelihood of undergoing transplantation was higher for HCC candidates than for other patients<sup>[72]</sup>, which produced a clear disadvantage for non-HCC patients<sup>[73]</sup>. For this reason, the "HCC-MELD" equation ( $1.27/\text{MELD} - 0.51/\log\text{AFP} + 4.59$ ) has been proposed<sup>[74]</sup>, which takes into account hepatic function and the log of the AFP value, and has been calibrated to the survival of non-HCC patients. This formula gives additional points to patients with HCC, not arbitrarily, but based on a calculation of the benefits of transplantation, in a manner similar to that for patients without HCC. Other authors<sup>[73]</sup>, with a similar aim, have studied and validated a new and promising model for allocation of patients using a large cohort in the United States and United Kingdom that includes: HCC size, HCC number, AFP value, and the classic MELD score calculated according to the following formula:  $\text{New MELD} = -37.8 + 1.9 \times \text{MELD} + 5.9$  (if HCC number  $\geq 2$ ) + 5.9 (if AFP level > 400 ng/mL) + 21.2 (if HCC size > 1 cm). This new model provides a dynamic and more accurate assessment of dropout than the use of the MELD exception, showing a distribution similar to that of the MELD for non-HCC patients. Both scores could be used in parallel for the management of WL patients with and without HCC.

## NEOADJUVANT TREATMENT OF PATIENTS ON THE WL (BRIDGING AND DOWNSTAGING TREATMENTS)

HCC patients who meet the MC and are included on the

WL should be monitored every 3 mo by CT/MRI and AFP level evaluation for the identification of those at high risk of dropout<sup>[75]</sup>. AFP progression while on the WL<sup>[66]</sup>, and more specifically an AFP increase of > 15 ng/mL per month, is the most relevant preoperative prognostic factor for low OS and DFS<sup>[76]</sup>. For patients with changes in tumor size and/or an increase of in the AFP level > 50 ng/mL, locoregional therapy (LRT) or removal of the patient from the WL should be performed, if necessary<sup>[77]</sup>.

### Bridging therapy

Bridging therapy is used for patients with HCC who meet the MC and are included on the WL but have the possibility of a delay in LT > 6 mo. Its purpose<sup>[78]</sup> is to prevent tumor progression<sup>[79]</sup>, reduce the recurrence of HCC after LT and increase posttransplant survival. As the waiting time for LT has progressively increased<sup>[79]</sup>, treatment of HCC in patients awaiting LT has become routine<sup>[80]</sup>. Bridging is not indicated for tumors that meet the current MC, except for those with a diameter greater than 3 cm or patients with more than 1 tumor, because these patients are more likely to have recurrence after LT<sup>[81]</sup>.

The most employed method of LRT for bridging therapy is percutaneous ablation<sup>[1]</sup>, which is frequently performed by radiofrequency (RF) and less often performed by ethanolization (ET) or surgery. ET and RF have similar effectiveness for tumors less than 2 cm, but with increased tumor size, RF is more effective and shows similar results to surgery. In lesions > 3 cm, ET failures increase; therefore, it is rarely used as bridging therapy<sup>[82,83]</sup>.

Patients with small solitary tumors and very well preserved liver function are the best candidates for surgical resection<sup>[1]</sup>, but tumor recurrence complicates 70% of cases at 5 years<sup>[6]</sup>. Certain favorable locations, such as peripheral tumors and left hepatic lobe location, may allow laparoscopic resection, which avoids the greater complexity of transplantation after laparotomic surgery. Resection may offer improved local tumor control and allows full microscopic analysis, with subsequent study of its biological aggressiveness, which

could lead to subsequent elective LT. Subsequent tumor recurrence after resection is an absolute indication for LT; this so-called salvage transplantation was first described by Majno *et al.*<sup>[84]</sup> in 2000. This procedure requires fewer donors and allows better management of the WL.

### Downstaging

Downstaging<sup>[78,79]</sup> is used to convert tumors that initially do not meet the transplant criteria, usually intermediate multinodular asymptomatic tumors (stage B of the BCLC)<sup>[6]</sup>, into tumors that meet the MC (the most frequent endpoint), UCSF criteria or the up-to-seven criteria, with the aim of including the patients on the WL once the tumor has decreased in size. Tumors with more favorable histology are more likely to respond to treatment and exhibit a good outcome after LT<sup>[85]</sup>. The eligibility criteria for downstaging should have an upper limit, which can be set as follows<sup>[85]</sup>: (1) one lesion > 5 cm and up to 8 cm; (2) two to three lesions with at least one lesion > 3 cm and not exceeding 5 cm, with a total tumor diameter up to 8 cm; or (3) four to five lesions with none > 3 cm, and a total tumor diameter up to 8 cm.

The LRT technique depends on each center, and the response is evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST) or the modified RECIST (mRECIST)<sup>[86]</sup>, which we will further discuss later. Once the treatment is completed, it is mandatory to follow the "ablate and wait policy"<sup>[81]</sup>, with close monitoring for at least 3 mo before inclusion on the WL<sup>[50,85]</sup> to evaluate the tumor's behavior and exclude aggressive tumors from LT; therefore, a total of six months will elapse until transplantation<sup>[81]</sup>.

Some authors<sup>[87]</sup> have attempted to perform a meta-analysis of HCC downstaging, which has been impossible due to many factors such as the great variability of the inclusion criteria protocols<sup>[79]</sup>, variability of post-treatment response assessment and absence of histological information on tumor biology<sup>[87]</sup>. At the moment, there is no evidence that patients submitted to downstaging followed by LT have a worse prognosis than those who initially meet the MC. Therefore, we must assume that those patients should be eligible for LT, as if they had been from the start<sup>[87]</sup>, and will show an excellent posttransplantation outcome<sup>[85]</sup>, reaching 5-year survival rates comparable to those of patients who meet the MC or UCSF criteria and do not require downstaging<sup>[75,88]</sup>.

Trans-arterial chemoembolization (TACE) is the form of LRT most often used for downstaging<sup>[75]</sup>, followed by RF ablation<sup>[89]</sup>. Chemoembolization improves the survival of stringently selected patients with unresectable HCC<sup>[90]</sup>. Posttransplant survival has shown a marked benefit in response to TACE, but this benefit was only seen in patients whose disease meets, but does not exceed, the MC<sup>[91]</sup>. TACE can reduce the percentage of posttransplant recurrence (17% with treatment vs 36% without treatment)<sup>[92]</sup>, and it is possible to verify its effectiveness using (18)FDG PET/CT to compare the

SUV before and after treatment<sup>[93]</sup>.

At the present time, there is no evidence demonstrating the superiority of one form of LRT over another, but merging the techniques of drug eluting beads-TACE and trans-arterial radio-embolization with Yttrium-90 and external beam conformal radiotherapy<sup>[78]</sup> is generally better tolerated than conventional techniques.

### Response criteria following downstaging with LRT

The efficacy of neo-adjuvant treatments should be evaluated<sup>[79]</sup> by the rate of dropout from the WL and, methodologically, with a 3-mo interval mRECIST<sup>[86]</sup> reassessment that considers not only the reduction in size, but the amount of tumor necrosis and the disappearance of any intratumoral arterial enhancement in conjunction with the initial and post-treatment AFP levels.

Patients presenting with an AFP level > 1000 ng/mL submitted to downstaging are a special problem because such high levels predict a greater risk of tumor recurrence and are considered the only factor in treatment failure<sup>[85]</sup>.

In these cases, a stable decrease in the AFP level to < 500 ng/mL is necessary in subsequent determinations until LT to consider the downstaging effective<sup>[50,94]</sup>. However, other authors<sup>[48]</sup> state that the level should be < 400 ng/mL because levels > 400 ng/mL in the immediate pretransplant period are a unique risk factor for recurrence after LRT<sup>[36]</sup>. This is because patients who did not show a reduction of the AFP level to ≤ 400 after downstaging had less intent-to-treat survival, and only the last pretransplant AFP value, not the original value (even if it was originally > 1000 ng/mL) or changes in the AFP level, independently predicted posttransplant survival<sup>[95]</sup>. Others have set the level to 100 ng/mL<sup>[96]</sup>, but in general, the mean AFP levels are higher in patients who do not achieve successful downstaging<sup>[97]</sup>. AFP levels are considered to play an important role in monitoring the response and/or tumor progression after LRT<sup>[25,98]</sup>.

Combined radiological and biological modifications permit documentation of the response to LRT in patients waiting for LT and are essential elements for further refining the selection criteria for potential liver recipients with HCC<sup>[94]</sup>. An AFP level ≥ 100 ng/mL, a maximum tumor size ≥ 7 cm and a lack of complete necrosis at LT after TACE were found to be independent predictors of HCC recurrence<sup>[46]</sup>. However, patients with maximum tumor size < 7 cm who achieve complete necrosis together with AFP levels < 100 ng/mL at LT may be the best candidates for LT following downstaging<sup>[46]</sup>.

In addition, an AFP slope > 15 ng/mL per month and mRECIST progression are unique independent risk factors for HCC recurrence and patient death regardless of whether the patient meets the MC<sup>[94]</sup>.

## CONCLUSION

Although the MC remain by far the standard and the most employed inclusion criteria for LT for HCC, in the



coming years, criteria will be consolidated that take into account not only data regarding the size/volume and number of tumors but also their biology, including AFP value and some of its published logarithmic models. Additionally, the AFP value will be considered in the allocation and prioritization of patients in the WL with the aforementioned new reform of the MELD-HCC system. Furthermore, the number of tumors, their volume and AFP levels will be important determinants for bridging and downstaging therapy and to evaluate the patient response. AFP values > 1000 ng/mL must be considered a sign of a bad prognosis and a questionable indication for LT unless the value can be reduced to < 400 ng/mL. Organ scarcity and the probability of recurrence following LT for HCC necessitate that all of these facts should be taken into account.

## REFERENCES

- Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]
- Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]
- Llovet JM, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, Brú C, Rodés J, Bruix J. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. *Hepatology* 1999; **29**: 62-67 [PMID: 9862851 DOI: 10.1002/hep.510290145]
- Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ. Cancer statistics, 2006. *CA Cancer J Clin* 2006; **56**: 106-130 [PMID: 16514137 DOI: 10.3322/canjclin.56.2.106]
- Organ Procurement and Transplantation Network and Scientific Registry of Transplant Recipients 2010 data report. *Am J Transplant* 2012; **12** Suppl 1: 1-156 [PMID: 22107249 DOI: 10.1111/j.1600-6143.2011.03886.x]
- European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]
- Sherman M. Epidemiology of hepatocellular carcinoma. *Oncology* 2010; **78** Suppl 1: 7-10 [PMID: 20616577 DOI: 10.1159/000315223]
- Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
- Bruix J, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 2014; **63**: 844-855 [PMID: 24531850 DOI: 10.1136/gutjnl-2013-306627]
- Sangiovanni A, Manini MA, Iavarone M, Romeo R, Forzenigo LV, Fraquelli M, Massironi S, Della Corte C, Ronchi G, Rumi MG, Biondetti P, Colombo M. The diagnostic and economic impact of contrast imaging techniques in the diagnosis of small hepatocellular carcinoma in cirrhosis. *Gut* 2010; **59**: 638-644 [PMID: 19951909 DOI: 10.1136/gut.2009.187286]
- Choi JY, Lee JM, Sirlin CB. CT and MR imaging diagnosis and staging of hepatocellular carcinoma: part II. Extracellular agents, hepatobiliary agents, and ancillary imaging features. *Radiology* 2014; **273**: 30-50 [PMID: 25247563 DOI: 10.1148/radiol.14132362]
- Ferda J, Ferdová E, Baxa J, Kreuzberg B, Daum O, Třeška V, Skalický T. The role of 18F-FDG accumulation and arterial enhancement as biomarkers in the assessment of typing, grading and staging of hepatocellular carcinoma using 18F-FDG-PET/CT with integrated dual-phase CT angiography. *Anticancer Res* 2015; **35**: 2241-2246 [PMID: 25862885]
- Takamori R, Wong LL, Dang C, Wong L. Needle-tract implantation from hepatocellular cancer: is needle biopsy of the liver always necessary? *Liver Transpl* 2000; **6**: 67-72 [PMID: 10648580 DOI: 10.1002/lt.500060103]
- Wang P, Meng ZQ, Chen Z, Lin JH, Ping B, Wang LF, Wang BH, Liu LM. Diagnostic value and complications of fine needle aspiration for primary liver cancer and its influence on the treatment outcome-a study based on 3011 patients in China. *Eur J Surg Oncol* 2008; **34**: 541-546 [PMID: 17764885 DOI: 10.1016/j.ejso.2007.07.013]
- Silva MA, Hegab B, Hyde C, Guo B, Buckels JA, Mirza DF. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. *Gut* 2008; **57**: 1592-1596 [PMID: 18669577 DOI: 10.1136/gut.2008.149062]
- Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403 [PMID: 11391528 DOI: 10.1053/jhep.2001.24563]
- Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-338 [PMID: 10518312 DOI: 10.1055/s-2007-1007122]
- Saraswat VA, Pandey G, Shetty S. Treatment algorithms for managing hepatocellular carcinoma. *J Clin Exp Hepatol* 2014; **4**: S80-S89 [PMID: 25755616 DOI: 10.1016/j.jceh.2014.05.004]
- Figueras J, Ibañez L, Ramos E, Jaurrieta E, Ortiz-de-Urbina J, Pardo F, Mir J, Loinaz C, Herrera L, López-Cillero P, Santoyo J. Selection criteria for liver transplantation in early-stage hepatocellular carcinoma with cirrhosis: results of a multicenter study. *Liver Transpl* 2001; **7**: 877-883 [PMID: 11679986 DOI: 10.1053/jlts.2001.27856]
- Bruix J. [Usefulness of the molecular profile in the diagnosis, prognosis and treatment of hepatocellular carcinoma]. *Gastroenterol Hepatol* 2014; **37** Suppl 2: 81-89 [PMID: 25087717 DOI: 10.1016/S0210-5705(14)70074-3]
- Xu X, Ke QH, Shao ZX, Wu J, Chen J, Zhou L, Zheng SS. The value of serum alpha-fetoprotein in predicting tumor recurrence after liver transplantation for hepatocellular carcinoma. *Dig Dis Sci* 2009; **54**: 385-388 [PMID: 18563566 DOI: 10.1007/s10620-008-0349-0]
- Mailey B, Artinyan A, Khalili J, Denitz J, Sanchez-Luege N, Sun CL, Bhatia S, Nissen N, Colquhoun SD, Kim J. Evaluation of absolute serum  $\alpha$ -fetoprotein levels in liver transplant for hepatocellular cancer. *Arch Surg* 2011; **146**: 26-33 [PMID: 21242442 DOI: 10.1001/archsurg.2010.295]
- Muscari F, Guinard JP, Kamar N, Peron JM, Otal P, Suc B. Impact of preoperative  $\alpha$ -fetoprotein level on disease-free survival after liver transplantation for hepatocellular carcinoma. *World J Surg* 2012; **36**: 1824-1831 [PMID: 22532309 DOI: 10.1007/s00268-012-1587-z]
- Chiao H, Yang CH, Frenette CT. Review on liver transplant for hepatocellular carcinoma. *Transl Cancer Res* 2013; **2**: 472-481
- Hameed B, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level > 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. *Liver Transpl* 2014; **20**: 945-951 [PMID: 24797281 DOI: 10.1002/lt.23904]
- Menon KV, Hakeem AR, Heaton ND. Review article: liver transplantation for hepatocellular carcinoma - a critical appraisal of the current worldwide listing criteria. *Aliment Pharmacol Ther* 2014; **40**: 893-902 [PMID: 25155143 DOI: 10.1111/apt.12922]
- Shirabe K, Itoh S, Yoshizumi T, Soejima Y, Taketomi A, Aishima S, Maehara Y. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma-with special reference to the serum levels of des-gamma-carboxy prothrombin. *J Surg Oncol* 2007; **95**: 235-240 [PMID: 17323337 DOI: 10.1002/jso.20655]
- Fujiki M, Takada Y, Ogura Y, Oike F, Kaido T, Teramukai S, Uemoto S. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for

- hepatocellular carcinoma. *Am J Transplant* 2009; **9**: 2362-2371 [PMID: 19656125 DOI: 10.1111/j.1600-6143.2009.02783.x]
- 29 **Kim HS**, Park JW, Jang JS, Kim HJ, Shin WG, Kim KH, Lee JH, Kim HY, Jang MK. Prognostic values of alpha-fetoprotein and protein induced by vitamin K absence or antagonist-II in hepatitis B virus-related hepatocellular carcinoma: a prospective study. *J Clin Gastroenterol* 2009; **43**: 482-488 [PMID: 19197197 DOI: 10.1097/MCG.0b013e318182015a]
- 30 **Bismuth H**, Chiche L, Adam R, Castaing D, Diamond T, Dennison A. Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. *Ann Surg* 1993; **218**: 145-151 [PMID: 8393649]
- 31 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]
- 32 **Belghiti J**, Durand F. Criteria for liver transplantation for hepatocellular carcinoma: what is an acceptable outcome? *Liver Int* 2011; **31** Suppl 1: 161-163 [PMID: 21205155 DOI: 10.1111/j.1478-3231.2010.02413.x]
- 33 **Washburn K**, Halff G. Hepatocellular carcinoma and liver transplantation. *Curr Opin Organ Transplant* 2011; **16**: 297-300 [PMID: 21505342 DOI: 10.1097/MOT.0b013e3283465756]
- 34 **Löhe F**, Angele MK, Gerbes AL, Löhns U, Jauch KW, Schauer RJ. Tumour size is an important predictor for the outcome after liver transplantation for hepatocellular carcinoma. *Eur J Surg Oncol* 2005; **31**: 994-999 [PMID: 16076546 DOI: 10.1016/j.ejso.2005.06.003]
- 35 **Freeman RB**, Mithoefer A, Ruthazer R, Nguyen K, Schore A, Harper A, Edwards E. Optimizing staging for hepatocellular carcinoma before liver transplantation: A retrospective analysis of the UNOS/OPTN database. *Liver Transpl* 2006; **12**: 1504-1511 [PMID: 16952174 DOI: 10.1002/lt.20847]
- 36 **Ciccarelli O**, Lai Q, Goffette P, Finet P, De Reyck C, Roggen F, Sempoux C, Doffagne E, Reding R, Lerut J. Liver transplantation for hepatocellular cancer: UCL experience in 137 adult cirrhotic patients. Alpha-fetoprotein level and locoregional treatment as refined selection criteria. *Transpl Int* 2012; **25**: 867-875 [PMID: 22716073 DOI: 10.1111/j.1432-2277.2012.01512.x]
- 37 **Toso C**, Trotter J, Wei A, Bigam DL, Shah S, Lancaster J, Grant DR, Greig PD, Shapiro AM, Kneteman NM. Total tumor volume predicts risk of recurrence following liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl* 2008; **14**: 1107-1115 [PMID: 18668667 DOI: 10.1002/lt.21484]
- 38 **Silva MF**, Sherman M. Criteria for liver transplantation for HCC: what should the limits be? *J Hepatol* 2011; **55**: 1137-1147 [PMID: 21718672 DOI: 10.1016/j.jhep.2011.05.012]
- 39 **Duffy JP**, Vardanian A, Benjamin E, Watson M, Farmer DG, Ghobrial RM, Lipshutz G, Yersiz H, Lu DS, Lassman C, Tong MJ, Hiatt JR, Busuttil RW. Liver transplantation criteria for hepatocellular carcinoma should be expanded: a 22-year experience with 467 patients at UCLA. *Ann Surg* 2007; **246**: 502-509; discussion 509-511 [PMID: 17717454 DOI: 10.1097/SLA.0b013e318148c704]
- 40 **Yao FY**, Xiao L, Bass NM, Kerlan R, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. *Am J Transplant* 2007; **7**: 2587-2596 [PMID: 17868066 DOI: 10.1111/j.1600-6143.2007.01965.x]
- 41 **Mazzaferro V**. Results of liver transplantation: with or without Milan criteria? *Liver Transpl* 2007; **13**: S44-S47 [PMID: 17969068 DOI: 10.1002/lt.21330]
- 42 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]
- 43 **Chan SC**, Fan ST, Chok KS, Cheung TT, Chan AC, Fung JY, Poon RT, Lo CM. Survival advantage of primary liver transplantation for hepatocellular carcinoma within the up-to-7 criteria with microvascular invasion. *Hepatol Int* 2012; **6**: 646-656 [PMID: 22016140 DOI: 10.1007/s12072-011-9318-3]
- 44 **D'Amico F**, Schwartz M, Vitale A, Tabrizian P, Roayaie S, Thung S, Guido M, del Rio Martin J, Schiano T, Cillo U. Predicting recurrence after liver transplantation in patients with hepatocellular carcinoma exceeding the up-to-seven criteria. *Liver Transpl* 2009; **15**: 1278-1287 [PMID: 19790142 DOI: 10.1002/lt.21842]
- 45 **Fan J**, Yang GS, Fu ZR, Peng ZH, Xia Q, Peng CH, Qian JM, Zhou J, Xu Y, Qiu SJ, Zhong L, Zhou GW, Zhang JJ. Liver transplantation outcomes in 1,078 hepatocellular carcinoma patients: a multi-center experience in Shanghai, China. *J Cancer Res Clin Oncol* 2009; **135**: 1403-1412 [PMID: 19381688 DOI: 10.1007/s00432-009-0584-6]
- 46 **Jang JW**, You CR, Kim CW, Bae SH, Yoon SK, Yoo YK, Kim DG, Choi JY. Benefit of downsizing hepatocellular carcinoma in a liver transplant population. *Aliment Pharmacol Ther* 2010; **31**: 415-423 [PMID: 19821808 DOI: 10.1111/j.1365-2036.2009.04167.x]
- 47 **Cillo U**, Vitale A, Grigoletto F, Gringeri E, D'Amico F, Valmasoni M, Brolese A, Zanusi G, Srsen N, Carraro A, Burra P, Farinati F, Angeli P, D'Amico DF. Intention-to-treat analysis of liver transplantation in selected, aggressively treated HCC patients exceeding the Milan criteria. *Am J Transplant* 2007; **7**: 972-981 [PMID: 17391137 DOI: 10.1111/j.1600-6143.2006.01719.x]
- 48 **Ravaioli M**, Grazi GL, Piscaglia F, Trevisani F, Cescon M, Ercolani G, Vivarelli M, Golfieri R, D'Errico Grigioni A, Panzini I, Morelli C, Bernardi M, Bolondi L, Pinna AD. Liver transplantation for hepatocellular carcinoma: results of down-staging in patients initially outside the Milan selection criteria. *Am J Transplant* 2008; **8**: 2547-2557 [PMID: 19032223 DOI: 10.1111/j.1600-6143.2008.02409.x]
- 49 **Volk ML**, Vijan S, Marrero JA. A novel model measuring the harm of transplanting hepatocellular carcinoma exceeding Milan criteria. *Am J Transplant* 2008; **8**: 839-846 [PMID: 18318783 DOI: 10.1111/j.1600-6143.2007.02138.x]
- 50 **Pomfret EA**, Washburn K, Wald C, Nalesnik MA, Douglas D, Russo M, Roberts J, Reich DJ, Schwartz ME, Miele L, Lee FT, Florman S, Yao F, Harper A, Edwards E, Freeman R, Lake J. Report of a national conference on liver allocation in patients with hepatocellular carcinoma in the United States. *Liver Transpl* 2010; **16**: 262-278 [PMID: 20209641 DOI: 10.1002/lt.21999]
- 51 **Ioannou GN**, Perkins JD, Carithers RL. Liver transplantation for hepatocellular carcinoma: impact of the MELD allocation system and predictors of survival. *Gastroenterology* 2008; **134**: 1342-1351 [PMID: 18471511 DOI: 10.1053/j.gastro.2008.02.013]
- 52 **Toso C**, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation for hepatocellular carcinoma utilizing the Scientific Registry of Transplant Recipients database. *Hepatology* 2009; **49**: 832-838 [PMID: 19152426 DOI: 10.1002/hep.22693]
- 53 **Witjes CD**, Willemssen FE, Verheij J, van der Veer SJ, Hansen BE, Verhoef C, de Man RA, Ijzermans JN. Histological differentiation grade and microvascular invasion of hepatocellular carcinoma predicted by dynamic contrast-enhanced MRI. *J Magn Reson Imaging* 2012; **36**: 641-647 [PMID: 22532493 DOI: 10.1002/jmri.23681]
- 54 **Chandarana H**, Robinson E, Hajdu CH, Drozhinin L, Babb JS, Taouli B. Microvascular invasion in hepatocellular carcinoma: is it predictable with pretransplant MRI? *AJR Am J Roentgenol* 2011; **196**: 1083-1089 [PMID: 21512074 DOI: 10.2214/AJR.10.4720]
- 55 **Fujita N**, Nishie A, Kubo Y, Asayama Y, Ushijima Y, Takayama Y, Moirita K, Shirabe K, Aishima S, Honda H. Hepatocellular carcinoma: clinical significance of signal heterogeneity in the hepatobiliary phase of gadoteric acid-enhanced MR imaging. *Eur Radiol* 2015; **25**: 211-220 [PMID: 25063395 DOI: 10.1007/s00330-014-3349-9]

- 56 **Hiraoka A**, Ochi H, Hidaka S. FDG positron emission tomography/computed tomography findings for prediction of early recurrence of hepatocellular carcinoma after surgical resection. *Exp Ther Med* 2010; **1**: 829-832 [DOI: 10.3892/etm.2010.126]
- 57 **Ochi H**, Hirooka M, Hiraoka A, Koizumi Y, Abe M, Sogabe I, Ishimaru Y, Furuya K, Miyagawa M, Kawasaki H, Michitaka K, Takada Y, Mochizuki T, Hiasa Y. (18)F-FDG-PET/CT predicts the distribution of microsatellite lesions in hepatocellular carcinoma. *Mol Clin Oncol* 2014; **2**: 798-804 [PMID: 25054048 DOI: 10.3892/mco.2014.328]
- 58 **Kornberg A**, Freesmeyer M, Bärthel E, Jandt K, Katenkamp K, Steenbeck J, Sappeler A, Habrecht O, Gottschild D, Settmacher U. 18F-FDG-uptake of hepatocellular carcinoma on PET predicts microvascular tumor invasion in liver transplant patients. *Am J Transplant* 2009; **9**: 592-600 [PMID: 19191771 DOI: 10.1111/j.1600-6143.2008.02516.x]
- 59 **Kornberg A**. Liver transplantation for hepatocellular carcinoma beyond Milan criteria: multidisciplinary approach to improve outcome. *ISRN Hepatol* 2014; **25**: 154-159 [DOI: 10.1155/2014/706945]
- 60 **DuBay D**, Sandroussi C, Sandhu L, Cleary S, Guba M, Catral MS, McGilvray I, Ghanekar A, Selzner M, Greig PD, Grant DR. Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criterion. *Ann Surg* 2011; **253**: 166-172 [PMID: 21294289 DOI: 10.1097/SLA.0b013e31820508f1]
- 61 **Young RS**, Aldiwani M, Hakeem AR, Nair A, Guthrie A, Wyatt J, Treanor D, Morris-Stiff G, Jones RL, Prasad KR. Pre-liver transplant biopsy in hepatocellular carcinoma: a potential criterion for exclusion from transplantation? *HPB (Oxford)* 2013; **15**: 418-427 [PMID: 23458127 DOI: 10.1111/hpb.12008]
- 62 **Lai Q**, Avolio AW, Manzia TM, Sorge R, Agnes S, Tisone G, Berloco PB, Rossi M. Combination of biological and morphological parameters for the selection of patients with hepatocellular carcinoma waiting for liver transplantation. *Clin Transplant* 2012; **26**: E125-E131 [PMID: 22192083 DOI: 10.1111/j.1399-0012.2011.01572.x]
- 63 **Duvoux C**, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, Francoz C, Compagnon P, Vanlemmens C, Dumortier J, Dharancy S, Gugenheim J, Bernard PH, Adam R, Radenne S, Muscari F, Conti F, Hardwigen J, Pageaux GP, Chazouillères O, Salame E, Hilleret MN, Lebray P, Abergel A, Debette-Gratien M, Kluger MD, Mallat A, Azoulay D, Cherqui D. Liver transplantation for hepatocellular carcinoma: a model including  $\alpha$ -fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012; **143**: 986-94.e3; quiz e14-5 [PMID: 22750200 DOI: 10.1053/j.gastro.2012.05.052]
- 64 **Varona MA**, Del Pino JM, Barrera M, Arranz J, Hernández BM, Perez HF, Padilla J, Fuentes JS, Aguirre A, Mendez S, Sanz P, Gianchandani R, Perera A, Soriano A. Hepatocellular carcinoma and liver transplantation: a 12-year experience. *Transplant Proc* 2009; **41**: 1005-1008 [PMID: 19376411 DOI: 10.1016/j.transproceed.2009.02.029]
- 65 **Varona MA**, Soriano A, Aguirre-Jaime A, Garrido S, Oton E, Diaz D, Portero J, Bravo P, Barrera MA, Perera A. Risk factors of hepatocellular carcinoma recurrence after liver transplantation: accuracy of the alpha-fetoprotein model in a single-center experience. *Transplant Proc* 2015; **47**: 84-89 [PMID: 25645778 DOI: 10.1016/j.transproceed.2014.12.013]
- 66 **Berry K**, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. *Liver Transpl* 2013; **19**: 634-645 [PMID: 23536495 DOI: 10.1002/lt.23652]
- 67 **Yang SH**, Suh KS, Lee HW, Cho EH, Cho JY, Cho YB, Kim IH, Yi NJ, Lee KU. A revised scoring system utilizing serum alpha-fetoprotein levels to expand candidates for living donor transplantation in hepatocellular carcinoma. *Surgery* 2007; **141**: 598-609 [PMID: 17462459 DOI: 10.1016/j.surg.2006.11.006]
- 68 **Akamatsu N**, Sugawara Y, Kokudo N. Living donor liver transplantation for patients with hepatocellular carcinoma. *Liver Cancer* 2014; **3**: 108-118 [PMID: 24945001 DOI: 10.1159/000343866]
- 69 **Kaido T**, Ogawa K, Mori A, Fujimoto Y, Ito T, Tomiyama K, Takada Y, Uemoto S. Usefulness of the Kyoto criteria as expanded selection criteria for liver transplantation for hepatocellular carcinoma. *Surgery* 2013; **154**: 1053-1060 [PMID: 24074704 DOI: 10.1016/j.surg.2013.04.056]
- 70 **Shirabe K**, Taketomi A, Morita K, Soejima Y, Uchiyama H, Kayashima H, Ninomiya M, Toshima T, Maehara Y. Comparative evaluation of expanded criteria for patients with hepatocellular carcinoma beyond the Milan criteria undergoing living-related donor liver transplantation. *Clin Transplant* 2011; **25**: E491-E498 [PMID: 21518000 DOI: 10.1111/j.1399-0012.2011.01463.x]
- 71 **Freeman RB**, Wiesner RH, Edwards E, Harper A, Merion R, Wolfe R. Results of the first year of the new liver allocation plan. *Liver Transpl* 2004; **10**: 7-15 [PMID: 14755772]
- 72 **Washburn K**, Edwards E, Harper A, Freeman R. Hepatocellular carcinoma patients are advantaged in the current liver transplant allocation system. *Am J Transplant* 2010; **10**: 1643-1648 [PMID: 20486906 DOI: 10.1111/j.1600-6143.2010.03127.x]
- 73 **Toso C**, Majno P, Berney T, Morel P, Mentha G, Combescure C. Validation of a dropout assessment model of candidates with/without hepatocellular carcinoma on a common liver transplant waiting list. *Transpl Int* 2014; **27**: 686-695 [PMID: 24649861 DOI: 10.1111/tri.12323]
- 74 **Vitale A**, Volk ML, De Feo TM, Burra P, Frigo AC, Ramirez Morales R, De Carlis L, Belli L, Colledan M, Fagioli S, Rossi G, Andorno E, Baccarani U, Regalia E, Vivarelli M, Donatascio M, Cillo U. A method for establishing allocation equity among patients with and without hepatocellular carcinoma on a common liver transplant waiting list. *J Hepatol* 2014; **60**: 290-297 [PMID: 24161408 DOI: 10.1016/j.jhep.2013.10.010]
- 75 **Bruix J**, Colombo M. Hepatocellular carcinoma: current state of the art in diagnosis and treatment. *Best Pract Res Clin Gastroenterol* 2014; **28**: 751 [PMID: 25260305 DOI: 10.1016/j.bpg.2014.08.010]
- 76 **Vibert E**, Azoulay D, Hoti E, Iacopinelli S, Samuel D, Salloum C, Lemoine A, Bismuth H, Castaing D, Adam R. Progression of alpha-fetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: a critical factor. *Am J Transplant* 2010; **10**: 129-137 [PMID: 20070666 DOI: 10.1111/j.1600-6143.2009.02750.x]
- 77 **Kneteman N**, Livraghi T, Madoff D, de Santibañez E, Kew M. Tools for monitoring patients with hepatocellular carcinoma on the waiting list and after liver transplantation. *Liver Transpl* 2011; **17** Suppl 2: S117-S127 [PMID: 21584926 DOI: 10.1002/lt.22334]
- 78 **Fujiki M**, Aucejo F, Choi M, Kim R. Neo-adjuvant therapy for hepatocellular carcinoma before liver transplantation: where do we stand? *World J Gastroenterol* 2014; **20**: 5308-5319 [PMID: 24833861 DOI: 10.3748/wjg.v20.i18.5308]
- 79 **Cescan M**, Cucchetti A, Ravaioli M, Pinna AD. Hepatocellular carcinoma locoregional therapies for patients in the waiting list. Impact on transplantability and recurrence rate. *J Hepatol* 2013; **58**: 609-618 [PMID: 23041304 DOI: 10.1016/j.jhep.2012.09.021]
- 80 **Raza A**, Sood GK. Hepatocellular carcinoma review: current treatment, and evidence-based medicine. *World J Gastroenterol* 2014; **20**: 4115-4127 [PMID: 24764650 DOI: 10.3748/wjg.v20.i15.4115]
- 81 **Roberts JP**, Venook A, Kerlan R, Yao F. Hepatocellular carcinoma: Ablate and wait versus rapid transplantation. *Liver Transpl* 2010; **16**: 925-929 [PMID: 20658555 DOI: 10.1002/lt.22103]
- 82 **Pompili M**, Francica G, Ponziani FR, Iezzi R, Avolio AW. Bridging and downstaging treatments for hepatocellular carcinoma in patients on the waiting list for liver transplantation. *World J Gastroenterol* 2013; **19**: 7515-7530 [PMID: 24282343 DOI: 10.3748/wjg.v19.i43.7515]
- 83 **Germani G**, Pleguezuelo M, Gurusamy K, Meyer T, Isgrò G, Burroughs AK. Clinical outcomes of radiofrequency ablation, percutaneous alcohol and acetic acid injection for hepatocellular carcinoma: a meta-analysis. *J Hepatol* 2010; **52**: 380-388 [PMID: 20149473 DOI: 10.1016/j.jhep.2009.12.004]
- 84 **Majno PE**, Sarasin FP, Mentha G, Hadengue A. Primary liver



- resection and salvage transplantation or primary liver transplantation in patients with single, small hepatocellular carcinoma and preserved liver function: an outcome-oriented decision analysis. *Hepatology* 2000; **31**: 899-906 [PMID: 10733546 DOI: 10.1053/he.2000.5763]
- 85 **Yao FY**, Kerlan RK, Hirose R, Davern TJ, Bass NM, Feng S, Peters M, Terrault N, Freise CE, Ascher NL, Roberts JP. Excellent outcome following down-staging of hepatocellular carcinoma prior to liver transplantation: an intention-to-treat analysis. *Hepatology* 2008; **48**: 819-827 [PMID: 18688876 DOI: 10.1002/hep.22412]
  - 86 **Llovet JM**, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, Schwartz M, Lotze M, Talwalkar J, Gores GJ. Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 698-711 [PMID: 18477802 DOI: 10.1093/jnci/djn134]
  - 87 **Sharr WW**, Chan SC, Lo CM. Section 3. Current status of downstaging of hepatocellular carcinoma before liver transplantation. *Transplantation* 2014; **97** Suppl 8: S10-S17 [PMID: 24849822 DOI: 10.1097/01.tp.0000446267.19148.21]
  - 88 **Clavien PA**, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A; OLT for HCC Consensus Group. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13**: e11-e22 [PMID: 22047762 DOI: 10.1016/S1470-2045(11)70175-9]
  - 89 **Chapman WC**, Majella Doyle MB, Stuart JE, Vachharajani N, Crippin JS, Anderson CD, Lowell JA, Shenoy S, Darcy MD, Brown DB. Outcomes of neoadjuvant transarterial chemoembolization to downstage hepatocellular carcinoma before liver transplantation. *Ann Surg* 2008; **248**: 617-625 [PMID: 18936575 DOI: 10.1097/SLA.0b013e31818a07d4]
  - 90 **Llovet JM**, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862]
  - 91 **Millonig G**, Graziadei IW, Freund MC, Jaschke W, Stadlmann S, Ladurner R, Margreiter R, Vogel W. Response to preoperative chemoembolization correlates with outcome after liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl* 2007; **13**: 272-279 [PMID: 17256758 DOI: 10.1002/lt.21033]
  - 92 **Porrett PM**, Peterman H, Rosen M, Sonnad S, Soulen M, Markmann JF, Shaked A, Furth E, Reddy KR, Olthoff K. Lack of benefit of pre-transplant locoregional hepatic therapy for hepatocellular cancer in the current MELD era. *Liver Transpl* 2006; **12**: 665-673 [PMID: 16482577]
  - 93 **Cascales Campos P**, Ramirez P, Gonzalez R, Febrero B, Pons JA, Miras M, Sanchez Bueno F, Robles R, Parrilla P. Value of 18-FDG-positron emission tomography/computed tomography before and after transarterial chemoembolization in patients with hepatocellular carcinoma undergoing liver transplantation: initial results. *Transplant Proc* 2011; **43**: 2213-2215 [PMID: 21839236 DOI: 10.1016/j.transproceed.2011.05.023]
  - 94 **Lai Q**, Avolio AW, Graziadei I, Otto G, Rossi M, Tisone G, Goffette P, Vogel W, Pitton MB, Lerut J. Alpha-fetoprotein and modified response evaluation criteria in solid tumors progression after locoregional therapy as predictors of hepatocellular cancer recurrence and death after transplantation. *Liver Transpl* 2013; **19**: 1108-1118 [PMID: 23873764 DOI: 10.1002/lt.23706]
  - 95 **Merani S**, Majno P, Kneteman NM, Berney T, Morel P, Mentha G, Toso C. The impact of waiting list alpha-fetoprotein changes on the outcome of liver transplant for hepatocellular carcinoma. *J Hepatol* 2011; **55**: 814-819 [PMID: 21334400 DOI: 10.1016/j.jhep.2010.12.040]
  - 96 **Bova V**, Miraglia R, Maruzzelli L, Vizzini GB, Luca A. Predictive factors of downstaging of hepatocellular carcinoma beyond the Milan criteria treated with intra-arterial therapies. *Cardiovasc Intervent Radiol* 2013; **36**: 433-439 [PMID: 22864644 DOI: 10.1007/s00270-012-0458-1]
  - 97 **Barakat O**, Wood RP, Ozaki CF, Ankoma-Sey V, Galati J, Skolkin M, Toombs B, Round M, Moore W, Miele L. Morphological features of advanced hepatocellular carcinoma as a predictor of downstaging and liver transplantation: an intention-to-treat analysis. *Liver Transpl* 2010; **16**: 289-299 [PMID: 20209588 DOI: 10.1002/lt.21994]
  - 98 **Riaz A**, Ryu RK, Kulik LM, Mulcahy MF, Lewandowski RJ, Minocha J, Ibrahim SM, Sato KT, Baker T, Miller FH, Newman S, Omary R, Abecassis M, Benson AB, Salem R. Alpha-fetoprotein response after locoregional therapy for hepatocellular carcinoma: oncologic marker of radiologic response, progression, and survival. *J Clin Oncol* 2009; **27**: 5734-5742 [PMID: 19805671 DOI: 10.1200/JCO.2009.23.1282]

**P- Reviewer:** Cao GW, Kaiser GM **S- Editor:** Wang JL  
**L- Editor:** A **E- Editor:** Liu SQ





## Treatment strategies for chronic hepatitis C prior to and following liver transplantation

Ryan B Perumpail, Thomas A Hahambis, Avin Aggarwal, Zobair M Younossi, Aijaz Ahmed

Ryan B Perumpail, Avin Aggarwal, Aijaz Ahmed, Division of Gastroenterology and Hepatology, Stanford University School of Medicine, Palo Alto, CA 94304, United States

Thomas A Hahambis, Gilead Sciences, Foster City, CA 94404, United States

Zobair M Younossi, Center for Liver Diseases, Department of Medicine, Inova Fairfax Hospital, Falls Church, VA 22042, United States

Zobair M Younossi, Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA 22042, United States

**Author contributions:** Perumpail RB prepared first and final draft, revised the final draft based on feedback from other authors; Hahambis TA prepared first and final draft with the first author; Aggarwal A, Younossi ZM and Ahmed A reviewed and revised each segment of the document and checked references for completeness.

**Conflict-of-interest statement:** Ryan B Perumpail and Avin Aggarwal have no conflict of interest; Thomas A Hahambis is Gilead Employee: Senior Medical Scientist, Hepatitis; Zobair M Younossi is Advisory Board and/or Consultant to Gilead, Abbvie, BMS, GSK, and Intercept; Aijaz Ahmed is Advisory Board: Gilead. Research Funding: Gilead.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Ryan B Perumpail, MD, Division of Gastroenterology and Hepatology, Stanford University School of Medicine, 750 Welch Road, Suite 210, Palo Alto, CA 94304, United States. [rperumpail@gmail.com](mailto:rperumpail@gmail.com)  
 Telephone: +1-650-4986091  
 Fax: +1-650-4985692

Received: August 20, 2015

Peer-review started: August 22, 2015

First decision: October 30, 2015

Revised: October 30, 2015

Accepted: December 17, 2015

Article in press: December 18, 2015

Published online: January 8, 2016

### Abstract

Hepatitis C virus (HCV)-related liver disease is the leading indication for liver transplantation (LT) worldwide. However, HCV is an independent predictor of lower survival following LT, and recurrence of HCV post-LT is virtually universal. The historic standard of care during the interferon era of HCV therapy was expectant management-initiation of antiviral therapy in the setting of documented disease progression following LT. With the advent of new direct acting antiviral (DAA) therapies for HCV, the paradigm of expectant treatment for recurrent HCV infection post-LT is shifting. The safety, tolerability, and efficacy of DAAs, even among the sickest patients with advanced liver disease, enables treatment of HCV in the pre-transplant setting among LT waitlist registrants. Finally, emerging data are supportive of preemptive therapy with DAAs in liver transplant recipients as the preferred approach. Expectant management of HCV following LT can rarely be justified in the modern era of HCV therapy.

**Key words:** Hepatitis C virus; Liver transplantation; Direct acting antivirals; Sustained virologic response

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The historic standard of care during the interferon era of hepatitis C virus (HCV) therapy was expectant management-initiation of antiviral therapy in the setting of documented disease progression following

liver transplantation. With the advent of new direct acting antiviral (DAA) therapies for HCV, the paradigm of expectant treatment for recurrent HCV infection post-liver transplantation (LT) is shifting. The safety, tolerability, and efficacy of DAAs, even among the sickest patients with advanced liver disease, enables treatment of HCV in the pre-transplant setting among LT waitlist registrants. Emerging data support preemptive therapy with DAAs in liver transplant recipients as the preferred approach.

Perumpail RB, Hahambis TA, Aggarwal A, Younossi ZM, Ahmed A. Treatment strategies for chronic hepatitis C prior to and following liver transplantation. *World J Hepatol* 2016; 8(1): 69-73 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i1/69.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i1.69>

## INTRODUCTION

Hepatitis C virus (HCV) infection afflicts an estimated 180 million people worldwide, or nearly 3% of the global population<sup>[1,2]</sup>. HCV results in 8000 to 13000 deaths annually in the United States<sup>[3]</sup>. To date, HCV remains the leading indication for liver transplantation (LT) in developed nations and represents 33% of patients currently on the LT waitlist<sup>[3,4]</sup>.

## NATURAL HISTORY OF HCV INFECTION BEFORE LT

Among 70% to 75% of patients, acute HCV infection is asymptomatic. The remaining minority of patients develops systemic symptoms, including weakness, malaise, anorexia, and, rarely, jaundice. Eighty-five percent of patients with acute HCV infection do not clear the infection without treatment and instead develop chronic infection<sup>[5]</sup>. Progression to cirrhosis or hepatocellular carcinoma occurs in between 15% to 40% of patients with chronic HCV<sup>[1]</sup>. Accelerated development of cirrhosis and end-stage liver disease ensue under certain conditions. Rate of progression to cirrhosis is impacted by age at exposure - higher risk with HCV exposure at advanced age; route of transmission - blood transfusion portends greater risk than injection drug use; duration of infection; HCV genotype; and coexisting illnesses, including human immunodeficiency virus infection, hepatitis B virus (HBV) infection, and alcoholic liver disease<sup>[6-10]</sup>.

## TREATMENT OF HCV INFECTION BEFORE LT

Although 5-year survival among patients with compensated cirrhosis due HCV ranges from 84% to 91%, there is a 20% risk of decompensation and a 10% risk of HCC<sup>[11,12]</sup>. Attainment of sustained virologic response (SVR) is associated with lower rates of hepatic

decompensation, HCC, and all-cause mortality<sup>[13]</sup>. Indeed, an international multicenter study demonstrated that patients with chronic HCV who achieve SVR have long-term survival comparable to that of the general population<sup>[14,15]</sup>. Moreover, recent data reveal improved long-term survival following LT among patients in whom HCV was eradicated prior to LT<sup>[16]</sup>. As a third of LT in the United States are performed for HCV-related liver disease<sup>[4]</sup> and HCV-positive recipients have worse outcomes following LT<sup>[17]</sup>, attaining pre-transplant SVR may yield significant improvements in patient outcomes. In the interferon era, HCV therapy was instituted with caution in patients with advanced liver disease due to the potential risk of hepatic decompensation. Now, with the advent of safe, well-tolerated, and efficacious direct acting antivirals (DAAs), a paradigm shift toward pre-transplant treatment of HCV is warranted. The shortage of donor livers in the United States, which results in substantial liver transplant waitlist mortality and dropout<sup>[18]</sup>, underscores the importance of treating HCV prior to LT. The significance of this shift is even greater in regions where the availability of LT is limited to only very sick patients<sup>[19]</sup>. Treatment of HCV pre-transplant stands not only to improve post-LT outcomes but also reduce the overall societal need for LT. Viral suppression in HBV has been shown to lead to regression of fibrosis<sup>[20,21]</sup>. Likewise, emerging data now reveals histological regression of fibrosis among patients with HCV who have achieved SVR<sup>[4]</sup>. As such, long-term virologic suppression of HCV may lead to disease reversal.

## LT FOR HCV

LT is optimal therapy for decompensated cirrhosis due to chronic HCV, but HCV reinfection poses challenging management issues that may arise either early or late after transplantation<sup>[22,23]</sup>.

## DONOR LIVER ALLOCATION FOR LT

In 2002, the model for end-stage liver disease (MELD) score shown to predict LT waitlist mortality was implemented as an allocation criterion for donor livers<sup>[24]</sup>. The goal is to improve survival and quality of life among patients with end-stage liver disease. LT has proven to be effective at achieving these goals. The benefits of LT are most established for patients with MELD scores of at least 15 or higher<sup>[25]</sup>. The MELD score necessary to receive a donor liver varies widely by United Network for Organ Sharing region. While patients with MELD scores in the mid-20s receive offers in some regions, MELD scores in the high-30s are commonly needed in other regions. Because offers are allocated to patients with higher MELD scores, concern has emerged about the possibility of a so-called "MELD purgatory" with pre-transplant treatment of HCV. Concern exists that certain patients may have delayed progression of liver disease after achieving SVR without substantial reversal or improvement in quality of life<sup>[26]</sup>. Proponents of this view

contend that post-LT treatment of HCV would alleviate this concern. We should be cognizant of the fact that up to 3000 potential liver transplant candidates are removed from the waitlist annually in the United States - half develop contraindications for LT while the wait for a potential donor and the other half die from complications of end-stage liver disease<sup>[27]</sup>. Therefore, necessitating changes in allocation policies to reduce waitlist mortality<sup>[28]</sup>. Therefore, deferring antiviral therapy from pre- to post-LT phase may not be safe. Morbidity and mortality associated with LT are low, but should be ignored with emerging DAA data supporting instituting treatment in the pre-transplant phase. Furthermore, most experts agree that fibrosing cholestatic hepatitis and compensated recurrent HCV infection following LT demonstrates relatively lower efficacy with DAA therapy<sup>[29,30]</sup>. The concerns regarding the use of HCV-positive allografts have been alleviated with more recent data suggesting that transplant outcomes for recipients who accept HCV-positive donor allografts may be comparable with those who receive HCV-negative allografts<sup>[31]</sup>. Emerging treatments to eradicate HCV have further improved the course of HCV-positive individuals, with improved efficacy and reduced side-effects. HCV-positive donors constitute 4.8% of HCV-positive LT recipients<sup>[32]</sup>. The use HCV-positive donor in HCV-negative recipients with the availability of DAAs needs to be studied further. Lastly, if LT is imminent in a Child-Turcotte-Pugh class C patient with MELD score > 35 or hepatocellular carcinoma patient with exception MELD points - it may be pragmatic to wait and institute antiviral therapy following LT<sup>[33]</sup>.

## NATURAL HISTORY OF HCV INFECTION FOLLOWING LT

Studies demonstrate worse outcomes post-LT among patients with recurrent HCV infection compared to patients transplanted for other causes of cirrhosis<sup>[23,34]</sup>. The natural history of HCV infection in liver transplant recipients is typically accelerated, partially due to concomitant administration of post-LT immunosuppression. Up to 20% of HCV-infected patients develop cirrhosis by 5 years following LT<sup>[23]</sup>. Recurrent disease ranges from asymptomatic mild hepatitis to severe chronic hepatitis and cirrhosis. Reinfection with HCV post-LT is virtually universal, occurring in over 95% of cases<sup>[22]</sup>.

## PREEMPTIVE TREATMENT OF HCV FOLLOWING TRANSPLANTATION

Historically, preemptive use of antiviral therapy post-LT was not advisable because of the increased rate of acute allograft rejection associated with interferon therapy<sup>[35]</sup>. However, with the emergence of safe and efficacious DAAs, the previous concern of interferon-related immunomodulation with allograft rejection and

poor tolerance due to anti-HCV therapy following LT is abating. None of the new DAAs have yet been approved by the United States Food and Drug Administration for use among patients following LT, but the powerful body of emerging literature suggests that approval may be expected in the near future<sup>[29,30]</sup>. Preemptive treatment of HCV in the post-LT setting may alleviate the need for re-transplantation.

## EXPECTANT TREATMENT OF HCV FOLLOWING TRANSPLANTATION

Despite being the previous standard of care in the interferon era, expectant management of HCV does not seem to have a role for the vast majority of patients in the era of DAAs. Delaying HCV therapy post-LT is not advisable due to the rapid progression of HCV-related liver damage and promising data regarding the use of DAAs.

## CONCLUSION

Advances in peri-transplant management of liver transplant recipients in the setting of chronic hepatitis C have resulted in long-term post-transplant survival rates approaching 90%<sup>[36]</sup>. Nevertheless, survival following LT remains lower among patients with HCV compared to those undergoing LT for liver disease related to other etiologies<sup>[17]</sup>. Attaining SVR pre-transplant reduces all-cause mortality, may decrease the need for LT, and may improve survival following LT<sup>[14,15]</sup>. The improvements in the efficacy of antiviral therapy against HCV infection with DAAs argue against the interferon-era paradigm of expectant use of antiviral therapy following LT. The decision between treating patients pre-transplant or preemptively in the early post-transplant setting should be individualized for each patient in the context of the regional waitlist trends and exception policies for LT. Despite advancements in LT, there remains a shortage of donor livers to meet the demands for LT in the United States. Treatment of patients on the LT waiting list may ultimately decrease the number of patients needing LT and help address the imbalance in supply and demand.

## REFERENCES

- 1 Wray CM, Davis AM. Screening for hepatitis C. *JAMA* 2015; **313**: 1855-1856 [PMID: 25965235 DOI: 10.1001/jama.2015.2833]
- 2 Chung RT, Baumert TF. Curing chronic hepatitis C--the arc of a medical triumph. *N Engl J Med* 2014; **370**: 1576-1578 [PMID: 24720678 DOI: 10.1056/NEJMp1400986]
- 3 Moyer VA. Screening for hepatitis C virus infection in adults: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2013; **159**: 349-357 [PMID: 23798026 DOI: 10.7326/0003-4819-159-5-201309030-00672]
- 4 Dhanasekaran R, Sanchez W, Mounajjed T, Wiesner RH, Watt KD, Charlton MR. Impact of fibrosis progression on clinical outcome in patients treated for post-transplant hepatitis C recurrence. *Liver Int* 2015; **35**: 2433-2441 [PMID: 26058570 DOI: 10.1111/liv.12890]
- 5 Tong MJ, el-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med* 1995; **332**:

- 1463-1466 [PMID: 7739682 DOI: 10.1056/NEJM199506013322202]
- 6 **Brechot C**, Nalpas B, Feitelson MA. Interactions between alcohol and hepatitis viruses in the liver. *Clin Lab Med* 1996; **16**: 273-287 [PMID: 8792072]
- 7 **Gordon SC**, Bayati N, Silverman AL. Clinical outcome of hepatitis C as a function of mode of transmission. *Hepatology* 1998; **28**: 562-567 [PMID: 9696025 DOI: 10.1002/hep.510280238]
- 8 **Marrone A**, Sallie R. Genetic heterogeneity of hepatitis C virus. The clinical significance of genotypes and quasispecies behavior. *Clin Lab Med* 1996; **16**: 429-449 [PMID: 8792081]
- 9 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIR, METAVIR, CLINIVIR, and DOSVIR groups. *Lancet* 1997; **349**: 825-832 [PMID: 9121257 DOI: 10.1016/S0140-6736(96)07642-8]
- 10 **Simmonds P**. Variability of hepatitis C virus. *Hepatology* 1995; **21**: 570-583 [PMID: 7531173 DOI: 10.1002/hep.1840210243]
- 11 **Fattovich G**, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, Nevens F, Solinas A, Mura D, Brouwer JT, Thomas H, Njapoum C, Casarin C, Bonetti P, Fuschi P, Basho J, Tocco A, Bhalla A, Galassini R, Noventa F, Schalm SW, Realdi G. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997; **112**: 463-472 [PMID: 9024300 DOI: 10.1053/gast.1997.v112.pm9024300]
- 12 **Serfaty L**, Aumaitre H, Chazouillères O, Bonnand AM, Rosmorduc O, Poupon RE, Poupon R. Determinants of outcome of compensated hepatitis C virus-related cirrhosis. *Hepatology* 1998; **27**: 1435-1440 [PMID: 9581703 DOI: 10.1002/hep.510270535]
- 13 **Veldt BJ**, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, Manns MP, Hansen BE, Schalm SW, Janssen HL. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. *Ann Intern Med* 2007; **147**: 677-684 [PMID: 18025443 DOI: 10.7326/0003-4819-147-10-200711200-00003]
- 14 **van der Meer AJ**, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Heathcote EJ, Manns MP, Kuske L, Zeuzem S, Hofmann WP, de Knecht RJ, Hansen BE, Janssen HL. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012; **308**: 2584-2593 [PMID: 23268517 DOI: 10.1001/jama.2012.144878]
- 15 **van der Meer AJ**, Wedemeyer H, Feld JJ, Dufour JF, Zeuzem S, Hansen BE, Janssen HL. Life expectancy in patients with chronic HCV infection and cirrhosis compared with a general population. *JAMA* 2014; **312**: 1927-1928 [PMID: 25387192 DOI: 10.1001/jama.2014.12627]
- 16 **Fortune BE**, Martinez-Camacho A, Kreidler S, Gralla J, Everson GT. Post-transplant survival is improved for hepatitis C recipients who are RNA negative at time of liver transplantation. *Transpl Int* 2015; **28**: 980-989 [PMID: 25818896 DOI: 10.1111/tri.12568]
- 17 **Forman LM**, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. *Gastroenterology* 2002; **122**: 889-896 [PMID: 11910340 DOI: 10.1053/gast.2002.32418]
- 18 **Charpentier KP**, Mavanur A. Removing patients from the liver transplant wait list: A survey of US liver transplant programs. *Liver Transpl* 2008; **14**: 303-307 [PMID: 18306339 DOI: 10.1002/lt.21353]
- 19 **Gentry SE**, Massie AB, Cheek SW, Lentine KL, Chow EH, Wickliffe CE, Dzebashvili N, Salvalaggio PR, Schnitzler MA, Axelrod DA, Segev DL. Addressing geographic disparities in liver transplantation through redistricting. *Am J Transplant* 2013; **13**: 2052-2058 [PMID: 23837931 DOI: 10.1111/ajt.12301]
- 20 **Chang TT**, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, Safadi R, Lee SS, Halota W, Goodman Z, Chi YC, Zhang H, Hindes R, Iloeje U, Beebe S, Kreter B. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. *Hepatology* 2010; **52**: 886-893 [PMID: 20683932 DOI: 10.1002/hep.23785]
- 21 **Marcellin P**, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, Washington MK, Germanidis G, Flaherty JF, Aguilar Schall R, Bornstein JD, Kitrinis KM, Subramanian GM, McHutchison JG, Heathcote EJ. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* 2013; **381**: 468-475 [PMID: 23234725 DOI: 10.1016/S0140-6736(12)61425-1]
- 22 **Ferrell LD**, Wright TL, Roberts J, Ascher N, Lake J. Hepatitis C viral infection in liver transplant recipients. *Hepatology* 1992; **16**: 865-876 [PMID: 1383115 DOI: 10.1002/hep.1840160403]
- 23 **Gane EJ**, Portmann BC, Naoumov NV, Smith HM, Underhill JA, Donaldson PT, Maertens G, Williams R. Long-term outcome of hepatitis C infection after liver transplantation. *N Engl J Med* 1996; **334**: 815-820 [PMID: 8596547 DOI: 10.1056/NEJM199603283341302]
- 24 **Wiesner R**, Edwards E, Freeman R, Harper A, Kim R, Kamath P, Kremers W, Lake J, Howard T, Merion RM, Wolfe RA, Krom R. Model for end-stage liver disease (MELD) and allocation of donor livers. *Gastroenterology* 2003; **124**: 91-96 [PMID: 12512033 DOI: 10.1053/gast.2003.50016]
- 25 **Åberg F**, Nordin A, Mäkisalo H, Isoniemi H. Who is too healthy and who is too sick for liver transplantation: external validation of prognostic scores and survival-benefit estimation. *Scand J Gastroenterol* 2015; **50**: 1144-1151 [PMID: 25865580 DOI: 10.3109/00365521.2015.1028992]
- 26 **Bonacci M**, Londoño MC, Esforzado N, Fornis X, Sotoca JM, Campistol JM. Antiviral treatment with sofosbuvir and simeprevir in a kidney transplant recipient with HCV-decompensated cirrhosis: viral eradication and removal from the liver transplant waiting list. *Transpl Int* 2015; **28**: 1345-1349 [PMID: 26073850 DOI: 10.1111/tri.12622]
- 27 **Massie AB**, Caffo B, Gentry SE, Hall EC, Axelrod DA, Lentine KL, Schnitzler MA, Gheorghian A, Salvalaggio PR, Segev DL. MELD Exceptions and Rates of Waiting List Outcomes. *Am J Transplant* 2011; **11**: 2362-2371 [PMID: 21920019 DOI: 10.1111/j.1600-6143.2011.03735.x]
- 28 **Massie AB**, Chow EK, Wickliffe CE, Luo X, Gentry SE, Mulligan DC, Segev DL. Early changes in liver distribution following implementation of Share 35. *Am J Transplant* 2015; **15**: 659-667 [PMID: 25693474 DOI: 10.1111/ajt.13099]
- 29 **Fornis X**, Charlton M, Denning J, McHutchison JG, Symonds WT, Brainard D, Brandt-Sarif T, Chang P, Kivett V, Castells L, Prieto M, Fontana RJ, Baumert TF, Coilly A, Londoño MC, Habersetzer F. Sofosbuvir compassionate use program for patients with severe recurrent hepatitis C after liver transplantation. *Hepatology* 2015; **61**: 1485-1494 [PMID: 25557906 DOI: 10.1002/hep.27681]
- 30 **Charlton M**, Gane E, Manns MP, Brown RS, Curry MP, Kwo PY, Fontana RJ, Gilroy R, Teperman L, Muir AJ, McHutchison JG, Symonds WT, Brainard D, Kirby B, Dvory-Sobol H, Denning J, Arterburn S, Samuel D, Fornis X, Terrault NA. Sofosbuvir and ribavirin for treatment of compensated recurrent hepatitis C virus infection after liver transplantation. *Gastroenterology* 2015; **148**: 108-117 [PMID: 25304641 DOI: 10.1053/j.gastro.2014.10.001]
- 31 **Patwardhan VR**, Curry MP. Reappraisal of the hepatitis C virus-positive donor in solid organ transplantation. *Curr Opin Organ Transplant* 2015; **20**: 267-275 [PMID: 25944236 DOI: 10.1097/MOT.0000000000000191]
- 32 **Northup PG**, Argo CK, Nguyen DT, McBride MA, Kumer SC, Schmitt TM, Pruett TL. Liver allografts from hepatitis C positive donors can offer good outcomes in hepatitis C positive recipients: a US National Transplant Registry analysis. *Transpl Int* 2010; **23**: 1038-1044 [PMID: 20444239 DOI: 10.1111/j.1432-2277.2010.01092.x]
- 33 **Charlton M**, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS, Fried MW, Terrault NA, O'Leary JG, Vargas HE, Kuo A, Schiff E, Sulkowski MS, Gilroy R, Watt KD, Brown K, Kwo P, Pungpapong S, Korenblat KM, Muir AJ, Teperman L, Fontana RJ, Denning J, Arterburn S, Dvory-Sobol H, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal N. Ledipasvir and sofosbuvir Plus Ribavirin for Treatment of HCV Infection in Patients With Advanced Liver Disease. *Gastroenterology* 2015; **149**: 649-659



- [PMID: 25985734 DOI: 10.1053/j.gastro.2015.05.010]
- 34 **Maor-Kendler Y**, Batts KP, Burgart LJ, Wiesner RH, Krom RA, Rosen CB, Charlton MR. Comparative allograft histology after liver transplantation for cryptogenic cirrhosis, alcohol, hepatitis C, and cholestatic liver diseases. *Transplantation* 2000; **70**: 292-297 [PMID: 10933151 DOI: 10.1097/00007890-200007270-00009]
  - 35 **Sperl J**, Petrasek J, Spicak J, Viklicky O. Acute rejection of non-functional allograft in kidney transplant recipients with hepatitis C treated with peginterferon-alpha 2a. *J Hepatol* 2008; **49**: 461-462; author reply 462-463 [PMID: 18644649 DOI: 10.1016/j.jhep.2008.06.002]
  - 36 **Ghobrial RM**, Farmer DG, Baquerizo A, Colquhoun S, Rosen HR, Yersiz H, Markmann JF, Drazan KE, Holt C, Imagawa D, Goldstein LI, Martin P, Busuttil RW. Orthotopic liver transplantation for hepatitis C: outcome, effect of immunosuppression, and causes of retransplantation during an 8-year single-center experience. *Ann Surg* 1999; **229**: 824-831; discussion 831-833 [PMID: 10363896 DOI: 10.1097/0000658-199906000-00009]

**P- Reviewer:** Kubota K, Zeng Z **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Liu SQ



Observational Study

## Adipokines, cytokines and body fat stores in hepatitis C virus liver steatosis

Emilio González-Reimers, Javier López-Prieto, Geraldine Quintero-Platt, Ricardo Pelazas-González, M Remedios Alemán-Valls, Onán Pérez-Hernández, M José de-la-Vega-Prieto, M Angeles Gómez-Rodríguez, Candelaria Martín-González, Francisco Santolaria-Fernández

Emilio González-Reimers, Javier López-Prieto, Geraldine Quintero-Platt, Ricardo Pelazas-González, M Remedios Alemán-Valls, Onán Pérez-Hernández, M José de-la-Vega-Prieto, M Angeles Gómez-Rodríguez, Candelaria Martín-González, Francisco Santolaria-Fernández, Servicio de Medicina Interna, Hospital Universitario de Canarias, Universidad de La Laguna, 38320 Canary Islands, Spain

**Author contributions:** González-Reimers E, López-Prieto J, Quintero-Platt G, Pelazas-González R, Alemán-Valls MR, Pérez-Hernández O, Martín-González C contributed to study conception and design; González-Reimers E, López-Prieto J, Quintero-Platt G, Alemán-Valls MR and Santolaria-Fernández F contributed to data acquisition, data analysis and interpretation, and writing of the article; López-Prieto J, Pelazas-González R and Pérez-Hernández O contributed to histomorphometrical analysis of the liver biopsies; de-la-Vega-Prieto MJ contributed to determination of cytokines and adipokines; Gómez-Rodríguez MA contributed to data acquisition and analysis of body composition by densitometry.

**Institutional review board statement:** The study was reviewed and approved by the Ethics Committee of the Hospital Universitario de Canarias (PI/07) and the Institutional Review Board from the third cycle studies of the University of la Laguna.

**Informed consent statement:** All the patients provided written informed consent before starting the study procedures.

**Conflict-of-interest statement:** The authors declare that there is no conflict of interest regarding this manuscript. No funding or institutional grants have been received for this study.

**Data sharing statement:** They are available upon request by emailing [egonrey@ull.es](mailto:egonrey@ull.es). There is no written informed consent for data sharing, but data are anonymized.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license,

which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Emilio González-Reimers, PhD, Servicio de Medicina Interna, Hospital Universitario de Canarias, Universidad de La Laguna, Ofra s/n, San Cristóbal de La Laguna, Tenerife, 38320 Canary Islands, Spain. [egonrey@ull.es](mailto:egonrey@ull.es)  
 Telephone: +34-92-2678600

Received: July 30, 2015

Peer-review started: July 31, 2015

First decision: September 29, 2015

Revised: October 19, 2015

Accepted: December 18, 2015

Article in press: December 21, 2015

Published online: January 8, 2016

### Abstract

**AIM:** To identify patients with or without liver steatosis and its severity in treatment-naïve patients affected by hepatitis C virus (HCV) infection.

**METHODS:** We included 56 HCV infected patients, and assessed the amount of liver fat by histomorphometry, and its relationships with fat and lean mass at different parts of the body (by densitometry), hormones [insulin, homeostatic model assessment (HOMA)], adipokines (resistin, adiponectin, leptin), and cytokines (tumor necrosis factor  $\alpha$ , interleukin-6).

**RESULTS:** Although the intensity of liver steatosis is related to trunk fat mass and HOMA, 33% of patients showed no liver steatosis, and this finding was not related to body mass index or genotype. Besides trunk

fat mass, no other factor was related to the presence or not of liver steatosis, or to the intensity of it, by multivariate analysis. Lean mass was not related to liver steatosis. Adiponectin levels were lower among patients. No differences were observed in leptin and resistin.

**CONCLUSION:** Steatosis in HCV infection is common (67.2%), and closely related to trunk fat, and insulin resistance, but not with leg fat mass or adipokines.

**Key words:** Resistin; Adiponectin; Insulin resistance; Proinflammatory cytokines; Leptin; Hepatitis C virus; Liver steatosis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Pathogenesis of liver steatosis in hepatitis C virus (HCV) infection is complex and is not fully understood. For unknown reasons some patients, despite having a high body mass index (BMI), do not develop liver steatosis, whereas others with normal BMI develop intense liver fat deposition. We analyse if body fat and lean mass composition, insulin resistance and adipokine profile may help to identify patients with or without liver steatosis and its severity in treatment-naïve HCV patients. Multivariate analysis showed that only trunk fat mass and insulin resistance were independently related to liver steatosis assessed on histomorphometrical grounds and its severity.

González-Reimers E, López-Prieto J, Quintero-Platt G, Pelazas-González R, Alemán-Valls MR, Pérez-Hernández O, de-la-Vega-Prieto MJ, Gómez-Rodríguez MA, Martín-González C, Santolaria-Fernández F. Adipokines, cytokines and body fat stores in hepatitis C virus liver steatosis. *World J Hepatol* 2016; 8(1): 74-82 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i1/74.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i1.74>

## INTRODUCTION

Non-alcoholic steatohepatitis is observed in several clinical conditions, especially diabetes and obesity. In steatohepatitis hepatocytes become laden with fat droplets that elicit an inflammatory response which may evolve to liver cirrhosis and hepatocarcinoma. In diabetes and obesity insulin deficiency and/or resistance lead to increased mobilization of fatty acids from adipose tissue to liver. In chronic hepatitis C virus (HCV) infection, steatosis and steatohepatitis are also observed and the pathogenesis is based on complex mechanisms: although HCV by itself especially genotype 3 may lead to liver steatosis, obesity and concomitant alcohol abuse are the main factors involved<sup>[1]</sup>. However, many HCV infected patients do not drink alcohol at all, but they may develop liver steatosis. Cytokine activation and increased lipid peroxidation may contribute both to liver steatosis and to the progression of simple liver steatosis

to steatohepatitis<sup>[2]</sup>.

The main source of liver fat accumulation is body fat stores<sup>[3]</sup>. In this scenario, fat tissue is not only the source of fatty acids, but also produces several proinflammatory cytokines which are of paramount importance in the progression of liver disease. However, adipose tissue is heterogeneous. For instance, trunk fat is associated with increased insulin resistance and an increased vascular risk<sup>[4]</sup>, whereas leg fat exerts opposite effects<sup>[5]</sup>, probably due to secretion of a different cytokine profile.

The association of liver steatosis with distribution of fat stores at different parts of the body in chronic HCV infection is not well known. This is an important issue, since the heterogeneous nature of fat tissue may lead to different adipokine secretion<sup>[6]</sup>. In fact, notable controversy exists regarding serum levels of different adipokines, such as adiponectin<sup>[7-9]</sup> or leptin<sup>[10,11]</sup> and their relationship with histological changes in chronic HCV infection. In a previous report which analysed a series of patients (different from those included in this study) we found that an increased waist circumference (> 102 cm for men and > 88 cm among women) was related to increased liver fat, but we also found that 38.8% of non-obese patients also showed intense fatty infiltration<sup>[12]</sup>, a result in accordance with other researchers, who have reported fatty liver among lean individuals<sup>[13]</sup>. Conversely, some HCV infected patients do not show liver steatosis, regardless of their body mass index. The mechanisms that underlie the lack of association in some cases between liver fat and body fat stores are unclear.

On the other hand, in a recent Indian study in a cohort of patients with steatohepatitis, 13% were lean patients<sup>[14]</sup>, and sarcopenia has been described as an independent risk factor for steatohepatitis<sup>[15]</sup>. In addition it has been shown that interleukin-6 (IL-6) a protean cytokine also produced by muscle<sup>[16]</sup> strongly modulates liver fat accumulation<sup>[17]</sup>. Therefore, given these observations, it is important to also analyse the relationship between lean mass and liver steatosis.

Based on these facts, in the present study we analyse the association of the degree of liver steatosis with fat and lean mass stores at different parts of the body, insulin resistance, and serum adipokine levels, in treatment-naïve patients affected by HCV infection. Since we have assessed liver steatosis on histological grounds, we also look for differences in cytokine and adipokine profile, fat and lean mass distribution among HCV patients who did not show liver steatosis and those who did, in order to shed light on the reasons why some HCV patients do not develop liver steatosis.

## MATERIALS AND METHODS

### Patients

We included 56 patients with (19 women) HCV infection, aged  $41.54 \pm 9.57$  years. Diagnostic criteria for HCV infection were the following: (1) presence of anti-HCV and/or HCV RNA by reverse transcriptase polymerase

**Table 1** Differences in biochemical variables, body mass index and total lean and total fat area between patients and controls

	Patients		Controls		
	<i>n</i>	<i>X</i> ± <i>SD</i> , median ( <i>IQ</i> range)	<i>n</i>	<i>X</i> ± <i>SD</i> , median ( <i>IQ</i> range)	
Insulin (μU/mL)	44	12.48 ± 15.65, 7.89 (4.63-14.32)	10	8.34 ± 4.34, 7.15 (5.08-10.63)	<i>Z</i> = 0.43; NS
Resistin (ng/mL)	44	4.97 ± 1.76, 4.90 (3.98-5.60)	10	4.28 ± 1.42, 4.97 (3.32-5.29)	<i>Z</i> = 0.88; NS
Adiponectin (ng/mL)	44	11.99 ± 8.30, 9.54 (6.04-17.16)	16	24.92 ± 21.84, 19.05 (13.53-21.58)	<i>Z</i> = 3.18; <i>P</i> = 0.001
Leptin (ng/mL)	44	12.25 ± 15.83, 4.23 (1.15-17.78)	10	18.41 ± 16.03, 12.89 (4.65-34.42)	<i>Z</i> = 1.78; NS
Tumor necrosis factor-α (pg/mL)	56	10.65 ± 4.14, 10.18 (7.15-13.08)	19	6.05 ± 1.90, 5.20 (4.40-8.00)	<i>Z</i> = 4.56; <i>P</i> < 0.001
Interleukin-6 (pg/mL)	53	4.28 ± 4.75, 2.0 (2.0-4.29)	19	5.90 ± 1.64, 5.0 (5.0-6.60)	<i>Z</i> = 2.97; <i>P</i> = 0.003
Body mass index (kg/m <sup>2</sup> )	56	24.19 ± 3.44	19	25.20 ± 3.42	<i>t</i> = 1.02; NS
Total fat mass (g)	50	19929 ± 11944	19	21443 ± 6393	<i>t</i> = 0.54; NS
Total lean mass (g)	50	48284 ± 8848	19	50131 ± 15796	<i>t</i> = 0.64; NS

Comparisons were made using non-parametric tests, such as Mann-Whitney's *U* test (*Z*) or parametric ones (Student's *t*-test). NS: Not significant.

chain reaction; and (2) Histology consistent with HCV. Most patients (43) were infected by HCV type 1 genotype, 5 by type 3 genotype, and 8 by type 4. All patients were recruited before treatment for virus C hepatitis was administered, and none of them were active drinkers. Liver function was still preserved: Liver function tests were normal, and none of them showed ascites or encephalopathy.

### Nutritional evaluation

After informed consent was obtained, 51 patients underwent assessment of fat and lean mass at different parts of the body, such as right and left arm, trunk, right and left leg, and total body, with a LUNAR PRODIGY ADVANCE device, General Electric, Piscataway, NJ, United States. We further calculated (using the protocol established by other authors<sup>[18]</sup>) the trunk fat/(right leg + left leg fat) index, as well as the indices fat mass/lean mass at each of the body compartments mentioned before. Body mass index [BMI, as weight (kg)/height (m)<sup>2</sup>] was also recorded.

### Biochemical assessment

Blood samples were taken at 8:00 am in fasting conditions. Routine laboratory evaluation was performed and these analyses included, among others, prothrombin activity, serum albumin and bilirubin. Samples were immediately frozen at -20 °C. We determined the following parameters-IL-6, by chemiluminescent assay interassay variation coefficient ranging 5.3%-7.5%, recovery = 85%-104%, diagnostic products corporation (DPC), Los Angeles, CA, United States; tumour necrosis factor α (TNF-α) by immunometric chemiluminescent assay (intra-assay variation coefficient ranging 4%-6.5%, interassay variation coefficient ranging 2.6%-3.6%, recovery 92%-112%, DPC, Los Angeles, CA, United States). We also determined serum insulin, by immunoanalysis (Chemiflex); interobserver variation coefficient = 1.9%-5.2%; intraobserver variation coefficient = 1.7%-4.2%; sensitivity = 1 μU/mL; recovery = 91.1%-101.6%; (Architect system, Abbott, Wiesbaden Germany), serum resistin, by ELISA (sensitivity = 0.033 ng/mL; intra-assay variation coefficient = 2.8%-3.4%; interassay variation coefficient ranging 5.1%-6.9%,

recovery = 85.2%-99.2%, Biovendor, Heidelberg, Germany), serum leptin, by ELISA (sensitivity = 0.2 ng/mL; intra-assay variation coefficient = 4.2%-7.6%; interassay variation coefficient ranging 4.4%-6.7%, recovery = 85.7%-98.0%, Biovendor, Heidelberg, Germany); serum adiponectin by ELISA (sensitivity = 26 ng/mL; intra-assay variation coefficient = 3.9%-5.9%; interassay variation coefficient ranging 6.3%-7%, recovery = 92.4%-102.9%, Biovendor, Heidelberg, Germany); insulin resistance was estimated by the homeostatic model assessment (HOMA).

Cytokine values were compared with those of a control group composed of 19 healthy hospital workers, seven of them women, aged 40.45 ± 3.57 years. As shown in Table 1, not all the variables were determined in all patients and controls.

All these data were recorded the day at which the patients underwent a liver biopsy before receiving active treatment against HCV infection.

### Histological assessment

The degree of liver steatosis was determined using software based on histomorphometry (LEICAQWin, version 3.0, Wetzlar, Germany). The specimens were stained with haematoxylin-eosin and Masson trichromic and were viewed at 40 ×. This protocol has been previously described<sup>[12]</sup>. The proportion of fatty area to total area in specimens was recorded. The Knodell index and the total amount of fibrous tissue determined by histomorphometry (using Masson trichromic stain) were also measured.

The study protocol was approved by the local ethical committee of our Hospital. All patients included gave their informed consent prior to their inclusion in the study, and the study conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

### Statistics analysis

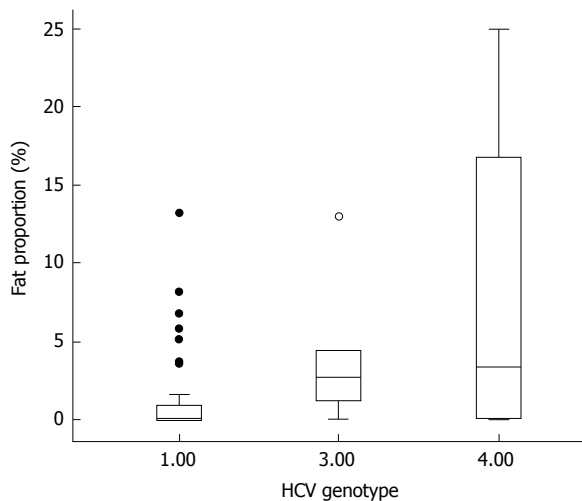
We tested for normal distribution using the Kolmogorov-Smirnov test. In order to compare means between two groups or between three or more groups, we used Student's *t* test and ANOVA, respectively. If the variables did not show a normal distribution, Mann-Whitney's *U* and Kruskal-Wallis tests were used to compare



**Table 2** Anthropometric measurements in patients with marked or less marked liver steatosis

	Steatosis over the median		Steatosis below the median		
	<i>n</i>	<i>X</i> ± <i>SD</i>	<i>n</i>	<i>X</i> ± <i>SD</i>	
Left arm fat mass (g)	27	1345.04 ± 871.98	24	783.30 ± 577.62	<i>t</i> = 2.68; <i>P</i> = 0.01
Right arm fat mass (g)	27	1396.97 ± 1084.44	24	852.37 ± 827.60	<i>t</i> = 2.00; <i>P</i> = 0.05
Trunk fat mass (g)	27	12673.68 ± 6077.05	24	7939.57 ± 5027.19	<i>t</i> = 3.01; <i>P</i> = 0.004
Left leg fat mass (g)	27	3919.72 ± 2533.87	24	2683.13 ± 2018.77	<i>t</i> = 1.91; NS
Right leg fat mass (g)	27	3948.29 ± 2626.75	24	2805.25 ± 1905.64	<i>t</i> = 1.76; NS
Total body fat mass (g)	27	23981.22 ± 12381.84	24	15733.66 ± 9812.41	<i>t</i> = 2.61; <i>P</i> = 0.012
Left arm lean mass (g)	26	2769.64 ± 783.77	24	2899.09 ± 941.35	<i>t</i> = 0.53; NS
Right arm lean mass (g)	26	2749.02 ± 819.68	24	3064.65 ± 1649.64	<i>t</i> = 0.87; NS
Trunk lean mass (g)	26	24458.49 ± 4791.16	24	23122.30 ± 4155.06	<i>t</i> = 1.05; NS
Left leg lean mass (g)	26	7469.77 ± 1684.70	24	7011.75 ± 1945.39	<i>t</i> = 0.89; NS
Right leg lean mass (g)	26	7651.20 ± 1664.56	24	7404.65 ± 1444.64	<i>t</i> = 0.56; NS
Total lean mass (g)	26	48592.11 ± 9723.42	24	47309.62 ± 8352.70	<i>t</i> = 0.50; NS
Body mass index (kg/m <sup>2</sup> )	28	25.55 ± 2.51	27	22.79 ± 3.76	<i>t</i> = 2.61; <i>P</i> = 0.012
Trunk fat/legs fat	27	1.85 ± 0.87	24	1.56 ± 0.42	<i>t</i> = 1.49; NS

NS: Not significant.

**Figure 1** Fat amount among the three hepatitis C virus genotypes included in this study. Patients with genotype 1 (the most frequent) show significantly less amount of fat than patients affected by genotype 3 or 4 (solid circles are outliers, and hollow circle, extreme values). HCV: Hepatitis C virus.

means. Correlations between quantitative variables were established using Spearman's *r* and Pearson's *r*. The  $\chi^2$  test was used to compare qualitative variables. We performed stepwise multiple regression analysis to establish which parameters liver steatosis depends on. All statistical analyses were performed using SPSS software (Chicago, Ill., United States).

## RESULTS

Liver steatosis was observed in 42 patients out of 56; in the remaining 14 patients, no steatosis at all was observed, and in 4 more, only very few small isolated fat droplets could be observed (fat amount < 0.05%). Median value of liver fat area was 0.20%, but 14 patients showed more than 5% of fat in their biopsies. Patients with genotype 1 showed significantly less steatosis than those with genotype 3 or 4 ( $Z = 2.17$ ;  $P$

= 0.03; Figure 1). Indeed, as shown in Figure 1, patients with genotype 3 or 4 showed higher values of liver fat (fat proportion = 6.66% ± 8.42%) when compared with those with genotype 1 (fat proportion = 1.40% ± 2.78%). Only 1 (out of 5) genotype 3 patient showed no steatosis at all, compared with 13 (out of 51) affected by non-3 genotype infection, but this association was not statistically significant ( $\chi^2 = 0.07$ ). No differences in liver fat were observed when HIV-coinfected patients were compared with non-co-infected ones ( $Z = 0.40$ ;  $P = 0.694$ ). Seven patients were diabetics, but although they showed a trend to more intense liver steatosis (6.66% ± 9.68%) than non-diabetics (2.05% ± 3.97%), this difference was not significant ( $Z = 1.31$ ;  $P > 0.20$ ). None of the diabetics showed no fat in their livers, but association between diabetes/no diabetes and presence or not of liver steatosis was not significant ( $P = 0.17$  by exact Fisher's test). No association was observed between viral load and proportion of liver fat.

Median proportion of fibrosis was 5.75% (interquartile range = 3.53%-8.88%). Twenty-one patients showed a Knodell index higher than 5, whereas 35 showed a Knodell index below 6.

### Relationship of liver steatosis with nutritional status

Patients with marked steatosis (over the median) showed increased BMI and greater fat mass, especially at the trunk ( $t = 3.01$ ,  $P = 0.004$ ), as shown in Table 2. In addition to the finding of a significantly higher BMI among those with liver steatosis over the median (Table 2), we also found that patients with BMI over 25 kg/m<sup>2</sup> had significantly more liver fat ( $Z = 2.25$ ;  $P = 0.031$ ). Only 22 patients were overweight, and only 3 of them were obese (BMI > 30 kg/m<sup>2</sup>). Three patients who were overweight showed no fat at all in their liver biopsies, vs 11 out of 33 with normal weight. This association was not statistically significant. Significant relationships were observed between fat parameters and liver steatosis, especially with trunk fat ( $r = 0.42$ ;  $P = 0.002$ ), right

**Table 3** Biochemical variables in patients with steatosis over the median or below the median

	Steatosis over the median		Steatosis below the median		
	<i>n</i>	<i>X</i> ± <i>SD</i> , median (IQ range)	<i>n</i>	<i>X</i> ± <i>SD</i> , median (IQ range)	
Insulin (μU/mL)	24	15.30 ± 19.42, 11.20 (6.59-16.46)	20	9.09 ± 8.72, 7.30 (3.87-11.62)	<i>Z</i> = 1.89; <i>P</i> = 0.059
HOMA	24	1645.68 ± 2828.28, 1068 (644-1524)	20	825.01 ± 801.41, 645.5 (327.8-1082.0)	<i>Z</i> = 2.15; <i>P</i> = 0.03
Resistin (ng/mL)	24	4.66 ± 0.96, 4.87 (4.19-5.37)	20	5.34 ± 2.38, 5.03 (3.88-6.12)	<i>Z</i> = 1.03; NS
Adiponectin (ng/mL)	24	11.77 ± 6.92, 11.18 (5.45-17.62)	20	12.26 ± 9.90, 8.38 (6.04-16.65)	<i>Z</i> = 0.21; NS
Leptin (ng/mL)	24	10.85 ± 12.45, 6.23 (1.35-17.20)	20	13.92 ± 19.35, 2.72 (0.79-32.89)	<i>Z</i> = 0.79; NS
Tumor necrosis factor-α (pg/mL)	28	11.31 ± 4.90, 11.20 (6.84-14.18)	28	10.00 ± 3.17, 9.56 (7.19-12.75)	<i>Z</i> = 1.00; NS
Interleukin (pg/mL)	25	5.06 ± 5.17, 2.0 (2.0-5.94)	28	3.59 ± 4.31, 2.0 (2.0-2.5)	<i>Z</i> = 1.14; NS
Cholesterol (mg/dL)	28	167 ± 36.82	28	174.2 ± 45.56	<i>t</i> = 0.65; NS
LDL cholesterol (mg/dL)	27	95.04 ± 34.17	28	103.86 ± 36.48	<i>t</i> = 1.01; NS
HDL cholesterol (mg/dL)	28	46.71 ± 14.87	28	42.86 ± 13.82	<i>t</i> = 0.92; NS
Triglycerides (mg/dL)	28	136.25 ± 114.27	28	145.96 ± 93.04	<i>t</i> = 0.35; NS

Comparisons were made using non-parametric tests, such as Mann-Whitney's *U* test (*Z*). NS: Not significant.

arm fat ( $r = 0.31$ ;  $P = 0.029$ ), left arm fat ( $r = 0.30$ ;  $P = 0.033$ ), and total fat ( $r = 0.34$ ;  $P = 0.016$ ). The significant relationship between liver steatosis and trunk fat was observed both among women ( $r = 0.50$ ;  $P = 0.04$ ) and men ( $r = 0.41$ ;  $P = 0.016$ ). In a similar way, BMI was related to liver steatosis both among women ( $r = 0.53$ ;  $P = 0.02$ ) and men ( $r = 0.36$ ;  $P = 0.032$ ). However, while liver steatosis was related to arm and leg fat mass among both women and men, the correlations were not statistically significant, possibly due to the relatively low number of cases. No relationship was observed between parameters related to lean mass and liver steatosis, but when the indices fat mass/lean mass were compared with liver steatosis, the results were similar to those obtained with fat parameters ( $r = 0.39$ ;  $P = 0.006$  for the trunk,  $r = 0.34$ ;  $P = 0.017$  for the left arm,  $r = 0.32$ ;  $P = 0.026$  for the right arm, and  $r = 0.34$ ;  $P = 0.016$  for total fat). Remarkably, no association was observed when leg fat mass was compared with liver steatosis. The ratio trunk fat/legs fat was not significantly different among patients with liver steatosis below or above the median. A significant correlation was observed between liver steatosis and BMI ( $r = 0.41$ ;  $P = 0.002$ ).

Trunk fat was the only variable that was selected ( $P = 0.011$ ) when a logistic regression analysis was done searching for the factors related to liver fat over or below the median values.

Similar results relative to fat mass at different parts of the body were observed when patients without liver steatosis (including those 4 with minimal steatosis) were compared with the remaining patients, although differences were less significant ( $t = 2.73$ ,  $P = 0.009$  for trunk fat,  $t = 2.34$ ,  $P = 0.023$  for left arm fat,  $t = 2.31$ ;  $P = 0.025$  for right arm fat) than when patients were classified according to the median values of liver fat. BMI was also significantly lower among those without liver steatosis ( $t = 2.43$ ;  $P = 0.023$ ). No differences at all were observed regarding lean mass variables. As with steatosis below or above the median, the only selected variable was trunk fat ( $P = 0.015$ ) when a logistic regression was performed to discern which

variables were independently related to the presence or absence of liver fat infiltration.

No associations were observed between the proportion of fibrosis in liver biopsy and any of the nutritional variables, but Knodell index was related both to fat mass variables (total fat,  $r = 0.37$ ;  $P = 0.007$ ; trunk fat,  $r = 0.32$ ;  $P = 0.024$ ); left arm and right arm fat,  $r = 0.47$  and  $r = 0.45$ ; respectively,  $P < 0.001$ ; left leg and right leg, ( $r = 0.31$  and  $r = 0.28$ , respectively,  $P < 0.05$  in both cases), as well as to some lean mass variables (trunk lean mass,  $r = 0.35$ ;  $P = 0.012$ ; left leg lean mass,  $r = 0.30$ ,  $P = 0.034$ ).

#### Relationship of liver steatosis with insulin resistance and adipokines

No differences were observed in any of the adipokines, HOMA, insulin, TNF-α, or IL-6 among patients with or without liver steatosis. Only HOMA, out of these parameters, was significantly higher among patients with liver fat over the median compared with those with liver fat below the median ( $Z = 2.15$ ;  $P = 0.032$ ); a similar trend that was not statistically significant ( $P = 0.059$ ) was observed with insulin (Tables 3 and 4, Figure 2).

Significant relationships were observed between liver steatosis (proportion of fat) and HOMA index ( $r = 0.30$ ;  $P = 0.046$ ). Serum insulin ( $r = 0.44$ ;  $P = 0.003$ ) and HOMA ( $r = 0.36$ ;  $P = 0.017$ ) were directly related to Knodell index, whereas no associations were observed between any of the adipokines and cytokines and the amount of fibrosis in the liver biopsies. Selecting only those patients with liver steatosis, a significant correlation was observed between IL-6 and amount of liver fat ( $r = 0.49$ ;  $P = 0.003$ ).

After introducing in a multiple regression analysis the fat variables which showed a significant relationship with liver steatosis in the univariate analysis, only trunk fat (beta = 0.37;  $P = 0.026$ ) was independently related to the amount of liver fat. In a similar way, trunk fat was the only selected variable when a logistic regression analysis was done searching for the factors related to liver fat over or below the median values (Table 5).

**Table 4** Correlations between body composition parameters and adipokines, proinflammatory cytokines and insulin resistance

	Leptin	Adipo-nectin	Insulin	HOMA	TNF- $\alpha$	IL-6	Resistin
Trunk fat	$\rho = 0.61, P < 0.001$		$\rho = 0.56, P < 0.001$	$\rho = 0.55, P < 0.001$			
Left leg fat	$\rho = 0.70, P < 0.001$		$\rho = 0.44, P = 0.005$	$\rho = 0.44, P = 0.005$			
Right leg fat	$\rho = 0.62, P < 0.001$		$\rho = 0.42, P = 0.006$	$\rho = 0.42, P = 0.006$			
Right arm fat	$\rho = 0.40, P = 0.011$		$\rho = 0.58, P < 0.001$	$\rho = 0.57, P < 0.001$			
Left arm fat	$\rho = 0.51, P < 0.001$		$\rho = 0.62, P < 0.001$	$\rho = 0.63, P < 0.001$			
Total fat	$\rho = 0.64, P < 0.001$		$\rho = 0.54, P < 0.001$	$\rho = 0.53, P < 0.001$			
Total lean		$\rho = -0.35, P = 0.032$			$\rho = -0.31, P = 0.029$		
Left arm lean		$\rho = -0.37, P = 0.02$			$\rho = -0.33, P = 0.021$		
Right arm lean		$\rho = -0.37, P = 0.02$			$\rho = -0.29, P = 0.04$		
Left leg lean							
Trunk lean		$\rho = -0.34, P = 0.021$			$\rho = -0.33, P = 0.021$	$\rho = -0.34, P = 0.018$	
Right leg lean					$\rho = -0.29, P = 0.039$		
Total fat/total lean	$\rho = 0.65, P < 0.001$		$\rho = -0.49, P = 0.001$	$\rho = 0.49, P = 0.001$			
Trunk fat/trunk lean	$\rho = 0.63, P < 0.001$		$\rho = -0.31, P = 0.036$	$\rho = 0.52, P < 0.001$		$\rho = 0.31, P = 0.036$	
Right arm fat/right arm lean	$\rho = 0.60, P < 0.001$		$\rho = 0.55, P < 0.001$	$\rho = 0.53, P < 0.001$			
Left arm fat/left arm lean	$\rho = 0.69, P < 0.001$		$\rho = 0.58, P = 0.001$	$\rho = 0.57, P < 0.001$			
Right leg fat/right leg lean	$\rho = 0.66, P < 0.001$		$\rho = 0.38, P = 0.016$	$\rho = 0.39, P = 0.014$			
Left leg fat/left leg lean	$\rho = 0.69, P < 0.001$		$\rho = 0.58, P < 0.001$	$\rho = 0.57, P < 0.001$			
High density lipoprotein cholesterol		$\rho = 0.56, P < 0.001$					$\rho = -0.41, P = 0.012$
Low density lipoprotein cholesterol	$\rho = 0.31, P = 0.046$						

Only the significant relationships are provided (Spearman's  $\rho$  test). TNF- $\alpha$ : Tumor necrosis factor  $\alpha$ ; IL-6: Interleukin-6.

**Table 5** Results of the logistic regression analysis performed in order to look for which parameters were independently associated with liver steatosis

		B	E.T.	Wald	Gl	Sig.	Exp (B)
Step 1	Trunk fat	0.000	0.000	6.157	1	0.013	1.000
	Constant	1.530	0.751	4.147	1	0.042	4.618

E.T.: Standard error; Gl: df (degrees of freedom); Sig.: Significance; Exp (B): Odd ratio.

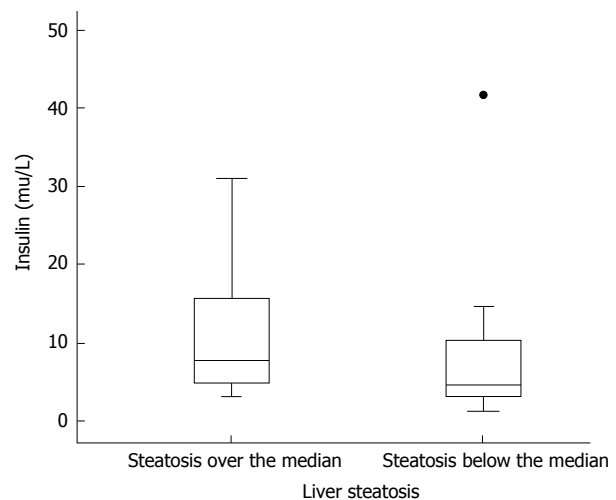
### Relationships of nutritional variables with insulin resistance and adipokines

Leptin, insulin and HOMA were strongly and directly related to fat parameters, as shown in Table 4 ( $r > 0.40$  in all the cases;  $P < 0.006$ ), but not to lean mass. On the contrary, adiponectin and TNF- $\alpha$  were inversely related to most of the lean mass parameters. Adiponectin was also inversely related to the trunk fat mass/leg fat mass index ( $r = -0.33$ ;  $P = 0.037$ ).

The fat/lean indices were also strongly related to leptin, insulin and HOMA, and also, to IL-6, in this last case only with the trunk fat/trunk lean mass index. No associations were observed between serum resistin and nutritional parameters (Table 4).

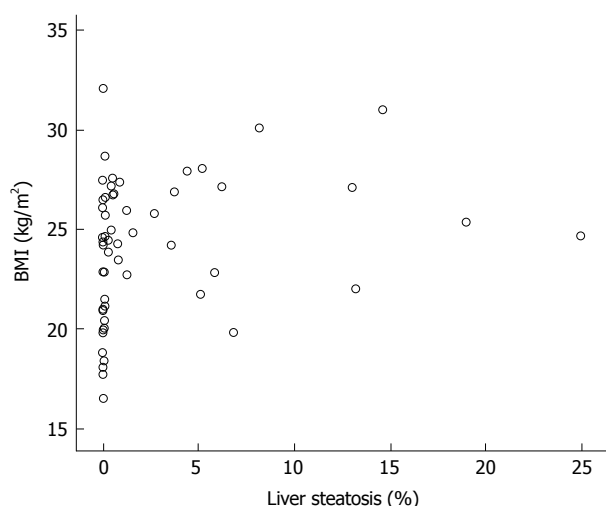
## DISCUSSION

We have found that liver steatosis is frequent among



**Figure 2** Serum insulin levels among patients with liver steatosis over the median (left) and below the median (right). Differences are not statistically significant, but there is a trend to higher values among patients with intense steatosis ( $P = 0.059$ ). Solid circle represents an outlier.

patients with HCV infection (67.86%), even surpassing the prevalence data (about 50%) reported by other authors<sup>[19]</sup>. This high proportion of patients with steatosis was observed despite a BMI that was not different - even slightly lower- than that of a control population. However, as expected, liver steatosis showed a significant relationship with BMI, but it is noteworthy that



**Figure 3** Scattergram showing the relation of body mass index with the amount of liver steatosis. Despite a significant relationship between both variables ( $\rho = 0.41$ ;  $P = 0.002$ ), as shown, some patients with BMI over 30 show no steatosis at all or only minimal amount of liver fat, in contrast with some others with BMI below 25 and marked steatosis in their livers. BMI: Body mass index.

some cases showed only minimal steatosis despite the fact that the patient was overweight. Some other cases showed considerable liver fat accumulation despite low BMI values (Figure 3), suggesting that factors other than BMI are involved in liver fat accumulation. This result is similar to that obtained by our group six years ago, in a different cohort of patients, in whom adiposity was assessed by waist circumference, triceps skinfold measurement, and BMI<sup>[12]</sup>.

We have also shown that liver steatosis in HCV-infected patients is associated with trunk fat. This has been also reported by other authors<sup>[20,21]</sup>, since, as mentioned above, it is generally accepted that trunk fat is associated with a more “noxious” adipokine secretion profile that is able to cause insulin resistance and a proinflammatory state. The opposite happens with peripheral fat. In this sense, we failed to find any relationship between liver steatosis and leg fat mass, as shown in Table 2. Therefore, in sharp contrast with trunk fat, which was clearly related to liver steatosis, liver fat accumulation seems to be independent of leg fat mass.

Regarding adipokines, adiponectin levels were significantly lower among patients than among controls, despite a similar BMI. Adiponectin was inversely related to lean mass, but not to fat mass or liver steatosis. However, it is important to highlight the inverse relationship between the trunk fat/leg fat ratio and adiponectin, fully in accordance with the observation of an inverse relationship between visceral fat and adiponectin levels in other settings<sup>[22]</sup>. Although there is little doubt about the protective role of adiponectin in steatohepatitis (it has been described that adiponectin antagonizes the effects of  $\text{TNF-}\alpha$ <sup>[23]</sup>), in the present study, there seems to be no association between adiponectin levels and liver steatosis, despite the fact that their serum levels are lower in HCV patients in comparison to controls.

This is not a universal finding. The studies on the levels of adiponectin in HCV-related steatohepatitis had been controversial<sup>[7-9,24-28]</sup>. It is also remarkable that we found, in accordance with the protective effect of adiponectin on vascular risk, a significant correlation between adiponectin and high density lipoprotein cholesterol ( $\rho = 0.56$ ;  $P < 0.001$ ), as other authors also did<sup>[29]</sup>.

We also failed to find differences in resistin and leptin between patients and controls, or when these adipokines were compared among patients with intense or less intense steatosis. Leptin, a fat derived cytokine, may promote fibrogenesis through up-regulation of  $\text{TGF-}\beta$ <sup>[30]</sup>, but also protects the liver from fat accumulation, by lowering the expression of SREBP-1<sup>[31]</sup>. These nearly opposite effects may explain, perhaps, disparate findings in relation to leptin levels in chronic HCV infection<sup>[32]</sup>. Indeed, there is also controversy regarding the levels of leptin in HCV-related steatohepatitis<sup>[10,11,33-35]</sup>.

Hyperinsulinaemia decreases synthesis of apoB-100, thus preventing very low density lipoproteins formation and leading to liver steatosis. Moreover, transcription of lipoprotein lipase is decreased by  $\text{TNF-}\alpha$ , leading to hypertriglyceridaemia<sup>[36]</sup>. Most of the results observed in this study sustain this hypothesis: We did find hyperinsulinemia and increased HOMA index in patients with more intense steatosis. This result is fully in accordance with the current knowledge, since insulin resistance leads to an ongoing lipolysis that overwhelms the liver capacity to metabolize them.

Genotype 3 infected patients usually show a more intense degree of steatosis, and it has been shown that it exerts a direct cytopathic effect on liver cell leading to steatosis<sup>[37]</sup>. Concordant with this, patients infected with genotype 3 showed a more intense liver steatosis than those genotype non-3 infected ones, but no significant differences were observed in nutritional anthropometric parameters among them. Also, although the number of patients infected with genotype 3 HCV was low, in one case no fat at all was observed in the liver, and this proportion was similar in HCV genotype non-3 patients. In fact, we have failed to find any difference in adipokine and/or cytokine profile between patients without fat and with fat in the liver. The only independent variable related to the intensity of liver steatosis or to the presence of liver steatosis was trunk fat. Lean mass parameters seem to play no role at all, and insulin resistance, assessed by HOMA, and IL-6 levels were also related to liver fat stores in the univariate analysis, being displaced by trunk fat mass in the multivariate analysis.

Therefore, we conclude that steatosis in chronic hepatitis C is a common event (67.86%), and is closely related to trunk fat, but not with leg fat mass; to insulin resistance, and to IL-6. The main factor involved is trunk fat, despite the normal BMI of the patients included in this study, and also despite the fact that at least 12 patients with BMI over 25 kg/m<sup>2</sup> showed no liver steatosis, or minimal amount of it, as shown in Figure 1. The reasons for this finding are unclear, and suggest that



factors other than BMI, HOMA or fat mass should be involved. The results here presented also do not support the hypothesis that lean mass plays a role in liver fat accumulation.

## ACKNOWLEDGMENTS

Authors are indebted to the nurses and staff of the Internal Medicine Unit and Infectious Diseases Section of the Hospital Universitario de Canarias.

## COMMENTS

### Background

Hepatitis C virus (HCV) infection is a common disease, ultimately leads to liver cirrhosis and hepatocarcinoma. Liver steatosis is an early finding in these patients. Mechanisms are poorly understood, although it is known that HCV genotype 3 may lead to steatosis. Possibly, trunk fat and some adipokines may be also involved.

### Research frontiers

There is a lot of controversy regarding the association of main adipokines, such as adiponectin or leptin, with liver steatosis, and their role in the progression of simple steatosis to liver inflammation. In addition, although there is general agreement in the association between obesity and liver steatosis, the relationship between fat distribution at different body compartments is not well defined. Moreover, there are some studies that also suggest a role of lean mass in liver steatosis.

### Innovations and breakthroughs

In this study the authors report that liver steatosis in chronic HCV infection is a common, but not universal event (67.86%). It is closely related to trunk fat and to interleukin (IL)-6, a cytokine that may be produced by trunk fat, but not with fat at the legs, and also to insulin resistance. However, there are still some unexplained results: The relationship between liver steatosis and trunk fat was observed despite the normal body mass index (BMI) of the patients included in this study, and also at least 12 patients with BMI over 25 kg/m<sup>2</sup> showed no liver steatosis, or minimal amount of it. In addition, their results also do not support the hypothesis that lean mass plays a role in liver fat accumulation.

### Applications

This study provides new data relative to the association of liver steatosis with several adipokines and inflammatory cytokines in HCV-infected patients. As mentioned above there is considerable controversy regarding levels of some of these cytokines in HCV-infected patients, and even opposite results have been reported by several groups. In addition, this study underscores the role of trunk fat in liver steatosis, despite normal BMI, and does not support to the hypothesis that lean mass could play a role.

### Terminology

Cytokines are small molecules with protean effects on inflammation and immune response, among many other effects on most organs. Tumor necrosis factor alpha is one of the first cytokines described, initially as the factor responsible for tumor-induced cachexia. IL-6 is a proinflammatory cytokine, that also bears an immunomodulatory effect. Adipokines are cytokines secreted by adipose tissue.

### Peer-review

In this manuscript, the authors described about effects of adipokines, cytokines, and body fats on liver steatosis in hepatitis C patients. The key results are very interesting to the readers of HCV and other hepatic diseases.

## REFERENCES

- 1 Woreta TA, Sutcliffe CG, Mehta SH, Brown TT, Higgins Y,

- Thomas DL, Torbenson MS, Moore RD, Sulkowski MS. Incidence and risk factors for steatosis progression in adults coinfecting with HIV and hepatitis C virus. *Gastroenterology* 2011; **140**: 809-817 [PMID: 21134375 DOI: 10.1053/j.gastro.2010.11.052]
- 2 James OF, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. *J Hepatol* 1998; **29**: 495-501 [PMID: 9765002 DOI: 10.1016/S0168-8278(98)80073-1]
- 3 Monto A, Alonzo J, Watson JJ, Grunfeld C, Wright TL. Steatosis in chronic hepatitis C: relative contributions of obesity, diabetes mellitus, and alcohol. *Hepatology* 2002; **36**: 729-736 [PMID: 12198667 DOI: 10.1053/jhep.2002.35064]
- 4 Maury E, Ehala-Aleksejev K, Guiot Y, Detry R, Vandenhoof A, Brichard SM. Adipokines oversecreted by omental adipose tissue in human obesity. *Am J Physiol Endocrinol Metab* 2007; **293**: E656-E665 [PMID: 17578888 DOI: 10.1152/ajpendo.00127.2007]
- 5 Snijder MB, Flyvbjerg A, Stehouwer CD, Frystyk J, Henry RM, Seidell JC, Heine RJ, Dekker JM. Relationship of adiposity with arterial stiffness as mediated by adiponectin in older men and women: the Hoorn Study. *Eur J Endocrinol* 2009; **160**: 387-395 [PMID: 19095778 DOI: 10.1530/EJE-08-0817]
- 6 Kim YL, Kim TK, Cheong ES, Shin DG, Choi GS, Jung J, Han KA, Min KW. Relation of absolute or relative adiposity to insulin resistance, retinol binding protein-4, leptin, and adiponectin in type 2 diabetes. *Diabetes Metab J* 2012; **36**: 415-421 [PMID: 23275935 DOI: 10.4093/dmj.2012.36.6.415]
- 7 Ashour E, Samy N, Sayed M, Imam A. The relationship between serum adiponectin and steatosis in patients with chronic hepatitis C genotype-4. *Clin Lab* 2010; **56**: 103-110 [PMID: 20476641]
- 8 Cua IH, Hui JM, Bandara P, Kench JG, Farrell GC, McCaughan GW, George J. Insulin resistance and liver injury in hepatitis C is not associated with virus-specific changes in adipocytokines. *Hepatology* 2007; **46**: 66-73 [PMID: 17596870 DOI: 10.1002/hep.21703]
- 9 Hung CH, Lee CM, Chen CH, Hu TH, Jiang SR, Wang JH, Lu SN, Wang PW. Association of inflammatory and anti-inflammatory cytokines with insulin resistance in chronic hepatitis C. *Liver Int* 2009; **29**: 1086-1093 [PMID: 19302182 DOI: 10.1111/j.1478-3231.2009.01991.x]
- 10 Tiftikci A, Atug O, Yilmaz Y, Eren F, Ozdemir FT, Yapali S, Ozdogan O, Celikel CA, Imeryuz N, Tozun N. Serum levels of adipokines in patients with chronic HCV infection: relationship with steatosis and fibrosis. *Arch Med Res* 2009; **40**: 294-298 [PMID: 19608019 DOI: 10.1016/j.arcmed.2009.04.008]
- 11 Testa R, Franceschini R, Giannini E, Caltadi A, Botta F, Fasoli A, Tenerelli P, Rolandi E, Barreca T. Serum leptin levels in patients with viral chronic hepatitis or liver cirrhosis. *J Hepatol* 2000; **33**: 33-37 [PMID: 10905583]
- 12 González-Reimers E, Castellano-Higuera A, Alemán-Valls R, Alvarez-Argüelles H, de la Vega-Prieto MJ, Abreu-González P, López-Prieto J, Santolaria-Fernández F, Valladares-Parrilla F. Relation between body fat and liver fat accumulation and cytokine pattern in non-alcoholic patients with chronic HCV infection. *Ann Nutr Metab* 2009; **55**: 351-357 [PMID: 19851063 DOI: 10.1159/000252351]
- 13 Feng RN, Du SS, Wang C, Li YC, Liu LY, Guo FC, Sun CH. Lean-non-alcoholic fatty liver disease increases risk for metabolic disorders in a normal weight Chinese population. *World J Gastroenterol* 2014; **20**: 17932-17940 [PMID: 25548491 DOI: 10.3748/wjg.v20.i47.17932]
- 14 Kumar R, Rastogi A, Sharma MK, Bhatia V, Garg H, Bihari C, Sarin SK. Clinicopathological characteristics and metabolic profiles of non-alcoholic fatty liver disease in Indian patients with normal body mass index: Do they differ from obese or overweight non-alcoholic fatty liver disease? *Indian J Endocrinol Metab* 2013; **17**: 665-671 [PMID: 23961483 DOI: 10.4103/2230-8210.113758]
- 15 Lee YH, Jung KS, Kim SU, Yoon HJ, Yun YJ, Lee BW, Kang ES, Han KH, Lee HC, Cha BS. Sarcopaenia is associated with NAFLD independently of obesity and insulin resistance: Nationwide surveys (KNHANES 2008-2011). *J Hepatol* 2015; **63**: 486-493 [PMID: 25772036 DOI: 10.1016/j.jhep.2015.02.051]
- 16 Bustamante M, Fernández-Verdejo R, Jaimovich E, Buvinic S. Electrical stimulation induces IL-6 in skeletal muscle through

- extracellular ATP by activating Ca(2+) signals and an IL-6 autocrine loop. *Am J Physiol Endocrinol Metab* 2014; **306**: E869-E882 [PMID: 24518675 DOI: 10.1152/ajpendo.00450.2013]
- 17 **Vida M**, Gavito AL, Pavón FJ, Bautista D, Serrano A, Suarez J, Arrabal S, Decara J, Romero-Cuevas M, Rodríguez de Fonseca F, Baixeras E. Chronic administration of recombinant IL-6 upregulates lipogenic enzyme expression and aggravates high-fat-diet-induced steatosis in IL-6-deficient mice. *Dis Model Mech* 2015; **8**: 721-731 [PMID: 26035386 DOI: 10.1242/dmm.019166]
  - 18 **Paniagua JA**, Escandell-Morales JM, Gil-Contreras D, Berral de la Rosa FJ, Romero-Jimenez M, Gómez-Urbano A, Sanchez-Lopez A, Bellido E, Poyato A, Calatayud B, Vidal-Puig AJ. Central obesity and altered peripheral adipose tissue gene expression characterize the NAFLD patient with insulin resistance: Role of nutrition and insulin challenge. *Nutrition* 2014; **30**: 177-185 [PMID: 24377452 DOI: 10.1016/j.nut.2013.07.017]
  - 19 **Lonardo A**, Adinolfi LE, Loria P, Carulli N, Ruggiero G, Day CP. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. *Gastroenterology* 2004; **126**: 586-597 [PMID: 14762795]
  - 20 **Adinolfi LE**, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; **33**: 1358-1364 [PMID: 11391523 DOI: 10.1053/jhep.2001.24432]
  - 21 **Brown TT**, Mehta SH, Sutcliffe C, Higgins Y, Torbenson MS, Moore RD, Thomas DL, Sulkowski MS. Hepatic steatosis associated with increased central body fat by dual-energy X-ray absorptiometry and uncontrolled HIV in HIV/hepatitis C co-infected persons. *AIDS* 2010; **24**: 811-817 [PMID: 20186036 DOI: 10.1097/QAD.0b013e3283333651]
  - 22 **Freitas P**, Carvalho D, Santos AC, Madureira AJ, Martinez E, Pereira J, Sarmento A, Medina JL. Adipokines, hormones related to body composition, and insulin resistance in HIV fat redistribution syndrome. *BMC Infect Dis* 2014; **14**: 347 [PMID: 24958357 DOI: 10.1186/1471-2334-14-347]
  - 23 **Masaki T**, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, Yoshimatsu H. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice. *Hepatology* 2004; **40**: 177-184 [PMID: 15239101 DOI: 10.1002/hep.20282]
  - 24 **Petit JM**, Minello A, Jooste V, Bour JB, Galland F, Duvillard L, Verges B, Olsson NO, Gambert P, Hillon P. Decreased plasma adiponectin concentrations are closely related to steatosis in hepatitis C virus-infected patients. *J Clin Endocrinol Metab* 2005; **90**: 2240-2243 [PMID: 15644404 DOI: 10.1210/jc.2004-1266]
  - 25 **Kara B**, Gunesacar R, Doran F, Kara IO, Akkiz H. Correlation of serum adiponectin levels and hepatic steatosis in hepatitis C virus genotype 1 infection. *Adv Ther* 2007; **24**: 972-982 [PMID: 18029322 DOI: 10.1007/BF02877701]
  - 26 **Aksöz K**, Unsal B, Kirci A, Alper E, Buyraç Z, Aslan F, Cekiç C, Cengiz O, Ozcan Ari F, Akpinar Z. The relationship between chronic HCV infection and the level of plasma adiponectin. *Turk J Gastroenterol* 2008; **19**: 254-257 [PMID: 19119485]
  - 27 **Siagris D**, Vafiadis G, Michalaki M, Lekkou A, Starakis I, Makri M, Margaritis V, Christofidou M, Tsamandas AC, Labropoulou-Karatza C. Serum adiponectin in chronic hepatitis C and B. *J Viral Hepat* 2007; **14**: 577-583 [PMID: 17650292 DOI: 10.1111/j.1365-2893.2007.00850.x]
  - 28 **Liu CJ**, Chen PJ, Jeng YM, Huang WL, Yang WS, Lai MY, Kao JH, Chen DS. Serum adiponectin correlates with viral characteristics but not histologic features in patients with chronic hepatitis C. *J Hepatol* 2005; **43**: 235-242 [PMID: 15964656 DOI: 10.1016/j.jhep.2005.02.044]
  - 29 **Takahara M**, Katakami N, Kishida K, Kaneto H, Funahashi T, Shimomura I, Matsunaga S, Kubo S, Fukamizu H, Otsuka A, Ichihara K, Nakamura T. Circulating adiponectin levels and their associated factors in young lean healthy Japanese women. *J Atheroscler Thromb* 2013; **20**: 57-64 [PMID: 22972430]
  - 30 **Wang J**, Leclercq I, Brymora JM, Xu N, Ramezani-Moghadam M, London RM, Brigstock D, George J. Kupffer cells mediate leptin-induced liver fibrosis. *Gastroenterology* 2009; **137**: 713-723 [PMID: 19375424 DOI: 10.1053/j.gastro.2009.04.011]
  - 31 **Myers MG**, Cowley MA, Münzberg H. Mechanisms of leptin action and leptin resistance. *Annu Rev Physiol* 2008; **70**: 537-556 [PMID: 17937601 DOI: 10.1146/annurev.physiol.70.113006.100707]
  - 32 **Kukla M**, Mazur W, Buldak RJ, Zwirska-Korczala K. Potential role of leptin, adiponectin and three novel adipokines--visfatin, chemerin and vaspin--in chronic hepatitis. *Mol Med* 2011; **17**: 1397-1410 [PMID: 21738955 DOI: 10.2119/molmed.2010.00105]
  - 33 **Giannini E**, Ceppa P, Botta F, Mastracci L, Romagnoli P, Comino I, Pasini A, Risso D, Lantieri PB, Icardi G, Barreca T, Testa R. Leptin has no role in determining severity of steatosis and fibrosis in patients with chronic hepatitis C. *Am J Gastroenterol* 2000; **95**: 3211-3217 [PMID: 11095344 DOI: 10.1111/j.1572-0241.2000.03294.x]
  - 34 **Myers RP**, Messous D, Poynard T, Imbert-Bismut F. Association between leptin, metabolic factors and liver histology in patients with chronic hepatitis C. *Can J Gastroenterol* 2007; **21**: 289-294 [PMID: 17505564]
  - 35 **Crespo J**, Rivero M, Fábrega E, Cayón A, Amado JA, García-Unzueta MT, Pons-Romero F. Plasma leptin and TNF-alpha levels in chronic hepatitis C patients and their relationship to hepatic fibrosis. *Dig Dis Sci* 2002; **47**: 1604-1610 [PMID: 12141823 DOI: 10.1023/A:1015835606718]
  - 36 **Sheikh MY**, Choi J, Qadri I, Friedman JE, Sanyal AJ. Hepatitis C virus infection: molecular pathways to metabolic syndrome. *Hepatology* 2008; **47**: 2127-2133 [PMID: 18446789 DOI: 10.1002/hep.22269]
  - 37 **Rubbia-Brandt L**, Quadri R, Abid K, Giostra E, Malé PJ, Mentha G, Spahr L, Zarski JP, Borisch B, Hadengue A, Negro F. Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. *J Hepatol* 2000; **33**: 106-115 [PMID: 10905593 DOI: 10.1016/S0168-8278(00)80166-X]

P- Reviewer: Jin B, Yun JW S- Editor: Qiu S

L- Editor: A E- Editor: Liu SQ





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 January 18; 8(2): 83-138







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 469 members, representing a team of worldwide experts in hepatology. They are from 53 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (1), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (98), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (19), Greece (11), Hungary (5), India (15), Indonesia (2), Iran (4), Israel (1), Italy (52), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (11), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (17), and United States (56).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*

Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*  
Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*

Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *São Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*



**Chile**

Luis A Videla, *Santiago*



## China

Guang-Wen Cao, Shanghai  
 En-Qiang Chen, Chengdu  
 Gong-Ying Chen, Hangzhou  
 Jin-lian Chen, Shanghai  
 Jun Chen, Changsha  
 Alfred Cheng, Hong Kong  
 Chun-Ping Cui, Beijing  
 Shuang-Suo Dang, Xi'an  
 Ming-Xing Ding, Jinhua  
 Zhi-Jun Duang, Dalian  
 He-Bin Fan, Wuhan  
 Xiao-Ming Fan, Shanghai  
 James Yan Yue Fung, Hong Kong  
 Yi Gao, Guangzhou  
 Zuo-Jiong Gong, Wuhan  
 Zhi-Yong Guo, Guangzhou  
 Shao-Liang Han, Wenzhou  
 Tao Han, Tianjin  
 Jin-Yang He, Guangzhou  
 Ming-Liang He, Hong Kong  
 Can-Hua Huang, Chengdu  
 Bo Jin, Beijing  
 Shan Jin, Hohhot  
 Hui-Qing Jiang, Shijiazhuang  
 Wan-Yee Joseph Lau, Hong Kong  
 Guo-Lin Li, Changsha  
 Jin-Jun Li, Shanghai  
 Qiang Li, Jinan  
 Sheng Li, Jinan  
 Zong-Fang Li, Xi'an  
 Xu Li, Guangzhou  
 Xue-Song Liang, Shanghai  
 En-Qi Liu, Xi'an  
 Pei Liu, Shenyang  
 Zhong-Hui Liu, Changchun  
 Guang-Hua Luo, Changzhou  
 Yi Lv, Xi'an  
 Guang-Dong Pan, Liuzhou  
 Wen-Sheng Pan, Hangzhou  
 Jian-Min Qin, Shanghai  
 Wai-Kay Seto, Hong Kong  
 Hong Shen, Changsha  
 Xiao Su, Shanghai  
 Li-Ping Sun, Beijing  
 Wei-Hao Sun, Nanjing  
 Xue-Ying Sun, Harbin  
 Hua Tang, Tianjin  
 Ling Tian, Shanghai  
 Eric Tse, Hong Kong  
 Guo-Ying Wang, Changzhou  
 Yue Wang, Beijing  
 Shu-Qiang Wang, Chengdu  
 Mary MY Wayne, Hong Kong  
 Hong-Shan Wei, Beijing  
 Danny Ka-Ho Wong, Hong Kong  
 Grace Lai-Hung Wong, Hong Kong  
 Bang-Fu Wu, Dongguan  
 Feng Wu, Chongqing  
 Xiong-Zhi Wu, Tianjin  
 Chun-Fang Xu, Suzhou  
 Rui-An Xu, Quanzhou  
 Rui-Yun Xu, Guangzhou  
 Wei-Li Xu, Shijiazhuang  
 Shi-Ying Xuan, Qingdao  
 Ming-Xian Yan, Jinan  
 Lv-Nan Yan, Chengdu  
 Jin Yang, Hangzhou  
 Ji-Hong Yao, Dalian  
 Winnie Yeo, Hong Kong

Zheng Zeng, Beijing  
 Qi Zhang, Hangzhou  
 Shi-Jun Zhang, Guangzhou  
 Xiao-Lan Zhang, Shijiazhuang  
 Xiao-Yong Zhang, Guangzhou  
 Xin-Chen Zhang, Harbin  
 Yong Zhang, Xi'an  
 Hong-Chuan Zhao, Hefei  
 Ming-Hua Zheng, Wenzhou  
 Yu-Bao Zheng, Guangzhou  
 Ren-Qian Zhong, Shanghai  
 Fan Zhu, Wuhan  
 Xiao Zhu, Dongguan



## Czech Republic

Kamil Vysloulzil, Olomouc



## Denmark

Henning Gronbaek, Aarhus  
 Christian Mortensen, Hvidovre



## Egypt

Ihab T Abdel-Raheem, Damanhour  
 NGB G Bader EL Din, Cairo  
 Hatem Elalfy, Mansoura  
 Mahmoud M El-Bendary, Mansoura  
 Mona El SH El-Raziky, Cairo  
 Mohammad El-Sayed, Cairo  
 Yasser M Fouad, Minia  
 Mohamed AA Metwally, Benha  
 Hany Shehab, Cairo  
 Mostafa M Sira, Shebin El-koom  
 Ashraf Taye, Minia  
 MA Ali Wahab, Mansoura



## France

Laurent Alric, Toulouse  
 Sophie Conchon, Nantes  
 Daniel J Felmlee, Strasbourg  
 Herve Lerat, Creteil  
 Dominique Salmon, Paris  
 Jean-Pierre Vartanian, Paris



## Germany

Laura E Buitrago-Molina, Hannover  
 Enrico N De Toni, Munich  
 Oliver Ebert, Muenchen  
 Rolf Gebhardt, Leipzig  
 Janine V Hartl, Regensburg  
 Sebastian Hinz, Kiel  
 Benjamin Juntermanns, Essen  
 Roland Kaufmann, Jena  
 Viola Knop, Frankfurt  
 Veronika Lukacs-Kornek, Homburg  
 Benjamin Maasoumy, Hannover  
 Jochen Mattner, Erlangen  
 Nadja M Meindl-Beinker, Mannheim  
 Ulf P Neumann, Aachen  
 Margarete Odenthal, Cologne  
 Yoshiaki Sunami, Munich

Christoph Roderburg, Aachen  
 Frank Tacke, Aachen  
 Yuchen Xia, Munich



## Greece

Alex P Betrosian, Athens  
 George N Dalekos, Larissa  
 Ioanna K Delladetsima, Athens  
 Nikolaos K Gatselis, Larissa  
 Stavros Gourgiotis, Athens  
 Christos G Savopoulos, Thessaloniki  
 Tania Siahaniidou, Athens  
 Emmanouil Sinakos, Thessaloniki  
 Nikolaos G Symeonidi, Thessaloniki  
 Konstantinos C Thomopoulos, Larissa  
 Konstantinos Tziomalos, Thessaloniki



## Hungary

Gabor Banhegyi, Budapest  
 Peter L Lakatos, Budapest  
 Maria Papp, Debrecen  
 Ferenc Sipos, Budapest  
 Zsolt J Tulassay, Budapest



## India

Deepak N Amarapurkar, Mumbai  
 Girish M Bhopale, Pune  
 Sibnarayan Datta, Tezpur  
 Nutan D Desai, Mumbai  
 Sorabh Kapoor, Mumbai  
 Jaswinder S Maras, New Delhi  
 Nabeen C Nayak, New Delhi  
 C Ganesh Pai, Manipal  
 Amit Pal, Chandigarh  
 K Rajeshwari, New Delhi  
 Anup Ramachandran, Vellore  
 D Nageshwar Reddy, Hyderabad  
 Shivaram P Singh, Cuttack  
 Ajith TA, Thrissur  
 Balasubramaniyan Vairappan, Pondicherry



## Indonesia

Cosmas RA Lesmana, Jakarta  
 Neneng Ratnasari, Yogyakarta



## Iran

Seyed M Jazayeri, Tehran  
 Sedigheh Kafi-Abad, Tehran  
 Iradj Maleki, Sari  
 Fakhraddin Naghibalhossaini, Shiraz



## Israel

Stephen DH Malnick, Rehovot



## Italy

Francesco Angelico, Rome

Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Andrea Galli, *Florence*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Salvatore Gruttadauria, *Palermo*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*



**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*  
 Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*

Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhito Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*



**Jordan**

Kamal E Bani-Hani, *Zarqa*



**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*



**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*



**Moldova**

Angela Peltec, *Chishinev*



**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*



**Nigeria**

CA Asabamaka Onyekwere, *Lagos*



**Pakistan**

Bikha Ram Devrajani, *Jamshoro*



**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*



**Poland**

Jacek Zielinski, *Gdansk*



**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*



**Qatar**

Reem Al Olaby, *Doha*



**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*



**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*



**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*



**Singapore**

Ser Yee Lee, *Singapore*



**South Korea**

Young-Hwa Chung, *Seoul*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonju*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*



**Spain**

Ivan G Marina, *Madrid*  
 Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*

José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodríguez-Frias, *Córdoba*  
 Manuel L Rodríguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Traperó-Marugán, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpaboon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*  
 Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*

Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



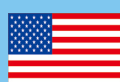
#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*



#### **United States**

Naim Alkhouri, *Cleveland*  
 Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*

Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Grigoriy E Gurvits, *New York*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Kusum K Kharbanda, *Omaha*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*





## Contents

Three issues per month Volume 8 Number 2 January 18, 2016

### TOPIC HIGHLIGHT

- 83 Hepatitis C virus infection and thyroid autoimmune disorders: A model of interactions between the host and the environment

*Pastore F, Martocchia A, Stefanelli M, Prunas P, Giordano S, Toussan L, Devito A, Falaschi P*

- 92 Chronic hepatitis C: This and the new era of treatment

*Bertino G, Ardiri A, Proiti M, Rigano G, Frazzetto E, Demma S, Ruggeri MI, Scuderi L, Malaguarnera G, Bertino N, Rapisarda V, Di Carlo I, Toro A, Salomone F, Malaguarnera M, Bertino E, Malaguarnera M*

### REVIEW

- 107 Hepatitis C virus and non-Hodgkin's lymphomas: Meta-analysis of epidemiology data and therapy options

*Pozzato G, Mazzaro C, Dal Maso L, Mauro E, Zorat F, Moratelli G, Bulian P, Serraino D, Gattei V*

### MINIREVIEWS

- 117 Hepatitis E virus infection in the liver transplant recipients: Clinical presentation and management

*Aggarwal A, Perumpail RB, Tummala S, Ahmed A*

- 123 Ribavirin: Past, present and future

*Loustaud-Ratti V, Debette-Gratien M, Jacques J, Alain S, Marquet P, Sautereau D, Rousseau A, Carrier P*

- 131 Hepatitis C and insulin action: An intimate relationship

*Knobler H, Malnick S*

## Contents

*World Journal of Hepatology*  
Volume 8 Number 2 January 18, 2016

### ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Jochen Mattner, MD, Associate Professor, Molecular Microbiology and Infection Immunology, University Clinic of Erlangen, 90154 Erlangen, Germany

### AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed Central, PubMed, Digital Object Identifier, Directory of Open Access Journals, and Scopus.

### FLYLEAF

#### I-IV Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
January 18, 2016

#### COPYRIGHT

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

#### SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

#### INSTRUCTIONS TO AUTHORS

Full instructions are available online at [http://www.wjgnet.com/1948-5182/g\\_info\\_20100316080002.htm](http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm)

#### ONLINE SUBMISSION

<http://www.wjgnet.com/esps/>

2016 Hepatitis C Virus: Global view

## Hepatitis C virus infection and thyroid autoimmune disorders: A model of interactions between the host and the environment

Francesca Pastore, Antonio Martocchia, Manuela Stefanelli, Pietro Prunas, Stefania Giordano, Lavinia Toussan, Antonio Devito, Paolo Falaschi

Francesca Pastore, Antonio Martocchia, Manuela Stefanelli, Pietro Prunas, Stefania Giordano, Lavinia Toussan, Antonio Devito, Paolo Falaschi, "Sapienza" University of Rome, Faculty of Medicine and Psychology, S. Andrea Hospital, 00189 Rome, Italy

Author contributions: All authors contributed to the manuscript.

Conflict-of-interest statement: No financial conflicts of interest or other relationships are present in the manuscript.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Francesca Pastore, MD, "Sapienza" University of Rome, Faculty of Medicine and Psychology, S. Andrea Hospital, Via di Grottarossa 1035/39, 00189 Rome, Italy. [francesca.past@virgilio.it](mailto:francesca.past@virgilio.it)  
Telephone: +39-6-33775467  
Fax: +39-6-33775401

Received: April 28, 2015  
Peer-review started: May 6, 2015  
First decision: October 14, 2015  
Revised: October 28, 2015  
Accepted: December 3, 2015  
Article in press: December 4, 2015  
Published online: January 18, 2016

### Abstract

The hepatitis C virus (HCV) infection is an important

public health problem and it is associated with hepatic and extrahepatic manifestations. Autoimmune thyroid diseases are common in HCV infected patients and the standard interferon-based treatment is associated with an increase of the immune-mediated thyroid damage. Recent evidence in the literature analyzed critical points of the mechanisms of thyroid damage, focusing on the balance between the two sides of the interaction: The environment (virus infection with potential cross-reaction) and the host (susceptibility genes with consistent immune response). The spectrum of antiviral treatment for chronic HCV infection is rapidly expanding for the development of dual or triple therapy. The availability of interferon-free combined treatment with direct antiviral agents for HCV is very promising, in order to ameliorate the patient compliance and to reduce the development of thyroid autoimmunity.

**Key words:** Hepatitis C virus; Thyroid autoimmunity; Interferon; Antiviral agents; Self-tolerance

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This review examines the relationship between the hepatitis C virus (HCV) infection and the thyroid autoimmunity, on the basis of recent evidence of the literature about the mechanisms of self tolerance and thyroid damage related to HCV. The advances in the HCV infection treatment have been discussed in the paper, with relevant clinical results.

Pastore F, Martocchia A, Stefanelli M, Prunas P, Giordano S, Toussan L, Devito A, Falaschi P. Hepatitis C virus infection and thyroid autoimmune disorders: A model of interactions between the host and the environment. *World J Hepatol* 2016; 8(2): 83-91 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/>

## INTRODUCTION

Hepatitis C virus (HCV) infection is a liver disease that may be associated with extra hepatic manifestations (EHM) (autoimmune disorders or malignant tumors), defining the HCV syndrome as result of multifactorial process with significant genetic predisposition and/or environmental triggering cofactors<sup>[1]</sup>.

More than 50% of HCV-positive patients have symptoms of at least one EHM during the course of the disease that can be the first and only clinical signs of a chronic hepatitis C<sup>[2]</sup>.

The loss of tolerance is the main mechanism that promotes autoimmune diseases and, particularly, autoimmune thyroid disorders (AITD)<sup>[3,4]</sup>, with autoantibodies (Abs) or T lymphocytes (humoral or cellular response) reacting with self-antigens (Ags) (Figure 1).

The clinical spectrum of AITD includes hyper- [Graves' disease (GD)] or hypo-function [Hashimoto's thyroiditis (HT)] of the gland. The Abs against the thyroglobulin (Tg) and the thyrotropin-stimulating hormone (TSH)-receptor (TSH-r) in patients with GD were firstly identified 50 years ago<sup>[5,6]</sup>. The Abs bind and activate the TSH receptor in GD, whereas antibody-dependent cellular cytotoxicity to thyroglobulin and thyroid peroxidase (TPO) and T cells mediated injury in HT. An immune-mediated mechanism is present in painful subacute thyroiditis (without significant anti-thyroid autoantibodies) and in drug-induced thyroiditis (interferons).

T cells CD4<sup>+</sup> are divided into regulatory T (Treg) cells and conventional T helper (Th) cells (with Th1 and Th2 lineages controlling cell-mediated and humoral immunity, respectively)<sup>[7-11]</sup>. In the central event of the immune response, the antigen-presenting cell (APC) presents the Ag bound to the human leukocyte antigen (HLA) class II to the CD4<sup>+</sup> T cell, through the T cell receptor and additional costimulations (engagement of B7 with CD28 and CD40 with CD40 ligand). The Ag recognition for CD8<sup>+</sup> T cells requires linear peptides that are processed and bound to HLA class I. The CD4<sup>+</sup>/CD8<sup>+</sup> ratio, the HLA system and the costimulation have been involved in initiation, progression, and maintenance of AITD<sup>[12]</sup>. Since activated T cells stimulate B cells to proliferate and secrete antibodies (IgG), B cell tolerance mechanisms are considered as a secondary mechanism<sup>[13]</sup>. Tregs suppress immune responses against self or non-self Ags, producing immunosuppressive cytokines [interleukin-10 (IL-10), and transforming growth factor  $\beta$  (TGF- $\beta$ )] and Tregs are dysfunctional in AITD patients<sup>[14,15]</sup>. Programmed death-1 negative co-stimulatory pathway mediate Treg activity, that is characterized by the expression of forkhead box protein 3 (FoxP3) and cytotoxic T-lymphocyte antigen 4 (CTLA-4).

At the peripheral site of chronic inflammation, the Th17 cells produce proinflammatory cytokines

(IL-17, IL-21 and IL-22), as it has been demonstrated in AITD<sup>[16,17]</sup>. Local immunosuppressive regulatory cytokines (TGF- $\beta$  and IL-10) may be involved in the maintenance of tolerance and prevention of AITD<sup>[18,19]</sup>. A decreased apoptosis of activated T cells, like in defects of interaction of Fas (CD95) and Fas ligand (Fas-L), has been studied in AITD<sup>[20]</sup>. The proportion of intrathyroidal natural killer T cell subset has been found lower in GD than in the peripheral blood of the same patients and of controls, contributing to the incomplete regulation of autoreactive T cells<sup>[13]</sup>.

## HOST-DEPENDENT FACTORS IN THYROID AUTOIMMUNITY

The aetiology of the AITD is unknown, but endogenous agents may predispose to the development to autoimmunity.

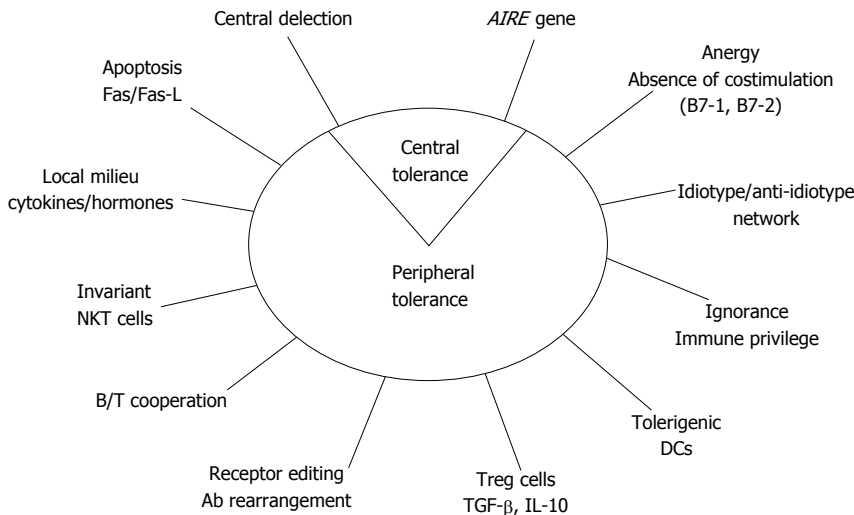
A genetic influence (the susceptibility genes) has been reported in the development of autoimmunity<sup>[21,22]</sup>. As matter of fact, the association with HLA class II molecules, the concordance studies in twins, the association with CTLA-4 and protein tyrosine phosphatase nonreceptor-type 22 and CD40 polymorphism (A/G49 and 1858C/T and CC genotype, respectively), the association of a microsatellite inside the *FoxP3* gene, the linkage with chromosomal locations (14q31, 18q21, 20q11, Xp11, Xq21, 6p, 13q32 and 12q22) and the presence of anti-thyroid Abs in siblings of probands with AITD have been observed<sup>[23-32]</sup>. Moreover, the HLA class II (DRB1\*0301) is also associated with chronic HCV infection<sup>[33]</sup>. Genome-wide association studies of autoimmune disease recently revealed multiple associations with the major immune cell subsets and uncovered insights into the control for regulatory Tregs<sup>[34]</sup>.

AITD clearly increases with age, resulting from changes in immune regulation (endogenous factor). A sexual dimorphism in AITD has been described<sup>[3]</sup>, with the highest ratio in females with HT (F:M = 4-10:1), suggesting an immunomodulatory role of sex steroids (respectively for androgens, estrogens and progesterone), mediated by specific receptor<sup>[35]</sup>. Males have an increased risk of advanced liver disease (cirrhosis and hepatocellular carcinoma) during HCV infection, in association with polymorphisms in sex steroid hormone synthesis and signaling<sup>[36,37]</sup>.

A blunted hypothalamic-pituitary-adrenal axis may be associated to susceptibility to autoimmune/inflammatory disease<sup>[38]</sup>, but no evidence of pituitary or adrenal involvement was present in a recent histopathologic study in HCV patients with thyroid disorders<sup>[39]</sup>.

The main targets of the immune response in AITD are the Tg (two 330-kDa monomers, with the highest "immunogenicity score"), the TSH-r (60 kDa for the A subunit) and the TPO (homodimer of two 107-kDa subunits); no supporting data, at the moment, for the sodium/iodide symporter (NIS) and the pendrin<sup>[9]</sup>. Specific Tg peptides (representing major T-cell epitopes





**Figure 1** Potential mechanisms for self-tolerance control. AIRE: Autoimmune regulator gene; DCs: Dendritic cells; TGF: Transforming growth factor; IL: Interleukin; Ab: Antibody; NKT cells: Natural killer T cells.

that can bind to the HLA-DRB-Arg74 pockets) and intron 1 polymorphism in the *TSH-r* gene (altering its splicing) has been associated with GD<sup>[40,41]</sup>. Cytotoxic CD8<sup>+</sup> T cells recognized Tg or TPO peptide epitopes associated to HLA-A2 molecules in patients with HT<sup>[11]</sup>.

Epigenetic modifications (including DNA methylation, histone modifications, and RNA interference by microRNA) can amplify a risk conferred by an inherited polymorphism resulting in a combined high risk for disease<sup>[42]</sup>.

## ENVIRONMENT AND VIRUS-DEPENDENT FACTORS IN THYROID AUTOIMMUNITY

Environmental risk factors include pollution, iodine intake (as in the cases of Jod-Basedow and Wolff-Chaikoff effect) and smoking. Stressful situations are well known inducers of AITD and, in particular, of hyperthyroidism<sup>[43]</sup>. Allostatic load during stress conditions is a well-known environmental factor favouring the development of AITD. A high number of drugs (lithium, amiodarone, interferons, anti-CD52 monoclonal antibody Campath-1H) may induce AITD<sup>[44-47]</sup>. In the past years, leukocyte-derived interferon (IFN) contaminated with  $\gamma$ -IFN demonstrated "*in vivo*" potent inducing properties of AITD in humans<sup>[48]</sup>.

The HCV is one of the most important viruses associated with autoimmune diseases (both chronic liver inflammation and EHM). HCV may interfere with the functions and mechanisms of self-recognition both on the immune system and thyroid cells<sup>[49,50]</sup>, where HCV may directly destroy thyroid tissue or mimic the structure of some components of thyroid gland, starting the autoimmune disease (Figure 2).

The HCV prevalence is about 5%, strongly associated with health inequity<sup>[51,52]</sup>. HCV structure consists of three structural (core, E1 and E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and

NS5B) and six main HCV-RNA genotypes<sup>[53]</sup>. HCV has a significant lymphotropism: In fact, the lymphoid tissue is a site for the persistence of the infection and chronic immune stimulus<sup>[54,55]</sup>. The chronic stimulation results in: AutoAbs production (clonal B lymphocyte expansion and Th2 response), anti-apoptotic effects (translocation with Bcl-2 activation and prolonged survival of lymphocytes), drive for autoimmunity (binding of protein E2 to CD81, that mediate attachment on hepatocytes), increased cytokine and chemokine secretion (IFN- $\gamma$  and Th1 response with IFN- $\gamma$  inducible chemokines such as C-X-C motif chemokine 10 or CXCL10, in order to stop viral spread; IL-8) and upregulation of CXCL10 by NS5a<sup>[56-59]</sup>. However, no association has been found between chronic hepatitis C with increased CXCL10 and AITD<sup>[60]</sup>.

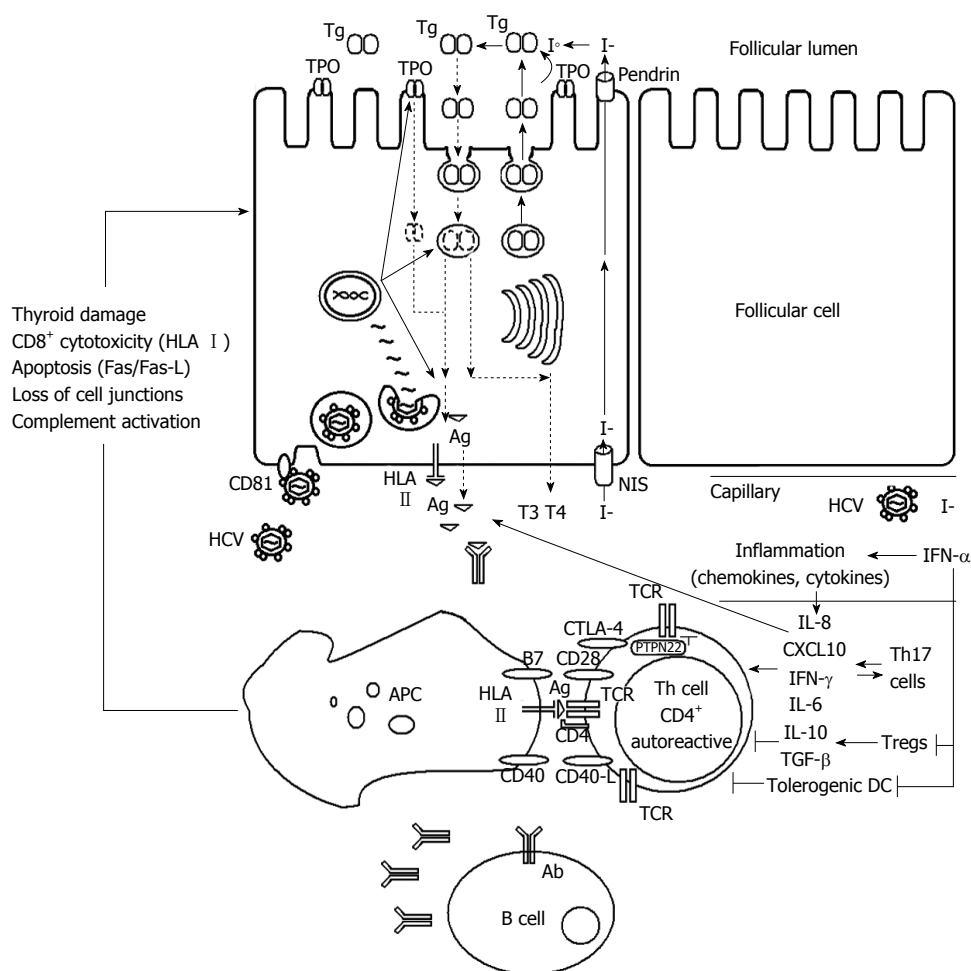
## DEVELOPMENT OF AITD DURING THE $\alpha$ -IFN TREATMENT FOR HCV CHRONIC HEPATITIS

The AITD during the  $\alpha$ -IFN treatment for viral chronic hepatitis is an interesting clinical model for autoimmunity, since it includes both environmental and endogenous factors, together interacting. The mechanisms responsible for AITD in HCV patients have not been elucidated.

In the autoimmune model, initiating (susceptibility genes/environmental stimuli) and modulating factors (sex hormones/neuroendocrine influences) are involved in the whole complex of the autoimmune processes.

Age, female gender and pre-existing positive Abs are well-known risk factors for the development of AITD in the IFN-treated HCV patients<sup>[61-64]</sup>.

HCV is associated with AITD (10%) and thyroid dysfunction (3%, with a hypothyroidism/hyperthyroidism ratio of about 2:1)<sup>[49,61-63,65-70]</sup>. AITD in patients with HCV are more frequent than in viral hepatitis B (5%) and in



**Figure 2** Development of thyroid autoimmunity in patients with chronic hepatitis C virus infection during interferon- $\alpha$  treatment. Ab: Antibody; Ag: Antigen; APC: Antigen presenting cell; CD: Cluster of differentiation; CTLA-4: Cytotoxic T-lymphocyte antigen 4; CXCL10: C-X-C motif chemokine; DC: Dendritic cell; HCV: Hepatitis C virus; HLA: Human leukocyte antigen; I-: Iodide; IFN: Interferon; IL: Interleukin; NIS: Sodium/iodide symporter; PTPN22: Protein tyrosine phosphatase nonreceptor-type 22; T3 and T4: Thyroid hormones; TCR: T cell receptor; Tg: Thyroglobulin; TGF: Transforming growth factor; Th: T helper; TPO: Thyroid peroxidase; Tregs: T regulatory cells.

controls (2%-4%)<sup>[11,66]</sup>.

The standard antiviral therapy with  $\alpha$ -IFN for HCV-related chronic hepatitis may exacerbate or induce underlying latent thyroid disorders, increasing the incidence of AITD and dysfunction to 20%-40% and 11%-15%, respectively<sup>[49,61-63,65,67,68,70-72]</sup>. The "de novo" appearance of anti-thyroid Abs and overt dysfunctions in euthyroid subjects have been demonstrated after the  $\alpha$ -IFN therapy, suggesting that this cytokine is a direct inducer of AITD<sup>[49,61,62,67,68,70,71]</sup>.

Recombinant  $\alpha$ -IFN administration induces an increase of endogenous  $\gamma$ -IFN and IL-6, supporting a sequence in the cytokine cascade that modulate the immune system and the neuroendocrine axis secretion<sup>[73]</sup>. At the thyroid level, IFNs ( $\alpha$ ,  $\beta$  and  $\gamma$ ) are inhibitors of iodide uptake and hormone release on thyrocytes<sup>[74]</sup>. At the pituitary level,  $\gamma$ -IFN and IL-6 do not change TSH release<sup>[75]</sup>, whereas at the hypothalamic level,  $\gamma$ -IFN stimulates somatostatin release<sup>[76]</sup> that suppresses TSH secretion. We examined the effect of  $\alpha$ -IFN (3 million IU i.m. 3 times a week) on hypothalamic-pituitary-thyroid (HPT) axis in patients with viral chronic hepatitis and negative anti-thyroid Abs

from a neuroendocrine point of view and we did not find a statistically relevant modification of thyroid hormones and TSH levels<sup>[77]</sup>.

A case of De Quervain's thyroiditis during  $\alpha$ -IFN therapy for HCV-related chronic hepatitis, with persisting negative anti-thyroid Abs after  $\alpha$ -IFN therapy, has been reported<sup>[78]</sup>. The common viral infections (Coxsackie virus, mumps, Epstein-Barr virus, adenovirus, cytomegalovirus) were negative, but we found an association with HLA-Bw35<sup>[79,80]</sup>. The patient presented the HCV, the typical HLA class I predisposition for the thyroid disease and an exogenous accelerating factor ( $\alpha$ -IFN therapy). During viral infections, APCs present antigens to Th cells, in the presence of cytokines (*i.e.*,  $\alpha$ -IFN, IL-12), inducing them to differentiate towards the Th1 phenotype that causes cell damage<sup>[81]</sup>.

In a second case report, a patient with HCV infection and negative anti-thyroid Abs before treatment but with the typical association for HT (HLA-DR5 antigen or HLA-DRB1.11/HLA-DRB1.12 alleles) in Caucasian developed HT during  $\alpha$ -IFN treatment<sup>[82]</sup>.

In a preliminary longitudinal (range 12-54 mo)

study in patients with chronic hepatitis C and absence of thyroid disorders at the baseline ( $n = 15$ ), the relationship between the HLA antigen susceptibility and the thyroid disorders during the  $\alpha$ -IFN treatment was evaluated, with respect to control subjects ( $n = 107$ )<sup>[83]</sup>. The HCV genotype was 1b (20%), 2a (60%) and 3a (20%), with the distribution (1b:2a:3a) of 1:3:1 and absence of mixed genotype. It is well known that the HLA-B35, -DR3 (DRB1.03 allele) and -DR5 (DRB1.11/HLA-DRB1.12 alleles) are commonly associated with De Quervain's thyroiditis, thyrotoxicosis/hyperthyroidism and hypothyroidism, respectively<sup>[80,84-92]</sup>. Arginine at position 74 of HLA-DRB1 chain (DRB-Arg74) may permit autoAg peptides to fit into the binding pocket, to be presented more efficiently to T cells<sup>[93]</sup>. On the other side, the HLA-A2 has been aspecifically associated with thyroid disorders (either hyper- or hypothyroidism) in patients with chronic hepatitis C during  $\alpha$ -IFN therapy<sup>[30]</sup>. The HLA-A2 antigen (class I molecule) is involved in the restricted presentation of HCV peptides by the APC to the CTL (response strongly increased by  $\alpha$ -IFN, with final outcome of target cell disruption both at the liver and thyroid gland level)<sup>[94-97]</sup>.

Forty percent of HCV patients presented a double positive HLA result (HLA-A2/B-35, HLA-A2/DRB1.03, HLA-A2/DRB1.11 or HLA-B35/DRB1.11) before the treatment and five patients with double positive HLA received the  $\alpha$ -IFN therapy. Four double positive HLA treated females developed clinical thyroid disorders, with the HLA system specifically associated with the particular kind of the thyroid disorder ( $P < 0.05$ ). The HLA-A2 was not specific for thyroid disorder, being present in hypothyroidism, in thyrotoxicosis as well as in thyroiditis. The relationship between the thyroid disorders and the HCV genotype did not reveal significant association. In our group with 40% double positive HLA pre-treatment, the overall development of thyroid disorders after  $\alpha$ -IFN was 36% (33% in patients with pre-treatment negative anti-thyroid Abs).

Previous studies have showed the association of AITD with female gender, older age and pre-existing positive anti-thyroid Abs, in  $\alpha$ -IFN treated patients with HCV-related chronic hepatitis<sup>[26,49,61,66,69,70]</sup>. Our results suggest that the HLA system is a strong susceptibility factor to the development of AITD, in particular, in the patients with two Ags together (the double association of HLA class I and/or II). Therefore, the examination of HLA (HLA-A2, -B35, -DRB1.03, -DRB1.11) in HCV patients before  $\alpha$ -IFN treatment may be a useful predictive tool to detect the predisposition to develop the specific AITD.

HCV virion attachment and entry in thyrocytes are mediated by CD81 (host) and E2 (virus), activating the local inflammatory response (as well as it occurs for hepatocytes). Moreover, HCV also replicates within the infected human thyroid cells *in vitro*<sup>[98]</sup>. The HCV infection of thyroid cells can trigger the autoimmune thyroiditis by induction of changes in self Ag expression, exposing of cryptic epitopes or molecular mimicry and

leading to production of the proinflammatory IL-8 (a contributor to bystander activation)<sup>[11]</sup>.

Even if important host effector molecules (such as the interferon-induced transmembrane proteins IFITM family of proteins) may act against HCV in the liver, restricting infection by targeting the endocytosed virion for lysosomal degradation<sup>[99]</sup>, at the moment, no data in the literature describe the role of IFITM in AITD.

The molecular mimicry is the mainly investigated mechanism of induction of autoimmunity and we analyzed the frequency of the sequence homology between the thyroid and the HCV. We found 62.5%-100% homology, when the conservative substitutions were included in the analysis (ten out of ten identical/conservative amino acids in the sequence), between the HCV polyprotein and five thyroid Ags (Tg, TPO, TSHr, NIS and pendrin). The homology was not restricted to a single HCV genotype, with the highest degree between the NIS and the HCV1a-NS4a protein. The Tg had the highest number of homologies with the different HCV genotypes. The length of ten amino acids is consistent with the presentation of the self/viral Ags with the HLA class I to CD8<sup>+</sup> lymphocytes (the HLA class II usually bind longer peptides)<sup>[100]</sup>.

The aberrant expression of HLA class II on thyroid cells (with costimulation) and the local inflammation (with cytokine release) result in activation of autoreactive T cells by bystander mechanisms. Systemic inflammation (cytokines and chemokines, like IL-8) plays an important role in the immunopathogenesis of thyroiditis and antagonize the antiviral effects of IFN, facilitating HCV persistence in thyrocytes. The absence of HCV clearance from thyrocytes perpetuates the chronic inflammation and autoimmunity.  $\alpha$ -IFN triggers AITD through an epigenetic mechanism involving variant of Tg and TSHr gene promoter<sup>[101,102]</sup>. Moreover,  $\alpha$ -IFN locally enhances the expression of TSH-r, Tg, TPO and HLA class I molecules on thyrocytes and the secretion of the potent proinflammatory IL-2 cytokine<sup>[11]</sup>.

$\alpha$ -IFN treatment for HCV-related chronic hepatitis acts an enhancer of AITD in susceptible patients. The standard dual therapy with pegylated  $\alpha$ -IFN (pegIFN)/ribavirin has been recently increased to a triple therapy, based on new direct-acting antiviral drugs [NS3/4A serine protease inhibitor (PI), such as telaprevir or boceprevir].

The monitoring of the patients during the treatment avoids the side effects (typically flu-like symptoms with pegIFN or anemia with ribavirin, or irritability, allergic reactions, severe fatigue, bacterial infections)<sup>[103,104]</sup>. Thyroid function tests should be examined every 3 mo during the  $\alpha$ -IFN based treatment<sup>[105,106]</sup>. Recently,  $\alpha$ -IFN-free combined treatment with direct antiviral agents for HCV has been developed with or without ribavirin, ameliorating the patient compliance and reducing the risk for thyroid autoimmunity development. These agents are second generation PI (simeprevir, grazoprevir), NS5A inhibitor (daclatasvir, ledipasvir, ombitasvir, elbasvir) and NS5B polymerase inhibitor (sofosbuvir,

paritaprevir, dasabuvir, beclabuvir, asunaprevir) that are strongly efficacious to eradicate the HCV infection (undetectable HCV-RNA after 24 wk from the beginning of therapy)<sup>[53,107-109]</sup>.

## CONCLUSION

In conclusion, the development of AITD in patients with chronic HCV-infection is a complex model for autoimmunity in which every component (the host and the environment) has a significant role.

The new approach with  $\alpha$ -IFN-free combined treatment for chronic HCV-infection with direct antiviral agents is very promising in order to ameliorate the patient compliance and to reduce the risk of development of AITD.

## REFERENCES

- 1 **Zignego AL**, Gragnani L, Piluso A, Sebastiani M, Giuggioli D, Fallahi P, Antonelli A, Ferri C. Virus-driven autoimmunity and lymphoproliferation: the example of HCV infection. *Expert Rev Clin Immunol* 2015; **11**: 15-31 [PMID: 25534977 DOI: 10.1586/1744666X.2015.997214]
- 2 **Jadali Z**, Alavian SM. Autoimmune diseases co-existing with hepatitis C virus infection. *Iran J Allergy Asthma Immunol* 2010; **9**: 191-206 [PMID: 21131699]
- 3 **Martocchia A**, Stefanelli M, Cola S, Falaschi P. Sex steroids in autoimmune diseases. *Curr Top Med Chem* 2011; **11**: 1668-1683 [PMID: 21463254 DOI: 10.2174/156802611796117595]
- 4 **Poletaev AB**, Stepanyuk VL, Gershwin ME. Integrating immunity: the immunusculus and self-reactivity. *J Autoimmun* 2008; **30**: 68-73 [PMID: 18191542 DOI: 10.1016/j.jaut.2007.11.012]
- 5 **Adams DD**, Purves HD. Abnormal responses in the assay of thyrotrophin. *Proc Univer Otago Med School* 1956; **34**: 11-12
- 6 **Roitt IM**, Doniach D, Campbell PN, Hudson RV. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). *Lancet* 1956; **271**: 820-821 [PMID: 13368530 DOI: 10.1016/S0140-6736(56)92249-8]
- 7 **Murphy KM**, Reiner SL. The lineage decisions of helper T cells. *Nat Rev Immunol* 2002; **2**: 933-944 [PMID: 12461566 DOI: 10.1038/nri954]
- 8 **Corthay A**. How do regulatory T cells work? *Scand J Immunol* 2009; **70**: 326-336 [PMID: 19751267 DOI: 10.1111/j.1365-3083.2009.02308.x]
- 9 **Abbas AK**, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1996; **383**: 787-793 [PMID: 8893001 DOI: 10.1038/383787a0]
- 10 **Bettelli E**, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of T(H)17 cells. *Nature* 2008; **453**: 1051-1057 [PMID: 18563156 DOI: 10.1038/nature07036]
- 11 **Watanabe M**, Nakamura Y, Matsuzuka F, Takamura Y, Miyauchi A, Iwatani Y. Decrease of intrathyroidal CD161+V $\alpha$ 24+V $\beta$ 11+ NKT cells in Graves' disease. *Endocr J* 2008; **55**: 199-203 [PMID: 18250538 DOI: 10.1507/endocrj.K07E-006]
- 12 **Nada AM**, Hammouda M. Immunoregulatory T cells, LFA-3 and HLA-DR in autoimmune thyroid diseases. *Indian J Endocrinol Metab* 2014; **18**: 574-581 [PMID: 25143920 DOI: 10.4103/2230-8210.137524]
- 13 **McLachlan SM**, Rapoport B. Breaking tolerance to thyroid antigens: changing concepts in thyroid autoimmunity. *Endocr Rev* 2014; **35**: 59-105 [PMID: 24091783 DOI: 10.1210/er.2013-1055]
- 14 **Glick AB**, Wodzinski A, Fu P, Levine AD, Wald DN. Impairment of regulatory T-cell function in autoimmune thyroid disease. *Thyroid* 2013; **23**: 871-878 [PMID: 23379353 DOI: 10.1089/thy.2012.0514]
- 15 **Rodríguez-Muñoz A**, Viales-Noyola M, Ramos-Levi A, Serrano-Somavilla A, González-Amaro R, Marazuela M. Levels of regulatory T cells CD69(+)NKG2D (+)IL-10 (+) are increased in patients with autoimmune thyroid disorders. *Endocrine* 2015; Epub ahead of print [PMID: 26100786]
- 16 **Steinman L**. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. *Nat Med* 2007; **13**: 139-145 [PMID: 17290272 DOI: 10.1038/nm1551]
- 17 **Li D**, Cai W, Gu R, Zhang Y, Zhang H, Tang K, Xu P, Katirai F, Shi W, Wang L, Huang T, Huang B. Th17 cell plays a role in the pathogenesis of Hashimoto's thyroiditis in patients. *Clin Immunol* 2013; **149**: 411-420 [PMID: 24211715 DOI: 10.1016/j.clim.2013.10.001]
- 18 **Vural P**, Degirmencioglu S, Erden S, Gelincik A. The relationship between transforming growth factor-beta1, vascular endothelial growth factor, nitric oxide and Hashimoto's thyroiditis. *Int Immunopharmacol* 2009; **9**: 212-215 [PMID: 19028605 DOI: 10.1016/j.intimp.2008.11.003]
- 19 **de la Vega JR**, Vilaplana JC, Biro A, Hammond L, Bottazzo GF, Mirakian R. IL-10 expression in thyroid glands: protective or harmful role against thyroid autoimmunity? *Clin Exp Immunol* 1998; **113**: 126-135 [PMID: 9697995]
- 20 **Shimaoka Y**, Hidaka Y, Okumura M, Takeoka K, Tada H, Amino N. Serum concentration of soluble Fas in patients with autoimmune thyroid diseases. *Thyroid* 1998; **8**: 43-47 [PMID: 9492152]
- 21 **Tomer Y**, Davies TF. Searching for the autoimmune thyroid disease susceptibility genes: from gene mapping to gene function. *Endocr Rev* 2003; **24**: 694-717 [PMID: 14570752 DOI: 10.1210/er.2002-0030]
- 22 **Weetman AP**. Autoimmune thyroid disease: propagation and progression. *Eur J Endocrinol* 2003; **148**: 1-9 [PMID: 12534350 DOI: 10.1530/eje.0.1480001]
- 23 **Ban Y**, Tozaki T, Tobe T, Ban Y, Jacobson EM, Concepcion ES, Tomer Y. The regulatory T cell gene FOXP3 and genetic susceptibility to thyroid autoimmunity: an association analysis in Caucasian and Japanese cohorts. *J Autoimmun* 2007; **28**: 201-207 [PMID: 17418529 DOI: 10.1016/j.jaut.2007.02.016]
- 24 **Bech K**, Lumpholtz B, Nerup J, Thomsen M, Platz P, Ryder LP, Svejgaard A, Siersbaek-Nielsen K, Hansen JM, Larsen JH. HLA antigens in Graves' disease. *Acta Endocrinol (Copenh)* 1977; **86**: 510-516 [PMID: 72471 DOI: 10.1530/acta.0.0860510]
- 25 **Brix TH**, Kyvik KO, Hegedus L. What is evidence of genetic factor in the aetiology of Graves' disease? A brief review. *Thyroid* 1998; **8**: 627-634 [DOI: 10.1089/thy.1998.8.627]
- 26 **Czaja AJ**, Carpenter HA, Santrach PJ, Moore SB. Immunologic features and HLA associations in chronic viral hepatitis. *Gastroenterology* 1995; **108**: 157-164 [PMID: 7806037 DOI: 10.1016/0016-5085(95)90020-9]
- 27 **Hall R**, Owen SG, Smart GA. Evidence for genetic predisposition to formation of thyroid autoantibodies. *Lancet* 1960; **2**: 187-188 [PMID: 14399065]
- 28 **Hunt PJ**, Marshall SE, Weetman AP, Bunce M, Bell JI, Wass JA, Welsh KI. Histocompatibility leucocyte antigens and closely linked immunomodulatory genes in autoimmune thyroid disease. *Clin Endocrinol (Oxf)* 2001; **55**: 491-499 [PMID: 11678832 DOI: 10.1046/j.1365-2265.2001.01356.x]
- 29 **Jacobson EM**, Huber AK, Akeno N, Sivak M, Li CW, Concepcion E, Ho K, Tomer Y. A CD40 Kozak sequence polymorphism and susceptibility to antibody-mediated autoimmune conditions: the role of CD40 tissue-specific expression. *Genes Immun* 2007; **8**: 205-214 [PMID: 17344890 DOI: 10.1038/sj.gene.6364375]
- 30 **Kakizaki S**, Takagi H, Murakami M, Takayama H, Mori M. HLA antigens in patients with interferon-alpha-induced autoimmune thyroid disorders in chronic hepatitis C. *J Hepatol* 1999; **30**: 794-800 [PMID: 10365804 DOI: 10.1016/S0168-8278(99)80131-7]
- 31 **Tomer Y**, Barbesino G, Greenberg DA, Concepcion E, Davies TF. A new Graves disease-susceptibility locus maps to chromosome 20q11.2. International Consortium for the Genetics of Autoimmune Thyroid Disease. *Am J Hum Genet* 1998; **63**: 1749-1756 [PMID: 9837828 DOI: 10.1086/302146]
- 32 **Yanagawa T**, Hidaka Y, Guimaraes V, Soliman M, DeGroot LJ. CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. *J Clin Endocrinol Metab* 1995; **80**: 41-45



- [PMID: 7829637 DOI: 10.1210/jc.80.1.41]
- 33 **Höhler T**, Gerken G, Notghi A, Knolle P, Lubjuhn R, Taheri H, Schneider PM, Meyer zum Büschenfelde KH, Rittner C. MHC class II genes influence the susceptibility to chronic active hepatitis C. *J Hepatol* 1997; **27**: 259-264 [PMID: 9288598]
  - 34 **Roederer M**, Quaye L, Mangino M, Beddall MH, Mahnke Y, Chattopadhyay P, Tosi I, Napolitano L, Terranova Barberio M, Menni C, Villanova F, Di Meglio P, Spector TD, Nestle FO. The genetic architecture of the human immune system: a bioresource for autoimmunity and disease pathogenesis. *Cell* 2015; **161**: 387-403 [PMID: 25772697 DOI: 10.1016/j.cell.2015.02.046]
  - 35 **Whitacre CC**. Sex differences in autoimmune disease. *Nat Immunol* 2001; **2**: 777-780 [PMID: 11526384 DOI: 10.1038/ni0901-777]
  - 36 **White DL**, Tavakoli-Tabasi S, Kuzniarek J, Pascua R, Ramsey DJ, El-Serag HB. Higher serum testosterone is associated with increased risk of advanced hepatitis C-related liver disease in males. *Hepatology* 2012; **55**: 759-768 [PMID: 21858849 DOI: 10.1002/hep.24618]
  - 37 **White DL**, Liu Y, Garcia J, El-Serag HB, Jiao L, Tsavachidis S, Franco LM, Lee JS, Tavakoli-Tabasi S, Moore D, Goldman R, Kuzniarek J, Ramsey DJ, Kanwal F, Marcelli M. Sex hormone pathway gene polymorphisms are associated with risk of advanced hepatitis C-related liver disease in males. *Int J Mol Epidemiol Genet* 2014; **5**: 164-176 [PMID: 25379136]
  - 38 **Sternberg EM**. Neuroendocrine regulation of autoimmune/inflammatory disease. *J Endocrinol* 2001; **169**: 429-435 [PMID: 11375112 DOI: 10.1677/joe.0.1690429]
  - 39 **Tran HA**, Reeves GE, Lyons TJ, Attia JR. Histopathologic findings of autoimmunity in thyroid, pituitary, and adrenal diseases in chronic hepatitis C postmortem cases. *Endocr Pract* 2010; **16**: 566-569 [PMID: 20150020 DOI: 10.4158/EP09359.OR]
  - 40 **Menconi F**, Huber A, Osman R, Concepcion E, Jacobson EM, Stefan M, David CS, Tomer Y. Tg.2098 is a major human thyroglobulin T-cell epitope. *J Autoimmun* 2010; **35**: 45-51 [PMID: 20303712 DOI: 10.1016/j.jaut.2010.01.004]
  - 41 **Yin X**, Latif R, Bahn R, Tomer Y, Davies TF. Influence of the TSH receptor gene on susceptibility to Graves' disease and Graves' ophthalmopathy. *Thyroid* 2008; **18**: 1201-1206 [PMID: 18925838 DOI: 10.1089/thy.2008.0098]
  - 42 **Tomer Y**. Mechanisms of autoimmune thyroid diseases: from genetics to epigenetics. *Annu Rev Pathol* 2014; **9**: 147-156 [PMID: 24460189 DOI: 10.1146/annurev-pathol-012513-104713]
  - 43 **Winsa B**, Adami HO, Bergström R, Gamstedt A, Dahlberg PA, Adamson U, Jansson R, Karlsson A. Stressful life events and Graves' disease. *Lancet* 1991; **338**: 1475-1479 [PMID: 1683917 DOI: 10.1016/0140-6736(91)92298-G]
  - 44 **Chiovato L**, Pinchera A. Stressful life events and Graves' disease. *Eur J Endocrinol* 1996; **134**: 680-682 [PMID: 8766933 DOI: 10.1530/eje.0.1340680]
  - 45 **Coles AJ**, Wing M, Smith S, Coraddu F, Greer S, Taylor C, Weetman A, Hale G, Chatterjee VK, Waldmann H, Compston A. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. *Lancet* 1999; **354**: 1691-1695 [PMID: 10568572 DOI: 10.1016/S0140-6736(99)02429-0]
  - 46 **Ruwhof C**, Drexhage HA. Iodine and thyroid autoimmune disease in animal models. *Thyroid* 2001; **11**: 427-436 [PMID: 11396701 DOI: 10.1089/105072501300176381]
  - 47 **Weetman AP**, McGregor AM. Autoimmune thyroid disease: further developments in our understanding. *Endocr Rev* 1994; **15**: 788-830 [PMID: 7705281 DOI: 10.1210/er.15.6.788]
  - 48 **Burman P**, Tötterman TH, Oberg K, Karlsson FA. Thyroid autoimmunity in patients on long term therapy with leukocyte-derived interferon. *J Clin Endocrinol Metab* 1986; **63**: 1086-1090 [PMID: 2944910 DOI: 10.1210/jcem-63-5-1086]
  - 49 **Hsieh MC**, Yu ML, Chuang WL, Shin SJ, Dai CY, Chen SC, Lin ZY, Hsieh MY, Liu JF, Wang LY, Chang WY. Virologic factors related to interferon-alpha-induced thyroid dysfunction in patients with chronic hepatitis C. *Eur J Endocrinol* 2000; **142**: 431-437 [PMID: 10802518 DOI: 10.1530/eje.0.1420431]
  - 50 **Lunel F**. Hepatitis C virus and autoimmunity: fortuitous association or reality? *Gastroenterology* 1994; **107**: 1550-1555 [PMID: 7523229 DOI: 10.1016/0016-5085(94)90564-9]
  - 51 **Shaheen MA**, Idrees M. Evidence-based consensus on the diagnosis, prevention and management of hepatitis C virus disease. *World J Hepatol* 2015; **7**: 616-627 [PMID: 25848486 DOI: 10.4254/wjh.v7.i3.616]
  - 52 **Smith B**, Falck-Ytter Y. Guidelines for the screening, care and treatment of persons with hepatitis C infection. WHO Library Cataloguing-in-Publication Data, 2014
  - 53 **Webster DP**, Klennerman P, Dusheiko GM. Hepatitis C. *Lancet* 2015; **385**: 1124-1135 [PMID: 25687730 DOI: 10.1016/S0140-6736(14)62401-6]
  - 54 **Ferri C**, Antonelli A, Mascia MT, Sebastiani M, Fallahi P, Ferrari D, Pileri SA, Zignego AL. HCV-related autoimmune and neoplastic disorders: the HCV syndrome. *Dig Liver Dis* 2007; **39** Suppl 1: S13-S21 [PMID: 17936215 DOI: 10.1016/S1590-8658(07)80005-3]
  - 55 **Calvaruso V**, Craxi A. Immunological alterations in hepatitis C virus infection. *World J Gastroenterol* 2013; **19**: 8916-8923 [PMID: 24379616 DOI: 10.3748/wjg.v19.i47.8916]
  - 56 **Rosa D**, Saletti G, De Gregorio E, Zorat F, Comar C, D'Oro U, Nuti S, Houghton M, Barnaba V, Pozzato G, Abrignani S. Activation of naïve B lymphocytes via CD81, a pathogenetic mechanism for hepatitis C virus-associated B lymphocyte disorders. *Proc Natl Acad Sci USA* 2005; **102**: 18544-18549 [PMID: 16339892 DOI: 10.1073/pnas.0509402102]
  - 57 **Petracca R**, Falugi F, Galli G, Norais N, Rosa D, Campagnoli S, Burgio V, Di Stasio E, Giardina B, Houghton M, Abrignani S, Grandi G. Structure-function analysis of hepatitis C virus envelope-CD81 binding. *J Virol* 2000; **74**: 4824-4830 [PMID: 10775621 DOI: 10.1128/JVI.74.10.4824-4830.2000]
  - 58 **Apolinario A**, Majano PL, Lorente R, Núñez O, Clemente G, García-Monzón C. Gene expression profile of T-cell-specific chemokines in human hepatocyte-derived cells: evidence for a synergistic inducer effect of cytokines and hepatitis C virus proteins. *J Viral Hepat* 2005; **12**: 27-37 [PMID: 15655045 DOI: 10.1111/j.1365-2893.2005.00540.x]
  - 59 **Akeno N**, Blackard JT, Tomer Y. HCV E2 protein binds directly to thyroid cells and induces IL-8 production: a new mechanism for HCV induced thyroid autoimmunity. *J Autoimmun* 2008; **31**: 339-344 [PMID: 18799285 DOI: 10.1016/j.jaut.2008.08.001]
  - 60 **Danilovic DL**, Mendes-Correa MC, Chammas MC, Zambrini H, Barros RK, Marui S. Thyroid disturbance related to chronic hepatitis C infection: role of CXCL10. *Endocr J* 2013; **60**: 583-590 [PMID: 23291435]
  - 61 **Broussole C**, Steineur MP, Bailly F, Zoulim F, Trépo C. [Hepatitis C virus infection and thyroid diseases]. *Rev Med Interne* 1999; **20**: 766-773 [PMID: 10522298 DOI: 10.1016/S0248-8663(00)88683-X]
  - 62 **Carella C**, Mazziotti G, Morisco F, Manganella G, Rotondi M, Tuccillo C, Sorvillo F, Caporaso N, Amato G. Long-term outcome of interferon-alpha-induced thyroid autoimmunity and prognostic influence of thyroid autoantibody pattern at the end of treatment. *J Clin Endocrinol Metab* 2001; **86**: 1925-1929 [PMID: 11344186 DOI: 10.1210/jcem.86.5.7459]
  - 63 **Fernandez-Soto L**, Gonzalez A, Escobar-Jimenez F, Vazquez R, Ocete E, Olea N, Salmeron J. Increased risk of autoimmune thyroid disease in hepatitis C vs hepatitis B before, during, and after discontinuing interferon therapy. *Arch Intern Med* 1998; **158**: 1445-1448 [PMID: 9665354 DOI: 10.1001/archinte.158.13.1445]
  - 64 **Huang MJ**, Tsai SL, Huang BY, Sheen IS, Yeh CT, Liaw YF. Prevalence and significance of thyroid autoantibodies in patients with chronic hepatitis C virus infection: a prospective controlled study. *Clin Endocrinol (Oxf)* 1999; **50**: 503-509 [PMID: 10468911 DOI: 10.1046/j.1365-2265.1999.00686.x]
  - 65 **Deutsch M**, Dourakis S, Manesis EK, Gioustozi A, Hess G, Horsch A, Hadziyannis S. Thyroid abnormalities in chronic viral hepatitis and their relationship to interferon alfa therapy. *Hepatology* 1997; **26**: 206-210 [PMID: 9214471 DOI: 10.1002/hep.510260127]
  - 66 **Ganne-Carrie N**, Medini A, Coderc E, Seror O, Christidis C,

- Grimbert S, Trinchet JC, Beaugrand M. Latent autoimmune thyroiditis in untreated patients with HCV chronic hepatitis: a case-control study. *J Autoimmun* 2000; **14**: 189-193 [PMID: 10677250 DOI: 10.1006/jaut.1999.0360]
- 67 **Marazuela M**, García-Buey L, González-Fernández B, García-Monzón C, Arranz A, Borque MJ, Moreno-Otero R. Thyroid autoimmune disorders in patients with chronic hepatitis C before and during interferon-alpha therapy. *Clin Endocrinol (Oxf)* 1996; **44**: 635-642 [PMID: 8759175 DOI: 10.1046/j.1365-2265.1996.751768.x]
- 68 **Marcellin P**, Pouteau M, Benhamou JP. Hepatitis C virus infection, alpha interferon therapy and thyroid dysfunction. *J Hepatol* 1995; **22**: 364-369 [PMID: 7608489 DOI: 10.1016/0168-8278(95)80291-6]
- 69 **Oppenheim Y**, Ban Y, Tomer Y. Interferon induced Autoimmune Thyroid Disease (AITD): a model for human autoimmunity. *Autoimmun Rev* 2004; **3**: 388-393 [PMID: 15288006 DOI: 10.1016/j.autrev.2004.03.003]
- 70 **Prummel MF**, Laurberg P. Interferon-alpha and autoimmune thyroid disease. *Thyroid* 2003; **13**: 547-551 [PMID: 12930598 DOI: 10.1089/105072503322238809]
- 71 **Rocco A**, Gargano S, Provenzano A, Nardone M, De Sanctis GM, Altavilla N, Chircu LV, Grimaldi F. Incidence of autoimmune thyroiditis in interferon-alpha treated and untreated patients with chronic hepatitis C virus infection. *Neuro Endocrinol Lett* 2001; **22**: 39-44 [PMID: 11335878]
- 72 **Tomer Y**, Blackard JT, Akeno N. Interferon alpha treatment and thyroid dysfunction. *Endocrinol Metab Clin North Am* 2007; **36**: 1051-1066; x-xi [PMID: 17983936 DOI: 10.1016/j.ecl.2007.07.001]
- 73 **Gisslinger H**, Gilly B, Woloszczuk W, Mayr WR, Havelec L, Linkesch W, Weissel M. Thyroid autoimmunity and hypothyroidism during long-term treatment with recombinant interferon-alpha. *Clin Exp Immunol* 1992; **90**: 363-367 [PMID: 1458673 DOI: 10.1111/j.1365-2249.1992.tb05852.x]
- 74 **Yamazaki K**, Kanaji Y, Shizume K, Yamakawa Y, Demura H, Kanaji Y, Obara T, Sato K. Reversible inhibition by interferons alpha and beta of 125I incorporation and thyroid hormone release by human thyroid follicles in vitro. *J Clin Endocrinol Metab* 1993; **77**: 1439-1441 [PMID: 8077347 DOI: 10.1210/jc.77.5.1439]
- 75 **McCann SM**, Lyson K, Karanth S, Gimeno M, Belova N, Kamat A, Rettori V. Mechanism of action of cytokines to induce the pattern of pituitary hormone secretion in infection. *Ann N Y Acad Sci* 1995; **771**: 386-395 [PMID: 8597416 DOI: 10.1111/j.1749-6632.1995.tb44697.x]
- 76 **Ryu SY**, Jeong KS, Yoon WK, Park SJ, Kang BN, Kim SH, Park BK, Cho SW. Somatostatin and substance P induced in vivo by lipopolysaccharide and in peritoneal macrophages stimulated with lipopolysaccharide or interferon-gamma have differential effects on murine cytokine production. *Neuroimmunomodulation* 2000; **8**: 25-30 [PMID: 10859485 DOI: 10.1159/000026449]
- 77 **Falaschi P**, D'Urso R, Proietti A, Martocchia A, Pastore R, Angelucci L. Effect of r-interferon alpha administration on hypothalamus-pituitary-thyroid axis in chronic hepatitis. *Life Sci* 1997; **60**: 43-50 [PMID: 8995531 DOI: 10.1016/S0024-3205(96)00587-5]
- 78 **Falaschi P**, Martocchia A, D'Urso R, Proietti A. Subacute thyroiditis during interferon-alpha therapy for chronic hepatitis C. *J Endocrinol Invest* 1997; **20**: 24-28 [PMID: 9075068 DOI: 10.1007/BF03347968]
- 79 **Hall R**. Subacute (De Quervain's) thyroiditis. In: Hall R, Besser GM. Fundamentals of clinical endocrinology. Churchill Livingstone: Edinburgh, 1989: 101
- 80 **Nyulassy S**, Hnilica P, Buc M, Guman M, Hirschová V, Stefanovic J. Subacute (de Quervain's) thyroiditis: association with HLA-Bw35 antigen and abnormalities of the complement system, immunoglobulins and other serum proteins. *J Clin Endocrinol Metab* 1977; **45**: 270-274 [PMID: 885992 DOI: 10.1210/jcem-45-2-270]
- 81 **Romagnani S**. Induction of TH1 and TH2 responses: a key role for the 'natural' immune response? *Immunol Today* 1992; **13**: 379-381 [PMID: 1418371 DOI: 10.1016/0167-5699(92)90083-J]
- 82 **Martocchia A**, Labbadia G, Paoletti V, Gargano S, Grossi A, Trabace S, Musca A, Falaschi P. Hashimoto's disease during interferon-alpha therapy in a patient with pre-treatment negative anti-thyroid autoantibodies and with the specific genetic susceptibility to the thyroid disease. *Neuro Endocrinol Lett* 2001; **22**: 49-52 [PMID: 11335880]
- 83 **Grimaldi F**, Martocchia A, Lulli P, Frugoni P, Fiore RF, Rossi C, Ferrari F, Labbadia G, Falaschi P. Frequenza degli alleli HLA nei pazienti affetti da epatite cronica HCV-correlata e predisposizione alla comparsa della patologia tiroidea immuno-mediata dopo trattamento antivirale. *Int Emerg Med* 2009; **4**: S84
- 84 **Ohsako N**, Tamai H, Sudo T, Mukuta T, Tanaka H, Kuma K, Kimura A, Sasazuki T. Clinical characteristics of subacute thyroiditis classified according to human leukocyte antigen typing. *J Clin Endocrinol Metab* 1995; **80**: 3653-3656 [PMID: 8530615]
- 85 **Chen QY**, Huang W, She JX, Baxter F, Volpe R, Maclaren NK. HLA-DRB1\*08, DRB1\*03/DRB3\*0101, and DRB3\*0202 are susceptibility genes for Graves' disease in North American Caucasians, whereas DRB1\*07 is protective. *J Clin Endocrinol Metab* 1999; **84**: 3182-3186 [PMID: 10487684]
- 86 **Dalton TA**, Bennett JC. Autoimmune disease and the major histocompatibility complex: therapeutic implications. *Am J Med* 1992; **92**: 183-188 [PMID: 1543203 DOI: 10.1016/0002-9343(92)90110-W]
- 87 **Heaward JM**, Allahabadia A, Daykin J, Carr-Smith J, Daly A, Armitage M, Dodson PM, Sheppard MC, Barnett AH, Franklyn JA, Gough SC. Linkage disequilibrium between the human leukocyte antigen class II region of the major histocompatibility complex and Graves' disease: replication using a population case control and family-based study. *J Clin Endocrinol Metab* 1998; **83**: 3394-3397 [PMID: 9768636 DOI: 10.1210/jc.83.10.3394]
- 88 **Kinney JS**, Hurwitz ES, Fishbein DB, Woolf PD, Pinsky PF, Lawrence DN, Anderson LJ, Holmes GP, Wilson CK, Loschen DJ. Community outbreak of thyrotoxicosis: epidemiology, immunogenetic characteristics, and long-term outcome. *Am J Med* 1988; **84**: 10-18 [PMID: 3257352 DOI: 10.1016/0002-9343(88)90002-2]
- 89 **Zamani M**, Spaepen M, Bex M, Bouillon R, Cassiman JJ. Primary role of the HLA class II DRB1\*0301 allele in Graves disease. *Am J Med Genet* 2000; **95**: 432-437 [PMID: 11146462 DOI: 10.1002/1096-8628(20001218)95]
- 90 **Farid NR**, Sampson L, Moens H, Barnard JM. The association of goitrous autoimmune thyroiditis with HLA-DR5. *Tissue Antigens* 1981; **17**: 265-268 [PMID: 6947505 DOI: 10.1111/j.1399-0039.1981.tb00700.x]
- 91 **Bogner U**, Badenhop K, Peters H, Schmieg D, Mayr WR, Usadel KH, Schleusener H. HLA-DR/DQ gene variation in nongoitrous autoimmune thyroiditis at the serological and molecular level. *Autoimmunity* 1992; **14**: 155-158 [PMID: 1363895 DOI: 10.1089/thy.2012.0507]
- 92 **Pocceco M**, Barbi E, De Campo C. [Autoimmune thyroid pathology. Study and follow-up of pediatric case reports]. *Pediatr Med Chir* 1986; **8**: 691-694 [PMID: 3496586]
- 93 **Menconi F**, Monti MC, Greenberg DA, Oashi T, Osman R, Davies TF, Ban Y, Jacobson EM, Concepcion ES, Li CW, Tomer Y. Molecular amino acid signatures in the MHC class II peptide-binding pocket predispose to autoimmune thyroiditis in humans and in mice. *Proc Natl Acad Sci USA* 2008; **105**: 14034-14039 [PMID: 18779568 DOI: 10.1073/pnas.0806584105]
- 94 **Sarobe P**, Huarte E, Lasarte JJ, López-Díaz de Cerio A, García N, Borrás-Cuesta F, Prieto J. Characterization of an immunologically conserved epitope from hepatitis C virus E2 glycoprotein recognized by HLA-A2 restricted cytotoxic T lymphocytes. *J Hepatol* 2001; **34**: 321-329 [PMID: 11281563 DOI: 10.1016/S0168-8278(00)00018-0]
- 95 **Vertuani S**, Bazzaro M, Gualandi G, Micheletti F, Marastoni M, Fortini C, Canella A, Marino M, Tomatis R, Traniello S, Gavioli R. Effect of interferon-alpha therapy on epitope-specific cytotoxic T lymphocyte responses in hepatitis C virus-infected individuals. *Eur*

- J Immunol* 2002; **32**: 144-154 [PMID: 11754355 DOI: 10.1002/1521-4141(200201)32:1]
- 96 **Brazillet MP**, Batteux F, Abehsira-Amar O, Nicoletti F, Charreire J. Induction of experimental autoimmune thyroiditis by heat-denatured porcine thyroglobulin: a Tc1-mediated disease. *Eur J Immunol* 1999; **29**: 1342-1352 [PMID: 10229102 DOI: 10.1002/(SICI)1521-4141(199904)29]
  - 97 **Iwatani Y**, Amino N, Hidaka Y, Kaneda T, Ichihara K, Tamaki H, Matsuzuka F, Fukata S, Kuma K, Miyai K. Decreases in alpha beta T cell receptor negative T cells and CD8 cells, and an increase in CD4+ CD8+ cells in active Hashimoto's disease and subacute thyroiditis. *Clin Exp Immunol* 1992; **87**: 444-449 [PMID: 1347493 DOI: 10.1111/j.1365-2249.1992.tb03017.x]
  - 98 **Blackard JT**, Kong L, Huber AK, Tomer Y. Hepatitis C virus infection of a thyroid cell line: implications for pathogenesis of hepatitis C virus and thyroiditis. *Thyroid* 2013; **7**: 863-870 [PMID: 2325973]
  - 99 **Narayana SK**, Helbig KJ, McCartney EM, Eyre NS, Bull RA, Eltahla A, Lloyd AR, Beard MR. The Interferon-induced Transmembrane Proteins, IFITM1, IFITM2, and IFITM3 Inhibit Hepatitis C Virus Entry. *J Biol Chem* 2015; **290**: 25946-25959 [PMID: 26354436]
  - 100 **Martocchia A**, Falaschi P. Amino acid sequence homologies between HCV polyprotein and thyroid antigens. *Intern Emerg Med* 2007; **2**: 65-67 [PMID: 17551693]
  - 101 **Stefan M**, Jacobson EM, Huber AK, Greenberg DA, Li CW, Skrabanek L, Conception E, Fadlalla M, Ho K, Tomer Y. Novel variant of thyroglobulin promoter triggers thyroid autoimmunity through an epigenetic interferon alpha-modulated mechanism. *J Biol Chem* 2011; **286**: 31168-31179 [PMID: 21757724 DOI: 10.1074/jbc.M111.247510]
  - 102 **Stefan M**, Wei C, Lombardi A, Li CW, Concepcion ES, Inabnet WB, Owen R, Zhang W, Tomer Y. Genetic-epigenetic dysregulation of thymic TSH receptor gene expression triggers thyroid autoimmunity. *Proc Natl Acad Sci USA* 2014; **111**: 12562-12567 [PMID: 25122677]
  - 103 **Fried MW**. Side effects of therapy of hepatitis C and their management. *Hepatology* 2002; **36**: S237-S244 [PMID: 12407599 DOI: 10.1002/hep.1840360730]
  - 104 **Hunyady B**, Kovács B, Battyáni Z. [Side-effects of pegylated interferon plus ribavirin therapy with or without protease inhibitor direct acting antiviral agents during treatment of chronic hepatitis C virus infection]. *Orv Hetil* 2011; **152**: 1997-2009 [PMID: 23259732 DOI: 10.1556/OH.2011.29266]
  - 105 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]
  - 106 **Bressler BL**, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; **38**: 639-644 [PMID: 12939590 DOI: 10.1053/jhep.2003.50350]
  - 107 **Feld JJ**, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, Weiland O, Aguilar H, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1594-1603 [PMID: 24720703 DOI: 10.1056/NEJMoa1315722]
  - 108 **Zeuzem S**, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourlière M, Sulkowski MS, Wedemeyer H, Tam E, Desmond P, Jensen DM, Di Bisceglie AM, Varunok P, Hassanein T, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1604-1614 [PMID: 24720679 DOI: 10.1056/NEJMoa1401561]
  - 109 **Ferenci P**, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A, Box TD, Younes Z, Enayati P, Green S, Baruch Y, Bhandari BR, Caruntu FA, Sepe T, Chulanov V, Janczewska E, Rizzardini G, Gervain J, Planas R, Moreno C, Hassanein T, Xie W, King M, Podsadecki T, Reddy KR. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *N Engl J Med* 2014; **370**: 1983-1992 [PMID: 24795200 DOI: 10.1056/NEJMoa1402338]

**P- Reviewer:** Sargsyants N **S- Editor:** Qi Y

**L- Editor:** A **E- Editor:** Liu SQ



## 2016 Hepatitis C Virus: Global view

# Chronic hepatitis C: This and the new era of treatment

Gaetano Bertino, Annalisa Ardiri, Maria Proiti, Giuseppe Rigano, Evelise Frazzetto, Shirin Demma, Maria Irene Ruggeri, Laura Scuderi, Giulia Malaguarnera, Nicoletta Bertino, Venerando Rapisarda, Isidoro Di Carlo, Adriana Toro, Federico Salomone, Mariano Malaguarnera, Emanuele Bertino, Michele Malaguarnera

Gaetano Bertino, Annalisa Ardiri, Maria Proiti, Giuseppe Rigano, Evelise Frazzetto, Shirin Demma, Laura Scuderi, Hepatology Unit - Department of Clinical and Experimental Medicine, University of Catania, 95123 Catania, Italy

Maria Irene Ruggeri, Internal Medicine Unit, ARNAS Civic Hospital, 90142 Palermo, Italy

Giulia Malaguarnera, Mariano Malaguarnera, Michele Malaguarnera, Research Centre "La Grande Senesce", University of Catania, 95100 Catania, Italy

Giulia Malaguarnera, Michele Malaguarnera, Department of Biomedical Sciences, University of Catania, 95100 Catania, Italy

Nicoletta Bertino, Emanuele Bertino, Faculty of Pharmacy, University of Catania, 95123 Catania, Italy

Venerando Rapisarda, Occupational Medicine Unit, Department of Clinical and Experimental Medicine, University of Catania, 95100 Catania, Italy

Isidoro Di Carlo, Adriana Toro, Department of Surgical Sciences, Organ Transplantation and Advanced Technologies, University of Catania, 95100 Catania, Italy

Federico Salomone, Gastroenterology Unit, Acireale Hospital, 95024 Acireale, Catania, Italy

**Author contributions:** All authors contributed to this paper.

**Conflict-of-interest statement:** No potential conflicts of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Gaetano Bertino, Professor, Hepatology Unit - Department of Clinical and Experimental Medicine, University of Catania, Policlinic - Via S. Sofia n. 78, 95123 Catania, Italy. [gaetanobertinounict@libero.it](mailto:gaetanobertinounict@libero.it)  
 Telephone: +39-09-53781573  
 Fax: +39-09-53781572

Received: April 23, 2015

Peer-review started: April 24, 2015

First decision: August 10, 2015

Revised: November 23, 2015

Accepted: December 17, 2015

Article in press: December 18, 2015

Published online: January 18, 2016

## Abstract

Over the last years it has started a real revolution in the treatment of chronic hepatitis C. This occurred for the availability of direct-acting antiviral agents that allow to reach sustained virologic response in approximately 90% of cases. In the near future further progress will be achieved with the use of pan-genotypic drugs with high efficacy but without side effects.

**Key words:** Direct-acting antiviral agents; Nucleoside inhibitors; Boceprevir; Sofosbuvir; Telaprevir; Hepatitis C; Simeprevir; Daclatasvir; Ledipasvir; Faldaprevir; Ritonavir; Ombitasvir; Dasabuvir

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This review analyzes the current therapies for chronic hepatitis C and the future challenges of the research. So it tries to give an update on the research of hepatitis C virus (HCV) infection, providing a critical view of the emerging therapies and their impact on the future management of HCV infection. Since novel



treatments for HCV infection are highly efficacious but costly, priority should be given to patients with advanced hepatic fibrosis, which is a disease that cannot be deferred.

Bertino G, Ardiri A, Proiti M, Rigano G, Frazzetto E, Demma S, Ruggeri MI, Scuderi L, Malaguarnera G, Bertino N, Rapisarda V, Di Carlo I, Toro A, Salomone F, Malaguarnera M, Bertino E, Malaguarnera M. Chronic hepatitis C: This and the new era of treatment. *World J Hepatol* 2016; 8(2): 92-106 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i2/92.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i2.92>

## INTRODUCTION

The hepatitis C virus (HCV), identified in the 70s but cloned in 1989, is a single-stranded RNA virus belonging to the family *Flaviviridae*.

HCV is the main cause of progressive liver diseases and a public health problem worldwide. It is estimated that approximately 150-180 million people in the world are living with chronic hepatitis<sup>[1,2]</sup>, 350 million of whom die each year from liver damage associated with the infection<sup>[3]</sup>.

About 80% of people infected with HCV develop chronic hepatitis, of which 20%-40% will develop liver cirrhosis or hepatocellular carcinoma (HCC) 20-30 years after infection.

As a consequence, chronic HCV infection is the major reason of liver transplantation in developed countries<sup>[4-7]</sup>.

According to the Global Burden Disease Study in Europe, the death rate for viral hepatitis is significantly higher than that for human immunodeficiency virus (HIV) and acquired immune deficiency syndrome; in particular in 2010, the number of deaths from viral hepatitis have been ten times bigger than that attributed to HIV. It is reasonable to think that this difference is due to the lack of effective therapies for HCV until a few years ago<sup>[8]</sup>.

HCV is also one of the main causes of death<sup>[9]</sup>. The virus causes both liver damage and extra-hepatic manifestations, many of these syndromes are associated with the ability of HCV to replicate in peripheral blood mononuclear cells (PBMCs); an example is the mixed cryoglobulinemia, which is by far the most common extrahepatic disease closely connected with the infection.

Recently it was shown that antiviral treatment is associated with improved renal and cardiovascular outcomes in patients with cryoglobulinemia<sup>[4,6,10,11]</sup>. Newly approved oral anti-HCV drugs are very safe and effective, but unfortunately their cost will force to choose a priority of treatment. The intent should therefore be to identify and treat patients with a higher risk of morbidity and mortality due to HCV.

The availability of these new oral treatments can definitely heal patients and consequently it will cause a significant reduction in health care costs<sup>[2]</sup>. The aim of

this review article is to give an update on the research of HCV infection, providing a critical view of the emerging therapies and their impact on the future management of HCV infection.

## Natural history of chronic hepatitis C

The natural history of chronic hepatitis C is partly defined. The primary HCV infection is completely asymptomatic in 60%-70% of cases, but in 80% of patients the infection becomes chronic and is characterized by the persistence of the viral genome in the blood for at least 6 mo from the onset of acute infection. In a variable proportion of people carrying the virus, especially in the presence of strong necro-inflammation and/or co-factors of liver damage, the disease can evolve from the condition of chronic hepatitis to cirrhosis and HCC.

There are several factors that can change the course, severity and progression of the disease, including age at the time of infection, route of infection, viral load, co-infection with other hepatitis viruses or HIV, alterations of immune status, and the coexistence of other hepatotoxic factors such as consumption of alcohol, iron overload, obesity, type 2 diabetes, resistance to insulin and genetic factors<sup>[12-14]</sup>.

Chronic HCV infection in about 20% of cases progresses up to hepatic cirrhosis, end-stage liver disease and HCC, generally after 20-30 years from primary infection.

The progression of chronic disease leads, through a mechanism of chronic damage, to the loss of organ function, for progressive deposition of fibrotic tissue and disruption of the parenchymal structure, and results in liver fibrosis and cirrhosis.

Cirrhosis changes the normal liver architecture, and furthermore itself represents the most important risk factor for the development of HCC, in part by acting as a cofactor accelerating the process triggered by a primary carcinogen (HCV), and specially by increased hepatocyte regeneration. Once HCV infection progresses to cirrhosis, there is a 1%-5% annual risk of HCC<sup>[12]</sup>.

The probability that a patient with compensated cirrhosis can evolve towards the decompensated form increases progressively over time.

Liver cirrhosis and its complications (portal hypertension and therefore esophageal varices, splenomegaly, ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, hepato-pulmonary syndrome and HCC) are burdened with high morbidity and mortality.

It is also known that different variables influence the progression of the disease, for which the prognosis changes individually and is very hard to define<sup>[12-14]</sup>.

Several studies have concluded that the eradication of HCV infection slows the progression of the disease, improves the survival, and reduces the incidence of liver failure and the risk of developing liver cancer<sup>[12-28]</sup>. The understanding of the natural history of chronic hepatitis C and its long-term consequences is essential to enable appropriate decisions on treatment, but unfortunately

the natural history of HCV infection is still the subject of much controversy. In fact, according to some authors the disease is relentlessly progressive, with a high probability to evolve to cirrhosis and HCC, while according to others the course is variable, and most patients die as a consequence of co-morbidities, not the infection itself.

Because the infection has a significant role in causing chronic hepatitis, cirrhosis and HCC, the goal of treatment is to cure HCV infection, and consequently to prevent its complications.

Although the viral RNA genome does not integrate into the host genome, the infection becomes persistent in the majority of patients, and about 70%-90% of the infected people fail to clear the virus once acquired.

It is widely known that the antiviral treatment and the achievement of a sustained virological response (SVR - defined as an absence of detectable HCV-RNA 12 mo after therapy is complete) are associated with regression of fibrosis and clinical improvement. However, despite treatment, HCV may persist in liver tissue and extrahepatic locations like PBMCs, leading to late relapse, defined as reappearance of viremia after SVR has been achieved<sup>[29,30]</sup>.

### **Treatment and SVR - what is the real purpose of antiviral therapy?**

As mentioned above the primary goal of HCV therapy is the complete eradication of the virus, which is the SVR.

SVR was traditionally defined as HCV-RNA undetectable in serum for at least 24 wk after the end of treatment (SVR24); however, recent data suggest that the assessment at 12 wk after treatment (SVR12) is sufficient for defining this result.

Follow-up studies document that more than 99% of patients who achieve an SVR remain HCV-RNA negative 4-5 years after the end of treatment, and no signs of hepatitis have been documented.

SVR represents the main goal of antiviral therapy, indeed once achieved, the SVR is considered effective in the long term because late recurrences are rare; the SVR is associated with long-term health benefits, including improved quality of life.

SVR reduces risk for progression to cirrhosis, HCC, liver transplantation and liver-related mortality, and also decreases extra-hepatic manifestations of HCV infection (for example, cryoglobulinemic vasculitis).

Moreover it seems reasonable to assume that a lasting biochemical and virological response induced by treatment can also lead to improved liver fibrosis<sup>[31-39]</sup>.

For decades the antiviral therapy of chronic HCV infection was based on the administration of interferon (IFN), initially as monotherapy and subsequently in combination with ribavirin (RBV). Dual therapy with "pegylated IFN (PEG-IFN) and RBV" achieves SVR rates of 40% to 50% in patients with genotype 1, and about 80% in those with genotypes 2, 3, 5 and 6; the results for genotype 4 are intermediates.

In 2011, the first direct-acting antivirals boceprevir

and telaprevir have been approved in combination with PEG-IFN and RBV. These drugs are protease inhibitors (PIs) and increase SVR rates in both naive patients and in experienced patients, compared to dual therapy<sup>[40-46]</sup>; however, they were dropped due to their significant toxicity.

With the advent of new oral antiviral regimens, with better efficacy and tolerability, and a shorter treatment duration, the number of patients that can be treated is expected to increase significantly, and also the SVR rates will improve to approximately 95% or plus<sup>[47]</sup>.

### **HCV and host: The HCV replication cycle and mechanisms of action of the new direct acting antiviral agents**

HCV is classified within the *Flaviviridae* family, as the only member of a distinct genus called *Hepacivirus*<sup>[48]</sup>.

The lack of detailed information on the viral replication cycle has significantly prevented the development of direct acting antivirals.

For decades the antiviral therapy of chronic HCV infection was based on the administration of IFN, initially alone and then in combination with RBV, but this regimen was effective in only 50% of patients with genotype 1, with significant side effects<sup>[49-54]</sup>.

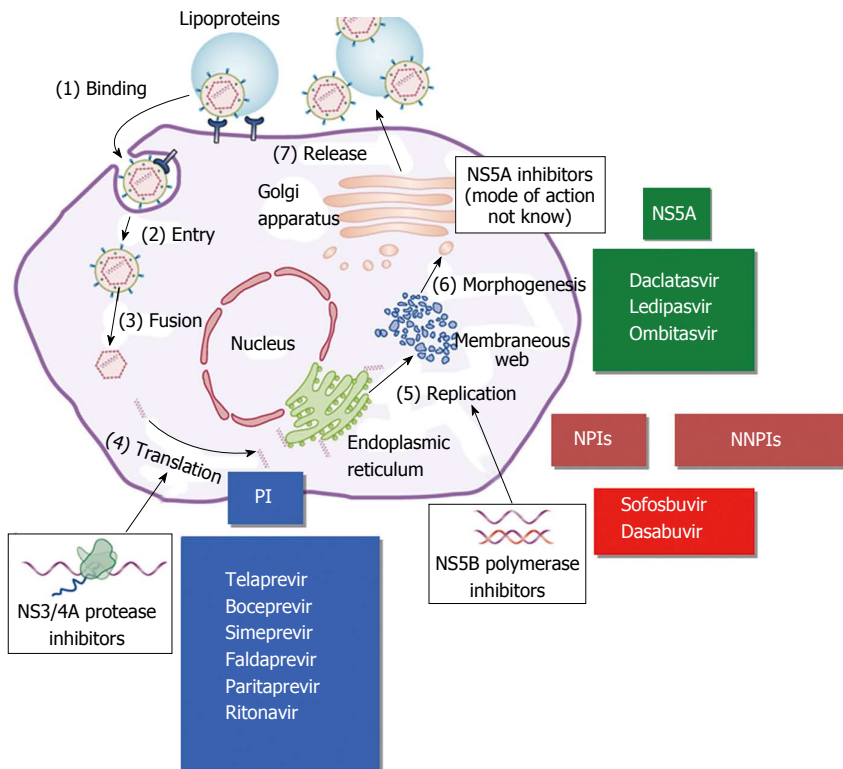
In the last decade the development of *in vitro* models of viral replication has thus represented a turning point for the understanding of the different stages of the replication cycle, and quickly has led to the design and introduction of direct acting antivirals (DAAs)<sup>[55]</sup>.

However, because of huge variability of the virus, new drugs cannot be administered as monotherapy because it would quickly lead to the selection of drug-resistant viral variants.

HCV indeed is characterized by an extremely high degree of variability. The genetic heterogeneity of HCV gives an adaptive advantage as the simultaneous presence of multiple genomic variants allows rapid selection of mutants that better adapt to environmental changes (for example resistance to drugs or the immune response); this genetic heterogeneity is the basis of chronic infection, and is probably involved in the phenomena of evasion of the immune response and in the limited efficacy of treatment<sup>[56-59]</sup>.

The HCV replication cycle occurs in the cytoplasm, and can be summarized as follows: (1) entry into the host cell and release of viral genomic RNA into the cytoplasm; (2) translation of RNA, processing of the viral polyprotein and formation of a replication complex associated with intracellular membrane; (3) using positive RNA for the synthesis of an intermediate negative RNA for the production of new positive RNA molecules with different destination; and (4) release of viral progeny into circulation from infected cells. The infectious viral structure is comprised of envelope glycoproteins in a lipid bilayer, that contain the viral core protein and RNA<sup>[60-63]</sup>.

After cell entry, the viral RNA is translated through the host machinery into a polyprotein, which is cleaved



**Figure 1** Hepatitis C virus replicative cycle and main targets for direct acting antiviral agents. Modified from Manns and Cornberg. *Lancet Infectious Diseases* 2013. PIs: Protease inhibitors; NPIs: Nucleoside polymerase inhibitors; NNPIs: Non-nucleoside polymerase inhibitors.

during and after translation by both host and viral-encoded proteases into 10 mature viral proteins, including several non-structural (NS) proteins. One of the viral proteases involved in this post-translational processing is a heterodimeric complex of the NS3 and NS4A proteins (NS3/NS4A). NS3 has the proteolytic activity and NS4A is a membrane protein that acts as a cofactor. Synthesis of new viral RNA occurs in a highly structured replication complex that consists of NS3, NS4A, NS4B, NS5A, and NS5B. NS5B is an RNA-dependent RNA polymerase that is essential for viral replication. NS5A has a presumptive role in the organization of the replication complex and in regulating replication. It is also involved in assembly of the viral particle that is released from the host cell (Figure 1)<sup>[64-69]</sup>.

### Therapies

Increased knowledge of the HCV replication cycle and genomic diversity has driven the development of antiviral agents specifically targeting well-conserved proteins required for efficient viral replication. Aside from PEG-IFN, HCV-specific therapeutic agents that have gained widespread use or reached late-stage clinical trials include NS3 PIs, nucleoside and nucleotide analogues, and other NS5B polymerase inhibitors.

### DAAs

After year of IFN-based therapy, the introduction of DAAs has increased the number of patients who respond to treatment, and has changed radically the treatment of chronic HCV genotype-1 infection<sup>[43,70-72]</sup>.

Thanks to the discovery of key viral replication targets such as the NS3/4A protease, NS5A, and the NS5B RNA polymerase, other potent antiviral inhibitors were licensed in 2014.

These new regimens include the addition of simeprevir (SMV) (a second-generation PI), daclatasvir (an NS5A inhibitor), and sofosbuvir (an uridine nucleotide prodrug NS5B polymerase inhibitor), in combination with PEG-IFN and RBV for 12-24 wk<sup>[73,74]</sup>.

The main targets of the DAAs are the HCV-encoded proteins that are vital to the viral replication. The DAAs have a high barrier to resistance and ideally, they should also be active against all HCV genotypes. Furthermore, these drugs are well tolerated and have few drug interactions.

There are four classes of DAAs, which are defined by their mechanism of action and therapeutic target<sup>[75]</sup> (Figure 2 and Table 1): (1) NS3/4A PIs; (2) NS5B nucleoside polymerase inhibitors (NPIs); (3) NS5B non-NPIs (NNPIs); and (4) NS5A inhibitors.

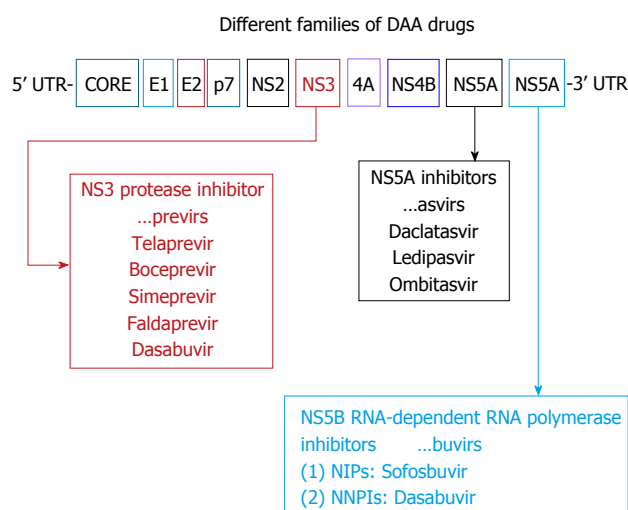
### NS3/4A PIS

NS3/4A PIs are drugs that inhibit the NS3/4A serine protease, an enzyme involved in post-translational processing and replication of HCV<sup>[76]</sup>.

There are two generation of PIs.

#### First-generation PIs (telaprevir and boceprevir)

The first-generation PIs telaprevir and boceprevir were the first DAAs available for the treatment of CHC<sup>[77]</sup>.



**Figure 2 Direct acting antiviral agents.** Modified from Alexopoulou *et al.*<sup>[121]</sup>. Interferon-based combination treatment for chronic hepatitis C in the era of direct acting antivirals. *Annals of Gastroenterology* 2015; 28: 55-65. NPIs: Nucleoside polymerase inhibitors; NNPIs: Non-nucleoside polymerase inhibitors; DAA: Direct acting antiviral.

The addition of PIs to PEG-IFN and RBV has become the new standard of care for the treatment of genotype 1 infection, and so, in 2011, has increased the efficacy of PEG-IFN and RBV in patients with chronic HCV genotype 1 infection.

Telaprevir and boceprevir were approved for the treatment of chronic HCV genotype 1 infection by the Food and Drug Administration (FDA) and European Medicines Agency in combination with PEG-IFN- $\alpha$  and RBV in adults with compensated liver disease, including cirrhosis, who are previously untreated or who have failed previous IFN and RBV therapy<sup>[78]</sup>.

Telaprevir and boceprevir are NS3/4A PIs, and they both have the same molecular target: The HCV NS3/4A serine protease.

They have an high antiviral potency only against genotypes 1 and 2, but a low barrier to resistance<sup>[79]</sup>.

Monotherapy with these agents resulted in the selection of drug resistant variants, so they should always be used in triple combinations together with PEG-IFN and RBV in a triple therapy regimen to reduce the frequencies of resistant mutants and viral breakthrough, and they can improve the SVR rates by 15% to 20% compared with PEG-IFN- $\alpha$  and RBV<sup>[42,43,80]</sup>.

Viral resistance may develop even in triple combinations with PEG-IFN and RBV, and due to this problem strict stopping rules are applied in triple therapy-based regimens.

Response to HCV therapy in genotype 1 can be predicted by identifying the single nucleotide polymorphisms located in the region of interleukin-28B (*IL-28B*) gene through genome-wide association studies.

High response rates have been reported in patients with CC genotype of *IL-28B* as compared to CT or TT *IL-28B*-genotype (70% vs 25%-30%). Testing for *IL-28B* genotype is thus a useful tool in the management

**Table 1 Classification of new antiviral drugs**

NS3/4A PIs	First-generation protease inhibitors
	Telaprevir
	Boceprevir
	Second-generation protease inhibitors
	Simeprevir
	Faldaprevir
	Paritaprevir
	Ritonavir
NS5B NPIs	Sofosbuvir
NS5B NNPIs	Dasabuvir
NS5A inhibitors	Daclatasvir
	Ledipasvir
	Ombitasvir

PIs: Protease inhibitors; NPIs: Nucleoside polymerase inhibitors; NNPIs: Non-nucleoside polymerase inhibitors.

of patients<sup>[81]</sup>.

First-generation PIs increase the number of patients with genotype 1 infection who respond to treatment, however, the side effect profiles of these triple combination therapies are not favourable, because it can cause clinically significant adverse events.

The most common side effects of telaprevir are anaemia, pruritis, nausea, diarrhoea, and anorectal discomfort. Around 4% of patients develop severe dermatitis, necessitating cessation of treatment.

Drug reactions like eosinophilia and systemic symptoms or Stevens-Johnson syndrome are rare, but have been reported. Boceprevir causes dysgeusia and anaemia<sup>[43]</sup>.

Several drug-drug interactions can occur, so the use of first-generation PIs has been significantly restricted<sup>[82]</sup>.

### Second-generation PIs

Second-generation PIs offer several benefits, for example, few drug-drug interactions and less frequent and less severe side effects.

In addition, second-generation PIs also appear to have increased efficacy against genotype 1 HCV<sup>[83]</sup>; as treatment options have progressed and improved, HCV- 1, HCV-2 and HCV-4 are considered to be easy to treat<sup>[84]</sup> but HCV genotype 3 infection has become the most difficult to treat.

**SMV:** SMV was the first available second-generation PI with antiviral activity against genotypes 1, 2, 4, 5 and 6<sup>[85]</sup>.

SMV is administered orally as a daily pill, and has limited drug-drug interactions.

No dose recommendation can be given for patients with Child-Pugh class B or C cirrhosis, because higher SMV exposure (particularly in Child-Pugh C patients) may be associated with increased frequency of adverse reactions. No dose adjustment is required in the setting of renal impairment, because SMV is eliminated by the liver<sup>[85]</sup>. SMV is well tolerated, and adverse reactions in patients receiving SMV in combination with PEG-IFN- $\alpha$



and RBV are rash, pruritus and nausea. Because SMV is an inhibitor of the transporters OATP1B1 and MRP2, mild, transient hyperbilirubinaemia not accompanied by changes in other liver parameters was observed in approximately 10% of cases. SMV is oxidatively metabolized by CYP3A subfamily, which consists mainly of hepatic and intestinal CYP3A4 metabolism<sup>[86]</sup>. Co-administration of SMV with inhibitors of cytochrome P450 3A (CYP3A) is not recommended.

In post-liver transplant patients with HCV infection, co-administration of SMV with cyclosporine resulted in significantly elevated SMV levels, so it is not recommended<sup>[87]</sup>. SMV can be safely administered with tacrolimus or sirolimus. SMV was approved by the FDA for genotype 1 treatment in November 2013 under the name of "OLYSIO", in Japan it was licensed in September 2013, finally in Europe in May 2014 (European Medical Agency approval).

In phase II of COSMOS trial, sofosbuvir (SOF; 400 mg daily) was administered in combination with SMV (SMV 150 mg daily) with or without RBV for 12 wk or 24 wk in genotype 1 patients. SVR12 rates were not different between 12 or 24 wk of treatment, with or without RBV, and comparing naive patients to experienced (95% vs 91%)<sup>[87,88]</sup>.

In this small study, the regimen SOF plus SMV with or without RBV was well tolerated; the most common side effects were headache, fatigue, and nausea, and only four (2%) patients discontinued treatment due to these events.

Although the results of this study are encouraging, due to the small number of patients and the future availability of other oral regimes with better antiviral efficacy and fewer side effects, this regimen should be considered as a second-line option.

Two phase III trials of SMV/SOF without RBV are ongoing (OPTIMIST-1 and -2)<sup>[89]</sup>. These studies provide us much bigger data about SOF/SIM regimen, and investigate the efficacy and safety of SMV 150 mg in combination with sofosbuvir 400 mg in HCV genotype 1 infected naïve or experienced patients, with and without cirrhosis.

SMV/SOF treatment led to high SVR12 rates in patients infected with HCV GT-1 subtype, regardless of treatment duration or the addition of RBV. SVR12 rates were high, regardless of baseline characteristics, including HCV GT-1 subtype, *IL-28B* allele, or Q80K polymorphism. On-treatment virologic response, including RVR, was not predictive of SVR. Two ongoing phase III trials are investigating SMV/SOF without RBV (OPTIMIST-1 and -2).

Baseline predictive factors significantly associated with virologic relapse were male sex, body weight  $\geq 75$  kg, *IL-28B* non-CC allele, cirrhosis, baseline HCV RNA  $\geq 800000$  IU/mL, and prior treatment failure. Current SOF regimens are highly efficacious, even in patients with multiple traditional negative predictors of diminished efficacy; SVR12 rates are comparatively lower in patients who have five or six negative predictors<sup>[90,91]</sup>.

The approval of the treatment scheme "SMV plus PEG-IFN/RBV" is based on a clinical trial program comprising three phase III studies, with more than 1000 patients with genotype 1.

The studies, QUEST-1, QUEST-2 and PROMISE, have evaluated the use of SMV in combination with PEG-IFN/RBV in naive patients (Quest-1 and 2)<sup>[92,93]</sup> and relapsed patients (PROMISE<sup>[94]</sup>) after an IFN-based treatment. All three studies have shown that SMV, in combination with PEG-IFN/RBV, gets significant SVR rates when compared to PEG-IFN/RBV.

A triple therapy with SMV, PEG-IFN and RBV has been recommended for genotype 1 also after the data of other four phase III trials: CONCERTO-1, -2, -3 and -4<sup>[95-98]</sup>.

**Faldaprevir:** Faldaprevir is one of the new-generation NS3/4A PIs in development. It is a pan-genotypic potent NS3/NS4 PI (antiviral activity against genotypes 1, 2, 4, 5 and 6 *in vitro*). It was used in genotype 1 infection in two combinations: (1) a triple regimen with faldaprevir, PEG-IFN and RBV for a total of 24 wk<sup>[98,99]</sup>; and (2) IFN-free regimens with faldaprevir and deleobuvir with or without RBV<sup>[100,101]</sup>.

In both combinations faldaprevir provides high SVR rates, but IFN-containing regimens registered most cases of breakthrough and relapse, while with the IFN-free combination of faldaprevir and deleobuvir with RBV, very encouraging results were obtained<sup>[102]</sup>.

Faldaprevir is administered orally, once a day. The most common adverse events are gastrointestinal dysfunction, rash and photosensitivity skin. Faldaprevir in combination with PEG-IFN and RBV appears to be associated with fewer adverse events than the first PIs telaprevir and boceprevir.

**Paritaprevir and ritonavir:** Paritaprevir is an HCV protease inhibitor that is given with low dose ritonavir for a pharmacologic boosting effect.

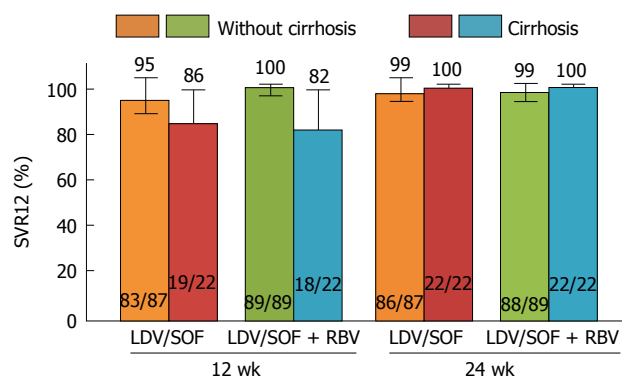
Ritonavir is a protease inhibitor that does not have anti-HCV activity but it is a pharmacoenhancer that is included to increase levels of paritaprevir through inhibition of CYP3A-mediated metabolism.

Paritaprevir and ritonavir are available as a fixed-dose combination with ombitasvir and given with the non-nucleoside NS5B inhibitor dasabuvir. This regimen is given with and without RBV for the treatment of HCV GT-1 subtype<sup>[103]</sup>.

## NS5A INHIBITORS

The NS5A is a multifunctional non-structural protein involved both in viral replication and in the assembly of HCV<sup>[104]</sup>. However, the precise molecular mechanisms of HCV NS5A inhibitors are unclear.

NS5A inhibitors have high antiviral activity against a lot of genotypes, but a low genetic barrier. They significantly reduce HCV-RNA levels and enhance SVR when given in conjunction with PEG-IFN and RBV. They



**Figure 3** ION-2 sub-analysis of cirrhosis vs without cirrhosis. Error bars represent 95% CIs. LDV: Ledipasvir; RBV: Ribavirin; SOF: Sofosbuvir; SVR12: Sustained virologic response at 12 wk post-treatment.

also result in very high SVR rates among patients with genotype 1 infection when given in combination with other direct-acting antivirals with or without RBV<sup>[105]</sup>.

### Daclatasvir

Daclatasvir is a pangenotypic NS5A inhibitor that is available for use in Europe. According to EASL guidelines daclatasvir should be administered orally (60 mg once daily) with low potential for drug-drug interactions. It is well tolerated. Dose adjustments are not needed in patients with Child B or C disease. Side effects with daclatasvir are fatigue, headache, and nausea. Little information has been released on daclatasvir drug-drug interactions.

In previously untreated patients infected with genotype 2 or 3, SVR was reported in 94%-100% of patients treated with the combination of daclatasvir plus sofosbuvir. In the ALLY-3 study<sup>[106]</sup>, 133 patients with genotype 3 infection were treated for 12 wk with 400 mg of sofosbuvir and 60 mg of daclatasvir for 12 wk. Ninety-one percent of previously untreated patients had an SVR compared with 86% of treatment-experienced patients.

In the COMMAND GT2/3 study, Dore *et al.*<sup>[107]</sup> compared the efficacy and safety of daclatasvir plus PEG-IFN- $\alpha$ -2a/RBV administered for either 12 or 16 wk with a standard 24-wk course of PEG-IFN- $\alpha$ -2a/RBV in HCV GT-2 or GT-3 subtype. Daclatasvir has been given with PEG-IFN- $\alpha$ -2a/RBV for 12 or 16 wk to previously untreated patients with genotype 2 or 3 infection. Around 83% of patients infected with genotype 2 and 70% of patients with genotype 3 infection have been reported to achieve SVR<sup>[107]</sup>. In another open-label study, the drug's effectiveness has been demonstrated<sup>[108]</sup>.

### Other NS5A inhibitors

Other NS5A inhibitors available in the United States are ledipasvir and ombitasvir, and they are each available in fixed-dose combinations with other direct-acting antivirals.

**Ledipasvir:** Ledipasvir is the first NS5A inhibitor

available in the United States. Ledipasvir and sofosbuvir are co-formulated in a single tablet in a fixed-dose combination (90 mg ledipasvir/400 mg sofosbuvir) that is administered once daily with or without food. This combination is well tolerated, and ledipasvir has the same drug interactions as sofosbuvir. This regimen is administered with or without RBV, depending on the patient population, in genotype 1 infection.

**Ombitasvir:** Ombitasvir (also known as ABT-267) is available as a fixed-dose combination with the PIs paritaprevir and ritonavir (12.5 mg ombitasvir/75 mg paritaprevir/50 mg ritonavir). This single tablet is administered orally with an additional drug: The non-nucleoside polymerase (NS5B) inhibitor dasabuvir<sup>[109,110]</sup>. This regimen is given with and without RBV in genotype 1 infection.

The combination ombitasvir-paritaprevir-ritonavir plus dasabuvir is generally well tolerated, and mild adverse effects are nausea, pruritus, insomnia, diarrhea, and asthenia<sup>[111,112]</sup>. Some of these symptoms may be attributable to RBV<sup>[113,114]</sup>.

The most important studies that evaluated treatment duration of ledipasvir/sofosbuvir treatment and its safety and efficacy (SVR12) in naive and treatment-experienced patients are ION-1, LONESTAR, and ION-2<sup>[115-117]</sup> (Figure 3).

## NS5B RNA-DEPENDENT RNA POLYMERASE INHIBITORS

NS5B is an RNA-dependent RNA polymerase involved in post-translational processing that is necessary for replication of HCV. The structure of NS5B is highly conserved across all HCV genotypes, so the drugs that inhibit NS5B have efficacy against all six genotypes.

There are two classes of polymerase inhibitors: NPIs and NNPIs. These two classes generally differ in specificity, according to their mode of action.

The NPIs mimic natural components and thus are incorporated into the nascent RNA chain, causing premature chain termination<sup>[118]</sup>. NNPIs act as allosteric inhibitors, and in fact they bind to one of four allosteric sites on the surface of NS5B.

### NPIs

NPIs have high antiviral efficacy across all genotypes, although they have a very high barrier to resistance.

**Sofosbuvir:** Sofosbuvir is the first NS5B NPI available in the United States.

Sofosbuvir is a pangenotypic NS5B polymerase inhibitor with a high barrier to resistance and favorable clinical pharmacology profile. It is administered orally as a 400 mg pill once a day, and has no food effect. Sofosbuvir is well tolerated, and the most commonly reported side effects of sofosbuvir and RBV, with or without PEG-IFN, are fatigue, headache, nausea,

insomnia, and anemia<sup>[74,119]</sup>.

Although renal clearance is the major form of elimination, in patients with mild or moderate renal impairment (glomerular filtration rate greater than 30 mL/min)<sup>[120]</sup>, any adjustment dose is not required.

No dose adjustment has been needed in patients with moderate (Child Pugh class B) or severe (Child Pugh class C) hepatic impairment.

Sofosbuvir has substantially less drug interactions than those observed with the HCV PIs. Sofosbuvir is a substrate of P-glycoprotein (P-gp), a drug transporter, so drugs that are potent intestinal P-gp inducers may decrease sofosbuvir levels. Thus, coadministration of sofosbuvir is not recommended with rifampin, rifabutin, rifapentine, St. John's wort, carbamazepine, phenytoin, phenobarbital, oxcarbazepine, or tipranavir/ritonavir.

Sofosbuvir was approved by FDA for genotype 1 in combination with PR, and in genotypes 2 and 3 in IFN free regimens in December 2013, in Canada during the same month and in Europe in January 2014 (European Medical Agency approval).

In the NEUTRINO study (an open-label, single-arm phase III registration trial) 327 treatment-naïve patients were treated with a regimen comprising sofosbuvir plus PR for 12 wk<sup>[119]</sup>. The overall patient population included mainly those infected with genotype 1 (89%) as well as a few patients infected with genotypes 4, 5 and 6; 17% of patients in this trial had cirrhosis. This sofosbuvir-based triple-therapy regimen resulted in a very high RVR, with the 4-wk RVR rate approaching 99%. The SVR rate for the entire trial population remained high at 90%, 12 wk after the end of treatment (with 99% of patients achieving virologic response at the end of treatment). Analyzing the groups based on viral genotype, patients with genotype 1 had an SVR rate of 89%, and the small number of patients with genotype 4, 5 and 6 had SVR rates between 96% and 100%. Overall, this sofosbuvir-based triple therapy regimen resulted in very high SVR rates across all genotypes that were evaluated. One important point from the NEUTRINO trial was the relative decrease in the overall response rate for patients with cirrhosis (SVR, 80%) compared with non-cirrhotics (SVR, 92%)<sup>[121]</sup>.

Other representative studies on genotype 1 are ELECTRON, QUANTUM, VALENCE and LONESTAR-2<sup>[122-125]</sup>.

Genotypes 2 and 3 have been studied together in three sofosbuvir phase III trials (FISSION, POSITRON, and FUSION)<sup>[119,122,126]</sup>.

Therapy with sofosbuvir-RBV for 12 wk in patients with HCV genotype 2 infection and for 24 wk in patients with HCV genotype 3 infection resulted in high rates of SVR<sup>[127]</sup>.

To date there are very few data on genotype 4 patients treated with sofosbuvir without PEG-IFN<sup>[128]</sup>. There are no data currently on treatment-experienced populations or any patients with genotypes 5 and 6<sup>[129]</sup>.

Sofosbuvir is used in various combinations with other antivirals for different indications: (1) with ledipasvir for HCV GT-1; (2) with SMV ( $\pm$  RBV) for HCV GT-1;

(3) with RBV for HCV GT-2, -3, -4, -5, and -6 infection (and among patients with any genotype awaiting liver transplant); and (4) with PEG-IFN and RBV for genotypes HCV GT-1 and -4.

### NNPIs

NNPIs bind to one of four allosteric sites on the surface of NS5B and cause a conformational change, making the enzyme ineffective. Despite the active site of NS5B is well conserved across all genotypes, and they should have a pan-genotype antiviral activity, NNIs have a more limited spectrum of activity specifically targeting against GT-1 (all NNPIs in clinical development have been optimized for GT-1). They have a low to moderate barrier to resistance variable toxicity profiles<sup>[130]</sup>. Consequently, this class of drug has been studied primarily as an adjunct to more potent compounds with higher barriers to resistance.

**Dasabuvir:** Dasabuvir is a non-nucleoside polymerase (NS5B) inhibitor administered with the fixed-dose combination ombitasvir-paritaprevir-ritonavir (12.5 mg ombitasvir/75 mg paritaprevir/50 mg ritonavir).

**ABT-450/ritonavir with ombitasvir (ABT-267) and dasabuvir (ABT-333):** TURQUOISE- II is a global, multi-center, randomized, open-label study evaluating the efficacy and safety of 12 or 24 wk of treatment with ABT-450/ritonavir (150/100 mg) co-formulated with ombitasvir (ABT-267) 25 mg, dosed once daily, and dasabuvir (ABT-333) 250 mg with RBV in adult patients with GT-1 HCV infection with compensated liver cirrhosis. Patients achieved SVR<sub>12</sub> rates of 91.8% and 95.9% in the 12 and 24-wk treatment arms, respectively<sup>[131]</sup>. In TURQUOISE- II, both cirrhotic non-responders and treatment-naïve cirrhotic subjects achieved higher SVR rates if they were genotype 1b-infected vs genotype 1a-infected. According to Asselah *et al.*<sup>[132]</sup> we support the efficacy and safety profile in GT-1 HCV cirrhotic patients, and in some cases the efficacy was demonstrated also in borderline compensated cirrhosis. However, current data in patients with cirrhosis and other HCV genotypes, such as genotype 3 and 4, are clearly an unfulfilled need. Another significant study is the PEARL- II<sup>[133]</sup>.

### New drugs: Cyclophilin A inhibitors

Cyclophilins (Cyp) are host proteins involved in the HCV lifecycle. CypA binds to the non-structural protein NS5A of HCV to promote replication of viral RNA, so molecules that are CypA antagonists, such as cyclosporines, are potent inhibitors of HCV replication. NS2, a non-structural protein of HCV involved in virus assembly, also plays an important role in the inhibitory effect of CypA inhibitors; NS2 modulates HCV sensitivity to cyclosporines and so NS2 may increase the inhibitory effect of cyclosporines on HCV replication<sup>[134,135]</sup>.

Alisporivir, is the first Cyp A inhibitor in clinical development. It is a cyclosporine analog without immunosuppressive properties, and due to its mechanism of

action, alisporivir is a pangenotypic antiviral, provides a high barrier for development of viral resistance, and does not permit cross-resistance to direct-acting antivirals.

This drug is also well tolerated. This drug has been used alone or in combination with PEG-IFN and RBV with very promising results<sup>[136,137]</sup>.

## GUIDELINES TREATMENTS HEPATITIS C

The treatment of CHC is performed following the American, European and Italian guidelines (AASLD, EASL, and AISF guidelines); this allows to optimize the therapy and customize it for various patient characteristics. Priority should be given to patients with advanced disease, patients with extrahepatic manifestations, HIV coinfection, post-liver transplantation recurrence and non-hepatic solid organ transplant recipients. Patients with mild disease can be treated with regimens containing PEG-IFN or deferred up to a worsening of the disease and the degree of liver fibrosis<sup>[138,139]</sup>.

## DISCUSSION AND CONCLUSION

Today, it can be anticipated that the future of HCV infection treatment seems very bright after the addition of first-generation HCV PIs as well as SMV and the first-in-kind HCV RNA polymerase inhibitor, "sofosbuvir", in the standard of care (*i.e.*, PEG-IFN/RBV). However, the real success of these drugs is very much dependent on careful monitoring of viral load and resistance, patterns of response to previous treatment, side effects and drug-drug interactions. Moreover, the logical meaning of novel emerging therapies must be to achieve high SVR and thorough clearance of the virus from treated patients. Nevertheless, the triple therapeutic regimens have several limitations. First, concomitant use of PEG-IFN plus RBV is essential to prevent the emergence of viral escape mutants and viral breakthrough during triple therapy. Second, triple therapy becomes less effective in prior null responders to PEG-IFN plus RBV and cannot be administered to patients who are contraindicated for PEG-IFN or RBV. To overcome these limitations, in the near future, many patients will be treated with two or more DAAs with or without IFN- $\alpha$  plus RBV based combination therapies. Currently, the approval of sofosbuvir- and SMV-based IFN-free regimens is an indication in this way. Triple and quadruple treatment regimens including multiple DAAs with or without PEG-IFN and RBV will likely be a suitable option for difficult-to-treat populations and for the prior null responders. All-oral IFN free regimens including drugs with a high genetic barrier to antiviral resistance (*e.g.*, NS5B inhibitors) and high antiviral efficacy (*e.g.*, NS3/4A PIs or NS5A inhibitors) may be a potent option for numerous patients contraindicated for PEG-IFN plus RBV. All oral regimens consisting of daclatasvir plus sofosbuvir once daily presented higher rates of SVR in untreated HCV GT-1, -2 and -3 infected patients and

in HCV GT-1 infected patients who had failed previous treatment with PIs. We hope that such combinational treatment strategies will become "the weapon" to treat the majority of HCV infected patients who represent the difficult population (*i.e.*, IL-28 polymorphism, HCV genotypes 1 and 4 subtypes, receipt of RBV, and the emergence of resistant variants) and will be more efficient to access the treatment in the near future. The testing of adenovirus vector based vaccines, which escalate the innate and acquired immune response against the most conserved regions of HCV genome in chimpanzees and humans, may be a promising therapeutic approach against HCV in the near future, although its fate still needs to be exploited fully in diverse HCV populations. One thing must be of special concern is whether the newly developed or being developed DAAs added in triple or quadruple therapies are safer or not than antiretroviral and traditional IFNs. Overall, the achievements in the field of HCV medicines may predict that we are near to complete elimination of HCV disease in the world<sup>[140]</sup>. The real challenges that our efforts must be directed are: (1) the effectiveness of IFN-free regimens in HCV-3, especially in cirrhotic non-responders; in this setting, combination with PEG-IFN is still possible; (2) the effectiveness of IFN-free regimens in decompensated cirrhosis are scarce in relation to the current correlation data between SVR and clinical outcome (literature confirms that the results of IFN-free regimens are good in compensated cirrhosis even if further clinical development is necessary in certain groups to improve SVR rates); (3) the development of new treatment strategies for patients who show resistance to new drugs; and (4) free-access to care<sup>[141]</sup>. In fact, many patients with CHC have mild disease and are currently excluded from the interferon-free treatment. In the near future we will inevitably prioritize this category in order to prevent progression to cirrhosis, decompensation and HCC.

## REFERENCES

- 1 **Davis GL**, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology* 2010; **138**: 513-521, 521.e1-6 [PMID: 19861128 DOI: 10.1053/j.gastro.2009.09.067]
- 2 **Razavi H**, Waked I, Sarrazin C, Myers RP, Idilman R, Calinas F, Vogel W, Mendes Correa MC, Hézode C, Lázaro P, Akarca U, Aleman S, Balik I, Berg T, Bihl F, Bilodeau M, Blasco AJ, Brandão Mello CE, Bruggmann P, Buti M, Calleja JL, Cheinquer H, Christensen PB, Clausen M, Coelho HS, Cramp ME, Dore GJ, Doss W, Duberg AS, El-Sayed MH, Ergör G, Esmat G, Falconer K, Félix J, Ferraz ML, Ferreira PR, Frankova S, García-Samaniego J, Gerstoft J, Gira JA, Gonçalves FL, Gower E, Gschwandler M, Guimarães Pessoa M, Hindman SJ, Hofer H, Husa P, Kåberg M, Kaita KD, Kautz A, Kaymakoglu S, Krajden M, Krarup H, Laleman W, Lavanchy D, Marinho RT, Marotta P, Mauss S, Moreno C, Murphy K, Negro F, Nemecek V, Örmeci N, Øvrehus AL, Parkes J, Pasini K, Peltekian KM, Ramji A, Reis N, Roberts SK, Rosenberg WM, Roudot-Thoraval F, Ryder SD, Sarmento-Castro R, Semela D, Sherman M, Shiha GE, Sievert W, Sperl J, Stärkel P, Stauber RE, Thompson AJ, Urbanek P, Van Damme P, van Thiel I, Van Vlierberghe H, Vandijck D, Wedemeyer H, Weis N,



- Wiegand J, Yosry A, Zekry A, Cornberg M, Müllhaupt B, Estes C. The present and future disease burden of hepatitis C virus (HCV) infection with today's treatment paradigm. *J Viral Hepat* 2014; **21** Suppl 1: 34-59 [PMID: 24713005 DOI: 10.1111/jvh.12248]
- 3 **Perz JF**, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; **45**: 529-538 [PMID: 16879891 DOI: 10.1016/j.jhep.2006.05.013]
- 4 **Lee MH**, Yang HI, Lu SN, Jen CL, You SL, Wang LY, Wang CH, Chen WJ, Chen CJ. Chronic hepatitis C virus infection increases mortality from hepatic and extrahepatic diseases: a community-based long-term prospective study. *J Infect Dis* 2012; **206**: 469-477 [PMID: 22811301 DOI: 10.1093/infdis/jis385]
- 5 **Malaguarnera M**, Scuderi L, Ardiri A, Malaguarnera G, Bertino N, Ruggeri IM, Carmela Greco G, Ozyalcin E, Bertino E, Bertino G. Type II Mixed Cryoglobulinemia in patients with Hepatitis C Virus: treatment with Pegylated-interferon and ribavirin. *Acta Medica Mediterr* 2015; **31**: 431
- 6 **van der Meer AJ**, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Heathcote EJ, Manns MP, Kuske L, Zeuzem S, Hofmann WP, de Knecht RJ, Hansen BE, Janssen HL. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012; **308**: 2584-2593 [PMID: 23268517 DOI: 10.1001/jama.2012.144878]
- 7 **Ly KN**, Xing J, Klevens RM, Jiles RB, Holmberg SD. Causes of death and characteristics of decedents with viral hepatitis, United States, 2010. *Clin Infect Dis* 2014; **58**: 40-49 [PMID: 24065331 DOI: 10.1093/cid/cit642]
- 8 **Cowie BC**, Allard N, MacLachlan JH. O86 European responses in focus: comparing viral hepatitis and hiv related deaths in europe 1990-2010 in the global burden of disease study 2010. *J Hepatol* 2014; **60** (1 Supplement): 35-36 [DOI: 10.1016/S0168-8278(14)60088-XS]
- 9 **Mahajan R**, Xing J, Liu SJ, Ly KN, Moorman AC, Rupp L, Xu F, Holmberg SD. Mortality among persons in care with hepatitis C virus infection: the Chronic Hepatitis Cohort Study (CHACS), 2006-2010. *Clin Infect Dis* 2014; **58**: 1055-1061 [PMID: 24523214 DOI: 10.1093/cid/ciu077]
- 10 **Hsu YC**, Lin JT, Ho HJ, Kao YH, Huang YT, Hsiao NW, Wu MS, Liu YY, Wu CY. Antiviral treatment for hepatitis C virus infection is associated with improved renal and cardiovascular outcomes in diabetic patients. *Hepatology* 2014; **59**: 1293-1302 [PMID: 24122848 DOI: 10.1002/hep.26892]
- 11 **Hsu CS**, Kao JH, Chao YC, Lin HH, Fan YC, Huang CJ, Tsai PS. Interferon-based therapy reduces risk of stroke in chronic hepatitis C patients: a population-based cohort study in Taiwan. *Aliment Pharmacol Ther* 2013; **38**: 415-423 [PMID: 23802888 DOI: 10.1111/apt.12391]
- 12 **Zabala V**, Tong M, Yu R, Ramirez T, Yalcin EB, Balbo S, Silbermann E, Deochand C, Nunez K, Hecht S, de la Monte SM. Potential contributions of the tobacco nicotine-derived nitrosamine ketone (NNK) in the pathogenesis of steatohepatitis in a chronic plus binge rat model of alcoholic liver disease. *Alcohol Alcohol* 2015; **50**: 118-131 [PMID: 25618784 DOI: 10.1093/alcalc/agu083]
- 13 **Caponnetto P**, Russo C, Di Maria A, Morjaria JB, Barton S, Guarino F, Basile E, Proiti M, Bertino G, Cacciola RR, Polosa R. Circulating endothelial-coagulative activation markers after smoking cessation: a 12-month observational study. *Eur J Clin Invest* 2011; **41**: 616-626 [PMID: 21198559 DOI: 10.1111/j.1365-2362.2010.02449.x]
- 14 **Bertino G**, Ardiri AM, Ali FT, Boemi PM, Cilio D, Di Prima P, Fisichella A, Ierna D, Neri S, Pulvirenti D, Urso G, Mauceri B, Valenti M, Bruno CM. Obesity and related diseases: an epidemiologic study in eastern Sicily. *Minerva Gastroenterol Dietol* 2006; **52**: 379-385 [PMID: 17108868]
- 15 **Westbrook RH**, Dusheiko G. Natural history of hepatitis C. *J Hepatol* 2014; **61**: S58-S68 [PMID: 25443346 DOI: 10.1016/j.jhep.2014.07.012]
- 16 **Bertino G**, Di Carlo I, Ardiri A, Calvagno GS, Demma S, Malaguarnera G, Bertino N, Malaguarnera M, Toro A, Malaguarnera M. Systemic therapies in hepatocellular carcinoma: present and future. *Future Oncol* 2013; **9**: 1533-1548 [PMID: 24106903 DOI: 10.2217/fon.13.171]
- 17 **Bertino G**, Demma S, Ardiri A, Proiti M, Gruttadauria S, Toro A, Malaguarnera G, Bertino N, Malaguarnera M, Malaguarnera M, Di Carlo I. Hepatocellular carcinoma: novel molecular targets in carcinogenesis for future therapies. *Biomed Res Int* 2014; **2014**: 203693 [PMID: 25089265 DOI: 10.1155/2014/203693]
- 18 **Bertino G**, Demma S, Ardiri A, Proiti M, Mangia A, Gruttadauria S, Toro A, Di Carlo I, Malaguarnera G, Bertino N, Malaguarnera M, Malaguarnera M. The immune system in hepatocellular carcinoma and potential new immunotherapeutic strategies. *Biomed Res Int* 2015; **2015**: 731469 [PMID: 25893197 DOI: 10.1155/2015/731469]
- 19 **Biondi A**, Malaguarnera G, Vacante M, Berretta M, D'Agata V, Malaguarnera M, Basile F, Drago F, Bertino G. Elevated serum levels of Chromogranin A in hepatocellular carcinoma. *BMC Surg* 2012; **12** Suppl 1: S7 [PMID: 23173843 DOI: 10.1186/1471-2482-12-S1-S7]
- 20 **Bertino G**, Ardiri A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS. Hepatocellular carcinoma serum markers. *Semin Oncol* 2012; **39**: 410-433 [PMID: 22846859 DOI: 10.1053/j.seminoncol.2012.05.001]
- 21 **Bertino G**, Neri S, Bruno CM, Ardiri AM, Calvagno GS, Malaguarnera M, Toro A, Malaguarnera M, Clementi S, Bertino N, Di Carlo I. Diagnostic and prognostic value of alpha-fetoprotein, des- $\gamma$ -carboxy prothrombin and squamous cell carcinoma antigen immunoglobulin M complexes in hepatocellular carcinoma. *Minerva Med* 2011; **102**: 363-371 [PMID: 22193346]
- 22 **Bertino G**, Ardiri AM, Calvagno GS, Bertino N, Boemi PM. Prognostic and diagnostic value of des- $\gamma$ -carboxy prothrombin in liver cancer. *Drug News Perspect* 2010; **23**: 498-508 [PMID: 21031166 DOI: 10.1358/dnp.2010.23.8]
- 23 **Bertino G**, Ardiri AM, Calvagno GS, Boemi PM. In chronic viral hepatitis without malignancy, abnormal serum carbohydrate 19-9 antigen levels are associated with liver disease severity and are related to different viral aetiology. *Dig Liver Dis* 2010; **42**: 458-459 [PMID: 19880358 DOI: 10.1016/j.dld.2009.09.011]
- 24 **Bertino G**, Ardiri AM, Santonocito MM, Boemi PM. Some patients with HCC haven't abnormal des-gamma-carboxy prothrombin and alpha-fetoprotein levels. *Panminerva Med* 2009; **51**: 133-134 [PMID: 19776714]
- 25 **Bertino G**, Ardiri AM, Boemi PM, Ierna D, Interlandi D, Caruso L, Minona E, Trovato MA, Vicari S, Li Destri G, Puleo S. A study about mechanisms of des-gamma-carboxy prothrombin's production in hepatocellular carcinoma. *Panminerva Med* 2008; **50**: 221-226 [PMID: 18927526]
- 26 **Mohd Hanafiah K**, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]
- 27 **Lavanchy D**. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011; **17**: 107-115 [PMID: 21091831 DOI: 10.1111/j.1469-0691.2010.03432.x]
- 28 **Thein HH**, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. *Hepatology* 2008; **48**: 418-431 [PMID: 18563841 DOI: 10.1002/hep.22375]
- 29 **Bruno S**, Stroffolini T, Colombo M, Bollani S, Benvenuto L, Mazzella G, Ascione A, Santantonio T, Piccinino F, Andreone P, Mangia A, Gaeta GB, Persico M, Fagioli S, Almasio PL. Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; **45**: 579-587 [PMID: 17326216 DOI: 10.1002/hep.21492]
- 30 **Zeuzem S**. Heterogeneous virologic response rates to interferon-based therapy in patients with chronic hepatitis C: who responds less well? *Ann Intern Med* 2004; **140**: 370-381 [PMID: 14996679 DOI: 10.7326/0003-4819-140-8-200404200-00009]

- 31 **Marcellin P**, Boyer N, Gervais A, Martinot M, Pouteau M, Castelnau C, Kilani A, Areias J, Auperin A, Benhamou JP, Degott C, Erlinger S. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. *Ann Intern Med* 1997; **127**: 875-881 [PMID: 9382365 DOI: 10.7326/0003-4819-127-10-199711150-00003]
- 32 **Poynard T**, Moussalli J, Munteanu M, Thabut D, Lebray P, Rudler M, Ngo Y, Thibault V, Mkada H, Charlotte F, Bismut FI, Deckmyn O, Benhamou Y, Valantin MA, Ratzu V, Katlama C. Slow regression of liver fibrosis presumed by repeated biomarkers after virological cure in patients with chronic hepatitis C. *J Hepatol* 2013; **59**: 675-683 [PMID: 23712051 DOI: 10.1016/j.jhep.2013.05.015]
- 33 **Maylin S**, Martinot-Peignoux M, Moucari R, Boyer N, Ripault MP, Cazals-Hatem D, Giuily N, Castelnau C, Cardoso AC, Asselah T, Féray C, Nicolas-Chanoine MH, Bedossa P, Marcellin P. Eradication of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Gastroenterology* 2008; **135**: 821-829 [PMID: 18593587 DOI: 10.1053/j.gastro.2008.05.044]
- 34 **Toccaceli F**, Laghi V, Capurso L, Koch M, Sereno S, Scuderi M. Long-term liver histology improvement in patients with chronic hepatitis C and sustained response to interferon. *J Viral Hepat* 2003; **10**: 126-133 [PMID: 12614469 DOI: 10.1046/j.1365-2893.2003.00403.x]
- 35 **Poynard T**, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, Ling MH, Albrecht J. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002; **122**: 1303-1313 [PMID: 11984517 DOI: 10.1053/gast.2002.33023]
- 36 **Shiratori Y**, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, Kuroki T, Nishiguchi S, Sata M, Yamada G, Fujiyama S, Yoshida H, Omata M. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; **132**: 517-524 [PMID: 10744587 DOI: 10.7326/0003-4819-132-7-200004040-00036]
- 37 **Backus LI**, Boothroyd DB, Phillips BR, Belperio P, Halloran J, Mole LA. A sustained virologic response reduces risk of all-cause mortality in patients with hepatitis C. *Clin Gastroenterol Hepatol* 2011; **9**: 509-516.e1 [PMID: 21397729 DOI: 10.1016/j.cgh.2011.03.004]
- 38 **Fontana RJ**, Sanyal AJ, Ghany MG, Lee WM, Reid AE, Naishadham D, Everson GT, Kahn JA, Di Bisceglie AM, Szabo G, Morgan TR, Everhart JE. Factors that determine the development and progression of gastroesophageal varices in patients with chronic hepatitis C. *Gastroenterology* 2010; **138**: 2321-2331, 2331.e1-2 [PMID: 20211180 DOI: 10.1053/j.gastro.2010.02.058]
- 39 **Veldt BJ**, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, Manns MP, Hansen BE, Schalm SW, Janssen HL. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. *Ann Intern Med* 2007; **147**: 677-684 [PMID: 18025443 DOI: 10.7326/0003-4819-147-10-200711200-00003]
- 40 **Alter HJ**, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. *Semin Liver Dis* 2000; **20**: 17-35 [PMID: 10895429]
- 41 **Seeff LB**. Natural history of chronic hepatitis C. *Hepatology* 2002; **36**: S35-S46 [PMID: 12407575 DOI: 10.1053/jhep.2002.36806]
- 42 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Snitkine V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]
- 43 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]
- 44 **Innes HA**, Hutchinson SJ, Allen S, Bhattacharyya D, Bramley P, Carman B, Delahooke TE, Dillon JF, Goldberg DJ, Kennedy N, Mills PR, Morris J, Morris J, Robertson C, Stanley AJ, Hayes P. Ranking predictors of a sustained viral response for patients with chronic hepatitis C treated with pegylated interferon and ribavirin in Scotland. *Eur J Gastroenterol Hepatol* 2012; **24**: 646-655 [PMID: 22433796 DOI: 10.1097/MEG.0b013e32835201a4]
- 45 **Bräu N**. Evaluation of the hepatitis C virus-infected patient: the initial encounter. *Clin Infect Dis* 2013; **56**: 853-860 [PMID: 23243172 DOI: 10.1093/cid/cis957]
- 46 **Trembling PM**, Tanwar S, Rosenberg WM, Dusheiko GM. Treatment decisions and contemporary versus pending treatments for hepatitis C. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 713-728 [PMID: 24019151 DOI: 10.1038/nrgastro.2013.163]
- 47 **Dore GJ**. The changing therapeutic landscape for hepatitis C. *Med J Aust* 2012; **196**: 629-632 [PMID: 22676877 DOI: 10.5694/mja11.11531]
- 48 **Liang TJ**, Rehermann B, Seeff LB, Hoofnagle JH. Pathogenesis, natural history, treatment, and prevention of hepatitis C. *Ann Intern Med* 2000; **132**: 296-305 [PMID: 10681285 DOI: 10.7326/0003-4819-132-4-200002150-00008]
- 49 **Bertino G**, Ardiri A, Boemi PM, Calvagno GS, Ruggeri IM, Speranza A, Santonocito MM, Ierna D, Bruno CM, Valenti M, Boemi R, Naimo S, Neri S. Epoetin alpha improves the response to antiviral treatment in HCV-related chronic hepatitis. *Eur J Clin Pharmacol* 2010; **66**: 1055-1063 [PMID: 20652232 DOI: 10.1007/s00228-010-0868-4]
- 50 **Neri S**, Bertino G, Petralia A, Giancarlo C, Rizzotto A, Calvagno GS, Mauceri B, Abate G, Boemi P, Di Pino A, Ignaccolo L, Vadalà G, Misseri M, Maiorca D, Mastro Simone G, Judica A, Palermo F. A multidisciplinary therapeutic approach for reducing the risk of psychiatric side effects in patients with chronic hepatitis C treated with pegylated interferon  $\alpha$  and ribavirin. *J Clin Gastroenterol* 2010; **44**: e210-e217 [PMID: 20838237 DOI: 10.1097/MCG.0b013e3181d88af5]
- 51 **Neri S**, Pulvirenti D, Bertino G. Psychiatric symptoms induced by antiviral therapy in chronic hepatitis C: comparison between interferon-alpha-2a and interferon-alpha-2b. *Clin Drug Investig* 2006; **26**: 655-662 [PMID: 17163300]
- 52 **Malaguarnera M**, Vacante M, Bertino G, Neri S, Malaguarnera M, Gargante MP, Motta M, Lupo L, Chisari G, Bruno CM, Pennisi G, Bella R. The supplementation of acetyl-L-carnitine decreases fatigue and increases quality of life in patients with hepatitis C treated with pegylated interferon- $\alpha$  2b plus ribavirin. *J Interferon Cytokine Res* 2011; **31**: 653-659 [PMID: 21923249 DOI: 10.1089/jir.2011.0010]
- 53 **Malaguarnera M**, Vacante M, Giordano M, Motta M, Bertino G, Pennisi M, Neri S, Malaguarnera M, Li Volti G, Galvano F. L-carnitine supplementation improves hematological pattern in patients affected by HCV treated with Peg interferon- $\alpha$  2b plus ribavirin. *World J Gastroenterol* 2011; **17**: 4414-4420 [PMID: 22110268]
- 54 **Malaguarnera G**, Pennisi M, Gagliano C, Vacante M, Malaguarnera M, Salomone S, Drago F, Bertino G, Caraci F, Nunnari G, Malaguarnera M. Acetyl-L-Carnitine Supplementation During HCV Therapy With Pegylated Interferon- $\alpha$  2b Plus Ribavirin: Effect on Work Performance; A Randomized Clinical Trial. *Hepat Mon* 2014; **14**: e11608 [PMID: 24910702 DOI: 10.5812/hepatmon.11608]
- 55 **Liang TJ**, Ghany MG. Current and future therapies for hepatitis C virus infection. *N Engl J Med* 2013; **368**: 1907-1917 [PMID: 23675659 DOI: 10.1056/NEJMra1213651]
- 56 **de Chasse B**, Navratil V, Tafforeau L, Hiet MS, Aublin-Gex A, Agaugué S, Meiffren G, Pradezynski F, Faria BF, Chantier T, Le Breton M, Pellet J, Davoust N, Mangeot PE, Chaboud A, Penin F, Jacob Y, Vidalain PO, Vidal M, André P, Rabourdin-Combe C, Lotteau V. Hepatitis C virus infection protein network. *Mol Syst Biol* 2008; **4**: 230 [PMID: 18985028 DOI: 10.1038/msb.2008.66]
- 57 **Li Q**, Brass AL, Ng A, Hu Z, Xavier RJ, Liang TJ, Elledge SJ.

- A genome-wide genetic screen for host factors required for hepatitis C virus propagation. *Proc Natl Acad Sci USA* 2009; **106**: 16410-16415 [PMID: 19717417 DOI: 10.1073/pnas.0907439106]
- 58 **Randall G**, Panis M, Cooper JD, Tellinghuisen TL, Sukhodolets KE, Pfeffer S, Landthaler M, Landgraf P, Kan S, Lindenbach BD, Chien M, Weir DB, Russo JJ, Ju J, Brownstein MJ, Sheridan R, Sander C, Zavolan M, Tuschl T, Rice CM. Cellular cofactors affecting hepatitis C virus infection and replication. *Proc Natl Acad Sci USA* 2007; **104**: 12884-12889 [PMID: 17616579 DOI: 10.1073/pnas.0704894104]
- 59 **Tai AW**, Benita Y, Peng LF, Kim SS, Sakamoto N, Xavier RJ, Chung RT. A functional genomic screen identifies cellular cofactors of hepatitis C virus replication. *Cell Host Microbe* 2009; **5**: 298-307 [PMID: 19286138 DOI: 10.1016/j.chom.2009.02.001]
- 60 **Hajarizadeh B**, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 553-562 [PMID: 23817321 DOI: 10.1038/nrgastro.2013.107]
- 61 **Moradpour D**, Penin F, Rice CM. Replication of hepatitis C virus. *Nat Rev Microbiol* 2007; **5**: 453-463 [PMID: 17487147 DOI: 10.1038/nrmicro1645]
- 62 **Ploss A**, Evans MJ. Hepatitis C virus host cell entry. *Curr Opin Virol* 2012; **2**: 14-19 [PMID: 22440961 DOI: 10.1016/j.coviro.2011.12.007]
- 63 **Bartenschlager R**, Penin F, Lohmann V, André P. Assembly of infectious hepatitis C virus particles. *Trends Microbiol* 2011; **19**: 95-103 [PMID: 21146993 DOI: 10.1016/j.tim.2010.11.005]
- 64 **Friedel CC**, Haas J. Virus-host interactomes and global models of virus-infected cells. *Trends Microbiol* 2011; **19**: 501-508 [PMID: 21855347 DOI: 10.1016/j.tim.2011.07.003]
- 65 **Katze MG**, Fornek JL, Palermo RE, Walters KA, Korth MJ. Innate immune modulation by RNA viruses: emerging insights from functional genomics. *Nat Rev Immunol* 2008; **8**: 644-654 [PMID: 18654572 DOI: 10.1038/nri2377]
- 66 **Panda D**, Cherry S. Cell-based genomic screening: elucidating virus-host interactions. *Curr Opin Virol* 2012; **2**: 784-792 [PMID: 23122855 DOI: 10.1016/j.coviro.2012.10.007]
- 67 **Berger KL**, Cooper JD, Heaton NS, Yoon R, Oakland TE, Jordan TX, Mateu G, Grakoui A, Randall G. Roles for endocytic trafficking and phosphatidylinositol 4-kinase III alpha in hepatitis C virus replication. *Proc Natl Acad Sci USA* 2009; **106**: 7577-7582 [PMID: 19376974 DOI: 10.1073/pnas.0902693106]
- 68 **Lupberger J**, Zeisel MB, Xiao F, Thumann C, Fofana I, Zona L, Davis C, Mee CJ, Turek M, Gorke S, Royer C, Fischer B, Zahid MN, Lavillette D, Fresquet J, Cosset FL, Rothenberg SM, Pietschmann T, Patel AH, Pessaux P, Doffoël M, Raffelsberger W, Poch O, McKeating JA, Brino L, Baumert TF. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. *Nat Med* 2011; **17**: 589-595 [PMID: 21516087 DOI: 10.1038/nm.2341]
- 69 **Reiss S**, Rebhan I, Backes P, Romero-Brey I, Erfle H, Matula P, Kaderali L, Poenisch M, Blankenburg H, Hiet MS, Longerich T, Diehl S, Ramirez F, Balla T, Rohr K, Kaul A, Bühler S, Pepperkok R, Lengauer T, Albrecht M, Eils R, Schirmacher P, Lohmann V, Bartenschlager R. Recruitment and activation of a lipid kinase by hepatitis C virus NS5A is essential for integrity of the membranous replication compartment. *Cell Host Microbe* 2011; **9**: 32-45 [PMID: 21238945 DOI: 10.1016/j.chom.2010.12.002]
- 70 **Poynard T**, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C, Albrecht J. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; **352**: 1426-1432 [PMID: 9807989 DOI: 10.1016/S0140-6736(98)07124-4]
- 71 **Mangia A**, Cenderello G, Orlandini A, Piazzolla V, Picciotto A, Zuin M, Ciano A, Brancaccio G, Forte P, Carretta V, Zignego AL, Minerva N, Brindici G, Marignani M, Baroni GS, Bertino G, Cuccorese G, Mottola L, Ripoli M, Pirisi M. Individualized treatment of genotype 1 naïve patients: an Italian multicenter field practice experience. *PLoS One* 2014; **9**: e110284 [PMID: 25340799 DOI: 10.1371/journal.pone.0110284]
- 72 **Asselah T**, Marcellin P. Interferon free therapy with direct acting antivirals for HCV. *Liver Int* 2013; **33** Suppl 1: 93-104 [PMID: 23286852 DOI: 10.1111/liv.12076]
- 73 **Kowdley KV**, Lawitz E, Crespo I, Hassanein T, Davis MN, DeMicco M, Bernstein DE, Afdhal N, Vierling JM, Gordon SC, Anderson JK, Hyland RH, Dvory-Sobol H, An D, Hindes RG, Albanis E, Symonds WT, Berrey MM, Nelson DR, Jacobson IM. Sofosbuvir with pegylated interferon alfa-2a and ribavirin for treatment-naïve patients with hepatitis C genotype-1 infection (ATOMIC): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2013; **381**: 2100-2107 [PMID: 23499440 DOI: 10.1016/S0140-6736(13)60247-0]
- 74 **Fried MW**, Buti M, Dore GJ, Flisiak R, Ferenci P, Jacobson I, Marcellin P, Manns M, Nikitin I, Poordad F, Sherman M, Zeuzem S, Scott J, Gilles L, Lenz O, Peeters M, Sekar V, De Smedt G, Beumont-Mauviel M. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: the randomized PILLAR study. *Hepatology* 2013; **58**: 1918-1929 [PMID: 23907700 DOI: 10.1002/hep.26641]
- 75 **Poordad F**, Dieterich D. Treating hepatitis C: current standard of care and emerging direct-acting antiviral agents. *J Viral Hepat* 2012; **19**: 449-464 [PMID: 22676357 DOI: 10.1111/j.1365-2893.2012.01617.x]
- 76 **Pockros PJ**. New direct-acting antivirals in the development for hepatitis C virus infection. *Therap Adv Gastroenterol* 2010; **3**: 191-202 [PMID: 21180601 DOI: 10.1177/1756283X10363055]
- 77 **Manzano-Robleda Md C**, Ornelas-Arroyo V, Barrientos-Gutiérrez T, Méndez-Sánchez N, Uribe M, Chávez-Tapia NC. Boceprevir and telaprevir for chronic genotype 1 hepatitis C virus infection. A systematic review and meta-analysis. *Ann Hepatol* 2015; **14**: 46-57 [PMID: 25536641]
- 78 **Pawlotsky JM**. Treatment failure and resistance with direct-acting antiviral drugs against hepatitis C virus. *Hepatology* 2011; **53**: 1742-1751 [PMID: 21374691 DOI: 10.1002/hep.24262]
- 79 **Ghany MG**, Nelson DR, Strader DB, Thomas DL, Seeff LB. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011; **54**: 1433-1444 [PMID: 21898493 DOI: 10.1002/hep.24641]
- 80 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]
- 81 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]
- 82 **Kiser JJ**, Burton JR, Everson GT. Drug-drug interactions during antiviral therapy for chronic hepatitis C. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 596-606 [PMID: 23817323 DOI: 10.1038/nrgastro.2013.106]
- 83 **Hunt D**, Pockros P. What are the promising new therapies in the field of chronic hepatitis C after the first-generation direct-acting antivirals? *Curr Gastroenterol Rep* 2013; **15**: 303 [PMID: 23250703 DOI: 10.1007/s11894-012-0303-3]
- 84 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]
- 85 **Sanford M**. Simeprevir: a review of its use in patients with chronic hepatitis C virus infection. *Drugs* 2015; **75**: 183-196 [PMID: 25559421 DOI: 10.1007/s40265-014-0341-2]
- 86 **Williams JA**, Ring BJ, Cantrell VE, Jones DR, Eckstein J, Ruterbories K, Hamman MA, Hall SD, Wrighton SA. Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. *Drug Metab Dispos* 2002; **30**: 883-891 [PMID: 12124305 DOI: 10.1124/



- dmd.30.8.883]
- 87 **Perumpail RB**, Wong RJ, Ha LD, Pham EA, Wang U, Luong H, Kumari R, Daugherty TJ, Higgins JP, Younossi ZM, Kim WR, Glenn JS, Ahmed A. Sofosbuvir and simeprevir combination therapy in the setting of liver transplantation and hemodialysis. *Transpl Infect Dis* 2015; **17**: 275-278 [PMID: 25641426 DOI: 10.1111/tid.12348]
  - 88 **Sulkowski M**, Jacobson IM, Ghalib R, Rodriguez-Torres M, Younossi Z, Corregidor A, Fevery B, Callewaert K, Symonds W, De La Rosa G, Picchio G, Ouwerkerk-Mahadevan S, Lambrecht T, Lawitz E. O7 Once-daily simeprevir (TMC435) plus sofosbuvir (GS-7977) with or without ribavirin in HCV genotype 1 prior null responders with Metavir F0-2: COSMOS study subgroup analysis. *J Hepatol* 2014; **60**: S4 [DOI: 10.1016/S0168-8278(14)60009-X]
  - 89 **Lawitz E**, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, Sulkowski MS, DeJesus E, Pearlman B, Rabinovitz M, Gitlin M, Lim JK, Pockros PJ, Fevery B, Lambrecht T, Ouwerkerk-Mahadevan S, Callewaert K, Symonds WT, Picchio G, Lindsay K, Beumont-Mauviel M, Jacobson IM. Simeprevir plus sofosbuvir with/without ribavirin in HCV genotype 1 prior null-responder/treatment-naïve patients (COSMOS study): primary endpoint (SVR12) results in patients with METAVIR F3-4 (Cohort 2). Abstract presented at: EASL - The International Liver Congress. 49th Annual Meeting of the European Association for the Study of the Liver. London (UK), 2014. [Accessed 2014 Jun 25]. Available from: URL: [http://www.natap.org/2014/EASL/EASL\\_26.htm](http://www.natap.org/2014/EASL/EASL_26.htm)
  - 90 **Foster GR**, Strasser S, Christensen C, Ma J, Bekele BN, Brainard DM, Symonds WT, McHutchison JG, Conway B, Crespo I, Zeuzem S. O66 Sofosbuvir-based regimens are associated with high SVR rates across genotypes and among patients with multiple negative predictive factors. *J Hepatol* 2014; **60**: S27 [DOI: 10.1016/S0168-8278(14)60068-4]
  - 91 **Pearlman BL**, Ehleben C, Perrys M. The combination of simeprevir and sofosbuvir is more effective than that of peginterferon, ribavirin, and sofosbuvir for patients with hepatitis C-related Child's class A cirrhosis. *Gastroenterology* 2015; **148**: 762-770.e2; quiz e11-12 [PMID: 25557952 DOI: 10.1053/j.gastro.2014.12.027]
  - 92 **Jacobson IM**, Dore GJ, Foster GR, Fried MW, Radu M, Rafalsky VV, Moroz L, Craxi A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Scott J, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2014; **384**: 403-413 [PMID: 24907225 DOI: 10.1016/S0140-6736(14)60494-3]
  - 93 **Manns M**, Marcellin P, Poordad F, de Araujo ES, Buti M, Horsmans Y, Janczewska E, Villamil F, Scott J, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2014; **384**: 414-426 [PMID: 24907224 DOI: 10.1016/S0140-6736(14)60538-9]
  - 94 **Forns X**, Lawitz E, Zeuzem S, Gane E, Bronowicki JP, Andreone P, Horban A, Brown A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, Scott J, De La Rosa G, Kalmeijer R, Sinha R, Beumont-Mauviel M. Simeprevir with peginterferon and ribavirin leads to high rates of SVR in patients with HCV genotype 1 who relapsed after previous therapy: a phase 3 trial. *Gastroenterology* 2014; **146**: 1669-79.e3 [PMID: 24602923 DOI: 10.1053/j.gastro.2014.02.051]
  - 95 **Hayashi N**, Izumi N, Kumada H, Okanoue T, Tsubouchi H, Yatsuhashi H, Kato M, Ki R, Komada Y, Seto C, Goto S. Simeprevir with peginterferon/ribavirin for treatment-naïve hepatitis C genotype 1 patients in Japan: CONCERTO-1, a phase III trial. *J Hepatol* 2014; **61**: 219-227 [PMID: 24727123 DOI: 10.1016/j.jhep.2014.04.004]
  - 96 **Izumi N**, Hayashi N, Kumada H, Okanoue T, Tsubouchi H, Yatsuhashi H, Kato M, Ki R, Komada Y, Seto C, Goto S. Once-daily simeprevir with peginterferon and ribavirin for treatment-experienced HCV genotype 1-infected patients in Japan: the CONCERTO-2 and CONCERTO-3 studies. *J Gastroenterol* 2014; **49**: 941-953 [PMID: 24626851 DOI: 10.1007/s00535-014-0949-8]
  - 97 **Kumada H**, Hayashi N, Izumi N, Okanoue T, Tsubouchi H, Yatsuhashi H, Kato M, Rito K, Komada Y, Seto C, Goto S. Simeprevir (TMC435) once daily with peginterferon- $\alpha$ -2b and ribavirin in patients with genotype 1 hepatitis C virus infection: The CONCERTO-4 study. *Hepatol Res* 2015; **45**: 501-513 [PMID: 24961662 DOI: 10.1111/hepr.12375]
  - 98 **Sulkowski MS**, Asselah T, Lalezari J, Ferenci P, Fainboim H, Leggett B, Bessone F, Mauss S, Heo J, Datsenko Y, Stern JO, Kukolj G, Scherer J, Nehmiz G, Steinmann GG, Böcher WO. Faldaprevir combined with pegylated interferon alfa-2a and ribavirin in treatment-naïve patients with chronic genotype 1 HCV: SILEN-C1 trial. *Hepatology* 2013; **57**: 2143-2154 [PMID: 23359516 DOI: 10.1002/hep.26276]
  - 99 **Nishiguchi S**, Sakai Y, Kuboki M, Tsunematsu S, Urano Y, Sakamoto W, Tsuda Y, Steinmann G, Omata M. Safety and efficacy of faldaprevir with pegylated interferon alfa-2a and ribavirin in Japanese patients with chronic genotype-1 hepatitis C infection. *Liver Int* 2014; **34**: 78-88 [PMID: 23944720 DOI: 10.1111/liv.12254]
  - 100 **Zeuzem S**, Asselah T, Angus P, Zarski JP, Larrey D, Müllhaupt B, Gane E, Schuchmann M, Lohse A, Pol S, Bronowicki JP, Roberts S, Arasteh K, Zoulim F, Heim M, Stern JO, Kukolj G, Nehmiz G, Haefner C, Boecher WO. Efficacy of the protease inhibitor BI 201335, polymerase inhibitor BI 207127, and ribavirin in patients with chronic HCV infection. *Gastroenterology* 2011; **141**: 2047-2055; quiz e14 [PMID: 21925126 DOI: 10.1053/j.gastro.2011.08.051]
  - 101 **Zeuzem S**, Soriano V, Asselah T, Bronowicki JP, Lohse AW, Müllhaupt B, Schuchmann M, Bourlière M, Buti M, Roberts SK, Gane EJ, Stern JO, Vinisko R, Kukolj G, Gallivan JP, Böcher WO, Mensa FJ. Faldaprevir and ledipasvir for HCV genotype 1 infection. *N Engl J Med* 2013; **369**: 630-639 [PMID: 23944300 DOI: 10.1056/NEJMoa1213557]
  - 102 **Kanda T**, Yokosuka O, Omata M. Antiviral therapy for "difficult-to-treat" hepatitis C virus-infected patients. *Chin Med J (Engl)* 2013; **126**: 4568-4574 [PMID: 24286427]
  - 103 **Hézode C**, Asselah T, Reddy KR, Hassanein T, Berenguer M, Fleischer-Stepniewska K, Marcellin P, Hall C, Schnell G, Pilot-Matias T, Mobashery N, Redman R, Vilchez RA, Pol S. Ombitasvir plus paritaprevir plus ritonavir with or without ribavirin in treatment-naïve and treatment-experienced patients with genotype 4 chronic hepatitis C virus infection (PEARL-I): a randomised, open-label trial. *Lancet* 2015; **385**: 2502-2509 [PMID: 25837829 DOI: 10.1016/S0140-6736(15)60159-3]
  - 104 **Tellinghuisen TL**, Foss KL, Treadaway J. Regulation of hepatitis C virion production via phosphorylation of the NS5A protein. *PLoS Pathog* 2008; **4**: e1000032 [PMID: 18369478 DOI: 10.1371/journal.ppat.1000032]
  - 105 **Ivachtchenko AV**, Mitkin OD, Yamanushkin PM, Kuznetsova IV, Bulanova EA, Shevkun NA, Koryakova AG, Karapetian RN, Bichko VV, Trifelenkov AS, Kravchenko DV, Vostokova NV, Veselov MS, Chufarova NV, Ivanenkov YA. Discovery of novel highly potent hepatitis C virus NS5A inhibitor (AV4025). *J Med Chem* 2014; **57**: 7716-7730 [PMID: 25148100 DOI: 10.1021/jm500951r]
  - 106 **Nelson DR**, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R, Oguchi G, Thuluvath PJ, Ortiz-Lasanta G, Rabinovitz M, Bernstein D, Bennett M, Hawkins T, Ravendran N, Sheikh AM, Varunok P, Kowdley KV, Hennicken D, McPhee F, Rana K, Hughes EA. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology* 2015; **61**: 1127-1135 [PMID: 25614962 DOI: 10.1002/hep.27726]
  - 107 **Dore GJ**, Lawitz E, Hézode C, Shafraan S, Ramji A, Tatum H, Taliani G, Tran A, Brunetto M, Zaltron S, Strasser S, Weis N, Ghesquiere W, Lee S, Larrey D, Pol S, Harley H, George J, Fung S, de Ledinghen V, Hagens P, Cohen D, Cooney E, Novello S, Hughes E. Daclatasvir combined with peginterferon alfa-2A and ribavirin for 12 or 16 weeks in patients with HCV genotype 2 or 3 infection: COMMAND GT2/3 STUDY. *J Hepatol* 2013; **58** (suppl



- 1): S570-571 [DOI: 10.1016/S0168-8278(13)61417-8]
- 108 **Sulkowski MS**, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hineostroza F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, McPhee F, Sherman D, Hindes R, Symonds W, Pasquinelli C, Graseola DM. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med* 2014; **370**: 211-221 [PMID: 24428467 DOI: 10.1056/NEJMoa1306218]
- 109 **Lawitz EJ**, Gruener D, Hill JM, Marbury T, Moorehead L, Mathias A, Cheng G, Link JO, Wong KA, Mo H, McHutchison JG, Brainard DM. A phase 1, randomized, placebo-controlled, 3-day, dose-ranging study of GS-5885, an NS5A inhibitor, in patients with genotype 1 hepatitis C. *J Hepatol* 2012; **57**: 24-31 [PMID: 22314425 DOI: 10.1016/j.jhep.2011.12.029]
- 110 **Poordad F**, Lawitz E, DeJesus E, Kowdley KN, Gaultier I, Cohen DE, Xie W, Larsen L, Pilot-Matias T, Koev G, Dumas D, Podsadecki T, Bernstein B. 1206 ABT-072 or ABT-333 combined with pegylated interferon/ribavirin after 3-day monotherapy in HCV genotype 1 (GT1)-infected treatment-naïve subjects: 12-week sustained virologic response (SVR12) and safety results. *J Hepatol* 2012; **56** Suppl 2: S478 [DOI: 10.1016/S0168-8278(12)61218-5]
- 111 **Feld JJ**, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, Weiland O, Aguilar H, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1594-1603 [PMID: 24720703 DOI: 10.1056/NEJMoa1315722]
- 112 **Zeuzem S**, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourlière M, Sulkowski MS, Wedemeyer H, Tam E, Desmond P, Jensen DM, Di Bisceglie AM, Varunok P, Hassanein T, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1604-1614 [PMID: 24720679 DOI: 10.1056/NEJMoa1401561]
- 113 **Ferenci P**, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A, Box TD, Younes Z, Enayati P, Green S, Baruch Y, Bhandari BR, Caruntu FA, Sepe T, Chulanov V, Janczewska E, Rizzardini G, Gervain J, Planas R, Moreno C, Hassanein T, Xie W, King M, Podsadecki T, Reddy KR. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *N Engl J Med* 2014; **370**: 1983-1992 [PMID: 24795200 DOI: 10.1056/NEJMoa1402338]
- 114 **Khatri A**, Menon RM, Marbury TC, Lawitz EJ, Podsadecki TJ, Mullally VM, Ding B, Awni WM, Bernstein BM, Dutta S. Pharmacokinetics and safety of co-administered paritaprevir plus ritonavir, ombitasvir, and dasabuvir in hepatic impairment. *J Hepatol* 2015; **63**: 805-812 [PMID: 26070406 DOI: 10.1016/j.jhep.2015.05.029]
- 115 **Jacobson IM**, Marcellin P, Mangia A, Kwo PY, Foster G, Buti M, Brau N, Muir AJ, Yang JC, Mo H, Ding X, Pang P, Symonds WT, McHutchison JG, Zeuzem S, Afdhal NH. Tu2038 All Oral Fixed-dose Combination Sofosbuvir/Ledipasvir With or Without Ribavirin for 12 or 24 Weeks in Treatment-Naïve Genotype 1 HCV-Infected Patients: The Phase 3 ION-1 Study. *J Hepatol* 2014; Supplement **60**: S523-S524 [DOI: 10.1016/S0016-5085(14)63284-4]
- 116 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Di Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]
- 117 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]
- 118 **Koch U**, Narjes F. Recent progress in the development of inhibitors of the hepatitis C virus RNA-dependent RNA polymerase. *Curr Top Med Chem* 2007; **7**: 1302-1329 [PMID: 17627559 DOI: 10.2174/156802607781212211]
- 119 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
- 120 **Kirby BJ**, Symonds WT, Kearney BP, Mathias AA. Pharmacokinetic, Pharmacodynamic, and Drug-Interaction Profile of the Hepatitis C Virus NS5B Polymerase Inhibitor Sofosbuvir. *Clin Pharmacokinet* 2015; **54**: 677-690 [PMID: 25822283 DOI: 10.1007/s40262-015-0261-7]
- 121 **Alexopoulou A**, Karayiannis P. Interferon-based combination treatment for chronic hepatitis C in the era of direct acting antivirals. *Ann Gastroenterol* 2015; **28**: 55-65 [PMID: 25608803]
- 122 **Gane EJ**, Stedman CA, Hyland RH, Ding X, Svarovskaia E, Symonds WT, Hindes RG, Berrey MM. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. *N Engl J Med* 2013; **368**: 34-44 [PMID: 23281974 DOI: 10.1056/NEJMoa1208953]
- 123 **Rodriguez-Torres M**, Lawitz E, Kowdley KV, Nelson DR, DeJesus E, McHutchison JG, Cornpropst MT, Mader M, Albanis E, Jiang D, Hebrner CM, Symonds WT, Berrey MM, Lalezari J. Sofosbuvir (GS-7977) plus peginterferon/ribavirin in treatment-naïve patients with HCV genotype 1: a randomized, 28-day, dose-ranging trial. *J Hepatol* 2013; **58**: 663-668 [PMID: 23183528 DOI: 10.1016/j.jhep.2012.11.018]
- 124 **Lawitz E**, Poordad F, Brainard DM, Hyland RH, An D, Dvory-Sobol H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir with peginterferon-ribavirin for 12 weeks in previously treated patients with hepatitis C genotype 2 or 3 and cirrhosis. *Hepatology* 2015; **61**: 769-775 [PMID: 25322962 DOI: 10.1002/hep.27567]
- 125 **Lawitz E**, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naïve and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; **383**: 515-523 [PMID: 24209977 DOI: 10.1016/S0140-6736(13)62121-2]
- 126 **Jacobson IM**, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]
- 127 **Zeuzem S**, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]
- 128 **Ruane PJ**, Ain D, Stryker R, Meshrekey R, Soliman M, Wolfe PR, Riad J, Mikhail S, Kersey K, Jiang D, Massetto B, Doehle B, Kirby BJ, Knox SJ, McHutchison JG, Symonds WT. Sofosbuvir plus ribavirin for the treatment of chronic genotype 4 hepatitis C virus infection in patients of Egyptian ancestry. *J Hepatol* 2015; **62**: 1040-1046 [PMID: 25450208 DOI: 10.1016/j.jhep.2014.10.044]
- 129 **Feld JJ**. Interferon-free strategies with a nucleoside/nucleotide analogue. *Semin Liver Dis* 2014; **34**: 37-46 [PMID: 24782257 DOI: 10.1055/s-0034-1371009]

- 130 **Au JS**, Pockros PJ. Novel therapeutic approaches for hepatitis C. *Clin Pharmacol Ther* 2014; **95**: 78-88 [PMID: 24126682 DOI: 10.1038/clpt.2013.206]
- 131 **Poordad F**, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, Shiffman ML, Wedemeyer H, Berg T, Yoshida EM, Forns X, Lovell SS, Da Silva-Tillmann B, Collins CA, Campbell AL, Podsadecki T, Bernstein B. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med* 2014; **370**: 1973-1982 [PMID: 24725237 DOI: 10.1056/NEJMoa1402869]
- 132 **Asselah T**, Bruno S, Craxi A. HCV cirrhosis at the edge of decompensation: will paritaprevir with ritonavir, ombitasvir, dasabuvir, and ribavirin solve the need for treatment? *J Hepatol* 2014; **61**: 1430-1433 [PMID: 25149112 DOI: 10.1016/j.jhep.2014.08.018]
- 133 **Andreone P**, Colombo MG, Enejosa JV, Koksai I, Ferenci P, Maieron A, Müllhaupt B, Horsmans Y, Weiland O, Reesink HW, Rodrigues L, Hu YB, Podsadecki T, Bernstein B. ABT-450, ritonavir, ombitasvir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. *Gastroenterology* 2014; **147**: 359-365.e1 [PMID: 24818763 DOI: 10.1053/j.gastro.2014.04.045]
- 134 **Binder M**, Quinkert D, Bochkarova O, Klein R, Kezmic N, Bartenschlager R, Lohmann V. Identification of determinants involved in initiation of hepatitis C virus RNA synthesis by using intergenotypic replicase chimeras. *J Virol* 2007; **81**: 5270-5283 [PMID: 17344294 DOI: 10.1128/JVI.00032-07]
- 135 **Paul D**, Romero-Brey I, Gouttenoire J, Stoitsova S, Krijnse-Locker J, Moradpour D, Bartenschlager R. NS4B self-interaction through conserved C-terminal elements is required for the establishment of functional hepatitis C virus replication complexes. *J Virol* 2011; **85**: 6963-6976 [PMID: 21543474 DOI: 10.1128/JVI.00502-11]
- 136 **Flisiak R**, Feinman SV, Jablkowski M, Horban A, Kryczka W, Pawlowska M, Heathcote JE, Mazzella G, Vandelli C, Nicolas-Métral V, Grosgrain P, Liz JS, Scalfaro P, Porchet H, Crabbé R. The cyclophilin inhibitor Debio 025 combined with PEG IFNalpha2a significantly reduces viral load in treatment-naïve hepatitis C patients. *Hepatology* 2009; **49**: 1460-1468 [PMID: 19353740 DOI: 10.1002/hep]
- 137 **Zeuzem S**, Flisiak R, Vierling JM, Mazur W, Mazzella G, Thongsawat S, Abdurakhmanov D, Van Kinh N, Calistru P, Heo J, Stanciu C, Gould M, Makara M, Hsu SJ, Buggisch P, Samuel D, Mutimer D, Nault B, Merz M, Bao W, Griffel LH, Brass C, Naoumov NV. Randomised clinical trial: alisporivir combined with peginterferon and ribavirin in treatment-naïve patients with chronic HCV genotype 1 infection (ESSENTIAL II). *Aliment Pharmacol Ther* 2015; **42**: 829-844 [PMID: 26238707 DOI: 10.1111/apt.13342]
- 138 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; **60**: 392-420 [PMID: 24331294 DOI: 10.1016/j.jhep.2013.11.003]
- 139 **AISF Guidelines e Position Papers**. 2015. Available from: URL: <http://www.webaisf.org/pubblicazioni/guidelines-e-position-papers.aspx>
- 140 **Shahid I**, ALMalki WH, Hafeez MH, Hassan S. Hepatitis C virus infection treatment: An era of game changer direct acting antivirals and novel treatment strategies. *Crit Rev Microbiol* 2014; 1-13 [PMID: 25373616 DOI: 10.3109/1040841X.2014.970123]
- 141 **Petta S**, Craxi A. Current and future HCV therapy: do we still need other anti-HCV drugs? *Liver Int* 2015; **35** Suppl 1: 4-10 [PMID: 25529081 DOI: 10.1111/liv.12714]

**P- Reviewer:** Abenavoli L, Han SY, Rodriguez-Frias F, Tovo CV

**S- Editor:** Ji FF **L- Editor:** Wang TQ **E- Editor:** Liu SQ



## Hepatitis C virus and non-Hodgkin's lymphomas: Meta-analysis of epidemiology data and therapy options

Gabriele Pozzato, Cesare Mazzaro, Luigino Dal Maso, Endri Mauro, Francesca Zorat, Giulia Moratelli, Pietro Bulian, Diego Serraino, Valter Gattei

Gabriele Pozzato, Francesca Zorat, Giulia Moratelli, Department of Medical and Surgical Sciences, University of Trieste, 34100 Trieste, Italy

Cesare Mazzaro, Pietro Bulian, Valter Gattei, Department of Oncology-Haematology, Centro di Riferimento Oncologico, IRCCS, 33081 Aviano, Italy

Luigino Dal Maso, Diego Serraino, Epidemiology and Biostatistics Units, Centro di Riferimento Oncologico, IRCCS, 33081 Aviano, Italy

Endri Mauro, Department of Internal Medicine, Pordenone General Hospital, 33170 Pordenone, Italy

**Author contributions:** Pozzato G and Mazzaro C designed the paper; Dal Maso L and Serraino D analyzed the data and performed the statistics; Bulian P and Gattei V performed the research; Zorat F, Moratelli G and Pozzato G wrote the manuscript; all authors contributed to this manuscript.

**Conflict-of-interest statement:** Authors declare no conflict of interests for this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Gabriele Pozzato, MD, Professor of Haematology, Department of Medical and Surgical Sciences, University of Trieste, Piazza Ospedale 1, 34100 Trieste, Italy. [g.pozzato@fmc.units.it](mailto:g.pozzato@fmc.units.it)  
 Telephone: +39-040-3992002  
 Fax: +39-040-3992560

Received: May 21, 2015  
 Peer-review started: May 22, 2015  
 First decision: July 10, 2015

Revised: October 9, 2015

Accepted: December 7, 2015

Article in press: December 8, 2015

Published online: January 18, 2016

### Abstract

Hepatitis C virus (HCV) is a global health problem affecting a large fraction of the world's population: This virus is able to determine both hepatic and extrahepatic diseases. Mixed cryoglobulinemia, a B-cell "benign" lymphoproliferative disorders, represents the most closely related as well as the most investigated HCV-related extrahepatic disorder. Since this virus is able to determine extrahepatic [non-Hodgkin's lymphoma (NHL)] as well as hepatic malignancies (hepatocellular carcinoma), HCV has been included among human cancer viruses. The most common histological types of HCV-associated NHL are the marginal zone, the lymphoplasmacytic and diffuse large cell lymphomas. The role of the HCV in the pathogenesis of the B-cell lymphoproliferative disorders is confirmed also by the responsiveness of the NHL to antiviral therapy. The purpose of this review is to provide an overview of the recent literature and a meta analysis of the epidemiology data, to explain the role of HCV in the development of NHL's lymphoma. Furthermore, the possibility to treat these HCV-related NHL with the antiviral therapy or with other therapeutic options, like chemotherapy, is also discussed.

**Key words:** Hepatitis C virus; Non-Hodgkin's lymphoma; Hepatitis C virus genotypes; Alpha-interferon

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The goal of this article is to review the epidemiological data from different countries to perform

an up-to-date meta-analysis of the risk to developing non-Hodgkin's lymphomas in hepatitis C virus (HCV)-infected patients. Finally, we highlighted the clinical and the biological data necessary to optimize the cure of the patients affected by HCV-positive non-Hodgkin's lymphomas.

Pozzato G, Mazzaro C, Dal Maso L, Mauro E, Zorat F, Moratelli G, Bulian P, Serraino D, Gattei V. Hepatitis C virus and non-Hodgkin's lymphomas: Meta-analysis of epidemiology data and therapy options. *World J Hepatol* 2016; 8(2): 107-116 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i2/107.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i2.107>

## INTRODUCTION

Non-Hodgkin's lymphomas (NHL) are neoplastic diseases of the lymphoid tissue. Given the high heterogeneity in terms of histological and clinical characteristics, anatomical location, and putative aetiologies, several causative factors have been reported including inherited or acquired immunodeficiency, exposure to some toxic substances (pesticides) or radiation, smoking habits, and, in the last few years, infectious factors. In fact, the Epstein-Barr virus (EBV) has been shown to be involved in the development of the Burkitt's lymphoma<sup>[1-3]</sup> and of other haematological malignancies (immunoblastic lymphoma, Hodgkin's disease, nasopharyngeal carcinoma), the human retrovirus HTLV-I in the T-cell leukemia-lymphoma<sup>[4,5]</sup>, and the double stranded DNA human herpes virus 8 in the Kaposi sarcoma<sup>[6,7]</sup>, primary effusion lymphoma and multicentric Castlemann disease<sup>[8]</sup>. But not only viruses are involved in pathogenesis of NHL, even the Gram-negative microaerophilic bacterium *Helicobacter Pylori* is thought to be the cause of gastric mucosa associated lymphoid (MALT) lymphoma<sup>[9,10]</sup>. However, although these infectious agents are widespread (EBV infects near 100% of all populations and remains in B-cell throughout the life span), only a very small fraction of virus-carriers develops lymphomas. This indicates the key role of some, not yet understood, host factors: Maybe genetic factors like HLA antigens or cytokine signalling pathways or acquired factors like exposure to toxic substances (ethanol, drugs, etc.) or immunosuppression secondary to therapy for rheumatological disorders or to chemotherapy for malignancies.

Hepatitis C virus (HCV) is a RNA virus belonging to the flaviviruses discovered in 1990 and involved in acute and chronic liver disease. The genome consists of a single-positive-stranded RNA molecule enveloped by a lipid bilayer within which two different glycoproteins are anchored<sup>[11]</sup>. The viral genome contains three distinct regions<sup>[12]</sup>: (1) a short 5' non-coding region with two domains: A stem-loop structure involved in HCV replication and the internal ribosome entry site the structure responsible for attachment of the ribosome

and polyprotein translation<sup>[13]</sup>; (2) A large, unique open reading frame of more than 9000 nucleotides, which encodes a single polyprotein precursor, that is cleaved co- and post-translationally to give the structural and non-structural viral proteins; and (3) The 3' non-translated region endowed with high variability in the length and structure. The HCV shows a high genetic diversity since, similarly to all RNA positive-strand viruses, the RNA-dependent RNA polymerase lacks a 3'-5' exonuclease proofreading activity for removal of the misincorporated bases. Therefore, the viral replication is error-prone, and this determines a large number of variants (quasispecies virus population)<sup>[14]</sup>. The frequency of the nucleotide mutations ranges from  $1.4 \times 10^3$  to  $1.9 \times 10^3$  substitutions per nucleotide per year. The HCV is classified into six genotypes with a different distribution by geographical region and between patient groups; each genotype contains a variable number of genetically distinct "subtypes". At the nucleotide level, the genotypes differ from each other by 31% to 33%, while subtypes from 20% to 25%<sup>[15]</sup>. The peculiar characteristic of HCV is the ability to infect not only the liver cells but also the lymphocytes<sup>[16]</sup> and, likely, other cells and tissues<sup>[17,18]</sup>. This is due on the fact that liver cells and lymphocytes share the same HCV receptor, *i.e.*, the CD81. The lymphotropism might explain the several extra hepatic manifestations of the chronic HCV infection<sup>[19-26]</sup>, among which mixed cryoglobulinemia (MC) is the most common<sup>[27-32]</sup>. MC is a disease characterised by the presence in the serum of immuno-complexes able to precipitate with cold temperature and to re-dissolve with rewarming<sup>[33]</sup>. The main clinical manifestations of this disease are the skin lesions (purpura) secondary to vasculitis, which is caused by the deposition of the cryoglobulins in the small and medium sized blood vessels<sup>[34]</sup>. In addition to skin lesions, MC may involve several organs and tissues, determining peripheral neuropathy and/or glomerulonephritis. Since cryoglobulins are the production of monoclonal B-cells and lymphoid infiltrates are present frequently in the bone marrow<sup>[35]</sup> of these patients, MC should be considered as a smouldering lymphoma. Accordingly, even the first cases of MC described by Melzer, later, by other researchers<sup>[36-38]</sup> developed lymphomas months or years after the onset of the symptoms of MC<sup>[39]</sup>. These reports suggest that chronic HCV infection induces clonal B-cell proliferation, which can evolve from a "benign" lymphoproliferative disorder to an overt malignant lymphoma<sup>[40]</sup>. Since, according to some estimates, near 170 million of people are carriers of the virus<sup>[41]</sup>, the clinical impact of the extrahepatic disorders, leading to neoplastic diseases of the hemopoietic system in addition to the liver diseases, makes HCV a major public health problem.

## THE EPIDEMIOLOGY OF HCV-POSITIVE NHL: META-ANALYSIS UP-DATING

The first studies, which described the association of



HCV and lymphoproliferative disorders, were performed recording the prevalence of anti-HCV antibodies in small-unselected groups of patients affected by lymphomas<sup>[42-46]</sup>. These preliminary reports excluded the association between HCV infection and Hodgkin's disease, while showed a strong association between NHL and HCV, especially in low-grade lymphomas. However, this association was found mainly in Italy and other researchers from the North of Europe did not confirm these findings. Therefore, some authors considered this relationship as due to the high prevalence of HCV in the Italian general population. In the following years, several studies addressed the possible association between HCV and NHL<sup>[47]</sup> and many papers have been published from different areas of the world. At present, more than 10000 cases of NHL have been screened for the presence of HCV infection and several meta-analyses on the relationship between HCV and lymphoma have been published<sup>[48,49]</sup>.

In this review, the most recent meta-analysis have been updated to include only the studies (until the end of 2011) with a control groups. Unfortunately, these control groups were heterogeneous, in fact, some papers included patients with hematological diseases other than NHL, other studies included cases with solid cancers, or cases undergoing an invasive procedure (like surgery or endoscopy) or population-based samples, other studies enrolled volunteer blood donors. Only recently, some authors designed these studies as case-control or as cohort studies with well-defined inclusion criteria. Therefore, these authors are able to estimate the odds ratios or the relative risks (RRs) adjusted for age, sex, and other confounding factors. In the present review, we discarded the studies including the patients with other lymphoproliferative diseases as control group since also these diseases might be correlated with HCV. In addition, we considered eligible for meta-analysis only the studies with at least one of the following requirements: (1) Sex- and age-adjusted RRs; (2) Cases and controls matched by age and sex; and (3) A measure of age and of the male/female ratio in both cases and controls.

If the authors did not provide RRs, we calculated the crude RRs (with 95% CIs) according to the Wald method, assuming the items 2 and 3 were available. In the analysis on NHL and HCV infection, we discarded the papers including less than 100 cases of NHL, while we included all the prospective studies (case-control or cohort studies) regardless of the number of NHL enrolled. Several problems of comparability were found in the retrieved studies since not all authors shared the definition of lymphoma. For instance, some authors included chronic lymphocytic leukemia (CLL) among NHL cases, whereas others did not. Since CLL patients show a prevalence of HCV infection lower than that observed in the general population, the inclusion or the exclusion of this very common lymphoproliferative disorder has a great impact on the epidemiological studies. In addition, the CLL cells and the small lymphocytic lymphoma (SLL) have the same immuno-phenotype<sup>[50,51]</sup>, but SLL was

included among NHL by all authors<sup>[51]</sup>. Most authors excluded the cases with human immunodeficiency virus (HIV) infection; therefore, we did not review the studies including HIV patients. To avoid bias, we discarded also the studies including only selected populations, non-representative of general population<sup>[52,53]</sup>. Another problem was the method of checking and confirming the HCV infection: In the first papers, most authors used only the enzyme-linked immunosorbent assay (ELISA) whereas, more recently, most authors used recombinant immunoblot assay (RIBA). To increase the complexity of the analysis, some authors enrolled only the patients with active HCV replication, *i.e.*, with detectable levels of serum HCV-RNA. Since the first generation ELISAs showed low sensitivity and specificity, in this review we included only the studies employing second or third generation ELISAs. However, we did not consider the detection of HCV-RNA as a requirement for including a study.

**Statistical methods:** We calculated the summary RR and corresponding 95%CI with the models of DerSimonian and Laird, which incorporate both within and between-study variability, as a weighted average of the estimated RRs, by giving each study a weight proportional to its precision. The heterogeneity among studies was evaluated using the *Q* statistics. The Begg's and Egger's asymmetry tests were used to assess the publication bias.

Figure 1 indicates the results of studies on HCV and NHL. The highest prevalence of HCV infection in the general population (over 20%) was found in Egypt. A rather high prevalence (5%-10%) was found in Italy and in Japan, while most countries (South Korea, Northern Europe, United States, Australia, and Canada) showed a prevalence below 5%. The 19 case-controls studies included in this review enrolled altogether 9038 cases and 12224 controls. The pooled RR from this large group was 2.4 (95%CI: 2.0-3.0), and most of them (11/19) showed a RRs significantly elevated (Figure 1). The RRs of the cohort studies was 2.0 (95%CI: 1.4-3.0). The overall RR estimation was 2.3 (95%CI: 1.8-2.9) with no significant heterogeneity between study designs. The different prevalence of the HCV infection in the control groups determined the great heterogeneity in the results. In fact, the studies performed in areas with a high HCV prevalence (above 5%) showed a more elevated RR (> 3) than those performed in areas with a low HCV prevalence (RR < 2). A significant heterogeneity emerged also for the publication period: In fact, the studies published up to 2003 indicated higher RRs when compared with the studies carried out thereafter. In addition, there are regional variations: people infected by HCV from Japan and from the Mediterranean basin show a relative risk of NHL from 2 to 4 times higher than people of Northern Europe<sup>[54]</sup>.

The mechanisms by which lymphoma is induced by HCV are still limited. The HCV-induced transformation process of B-cell may occur in three ways: (1) Chronic stimulation of B-Cell Receptor or other receptors placed

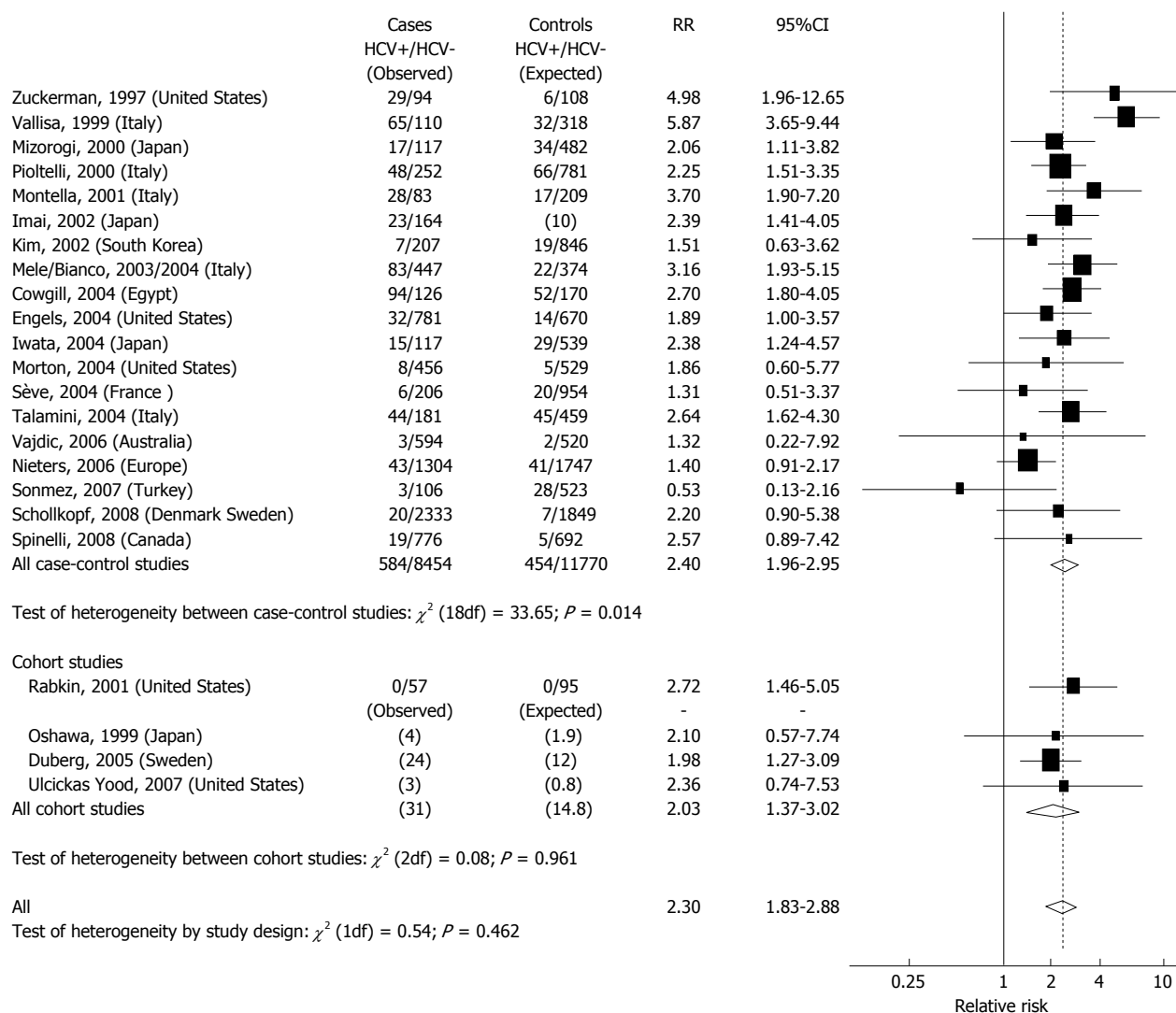


Figure 1 Relative risk estimates and corresponding 95%CI of non-Hodgkin's lymphoma by hepatitis C virus seropositivity in case-control and cohort studies.

on the surface of B-cells by the viral antigens (in absence of cell infection) with secondary proliferation; (2) Infection and persistent replication of HCV inside B-cells with oncogenic effects by some viral proteins; and (3) Temporary intracellular virus replication with damage of B-cells<sup>[55]</sup>. However, since an active replication of HCV in human B or T lymphocytes *in vivo* (with evidence of the HCV-RNA negative strands) has never been demonstrated, a direct oncogenic effect by HCV inside B cells is unlikely. In addition, viral proteins, indicative of active replication, could never be demonstrated in the neoplastic lymphoid tissue of the HCV-NHL. Based on these considerations, it is likely that the neoplastic transformation is determined by the chronic antigen stimulation of B cells by viral surface proteins<sup>[56]</sup>. There are several experimental data supporting this theory: (1) the B lymphocytes from HCV patients show a higher level of activation markers<sup>[56]</sup> than normal lymphocyte; and (2) the long-term exposure to the epitopes of HCV lead to selection and expansion of a oligoclonal B-cells, which evolve in clonal B-cells and finally in an overt HCV-

NHL.

In conclusions, HCV infection seems to be associated with a 2.5-fold increase in the risk of developing NHL. The fraction of the NHL secondary to HCV infection may be 10%-15% in areas where HCV prevalence is high, but it is smaller in the countries of low prevalence. Based on epidemiological and experimental evidence, IARC recently concluded that there was sufficient evidence in humans to indicate the HCV infection as a cause of non-Hodgkin lymphomas, in addition to the previously recognized causal association with hepatocellular carcinoma<sup>[57]</sup>.

## THE THERAPY OF HCV-POSITIVE NHL

As previously indicated, the HCV-positive NHL are heterogeneous in terms of histological features and clinical aspects. The most common HCV-related NHL are indolent lymphomas (marginal-zone), but several aggressive and, rarely, very aggressive NHL are reported. Since the relationship between viral

replication and monoclonal lympho-proliferation is by now consolidated, the antiviral therapy could appear to be an attractive therapeutic option, in analogy to the antibiotic therapy employed to treat MALT lymphoma associated with *Helicobacter Pylori* infection<sup>[7]</sup>. However, before starting antiviral treatment of "bona fide" HCV-related NHL, several points should be taken in consideration, including: (1) Is the NHL really related with HCV infection? How the haematologist can be sure that a given NHL is HCV-associated? (2) Which is the best therapeutic approach? (3) Is the chemotherapy safe? In the case of the need to plan a chemotherapy, are the HCV-positive NHL exposed to higher risks than HCV-negative cases? and (4) The outcome of the HCV + NHL is the same as compared with the HCV-NHL with the same histotype?

The HCV-related NHL show some typical, histological, clinical, laboratory and molecular characteristics. The most common histological types of HCV+NHL are lymphoplasmacytic, primary nodal marginal zone, splenic marginal zone, MALT marginal zone, while other histotypes are less closely associated with HCV<sup>[58-61]</sup>. The clinical course of the disease is generally indolent. The most common feature of true HCV + NHL is the longstanding presence of MC<sup>[62]</sup>, and the late appearance of overt NHL, often after years from the onset of the clinical symptoms of MC. From a biological point of view, the HCV-related NHL often show a monoclonal IgMk component and the presence of several auto-antibodies (mainly anti-thyroid). From a molecular point of view, these patients use a restricted *IgHV* gene repertoire<sup>[63]</sup>, with a strong bias for the IGHV1-69 and V3-A27<sup>[64,65]</sup>. In addition, the same set of V region genes, VH1-69 and Vk3 -A27 encode for the monoclonal IgMk component (if present). Finally the bcl-2/IgH translocation has been described in some studies, although not confirmed by others, present in HCV + NHL, at least of the lymphoplasmacytic subtype<sup>[66-68]</sup>.

To choose the best therapeutic strategy several factors should be taken in consideration. Firstly the tumour burden: If there are large or huge nodal or extra nodal masses, chemotherapy becomes the first choice; on the contrary, if the tumour burden is low (confined to enlarged spleen and mild lymphoid infiltration of bone marrow) antiviral therapy is more indicated. A second factor to be considered is the course of the disease: If the course is indolent and lymphoma discovered occasionally during the follow-up of the patient, the antiviral therapy is more attractive, while if the patient show progressive and rapid node or spleen enlargement, chemotherapy is again the best choice. A third factor should be always taken in consideration, *i.e.*, the presence or the absence of a chronic liver disease (CLD), and, if present, the severity of such a CLD. This means that the patient should undergo a complete hepatological evaluation including ultrasonography and, if indicated, endoscopy and liver biopsy. If the patient is affected by chronic C hepatitis without evidence of cirrhosis, the antiviral therapy should be indicated, while an advanced chronic

liver disease with severe portal hypertension could be a contra-indication for antiviral and chemotherapy as well. A fourth factor to be considered is the presence and the quality of clinical symptoms. In fact the symptoms could be tumour-related (fever, weight loss, asthenia, *etc.*) or MC-related (vasculitis, neuropathy, arthralgias, *etc.*), in the former case chemotherapy is indicated while in the latter antiviral treatment could be the right choice. Finally, some specific contra-indications to antiviral therapy should be considered, often not familiar to haematologists<sup>[69]</sup>, like deep depression<sup>[70,71]</sup> or immunological disorders<sup>[72]</sup>.

The presence of HCV replication, *i.e.*, detectable levels of HCV-RNA, without liver disease, cannot be considered a contra-indication for chemotherapy. In fact, the experience in the treatment of HCV + cryoglobulinemia<sup>[73]</sup> shows that when these patients undergo either anti-CD20 therapy, or other intensive immunosuppressive treatment, though a mild elevation of HCV-RNA levels has been noticed, the liver function never worsens. On the contrary, some author reported a mild improvement in some cases. Despite few papers focused on this topic, the literature data confirm this point of view: Faggioli *et al.*<sup>[74]</sup>, in a small series of cases, did not detect any acute hepatitis due to the reactivation of HCV replication. These data were confirmed by other authors in larger cohorts of patients: Takai *et al.*<sup>[75]</sup> found that, after chemotherapy, the fraction of NHL patients who developed liver function test alterations was higher in non-hepatitis virus carriers (12%) than in HCV-bearing patients (10%), while a significant proportion of HBsAg carriers (36%) showed post-chemotherapy liver injuries. To further confirm these data, Visco *et al.*<sup>[76]</sup>, during the follow-up of 136 HCV-positive diffuse large cell lymphomas, found that only 5 cases (4%) discontinued the chemotherapy due to severe liver function impairment. It is noteworthy that 9 cases (7%) of the series had liver cirrhosis, and 26 cases had chronic hepatitis (19%). Altogether, this means that even in presence of HCV-related chronic liver disease, chemotherapy is feasible with a reasonable margin of safety.

Contradictory data on the outcome of HCV-positive NHL are present in the literature. A first Japanese paper of Tomita *et al.*<sup>[77]</sup> showed that the cases affected by HCV-positive aggressive NHL have the same prognosis as HCV-negative aggressive NHL, at least in the subjects without an advanced chronic liver disease. On the contrary, Besson *et al.*<sup>[78]</sup>, grouping together two large GELA studies (NHL93 and NHL98), found that the proportion of patients with high and high-intermediate IPI was higher among HCV-positive patients, and that, at 2 years, the OS and PFS of HCV-positive cases was 56% vs 80% and 53% vs 75%, respectively. These surprising results could be explained, at least in part, by taking into account a possible selection bias of cases. In fact, the prevalence of HCV in these two cohorts of cases affected by NHL is largely lower (0.46%) of that found in the general population of France, where the

**Table 1** Main studies of antiviral therapy in patients with hepatitis C virus infection and non-Hodgkin's lymphoma (reports with single cases were discarded)

Ref.	n	Lymphoma histology (n)	Disease sites BM-S-LN-PB	MC type II (n)	Antiviral therapy (n)	SVR (n)	NHL response (n)	Response duration (mo)
Mazzaro <i>et al</i> <sup>[94]</sup>	6	LPL (6)	6-0-2-0	4	IFN (6)	4	CR (3) PR (1)	12 (8-18)
Moccia <i>et al</i> <sup>[95]</sup>	3	SMZL (3)	1-3-0-0	NR	IFN (3)	2	CR (2) NR (1)	24 (5-40)
Hermine <i>et al</i> <sup>[80]</sup>	9	SLVL (9)	6-9-5-9	6	IFN (7) IFN-RBV (2)	7	CR (7) PR (1) NR (1)	27 (15-40)
Arcaini <i>et al</i> <sup>[96]</sup>	4	SMZL (4)	4-4-1-2	NR	IFN + RBV (4)	3	CR (2) PR (1)	36 (1-16)
Kelaïdi <i>et al</i> <sup>[82]</sup>	8	SMZL (4) MZL/MALT (4)	7-6-2-6	8	IFN (2) IFN + RBV (6)	5	CR (5) PR (1)	6
Pitini <i>et al</i> <sup>[97]</sup>	2	SMZL (2)	2-2-1-2	NR	IFN (2)	2	CR (2)	9
Saadoun <i>et al</i> <sup>[83]</sup>	18	SLVL (18)	10-18-8-10	18	IFN (8) IFN + RBV (10)	14	CR (14) PR (4)	62
Tursi <i>et al</i> <sup>[89]</sup>	16	MZL/MALT (16)	NR	NR	IFN + RBV (16)	11	CR (11)	NotR
Vallisa <i>et al</i> <sup>[86]</sup>	13	SMZL (4) MALT (4) FL (1) LPL (4)	5-4-0-6	5	PEG-IFN + RBV (13)	7	CR (7) PR (2)	14 (2-24)
Mazzaro <i>et al</i> <sup>[85]</sup>	18	SLVL (1), FL (1), LPL (16)	16-2-2-16	13	IFN + RBV (8) PEG-IFN + RBV (10)	3 6	CR (3) PR (2) CR (6) PR (2)	18 (8-32)
Paulli <i>et al</i> <sup>[98]</sup>	2	MZL/MALT (2)	Cutaneous	2	PEG-IFN + RBV	2	CR (1) PR (1)	NotR
Pellicelli <i>et al</i> <sup>[88]</sup>	9	SMZL (3) MZL (4) FL (2)	NR	4	PEG-IFN + RBV (9)	7	CR (5) PR (2)	12

MZL: Marginal zone lymphoma; SMZL: Splenic marginal zone lymphoma; SLVL: Splenic lymphoma with villous lymphocytes; FL: Follicular lymphoma; LPL: Lymphoplasmacytic lymphoma; BM: Bone marrow; S: Spleen; LN: Lymph nodes; PB: Peripheral blood; IFN: Alfa2a/Alfa2b interferon 3 times a week; RBV: Ribavirin; PEG-IFN: Pegylated alfa2a/alfa2b interferon; CR: Complete remission; PR: Partial remission; NR: No response; SVR: Sustained virological response; NotR: Not reported.

prevalence is 2.8%. Since, as previously indicated, the prevalence of HCV-infection is always higher in NHL than in the general population<sup>[79]</sup>, the very low number of HCV-positivity in the two groups of patients indicates the possibility of a selective enrolment in the trial of high-risk HCV-positive cases only, while standard- or low-risk cases were discarded. Nearly at the same time, Visco *et al*<sup>[76]</sup> following his large cohort of HCV-positive NHL showed that the OS and PFS of HCV-positive cases were similar to HCV-negatives. The question is still open and further controlled clinical trials should be needed to have definitive answers.

As shown in Table 1, antiviral therapy of HCV-NHL yielded different outcomes, according to the various authors. Since the number of cases is usually rather limited, several histotypes were enrolled with obvious different response rates, which makes published data are often contradictory. Moreover, several authors included cases with liver disease while others excluded these cases, and, finally, the presence of cryoglobulinemia is scattered among the cases and not recorded by all the authors. From 1996 to 2011, only 112 cases of HCV-positive NHL underwent antiviral therapy, the first three groups have been treated with interferon alone, thereafter with interferon and ribavirin and the last three groups with PEG-interferon (PEG-IFN) and ribavirin. The different antiviral power of these three regimens increases the difficult to interpret the results. In the first studies, the complete remission of the lymphomas was obtained in large fractions of patients (75% range: 64% to 84%), but most cases relapsed within few months. Much better results were achieved in the patients affected by splenic lymphoma with villous lymphocytes<sup>[80]</sup>, in fact all HCV-positive cases entered complete remission upon treatment with interferon alone or with interferon and ribavirin, while

HCV-negative lymphomas with villous lymphocytes controls did not benefit from antiviral therapy. The results obtained by Hermine *et al*<sup>[80]</sup> were confirmed subsequently by other studies<sup>[81-83]</sup>. These results suggest to perform a systematic screening for HCV in the patients affected by the marginal-zone lymphomas, since in the HCV-RNA positive cases, the antiviral therapy should be considered the treatment of choice. Several studies have documented the regression of different histotypes of NHL after antiviral treatment, such as lymphoplasmacytic lymphoma<sup>[84-86]</sup>, mantle-cell lymphoma<sup>[87]</sup>, nodal marginal zone lymphomas<sup>[88]</sup> or extranodal marginal zone lymphoma of MALT tissue (MALT lymphomas)<sup>[89]</sup>. In the last three published studies all the patients were treated with the same antiviral regimen (PEG-IFN plus ribavirin), allowing better interpretation of the homogeneous results. In all three papers the haematological response significantly ( $P < 0.005$ ) correlates to the disappearance of HCV-RNA, and the sustained virological response was more frequently obtained in patients with genotype 2 or 3 more than genotype 1 or 4, which are usually found in HCV-chronic hepatitis without NHL. Given the high antiviral power of the treatment, the relapse rate is lower in these three studies (30%) than that previously recorded. At present, no data are available on the triple therapy in HCV-NHL.

## CONCLUSION

In addition of acute and chronic liver diseases, the HCV infection determines many extra hepatic manifestations. Among them, the ability of the virus to interact with B cells leads to antigen-driven B-cell transformation, which ultimately may determine MC and finally a frank NHL. Based on clinical and biological considerations, the antiviral therapy should be considered as the treatment



of choice in HCV-associated lymphomas, especially in the presence of MC. However, the traditional antiviral therapy, based on PEG-IFN plus RIBA, is fading given the low efficacy and the numerous and severe side effects. At present, a new era is born for the management of HCV infection: The new strong direct antiviral agents<sup>[90-93]</sup> opened the gate for a complete eradication of viral infection. These new drugs, described as lacking in side effects, can be used even in heavily pretreated patients or in cases with advanced liver disease with high possibility of success. It is likely that these new treatment options will be able to reduce drastically the number of the chronic carriers of HCV, as consequence, the number of HCV-related NHL.

## REFERENCES

- 1 Vereide D, Sugden B. Proof for EBV's sustaining role in Burkitt's lymphomas. *Semin Cancer Biol* 2009; **19**: 389-393 [PMID: 19628040 DOI: 10.1016/j.semcancer.2009.07.006]
- 2 Hecht JL, Aster JC. Molecular biology of Burkitt's lymphoma. *J Clin Oncol* 2000; **18**: 3707-3721 [PMID: 11054444]
- 3 Kennedy G, Komano J, Sugden B. Epstein-Barr virus provides a survival factor to Burkitt's lymphomas. *Proc Natl Acad Sci USA* 2003; **100**: 14269-14274 [PMID: 14603034 DOI: 10.1073/pnas.2336099100]
- 4 Gallo RC. Research and discovery of the first human cancer virus, HTLV-I. *Best Pract Res Clin Haematol* 2011; **24**: 559-565 [PMID: 22127321 DOI: 10.1016/j.beha.2011.09.012]
- 5 Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Golde D, Gallo RC. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. *Science* 1982; **218**: 571-573 [PMID: 6981847]
- 6 Cai Q, Verma SC, Lu J, Robertson ES. Molecular biology of Kaposi's sarcoma-associated herpesvirus and related oncogenesis. *Adv Virus Res* 2010; **78**: 87-142 [PMID: 21040832 DOI: 10.1016/B978-0-12-385032-4.00003-3]
- 7 Wen KW, Damania B. Kaposi sarcoma-associated herpesvirus (KSHV): molecular biology and oncogenesis. *Cancer Lett* 2010; **289**: 140-150 [PMID: 19651473]
- 8 Leroy S, Moshous D, Cassar O, Reguerre Y, Byun M, Pedergnana V, Canioni D, Gessain A, Oksenhendler E, Fieschi C, Mahlaoui N, Rivière JP, Herbigneaux RM, Muszlak M, Arnaud JP, Fischer A, Picard C, Blanche S, Plancoulaine S, Casanova JL. Multicentric Castleman disease in an HHV8-infected child born to consanguineous parents with systematic review. *Pediatrics* 2012; **129**: e199-e203 [PMID: 22157133 DOI: 10.1542/peds.2010-2739]
- 9 Wotherspoon AC, Doglioni C, Isaacson PG. Low-grade gastric B-cell lymphoma of mucosa-associated lymphoid tissue (MALT): a multifocal disease. *Histopathology* 1992; **20**: 29-34 [PMID: 1737623]
- 10 Roggero E, Zucca E, Pinotti G, Pascarella A, Capella C, Savio A, Pedrinis E, Paterlini A, Venco A, Cavalli F. Eradication of *Helicobacter pylori* infection in primary low-grade gastric lymphoma of mucosa-associated lymphoid tissue. *Ann Intern Med* 1995; **122**: 767-769 [PMID: 7717599 DOI: 10.7326/0003-4819-122-10-199505150-00006]
- 11 Brass V, Moradpour D, Blum HE. Molecular virology of hepatitis C virus (HCV): 2006 update. *Int J Med Sci* 2006; **3**: 29-34 [PMID: 16614739]
- 12 Hollinger FB. NANBH viruses. In: Hollinger FB, Robinson WS, Purcell RH, Gerin JL, Ticehurst J. Viral hepatitis, biological and clinical features, specific diagnosis and prophylaxis. New York: Raven Press, 1991: 139-173
- 13 Reed KE, Rice CM. Overview of hepatitis C virus genome structure, polyprotein processing, and protein properties. *Curr Top Microbiol Immunol* 2000; **242**: 55-84 [PMID: 10592656]
- 14 Martell M, Esteban JI, Quer J, Vargas V, Esteban R, Guardia J, Gómez J. Dynamic behavior of hepatitis C virus quasispecies in patients undergoing orthotopic liver transplantation. *J Virol* 1994; **68**: 3425-3436 [PMID: 8151804]
- 15 Simmonds P, Bukh J, Combet C, Deléage G, Enomoto N, Feinstone S, Halfon P, Inchauspé G, Kuiken C, Maertens G, Mizokami M, Murphy DG, Okamoto H, Pawlotsky JM, Penin F, Sablon E, Shin-I T, Stuyver LJ, Thiel HJ, Viazov S, Weiner AJ, Widell A. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology* 2005; **42**: 962-973 [PMID: 16149085 DOI: 10.1002/hep.20819]
- 16 Zignego AL, Macchia D, Monti M, Thiers V, Mazzetti M, Foschi M, Maggi E, Romagnani S, Gentilini P, Bréchet C. Infection of peripheral mononuclear blood cells by hepatitis C virus. *J Hepatol* 1992; **15**: 382-386 [PMID: 1332999]
- 17 Lerat H, Berby F, Trabaud MA, Vidalin O, Major M, Trépo C, Inchauspé G. Specific detection of hepatitis C virus minus strand RNA in hematopoietic cells. *J Clin Invest* 1996; **97**: 845-851 [PMID: 8609243 DOI: 10.1172/JCI118485]
- 18 Crovatto M, Pozzato G, Zorat F, Pussini E, Nascimben F, Baracetti S, Grando MG, Mazzaro C, Reitano M, Modolo ML, Martelli P, Spada A, Santini G. Peripheral blood neutrophils from hepatitis C virus-infected patients are replication sites of the virus. *Haematologica* 2000; **85**: 356-361 [PMID: 10756359]
- 19 Cosserat J, Cacoub P, Blétry O. Immunological disorders in C virus chronic hepatitis. *Nephrol Dial Transplant* 1996; **11** Suppl 4: 31-35 [PMID: 8918749]
- 20 Andreone P, Gramenzi A, Cursaro C, Bernardi M, Zignego AL. Monoclonal gammopathy in patients with chronic hepatitis C virus infection. *Blood* 1996; **88**: 1122 [PMID: 8704223]
- 21 Ganne-Carrie N, Medini A, Coderc E, Seror O, Christidis C, Grimbret S, Trinchet JC, Beaugrand M. Latent autoimmune thyroiditis in untreated patients with HCV chronic hepatitis: a case-control study. *J Autoimmun* 2000; **14**: 189-193 [PMID: 10677250 DOI: 10.1006/jaut.1999.0360]
- 22 Ramos-Casals M, Garcia-Carrasco M, Cervera R, Rosas J, Trejo O, de la Red G, Sánchez-Tapias JM, Font J, Ingelmo M. Hepatitis C virus infection mimicking primary Sjögren syndrome. A clinical and immunologic description of 35 cases. *Medicine (Baltimore)* 2001; **80**: 1-8 [PMID: 11204499]
- 23 Koike K, Moriya K, Ishibashi K, Yotsuyanagi H, Shintani Y, Fujie H, Kurokawa K, Matsuura Y, Miyamura T. Sialadenitis histologically resembling Sjögren syndrome in mice transgenic for hepatitis C virus envelope genes. *Proc Natl Acad Sci USA* 1997; **94**: 233-236 [PMID: 8990191]
- 24 Pilli M, Penna A, Zerbini A, Vescovi P, Manfredi M, Negro F, Carrozzo M, Mori C, Giuberti T, Ferrari C, Missale G. Oral lichen planus pathogenesis: A role for the HCV-specific cellular immune response. *Hepatology* 2002; **36**: 1446-1452 [PMID: 12447871]
- 25 Silvestri F, Barillari G, Fanin R, Zaja F, Infanti L, Patriarca F, Baccarani M, Pipan C, Falasca E, Botta GA. Risk of hepatitis C virus infection, Waldenström's macroglobulinemia, and monoclonal gammopathies. *Blood* 1996; **88**: 1125-1126 [PMID: 8704227]
- 26 Santini GF, Crovatto M, Modolo ML, Martelli P, Silvia C, Mazzi G, Franzin F, Moretti M, Tulissi P, Pozzato G. Waldenström macroglobulinemia: a role of HCV infection? *Blood* 1993; **82**: 2932 [PMID: 8219244]
- 27 Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992; **327**: 1490-1495 [PMID: 1383822 DOI: 10.1056/NEJM199211193272104]
- 28 Ferri C, Greco F, Longombardo G, Palla P, Moretti A, Marzo E, Mazzoni A, Pasero G, Bombardieri S, Highfield P. Association between hepatitis C virus and mixed cryoglobulinemia [see comment]. *Clin Exp Rheumatol* 1991; **9**: 621-624 [PMID: 1662567]
- 29 Misiani R, Bellavita P, Fenili D, Borelli G, Marchesi D, Massazza M, Vendramin G, Comotti B, Tanzi E, Scudeller G. Hepatitis C virus infection in patients with essential mixed cryoglobulinemia. *Ann Intern Med* 1992; **117**: 573-577 [PMID: 1326246 DOI: 10.732]

- 6/0003-4819-117-7-573]
- 30 **Ferri C**, La Civita L, Longombardo G, Zignego AL. Hepatitis C virus and mixed cryoglobulinaemia. *Br J Rheumatol* 1994; **33**: 301 [PMID: 7512423]
- 31 **Mazzaro C**, Tulissi P, Moretti M, Mazzoran L, Pussini E, Crovatto M, Santini GF, Pozzato G. Clinical and virological findings in mixed cryoglobulinaemia. *J Intern Med* 1995; **238**: 153-160 [PMID: 7629483]
- 32 **Adinolfi LE**, Utili R, Attanasio V, Zampino R, Ragone E, Tripodi MF, Ruggiero G. Epidemiology, clinical spectrum and prognostic value of mixed cryoglobulinaemia in hepatitis C virus patients: a prospective study. *Ital J Gastroenterol* 1996; **28**: 1-9 [PMID: 8743066]
- 33 **Meltzer M**, Franklin EC, Elias K, McCluskey RT, Cooper N. Cryoglobulinemia--a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. *Am J Med* 1966; **40**: 837-856 [PMID: 4956871]
- 34 **Grey HM**, Kohler PF. Cryoimmunoglobulins. *Semin Hematol* 1973; **10**: 87-112 [PMID: 4633223]
- 35 **Perl A**, Gorevic PD, Ryan DH, Condemni JJ, Ruskowski RJ, Abraham GN. Clonal B cell expansions in patients with essential mixed cryoglobulinaemia. *Clin Exp Immunol* 1989; **76**: 54-60 [PMID: 2786780]
- 36 **Invernizzi F**, Galli M, Serino G, Monti G, Meroni PL, Granatieri C, Zanussi C. Secondary and essential cryoglobulinemias. Frequency, nosological classification, and long-term follow-up. *Acta Haematol* 1983; **70**: 73-82 [PMID: 6408882]
- 37 **Gorevic PD**, Kassab HJ, Levo Y, Kohn R, Meltzer M, Prose P, Franklin EC. Mixed cryoglobulinemia: clinical aspects and long-term follow-up of 40 patients. *Am J Med* 1980; **69**: 287-308 [PMID: 6996482]
- 38 **Monteverde A**, Rivano MT, Allegra GC, Monteverde AI, Zigrossi P, Baglioni P, Gobbi M, Falini B, Bordin G, Pileri S. Essential mixed cryoglobulinemia, type II: a manifestation of a low-grade malignant lymphoma? Clinical-morphological study of 12 cases with special reference to immunohistochemical findings in liver frozen sections. *Acta Haematol* 1988; **79**: 20-25 [PMID: 3124457]
- 39 **Silvestri F**, Pipan C, Barillari G, Zaja F, Fanin R, Infanti L, Russo D, Falasca E, Botta GA, Baccarani M. Prevalence of hepatitis C virus infection in patients with lymphoproliferative disorders. *Blood* 1996; **87**: 4296-4301 [PMID: 8639788]
- 40 **Mazzaro C**, Zagonel V, Monfardini S, Tulissi P, Pussini E, Fanni M, Sorio R, Bortolus R, Crovatto M, Santini G, Tiribelli C, Sasso F, Masutti R, Pozzato G. Hepatitis C virus and non-Hodgkin's lymphomas. *Br J Haematol* 1996; **94**: 544-550 [PMID: 8790157]
- 41 **Averhoff FM**, Glass N, Holtzman D. Global burden of hepatitis C: considerations for healthcare providers in the United States. *Clin Infect Dis* 2012; **55** Suppl 1: S10-S15 [PMID: 22715208 DOI: 10.1093/cid/cis361]
- 42 **Ferri C**, Caracciolo F, La Civita L, Monti M, Longombardo G, Greco F, Zignego AL. Hepatitis C virus infection and B-cell lymphomas. *Eur J Cancer* 1994; **30A**: 1591-1592 [PMID: 7833125]
- 43 **Ferri C**, La Civita L, Caracciolo F, Zignego AL. Non-Hodgkin's lymphoma: possible role of hepatitis C virus. *JAMA* 1994; **272**: 355-356 [PMID: 8028163 DOI: 10.1001/jama.1994.03520050033021]
- 44 **Pozzato G**, Mazzaro C, Crovatto M, Modolo ML, Ceselli S, Mazzi G, Sulfaro S, Franzin F, Tulissi P, Moretti M. Low-grade malignant lymphoma, hepatitis C virus infection, and mixed cryoglobulinemia. *Blood* 1994; **84**: 3047-3053 [PMID: 7949176]
- 45 **Ferri C**, Caracciolo F, Zignego AL, La Civita L, Monti M, Longombardo G, Lombardini F, Greco F, Capochiani E, Mazzoni A. Hepatitis C virus infection in patients with non-Hodgkin's lymphoma. *Br J Haematol* 1994; **88**: 392-394 [PMID: 7803287]
- 46 **Negri E**, Little D, Boiocchi M, La Vecchia C, Franceschi S. B-cell non-Hodgkin's lymphoma and hepatitis C virus infection: a systematic review. *Int J Cancer* 2004; **111**: 1-8 [PMID: 15185336 DOI: 10.1002/ijc.20205]
- 47 **Gisbert JP**, García-Buey L, Arranz R, Blas C, Pinilla I, Khorrami S, Acevedo A, Borque MJ, Pajares JM, Fernández-Rañada JM, Moreno-Otero R. The prevalence of hepatitis C virus infection in patients with non-Hodgkin's lymphoma. *Eur J Gastroenterol Hepatol* 2004; **16**: 135-138 [PMID: 15075985]
- 48 **Matsuo K**, Kusano A, Sugumar A, Nakamura S, Tajima K, Mueller NE. Effect of hepatitis C virus infection on the risk of non-Hodgkin's lymphoma: a meta-analysis of epidemiological studies. *Cancer Sci* 2004; **95**: 745-752 [PMID: 15471561 DOI: 10.1111/j.1349-7006.2004.tb03256.x]
- 49 **Dal Maso L**, Franceschi S. Hepatitis C virus and risk of lymphoma and other lymphoid neoplasms: a meta-analysis of epidemiologic studies. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 2078-2085 [PMID: 17119031 DOI: 10.1158/1055-9965]
- 50 **Flanagan MB**, Sathanoori M, Surti U, Soma L, Swerdlow SH. Cytogenetic abnormalities detected by fluorescence in situ hybridization on paraffin-embedded chronic lymphocytic leukemia/small lymphocytic lymphoma lymphoid tissue biopsy specimens. *Am J Clin Pathol* 2008; **130**: 620-627 [PMID: 18794056 DOI: 10.1309/H9AREV6E2JTMEC6J]
- 51 **Campo E**, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood* 2011; **117**: 5019-5032 [PMID: 21300984 DOI: 10.1182/blood-2011-01-293050]
- 52 **Amin J**, Dore GJ, O'Connell DL, Bartlett M, Tracey E, Kaldor JM, Law MG. Cancer incidence in people with hepatitis B or C infection: a large community-based linkage study. *J Hepatol* 2006; **45**: 197-203 [PMID: 16684579 DOI: 10.1016/j.jhep.2006.02.014]
- 53 **Giordano TP**, Henderson L, Landgren O, Chiao EY, Kramer JR, El-Serag H, Engels EA. Risk of non-Hodgkin lymphoma and lymphoproliferative precursor diseases in US veterans with hepatitis C virus. *JAMA* 2007; **297**: 2010-2017 [PMID: 17488966 DOI: 10.1001/jama.297.18.2010]
- 54 **IARC**. Monographs on the Evaluation of carcinogenic risks to Humans Volume 100 Part B: A review of human carcinogens: Biological agents. IARC Press: Lyon, 2012. Available from: URL: <http://monographs.iarc.fr/ENG/Monographs/vol100B/mono100B.pdf>
- 55 **Sung VM**, Shimodaira S, Doughty AL, Picchio GR, Can H, Yen TS, Lindsay KL, Levine AM, Lai MM. Establishment of B-cell lymphoma cell lines persistently infected with hepatitis C virus in vivo and in vitro: the apoptotic effects of virus infection. *J Virol* 2003; **77**: 2134-2146 [PMID: 12525648 DOI: 10.1128/JVI.77.3.2134-2146.2003]
- 56 **Rosa D**, Saletti G, De Gregorio E, Zorat F, Comar C, D'Oro U, Nuti S, Houghton M, Barnaba V, Pozzato G, Abrignani S. Activation of naïve B lymphocytes via CD81, a pathogenetic mechanism for hepatitis C virus-associated B lymphocyte disorders. *Proc Natl Acad Sci USA* 2005; **102**: 18544-18549 [PMID: 16339892 DOI: 10.1073/pnas.0509402102]
- 57 **Franceschi S**, Lise M, Trépo C, Berthillon P, Chuang SC, Nieters A, Travis RC, Vermeulen R, Overvad K, Tjønneland A, Olsen A, Bergmann MM, Boeing H, Kaaks R, Becker N, Trichopoulos A, Lagiou P, Bamia C, Palli D, Sieri S, Panico S, Tumino R, Sacerdote C, Bueno-de-Mesquita B, Peeters PH, Rodríguez L, Barroso LL, Dorronsoro M, Sánchez MJ, Navarro C, Barricarte A, Regnér S, Borgquist S, Melin B, Hallmans G, Khaw KT, Wareham N, Rinaldi S, Hainaut P, Riboli E, Vineis P. Infection with hepatitis B and C viruses and risk of lymphoid malignancies in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 208-214 [PMID: 21098651 DOI: 10.1158/1055-9965.EPI-10-0889]
- 58 **Gasparotto D**, De Re V, Boiocchi M. Hepatitis C virus, B-cell proliferation and lymphomas. *Leuk Lymphoma* 2002; **43**: 747-751 [PMID: 12153160 DOI: 10.1080/10428190290016845]
- 59 **Zuckerman E**, Zuckerman T, Levine AM, Douer D, Gutekunst K, Mizokami M, Qian DG, Velankar M, Nathwani BN, Fong TL. Hepatitis C virus infection in patients with B-cell non-Hodgkin lymphoma. *Ann Intern Med* 1997; **127**: 423-428 [PMID: 9312998 DOI: 10.7326/0003-4819-127-6-199709150-00002]
- 60 **Khouri T**, Chen S, Adar T, Jacob EO, Mizrahi M. Hepatitis C

- infection and lymphoproliferative disease: accidental comorbidities? *World J Gastroenterol* 2014; **20**: 16197-16202 [PMID: 25473174 DOI: 10.3748/wjg.v20.i43.16197]
- 61 **Rasul I**, Shepherd FA, Kamel-Reid S, Krajden M, Pantalony D, Heathcote EJ. Detection of occult low-grade b-cell non-Hodgkin's lymphoma in patients with chronic hepatitis C infection and mixed cryoglobulinemia. *Hepatology* 1999; **29**: 543-547 [PMID: 9918933 DOI: 10.1002/hep.510290224]
  - 62 **Newkirk MM**, Mageed RA, Jefferis R, Chen PP, Capra JD. Complete amino acid sequences of variable regions of two human IgM rheumatoid factors, BOR and KAS of the Wa idiotype family, reveal restricted use of heavy and light chain variable and joining region gene segments. *J Exp Med* 1987; **166**: 550-564 [PMID: 2439644]
  - 63 **Ivanovski M**, Silvestri F, Pozzato G, Anand S, Mazzaro C, Burrone OR, Efremov DG. Somatic hypermutation, clonal diversity, and preferential expression of the VH 51p1/VL kv325 immunoglobulin gene combination in hepatitis C virus-associated immunocytomas. *Blood* 1998; **91**: 2433-2442 [PMID: 9516143]
  - 64 **Marasca R**, Vaccari P, Luppi M, Zucchini P, Castelli I, Barozzi P, Cuoghi A, Torelli G. Immunoglobulin gene mutations and frequent use of VH1-69 and VH4-34 segments in hepatitis C virus-positive and hepatitis C virus-negative nodal marginal zone B-cell lymphoma. *Am J Pathol* 2001; **159**: 253-261 [PMID: 11438472 DOI: 10.1016/S0002-9440(10)61691-4]
  - 65 **Perotti M**, Ghidoli N, Altara R, Diotti RA, Clementi N, De Marco D, Sassi M, Clementi M, Burioni R, Mancini N. Hepatitis C virus (HCV)-driven stimulation of subfamily-restricted natural IgM antibodies in mixed cryoglobulinemia. *Autoimmun Rev* 2008; **7**: 468-472 [PMID: 18558364 DOI: 10.1016/j.autrev.2008.03.008]
  - 66 **Zignego AL**, Giannelli F, Marrocchi ME, Mazzocca A, Ferri C, Giannini C, Monti M, Caini P, Villa GL, Laffi G, Gentilini P. T(14; 18) translocation in chronic hepatitis C virus infection. *Hepatology* 2000; **31**: 474-479 [PMID: 10655273 DOI: 10.1002/hep.510310230]
  - 67 **Zignego AL**, Ferri C, Giannelli F, Giannini C, Caini P, Monti M, Marrocchi ME, Di Pietro E, La Villa G, Laffi G, Gentilini P. Prevalence of bcl-2 rearrangement in patients with hepatitis C virus-related mixed cryoglobulinemia with or without B-cell lymphomas. *Ann Intern Med* 2002; **137**: 571-580 [PMID: 12353944 DOI: 10.7326/0003-4819-137-7-200210010-00008]
  - 68 **Zuckerman E**, Zuckerman T, Sahar D, Streichman S, Attias D, Sabo E, Yeshurun D, Rowe J. bcl-2 and immunoglobulin gene rearrangement in patients with hepatitis C virus infection. *Br J Haematol* 2001; **112**: 364-369 [PMID: 11167830 DOI: 10.1046/j.1365-2141.2001.02573.x]
  - 69 **Cooper C**, Lester R, Thorlund K, Druyts E, El Khoury AC, Yaya S, Mills EJ. Direct-acting antiviral therapies for hepatitis C genotype 1 infection: a multiple treatment comparison meta-analysis. *QJM* 2013; **106**: 153-163 [PMID: 23159839 DOI: 10.1093/qjmed/hcs214]
  - 70 **Loftis JM**, Patterson AL, Wilhelm CJ, McNett H, Morasco BJ, Huckans M, Morgan T, Saperstein S, Asghar A, Hauser P. Vulnerability to somatic symptoms of depression during interferon-alpha therapy for hepatitis C: a 16-week prospective study. *J Psychosom Res* 2013; **74**: 57-63 [PMID: 23272989 DOI: 10.1016/j.jpsychores.2012.10.012]
  - 71 **Schäfer A**, Scheurlen M, Kraus MR. [Managing psychiatric side effects of antiviral therapy in chronic hepatitis C]. *Z Gastroenterol* 2012; **50**: 1108-1113 [PMID: 23059806 DOI: 10.1055/s-0031-1281682]
  - 72 **Tran HA**, Malcolm Reeves GE, Gibson R, Attia JR. Development of thyroid diseases in the treatment of chronic hepatitis C with alpha-interferon may be a good prognosticator in achieving a sustained virological response: a meta-analysis. *J Gastroenterol Hepatol* 2009; **24**: 1163-1168 [PMID: 19682190 DOI: 10.1111/j.1440-1746.2009.05874.x]
  - 73 **Ferri C**, Cacoub P, Mazzaro C, Roccatello D, Scaini P, Sebastiani M, Tavoni A, Zignego AL, De Vita S. Treatment with rituximab in patients with mixed cryoglobulinemia syndrome: results of multicenter cohort study and review of the literature. *Autoimmun Rev* 2011; **11**: 48-55 [PMID: 21821153 DOI: 10.1016/j.autrev.2011.07.005]
  - 74 **Faggioli P**, De Paschale M, Tocci A, Luoni M, Fava S, De Paoli A, Tosi A, Cassi E. Acute hepatic toxicity during cyclic chemotherapy in non Hodgkin's lymphoma. *Haematologica* 1997; **82**: 38-42 [PMID: 9107080]
  - 75 **Takai S**, Tsurumi H, Ando K, Kasahara S, Sawada M, Yamada T, Hara T, Fukuno K, Takahashi T, Oyama M, Onishi H, Tomita E, Takami T, Imawari M, Moriawaki H. Prevalence of hepatitis B and C virus infection in haematological malignancies and liver injury following chemotherapy. *Eur J Haematol* 2005; **74**: 158-165 [PMID: 15654908 DOI: 10.1111/j.1600-0609.2004.00376.x]
  - 76 **Visco C**, Arcaini L, Brusamolino E, Burcheri S, Ambrosetti A, Merli M, Bonoldi E, Chilosi M, Viglio A, Lazzarino M, Pizzolo G, Rodeghiero F. Distinctive natural history in hepatitis C virus positive diffuse large B-cell lymphoma: analysis of 156 patients from northern Italy. *Ann Oncol* 2006; **17**: 1434-1440 [PMID: 16766591 DOI: 10.1093/annonc/mdl131]
  - 77 **Tomita N**, Kodama F, Takabayashi M, Kawano T, Yamaji S, Fujimaki K, Fujisawa S, Kanamori H, Motomura S, Ishigatsubo Y. Clinical features and outcome in HCV-positive aggressive non-Hodgkin's lymphoma. *Leuk Lymphoma* 2003; **44**: 1159-1164 [PMID: 12916868 DOI: 10.1080/1042819031000083055]
  - 78 **Besson C**, Canioni D, Lepage E, Pol S, Morel P, Lederlin P, Van Hoof A, Tilly H, Gaulard P, Coiffier B, Gisselbrecht C, Brousse N, Reyes F, Hermine O. Characteristics and outcome of diffuse large B-cell lymphoma in hepatitis C virus-positive patients in LNH 93 and LNH 98 Groupe d'Etude des Lymphomes de l'Adulte programs. *J Clin Oncol* 2006; **24**: 953-960 [PMID: 16418500 DOI: 10.1200/JCO.2005.01.5016]
  - 79 **Sève P**, Renaudier P, Sascio AJ, Dumontet C, Salles G, Coiffier B, Zoulim F, Broussolle C, Trépo C. Hepatitis C virus infection and B-cell non-Hodgkin's lymphoma: a cross-sectional study in Lyon, France. *Eur J Gastroenterol Hepatol* 2004; **16**: 1361-1365 [PMID: 15618846]
  - 80 **Hermine O**, Lefrère F, Bronowicki JP, Mariette X, Jondeau K, Eclache-Saudreau V, Delmas B, Valensi F, Cacoub P, Brechot C, Varet B, Troussard X. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. *N Engl J Med* 2002; **347**: 89-94 [PMID: 12110736 DOI: 10.1056/NEJMoa013376]
  - 81 **Vallisa D**, Berté R, Rocca A, Civardi G, Giangregorio F, Ferrari B, Sbolli G, Cavanna L. Association between hepatitis C virus and non-Hodgkin's lymphoma, and effects of viral infection on histologic subtype and clinical course. *Am J Med* 1999; **106**: 556-560 [PMID: 10335728 DOI: 10.1016/S0002-9343(99)00069-8]
  - 82 **Kelaidi C**, Rollet F, Park S, Tulliez M, Christoforov B, Calmus Y, Podevin P, Bouscary D, Sogni P, Blanche P, Dreyfus F. Response to antiviral treatment in hepatitis C virus-associated marginal zone lymphomas. *Leukemia* 2004; **18**: 1711-1716 [PMID: 15284859 DOI: 10.1038/sj.leu.2403443]
  - 83 **Saadoun D**, Suarez F, Lefrère F, Valensi F, Mariette X, Aouba A, Besson C, Varet B, Troussard X, Cacoub P, Hermine O. Splenic lymphoma with villous lymphocytes, associated with type II cryoglobulinemia and HCV infection: a new entity? *Blood* 2005; **105**: 74-76 [PMID: 15353484 DOI: 10.1182/blood-2004-05-1711]
  - 84 **Patriarca F**, Silvestri F, Fanin R, Zaja F, Sperotto A, Baccarani M. Long-lasting complete remission of hepatitis C virus (HCV) infection and HCV-associated immunocytoma with alpha-interferon treatment. *Br J Haematol* 2001; **112**: 370-372 [PMID: 11167831 DOI: 10.1046/j.1365-2141.2001.02571.x]
  - 85 **Mazzaro C**, De Re V, Spina M, Dal Maso L, Festini G, Comar C, Tirelli U, Pozzato G. Pegylated-interferon plus ribavirin for HCV-positive indolent non-Hodgkin lymphomas. *Br J Haematol* 2009; **145**: 255-257 [PMID: 19239472 DOI: 10.1111/j.1365-2141.2008.07565.x]
  - 86 **Vallisa D**, Bernuzzi P, Arcaini L, Sacchi S, Callea V, Marasca R, Lazzaro A, Trabacchi E, Anselmi E, Arcari AL, Moroni C, Berté R, Lazzarino M, Cavanna L. Role of anti-hepatitis C virus (HCV) treatment in HCV-related, low-grade, B-cell, non-Hodgkin's



- lymphoma: a multicenter Italian experience. *J Clin Oncol* 2005; **23**: 468-473 [PMID: 15659492 DOI: 10.1200/JCO.2005.06.008]
- 87 **Levine AM**, Shimodaira S, Lai MM. Treatment of HCV-related mantle-cell lymphoma with ribavirin and pegylated interferon Alfa. *N Engl J Med* 2003; **349**: 2078-2079 [PMID: 14627800 DOI: 10.1056/NEJM200311203492121]
- 88 **Pellicelli AM**, Marignani M, Zoli V, Romano M, Morrone A, Nosotti L, Barbaro G, Picardi A, Gentilucci UV, Remotti D, D' Ambrosio C, Furlan C, Mecenate F, Mazzoni E, Majolino I, Villani R, Andreoli A, Barbarini G. Hepatitis C virus-related B cell subtypes in non Hodgkin's lymphoma. *World J Hepatol* 2011; **3**: 278-284 [PMID: 22125661 DOI: 10.4254/wjh.v3.i11.278]
- 89 **Tursi A**, Brandimarte G, Torello M. Disappearance of gastric mucosa-associated lymphoid tissue in hepatitis C virus-positive patients after anti-hepatitis C virus therapy. *J Clin Gastroenterol* 2004; **38**: 360-363 [PMID: 15087696]
- 90 **Rodriguez-Torres M**, Lawitz E, Kowdley KV, Nelson DR, Dejesus E, McHutchison JG, Cornpropst MT, Mader M, Albanis E, Jiang D, Hebnar CM, Symonds WT, Berrey MM, Lalezari J. Sofosbuvir (GS-7977) plus peginterferon/ribavirin in treatment-naïve patients with HCV genotype 1: a randomized, 28-day, dose-ranging trial. *J Hepatol* 2013; **58**: 663-668 [PMID: 23183528 DOI: 10.1016/j.jhep.2012.11.018]
- 91 **Jacobson IM**, Dore GJ, Foster GR, Fried MW, Radu M, Rafalsky VV, Moroz L, Craxi A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Scott J, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2014; **384**: 403-413 [PMID: 24907225 DOI: 10.1016/S0140-6736(14)60494-3]
- 92 **Suzuki F**, Toyota J, Ikeda K, Chayama K, Mochida S, Hayashi N, Ishikawa H, Miyagoshi H, Hu W, McPhee F, Hughes EA, Kumada H. A randomized trial of daclatasvir with peginterferon alfa-2b and ribavirin for HCV genotype 1 infection. *Antivir Ther* 2014; **19**: 491-499 [PMID: 24451122 DOI: 10.3851/IMP2730]
- 93 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]
- 94 **Mazzaro C**, Franzin F, Tulissi P, Pussini E, Crovatto M, Carniello GS, Efremov DG, Burrone O, Santini G, Pozzato G. Regression of monoclonal B-cell expansion in patients affected by mixed cryoglobulinemia responsive to alpha-interferon therapy. *Cancer* 1996; **77**: 2604-2613 [PMID: 8640712]
- 95 **Moccia F**, Tognoni E, Boccaccio P. The relationship between splenic marginal zone B-cell lymphoma and chronic liver disease associated with hepatitis C virus infection. *Ann Ital Med Int* 1999; **14**: 288-293 [PMID: 10638021]
- 96 **Arcaini L**, Paulli M, Boveri E, Vallisa D, Bernuzzi P, Orlandi E, Incardona P, Brusamolino E, Passamonti F, Burcheri S, Schena C, Pascutto C, Cavanna L, Magrini U, Lazzarino M. Splenic and nodal marginal zone lymphomas are indolent disorders at high hepatitis C virus seroprevalence with distinct presenting features but similar morphologic and phenotypic profiles. *Cancer* 2004; **100**: 107-115 [PMID: 14692030 DOI: 10.1002/cncr.11893]
- 97 **Pitini V**, Arrigo C, Righi M, Scaffidi M, Sturniolo G. Systematic screening for HCV infection should be performed in patients with splenic marginal zone lymphoma. *Br J Haematol* 2004; **124**: 252-253 [PMID: 14687039 DOI: 10.1046/j.1365-2141.2003.04751.x]
- 98 **Paulli M**, Arcaini L, Lucioni M, Boveri E, Capello D, Passamonti F, Merli M, Rattotti S, Rossi D, Riboni R, Berti E, Magrini U, Bruno R, Gaidano G, Lazzarino M. Subcutaneous 'lipoma-like' B-cell lymphoma associated with HCV infection: a new presentation of primary extranodal marginal zone B-cell lymphoma of MALT. *Ann Oncol* 2010; **21**: 1189-1195 [PMID: 19858084 DOI: 10.1093/annonc/mdp454]

**P- Reviewer:** Kim SJ, Yamakawa M **S- Editor:** Ji FF

**L- Editor:** A **E- Editor:** Liu SQ





## Hepatitis E virus infection in the liver transplant recipients: Clinical presentation and management

Avin Aggarwal, Ryan B Perumpail, Swetha Tummala, Aijaz Ahmed

Avin Aggarwal, Ryan B Perumpail, Swetha Tummala, Aijaz Ahmed, Division of Gastroenterology and Hepatology, Stanford University School of Medicine, Stanford, CA 94305, United States

Author contributions: All authors contributed to the manuscript.

Conflict-of-interest statement: We declare that we have no conflicts of interest.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Aijaz Ahmed, MD, Associate Professor, Medical Director Liver Transplant Program, Division of Gastroenterology and Hepatology, Stanford University School of Medicine, 750 Welch Road, Suite 210, Palo Alto, Stanford, CA 94304, United States. [aijazahmed@stanford.edu](mailto:aijazahmed@stanford.edu)  
Telephone: +1-650-4986091  
Fax: +1-650-4985692

Received: October 7, 2015

Peer-review started: October 7, 2015

First decision: November 6, 2015

Revised: December 19, 2015

Accepted: January 5, 2016

Article in press: January 7, 2016

Published online: January 18, 2016

and prevalence of HEV infection in this population remains unclear but is certainly greater than historical estimates. Identifying acute HEV infection in this population is imperative for choosing the right course of management as it is very difficult to distinguish histologically from acute rejection on liver biopsy. Current suggested approach to manage acute HEV involves modifying immunosuppression, especially discontinuing calcineurin inhibitors which are the preferred immunosuppressive agents post-orthotopic liver transplantation. The addition of ribavirin monotherapy has shown promising success rates in clearing HEV infection and is used commonly in reported cases.

**Key words:** Chronic hepatitis E infection; Solid organ transplant; Immunosuppression; Ribavirin; Hepatitis E virus; Orthotopic liver transplantation

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatitis E virus (HEV) is an emerging pathogen in developed countries and an important cause of graft hepatitis in the post-orthotopic liver transplantation population that is often misdiagnosed either due to low index of suspicion or due to poor diagnostic assays. We recommend mandatory HEV testing in such cases, and careful treatment with modification of immunosuppression, especially switching from calcineurin inhibitors to a different class. Ribavirin has shown to be increasingly successful in treating HEV infection and preventing graft failure from acute HEV infection, if diagnosed early.

### Abstract

Hepatitis E virus (HEV) is an emerging pathogen and an increasingly recognized cause of graft hepatitis, especially in the post-orthotopic liver transplantation immunocompromised population. The exact incidence

Aggarwal A, Perumpail RB, Tummala S, Ahmed A. Hepatitis E virus infection in the liver transplant recipients: Clinical presentation and management. *World J Hepatol* 2016; 8(2): 117-122 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i2/117.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i2.117>

## INTRODUCTION

Hepatitis E virus (HEV) is one of the major causes of acute viral hepatitis globally. HEV genotypes vary globally in terms of transmission and pathogenicity (Table 1). There have been large scale epidemics of HEV across the low and middle income countries of Asia and Africa as well as sporadic cases in the same geographical regions<sup>[1]</sup>. More recently, HEV has been identified as an emerging pathogen in developed countries as well, particularly among immunosuppressed solid organ transplant recipients.

The overall prevalence varies greatly among existing studies, which are primarily from Europe. In the United States, diagnoses of symptomatic autochthonous HEV infection are very rare compared to the number of cases reported in several European countries. The annual incidence of *de-novo* HEV infection in the United States is reported to be approximately 0.7%<sup>[2]</sup>. However, it is interesting that serological evidence of HEV exposure is more common than expected in a low endemic area like the United States (around 21%)<sup>[3,4]</sup>. In general, HEV seroprevalence was found to be higher in liver transplant recipients, particularly those with liver cirrhosis (7.4% and 32.1%, respectively)<sup>[5]</sup>. Whether cirrhosis is a predisposing factor for HEV or whether HEV infection may play a role in the pathogenesis of cirrhosis, remains controversial.

HEV has been identified as a cause of graft hepatitis in liver transplant recipients. The true frequency and clinical importance of HEV infections after liver transplantation is still unclear<sup>[6]</sup>. A study conducted in France estimated pre-transplant anti-HEV IgG prevalence as 29% increasing regularly with age from 7% in children < 15 years old to 49% for adults > 60 years old<sup>[7]</sup>. On follow-up, the annual incidence of HEV infection post-transplantation was 2.1% in previously seronegative patients, and it was much higher than that those found in other areas of the world. In previously seropositive patients, the annual incidence of post-transplantation re-infections detected by HEV RNA was 3.3%, an incidence similar to that of *de novo* infection<sup>[8]</sup>. Another group in the Netherlands retrospectively estimated HEV prevalence in a cohort of 285 adult liver transplant recipients and found 274 (96.1%) to be negative for all HEV parameters (HEV RNA, IgM/IgG). The prevalence of acquired *de novo* HEV hepatitis in this cohort was 1%-2% after transplantation. Therefore, despite low prevalence, chronic hepatitis E needs to be considered in the differential diagnosis of graft hepatitis<sup>[9,10]</sup>.

## PRESENTATION

HEV in most individuals is known as self-limiting, acute, icteric hepatitis which recovers without sequelae in most cases. Case fatality rates in the general population can vary from 0.1% to 3.0%<sup>[11]</sup>. However, pregnant women often have worse outcomes with more likeli-

hood of progression to fulminant liver failure and a case fatality rate of 10%-20% or higher, especially in developing countries<sup>[12]</sup>. Although the usual outcome of HEV is favorable, in a minority of cases, fulminant liver failure often leads to liver transplantation have been well described, many in non-endemic areas and autochthonous without any evidence of foreign exposure. HEV testing thus should be performed during the initial evaluation of every acute liver failure regardless of epidemiological context<sup>[13,14]</sup>.

## HEV IN SOLID ORGAN TRANSPLANT RECIPIENTS

In patients with chronic liver disease, acute viral hepatitis from HEV can worsen rapidly to a syndrome called acute on chronic liver failure leading to very high mortality (0%-67% with a median of 34%)<sup>[1]</sup>. In immunocompromised individuals, HEV can take up a more chronic course with prolonged viremia<sup>[15]</sup>. Those with solid organ transplant have been studied the most with overall chronic HEV infections reported in up to 50%-60% of organ transplant recipients<sup>[16]</sup>. The chronic HEV infection usually manifests as mild elevation in liver enzymes without clinical signs of overt hepatitis. However rapid fibrosis progression causing cirrhosis within 1-2 years of infection and graft failure is seen in some cases<sup>[9,16,17]</sup>. Prospective study by Kamar *et al.*<sup>[18]</sup> evaluated evolution of liver fibrosis in chronic HEV infected 16 organ transplant patients by sequential liver biopsies. Three out of 16 patients progressed to cirrhosis and two out of these died from decompensated cirrhosis<sup>[18]</sup>. The same group looked at virological and immunological factors associated with viral persistence leading to chronic infection in solid organ transplant (SOT) patients. The patients that had progressive liver fibrosis were found to have less quasispecies diversification during the first year than patients without liver fibrosis progression. This along with a weak inflammatory response [low serum concentrations of interleukin-1 (IL-1) receptor antagonist and soluble IL-2 receptor] and high serum concentrations of the chemokines involved in leukocyte recruitment to the liver in the acute phase were associated with persistent HEV infection<sup>[19]</sup>. HEV related extra-hepatic manifestations like neurological symptoms, kidney injuries and hematological disorders have also been reported<sup>[20]</sup>. Most of chronic HEV infection cases observed belonged to genotype 3, however there are recent reports of genotype 4 infections as well<sup>[21,22]</sup>.

## HIGH INDEX OF SUSPICION

A high index of suspicion is needed in patients with graft hepatitis of unclear etiology since graft failure can result from missed chronic HEV infection. Cases where re-transplantation was done as a last resort have been described<sup>[23]</sup>. Similarly, in allo-hematopoietic stem cell transplant recipients, liver enzyme abnormalities are

**Table 1** Hepatitis E virus genotypic characteristics

Characteristics	Genotype 1	Genotype 2	Genotype 3	Genotype 4
Geographic location	Africa and Asia	Mexico and West Africa	Developed countries	China, Taiwan, Japan
Transmission	Water-borne, fecal oral, person to person	Water-borne, fecal oral, person to person	Food-borne	Food-borne
Group at high risk for infection	Young adults	Young adults	Older adults (> 40 yr) and males. Immuno-compromised persons	Young adults
Zoonotic transmission	No	No	Yes	Yes
Chronic infection	No	No	Yes	Yes
Occurrence of outbreaks	Common	Smaller scale outbreaks	Uncommon	Uncommon

Adapted from centers of disease control and prevention (<http://www.cdc.gov/hepatitis/hev/hevfaq.htm>).

often attributed to hepatic graft vs host disease or drug induced liver injury and possibility of HEV infection is overlooked<sup>[24]</sup>. Presence of anti-HEV antibodies may not protect against re-infection, especially in low concentrations (< 7 World Health Organization units/mL)<sup>[8]</sup>.

A study in France compared SOT recipients who developed chronic HEV infection with those who cleared infection. In general acute aminotransferase levels were higher in those who cleared their infection. Also levels of IgM, IgG anti-HEV antibodies and HEV RNA during acute infection phase were not predictive of whether or not the infection will become chronic. In acute phase itself, only 24% had abnormal bilirubin levels<sup>[25]</sup>. This further emphasizes that an acute HEV infection can be easily missed unless clinician had a high index of suspicion. Now there is increasingly common recognition of HEV and this emerging pathogen is coming within the spectrum of differential diagnosis of US physicians.

## INADEQUACY OF AVAILABLE DIAGNOSTIC ASSAYS

Currently available antibody assays have shown low and variable sensitivity<sup>[26,27]</sup>. In severely immuno-compromised person, anti-HEV IgG detection could be false negative. Comparison of two commercially available assays (Adaltis and Wantai) showed a wide discrepancy in results. Anti-HEV IgG positivity among both assays was wide (10.9% vs 31.3%,  $P = 0.005$ ). On immunoblot, specificity of both assays remained 80%-86%. For anti-HEV IgM testing, both assays were concordant for 97% of the serum samples<sup>[28]</sup>.

Also there was a considerable variability in the accuracy of PCR tests assays used in various studies from Europe from where most of our data regarding HEV infection has been derived<sup>[29]</sup>.

The testing for HEV hence should be done during initial evaluation of graft dysfunction irrespectively since histological appearance on liver biopsy may not clearly distinguish rejection and acute viral hepatitis.

Early diagnosis of HEV should lead to prompt treatment particularly adjusting the immunosuppressive drug regimen as some drugs have been shown to exert opposing effects on HEV replication<sup>[30]</sup>.

## MANAGEMENT

### Modification of immunosuppressive regimen

Immunosuppressive therapy has been proposed to be a key factor for developing chronic hepatitis E in organ transplant recipients<sup>[31]</sup> and is often attributed to diminished antiviral immunity. However, the effect of various immunosuppressive agents on HEV replication is lesser known. Role of steroids is particularly important in the setting of liver transplantation as it is known that steroid boluses used to treat acute rejection in HCV patients can increase the severity of HCV recurrence and viral load. Wang *et al.*<sup>[30]</sup> studied the different immunosuppressants in two HEV replication models. They demonstrated that steroid (prednisone and dexamethasone) did not affect viral replication. It was also demonstrated that calcineurin inhibitors (CsA and FK506) promoted HEV infection. In fact the use of FK506 was found to be the main predictive factor for chronic hepatitis E in organ recipients in another study by Kamar *et al.*<sup>[16,18]</sup>. On the other hand mycophenolic acid/mycophenolate mofetil (MPA/MMF) suppressed viral infection in replica model. The clinical benefit was demonstrated in heart transplant recipients where MMF containing regimens were assumed to play a role in more frequent HEV clearance<sup>[17]</sup>. These were *in vitro* studies and will need further validation with randomized controlled clinical trials. Nevertheless the results provide valuable reference for the management of immunosuppression in these patients.

## ROLE OF PEGYLATED- INTERFERON ALPHA

Pegylated-interferon alpha (Peg-IFN $\alpha$ ) is a strong immune-stimulatory drug that is being already used for the treatment of chronic hepatitis B and C infections. However Peg-IFN $\alpha$  is suggested to induce allogenic immunity, leading to transplant rejection in patients after solid organ transplantation which possibly limits its use in the treatment of chronic hepatitis E.

Successful use of Peg-IFN $\alpha$  therapy for chronic HEV has been reported in a patient with hemodialysis dependent end stage renal disease after failed renal transplant after 3 mo of therapy and achievement of

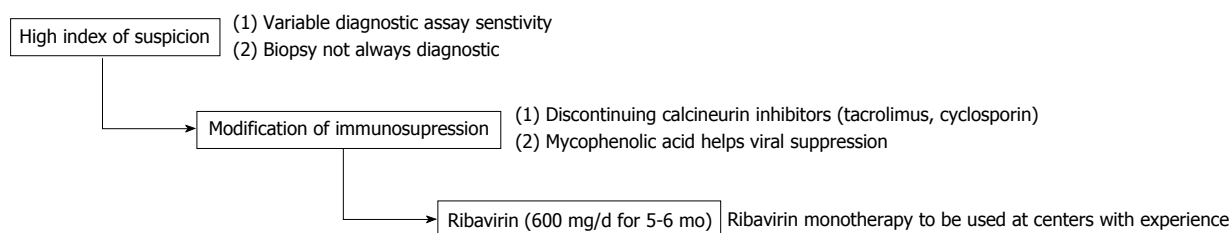


Figure 1 Key steps in the management of hepatitis E virus.

sustained viral response (SVR) at 6 mo<sup>[32]</sup>. The use of Peg-IFN $\alpha$  in post-orthotopic liver transplantation (OLT) patients was successful in achieving SVR, albeit with variable and longer course of therapy<sup>[33,34]</sup>. A recent systemic review found total 8 patients treated with Peg-IFN. SVR was achieved in 6 out of 8 patients (75%) after cessation of therapy, but only 2 out of 8 patients (25%) achieved SVR at or greater than 6 mo. Also 2 patients experienced acute rejection of their transplant organ during treatment<sup>[35]</sup>. This suggests the use of other antiviral agents like ribavirin preferable option, especially in post-OLT chronic HEV.

## ROLE OF RIBAVIRIN

Despite a clear benefit to manipulating immunosuppressive regimens, a substantial proportion of patients are still not able to clear the virus and rapidly progress toward chronic hepatitis<sup>[16]</sup>. Although no proven medication is available, the use of ribavirin monotherapy as an off label drug is gaining acceptance for treating hepatitis E. There is not enough data to recommend treatment with role of ribavirin (RBV) for adult liver transplant recipients, although this has been previously well studied in other SOT populations including lung<sup>[36]</sup>, heart<sup>[17]</sup>, kidney<sup>[37,38]</sup>, and kidney-pancreas<sup>[39]</sup> transplantation. A large retrospective multicenter case series to assess the effects of RBV as monotherapy for SOT was done by Kamar *et al*<sup>[40]</sup>. It included 59 SOT patients (37 kidney, 10 liver, 5 heart, 5 kidney pancreas, and 2 lung) with prolonged HEV viremia. Fifty-four out of 59 had genotyping performed and were HEV genotype 3. Ninety-five percent had HEV clearance with RBV median therapy duration of 3 mo (1-18 mo). SVR measured as undetectable serum HEV RNA at 6 mo after therapy cessation was observed in 46 out of 59 (78%) patients<sup>[40]</sup>. Recently, there have been several case reports of RBV monotherapy for post orthotopic liver transplant, the earliest case reporting SVR-8 following 16 wk therapy<sup>[41]</sup>. Pischke *et al*<sup>[42]</sup> demonstrated successful HEV clearance with RBV monotherapy at 600 mg daily for 5 mo in 11 liver transplant patients.

There have been small number of patients who were non responders to antiviral therapy. One of the identified mutations is G1634R mutation in viral polymerase that was detected in HEV RNA of non-responders. Although there was no resistance to RBV in mutated HEV *in vitro*, but this mutant form of a sub-genomic replicon

of genotype 3 HEV replicated more efficiently *in vitro* than the non-mutant strains. Similar results were seen for infectious virus in competition assays<sup>[43]</sup>. Also, interestingly a higher lymphocyte count at the time of RBV initiation was associated with a greater likelihood of achieving SVR<sup>[40]</sup>.

The exact mode of action of RBV against HEV is not known but successful clearance of both HEV genotype 1 and 3 indicate broad antiviral activity across genotypes<sup>[42]</sup>. However the standard dose and duration of RBV is yet to be determined. Successful outcomes with RBV monotherapy along with tailoring of immunosuppression regimen could provide an acceptable management approach to post OLT HEV. A beneficial effect of combining ribavirin with MPA was seen *in vitro* as well<sup>[30]</sup>.

## CONCLUSION

HEV is an emerging pathogen and an increasingly recognized cause of graft hepatitis especially in the post-OLT immunocompromised population. The exact incidence and prevalence of HEV infection in this population might be unclear but certainly more than historical estimates. Identifying acute HEV infection in this population is imperative for choosing the right course of management as it is very difficult to distinguish histologically from acute rejection on liver biopsy. The current suggested approach to manage acute HEV involves modifying immunosuppression, especially discontinuing calcineurin inhibitors which are the preferred immunosuppressive agents post-OLT. Along with immunosuppression modification, addition of RBV monotherapy has shown promising success rate in clearing HEV infection with current studies suggest using RBV 600 mg/d for a minimum of 5-6 mo successfully with a high SVR rate. We recommend maintaining high index of suspicion and mandatory confirmatory testing for HEV infection in post-OLT hepatitis with careful use of RBV in cases of established diagnosis (Figure 1).

## REFERENCES

- 1 Kumar A, Saraswat VA. Hepatitis E and Acute-on-Chronic Liver Failure. *J Clin Exp Hepatol* 2013; **3**: 225-230 [PMID: 25755504 DOI: 10.1016/j.jceh.2013.08.013]
- 2 Faramawi MF, Johnson E, Chen S, Pannala PR. The incidence of hepatitis E virus infection in the general population of the USA. *Epidemiol Infect* 2011; **139**: 1145-1150 [PMID: 20854712 DOI: 10.1017/S0950268810002177]



- 3 **Kuniholm MH**, Purcell RH, McQuillan GM, Engle RE, Wasley A, Nelson KE. Epidemiology of hepatitis E virus in the United States: results from the Third National Health and Nutrition Examination Survey, 1988-1994. *J Infect Dis* 2009; **200**: 48-56 [PMID: 19473098 DOI: 10.1086/599319]
- 4 **Nelson KE**, Kmush B, Labrique AB. The epidemiology of hepatitis E virus infections in developed countries and among immunocompromised patients. *Expert Rev Anti Infect Ther* 2011; **9**: 1133-1148 [PMID: 22114964 DOI: 10.1586/eri.11.138]
- 5 **Riveiro-Barciela M**, Buti M, Homs M, Campos-Varela I, Cantarell C, Crespo M, Castells L, Tabernero D, Quer J, Esteban R, Rodriguez-Frías F. Cirrhosis, liver transplantation and HIV infection are risk factors associated with hepatitis E virus infection. *PLoS One* 2014; **9**: e103028 [PMID: 25068388 DOI: 10.1371/journal.pone.0103028]
- 6 **Behrendt P**, Steinmann E, Manns MP, Wedemeyer H. The impact of hepatitis E in the liver transplant setting. *J Hepatol* 2014; **61**: 1418-1429 [PMID: 25195557 DOI: 10.1016/j.jhep.2014.08.047]
- 7 **Buffaz C**, Scholtes C, Dron AG, Chevallier-Queyron P, Ritter J, André P, Ramière C. Hepatitis E in liver transplant recipients in the Rhône-Alpes region in France. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 1037-1043 [PMID: 24445407 DOI: 10.1007/s10096-013-2042-2]
- 8 **Abravanel F**, Lhomme S, Chapuy-Regaud S, Mansuy JM, Muscari F, Sallusto F, Rostaing L, Kamar N, Izopet J. Hepatitis E virus reinfections in solid-organ-transplant recipients can evolve into chronic infections. *J Infect Dis* 2014; **209**: 1900-1906 [PMID: 24436450 DOI: 10.1093/infdis/jiu032]
- 9 **Pischke S**, Suneetha PV, Baechlein C, Barg-Hock H, Heim A, Kamar N, Schlue J, Strassburg CP, Lehner F, Raupach R, Bremer B, Magerstedt P, Cornberg M, Seehusen F, Baumgaertner W, Klempnauer J, Izopet J, Manns MP, Grummer B, Wedemeyer H. Hepatitis E virus infection as a cause of graft hepatitis in liver transplant recipients. *Liver Transpl* 2010; **16**: 74-82 [PMID: 19866448 DOI: 10.1002/lt.21958]
- 10 **Haagsma EB**, Niesters HG, van den Berg AP, Riezebos-Brilman A, Porte RJ, Vennema H, Reimerink JH, Koopmans MP. Prevalence of hepatitis E virus infection in liver transplant recipients. *Liver Transpl* 2009; **15**: 1225-1228 [PMID: 19790147 DOI: 10.1002/lt.21819]
- 11 **Mushahwar IK**. Hepatitis E virus: molecular virology, clinical features, diagnosis, transmission, epidemiology, and prevention. *J Med Virol* 2008; **80**: 646-658 [PMID: 18297720 DOI: 10.1002/jmv.21116]
- 12 **Navaneethan U**, Al Mohajer M, Shata MT. Hepatitis E and pregnancy: understanding the pathogenesis. *Liver Int* 2008; **28**: 1190-1199 [PMID: 18662274 DOI: 10.1111/j.1478-3231.2008.01840.x]
- 13 **Ohnishi S**, Kang JH, Maekubo H, Takahashi K, Mishihiro S. A case report: two patients with fulminant hepatitis E in Hokkaido, Japan. *Hepatol Res* 2003; **25**: 213-218 [PMID: 12644058 DOI: 10.1016/S1386-6346(03)00009-3]
- 14 **Aherfi S**, Borentain P, Raissouni F, Le Goffic A, Guisset M, Renou C, Grimaud JC, Hardwigen J, Garcia S, Botta-Fridlund D, Nafati C, Motte A, Le Treut YP, Colson P, Gerolami R. Liver transplantation for acute liver failure related to autochthonous genotype 3 hepatitis E virus infection. *Clin Res Hepatol Gastroenterol* 2014; **38**: 24-31 [PMID: 24462173 DOI: 10.1016/j.clinre.2013.05.013]
- 15 **Wedemeyer H**, Pischke S, Manns MP. Pathogenesis and treatment of hepatitis e virus infection. *Gastroenterology* 2012; **142**: 1388-1397.e1 [PMID: 22537448]
- 16 **Kamar N**, Garrouste C, Haagsma EB, Garrigue V, Pischke S, Chauvet C, Dumortier J, Cannesson A, Cassuto-Viguier E, Thervet E, Conti F, Lebray P, Dalton HR, Santella R, Kanaan N, Essig M, Mousson C, Radenne S, Roque-Afonso AM, Izopet J, Rostaing L. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology* 2011; **140**: 1481-1489 [PMID: 21354150 DOI: 10.1053/j.gastro.2011.02.050]
- 17 **Pischke S**, Stiefel P, Franz B, Bremer B, Suneetha PV, Heim A, Ganzenmueller T, Schlue J, Horn-Wichmann R, Raupach R, Darnedde M, Scheibner Y, Taubert R, Haverich A, Manns MP, Wedemeyer H, Bara CL. Chronic hepatitis e in heart transplant recipients. *Am J Transplant* 2012; **12**: 3128-3133 [PMID: 22823202 DOI: 10.1111/j.1600-6143.2012.04200.x]
- 18 **Kamar N**, Abravanel F, Selves J, Garrouste C, Esposito L, Lavyssière L, Cointault O, Ribes D, Cardeau I, Nogier MB, Mansuy JM, Muscari F, Peron JM, Izopet J, Rostaing L. Influence of immunosuppressive therapy on the natural history of genotype 3 hepatitis-E virus infection after organ transplantation. *Transplantation* 2010; **89**: 353-360 [PMID: 20145528 DOI: 10.1097/TP.0b013e3181c4096c]
- 19 **Lhomme S**, Abravanel F, Dubois M, Sandres-Saune K, Rostaing L, Kamar N, Izopet J. Hepatitis E virus quasiespecies and the outcome of acute hepatitis E in solid-organ transplant patients. *J Virol* 2012; **86**: 10006-10014 [PMID: 22761386 DOI: 10.1128/JVI.01003-12]
- 20 **Kamar N**, Rostaing L, Izopet J. Hepatitis E virus infection in immunosuppressed patients: natural history and therapy. *Semin Liver Dis* 2013; **33**: 62-70 [PMID: 23564390 DOI: 10.1055/s-0033-1338115]
- 21 **Geng Y**, Zhang H, Huang W, J Harrison T, Geng K, Li Z, Wang Y. Persistent hepatitis e virus genotype 4 infection in a child with acute lymphoblastic leukemia. *Hepat Mon* 2014; **14**: e15618 [PMID: 24596581]
- 22 **Perumpail RB**, Ahmed A, Higgins JP, So SK, Cochran JL, Drobeniuc J, Mixson-Hayden TR, Teo CG. Fatal Accelerated Cirrhosis after Imported HEV Genotype 4 Infection. *Emerg Infect Dis* 2015; **21**: 1679-1681 [PMID: 26291424 DOI: 10.3201/eid2109.150300]
- 23 **Liu X**, Shen T, Wang Z, Zhuang L, Zhang W, Yu J, Wu J, Zheng S. Hepatitis E virus infection results in acute graft failure after liver transplantation: a case report. *J Infect Dev Ctries* 2014; **8**: 245-248 [PMID: 24518638 DOI: 10.3855/jidc.3638]
- 24 **van der Eijk AA**, Pas SD, Cornelissen JJ, de Man RA. Hepatitis E virus infection in hematopoietic stem cell transplant recipients. *Curr Opin Infect Dis* 2014; **27**: 309-315 [PMID: 24977683 DOI: 10.1097/QCO.0000000000000076]
- 25 **Legrand-Abravanel F**, Kamar N, Sandres-Saune K, Garrouste C, Dubois M, Mansuy JM, Muscari F, Sallusto F, Rostaing L, Izopet J. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. *J Infect Dis* 2010; **202**: 835-844 [PMID: 20695798 DOI: 10.1086/655899]
- 26 **Bendall R**, Ellis V, Ijaz S, Ali R, Dalton H. A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. *J Med Virol* 2010; **82**: 799-805 [PMID: 20336757 DOI: 10.1002/jmv.21656]
- 27 **Reuter S**, Oette M, Wilhelm FC, Beggel B, Kaiser R, Balduin M, Schweitzer F, Verheyen J, Adams O, Lengauer T, Fätkenheuer G, Pfister H, Häussinger D. Prevalence and characteristics of hepatitis B and C virus infections in treatment-naïve HIV-infected patients. *Med Microbiol Immunol* 2011; **200**: 39-49 [PMID: 20853118 DOI: 10.1007/s00430-010-0172-z]
- 28 **Rossi-Tamisier M**, Moal V, Gerolami R, Colson P. Discrepancy between anti-hepatitis E virus immunoglobulin G prevalence assessed by two assays in kidney and liver transplant recipients. *J Clin Virol* 2013; **56**: 62-64 [PMID: 23089569 DOI: 10.1016/j.jcv.2012.09.010]
- 29 **Baylis SA**, Hanschmann KM, Blümel J, Nübling CM. Standardization of hepatitis E virus (HEV) nucleic acid amplification technique-based assays: an initial study to evaluate a panel of HEV strains and investigate laboratory performance. *J Clin Microbiol* 2011; **49**: 1234-1239 [PMID: 21307208 DOI: 10.1128/JCM.02578-10]
- 30 **Wang Y**, Zhou X, Debing Y, Chen K, Van Der Laan LJ, Neyts J, Janssen HL, Metselaar HJ, Peppelenbosch MP, Pan Q. Calcineurin inhibitors stimulate and mycophenolic acid inhibits replication of hepatitis E virus. *Gastroenterology* 2014; **146**: 1775-1783 [PMID: 24582714 DOI: 10.1053/j.gastro.2014.02.036]
- 31 **Kamar N**, Selves J, Mansuy JM, Ouezzani L, Péron JM, Guitard J, Cointault O, Esposito L, Abravanel F, Danjoux M, Durand D, Vinel

- JP, Izopet J, Rostaing L. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med* 2008; **358**: 811-817 [PMID: 18287603 DOI: 10.1056/NEJMoa0706992]
- 32 **Kamar N**, Abravanel F, Garrouste C, Cardeau-Desangles I, Mansuy JM, Weclawiak H, Izopet J, Rostaing L. Three-month pegylated interferon-alpha-2a therapy for chronic hepatitis E virus infection in a haemodialysis patient. *Nephrol Dial Transplant* 2010; **25**: 2792-2795 [PMID: 20494897 DOI: 10.1093/ndt/gfq282]
- 33 **Haagsma EB**, Riezebos-Brilman A, van den Berg AP, Porte RJ, Niesters HG. Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon alpha-2b. *Liver Transpl* 2010; **16**: 474-477 [PMID: 20373458 DOI: 10.1002/lt.22014]
- 34 **Kamar N**, Rostaing L, Abravanel F, Garrouste C, Esposito L, Cardeau-Desangles I, Mansuy JM, Selves J, Peron JM, Ota P, Muscari F, Izopet J. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. *Clin Infect Dis* 2010; **50**: e30-e33 [PMID: 20113176 DOI: 10.1086/650488]
- 35 **Peters van Ton AM**, Gevers TJ, Drenth JP. Antiviral therapy in chronic hepatitis E: a systematic review. *J Viral Hepat* 2015; **22**: 965-973 [PMID: 25760481 DOI: 10.1111/jvh.12403]
- 36 **Riezebos-Brilman A**, Puchhammer-Stöckl E, van der Weide HY, Haagsma EB, Jaksch P, Bejvl I, Niesters HG, Verschuuren EA. Chronic hepatitis E infection in lung transplant recipients. *J Heart Lung Transplant* 2013; **32**: 341-346 [PMID: 23415316 DOI: 10.1016/j.healun.2012.11.027]
- 37 **Moal V**, Motte A, Kaba M, Gerolami R, Berland Y, Colson P. Hepatitis E virus serological testing in kidney transplant recipients with elevated liver enzymes in 2007-2011 in southeastern France. *Diagn Microbiol Infect Dis* 2013; **76**: 116-118 [PMID: 23608351 DOI: 10.1016/j.diagmicrobio.2013.02.017]
- 38 **de Niet A**, Zaaijer HL, ten Berge I, Weegink CJ, Reesink HW, Beuers U. Chronic hepatitis E after solid organ transplantation. *Neth J Med* 2012; **70**: 261-266 [PMID: 22859417]
- 39 **Mallet V**, Nicand E, Sultanik P, Chakvetadze C, Tessé S, Thervet E, Mouthon L, Sogni P, Pol S. Brief communication: case reports of ribavirin treatment for chronic hepatitis E. *Ann Intern Med* 2010; **153**: 85-89 [PMID: 20547886 DOI: 10.7326/0003-4819-153-2-201007200-00257]
- 40 **Kamar N**, Izopet J, Tripon S, Bismuth M, Hillaire S, Dumortier J, Radenne S, Coilly A, Garrigue V, D'Alteroche L, Buchler M, Couzi L, Lebray P, Dharancy S, Minello A, Hourmant M, Roque-Afonso AM, Abravanel F, Pol S, Rostaing L, Mallet V. Ribavirin for chronic hepatitis E virus infection in transplant recipients. *N Engl J Med* 2014; **370**: 1111-1120 [PMID: 24645943 DOI: 10.1056/NEJMoa1215246]
- 41 **Klein F**, Neuhaus R, Hofmann J, Rudolph B, Neuhaus P, Bahra M. Successful Treatment of Chronic Hepatitis E After an Orthotopic Liver Transplant With Ribavirin Monotherapy. *Exp Clin Transplant* 2015; **13**: 283-286 [PMID: 24779678]
- 42 **Pischke S**, Hardtke S, Bode U, Birkner S, Chatzikyrkou C, Kauffmann W, Bara CL, Gottlieb J, Wenzel J, Manns MP, Wedemeyer H. Ribavirin treatment of acute and chronic hepatitis E: a single-centre experience. *Liver Int* 2013; **33**: 722-726 [PMID: 23489973 DOI: 10.1111/liv.12114]
- 43 **Debing Y**, Gisa A, Dallmeier K, Pischke S, Bremer B, Manns M, Wedemeyer H, Suneetha PV, Neyts J. A mutation in the hepatitis E virus RNA polymerase promotes its replication and associates with ribavirin treatment failure in organ transplant recipients. *Gastroenterology* 2014; **147**: 1008-1011.e7; quiz e15-16 [PMID: 25181691]

**P- Reviewer:** Ajith TA, Arias J, Chiang TA, Sazci A, Wang K, Waisberg J, Zhang XC

**S- Editor:** Qi Y **L- Editor:** A **E- Editor:** Liu SQ



## Ribavirin: Past, present and future

Véronique Loustaud-Ratti, Marilyne Debette-Gratien, Jérémie Jacques, Sophie Alain, Pierre Marquet, Denis Sautereau, Annick Rousseau, Paul Carrier

Véronique Loustaud-Ratti, Marilyne Debette-Gratien, Jérémie Jacques, Denis Sautereau, Paul Carrier, Fédération Hépatologie, Service d'Hépatogastroentérologie, CHU Limoges, 87042 Limoges, France

Véronique Loustaud-Ratti, Marilyne Debette-Gratien, Pierre Marquet, Annick Rousseau, Paul Carrier, Université de Limoges, UMR 850 INSERM, 87025 Limoges, France

Sophie Alain, Service de Bactériologie Virologie, CHU Limoges, 87042 Limoges, France

Sophie Alain, U1092 INSERM, Université de Limoges, CHU Limoges, 87042 Limoges, France

Pierre Marquet, Service de Pharmacologie, CHU Limoges, 87042 Limoges, France

**Author contributions:** Loustaud-Ratti V and Carrier P wrote the manuscript; Debette-Gratien M, Jacques J, Alain S, Marquet P, Sautereau D and Rousseau A read the manuscript and conducted a critical analysis.

**Conflict-of-interest statement:** The authors have no conflict of interest concerning this work.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Véronique Loustaud-Ratti, Professor, Fédération Hépatologie, Service d'Hépatogastroentérologie, CHU Limoges, 2 Avenue Martin Luther King, 87042 Limoges, France. [veronique.loustaud-ratti@unilim.fr](mailto:veronique.loustaud-ratti@unilim.fr)  
 Telephone: +33-5-55058484  
 Fax: +33-5-55056767

Received: September 2, 2015

Peer-review started: September 5, 2015

First decision: October 16, 2015

Revised: November 6, 2015

Accepted: December 29, 2015

Article in press: January 4, 2016

Published online: January 18, 2016

### Abstract

Before the advent of direct acting antiviral agents (DAAs) ribavirin, associated to pegylated-interferon played a crucial role in the treatment of chronic hepatitis C, preventing relapses and breakthroughs. In the present era of new potent DAAs, a place is still devoted to the drug. Ribavirin associated with sofosbuvir alone is efficient in the treatment of most cases of G2 infected patients. All options currently available for the last difficult-to-treat cirrhotic G3 patients contain ribavirin. Reducing treatment duration to 12 wk in G1 or G4 cirrhotic compensated patients is feasible thanks to ribavirin. Retreating patients with acquired anti NS5A resistance-associated variants using ribavirin-based strategies could be useful. The addition of ribavirin with DAAs combinations however, leads to more frequent but mild adverse events especially in cirrhotic patients. Preliminary data with interferon-free second generation DAAs combinations without ribavirin suggest that future of the drug is jeopardized even in difficult-to-treat patients: The optimization of ribavirin dosage according to an early monitoring of blood levels has been suggested to be relevant in double therapy with peginterferon or sofosbuvir but not with very potent combinations of more than two DAAs.

**Key words:** Ribavirin; Hepatitis C; Peginterferon; Direct acting antiviral agents

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Ribavirin plays a crucial role when associated with peginterferon, preventing relapses and

breakthroughs and doubling the support vector regression rate. Its antiviral effect is weak and ribavirin could enhance the response of interferon-stimulated genes in the combination. Ribavirin is still useful in the era of approved new direct acting antiviral agents (DAAs), in order to shorter treatment duration in genotype 1 or 4 cirrhotic patients, in all options available for genotype 3 cirrhotic patients, and as the only drug associated with sofosbuvir in genotype 2. Preliminary data with interferon-free second generation DAAs combinations without ribavirin suggest that future of the drug is jeopardized.

Loustaud-Ratti V, Debette-Gratien M, Jacques J, Alain S, Marquet P, Sautereau D, Rousseau A, Carrier P. Ribavirin: Past, present and future. *World J Hepatol* 2016; 8(2): 123-130 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i2/123.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i2.123>

## INTRODUCTION

Before the advent of direct acting antiviral agents (DAAs) ribavirin played a crucial role in the treatment of chronic hepatitis C associated to pegylated interferon<sup>[1]</sup>. This combination is still relevant in many parts of the world which do not have access to new therapies because of cost issues<sup>[2]</sup>. Although the role of ribavirin in the era of DAAs will probably decrease in the future with the arrival of second generation drugs, it remains essential in strategies decreasing treatment duration or in some difficult situations. The goal of this review is to briefly recall the recent past of ribavirin and consider its present and potential future.

## RIBAVIRIN: MECHANISMS OF ACTION

So far, multiple mechanisms of action of ribavirin have been described. The antiviral mechanism is probably the best documented, the erroneous incorporation of ribavirin triphosphate into replicating RNA strands inhibiting chain elongation<sup>[3]</sup>. *In vitro*, in the hepatitis C virus (HCV) RNA replication system, ribavirin reduces HCV replicon colony-forming efficiency in a dose-dependent manner, reinforcing this hypothesis<sup>[4]</sup>. The inhibition *via* inosine monophosphate dehydrogenase of the *de novo* synthesis of GTP, required for the synthesis of viral RNA, is another but probably weak potential mechanism of action<sup>[5]</sup>. However, the mutagenesis hypothesis remains controversial<sup>[6]</sup>. The last most attractive mechanism of action is that ribavirin could enhance the response of interferon-stimulated genes making cells more sensitive to exogenous interferon and increasing the production of endogenous interferon<sup>[7]</sup>.

Two phases of plasma HCV RNA decline in patients treated with peginterferon and ribavirin have been described: A rapid first phase in the first two days<sup>[8]</sup> reflecting the genesis and release of new virions and a slower second phase corresponding to the elimination

of infected cells. The impact of the first-phase decline is weak (0.5 log) and goes unnoticed during double therapy, but is enhanced in patients treated with ribavirin alone<sup>[9]</sup>. The second slope probably reflects the interferon-stimulated genes' response and the production of endogenous interferon.

Multiscale models recently considered the possible effects of DAAs on intracellular HCV RNA production, degradation, assembly and secretion as virus into the circulation<sup>[10]</sup>. The first-phase decline represents the viral clearance. The second represents the loss of intracellular viral RNA by export and degradation as well as the elimination of infected cells. The third represents a combination of the reduction in intracellular viral RNA production and the elimination of infected cells. Nowadays, there are no data available on the role of ribavirin in this setting, but we may imagine that ribavirin might impact the second- and the third-phase decline.

## PAST OF RIBAVIRIN: COMBINATION THERAPY PEGINTERFERON AND RIBAVIRIN

### Clinical history

Ribavirin, a guanosine analog is active against many DNA and RNA viruses and has clinical applications in respiratory syncytial infection in children, and Lassa Fever infection<sup>[11,12]</sup>. Di Bisceglie *et al*<sup>[13]</sup> first showed that ribavirin could double the efficiency of standard alfa interferon. A similar synergy was observed with the association of peginterferon and ribavirin<sup>[14,15]</sup>, ribavirin impacting favourably the number of relapses and breakthroughs<sup>[16]</sup>. A total daily dose of ribavirin during the first three months > 10.6 mg/kg of body weight was predictive of sustained virological response (SVR)<sup>[14,17]</sup> and ribavirin had to be administered for the total duration of treatment<sup>[16]</sup>. A pilot study also showed, that the use of high doses of ribavirin early during treatment led to high sustained virological rates<sup>[18]</sup>. The same team proposed to optimize the dose of ribavirin using a formula based on renal function and body weight<sup>[19]</sup>.

### Pharmacokinetics of ribavirin

Ribavirin is a drug typically adapted for therapeutic drug monitoring: Long half-life, large inter-individual variability of the dose-concentration relationship, and narrow therapeutic zone. After the first oral dose, a rapid absorption phase is observed with a maximum concentration at 1.5 h, followed by a rapid distribution phase (half-life of 3.7 h), and a long elimination phase of about 100 h post-dose<sup>[20]</sup>. The monitoring of ribavirin plasma concentrations during double therapy initially used trough concentrations at week 4 and week 8 of treatment<sup>[21,22]</sup>. However, trough concentrations had a lower influence than the genotype and the viral load on SVR<sup>[21]</sup>.

We secondly showed that ribavirin plasma exposure



after the first dose [*i.e.*, measured by the interdose area under the concentration curve, area under the curve (AUC<sub>0-12h</sub>) or abbreviated AUC<sub>0-4h</sub>] was strongly linked to SVR and was probably a more relevant tool<sup>[23]</sup>. Using receiver operating characteristic curve analysis, we defined an AUC<sub>0-4h</sub> threshold of 1755 µg/h per litre at day 0 as a target for ribavirin early dose adjustment, AUC<sub>0-4h</sub> being estimated using 3 blood samples (0.5, 1 and 2 h after the first dose) and Bayesian estimation. When comparing adapted and non-adapted patients with a suboptimal exposure to ribavirin at day 0 (*i.e.*, D0 AUC<sub>0-4h</sub> < 1755 µg/h per litre), the difference of SVR reached nearly 30%, enhancing the benefit of adapted dose in this population (unpublished results).

#### ***Ribavirin and anemia during peginterferon and ribavirin treatment***

Ribavirin-induced haemolytic anaemia is a frequent adverse event leading to drug discontinuation in 36% of the cases in real-life studies<sup>[24]</sup>, even if this anemia is reversible and dose-dependent. Medullar regeneration is partially prevented by various degrees of bone marrow suppression due to interferon impact. The prevalence of anaemia is high, with Hb level < 11 g/dL in 30% and < 10 g/dL in 9% to 13% of the patients<sup>[14,15]</sup> with 10% to 15% of the patients presenting with an Hb decline of more than 5 g/dL. Erythropoietin has been shown to improve the ribavirin treatment maintenance and tolerance<sup>[25,26]</sup> but did not prove its impact on SVR.

## **PRESENT OF RIBAVIRIN: TREATMENT WITH NEW DAAS**

Interferon-free regimens DAAs currently approved by FDA and EMEA are used in combinations: Pangenotypic polymerase inhibitor sofosbuvir (Sovaldi®) associated with NS5A inhibitors ledipasvir (associated with sofosbuvir: Harvoni®) or daclatasvir (Daklinza®) (genotype 1, 3, 4), or with a protease inhibitor simeprevir (Olysio®) (genotype 1, 4); triple combination paritaprevir boosted with ritonavir (protease inhibitor), ombitasvir (NS5a inhibitor) (Viekirax®) and quadruple combination of paritaprevir, ritonavir, ombitasvir and dasabuvir (Exviera®) a polymerase inhibitor are also available for genotype 1, 4 patients.

Most of the time, these regimens give more than 90% SVR rate without the addition of ribavirin. However, ribavirin is still relevant in some circumstances.

#### ***Ribavirin and sofosbuvir alone are efficient in the treatment of most cases of G2 infected patients***

Sofosbuvir and ribavirin combination is recommended in both European Association for the Study of the Liver (EASL) and French guidelines in G2 patients for 12 wk mainly<sup>[27]</sup> except for cirrhotic experienced-patients (24 wk)<sup>[28]</sup>. In this particular population, the only way to reduce treatment duration to 12 wk with similar SVR (95% to 100%) is to add peginterferon<sup>[29,30]</sup>.

#### ***Nowadays, ribavirin remains essential for the last difficult-to-treat cirrhotic G3 patients***

HCV G3 patients were first treated with sofosbuvir and ribavirin for 24 wk in phase III trials; response rates were 91% in patients without cirrhosis and only 68% in patients with cirrhosis, respectively<sup>[27]</sup>. Recently, the Boson study showed the potential superiority of a peginterferon sofosbuvir and ribavirin regimen for 12 wk with a 91% to 86% SVR in naive and pre-treated cirrhotic patients respectively<sup>[30]</sup>. Another strategy using sofosbuvir daclatasvir without ribavirin for 12 wk in G3 cirrhotic patients led to a weak 63% rate of SVR<sup>[31]</sup>. Results of the French initial authorization for new DAAs are in favour of a 24-wk treatment but the sofosbuvir daclatasvir and ribavirin strategy for 12 wk was not available<sup>[32]</sup>. This option could be a pertinent alternative to the 24-wk sofosbuvir daclatasvir association. Currently, EASL and French expert advices recommend treating patients with sofosbuvir daclatasvir for 24 wk, in the absence of the results of a new trial evaluating sofosbuvir daclatasvir ribavirin for 12 wk.

To sum up, all options currently available for cirrhotic G3 patients contain ribavirin and we have to wait for the results of new associations like sofosbuvir and the pangenotypic GS 5816 (astral 3 waiting results) or more sophisticated triple strategies like grazoprevir elbasvir and sofosbuvir<sup>[33]</sup>.

#### ***Ribavirin is still necessary for G1a patients treated with ritonavir-boosted paritaprevir, ombitasvir and dasabuvir***

The approval of the triple combination of ritonavir-boosted paritaprevir, ombitasvir and dasabuvir in patients infected with G1 was supported by six phase III clinical trials. In PEARL-IV, in patients infected with subtype 1a, the SVR rates were 97% and 90% with and without ribavirin respectively, suggesting that, unlike for G1b, ribavirin is needed in the 12-wk regimen for this subtype<sup>[34]</sup>. Moreover, considering treatment-experienced cirrhotic patients with subtype 1a infection a 24 wk-treatment duration with ribavirin was needed<sup>[35]</sup>.

#### ***Reducing treatment duration to 12 wk in G1 or G4 cirrhotic patients is feasible thanks to ribavirin***

##### **In compensated and decompensated cirrhosis:**

Recent data suggest that the addition of ribavirin allows the treatment duration to be limited to 12 wk in patients with advanced liver disease, including patients with compensated cirrhosis (especially if they are treatment-experienced), patients with decompensated cirrhosis and subjects in pre- and post-liver transplant setting.

#### ***Twelve weeks with ribavirin or 24 wk without ribavirin are equivalent in compensated cirrhosis:***

In the Sirius study<sup>[36]</sup>, ledipasvir-sofosbuvir plus ribavirin for 12 wk and ledipasvir-sofosbuvir for 24 wk provided similar high SVR12 rates in previous non-responders with HCV G1 and compensated cirrhosis. The shorter regimen, when given with ribavirin, might, therefore,

be useful to treat experienced patients with cirrhosis in case of no contra-indications to ribavirin.

Of note, in cirrhotic pre-treated patients with platelet count  $< 75000/\text{mm}^3$ , the SVR rate is suboptimal (84%)<sup>[37]</sup> and EASL guidelines recommend to extend the ribavirin-associated regimen to 24 wk in this subgroup.

In a post-hoc analysis of data from seven clinical trials which evaluated the efficacy and safety of the fixed-dose combination of ledipasvir and sofosbuvir, with and without ribavirin in 513 treatment-naïve and previously treated patients with G1 HCV compensated cirrhosis, Reddy *et al*<sup>[37]</sup> suggested the usefulness of ribavirin in the subpopulation of treatment-experienced patients receiving 12 wk of treatment (SVR12 rate of 90% vs 96% with ribavirin).

Finally in the hepather cohort<sup>[38]</sup>, difficult-to treat G1 (88% cirrhotics) patients receiving sofosbuvir daclatasvir and ribavirin achieved a SVR4 of 100% not different from sofosbuvir daclatasvir for 24 wk (SVR4 95%). In a multivariate analysis, factors associated with SVR in cirrhotics were the addition of ribavirin (OR = 6.3;  $P = 0.057$ ) and a treatment-duration of 24 wk (OR = 4.3;  $P = 0.008$ ).

Similarly, results from the same cohort study showed a benefit in the pre-treated cirrhotic population infected with G4 and receiving sofosbuvir daclatasvir or sofosbuvir simeprevir, with ribavirin<sup>[39]</sup>.

**Same results are observed in decompensated cirrhosis in the pre and post-transplant setting except for Child Pugh C patients:** The association of sofosbuvir ledipasvir and ribavirin for 12 wk in the pre and post transplant setting led to more than 85% to 95% SVR in cirrhotic patients<sup>[40,41]</sup>. However, in one study, the response rate was much lower (under 60%) in Child Pugh C patients suggesting a prolongation of treatment course to 24 wk<sup>[42]</sup>.

#### ***In non-cirrhotic G1 patients, ribavirin does not help to reduce treatment duration under 8 wk***

Among previously untreated patients with HCV G1 infection and without cirrhosis in the phase III ION 3 study, the 8-wk ledipasvir-sofosbuvir regimen showed no inferiority to the 12-wk regimen<sup>[43]</sup>. One interesting hypothesis could have been to further reduce the treatment duration by adding ribavirin to the combination.

However, in the electron study, among treatment-naïve patients receiving 6 wk of sofosbuvir, ledipasvir and ribavirin, only 17 of 25 (68%) achieved an SVR12. The addition of ribavirin in this setting does not seem to be an appropriate strategy<sup>[44]</sup>.

#### ***Retreating patients with acquired anti NS5A resistance-associated variants using ribavirin-based strategies could be useful***

In Reddy's study<sup>[37]</sup>, 91% of G1 cirrhotic patients with NS5A resistance-associated variants (RAVs) at baseline and treated with sofosbuvir-ledipasvir achieved SVR12 (95%CI: 84-96), as compared with 98% (407 of 417)

of those without baseline NS5A RAVs (95%CI: 96-99). This difference appeared to be mitigated by the addition of ribavirin to the regimen (88% of SVR without vs 94% with ribavirin).

#### ***The addition of ribavirin with DAAs combinations leads to more frequent but mild adverse events***

In the main studies comparing interferon-free DAAs combinations with or without ribavirin for 12 wk, adverse events (AEs) were significantly higher (about 10%) when ribavirin was included in the strategy: Particularly fatigue, insomnia, pruritus, cough and of course all grades of anemia but only 5% of grade 3 and 4. Treatment discontinuation due to AEs (4%) was slightly more frequent. However, these AEs were not significantly higher in compensated cirrhotic patients when a 12-wk regimen with ribavirin was compared to a 24-wk regimen without ribavirin<sup>[36]</sup>. Erythropoietin (EPO) was not used except in advanced cirrhotic disease and reduction of ribavirin dosage (9%) was most of the time sufficient with no impact on SVR<sup>[45]</sup>.

Of course, these AEs were more tolerable than in regimens including interferon, and even more than in triple therapy with first generation protease inhibitors.

#### ***There is probably no more place for ribavirin dose adjustment during treatment with DAAs***

In the NIAID SPARE trial, Rower *et al*<sup>[46]</sup>, showed that ribavirin-monophosphate concentrations in red blood cells at day 14 were related to anaemia and SVR. A therapeutic range was identified for ribavirin-monophosphate in persons with HCV G1 disease receiving 24 wk of sofosbuvir plus ribavirin, suggesting a potential pharmacological basis for individualized ribavirin dosing in this interferon-free regimen. However, Jacobson *et al*<sup>[45]</sup> showed in cirrhotic G1 patients, that ribavirin dose reduction due to anemia in the triple Abbvie combination (10% of the cohort) did not impact the SVR. One may hypothesize that the monitoring of ribavirin dose in G1 patients will not be useful when using at least two very potent new DAAs, unlike what was observed with the association of peginterferon and ribavirin or sofosbuvir and ribavirin.

## **FUTURE**

#### ***Preliminary data with second generation interferon-free DAAs combinations without ribavirin suggest that ribavirin future is jeopardized even in difficult-to-treat patients***

**New double combinations:** Grazoprevir elbasvir without ribavirin for 12 wk is efficient in difficult-to-treat G1 and G4 patients. In a phase II study (C-Worthy), high SVR12 rates were achieved irrespective of the use of ribavirin or of the extension of treatment duration from 12 to 18 wk in two cohorts of G1 patients, *i.e.*, cohort 1, naïve cirrhotic patients and cohort 2 previous null responders with or without cirrhosis. The SVR rate without ribavirin was 97% and 91% in the two

cohorts respectively<sup>[29]</sup>. In the Edge study, considering G1 and 4 patients (35% cirrhosis), the association of grazoprevir elbasvir gave similar results with and without ribavirin for a 12- or 16-wk duration (92% to 97%). Interestingly however, SVR rates were higher for the 16 wk + ribavirin arm regardless the status of the patient, the presence of cirrhosis and the presence of NS5A mutation (97%)<sup>[47]</sup> suggesting a small residual role of ribavirin. In a phase II preliminary study, the same combination without ribavirin was effective and well tolerated in G1 Child B-cirrhotic patients<sup>[48]</sup> leading to a 90% SVR. The combination of grazoprevir and elbasvir was useless or suboptimal for G3 and G2 patients respectively even with the addition of ribavirin and G5 patients, interestingly, still needed ribavirin<sup>[49,50]</sup>.

The sofosbuvir GS-5816 (pangenotypic NS5a inhibitor) combination without ribavirin was clearly efficient in G3 non cirrhotic patients (100% SVR) and more efficient than other previous combinations in experienced cirrhotic patients (88%). However in the latter case, the addition of ribavirin seemed to bring a mild benefit (96% of SVR)<sup>[51]</sup>.

#### Multiple DAAs combinations without ribavirin in difficult-to-treat patients:

In G1 naive or pre-treated cirrhotic patients, the association of daclatasvir NS5A pangenotypic inhibitor, asunaprevir NS3 protease inhibitor and beclabuvir NS5B non nucleosidic polymerase inhibitor without ribavirin, gave high response rates in naive patients (93%). However, ribavirin could still be useful in pre-treated patients (93% vs 87% SVR with and without ribavirin, respectively)<sup>[52]</sup>.

In G3 cirrhotic patients, preliminary results showed that the association of grazoprevir elbasvir sofosbuvir without ribavirin gave a 91% SVR suggesting that this combination could be an ideal strategy for these difficult to treat population<sup>[33]</sup>. Of course, these results have to be confirmed.

#### Renal insufficiency: It will be soon possible to avoid ribavirin

Ribavirin use is problematic in this setting due to the management of severe anemia and the delicate dose adjustment which is not standardized (200 mg × 3/wk to 200 mg/d) and requires ribavirin concentration measurement especially in hemodialysis.

Today, no DAA association is recommended in patients with estimated glomerular filtration rate < 30 mL/mn, especially because the key tool of the approved associations, sofosbuvir and its main metabolite are eliminated by the kidney and the appropriate dosing is not known. Preliminary studies however showed that the simeprevir sofosbuvir (200 mg/d) association without ribavirin gave a SVR rate of 88% to 100% with a quite good tolerance<sup>[53,54]</sup>.

The paritaprevir/ritonavir ombitasvir dasabuvir combination was also very efficient (100% response) but G1a subtype still needed ribavirin<sup>[55]</sup>.

Finally, in the largest study so far, out of 226 G1

patients with severe renal insufficiency, 191 with chronic kidney disease stage 5 and 179 hemodialysed showed a 99% SVR when treated with grazoprevir elbasvir for 12 wk without ribavirin with an excellent tolerance<sup>[56]</sup>.

These encouraging results will probably lead us to treat hemodialysed patients if no transplant perspective is envisaged, or before kidney transplantation, as HCV negatively impacts these patients' prognosis.

## CONCLUSION

Even if new DAAs are cost-effective, at their current prices, they are not cost-saving, and the addition of ribavirin with approved DAAs interferon-free regimens is probably the best option to decrease treatment duration without impacting SVR. The next step of course is one pill of DAAs a day without ribavirin to treat all patients whatever the stage of the disease or the genotype, with no side effects and for the shortest treatment duration possible. Even if second generation drugs do not yet fulfil all the criteria and probably will not for the next 5 years, they dangerously jeopardize ribavirin future.

## ACKNOWLEDGMENTS

We thank Céline Rigaud for her help and Sarah Demai for her proofreading of English.

## REFERENCES

- 1 European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]
- 2 Hoofnagle JH, Sherker AH. Therapy for hepatitis C--the costs of success. *N Engl J Med* 2014; **370**: 1552-1553 [PMID: 24725236 DOI: 10.1056/NEJMe1401508]
- 3 Dixit NM, Perelson AS. The metabolism, pharmacokinetics and mechanisms of antiviral activity of ribavirin against hepatitis C virus. *Cell Mol Life Sci* 2006; **63**: 832-842 [PMID: 16501888 DOI: 10.1007/s00018-005-5455-y]
- 4 Zhou S, Liu R, Baroudy BM, Malcolm BA, Reyes GR. The effect of ribavirin and IMPDH inhibitors on hepatitis C virus subgenomic replicon RNA. *Virology* 2003; **310**: 333-342 [PMID: 12781720]
- 5 Markland W, McQuaid TJ, Jain J, Kwong AD. Broad-spectrum antiviral activity of the IMP dehydrogenase inhibitor VX-497: a comparison with ribavirin and demonstration of antiviral additivity with alpha interferon. *Antimicrob Agents Chemother* 2000; **44**: 859-866 [PMID: 10722482]
- 6 Chevaliez S, Brillet R, Lázaro E, Hézode C, Pawlotsky JM. Analysis of ribavirin mutagenicity in human hepatitis C virus infection. *J Virol* 2007; **81**: 7732-7741 [PMID: 17494069 DOI: 10.1128/JVI.00382-07]
- 7 Feld JJ, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, Schweigler LM, Theodore D, Zacks SL, Liang TJ, Fried MW. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. *Hepatology* 2007; **46**: 1548-1563 [PMID: 17929300 DOI: 10.1002/hep.21853]
- 8 Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 1998; **282**: 103-107 [PMID: 9756471]
- 9 Pawlotsky JM, Dahari H, Neumann AU, Hezode C, Germanidis G,



- Lonjon I, Castera L, Dhumeaux D. Antiviral action of ribavirin in chronic hepatitis C. *Gastroenterology* 2004; **126**: 703-714 [PMID: 14988824]
- 10 **Rong L**, Perelson AS. Mathematical analysis of multiscale models for hepatitis C virus dynamics under therapy with direct-acting antiviral agents. *Math Biosci* 2013; **245**: 22-30 [PMID: 23684949 DOI: 10.1016/j.mbs.2013.04.012]
- 11 **Snell NJ**. Ribavirin--current status of a broad spectrum antiviral agent. *Expert Opin Pharmacother* 2001; **2**: 1317-1324 [PMID: 11585000 DOI: 10.1517/14656566.2.8.1317]
- 12 **Murata Y**, Falsey AR. Respiratory syncytial virus infection in adults. *Antivir Ther* 2007; **12**: 659-670 [PMID: 17944273]
- 13 **Di Bisceglie AM**, Conjeevaram HS, Fried MW, Sallie R, Park Y, Yurdaydin C, Swain M, Kleiner DE, Mahaney K, Hoofnagle JH. Ribavirin as therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1995; **123**: 897-903 [PMID: 7486483]
- 14 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749]
- 15 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 12324553 DOI: 10.1056/NEJMoa020047]
- 16 **Bronowicki JP**, Ouzan D, Asselah T, Desmorat H, Zarski JP, Foucher J, Bourlière M, Renou C, Tran A, Melin P, Hézode C, Chevalier M, Bouvier-Alias M, Chevaliez S, Montestruc F, Lonjon-Domanec I, Pawlotsky JM. Effect of ribavirin in genotype 1 patients with hepatitis C responding to pegylated interferon alfa-2a plus ribavirin. *Gastroenterology* 2006; **131**: 1040-1048 [PMID: 17030174 DOI: 10.1053/j.gastro.2006.07.022]
- 17 **McHutchison JG**, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**: 1061-1069 [PMID: 12360468]
- 18 **Lindahl K**, Stahle L, Bruchfeld A, Schvarcz R. High-dose ribavirin in combination with standard dose peginterferon for treatment of patients with chronic hepatitis C. *Hepatology* 2005; **41**: 275-279 [PMID: 15660393 DOI: 10.1002/hep.20563]
- 19 **Lindahl K**, Hörnfeld E, Ståhle L, Carlsson T, Weiland O, Parke Å, Schvarcz R. High-Dose Ribavirin Enhances Early Virological Response in Hepatitis C Genotype 1-Infected Patients. *Ther Drug Monit* 2015; **37**: 745-750 [PMID: 25811342 DOI: 10.1097/FTD.0000000000000210]
- 20 **Glue P**. The clinical pharmacology of ribavirin. *Semin Liver Dis* 1999; **19** Suppl 1: 17-24 [PMID: 10349689]
- 21 **Jen J**, Laughlin M, Chung C, Heft S, Affrime MB, Gupta SK, Glue P, Hajian G. Ribavirin dosing in chronic hepatitis C: application of population pharmacokinetic-pharmacodynamic models. *Clin Pharmacol Ther* 2002; **72**: 349-361 [PMID: 12386637 DOI: 10.1067/mcp.2002.127112]
- 22 **Maynard M**, Pradat P, Gagnieu MC, Souvignet C, Trepo C. Prediction of sustained virological response by ribavirin plasma concentration at week 4 of therapy in hepatitis C virus genotype 1 patients. *Antivir Ther* 2008; **13**: 607-611 [PMID: 18672540]
- 23 **Loustaud-Ratti V**, Alain S, Rousseau A, Hubert IF, Sauvage FL, Marquet P, Denis F, Lunel F, Calès P, Lefebvre A, Fauchais AL, Liozon E, Vidal E. Ribavirin exposure after the first dose is predictive of sustained virological response in chronic hepatitis C. *Hepatology* 2008; **47**: 1453-1461 [PMID: 18435468 DOI: 10.1002/hep.22217]
- 24 **Gaeta GB**, Precone DF, Felaco FM, Bruno R, Spadaro A, Stornaiuolo G, Stanzione M, Ascione T, De Sena R, Campanone A, Filice G, Piccinino F. Premature discontinuation of interferon plus ribavirin for adverse effects: a multicentre survey in 'real world' patients with chronic hepatitis C. *Aliment Pharmacol Ther* 2002; **16**: 1633-1639 [PMID: 12197842]
- 25 **Dieterich DT**, Wasserman R, Bräu N, Hassanein TI, Bini EJ, Bowers PJ, Sulkowski MS. Once-weekly epoetin alfa improves anemia and facilitates maintenance of ribavirin dosing in hepatitis C virus-infected patients receiving ribavirin plus interferon alfa. *Am J Gastroenterol* 2003; **98**: 2491-2499 [PMID: 14638354 DOI: 10.1111/j.1572-0241.2003.08700.x]
- 26 **Afdhal NH**, Dieterich DT, Pockros PJ, Schiff ER, Shiffman ML, Sulkowski MS, Wright T, Younossi Z, Goon BL, Tang KL, Bowers PJ. Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. *Gastroenterology* 2004; **126**: 1302-1311 [PMID: 15131791]
- 27 **Zeuzem S**, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]
- 28 **Jacobson IM**, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]
- 29 **Lawitz E**, Poordad F, Brainard DM, Hyland RH, An D, Dvory-Sobol H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir with peginterferon-ribavirin for 12 weeks in previously treated patients with hepatitis C genotype 2 or 3 and cirrhosis. *Hepatology* 2015; **61**: 769-775 [PMID: 25322962 DOI: 10.1002/hep.27567]
- 30 **Foster GR**, Pianko S, Brown A, Forton D, Nahass RG, George J, Barnes E, Brainard DM, Massetto B, Lin M, Han B, McHutchison JG, Subramanian GM, Cooper C, Agarwal K. Efficacy of Sofosbuvir Plus Ribavirin With or Without Peginterferon-Alfa in Patients With Hepatitis C Virus Genotype 3 Infection and Treatment-Experienced Patients With Cirrhosis and Hepatitis C Virus Genotype 2 Infection. *Gastroenterology* 2015; **149**: 1462-1470 [PMID: 26248087 DOI: 10.1053/j.gastro.2015.07.043]
- 31 **Nelson DR**, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R, Oguchi G, Thuluvath PJ, Ortiz-Lasanta G, Rabinovitz M, Bernstein D, Bennett M, Hawkins T, Ravendhran N, Sheikh AM, Varunok P, Kowdley KV, Hennicken D, McPhee F, Rana K, Hughes EA. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology* 2015; **61**: 1127-1135 [PMID: 25614962 DOI: 10.1002/hep.27726]
- 32 **Hezode C**, De Ledinghen V, Fontaine H, Zoulim F, Lebray P, Boyer N, Larrey D, Silvain C, Botta-Fridlund D, Leroy V, Bourlière M, D'alteroche L, Hubert-Fouchard I, Guyader D, Rosa I, Nguyen-Khac E, Di Martino V, Carrat F, Fedchuk L, Akremi R, Bennai Y, Bronowicki J. Daclatasvir plus sofosbuvir with or without ribavirin in patients with hcv genotype 3 infection: interim analysis of a french multicenter compassionate use program, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract LP05
- 33 **Poordad F**, Lawitz E, Gutierrez JA, Evans B, Howe A, Feng HP, Li JJ, Hwang P, Robertson M, Wahl J, Barr E, Haber B. 2c-swift: grazoprevir/elbasvir sofosbuvir in cirrhotic and noncirrhotic, treatment-naïve patients with hepatitis c virus genotype 1 infection, for durations of 4, 6 or 8 weeks and genotype 3 infection for durations of 8 or 12 weeks, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract O006
- 34 **Ferenci P**, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A, Box TD, Younes Z, Enayati P, Green S, Baruch Y, Bhandari BR, Caruntu FA, Sepe T, Chulanov V, Janczewska E, Rizzardini G, Gervain J, Planas R, Moreno C, Hassanein T, Xie W, King M, Podsadecki T, Reddy KR. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin



- for HCV. *N Engl J Med* 2014; **370**: 1983-1992 [PMID: 24795200 DOI: 10.1056/NEJMoa1402338]
- 35 **Zeuzem S**, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourlière M, Sulkowski MS, Wedemeyer H, Tam E, Desmond P, Jensen DM, Di Bisceglie AM, Varunok P, Hassanein T, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1604-1614 [PMID: 24720679 DOI: 10.1056/NEJMoa1401561]
  - 36 **Bourlière M**, Bronowicki JP, de Ledinghen V, Hézode C, Zoulim F, Mathurin P, Tran A, Larrey DG, Ratzliff V, Alric L, Hyland RH, Jiang D, Doehle B, Pang PS, Symonds WT, Subramanian GM, McHutchison JG, Marcellin P, Habersetzer F, Guyader D, Grangé JD, Loustaud-Ratti V, Serfaty L, Metivier S, Leroy V, Abergel A, Pol S. Ledipasvir-sofosbuvir with or without ribavirin to treat patients with HCV genotype 1 infection and cirrhosis non-responsive to previous protease-inhibitor therapy: a randomised, double-blind, phase 2 trial (SIRIUS). *Lancet Infect Dis* 2015; **15**: 397-404 [PMID: 25773757 DOI: 10.1016/S1473-3099(15)70050-2]
  - 37 **Reddy KR**, Bourlière M, Sulkowski M, Omata M, Zeuzem S, Feld JJ, Lawitz E, Marcellin P, Welzel TM, Hyland R, Ding X, Yang J, Knox S, Pang P, Dvory-Sobol H, Subramanian GM, Symonds W, McHutchison JG, Mangia A, Gane E, Mizokami M, Pol S, Afdhal N. Ledipasvir and sofosbuvir in patients with genotype 1 hepatitis C virus infection and compensated cirrhosis: An integrated safety and efficacy analysis. *Hepatology* 2015; **62**: 79-86 [PMID: 25846144 DOI: 10.1002/hep.27826]
  - 38 **Pol S**, Bourlière M, Lucier S, De Ledinghen V, Zoulim F, Dorival-Mouly C. Safety and efficacy of the combination daclatasvir-sofosbuvir in hcv genotype 1-mono-infected patients from the french observational cohort anrs co22 hepather. 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract L03
  - 39 **Fontaine H**, Hézode C, Zoulim F, Samuel D, Bourlière M, Haour G. Efficacy of the oral sofosbuvir-based combinations in hcv genotype 4-mono-infected patients from the french observational cohort anrs co22 hepather. 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract LP28
  - 40 **Flamm SL**, Everson GT, Charlton M, Denning JM, Arterburn S, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal NH. Ledipasvir/Sofosbuvir with Ribavirin for the Treatment of HCV in Patients with Decompensated Cirrhosis: Preliminary Results of a Prospective, Multicenter Study. 65th Annual Meeting of the American Association for the Study of Liver diseases; 2015 November 7-11; Boston, USA. 2014: Abstract 239
  - 41 **Coilly A**, Fougereou C, De Ledinghen V, Housset-Debry P, Duvoux C, Di Martino V. The association of sofosbuvir and daclatasvir for treating severe recurrence of hcv infection after liver transplantation: results from a large french prospective multicentric anrs co23 cupilt cohort, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract L08
  - 42 **Poordad F**, Schiff ER, Vierling JM, Landis C, Fontana RJ, Yang R, McPhee F, Hughes E, Noviello S, Swenson ES. Daclatasvir, sofosbuvir, and ribavirin combination for hcv patients with advanced cirrhosis or posttransplant recurrence: phase 3 ally-1 study. 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract L08
  - 43 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Di Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]
  - 44 **Gane EJ**, Stedman CA, Hyland RH, Ding X, Svarovskaia E, Subramanian GM, Symonds WT, McHutchison JG, Pang PS. Efficacy of nucleotide polymerase inhibitor sofosbuvir plus the NS5A inhibitor ledipasvir or the NS5B non-nucleoside inhibitor GS-9669 against HCV genotype 1 infection. *Gastroenterology* 2014; **146**: 736-743.e1 [PMID: 24262278 DOI: 10.1053/j.gastro.2013.11.007]
  - 45 **Jacobson Ira M**, Forns X, Zeuzem S, Hezode C, Shiffman ML, Pol S, Berenguer M, Fried MW, Agarwal K, Kowdley KV, Lovell SS, Abunimeh M, Trinh R, McGovern BH, Craxi A. Characteristics of HCV-Infected Patients with Cirrhosis Requiring Ribavirin Dose Reduction During Treatment with Direct-Acting Antivirals. 65th Annual Meeting of the American Association for the Study of Liver diseases; 2014 November 7-11; Boston, USA. 2015: Poster 1973
  - 46 **Rowe JE**, Meissner EG, Jirmerson LC, Osinusi A, Sims Z, Petersen T, Bushman LR, Wolfe P, McHutchison JG, Kottlilil S, Kiser JJ. Serum and cellular ribavirin pharmacokinetic and concentration-effect analysis in HCV patients receiving sofosbuvir plus ribavirin. *J Antimicrob Chemother* 2015; **70**: 2322-2329 [PMID: 25971261 DOI: 10.1093/jac/dkv122]
  - 47 **Kwo P**, Gane E, Peng CY, Pearlman B, Vireling J, Serfaty L, Buti M, Shafran S, Stryzak P, Lin L, Gress J, Robertson M, Wahl J, Barr E, Haber B. Efficacy and safety of grazoprevir/elbasvir +/- rbv for 12 weeks in patients with hcv g1 or g4 infection who previously failed peginterferon/rbv: c-edge treatment-experienced trial, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Poster P886
  - 48 **Jacobson IM**, Poordad F, Firpi-Morell R, Everson GT, Verna EC, Bhanja S, Zhang B, Caro L, Wahl J, Robertson M, Barr E, Charles ED. Efficacy and safety of grazoprevir and elbasvir in hepatitis c genotype 1-infected patients with child-pugh class b cirrhosis (c-salt part a), 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract O008
  - 49 **Brown A**, Hézode C, Zuckerman E, Foster G, Zekry A, Roberts S, Howe A, Durkan C, Badshah C, Zhang B, Robertson M, Wahl J, Barr E, Haber B. C-scape: efficacy and safety of 12 weeks of grazoprevir +/- elbasvir +/- ribavirin in patients with hcv gt2, 4, 5 or 6 infection, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Poster P0771
  - 50 **Gane E**, Nahass R, Luketic V, Hwang P, Robertson M, Wahl J, Barr E, Haber B. Efficacy of 12 or 18 weeks of grazoprevir plus elbasvir with ribavirin in treatment-naïve, noncirrhotic hcv genotype 3-infected patients, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Poster P0776
  - 51 **Pianko S**, Flamm SL, Shiffman ML, Kumar S, Strasser SI, Dore GJ, McNally J, Brainard DM, Han L, Doehle B, Mogalian E, McHutchison JG, Reddy KR, Roberts SK. High Efficacy of Treatment with Sofosbuvir+GS-5816 ±Ribavirin for 12 Weeks in Treatment Experienced Patients with Genotype 1 or 3 HCV Infection, 65th Annual Meeting of the American Association for the Study of Liver diseases; 2014 November 7-11; Boston, USA. 2015: Abstract 197
  - 52 **Muir AJ**, Poordad F, Lalezari J, Everson G, Dore GJ, Herring R, Sheikh A, Kwo P, Hézode C, Pockros PJ, Tran A, Yozviak J, Reau N, Ramji A, Stuart K, Thompson AJ, Vierling J, Freilich B, Cooper J, Ghesquiere W, Yang R, McPhee F, Hughes EA, Swenson ES, Yin PD. Daclatasvir in combination with asunaprevir and beclabuvir for hepatitis C virus genotype 1 infection with compensated cirrhosis. *JAMA* 2015; **313**: 1736-1744 [PMID: 25942724 DOI: 10.1001/jama.2015.3868]
  - 53 **Czul F**, Schiff E, Peyton A, Levy C, Hernandez M, Jeffers L, C O'Brien1, P Martin1 KR. Bhamidimarri1, First ribavirin-free sofosbuvir and simeprevir treatment of hepatitis c genotype 1 patients with severe renal impairment (gfr < 30 mL/min r dialysis), 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Poster P878
  - 54 **Nazario HE**, Ndungu M, Modi A. Safety and efficacy of sofosbuvir simeprevir without ribavirin in hepatitis c genotype

- 1-infected patients with end-stage renal disease or  $\text{gfr} < 30 \text{ mL/min}$ , 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Poster P802
- 55 **Pockros PJ**, Reddy KR, Mantry PS, Cohen E, Bennett M, Sulkowski MS, Bernstein D, Podsadecki T, Cohen D, Shulman NS, Wang D, Khatri A, Abunimeh M, Lawitz E. Safety of ombitasvir/paritaprevir/ritonavir plus dasabuvir for treating hcv gt1 infection in patients with severe renal impairment or end-stage renal disease: the ruby-i study, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract L01
- 56 **Roth D**, Nelson DR, Bruchfeld A, Liapakis A, Silva M, Monsour H, Martin P, Pol S, Londoño MC, Hassanein T, Zamor PJ, Zuckerman E, Wan S, Jackson B, Nguyen BY, Robertson M, Barr E, Wahl J, Greaves W. Grazoprevir plus elbasvir in treatment-naive and treatment-experienced patients with hepatitis C virus genotype 1 infection and stage 4-5 chronic kidney disease (the C-SURFER study): a combination phase 3 study. *Lancet* 2015; **386**: 1537-1545 [PMID: 26456905 DOI: 10.1016/S0140-6736(15)00349-9]

**P- Reviewer:** Mattner J, Rajeshwari K, Urganci N **S- Editor:** Qiu S  
**L- Editor:** A **E- Editor:** Liu SQ



## Hepatitis C and insulin action: An intimate relationship

Hilla Knobler, Stephen Malnick

Hilla Knobler, Diabetes and Metabolic Disease Unit, Kaplan Medical Center, Rehovot 76100, Israel

Stephen Malnick, Department of Internal Medicine C, Kaplan Medical Center, Rehovot 76100, Israel

**Author contributions:** Both authors contributed equally to this work.

**Conflict-of-interest statement:** None of the authors has any conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Hilla Knobler, MD, Diabetes and Metabolic Disease Unit, Kaplan Medical Center, Pasternak St., Rehovot 76100, Israel. [knobler@inter.net.il](mailto:knobler@inter.net.il)  
 Telephone: +972-8-9441650  
 Fax: +972-8-9441912

Received: July 6, 2015  
 Peer-review started: July 11, 2015  
 First decision: September 16, 2015  
 Revised: December 10, 2015  
 Accepted: December 29, 2015  
 Article in press: January 4, 2016  
 Published online: January 18, 2016

### Abstract

Chronic hepatitis C virus (HCV) infection has been shown to be linked to a higher prevalence of type 2 diabetes compared with the general population or with patients with chronic hepatitis B infection and diabetes is the most common extra-hepatic manifestation of HCV. The HCV-diabetes association is due to insulin resistance (IR) that occurs early in the course of the

disease even in patients without or with minimal fibrosis. The mechanisms for HCV-induced IR are only partly understood and include a direct inhibitory effect of HCV on insulin signaling pathway. IR in chronic HCV results in an increased progression rate of hepatic fibrosis, cirrhosis and hepatocellular carcinoma. Some but not all studies found that IR reduces the response rate to interferon/ribavirin therapy. Whether IR affects the response to the new direct-acting antiviral treatments is still unknown.

**Key words:** Hepatitis C; Type 2 diabetes; Antiviral therapy; Insulin resistance; Insulin signaling

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Chronic hepatitis C virus (HCV) infection is associated with a higher prevalence of diabetes as compared to either the general population or patients with chronic hepatitis B infections. HCV hepatitis is linked to insulin resistance (IR) early in the disease course, mediated partly by direct inhibitory effect of the viral proteins on insulin signaling. The presence of IR is associated with an increased rate of disease progression to fibrosis, cirrhosis and hepatocellular carcinoma. Interferon and ribavirin treatment of HCV hepatitis may be less successful in the presence of IR. The effect of IR on the new direct-acting antiviral treatment is unclear.

Knobler H, Malnick S. Hepatitis C and insulin action: An intimate relationship. *World J Hepatol* 2016; 8(2): 131-138 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i2/131.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i2.131>

### INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a major healthcare problem worldwide with between 130-170 million people infected<sup>[1,2]</sup>. In addition, there are extra-

hepatic manifestations of HCV infection including mixed cryoglobulinemia, thyroid disorders and other autoimmune-mediated diseases<sup>[3]</sup>, but several studies published since 1994 provide evidence that diabetes mellitus (DM) maybe the most common extra-hepatic disease associated with chronic HCV.

## THE ASSOCIATION BETWEEN HCV AND DIABETES

The first studies that demonstrated an association between HCV and DM evaluated patients at advanced stage of liver disease necessitating liver transplantation and revealed that diabetes occurred in 50% and 62% of patients whose liver failure was HCV-related compared to 9% in patients whose liver failure was related to other causes<sup>[4,5]</sup>. These unexpected results were confirmed by studies from many parts of the world demonstrating that the increased prevalence of diabetes in HCV patients is unique and is significantly different compared to hepatitis B virus (HBV) infection<sup>[6-8]</sup>.

Many of these additional studies, however, also included patients with cirrhosis - a condition that by itself is known to lead to impaired glucose tolerance<sup>[9,10]</sup>.

### *Diabetes in non-cirrhotic HCV patients*

In order to avoid the confounding effect of cirrhosis on glucose metabolism, we designed a study conducted in patients without liver cirrhosis that included 45 patients with chronic HCV, 88 patients with chronic HBV infection and 90 healthy individuals<sup>[11]</sup>. Diabetes status was based on an oral glucose tolerance test (OGTT). We found that 33% of HCV patients had type 2 diabetes compared to 12% of patients with chronic HBV infection and 6% of a healthy control cohort. We have reported that HCV patients with diabetes had a higher incidence of a family history of diabetes as compared to HCV patients without diabetes ( $P < 0.001$ ). In addition on comparing liver biopsies from HCV patients with diabetes to those with HCV and no diabetes there was a significantly higher inflammatory activity, fibrosis grade and more steatosis.

### *Large cohort studies evaluating the relationship between HCV and diabetes*

The National Health and Nutrition Examination Survey (NHANES III) evaluated 9841 community-dwelling subjects and found that 8% of this population had type 2 diabetes and 2% were anti-HCV positive. The odds ratio (OR) for type 2 DM in those over 40 years of age after adjusting for sex, body mass index (BMI), ethnicity, poverty index, and previous drug or alcohol use was 3.77 (95%CI: 1.80-7.87)<sup>[12]</sup>. There was no increased risk for DM in those with chronic HBV infection. Although liver biopsies were not performed in these patients, there were no clinical signs of chronic liver disease. A large study of consecutive chronic HCV patients from Spain, found a 3-fold increase in the prevalence of glucose abnormalities in non-cirrhotic HCV+ compared

with HCV- subjects<sup>[13]</sup> but not in cirrhotic patients. Furthermore, multivariate analysis of chronic HCV patients without cirrhosis found that HCV infection was an independent determinant of glucose abnormalities, OR of 4.26 (95%CI: 2.03-8.93). In the Atherosclerosis Risk in Communities study, with a follow-up of 9-years pre-existing HCV infection was found to be a significant risk factor for developing diabetes in aged patients or those with a high BMI. This finding was strikingly robust with a relative hazard of 11.58 (95%CI: 1.39-96.6)<sup>[14]</sup>. Two meta-analyses including 47 cross-sectional and cohort studies found that HCV was associated with DM with an OR of 1.7<sup>[15,16]</sup> with an excess risk observed in comparison to HBV-infected controls.

However a recent additional report based on NHANES data 1999-2010 survey evaluated 15128 participants with known HCV and glucose status and did not find an association between HCV status and diabetes/pre-diabetes<sup>[17]</sup>. The reasons for this discrepancy are not entirely clear however the number of patients who were HCV positive was relatively small (1.7% were anti-HCV+ and 1.1% were HCV RNA+) and OGTT was not performed. Another factor that can reduce the strength of the association between HCV infection and diabetes in this recent United States survey is the increase within the rate of obesity and consequently obesity-induced diabetes that may dilute the effect of HCV.

Taken together, the vast majority of studies suggest that chronic hepatitis C is specifically associated with type 2 diabetes and the association is strongest in patients with additional risk factors such as older age and positive family history of diabetes implying that HCV leads to diabetes particularly in susceptible hosts.

### *Interferon-induced diabetes*

Interferon treatment that was until recently the cornerstone of HCV treatment was shown to induce a distinct form of diabetes. However this is a relatively rare complication that in contrast to the common form of HCV-related type 2 diabetes described above, has an abrupt onset, necessitates insulin treatment from onset and is mediated by an autoimmune process manifested by a very high titer of pancreatic autoantibodies<sup>[18]</sup>.

## PATHOGENESIS OF HEPATITIS C ASSOCIATED DIABETES

### *HCV and insulin resistance*

There is substantial evidence that insulin resistance (IR), that has a pivotal role in the pathogenesis of type 2 diabetes, develops early in the course of HCV infection<sup>[19-21]</sup>. A study of 260 subjects with HCV with assorted stages of fibrosis compared with 137 healthy volunteer in which IR was measured by the homeostasis model assessment-IR (HOMA-IR), found significant IR even in the sub-group of 121 patients with only stage 0 or 1 of hepatic fibrosis. However, although IR was detected even in subjects with minimal or no fibrosis,



more advanced fibrosis was associated with increased HOMA-IR<sup>[19]</sup>. Other studies confirmed these findings and showed a correlation between the degree of fibrosis and IR<sup>[20,22]</sup>. By using the gold standard measurement of IR, the hyperinsulinemic - euglycemic clamp it was shown that IR occurred mainly in the periphery, *i.e.*, in muscles and not in the liver and was related to viral load but not to liver fat content<sup>[23]</sup>. The notion that HCV has a direct effect on insulin sensitivity that is not mediated by virus-induced steatosis is also supported by a transgenic mice model which expresses the HCV core protein in the liver. IR was detected as early as 1 mo of age while hepatic steatosis developed after 3 mo<sup>[24]</sup>. In a landmark study, Aytug *et al*<sup>[25]</sup> evaluated liver specimens obtained from non-obese non-diabetic HCV patients compared to controls and their data not only confirmed the existence of HCV-induced IR but also revealed a specific impairment of insulin - stimulated IRS-1/PI3 kinase signaling pathway in HCV patients, a pathway that is responsible for insulin metabolic effects.

### IR and HCV genotypes

The relationship between IR and HCV genotype is still controversial. In a study of Hui *et al*<sup>[19]</sup> patients with genotype 3 had significantly lower HOMA-IR compared with other genotypes and this association remained significant even after adjusting for other variables. In another large study of 275 non-diabetic treatment-naïve HCV patients, HOMA-IR was significantly higher in non-3 genotype compared with genotype 3. However in non-obese patients with minimal fibrosis, using a cut-off level of HOMA > 3 as indicating IR, there was no significant effect of genotypes<sup>[26]</sup>. In another smaller study of 44 patients that used a cut-off level of HOMA  $\geq 2$  as indicating IR, the prevalence of IR was similarly high, 65% and 57% in genotype 1 and genotype 3, respectively<sup>[27]</sup>. However it is important to emphasize that the usage of these HOMA-IR criteria to define IR is problematic since there are no acceptable absolute cut-off levels.

### The underlying mechanisms for HCV-induced IR

**Tumor necrosis factor alpha:** The role of the cytokine tumor necrosis factor alpha (TNF- $\alpha$ ) in HCV-induced IR is supported by several studies (for review<sup>[28]</sup>). TNF- $\alpha$  producing cells, the majority of which are derived from macrophage/Kupfer cell lineage, are increased in HCV infection; and TNF- $\alpha$  activation was found to be significantly associated with the inflammatory process<sup>[29]</sup>. TNF- $\alpha$  also has an important inhibitory role on the insulin signaling pathway and the mechanism is mediated by activating serine/threonine (Ser/Thr) kinases that phosphorylate the insulin receptor substrate (IRS) protein, and uncoupling it from both upstream and downstream effectors<sup>[30]</sup>. TNF- $\alpha$  induces IR also by indirect mechanisms such as increasing lipolysis leading to increased serum free fatty acids and regulating expression of several adipocyte genes that modulate insulin sensitivity<sup>[31]</sup>. TNF- $\alpha$  binds to two distinct cell

surface receptors, TNFR-1 and TNFR-2 that undergo proteolytic cleavage producing soluble receptors sTNFR1 and sTNFR2. Serum levels of TNF- $\alpha$  and sTNFR were increased in HCV-infected patients compared with controls<sup>[32]</sup>. When serum sTNFR were measured in non-cirrhotic HCV patients with and without diabetes, non-HCV patients with type 2 diabetes and controls, a marked increase of sTNFR was found in the HCV-diabetes<sup>+</sup> group compared to HCV patients without diabetes, and non-HCV patients with type 2 DM<sup>[33]</sup>. A significant correlation was found between the degree of liver inflammation and sTNFR<sup>[29]</sup>. The role of TNF- $\alpha$  in HCV-induced IR is supported by the finding that anti TNF- $\alpha$  antibody administration restored insulin sensitivity in a transgenic mice model that specifically expressed the HCV core protein in the liver<sup>[24]</sup>.

However, increased levels of TNF- $\alpha$  are also present in other chronic liver diseases and thus cannot fully account for the unique association between HCV and IR. Therefore direct effects of HCV proteins on insulin signaling have been also considered.

### Direct effects of HCV proteins on insulin signaling

In human hepatoma cells, HCV core protein up-regulates suppressor cytokine signaling (SOCS)-3, which is known to inhibit insulin signaling by causing ubiquitination of IRS1 and IRS2 proteins<sup>[34]</sup>. These defects were not detected in SOCS3<sup>-/-</sup> mouse embryonic fibroblasts cells or in the presence of an inhibitor of proteasomal proteolysis<sup>[34]</sup>. We have reported several impairments of the insulin signaling cascade linked to the proteasomal degradation of IRS-1 protein<sup>[35]</sup>. Additionally we found that the core protein impaired insulin ability to inhibit the expression of the target gene insulin growth factor binding protein-1.

HCV can also inhibit insulin signaling by dephosphorylation of AKT involving the endoplasmic reticulum stress signal inducing over-expression of protein phosphatase 2A<sup>[36]</sup>. Taken together these data imply a direct effect of HCV core protein in inhibiting insulin signaling pathway.

## DOES ERADICATION OF HCV

### AMELIORATE IR?

A recent study of 8 normoglycemic men with chronic HCV infection that used the hyperinsulinemic-euglycemic clamp that provides a direct measurement of peripheral insulin sensitivity, showed that viral clearance led to improvement in glycemic control and to insulin sensitivity that become comparable to 15 matched HCV-negative controls<sup>[37]</sup>. A larger earlier study, using the surrogate marker HOMA-IR also showed that in HCV patients who were sustained responders HOMA-IR decreased while in it did not change in nonresponders and relapsers<sup>[38]</sup>. However, another study showed that HCV therapy improved IR regardless of virologic response but the response was greatly influenced by BMI

changes and interferon use making data interpretation difficult<sup>[39]</sup>.

## THE EFFECT OF IR AND DIABETES ON THE CLINICAL OUTCOME OF HCV

The link between HCV infection and IR and diabetes is complex. IR appears at an early stage of chronic HCV infection as discussed above and results in an increased rate of progression of hepatic fibrosis and the complications of cirrhosis including hepatocellular carcinoma (HCC)<sup>[40]</sup>.

IR is also related to obesity and type 2 diabetes and both of these conditions are known to be risk factors for HCC leading to about 2-fold increased prevalence<sup>[41,42]</sup>. The rise in HCV infection and HCV-induced IR together with increased obesity-induced IR may partly explain the marked increase in HCC in the last decades<sup>[43]</sup>.

The compensatory hyperinsulinemia that occurs in IR can lead to fibrogenesis. In human hepatic stellate cells (HSC), incubation with insulin and insulin growth factor (IGF)-1 led to increased HSC proliferation and type 1 collagen gene expression<sup>[44]</sup>. The increased IGF-1 levels that occur in the IR state is also one of the mechanisms for IR-associated malignancy and particularly HCC and changes in the expression pattern of IGF system components were found in human hepatoma cell lines and in animal models<sup>[45]</sup>.

In a recent systemic review of 14 studies including 3695 participants with HCV infection, the relative risk for fibrosis was 2.26 (95%CI: 1.52-3.06) for genotype 1, but the association was not significant for genotype 3<sup>[46]</sup>. HCV is also intimately related to hepatic steatosis<sup>[47,48]</sup> and steatosis is much more common in patients infected with HCV than in other liver diseases. This association is most marked for genotype 3<sup>[49]</sup>. Steatosis is also linked to HCC and in two lines of transgenic mice expressing the HCV core protein, HCC developed within fat-containing adenomas<sup>[50]</sup>.

## THE EFFECT OF IR AND DIABETES ON THE RESPONSE TO THERAPY

It has been shown that patients with high IR have a slower rate of decline in the viral load of HCV RNA compared to patients with low IR, even in the first 24 h of treatment<sup>[51]</sup>. In addition, there is an association between a high degree of IR and a low rate of rapid viral response in genotypes 1<sup>[52]</sup>, 3<sup>[53]</sup> and 4<sup>[54]</sup>. Several studies have shown that IR is associated with a higher likelihood of not achieving sustained virological response (SVR)<sup>[52-56]</sup>. A study from Spain of 159 patients with chronic HCV hepatitis found that those with a SVR had lower baseline HOMA scores compared to those patients who did not achieve a SVR<sup>[57]</sup>. The Virahep-C study which included both Caucasian and African-Americans found that IR and interferon dose were negatively associated with SVR<sup>[56]</sup>. The patients in this study had a high degree of obesity

and IR as compared to other published reports. These studies have used HOMA to assess insulin sensitivity, a surrogate measure of IR although this technique is less precise than more direct measurements such as the insulin suppression test<sup>[58]</sup>. Furthermore IR can change over time with in patients with chronic HCV infection<sup>[59]</sup>. When IR was directly assessed by means of an insulin suppression test in a cohort of 50 non-cirrhotic, non-diabetic patients with chronic HCV infection, SVR was not associated with insulin sensitivity<sup>[39]</sup>. The steady state plasma glucose level decreased during anti-viral therapy but was not statistically significant between those patients achieving SVR and those not achieving SVR during and after treatment<sup>[39]</sup>. IR often progresses to diabetes but in a study that evaluated SVR and the development of diabetes or impaired glucose tolerance, no such correlation was found during a median follow up of 8 years<sup>[60]</sup>. In 2011, two meta-analyses were published that examined the effect of IR on SVR including fourteen studies with more than 2700 patients<sup>[61,62]</sup>. The studies that did not find an association between IR and SVR had a baseline HOMA value of less than 3 and a low prevalence of advanced fibrosis. This suggests that the HOMA value may be predictive of response to antiviral treatment in those patients with advanced liver disease. These inconsistent data may be partly due the interplay between the baseline characteristics of the patients and the effect of the HCV virus on insulin sensitivity. Notably, about 25%-30% of the United States population have metabolic features of HCV-independent IR<sup>[63]</sup>.

## TARGETING IR AS PART OF HCV TREATMENT

In view of the link between IR and the progression of HCV hepatitis and the possible influence of IR on treatment, attention has been drawn to improving the metabolic factors related to IR before or during anti-viral treatment.

### *Lifestyle modification*

A 24 wk lifestyle and dietary intervention was shown to reduce BMI and HOMA in obese patients with chronic HCV hepatitis<sup>[64]</sup>. A 3-mo trial of a low calorie diet before starting anti-viral therapy has been shown to result in a higher end-of- treatment viral response in patients with type 1 chronic HCV hepatitis together with an improvement in IR.

### *Metformin*

Metformin is an insulin sensitizer that mainly decreases hepatic glucose production. An attempt to add metformin to treatment with peg-interferon-2a and ribavirin led to decreased HOMA-IR and viral load, together with an improvement in the SVR, but this effect was observed only in females<sup>[65]</sup>. In another study metformin administration led to an increase in SVR in both male and female HCV patients with genotype 1 treated by

pegylated interferon and ribavirin<sup>[66]</sup>.

### Thiazolidinediones

Thiazolidinediones produce an increase in insulin sensitivity *via* activation of the peroxisome proliferator-activated receptor- $\gamma$  in adipocytes and skeletal muscle<sup>[67]</sup>. Pioglitazone has been shown to produce an increase in SVR in patients with genotype 4 and IR but not in patients with genotype 1<sup>[68]</sup>. Another study of pioglitazone added to pegylated interferon-2a and ribavirin in non-diabetic HCV patients who previously did not respond to this treatment and who had HOMA > 2, was terminated after none of the first five patients achieved a 12 wk viral response, despite an improvement in IR in some of them<sup>[69]</sup>.

In a recent small study of patients with HCC, in a sub-group analysis of diabetic over-weight patients, the addition of pioglitazone to curative treatment resulted in reduced HCC recurrence<sup>[70]</sup>.

## THE EFFECT OF DIABETES ON THE RESPONSE TO THE DIRECT-ACTING ANTI-VIRAL TREATMENTS

The recently approved sofosbuvir, simeprevir, ledipasvir, and the combination of paritaprevir, ombitasvir and dasabuvir have ushered in the era of interferon-free therapy for HCV hepatitis. These direct-acting anti-viral treatments (DAA) achieve SVRs of more than 90% for most treatment groups<sup>[71]</sup>. With such an effective treatment available it is likely that the effect of IR will be less evident. However, a recent preliminary report suggests that metabolic factors such as diabetes and hyperlipidemia still compromise the effect of DAA treatment. This was based on the results of a recent study that examined SVR at 12-wk in 54 non-Caucasian populations in the United States, 65% of whom were Hispanic and 24% had diabetes. SVR in this study was 81% which is lower than the rate reported in previous studies. A pre-treatment glucose level of less than 126 mg/dL was shown to be linked to a higher rate of SVR<sup>[72]</sup>. Further studies are needed to evaluate the effect of IR and diabetes on the response to DAA treatment.

Although the future of treatment of HCV hepatitis will undoubtedly be oral, once-daily pangenotypic therapy with a nearly 100% SVR, in 2015 there is still a place for treatment of HCV hepatitis with interferon-containing regimens.

For patients with genotypes 2-6 peginterferon and ribavirin is still effective treatment. For patients with genotype 2, 24 wk of treatment is sufficient and an SVR of 85%-90% is achieved<sup>[73]</sup>. Interferon has an important role in the treatment of genotype 3, including a regimen with sofosbuvir<sup>[74]</sup>, and in the treatment of genotype 4 with an SVR of 43%-70% and 60%-85% SVR for genotype 6<sup>[75]</sup>.

In addition for many economically-constrained health services and patients who are self-funding, the

cost of the DAAs is prohibitive, and treatment with interferon will remain an option for the near future<sup>[76]</sup>.

## CONCLUSION

IR is intimately related to HCV infection based on numerous studies in animal models and humans resulting in increased prevalence of type 2 diabetes in HCV patients. The underlying mechanisms are only partly understood and recent data suggest a direct inhibitory effect of the virus on insulin signaling pathway. IR was shown by several, but not all studies, to have a deleterious effect on the clinical course of chronic HCV infection and the inconsistency maybe explained by differences in the baseline characteristics of the patients. Small studies suggest that life-style intervention and metformin may increase SVR rate but further studies are needed to confirm these findings. The effect of IR in the DAA drugs era is still unclear.

## REFERENCES

- 1 **Choo QL**, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**: 359-362 [PMID: 2523562]
- 2 **Ansaldi F**, Orsi A, Sticchi L, Bruzzone B, Icardi G. Hepatitis C virus in the new era: perspectives in epidemiology, prevention, diagnostics and predictors of response to therapy. *World J Gastroenterol* 2014; **20**: 9633-9652 [PMID: 25110404 DOI: 10.3748/wjg.v20.i29.9633]
- 3 **Cacoub P**, Renou C, Rosenthal E, Cohen P, Louri I, Loustaud-Ratti V, Yamamoto AM, Camproux AC, Hausfater P, Musset L, Veyssier P, Raguin G, Piette JC. Extrahepatic manifestations associated with hepatitis C virus infection. A prospective multicenter study of 321 patients. The GERMIVIC. Groupe d'Etude et de Recherche en Medecine Interne et Maladies Infectieuses sur le Virus de l'Hepate C. *Medicine* (Baltimore) 2000; **79**: 47-56 [PMID: 10670409]
- 4 **Allison ME**, Wreghitt T, Palmer CR, Alexander GJ. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. *J Hepatol* 1994; **21**: 1135-1139 [PMID: 7699240]
- 5 **Knobler H**, Stagnaro-Green A, Wallenstein S, Schwartz M, Roman SH. Higher incidence of diabetes in liver transplant recipients with hepatitis C. *J Clin Gastroenterol* 1998; **26**: 30-33 [PMID: 9492860 DOI: 10.1097/00004836-199801000-00009]
- 6 **Fraser GM**, Harman I, Meller N, Niv Y, Porath A. Diabetes mellitus is associated with chronic hepatitis C but not chronic hepatitis B infection. *Isr J Med Sci* 1996; **32**: 526-530 [PMID: 8756978]
- 7 **Ozyilkan E**, Arslan M. Increased prevalence of diabetes mellitus in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 1996; **91**: 1480-1481 [PMID: 8678039]
- 8 **Grimbert S**, Valensi P, Lévy-Marchal C, Perret G, Richardet JP, Raffoux C, Trinchet JC, Beaugrand M. High prevalence of diabetes mellitus in patients with chronic hepatitis C. A case-control study. *Gastroenterol Clin Biol* 1996; **20**: 544-548 [PMID: 8881566]
- 9 **Kruszynska YT**, Home PD, McIntyre N. Relationship between insulin sensitivity, insulin secretion and glucose tolerance in cirrhosis. *Hepatology* 1991; **14**: 103-111 [PMID: 2066059]
- 10 **Nolte W**, Hartmann H, Ramadori G. Glucose metabolism and liver cirrhosis. *Exp Clin Endocrinol Diabetes* 1995; **103**: 63-74 [PMID: 7553077 DOI: 10.1055/s-0029-1211331]
- 11 **Knobler H**, Schihmanter R, Zifroni A, Fenakel G, Schattner A. Increased risk of type 2 diabetes in noncirrhotic patients with chronic hepatitis C virus infection. *Mayo Clin Proc* 2000; **75**:



- 355-359 [PMID: 10761489 DOI: 10.4065/75.4.355]
- 12 **Mehta SH**, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. *Ann Intern Med* 2000; **133**: 592-599 [PMID: 11033586 DOI: 10.7326/0003-4819-133-8-200010170-00009]
  - 13 **Lecube A**, Hernández C, Genescà J, Esteban JI, Jardí R, Simó R. High prevalence of glucose abnormalities in patients with hepatitis C virus infection: a multivariate analysis considering the liver injury. *Diabetes Care* 2004; **27**: 1171-1175 [PMID: 15111540 DOI: 10.2337/diacare.27.5.1171]
  - 14 **Mehta SH**, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J, Szklo M, Thomas DL. Hepatitis C virus infection and incident type 2 diabetes. *Hepatology* 2003; **38**: 50-56 [PMID: 12829986 DOI: 10.1053/jhep.2003.50291]
  - 15 **Naing C**, Mak JW, Ahmed SI, Maung M. Relationship between hepatitis C virus infection and type 2 diabetes mellitus: meta-analysis. *World J Gastroenterol* 2012; **18**: 1642-1651 [PMID: 22529694 DOI: 10.3748/wjg.v18.i14.1642]
  - 16 **White DL**, Ratzliff V, El-Serag HB. Hepatitis C infection and risk of diabetes: a systematic review and meta-analysis. *J Hepatol* 2008; **49**: 831-844 [PMID: 18814931 DOI: 10.1016/j.jhep.2008.08.006]
  - 17 **Ruhl CE**, Menke A, Cowie CC, Everhart JE. Relationship of hepatitis C virus infection with diabetes in the U.S. population. *Hepatology* 2014; **60**: 1139-1149 [PMID: 24500979 DOI: 10.1002/hep.27047]
  - 18 **Zornitzki T**, Malnick S, Lysy L, Knobler H. Interferon therapy in hepatitis C leading to chronic type 1 diabetes. *World J Gastroenterol* 2015; **21**: 233-239 [PMID: 25574096 DOI: 10.3748/wjg.v21.i1.233]
  - 19 **Hui JM**, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. *Gastroenterology* 2003; **125**: 1695-1704 [PMID: 14724822 DOI: 10.1053/j.gastro.2003.08.032]
  - 20 **Petit JM**, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, Brun JM, Hillon P. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. *J Hepatol* 2001; **35**: 279-283 [PMID: 11580152 DOI: 10.1016/S0168-8278(01)00143-X]
  - 21 **Sougleri M**, Labropoulou-Karatza C, Paraskevopoulou P, Fragopanagou H, Alexandrides T. Chronic hepatitis C virus infection without cirrhosis induces insulin resistance in patients with alpha-thalassaemia major. *Eur J Gastroenterol Hepatol* 2001; **13**: 1195-1199 [PMID: 11711776 DOI: 10.1097/00042737-200110000-00012]
  - 22 **Hickman IJ**, Powell EE, Prins JB, Clouston AD, Ash S, Purdie DM, Jonsson JR. In overweight patients with chronic hepatitis C, circulating insulin is associated with hepatic fibrosis: implications for therapy. *J Hepatol* 2003; **39**: 1042-1048 [PMID: 14642624 DOI: 10.1016/S0168-8278(03)00463-X]
  - 23 **Milner KL**, van der Poorten D, Trenell M, Jenkins AB, Xu A, Smythe G, Dore GJ, Zekry A, Weltman M, Fragomeli V, George J, Chisholm DJ. Chronic hepatitis C is associated with peripheral rather than hepatic insulin resistance. *Gastroenterology* 2010; **138**: 932-941.e1-3 [PMID: 19962985 DOI: 10.1053/j.gastro.2009.11.050]
  - 24 **Shintani Y**, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; **126**: 840-848 [PMID: 14988838 DOI: 10.1053/j.gastro.2003.11.056]
  - 25 **Aytug S**, Reich D, Sapir LE, Bernstein D, Begum N. Impaired IRS-1/PI3-kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. *Hepatology* 2003; **38**: 1384-1392 [PMID: 14647049 DOI: 10.1016/j.hep.2003.09.012]
  - 26 **Tsochatzis E**, Manolakopoulos S, Papatheodoridis GV, Hadziyannis E, Triantos C, Zisimopoulos K, Goulis I, Tzourmakliotis D, Akriviadis E, Manesis EK, Archimandritis AJ. Serum HCV RNA levels and HCV genotype do not affect insulin resistance in nondiabetic patients with chronic hepatitis C: a multicentre study. *Aliment Pharmacol Ther* 2009; **30**: 947-954 [PMID: 19604179 DOI: 10.1111/j.1365-2036.2009.04094.x]
  - 27 **Péres DP**, Cheinquer H, Wolf FH, Cheinquer N, Falavigna M, Péres LD. Prevalence of insulin resistance in chronic hepatitis C genotype 1 and 3 patients. *Ann Hepatol* 2013; **12**: 871-875 [PMID: 24114816]
  - 28 **Knobler H**, Schattner A. TNF- $\alpha$ , chronic hepatitis C and diabetes: a novel triad. *QJM* 2005; **98**: 1-6 [PMID: 15625348 DOI: 10.1093/qjmed/hci001]
  - 29 **Zylberberg H**, Rimaniol AC, Pol S, Masson A, De Groote D, Berthelot P, Bach JF, Bréchet C, Zavala F. Soluble tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. *J Hepatol* 1999; **30**: 185-191 [PMID: 10068094 DOI: 10.1016/S0168-8278(99)80060-9]
  - 30 **Zick Y**. Uncoupling insulin signalling by serine/threonine phosphorylation: a molecular basis for insulin resistance. *Biochem Soc Trans* 2004; **32**: 812-816 [PMID: 15494022 DOI: 10.1042/BST0320812]
  - 31 **Ruan H**, Hacohen N, Golub TR, Van Parijs L, Lodish HF. Tumor necrosis factor- $\alpha$  suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes: nuclear factor- $\kappa$ B activation by TNF- $\alpha$  is obligatory. *Diabetes* 2002; **51**: 1319-1336 [PMID: 11978627 DOI: 10.2337/diabetes.51.5.1319]
  - 32 **Itoh Y**, Okanoue T, Ohnishi N, Sakamoto M, Nishioji K, Nakagawa Y, Minami M, Murakami Y, Kashima K. Serum levels of soluble tumor necrosis factor receptors and effects of interferon therapy in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 1999; **94**: 1332-1340 [PMID: 10235215 DOI: 10.1111/j.1572-0241.1999.01083.x]
  - 33 **Knobler H**, Zornitzki T, Sandler A, Haran N, Ashur Y, Schattner A. Tumor necrosis factor- $\alpha$ -induced insulin resistance may mediate the hepatitis C virus-diabetes association. *Am J Gastroenterol* 2003; **98**: 2751-2756 [PMID: 14687828 DOI: 10.1111/j.1572-0241.2003.08728.x]
  - 34 **Kawaguchi T**, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, Taniguchi E, Kumemura H, Hanada S, Maeyama M, Baba S, Koga H, Kumashiro R, Ueno T, Ogata H, Yoshimura A, Sata M. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 2004; **165**: 1499-1508 [PMID: 15509521 DOI: 10.1016/S0002-9440(10)63408-6]
  - 35 **Alberstein M**, Zornitzki T, Zick Y, Knobler H. Hepatitis C core protein impairs insulin downstream signalling and regulatory role of IGFBP-1 expression. *J Viral Hepat* 2012; **19**: 65-71 [PMID: 22187946 DOI: 10.1111/j.1365-2893.2011.01447.x]
  - 36 **Bernsmeier C**, Duong FH, Christen V, Pugnale P, Negro F, Terracciano L, Heim MH. Virus-induced over-expression of protein phosphatase 2A inhibits insulin signalling in chronic hepatitis C. *J Hepatol* 2008; **49**: 429-440 [PMID: 18486982 DOI: 10.1016/j.jhep.2008.04.007]
  - 37 **Milner KL**, Jenkins AB, Trenell M, Tid-Ang J, Samocha-Bonet D, Weltman M, Xu A, George J, Chisholm DJ. Eradicating hepatitis C virus ameliorates insulin resistance without change in adipose depots. *J Viral Hepat* 2014; **21**: 325-332 [PMID: 24716635 DOI: 10.1111/jvh.12143]
  - 38 **Kawaguchi T**, Ide T, Taniguchi E, Hirano E, Itou M, Sumie S, Nagao Y, Yanagimoto C, Hanada S, Koga H, Sata M. Clearance of HCV improves insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate 1 and 2. *Am J Gastroenterol* 2007; **102**: 570-576 [PMID: 17222321 DOI: 10.1111/j.1572-0241.2006.01038.x]
  - 39 **Brandman D**, Bacchetti P, Ayala CE, Maher JJ, Khalili M. Impact of insulin resistance on HCV treatment response and impact of HCV treatment on insulin sensitivity using direct measurements of insulin action. *Diabetes Care* 2012; **35**: 1090-1094 [PMID: 22399695 DOI: 10.2337/dc11-1837]
  - 40 **Mangia A**, Ripoli M. Insulin resistance, steatosis and hepatitis C virus. *Hepatol Int* 2013; **7** Suppl 2: 782-789 [PMID: 24587848 DOI: 10.1007/s12072-013-9460-1]
  - 41 **Hassan MM**, Abdel-Wahab R, Kaseb A, Shalaby A, Phan AT, El-



- Serag HB, Hawk E, Morris J, Singh Raghav KP, Lee JS, Vauthey JN, Bortus G, Torres HA, Amos CI, Wolff RA, Li D. Obesity Early in Adulthood Increases Risk but Does Not Affect Outcomes of Hepatocellular Carcinoma. *Gastroenterology* 2015; **149**: 119-129 [PMID: 25836985 DOI: 10.1053/j.gastro.2015.03.044]
- 42 **El-Serag HB**, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460-468 [PMID: 14762783 DOI: 10.1053/j.gastro.2003.10.065]
- 43 **El-Serag HB**. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004; **127**: S27-S34 [PMID: 15508094 DOI: 10.1053/j.gastro.2004.09.013]
- 44 **Svegliati-Baroni G**, Ridolfi F, Di Sario A, Casini A, Marucci L, Gaggiotti G, Orlandoni P, Macarri G, Perego L, Benedetti A, Folli F. Insulin and insulin-like growth factor-1 stimulate proliferation and type I collagen accumulation by human hepatic stellate cells: differential effects on signal transduction pathways. *Hepatology* 1999; **29**: 1743-1751 [PMID: 10347117 DOI: 10.1002/hep.510290632]
- 45 **Alexia C**, Fallot G, Lasfer M, Schweizer-Groyer G, Groyer A. An evaluation of the role of insulin-like growth factors (IGF) and of type-I IGF receptor signalling in hepatocarcinogenesis and in the resistance of hepatocarcinoma cells against drug-induced apoptosis. *Biochem Pharmacol* 2004; **68**: 1003-1015 [PMID: 15313394 DOI: 10.1016/j.bcp.2004.05.029]
- 46 **Patel S**, Jinjuvadia R, Patel R, Liangpunsakul S. Insulin Resistance is Associated With Significant Liver Fibrosis in Chronic Hepatitis C Patients: A Systemic Review and Meta-Analysis. *J Clin Gastroenterol* 2016; **50**: 80-84 [PMID: 26302498 DOI: 10.1097/MCG.0000000000000400]
- 47 **Dev A**, Patel K, McHutchison JG. Hepatitis C and steatosis. *Clin Liver Dis* 2004; **8**: 881-892, ix [PMID: 15464660 DOI: 10.1016/j.cld.2004.06.007]
- 48 **Abenavoli L**, Masarone M, Peta V, Milic N, Kobylak N, Rouabhia S, Persico M. Insulin resistance and liver steatosis in chronic hepatitis C infection genotype 3. *World J Gastroenterol* 2014; **20**: 15233-15240 [PMID: 25386071 DOI: 10.3748/wjg.v20.i41.15233]
- 49 **Lonardo A**, Adinolfi LE, Loria P, Carulli N, Ruggiero G, Day CP. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. *Gastroenterology* 2004; **126**: 586-597 [PMID: 14762795 DOI: 10.1053/j.gastro.2003.11.020]
- 50 **Moriya K**, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. *Nat Med* 1998; **4**: 1065-1067 [PMID: 9734402 DOI: 10.1038/2053]
- 51 **Bortoletto G**, Scribano L, Realdon S, Marcolongo M, Mirandola S, Franceschini L, Bonisegna S, Noventa F, Plebani M, Martines D, Alberti A. Hyperinsulinaemia reduces the 24-h virological response to PEG-interferon therapy in patients with chronic hepatitis C and insulin resistance. *J Viral Hepat* 2010; **17**: 475-480 [PMID: 19878535 DOI: 10.1111/j.1365-2893.2009.01204.x]
- 52 **Grasso A**, Malfatti F, De Leo P, Martines H, Fabris P, Toscanini F, Anselmo M, Menardo G. Insulin resistance predicts rapid virological response in non-diabetic, non-cirrhotic genotype 1 HCV patients treated with peginterferon alpha-2b plus ribavirin. *J Hepatol* 2009; **51**: 984-990 [PMID: 19695729 DOI: 10.1016/j.jhep.2009.07.008]
- 53 **Fattovich G**, Covolo L, Pasino M, Perini E, Rossi L, Brocco G, Guido M, Cristofori C, Belotti C, Puoti M, Gaeta GB, Santantonio T, Raimondo G, Bruno R, Minola E, Negro F, Donato F. The homeostasis model assessment of the insulin resistance score is not predictive of a sustained virological response in chronic hepatitis C patients. *Liver Int* 2011; **31**: 66-74 [PMID: 20840397 DOI: 10.1111/j.1478-3231.2010.02343.x]
- 54 **Khattab M**, Eslam M, Sharwae MA, Shatat M, Ali A, Hamdy L. Insulin resistance predicts rapid virologic response to peginterferon/ribavirin combination therapy in hepatitis C genotype 4 patients. *Am J Gastroenterol* 2010; **105**: 1970-1977 [PMID: 20234345 DOI: 10.1038/ajg.2010.110]
- 55 **Moucari R**, Ripault MP, Martinot-Peignoux M, Voitot H, Cardoso AC, Stern C, Boyer N, Maylin S, Nicolas-Chanoine MH, Vidaud M, Valla D, Bedossa P, Marcellin P. Insulin resistance and geographical origin: major predictors of liver fibrosis and response to peginterferon and ribavirin in HCV-4. *Gut* 2009; **58**: 1662-1669 [PMID: 19671541 DOI: 10.1136/gut.2009.185074]
- 56 **Conjeevaram HS**, Kleiner DE, Everhart JE, Hoofnagle JH, Zacks S, Afdhal NH, Wahed AS. Race, insulin resistance and hepatic steatosis in chronic hepatitis C. *Hepatology* 2007; **45**: 80-87 [PMID: 17187406 DOI: 10.1002/hep.21455]
- 57 **Romero-Gómez M**, Del Mar Vitoria M, Andrade RJ, Salmerón J, Diago M, Fernández-Rodríguez CM, Corpas R, Cruz M, Grande L, Vázquez L, Muñoz-De-Rueda P, López-Serrano P, Gila A, Gutiérrez ML, Pérez C, Ruiz-Extremera A, Suárez E, Castillo J. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; **128**: 636-641 [PMID: 15765399 DOI: 10.1053/j.gastro.2004.12.049]
- 58 **Lam KD**, Bacchetti P, Abbasi F, Ayala CE, Loeb SM, Shah V, Wen MJ, Reaven GM, Maher JJ, Khalili M. Comparison of surrogate and direct measurement of insulin resistance in chronic hepatitis C virus infection: impact of obesity and ethnicity. *Hepatology* 2010; **52**: 38-46 [PMID: 20578127 DOI: 10.1002/hep.23670]
- 59 **Park SK**, Cho YK, Park JH, Kim HJ, Park DI, Sohn CI, Jeon WK, Kim BI. Change of insulin sensitivity in hepatitis C patients with normal insulin sensitivity: a 5-year prospective follow-up study variation of insulin sensitivity in HCV patients. *Intern Med J* 2010; **40**: 503-511 [PMID: 19712201 DOI: 10.1111/j.1445-5994.2009.02042.x]
- 60 **Giordanino C**, Bugianesi E, Smedile A, Ciancio A, Abate ML, Olivero A, Pellicano R, Cassader M, Gambino R, Bo S, Ciccone G, Rizzetto M, Saracco G. Incidence of type 2 diabetes mellitus and glucose abnormalities in patients with chronic hepatitis C infection by response to treatment: results of a cohort study. *Am J Gastroenterol* 2008; **103**: 2481-2487 [PMID: 18702647 DOI: 10.1111/j.1572-0241.2008.02002.x]
- 61 **Eslam M**, Aparcero R, Kawaguchi T, Del Campo JA, Sata M, Khattab MA, Romero-Gomez M. Meta-analysis: insulin resistance and sustained virological response in hepatitis C. *Aliment Pharmacol Ther* 2011; **34**: 297-305 [PMID: 21623851 DOI: 10.1111/j.1365-2036.2011.04716.x]
- 62 **Deltenre P**, Louvet A, Lemoine M, Mourad A, Fartoux L, Moreno C, Henrion J, Mathurin P, Serfaty L. Impact of insulin resistance on sustained response in HCV patients treated with pegylated interferon and ribavirin: a meta-analysis. *J Hepatol* 2011; **55**: 1187-1194 [PMID: 21703195 DOI: 10.1016/j.jhep.2011.03.010]
- 63 **Falkner B**, Cossrow ND. Prevalence of metabolic syndrome and obesity-associated hypertension in the racial ethnic minorities of the United States. *Curr Hypertens Rep* 2014; **16**: 449 [PMID: 24819559 DOI: 10.1007/s11906-014-0449-5]
- 64 **Pattullo V**, Duarte-Rojo A, Soliman W, Vargas-Vorackova F, Sockalingam S, Fantus IG, Allard J, Heathcote J. A 24-week dietary and physical activity lifestyle intervention reduces hepatic insulin resistance in the obese with chronic hepatitis C. *Liver Int* 2013; **33**: 410-419 [PMID: 23278982 DOI: 10.1111/liv.12041]
- 65 **Romero-Gómez M**, Diago M, Andrade RJ, Calleja JL, Salmerón J, Fernández-Rodríguez CM, Solà R, García-Samaniego J, Herreras JM, De la Mata M, Moreno-Otero R, Nuñez O, Oliveira A, Durán S, Planas R. Treatment of insulin resistance with metformin in naïve genotype 1 chronic hepatitis C patients receiving peginterferon alfa-2a plus ribavirin. *Hepatology* 2009; **50**: 1702-1708 [PMID: 19845037 DOI: 10.1002/hep.23206]
- 66 **Yu JW**, Sun LJ, Zhao YH, Kang P, Yan BZ. The effect of metformin on the efficacy of antiviral therapy in patients with genotype 1 chronic hepatitis C and insulin resistance. *Int J Infect Dis* 2012; **16**: e436-e441 [PMID: 22486858 DOI: 10.1016/j.ijid.2012.02.004]
- 67 **Harrison SA**. Liver disease in patients with diabetes mellitus. *J Clin Gastroenterol* 2006; **40**: 68-76 [PMID: 16340637 DOI: 10.1097/01.mcj.0000190774.91875.d2]
- 68 **Harrison SA**, Hamzeh FM, Han J, Pandya PK, Sheikh MY,

- Vierling JM. Chronic hepatitis C genotype 1 patients with insulin resistance treated with pioglitazone and peginterferon alpha-2a plus ribavirin. *Hepatology* 2012; **56**: 464-473 [PMID: 22334369 DOI: 10.1002/hep.25661]
- 69 **Overbeck K**, Genné D, Golay A, Negro F. Pioglitazone in chronic hepatitis C not responding to pegylated interferon-alpha and ribavirin. *J Hepatol* 2008; **49**: 295-298 [PMID: 18555553 DOI: 10.1016/j.jhep.2008.03.033]
- 70 **Sumie S**, Kawaguchi T, Kawaguchi A, Kuromatsu R, Nakano M, Satani M, Yamada S, Okamura S, Yonezawa Y, Kakuma T, Torimura T, Sata M. Effect of pioglitazone on outcome following curative treatment for hepatocellular carcinoma in patients with hepatitis C virus infection: A prospective study. *Mol Clin Oncol* 2015; **3**: 115-120 [PMID: 25469280 DOI: 10.3892/mco.2014.435]
- 71 **Pawlotsky JM**, Feld JJ, Zeuzem S, Hoofnagle JH. From non-A, non-B hepatitis to hepatitis C virus cure. *J Hepatol* 2015; **62**: S87-S99 [PMID: 25920094 DOI: 10.1016/j.jhep.2015.02.006]
- 72 **Nasrollah L**, Backstedt DW, Pedersen MR, Choi M, Seetharam AB. Tu1022 Diabetes and hyperlipidemia compromise practical effectiveness of direct acting antiviral HCV therapy in minority populations. *Gastroenterology* 2015; **148** (Suppl 1): S-1087 [DOI: 10.1016/S0016-5085(15)33711-2]
- 73 **Webster DP**, Klenerman P, Dusheiko GM. Hepatitis C. *Lancet* 2015; **385**: 1124-1135 [PMID: 25687730 DOI: 10.1016/S0140-6736(14)62401-6]
- 74 **Gondeau C**, Pageaux GP, Larrey D. Hepatitis C virus infection: Are there still specific problems with genotype 3? *World J Gastroenterol* 2015; **21**: 12101-12113 [PMID: 26576095 DOI: 10.3748/wjg.v21.i42.12101]
- 75 **Antaki N**, Craxi A, Kamal S, Moucari R, Van der Merwe S, Haffar S, Gadano A, Zein N, Lai CL, Pawlotsky JM, Heathcote EJ, Dusheiko G, Marcellin P. The neglected hepatitis C virus genotypes 4, 5 and 6: an international consensus report. *Liver Int* 2010; **30**: 342-355 [PMID: 20015149 DOI: 10.1111/j.1478-3231.2009.02188.x]
- 76 **Lim SG**. Chronic hepatitis C genotype 1 treatment roadmap for resource constrained settings. *World J Gastroenterol* 2015; **21**: 1972-1981 [PMID: 25684966 DOI: 10.3748/wjg.v21.i6.1972]

**P- Reviewer:** Kovacs SJ, Liang J **S- Editor:** Song XX

**L- Editor:** A **E- Editor:** Liu SQ





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

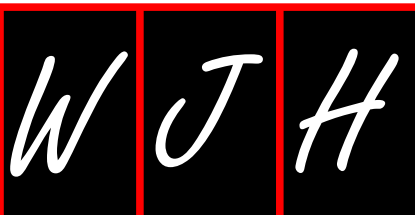


# World Journal of *Hepatology*

*World J Hepatol* 2016 January 28; 8(3): 139-206







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 469 members, representing a team of worldwide experts in hepatology. They are from 53 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (1), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (98), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (19), Greece (11), Hungary (5), India (15), Indonesia (2), Iran (4), Israel (1), Italy (52), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (11), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (17), and United States (56).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*

Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*  
Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*

Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *São Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*



**Chile**

Luis A Videla, *Santiago*



## China

Guang-Wen Cao, Shanghai  
 En-Qiang Chen, Chengdu  
 Gong-Ying Chen, Hangzhou  
 Jin-lian Chen, Shanghai  
 Jun Chen, Changsha  
 Alfred Cheng, Hong Kong  
 Chun-Ping Cui, Beijing  
 Shuang-Suo Dang, Xi'an  
 Ming-Xing Ding, Jinhua  
 Zhi-Jun Duang, Dalian  
 He-Bin Fan, Wuhan  
 Xiao-Ming Fan, Shanghai  
 James Yan Yue Fung, Hong Kong  
 Yi Gao, Guangzhou  
 Zuo-Jiong Gong, Wuhan  
 Zhi-Yong Guo, Guangzhou  
 Shao-Liang Han, Wenzhou  
 Tao Han, Tianjin  
 Jin-Yang He, Guangzhou  
 Ming-Liang He, Hong Kong  
 Can-Hua Huang, Chengdu  
 Bo Jin, Beijing  
 Shan Jin, Hohhot  
 Hui-Qing Jiang, Shijiazhuang  
 Wan-Yee Joseph Lau, Hong Kong  
 Guo-Lin Li, Changsha  
 Jin-Jun Li, Shanghai  
 Qiang Li, Jinan  
 Sheng Li, Jinan  
 Zong-Fang Li, Xi'an  
 Xu Li, Guangzhou  
 Xue-Song Liang, Shanghai  
 En-Qi Liu, Xi'an  
 Pei Liu, Shenyang  
 Zhong-Hui Liu, Changchun  
 Guang-Hua Luo, Changzhou  
 Yi Lv, Xi'an  
 Guang-Dong Pan, Liuzhou  
 Wen-Sheng Pan, Hangzhou  
 Jian-Min Qin, Shanghai  
 Wai-Kay Seto, Hong Kong  
 Hong Shen, Changsha  
 Xiao Su, Shanghai  
 Li-Ping Sun, Beijing  
 Wei-Hao Sun, Nanjing  
 Xue-Ying Sun, Harbin  
 Hua Tang, Tianjin  
 Ling Tian, Shanghai  
 Eric Tse, Hong Kong  
 Guo-Ying Wang, Changzhou  
 Yue Wang, Beijing  
 Shu-Qiang Wang, Chengdu  
 Mary MY Wayne, Hong Kong  
 Hong-Shan Wei, Beijing  
 Danny Ka-Ho Wong, Hong Kong  
 Grace Lai-Hung Wong, Hong Kong  
 Bang-Fu Wu, Dongguan  
 Feng Wu, Chongqing  
 Xiong-Zhi Wu, Tianjin  
 Chun-Fang Xu, Suzhou  
 Rui-An Xu, Quanzhou  
 Rui-Yun Xu, Guangzhou  
 Wei-Li Xu, Shijiazhuang  
 Shi-Ying Xuan, Qingdao  
 Ming-Xian Yan, Jinan  
 Lv-Nan Yan, Chengdu  
 Jin Yang, Hangzhou  
 Ji-Hong Yao, Dalian  
 Winnie Yeo, Hong Kong

Zheng Zeng, Beijing  
 Qi Zhang, Hangzhou  
 Shi-Jun Zhang, Guangzhou  
 Xiao-Lan Zhang, Shijiazhuang  
 Xiao-Yong Zhang, Guangzhou  
 Xin-Chen Zhang, Harbin  
 Yong Zhang, Xi'an  
 Hong-Chuan Zhao, Hefei  
 Ming-Hua Zheng, Wenzhou  
 Yu-Bao Zheng, Guangzhou  
 Ren-Qian Zhong, Shanghai  
 Fan Zhu, Wuhan  
 Xiao Zhu, Dongguan



## Czech Republic

Kamil Vyslouzil, Olomouc



## Denmark

Henning Gronbaek, Aarhus  
 Christian Mortensen, Hvidovre



## Egypt

Ihab T Abdel-Raheem, Damanshour  
 NGB G Bader EL Din, Cairo  
 Hatem Elalfy, Mansoura  
 Mahmoud M El-Bendary, Mansoura  
 Mona El SH El-Raziky, Cairo  
 Mohammad El-Sayed, Cairo  
 Yasser M Fouad, Minia  
 Mohamed AA Metwally, Benha  
 Hany Shehab, Cairo  
 Mostafa M Sira, Shebin El-koom  
 Ashraf Taye, Minia  
 MA Ali Wahab, Mansoura



## France

Laurent Alric, Toulouse  
 Sophie Conchon, Nantes  
 Daniel J Felmlee, Strasbourg  
 Herve Lerat, Creteil  
 Dominique Salmon, Paris  
 Jean-Pierre Vartanian, Paris



## Germany

Laura E Buitrago-Molina, Hannover  
 Enrico N De Toni, Munich  
 Oliver Ebert, Muenchen  
 Rolf Gebhardt, Leipzig  
 Janine V Hartl, Regensburg  
 Sebastian Hinz, Kiel  
 Benjamin Juntermanns, Essen  
 Roland Kaufmann, Jena  
 Viola Knop, Frankfurt  
 Veronika Lukacs-Kornek, Homburg  
 Benjamin Maasoumy, Hannover  
 Jochen Mattner, Erlangen  
 Nadja M Meindl-Beinker, Mannheim  
 Ulf P Neumann, Aachen  
 Margarete Odenthal, Cologne  
 Yoshiaki Sunami, Munich

Christoph Roderburg, Aachen  
 Frank Tacke, Aachen  
 Yuchen Xia, Munich



## Greece

Alex P Betrosian, Athens  
 George N Dalekos, Larissa  
 Ioanna K Delladetsima, Athens  
 Nikolaos K Gatselis, Larissa  
 Stavros Gourgiotis, Athens  
 Christos G Savopoulos, Thessaloniki  
 Tania Siahaniidou, Athens  
 Emmanouil Sinakos, Thessaloniki  
 Nikolaos G Symeonidi, Thessaloniki  
 Konstantinos C Thomopoulos, Larissa  
 Konstantinos Tziomalos, Thessaloniki



## Hungary

Gabor Banhegyi, Budapest  
 Peter L Lakatos, Budapest  
 Maria Papp, Debrecen  
 Ferenc Sipos, Budapest  
 Zsolt J Tulassay, Budapest



## India

Deepak N Amarapurkar, Mumbai  
 Girish M Bhopale, Pune  
 Sibnarayan Datta, Tezpur  
 Nutan D Desai, Mumbai  
 Sorabh Kapoor, Mumbai  
 Jaswinder S Maras, New Delhi  
 Nabeen C Nayak, New Delhi  
 C Ganesh Pai, Manipal  
 Amit Pal, Chandigarh  
 K Rajeshwari, New Delhi  
 Anup Ramachandran, Vellore  
 D Nageshwar Reddy, Hyderabad  
 Shivaram P Singh, Cuttack  
 Ajith TA, Thrissur  
 Balasubramaniyan Vairappan, Pondicherry



## Indonesia

Cosmas RA Lesmana, Jakarta  
 Neneng Ratnasari, Yogyakarta



## Iran

Seyed M Jazayeri, Tehran  
 Sedigheh Kafi-Abad, Tehran  
 Iradj Maleki, Sari  
 Fakhraddin Naghibalhossaini, Shiraz



## Israel

Stephen DH Malnick, Rehovot



## Italy

Francesco Angelico, Rome

Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Andrea Galli, *Florence*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Salvatore Gruttadauria, *Palermo*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*



**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*  
 Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*

Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhito Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*



**Jordan**

Kamal E Bani-Hani, *Zarqa*



**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*



**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*



**Moldova**

Angela Peltec, *Chishinev*



**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*



**Nigeria**

CA Asabamaka Onyekwere, *Lagos*



**Pakistan**

Bikha Ram Devrajani, *Jamshoro*



**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*



**Poland**

Jacek Zielinski, *Gdansk*



**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*



**Qatar**

Reem Al Olaby, *Doha*



**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*



**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*



**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*



**Singapore**

Ser Yee Lee, *Singapore*



**South Korea**

Young-Hwa Chung, *Seoul*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonju*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*



**Spain**

Ivan G Marina, *Madrid*  
 Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*

José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodríguez-Frias, *Córdoba*  
 Manuel L Rodríguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Traperó-Marugán, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpaboon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*  
 Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*

Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



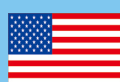
#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*



#### **United States**

Naim Alkhouri, *Cleveland*  
 Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*

Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Grigoriy E Gurvits, *New York*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Kusum K Kharbanda, *Omaha*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*





## Contents

Three issues per month Volume 8 Number 3 January 28, 2016

### TOPIC HIGHLIGHT

- 139 Advances in hepatitis C therapy: What is the current state - what come's next?  
*Zopf S, Kremer AE, Neurath MF, Siebler J*

### REVIEW

- 148 Management of immunosuppressant agents following liver transplantation: Less is more  
*Ascha MS, Ascha ML, Hanouneh IA*
- 162 Innate immunity and hepatocarcinoma: Can toll-like receptors open the door to oncogenesis?  
*Lopes JAG, Borges-Canha M, Pimentel-Nunes P*
- 183 Sofosbuvir treatment and hepatitis C virus infection  
*Nakamura M, Kanda T, Haga Y, Sasaki R, Wu S, Nakamoto S, Yasui S, Arai M, Imazeki F, Yokosuka O*

### MINIREVIEWS

- 191 Ablation techniques for primary and metastatic liver tumors  
*Ryan MJ, Willatt J, Majdalany BS, Kielar AZ, Chong S, Ruma JA, Pandya A*

### ORIGINAL ARTICLE

#### Observational Study

- 200 Cirrhotic cardiomyopathy: Isn't stress evaluation always required for the diagnosis?  
*Barbosa M, Guardado J, Marinho C, Rosa B, Quelhas I, Lourenço A, Cotter J*

## ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Mohammad K Parvez, PhD, Assistant Professor, Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 22451, Saudi Arabia

## AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

INDEXING/  
ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

## FLYLEAF

I-IV Editorial Board

EDITORS FOR  
THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
January 28, 2016

## COPYRIGHT

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

## SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

## INSTRUCTIONS TO AUTHORS

Full instructions are available online at [http://www.wjgnet.com/1948-5182/g\\_info\\_20100316080002.htm](http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm)

## ONLINE SUBMISSION

<http://www.wjgnet.com/esps/>

## 2016 Hepatitis C Virus: Global view

# Advances in hepatitis C therapy: What is the current state - what come's next?

Steffen Zopf, Andreas E Kremer, Markus F Neurath, Juergen Siebler

Steffen Zopf, Andreas E Kremer, Markus F Neurath, Juergen Siebler, Medical Department 1, University of Erlangen-Nuremberg, 91054 Erlangen, Germany

**Author contributions:** Zopf S, Kremer AE, Neurath MF and Siebler J made substantial contributions to conception and design of the manuscript; Zopf S and Kremer AE wrote the paper; Neurath MF and Siebler J critically revised the manuscript.

**Conflict-of-interest statement:** The authors have no conflict of interest to report.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Steffen Zopf, MD, Medical Department 1, University of Erlangen-Nuremberg, Ulmenweg 18, 91054 Erlangen, Germany. [steffen.zopf@uk-erlangen.de](mailto:steffen.zopf@uk-erlangen.de)  
Telephone: +49-9131-8535000  
Fax: +49-9131-8535207

Received: April 29, 2015

Peer-review started: May 8, 2015

First decision: September 8, 2015

Revised: December 15, 2015

Accepted: January 5, 2016

Article in press: January 7, 2016

Published online: January 28, 2016

## Abstract

Chronic hepatitis C virus (HCV) infection affects 80-160 million people worldwide and is one of the leading causes of chronic liver disease. It is only a few years ago that standard treatment regimes were based on

pegylated interferon alpha and ribavirin. However, treatment of HCV has undergone a revolutionary change in recent years. The admission of the nucleotide polymerase inhibitor Sofosbuvir enabled an interferon-free regimen with direct antiviral agents (DAA). Meanwhile seven DAAs are available and can be applied in several combinations for 8 to 24 wk depending on HCV genotype and patient characteristics such as cirrhosis and chronic renal failure. High rates of sustained virological response (SVR) rates can be achieved with these novel drugs. Even in difficult to treat populations such as patients with liver cirrhosis, HCV-human immunodeficiency virus co-infections, after liver transplantation, or with chronic kidney disease comparable high rates of SVR can be achieved. The anticipated 2<sup>nd</sup> generation DAAs are strikingly effective in patients so far classified as difficult to treat including decompensated liver cirrhosis or post-transplant patients. These 2<sup>nd</sup> generations DAAs will have higher resistance barriers, higher antiviral effects and a pan-genotypic spectrum. This review highlights the current state of the art of antiviral treatment in hepatitis C and gives an outlook for upcoming therapies.

**Key words:** Hepatitis C virus; Direct antiviral agents; Sustained virological response; Liver transplantation; Renal impairment; Cirrhosis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Treatment of chronic hepatitis C virus (HCV) infections has undergone a revolutionary change in recent years. This review highlights the current state of the art of antiviral treatment in chronic hepatitis C infections and gives an outlook for upcoming therapies. Difficult to treat populations such as patients with decompensated liver cirrhosis, HCV-human immunodeficiency virus co-infections, after liver transplantation and patients with renal impairment or on hemodialysis are highlighted.

Zopf S, Kremer AE, Neurath MF, Siebler J. Advances in hepatitis C therapy: What is the current state - what comes next? *World J Hepatol* 2016; 8(3): 139-147 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i3/139.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i3.139>

## INTRODUCTION

Chronic hepatitis C virus (HCV) infection is one of the leading causes of chronic liver disease worldwide<sup>[1]</sup>. Worldwide 80-160 million people are estimated to be chronically infected with HCV<sup>[2]</sup>. The long-term follow of chronic HCV are highly variable, ranging from minimal histological changes to extensive fibrosis with or without cirrhosis<sup>[2]</sup>.

The primary goal of HCV therapy is to achieve eradication of the HCV which is currently determined by a sustained virological response (SVR) as surrogate marker. SVR is defined as undetectable HCV RNA 12 wk (SVR 12) or 24 wk (SVR 24) after end of treatment.

In recent years antiviral therapy has experienced a tremendous progress. It is only a few years ago that standard treatment regimes were based on pegylated interferon alpha and ribavirin (P/R). These therapies were associated with many adverse effects, long treatment durations of usually 48 wk and low SVR rates<sup>[3,4]</sup>. In 2011, the two protease inhibitors boceprevir and telaprevir were approved for treatment of genotype 1. Due to their comparatively lower antiviral effectiveness and rapid resistance development both drugs were used only as triple-therapy regimen in combination with pegylated interferon alpha and ribavirin<sup>[5-8]</sup>.

Since 2014 several direct acting antivirals (DAAs) have been approved enabling interferon-free antiviral treatments with high SVR rates.

The decoding of the HCV life cycle and the resolution of crystal structure of the relevant viral proteins enabled the development of many DAAs. The currently approved DAAs consist of three groups. The first group is directed against the viral protease NS3/4A (protease inhibitors; name ending on -previr), the second against the viral RNA-dependent RNA-polymerase NS5B (polymerase inhibitors, name ending on -buvir) and the third against the viral protein which is involved in the formation of the replicon complex NS5A (NS5A-inhibitors, name ending on -asvir)<sup>[9]</sup>.

## DAAs SUBSTANCES

### Sofosbuvir

Sofosbuvir (SOF) is an inhibitor of the NS5B-polymerase. As nucleotide analogue it causes chain termination during replication. SOF has a pan-genotypic effectiveness and a high resistance barrier. It is taken once daily with a good tolerability. No cross resistances with other substances have been reported. Drug interactions were described only for strong inducers of the gut transporters P-gp and

BCRP (e.g., rifampicin, St John's wort, carbamazepin, phenytoin)<sup>[10]</sup>.

### Simeprevir

Simeprevir (SMV) is a second-generation protease inhibitor. In addition to the activity against genotype 1 a simeprevir has clinically relevant antiviral effects against genotypes 4 and 6. Similar to boceprevir it needs to be taken only once a day. Adverse effects reported in clinical studies consisted mainly of skin lesions with or without itching, nausea and dyspnea. Of note, the variant (RAV) Q80K exhibits a preexisting resistance against simeprevir resulting in treatment failure of patients with genotype 1a<sup>[11]</sup>.

As SMV is metabolized by hepatic CYP3A4, inhibitors and inducers of CYP3A4 may affect plasma levels of SMV<sup>[12]</sup>.

### Daclatasvir

Daclatasvir (DCV) is a NS5A-inhibitor. DCV has a high antiviral activity against genotypes 1 to 4 *in vivo* and *in vitro* also against genotypes 5 and 6. On the other side the resistance barrier is relatively low. In case of treatment failure resistance-associated resistances (RAVs) may be selected, which remain detectable after end of treatment<sup>[13]</sup>. The influence of these RAVs on following therapies has not been systematically investigated so far. In studies using P/R the combination of DCV plus P/R showed no additional adverse effects<sup>[14]</sup>. Similar to SMV, DCV is also metabolized by CYP3A4.

### Ledipasvir

Ledipasvir (LDV) represents another NS5A-inhibitor with antiviral activity particularly against genotype 1 and partially against other genotypes such as 4-6. LDV is only available in a fix dose combination with SOF. The most commonly reported adverse effects were headache and fatigue. As with DCV, RAVs have been detected during therapy and were not clinically relevant due to the strong antiviral effect of SOF<sup>[15]</sup>.

*In vitro*, no detectable metabolism of ledipasvir was observed by human CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Evidence of slow oxidative metabolism *via* an unknown mechanism has been observed. Biliary excretion of unchanged ledipasvir is a major route of elimination, with renal excretion being a minor pathway (approximately 1%).

### Paritaprevir/ritonavir + ombitasvir ± dasabuvir

The combination of paritaprevir/ritonavir (PTV/r) + ombitasvir (OBV) ± dasabuvir (DSV) is referred to as 3D. PTV is an NS3/4A-inhibitor, which is boosted by r to optimize its pharmacokinetics. OBV is an NS5A-inhibitor, while DSV is a non-nucleotide polymerase-inhibitor. PTV/r and OBV are available at fix dose combinations and have antiviral activity against genotypes 1 and 4, while DSV is only effective against genotype 1. Registered adverse effects were pruritus in clinical study cohorts



**Table 1** Current recommendations of antiviral regimens depending on the genotype

DAA-regime	HCV-genotype						
	1a	1b	2	3	4	5	6
SOF + R	(x)	(x)	x	x	(x)	(x)	(x)
SOF + SMV ± R	x	x			x		
SOF + DCV <sup>1</sup> ± R	x	x	(x)	x	x		
SOF + LDV ± R	x	x		(x)	x	x	x
OBV + PTV/r ± DSV ± R (3D)	x	x					
OBV + PTV/r ± R (2D)					x		

<sup>1</sup>DCV, no approval in the United States. DAA: Direct antiviral agents; LDV: Ledipasvir; SMV: Simeprevir; DCV: Daclatasvir; SOF: Sofosbuvir; OBV: Ombitasvir; PTV: Paritaprevir; HCV: Hepatitis C virus; DSV: Dasabuvir; R: Ribavirin; r: Ritonavir.

without ribavirin and in very few patient increased transaminases as well as elevations of bilirubin. As PTV/r is metabolized by CYP3A4 interactions with several other drugs may occur. There are existing cross-resistances to other protease-inhibitors and NS5A-inhibitors while DSV has a low resistance-barrier<sup>[16]</sup>.

## DAA COMBINATION REGIMENS

Of the approved DAAs the following combinations have been studied in clinical trials (Table 1).

SOF is the so-called backbone of most combinations, as it has a high resistance barrier and a pan-genotypic activity. In contrast, the high antiviral activity is achieved by the combination of the various groups of substances in the 3D-regime. In all regimes the addition of R is possible and may be useful for defined patient groups.

### SOF + R

This combination has high SVR-rates (86%-97%) in genotype 2 patients<sup>[10,17]</sup>. In genotype 1, however, SVR-rates were inconsistently in phase 2 studies. Especially in difficult to treat patients, *e.g.*, with cirrhosis, SVR-rates were unsatisfactory (SVR 10%-84%)<sup>[18]</sup>. Furthermore, genotype 3 treatment efficacy during a 12-wk regimen was low with SVR rates between 30% to 56%<sup>[10,17]</sup>. However, a significant increase in SVR-rates up to 85% could be achieved by extending the treatment duration to 24 wk in patients with genotype 3. Existence of cirrhosis was associated with poorer SVR-rates in genotype 3 patients (SVR 68% with cirrhosis and pre-treatment with P/R vs SVR 91% without cirrhosis)<sup>[19]</sup>. Smaller studies treating genotype 4 patients for 12 and 24 wk, respectively, have shown SVR-rates in therapy-naïve patients of 79% and 100%, respectively, and in pretreated patients 59% and 87%, respectively<sup>[20]</sup>.

### SOF + SMV ± R

The COSMOS-study, a phase II trial, analyzed the efficacy of SOF + SMV with and without R in patients with HCV genotype 1. This study consisted of two cohorts of which the first represented patients with null-response to P/R but without advanced fibrosis or

cirrhosis. The second cohort included patients with advanced fibrosis (F3) or cirrhosis. Patients were treated for 12 or 24 wk with and without R. In the first cohort a cumulative SVR of 90% was observed, while in the second cohort an even higher SVR of 94% could be achieved. Neither the extension to a 24-wk treatment nor the addition of R were of any advantage in this study<sup>[12]</sup>.

These results could be confirmed by two big observational-studies. The TRIO-trial was able to demonstrate a higher SVR in genotype 1b, compared to 1a (92% vs 80%)<sup>[21]</sup>. The TARGET-study with 883 genotype 1 patients (54% cirrhosis) treated with SOF + SMV ± R presented in an interim analysis a SVR4-rate of 93% in 98 patients without cirrhosis and in patients a SVR4 of 85% in 124 cirrhotics<sup>[22]</sup>.

Meanwhile results of two phase-III study entitled OPTIMIST 1 and 2 have been presented. In the OPTIMIST 1-study 310 naïve or pretreated genotype 1 patients without cirrhosis were treated with SOF + SMV for 8 or 12 wk. Patients treated for 12 wk achieved SVR-rates of 97% and those treated only for 8 wk 83%<sup>[23]</sup>. The OPTIMIST 2-study investigated 103 naïve or pretreated genotype 1 patients with cirrhosis being treated for 12 wk with SOF + SMV resulting in a SVR of 83%<sup>[24]</sup>.

### SOF + DCV ± R

The combination of SOF + DCV was investigated in treatment-naïve patients with genotypes 1, 2 and 3 without cirrhosis and in genotype 1-patients with treatment-failure of a protease-inhibitor based therapy. The treatment response in GT1 was investigated in different groups in a phase II -study. The results showed high SVR-rates between 93%-100% regardless of treatment duration and addition of R. In pretreated genotype 1 patients only 24 wk of therapy were evaluated with or without R. This regimen resulted in SVR-rates of 95%-100%. The ALLY-1 study investigated SOF + DCV + R for 12 wk in patients with cirrhosis (*n* = 60) or after orthotopic liver-transplantation (OLT) (*n* = 53). For genotype 1, patients with cirrhosis achieved a SVR rate of 82%, whereas the SVR rate after OLT was even higher with 95%<sup>[25]</sup>.

Initially only a 24-wk treatment was evaluated in genotype 2 and 3 patients. This lead to SVR rates of 92% in genotype 2 and 89% in genotype 3 patients<sup>[26]</sup>. In the ALLY-3 study genotype 3 patients were treated with SOF + DCV for 12 wk without R. Patients without cirrhosis achieved independent of pretreatment high SVR-rates of 97% in naïve and 94% in pretreated patients. In case of cirrhosis SVR rates were lower with only 58% to 69%<sup>[27]</sup>. The combination of SOF and DCV has an antiviral effectiveness in genotype 4 but results of studies are lacking.

### SOF + LDV ± R

For this fixe dose combination profound phase 3 study data exists. Studies were performed on 1.952 patients

**Table 2 Overview on clinical studies using the NSSB inhibitor sofosbuvir**

Study	Patient population	Therapy	Duration (wk)	SVR	Comments
ION-1	First-line therapy	SOF + LDV	12	99%	
		SOF + LDV + R	12	97%	
		SOF + LDV	24	98%	
		SOF + LDV + R	24	99%	
ION-2	Re-therapy	SOF + LDV	12	94%	86% cirrhotic
		SOF + LDV + R	12	96%	82% cirrhotic
		SOF + LDV	24	99%	100% cirrhotic
		SOF + LDV + R	24	99%	86% cirrhotic
ION-3	First-line therapy	SOF + LDV	8	94%	Only non-cirrhotic
		SOF + LDV + R	8	93%	Only non-cirrhotic
		SOF + LDV	12	95%	Only non-cirrhotic

SOF: Sofosbuvir; LDV: Ledipasvir; R: Ribavirin; SVR: Sustained virological response.

**Table 3 Overview on clinical studies using the combination of paritaprevir/ritonavir + ombitasvir ± dasabuvir**

Study	Patient population	Therapy	Duration (wk)	SVR	Comments
SAPPHIRE- I	First-line therapy	OBV + PTV/r + DSV + R	12	96%	No cirrhosis
SAPPHIRE- II	Re-therapy	OBV + PTV/r + DSV + R	12	96%	No cirrhosis
TURQUOISE- II	Cirrhosis	OBV + PTV/r + DSV + R	12	92%	GT1a 89%
					GT1b 99%
		OBV + PTV/r + DSV + R	24	96%	GT1a 95%
PEARL- II	Re-therapy, GT1b	OBV + PTV/r + DSV	12	100%	No cirrhosis
		OBV + PTV/r + DSV + R	12	97%	No cirrhosis
PEARL- III	First-line therapy, GT1b	OBV + PTV/r + DSV	12	99%	No cirrhosis
		OBV + PTV/r + DSV + R	12	99%	No cirrhosis
PEARL- IV	First-line therapy, GT1a	OBV + PTV/r + DSV	12	90%	No cirrhosis
		OBV + PTV/r + DSV + R	12	97%	No cirrhosis

OBV: Ombitasvir; PTV: Paritaprevir; DSV: Dasabuvir; R: Ribavirin; r: Ritonavir; SVR: Sustained virological response.

with genotype 1. The detailed results of the ION-studies are shown in Table 2. In all treatment-groups high rates of SVR were observed. In treatment-naïve patients there was neither advantage of treatment for more than 12 wk nor addition of R even in cirrhotic patients<sup>[28]</sup>. In contrast, pretreated patients with cirrhosis achieved higher SVR-rates after treatment for 24 wk. In these patients high SVR-rates could also be achieved by adding R to a 12 wk antiviral treatment<sup>[15]</sup>.

Treatment-naïve genotype 1-patients without cirrhosis achieved a SVR of 94% after only 8 wk of treatment. The higher number of relapses in this cohort were patients with a viral load above  $6 \times 10^6$  IU/mL<sup>[29]</sup>.

For genotypes 3 and 6 data from a small study showed also a high antiviral effectiveness for SOF/LDV + R. Genotype 3 treatment-naïve patients with cirrhosis achieved 100% SVR, pretreated patients with cirrhosis 89% SVR. Without addition of R only 64% of the treatment-naïve cirrhotic patients achieved SVR<sup>[30,31]</sup>. In genotype 6 SOF/LDV without R resulted in SVR-rates of 96%<sup>[31]</sup>.

### OBV + PTV/r ± DSV ± R (3D)

The 3D-regimen achieved high SVR-rates in several phase III-studies with a total of 1577 genotype 1

patients. The detailed results of these studies are shown in Table 3. The 3D-regimen achieved in genotype 1 patients without cirrhosis in the SAPPHIRE-study high SVR-rates of 96% regardless of a prior therapy<sup>[32,33]</sup>.

Due to a weaker antiviral activity of 3D in genotype 1a the PEARL-studies (which did not include cirrhotic patients) were performed separately for both subtypes. The treatment-regimes differed regarding the addition of R. In genotype 1b nearly all patients achieved SVR without R regardless of pretreatment indicating that R can be omitted for this patient population<sup>[16,34]</sup>. In contrast, genotype 1a patients exhibited higher SVR rates by addition of R compared to those being treated without R (97% vs 90%)<sup>[16]</sup>.

Genotype 1 patients with cirrhosis were investigated in the TURQUOISE- II -study. Here, 3D + R was admitted for 12 or 24 wk. For genotype 1a extension of treatment from 12 to 24 wk resulted in higher SVR-rates (89% vs 95%), whereas for genotype 1b nearly all patients were cured by a 12-wk treatment<sup>[35]</sup>.

In genotype 4 DSV was omitted due to lack of antiviral activity. Therefore, a combination of OBV + PTV/r with and without R was tested for 12 wk in the PEARL-I-study. Addition of R resulted in a SVR rate of 100%, whereas without R only 91% of patients

achieved SVR<sup>[36]</sup>.

Current treatment recommendations have been published in the AASLD and EASL guidelines. These take into account the specific conditions in different countries in terms of availability of DAAs<sup>[2,37]</sup>.

So far there are currently sufficient IFN-free DAA-regimes with excellent SVR-rates, in particular for genotype 1 patients. Unresolved issues represent patients with relapse after DAA-regimen as they exhibit RAVs and cirrhotic patients with genotype 3 as SVR rates remain unsatisfactory for this population. Current antiviral studies address these challenges and in the near future we expect efficient regimens for the remaining difficult to treat HCV patients.

## DIFFICULT TO TREAT POPULATIONS

### Cirrhotic patients

Liver cirrhosis is the most important negative predictor of SVR in DAA therapies. In nearly all regimens treatment efficacy is lower compared to non-cirrhotic patients. In pivotal studies on cirrhotic patients for the 3D-regime and SOF/LDV SVR could be increased by treatment extension to 24 wk and addition of ribavirin. It should be noted that only compensated patients with Child Pugh stage A were included in these studies. Recently, in a prospective study 108 patients with decompensated cirrhosis with Child Pugh stage B and C were treated with SOF/LDV + R for 12 or 24 wk. SVR was achieved in 87% of patients with Child Pugh B and 89% with Child Pugh C indicating that this therapy regimen is safe and effective even in decompensated liver cirrhosis. Of note, an improvement of liver function was observed during and after therapy<sup>[38]</sup>.

### Liver transplantation

After liver transplantation of patients with chronic HCV infections a reinfection is common. Due to immuno-suppression an accelerated progression of fibrosis in the transplant is often observed. Pre-treatment with SOF + R before transplantation in 61 patients with HCC within Milan criteria and compensated HCV-induced cirrhosis prevented a reinfection of the graft in 70%<sup>[39]</sup>. After liver transplantation a different study using the 3D-regime + R resulted in a SVR of 97% (33 out of 34 patients)<sup>[40]</sup>. Using SOF/LDV + R for 12 or 24 wk in 223 patients after liver transplantation similar high SVR rates could be achieved in patients without cirrhosis (96%-98%) or Child Pugh A cirrhosis (96%). However, in case of decompensated cirrhosis SVR rates were lower (Child Pugh B: 83%-85%, Child Pugh C: 60%-67%)<sup>[41]</sup>.

### Human immunodeficiency virus-HCV co-infection

Human immunodeficiency virus (HIV)-HCV co-infections result in a faster progression of fibrosis compared to mono-infections. In a variety of studies with similar designs comparable treatment responses were found. Thus, HIV-HCV co-infected patients can be treated equal to mono-infected patients. To give an example

the combination-therapy with SOF/LDV achieved SVR-rate of 98% in GT1 first line therapy<sup>[42]</sup>. It should be noted that possible drug-drug interactions between HCV regimes and antiretroviral substances may occur.

### Renal impairment

Due to its renal elimination SOF may only be given to patients with a glomerular filtration rate above 30 mL/min per 1.73 m<sup>2</sup>. Patients with severe renal impairment (glomerular filtration rate < 30 mL/min per 1.73 m<sup>2</sup>) or chronic renal failure undergoing dialysis therefore require other antiviral regimens.

Pharmacokinetic data showed the possibility of using OBV + PTV/r + DSV ± R (3D) in patients with severe renal impairment and chronic renal failure. Serum levels of the 3D substances were comparable to HCV-patients without renal impairment. SVR12 data are outstanding but all finished patients (10 out of 20) presented SVR4<sup>[43]</sup>.

Another placebo-controlled study with Grazoprevir (100 mg) plus Elbasvir (50 mg) for 12 wk in 122 HCV GT 1 patients with renal impairment (75% under dialysis) presented SVR12 rates of 94%, representing a potential future treatment regimen in this difficult to treat patient population<sup>[44]</sup>.

## FUTURE HCV TREATMENT OPTIONS

Future developments consist of second generation DAAs. The protease inhibitors of the second generation will exhibit a better resistance barrier and broader spectrum of activity against various genotypes of HCV, in particular subtype 1a (e.g., Grazoprevir, Sovaprevir) and pan-genotypic (e.g., ABT-493, GS-9857). Moreover, these substances do not have complete cross-resistances against associated RAVs against first-generation protease inhibitors (e.g., ABT-493)<sup>[45]</sup>.

The second generation NS5A-inhibitors will have higher resistance barriers, higher antiviral effects and a pan-genotypic spectrum (e.g., Elbasvir, Samatasvir, GS-5816, MK-8408, ABT-530). ABT-530 presented *in vitro* high antiviral effectiveness against frequent NS5A-RAVs<sup>[45]</sup>. Furthermore, novel drugs will be used as combination-regimes. Combinations of these protease inhibitors and NS5A-inhibitors could achieve similar SVR-rates than previous regimes based on nucleotide NS5B-polymerase inhibitors (NUC). It is likely that shorter treatment durations may be achieved using these regimens. A recently presented study using Grazoprevir/Elbasvir + SOF (C-SWIFT) in treatment-naïve genotype 1 and 3 patients with and without cirrhosis investigated the antiviral effectiveness in terms of treatment duration. In genotype 1 non-cirrhotic patients achieved SVR in only 33% after 4 wk of treatment. In contrast, an SVR-rate of 87% was achieved after 6 wk of treatment. Cirrhotic patients with HCV-genotype 1 achieved SVR in 80% after 6 wk and SVR in 94% after 8 wk of treatment. In genotype 3 non-cirrhotic patients were treated for 8 and 12 wk resulting in SVR-rates of 93% and 100%.

**Table 4 Overview on clinical studies using future antiviral drugs and combinations**

Substances (study)	RBV	Genotype	Population	Duration (wk)	Phase	Results (SVR)
SOF + GS-5816 (NS5A) <sup>[47]</sup>	±	1	TN, NCi	8	II	81% - R; 90% + R; (n = 60)
	±	2	TN, NCi	8	II	88% - R; 88% + R; (n = 52)
	±	3	TN, NCi	8	II	96% - R; 100% + R; (n = 53)
	±	1	TE, Ci, NCi	12	II	NCi: 100% (n = 38); Ci: 100% - R, 90% + R (n = 17)
	±	3	TE, Ci, NCi	12	II	NCi: 100% (n = 53); Ci: 88% - R, 96% + R (n = 52)
SOF + ACH-3102 (NS5A) (PROXY) <sup>[48]</sup>	-	1	TN, NCi	6 and 8	II	100% after 6 wk (n = 12) or 8 wk (n = 12)
SOF/LDV + Vedroprevir (SYNERGY) <sup>[49]</sup>	-	1	TN, NCi	6	II	95% (n = 20)
SOF/LDV + GS 9669 (non-NUC-NS5B) (SYNERGY) <sup>[49]</sup>	-	1	TN, NCi	6	II	95% (n = 20)
SOF + Grazoprevir + Elbasvir (C-SWIFT) <sup>[46]</sup>	-	1, 3	TN, NCi	4 or 6 (GT1)	II	SVR8: GT1: 39% after 4 wk (n = 31); 87% after 6 wk (n = 30)
				8 or 12 (GT3)		GT3: 100% after 8 and 12 wk (n = 15/14)
			TN, Ci	6 or 8 (GT1)	II	SVR8: GT1: 80% after 6 wk (n = 20); 89% after 8 wk (n = 21)
				12 (GT3)		GT3: 90% (n = 12)
Grazoprevir + Elbasvir (C-WORTHY) <sup>[50]</sup>	±	1	TN, NCi	8 or 12	II	8 wk GT1a: 80%
						12 wk: 98% - R; 93% + R
			TN, Ci	12 or 18	II	12 wk: 97% - R; 90% + R
						18 wk: 94% - R; 97% + R
			TE, Ci, NCi	12 or 18	II	12 wk: 91% - R; 94% + R
						18 wk: 97% - R; 100% + R
SMV + Samatasvir (HELIX-1) <sup>[51]</sup>	+	1b, 4	TN, NCi	12	II	SVR4: GT1b: 80% (n = 84)
						GT4: 100% (n = 9)
Asunaprevir + DCV + Beclabuvir (UNITY 1) <sup>[52]</sup>	-	1	TN, TE, NCi	12	III	TN: 91%; TE: 89%
Asunaprevir + DCV + Beclabuvir (UNITY 2) <sup>[53]</sup>	±	1	TN, TE, Ci	12	III	90% - R; 96% + R

TN: Therapy-naïve; TE: Therapy-experienced; Ci: Cirrhosis; NCi: No cirrhosis; GT: Hepatitis C virus-genotype; SOF: Sofosbuvir; LDV: Ledipasvir; R: Ribavirin; SVR: Sustained virological response; SMV: Simeprevir; DCV: Daclatasvir.

Cirrhotic genotype 3 patients being treated only for 12 wk achieved a SVR-rate of 91%<sup>[46]</sup>. These future substances will enable a shortened treatment time and increase the antiviral activity in individual populations. A selection of future therapies and their phase II - III trial results are presented in Table 4<sup>[46-53]</sup>.

As of mid 2016, the 2<sup>nd</sup> generations DAAs are expected to be available. These therapy regimes will have pan-genotypic and high antiviral effectiveness as well as a better resistance profile. It is likely that R will become dispensable and treatment duration may be reduced to 6-8 wk for the majority of patients. In addition, difficult to treat populations (e.g., genotype 3 with cirrhosis) may achieve higher rates of SVR.

However, in order to significantly lower HCV-induced morbidity and mortality, a higher proportion of infected patients would have to be treated. This will only be possible by an increased screening of risk populations and customized pricing.

## REFERENCES

- 1 Lavanchy D. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011; **17**: 107-115 [PMID: 21091831 DOI: 10.1111/j.1469-0691.2010.03432.x]
- 2 European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2014. *J Hepatol* 2014; **61**: 373-395 [PMID: 24818984 DOI: 10.1016/j.jhep.2014.05.001]
- 3 Antaki N, Craxi A, Kamal S, Moucari R, Van der Merwe S, Haffar S, Gadano A, Zein N, Lai CL, Pawlotsky JM, Heathcote EJ, Dusheiko G, Marcellin P. The neglected hepatitis C virus genotypes 4, 5 and 6: an international consensus report. *Liver Int* 2010; **30**: 342-355 [PMID: 20015149 DOI: 10.1111/j.1478-3231.2009.02188.x]
- 4 European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]
- 5 Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]
- 6 Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]
- 7 Poordad F, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]
- 8 Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; **364**: 2417-2428 [PMID: 21696308 DOI: 10.1056/NEJMoa1013086]
- 9 Rupp D, Bartenschlager R. Targets for antiviral therapy of hepatitis C. *Semin Liver Dis* 2014; **34**: 9-21 [PMID: 24782254 DOI: 10.1055/s-0034-1371006]



- 10 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
- 11 **Lenz O**, Verbinen T, Fevery B, Tambuyzer L, Vijgen L, Peeters M, Buelens A, Ceulemans H, Beumont M, Picchio G, De Meyer S. Virology analyses of HCV isolates from genotype 1-infected patients treated with simeprevir plus peginterferon/ribavirin in Phase IIb/III studies. *J Hepatol* 2015; **62**: 1008-1014 [PMID: 25445400 DOI: 10.1016/j.jhep.2014.11.032]
- 12 **Lawitz E**, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, DeJesus E, Pearlman B, Rabinovitz M, Gitlin N, Lim JK, Pockros PJ, Scott JD, Fevery B, Lambrecht T, Ouwkerk-Mahadevan S, Callewaert K, Symonds WT, Picchio G, Lindsay KL, Beumont M, Jacobson IM. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naïve patients: the COSMOS randomised study. *Lancet* 2014; **384**: 1756-1765 [PMID: 25078309 DOI: 10.1016/S0140-6736(14)61036-9]
- 13 **Karino Y**, Toyota J, Ikeda K, Suzuki F, Chayama K, Kawakami Y, Ishikawa H, Watanabe H, Hernandez D, Yu F, McPhee F, Kumada H. Characterization of virologic escape in hepatitis C virus genotype 1b patients treated with the direct-acting antivirals daclatasvir and asunaprevir. *J Hepatol* 2013; **58**: 646-654 [PMID: 23178977 DOI: 10.1016/j.jhep.2012.11.012]
- 14 **Hézode C**, Hirschfield CM, Ghesquiere W, Sievert W, Rodriguez-Torres M, Shafraan SD, Thuluvath PJ, Tatum HA, Waked I, Esmat G, Lawitz EJ, Rustgi VK, Pol S, Weis N, Pockros PJ, Bourlière M, Serfaty L, Vierling JM, Fried MW, Weiland O, Brunetto MR, Everson GT, Zeuzem S, Kwo PY, Sulkowski M, Bräu N, Hernandez D, McPhee F, Wind-Rotolo M, Liu Z, Noviello S, Hughes EA, Yin PD, Schnittman S. Daclatasvir plus peginterferon alfa and ribavirin for treatment-naïve chronic hepatitis C genotype 1 or 4 infection: a randomised study. *Gut* 2015; **64**: 948-956 [PMID: 25080450 DOI: 10.1136/gutjnl-2014-307498]
- 15 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]
- 16 **Ferenci P**, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A, Box TD, Younes Z, Enayati P, Green S, Baruch Y, Bhandari BR, Caruntu FA, Sepe T, Chulanov V, Janczewska E, Rizzardini G, Gervain J, Planas R, Moreno C, Hassanein T, Xie W, King M, Podsadecki T, Reddy KR. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *N Engl J Med* 2014; **370**: 1983-1992 [PMID: 24795200 DOI: 10.1056/NEJMoa1402338]
- 17 **Jacobson IM**, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]
- 18 **Gane EJ**, Stedman CA, Hyland RH, Ding X, Svarovskaia E, Symonds WT, Hindes RG, Berrey MM. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. *N Engl J Med* 2013; **368**: 34-44 [PMID: 23281974 DOI: 10.1056/NEJMoa1208953]
- 19 **Zeuzem S**, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]
- 20 **Ruane PJ**, Ain D, Stryker R, Meshrekey R, Soliman M, Wolfe PR, Riad J, Mikhail S, Kersey K, Jiang D, Massetto B, Doehle B, Kirby BJ, Knox SJ, McHutchison JG, Symonds WT. Sofosbuvir plus ribavirin for the treatment of chronic genotype 4 hepatitis C virus infection in patients of Egyptian ancestry. *J Hepatol* 2015; **62**: 1040-1046 [PMID: 25450208 DOI: 10.1016/j.jhep.2014.10.044]
- 21 **Dieterich D**, Bacon BR, Flamm SL, Kowdley KV, Milligan S, Tsai N, Younossi Z, Lawitz E. O046: Evaluation of sofosbuvir and simeprevir-based regimens in the TRIO network: academic and community treatment of a real-world, heterogeneous population. *Hepatology* 2014; **60** Suppl 1: 220a-220a
- 22 **Jensen DM**, O'Leary JG, Pockros PJ, Sherman KE, Kwo PY, Mailliard ME, Kowdley KV, Muir AJ, Dickson RC, Ramani A, Manns MP, Lok AS, Akuskevich L, Nelson DR, Fried MW. O045: Safety and Efficacy of Sofosbuvir-Containing Regimens for Hepatitis C: Real-World Experience in a Diverse, Longitudinal Observational Cohort. *Hepatology* 2014; **60** Suppl 1: 219a-220a
- 23 **Kwo P**, Gitlin N, Nahass R, Bernstein D, Rojter S, Schiff E, Davis M, Ruane PJ, Younes Z, Kalmeijer R, Peeters M, Lenz O, Fevery B, De La Rosa G, Scott J, Sinha R, Witek J. LP14: A Phase 3, Randomised, Open-Label Study to Evaluate the Efficacy and Safety of 8 and 12 Weeks of Simeprevir (Smv) Plus Sofosbuvir (Sof) in Treatment-Naïve and -Experienced Patients with Chronic Hcv Genotype 1 Infection without Cirrhosis: Optimist-1. *J hepatol* 2015; **62** Suppl 2: S270-S270 [DOI: 10.1016/S0168-8278(15)3016-8-9]
- 24 **Lawitz E**, Matusow G, DeJesus E, Yoshida E, Felizarta F, Ghalib R, Godofsky E, Herring R, Poleynd G, Sheikh A, Tobias H, Kugelman M, Kalmeijer R, Peeters M, Lenz O, Fevery B, De La Rosa G, Scott J, Sinha R, Witek J. LP04: A Phase 3, Open-Label, Single-Arm Study to Evaluate the Efficacy and Safety of 12 Weeks of Simeprevir (Smv) Plus Sofosbuvir (Sof) in Treatment-Naïve or -Experienced Patients with Chronic Hcv Genotype 1 Infection and Cirrhosis: Optimist-2. *J hepatol* 2015; **62** Suppl 2: S264-S265 [DOI: 10.1016/S0168-8278(15)30158-6]
- 25 **Poordad F**, Schiff E, Vierling J, Landis C, Fontana R, Yang R, McPhee F, Hughes E, Noviello S, Swenson E. Daclatasvir, sofosbuvir, and ribavirin combination for HCV patients with advanced cirrhosis or post-transplant recurrence: phase 3 ALLY-1 study. *J Viral Hepatitis* 2015; **22**: 30-31
- 26 **Sulkowski MS**, Jacobson IM, Nelson DR. Daclatasvir plus sofosbuvir for HCV infection. *N Engl J Med* 2014; **370**: 1560-1561 [PMID: 24738674 DOI: 10.1056/NEJMoa1401726]
- 27 **Nelson DR**, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R, Oguchi G, Thuluvath PJ, Ortiz-Lasanta G, Rabinovitz M, Bernstein D, Bennett M, Hawkins T, Ravendhran N, Sheikh AM, Varunok P, Kowdley KV, Hennicken D, McPhee F, Rana K, Hughes EA. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology* 2015; **61**: 1127-1135 [PMID: 25614962 DOI: 10.1002/hep.27726]
- 28 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]
- 29 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Di Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]
- 30 **Gane EJ**, Hyland RH, An D, Pang PS, Symonds WT, McHutchison

- JG, Stedman CA. O006: Sofosbuvir/Ledipasvir Fixed Dose Combination Is Safe and Effective in Difficult-to-Treat Populations Including Genotype-3 Patients, Decompensated Genotype-1 Patients, and Genotype-1 Patients with Prior Sofosbuvir Treatment Experience. *J hepatol* 2014; **60**: S3-S4 [DOI: 10.1016/S0168-8278(14)60008-8]
- 31 **Gane EJ**, Hyland RH, An D, Svarovskaia ES, Pang PS, Symonds WT, McHutchison JG, Stedman CA. High Efficacy of LDV/SOF Regimens for 12 Weeks for Patients with HCV Genotype 3 or 6 Infection. *Hepatology* 2014; **60**: 1274a-1275a
  - 32 **Feld JJ**, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, Weiland O, Aguilar H, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1594-1603 [PMID: 24720703 DOI: 10.1056/NEJMoa1315722]
  - 33 **Zeuzem S**, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourliere M, Sulkowski MS, Wedemeyer H, Tam E, Desmond P, Jensen DM, Di Bisceglie AM, Varunok P, Hassanein T, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1604-1614 [PMID: 24720679 DOI: 10.1056/NEJMoa1401561]
  - 34 **Andreone P**, Colombo MG, Enejosa JV, Koksai I, Ferenci P, Maieron A, Mühlhaupt B, Horsmans Y, Weiland O, Reesink HW, Rodrigues L, Hu YB, Podsadecki T, Bernstein B. ABT-450, ritonavir, ombitasvir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. *Gastroenterology* 2014; **147**: 359-365.e1 [PMID: 24818763 DOI: 10.1053/j.gastro.2014.04.045]
  - 35 **Poordad F**, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, Shiffman ML, Wedemeyer H, Berg T, Yoshida EM, Forns X, Lovell SS, Da Silva-Tillmann B, Collins CA, Campbell AL, Podsadecki T, Bernstein B. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med* 2014; **370**: 1973-1982 [PMID: 24725237 DOI: 10.1056/NEJMoa1402869]
  - 36 **Hézode C**, Asselah T, Reddy KR, Hassanein T, Berenguer M, Fleischner-Stepniowska K, Marcellin P, Hall C, Schnell G, Pilot-Matias T, Mobashery N, Redman R, Vilchez RA, Pol S. Ombitasvir plus paritaprevir plus ritonavir with or without ribavirin in treatment-naïve and treatment-experienced patients with genotype 4 chronic hepatitis C virus infection (PEARL-I): a randomised, open-label trial. *Lancet* 2015; **385**: 2502-2509 [PMID: 25837829 DOI: 10.1016/S0140-6736(15)60159-3]
  - 37 **AASLD/IDSA HCV Guidance Panel**. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology* 2015; **62**: 932-954 [PMID: 26111063 DOI: 10.1002/hep.27950]
  - 38 **Flamm SL**, Everson GT, Charlton M, Denning JM, Arterburn S, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal NH. O239: Ledipasvir/Sofosbuvir with Ribavirin for the Treatment of HCV in Patients with Decompensated Cirrhosis: Preliminary Results of a Prospective, Multicenter Study. *Hepatology* 2014; **60** Suppl 1: 320a-321a
  - 39 **Curry MP**, Forns X, Chung RT, Terrault NA, Brown R, Fenkel JM, Gordon F, O'Leary J, Kuo A, Schiano T, Everson G, Schiff E, Befeler A, Gane E, Saab S, McHutchison JG, Subramanian GM, Symonds WT, Denning J, McNair L, Arterburn S, Svarovskaia E, Moonka D, Afdhal N. Sofosbuvir and ribavirin prevent recurrence of HCV infection after liver transplantation: an open-label study. *Gastroenterology* 2015; **148**: 100-107.e1 [PMID: 25261839 DOI: 10.1053/j.gastro.2014.09.023]
  - 40 **Kwo PY**, Mantry PS, Coakley E, Te HS, Vargas HE, Brown R, Gordon F, Levitsky J, Terrault NA, Burton JR, Xie W, Setze C, Badri P, Pilot-Matias T, Vilchez RA, Forns X. An interferon-free antiviral regimen for HCV after liver transplantation. *N Engl J Med* 2014; **371**: 2375-2382 [PMID: 25386767 DOI: 10.1056/NEJMoa1408921]
  - 41 **Charlton M**, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS, Fried MW, Terrault NA, O'Leary JG, Vargas HE, Kuo A, Schiff E, Sulkowski MS, Gilroy R, Watt KD, Brown K, Kwo P, Pungpapong S, Korenblat KM, Muir AJ, Teperman L, Fontana RJ, Denning J, Arterburn S, Dvory-Sobol H, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal N. Ledipasvir and Sofosbuvir Plus Ribavirin for Treatment of HCV Infection in Patients With Advanced Liver Disease. *Gastroenterology* 2015; **149**: 649-659 [PMID: 25985734 DOI: 10.1053/j.gastro.2015.05.010]
  - 42 **Osinusi A**, Townsend K, Kohli A, Nelson A, Seamon C, Meissner EG, Bon D, Silk R, Gross C, Price A, Sajadi M, Sidharthan S, Sims Z, Herrmann E, Hogan J, Teferi G, Talwani R, Proschan M, Jenkins V, Kleiner DE, Wood BJ, Subramanian GM, Pang PS, McHutchison JG, Polis MA, Fauci AS, Masur H, Kottlil S. Virologic response following combined ledipasvir and sofosbuvir administration in patients with HCV genotype 1 and HIV co-infection. *JAMA* 2015; **313**: 1232-1239 [PMID: 25706232 DOI: 10.1001/jama.2015.1373]
  - 43 **Pockros PJ**, Reddy KR, Mantry PS, Cohen E, Bennett M, Sulkowski MS, Bernstein D, Podsadecki T, Cohen D, Shulman NS, Wang D, Khatri A, Abunimeh M, Lawitz E. L01: Safety of Ombitasvir/Paritaprevir/Ritonavir Plus Dasabuvir for Treating Hcv Gt1 Infection in Patients with Severe Renal Impairment or End-Stage Renal Disease: The Ruby-I Study. *J hepatol* 2015; **62** Suppl 2: S257-S257 [DOI: 10.1016/S0168-8278(15)30147-1]
  - 44 **Roth D**, Nelson DR, Bruchfeld A, Liapakis A, Silva M, Monsour H, Martin P, Pol S, Londoño MC, Hassanein T, Zamor PJ, Zuckerman E, Wan S, Jackson B, Nguyen BY, Robertson M, Barr E, Wahl J, Greaves W. Grazoprevir plus elbasvir in treatment-naïve and treatment-experienced patients with hepatitis C virus genotype 1 infection and stage 4-5 chronic kidney disease (the C-SURFER study): a combination phase 3 study. *Lancet* 2015; **386**: 1537-1545 [PMID: 26456905 DOI: 10.1016/S0140-6736(15)00349-9]
  - 45 **Ng T**, Pilot-Matias T, Lu LJ, Reisch T, Dekhtyar T, Krishnan P, Beyer J, Tripathi R, Pithawalla RB, Asatryan A, Campbell AL, Kort J, Collins C. P1946: A Next Generation HCV DAA Combination: Potent, Pangenotypic Inhibitors ABT-493 and ABT-530 with High Barriers to Resistance. *Hepatology* 2014; **60** Suppl 1: 1142a-1142a
  - 46 **Poordad F**, Lawitz E, Gutierrez J, Evans B, Howe A, Feng H, Li J, Hwang P, Robertson M, Wahl J, Barr E, Haber B. O006: C-swift: grazoprevir/elbasvir sofosbuvir in cirrhotic and noncirrhotic, treatment-naïve patients with hepatitis C virus genotype 1 infection, for durations of 4, 6 or 8 weeks and genotype 3 infection for durations of 8 or 12 weeks. *J hepatol* 2015; **62** Suppl 2: S192-S193 [DOI: 10.1016/S0168-8278(15)30013-1]
  - 47 **Pianko S**, Flamm SL, Shiffman ML, Kumar S, Strasser SI, Dore GJ, McNally J, Brainard DM, Han LL, Doehle B, Mogalian E, McHutchison JG, Reddy KR, Roberts SK. O197: High Efficacy of Treatment with Sofosbuvir GS-5816 +/- Ribavirin for 12 Weeks in Treatment Experienced Patients with Genotype 1 or 3 HCV Infection. *Hepatology* 2014; **60** Suppl 1: 297a-298a
  - 48 **Gane E**, Schwabe C, Mader M, Suri V, Donohue M, Huang M, Hui J, Yang J, Robison H, Apelian D, Kocinsky H. LP06: Sustained Virologic Response after Ach-3102 and Sofosbuvir Treatment for 8 or 6 Weeks: A Phase 2 "Proxy" Study. *J hepatol* 2015; **62** Suppl 2: S266-S266 [DOI: 10.1016/S0168-8278(15)30160-4]
  - 49 **Kohli A**, Osinusi A, Sims Z, Nelson A, Meissner EG, Barrett LL, Bon D, Marti MM, Silk R, Kotb C, Gross C, Jolley TA, Sidharthan S, Petersen T, Townsend K, Egerson D, Kapoor R, Spurlin E, Sneller M, Proschan M, Herrmann E, Kwan R, Teferi G, Talwani R, Diaz G, Kleiner DE, Wood BJ, Chavez J, Abbott S, Symonds WT, Subramanian GM, Pang PS, McHutchison J, Polis MA, Fauci AS, Masur H, Kottlil S. Virological response after 6 week triple-drug regimens for hepatitis C: a proof-of-concept phase 2A cohort study. *Lancet* 2015; **385**: 1107-1113 [PMID: 25591505 DOI: 10.1016/S0140-6736(14)61228-9]
  - 50 **Lawitz E**, Gane E, Pearlman B, Tam E, Ghesquiere W, Guyader D, Alric L, Bronowicki JP, Lester L, Sievert W, Ghalib R, Balart L, Sund F, Shagging M, Dutko F, Shaughnessy M, Hwang P, Howe AY, Wahl J, Robertson M, Barr E, Haber B. Efficacy and safety of 12 weeks versus 18 weeks of treatment with grazoprevir (MK-5172) and elbasvir (MK-8742) with or without ribavirin for hepatitis C

virus genotype 1 infection in previously untreated patients with cirrhosis and patients with previous null response with or without cirrhosis (C-WORTHY): a randomised, open-label phase 2 trial. *Lancet* 2015; **385**: 1075-1086 [PMID: 25467591 DOI: 10.1016/S0140-6736(14)61795-5]

- 51 **Lawitz E**, Rodriguez-Torres M, Nguyen T, Sheikh A, Tobias H, Galati J, Hill J, Lok A, Nelson D, Patrick GD, Chen J, Frank D, Zhou XJ, Sullivan-Bolyai Z, Mayers D. P1222: A Phase Ii Study of Samatasvir (Idx719) in Combination with Simeprevir and Ribavirin in Treatment-Naive Hcv-Infected Subjects with Genotypes 1b and 4 (Helix-1 Study). *J hepatol* 2014; **60**: S495-S496 [DOI: 10.1016/S0168-8278(14)61382-9]
- 52 **Poordad F**, Sievert W, Mollison L, Bennett M, Tse E, Bräu N, Levin J, Sepe T, Lee SS, Angus P, Conway B, Pol S, Boyer N,

Bronowicki JP, Jacobson I, Muir AJ, Reddy KR, Tam E, Ortiz-Lasanta G, de Ledinghen V, Sulkowski M, Boparai N, McPhee F, Hughes E, Swenson ES, Yin PD. Fixed-dose combination therapy with daclatasvir, asunaprevir, and beclabuvir for noncirrhotic patients with HCV genotype 1 infection. *JAMA* 2015; **313**: 1728-1735 [PMID: 25942723 DOI: 10.1001/jama.2015.3860]

- 53 **Muir AJ**, Poordad F, Lalezari J, Everson G, Dore GJ, Herring R, Sheikh A, Kwo P, Hézode C, Pockros PJ, Tran A, Yozviak J, Reau N, Ramji A, Stuart K, Thompson AJ, Vierling J, Freilich B, Cooper J, Ghesquiere W, Yang R, McPhee F, Hughes EA, Swenson ES, Yin PD. Daclatasvir in combination with asunaprevir and beclabuvir for hepatitis C virus genotype 1 infection with compensated cirrhosis. *JAMA* 2015; **313**: 1736-1744 [PMID: 25942724 DOI: 10.1001/jama.2015.3868]

**P- Reviewer:** Lee SW, Sanz-Cameno P, Stasi C

**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Liu SQ



## Management of immunosuppressant agents following liver transplantation: Less is more

Mustafa S Ascha, Mona L Ascha, Ibrahim A Hanouneh

Mustafa S Ascha, Mona L Ascha, Ibrahim A Hanouneh, Department of Gastroenterology and Hepatology, Digestive Disease Institute, Cleveland Clinic, Cleveland, OH 44195, United States

**Author contributions:** Ascha MS participated in writing and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted; Ascha ML participated in critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted; Hanouneh IA participated in critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted.

**Conflict-of-interest statement:** All of the authors have no significant conflicts of interest with any companies or organization whose products or services may be discussed in this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Ibrahim A Hanouneh, MD, Department of Gastroenterology and Hepatology, Digestive Disease Institute, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, United States. [hanouni2@ccf.org](mailto:hanouni2@ccf.org)  
 Telephone: +1-216-4441762  
 Fax: +1-216-4446302

Received: July 1, 2015  
 Peer-review started: July 1, 2015  
 First decision: August 31, 2015  
 Revised: December 13, 2015  
 Accepted: January 5, 2016  
 Article in press: January 7, 2016  
 Published online: January 28, 2016

### Abstract

Immunosuppression in organ transplantation was revolutionary for its time, but technological and population changes cast new light on its use. First, metabolic syndrome (MS) is increasing as a public health issue, concomitantly increasing as an issue for post-orthotopic liver transplantation patients; yet the medications regularly used for immunosuppression contribute to dysfunctional metabolism. Current mainstay immunosuppression involves the use of calcineurin inhibitors; these are potent, but nonspecifically disrupt intracellular signaling in such a way as to exacerbate the impact of MS on the liver. Second, the impacts of acute cellular rejection and malignancy are reviewed in terms of their severity and possible interactions with immunosuppressive medications. Finally, immunosuppressive agents must be considered in terms of new developments in hepatitis C virus treatment, which undercut what used to be inevitable viral recurrence. Overall, while traditional immunosuppressive agents remain the most used, the specific side-effect profiles of all immunosuppressants must be weighed in light of the individual patient.

**Key words:** Immunosuppression; Orthotopic liver transplantation; Metabolic syndrome; Acute cellular rejection; Hepatitis C virus

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The use of immunosuppressive agents is reviewed in the context of the modern post-orthotopic liver transplantation population. The side effects of mainstay immunosuppressive strategies exacerbate some patient pathologies, and combinations of different immunosuppressants could be more specifically tailored to patient needs. Acute cellular rejection and malignant complications are also discussed with respect to im-



munosuppressive strategies. Finally, hepatitis C virus and its impact on immunosuppression is re-evaluated in light of recent developments in viral clearance.

Ascha MS, Ascha ML, Hanounch IA. Management of immunosuppressant agents following liver transplantation: Less is more. *World J Hepatol* 2016; 8(3): 148-161 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i3/148.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i3.148>

## INTRODUCTION

The use of immunosuppression in organ transplantation was revolutionary for its time, and its results were quickly embraced for their efficacy at suppressing host rejection of a graft.

However, given the increasing impact of metabolic syndrome (MS) as a public health issue<sup>[1]</sup>, immunosuppression and its side effects may pose a greater risk to patients than rejection of a newly-transplanted organ. In fact, metabolic complications of immunosuppressive therapy were at one point the leading cause of morbidity and mortality for patients following orthotopic liver transplantation (OLT)<sup>[2]</sup>. Reduction of immunosuppression is a widely-recognized strategy to addressing this issue<sup>[3]</sup>.

Cardiovascular disease (CVD) and renal disease account for 19.3% and 6.8% of nonhepatic causes of death in post-OLT patients, respectively<sup>[4]</sup>. In patients who survive at least 3 years, non-hepatic cause of death accounts for 58% of all-cause mortality post-OLT<sup>[5]</sup>. In their evaluation of liver transplantation as a cardiovascular risk factor, Madhwal *et al*<sup>[6]</sup> (2012) found that up to two-thirds of patients develop MS after OLT. Clearly, the trend towards post-transplant metabolic disturbances must be addressed; the first place to start is optimizing post-transplant immunosuppressive therapies<sup>[7]</sup>.

Further, with recent advances in the treatment of hepatitis C virus (HCV)<sup>[8,9]</sup>, long-term metabolic complications posed by immunosuppression must be weighed more heavily against the immediate issue of organ rejection. Just as the introduction of immunosuppression made longer-term complications a new focus in transplantation, eradication of this chronic liver infection leaves room to focus on metabolic issues.

## BRIEF OVERVIEW OF LIVER IMMUNOLOGY: THE LIVER IS IMMUNOPRIVILEGED

From a physiological standpoint, the liver is one of the first organs exposed to the absorbed contents of the external environment. Handling of newly-acquired blood content immediately after absorption from the external environment necessitates that the liver maintain its own unique balance of immunity vs tolerance. The

special status of the liver as immunoprivileged is well-recognized; for example, there is a paucity of hepatic B- and T-cell mediated autoimmune disease, and some autoimmune hepatitis liver markers are found in healthy and ill people alike<sup>[10]</sup>. Further, transplant tolerance is known to occur at greater frequency for liver transplant recipients, compared to other vascularized organ recipients<sup>[11]</sup>. At the same time, there remains much to be learned about liver immunology. For example, the role of humoral alloreactivity in ABO-compatible liver transplantation is still being elucidated<sup>[12-14]</sup>.

The liver is rich with parenchymal hepatocytes, but also contains non-parenchymal immune cells that serve as a first barrier to antigens arriving from portal circulation. Hepatic nonparenchymal cells include the largest population of fixed resident macrophages in the body, Kupffer cells, as well as other reticuloendothelial cells<sup>[15]</sup>. The parenchymal hepatocytes further contribute to immunity by secreting 80%-90% of complement components and pathogen-recognition receptors (PRR), as well as synthesizing membrane-bound PRRs to catch portal antigens<sup>[16]</sup>.

Along with antigens arriving from portal circulation, about 10<sup>8</sup> peripheral blood lymphocytes pass through the liver every 24 h<sup>[17]</sup>. These cells are squeezed through fenestrated capillaries that may open a window to T-cell priming<sup>[18]</sup>. The status of the liver as a major reservoir of immune cells has major implications, then, both as far as catching portal circulation antigens as well as immunomodulation.

For example, the privileged status of the liver might be used in the future to induce complete graft tolerance. As a promising example, animal models have shown a lifetime tolerance to liver grafts: In 20% of outbred pig liver recipients, lifetime tolerance can be achieved without the use of immunosuppressants<sup>[19]</sup>. The possibility of lifetime tolerance in humans, too, remains optimistic. Ramos *et al*<sup>[20]</sup> (1995) review their experience withdrawing immunosuppressive therapy after witnessing patient noncompliance with immunosuppression. They were able to accomplish immunosuppression-free tolerance in 16 patients for 3 to 19 mo, continuing efforts to completely wean 28 patients, but failed in 15 patients without any graft loss or demonstrable loss of function due to rejection.

Not only is the liver self-protective, but there is evidence of immunocompetence conferred to other organs: Simpson *et al*<sup>[21]</sup> (2006) showed that patients who receive combined same-donor liver and kidney transplants are immunoprotected compared to patients who receive kidney transplant after liver transplant. The authors speculate that the identical genotypes of the transplanted organs facilitates immunoprotective effects. There is, however, conflicting data: Katznelson *et al*<sup>[22]</sup> (1996), comparing incidence of acute rejection between 248 combined liver and kidney transplantations to a control group of 206 kidney-alone transplantations, found that 3-year survival rates are not significantly different.

Despite a key role in immunoregulation generally, human leukocyte antigen (HLA) histocompatibility has little clinical significance to liver allograft outcome<sup>[23,24]</sup>. On the other hand, HLA compatibility may play a more subtle role in OLT than we see clinically, where immuno-suppressive regimens may paint broad enough strokes to obfuscate nuances. Neumann *et al.*<sup>[25]</sup> (2003) report that HLA compatibility is associated with significantly less acute rejection, but no difference in graft survival. This peculiar behavior of the liver compared to other organs may further reflect the possibility that acute rejection is not as harmful to OLT as previously thought. A better understanding of the mechanisms of liver immunology is necessary to identify how to maximize the utility of histocompatibility<sup>[26]</sup>.

## IMMUNOSUPPRESSANT AGENT OVERVIEW

There are three signal pathways targeted by immuno-suppressive agents. The first is calcineurin-mediated nuclear factor of activated T-cells activation *via* the T-cell receptor (TCR) and CD3 meeting an antigen presented on an major histocompatibility complex (MHC) protein; the second is a B7/CD28 costimulatory signal required for TCR-MHC complex synapsing; and the third signal is mediated by interleukin 2 (IL-2) as a ligand to CD25, through adaptor proteins JAK3 and PI-3K to mechanistic target of rapamycin (mTOR) regulation of cyclin-dependent kinases and cyclins to control the cell cycle<sup>[27]</sup>.

Figure 1 depicts the process of an antigen-presenting cell (APC) travelling to a lymphoid organ, where it meets T cells in the paracortex. A native T-cell interacts with the APC, and if a set of several interactions and conditions are met, then the native T-cell replicates many times. This is called T-cell activation, and T-cell clonal expansion, and creates numerous effector T-cells that are specific to the antigen originally presented by the APC. These effector T-cells proceed to leave the lymph nodes and head back to the liver, where they can detect antigen and effect an immune response.

Many current immunosuppressive drugs target either those extracellular interactions or the intracellular signals that are highlighted in Figure 1. Calcineurin inhibitors (CNIs) prevent T-cell activation *via* an intracellular pathway, for example; on the other hand, the anti-IL-2 receptor antibodies basiliximab and daclizumab prevent IL-2 receptor activation<sup>[28]</sup>.

Immunosuppressant drugs can function as depleting agents, or as non-depleting agents. Depleting immuno-suppressive therapies cause destruction of T cells and/or B cells<sup>[27]</sup>, whereas non-depleting agents affect the immune system by preventing immune cell proliferation.

CNIs have been the mainstay of immunosuppression since they were discovered, and increased 1-year patient and graft survival to greater than 80%<sup>[29,30]</sup>. CNIs serve to prevent transcription of the autocrine factor IL-2, preventing cell proliferation. These drugs halt the phosphatase activity of calcineurin, which is an

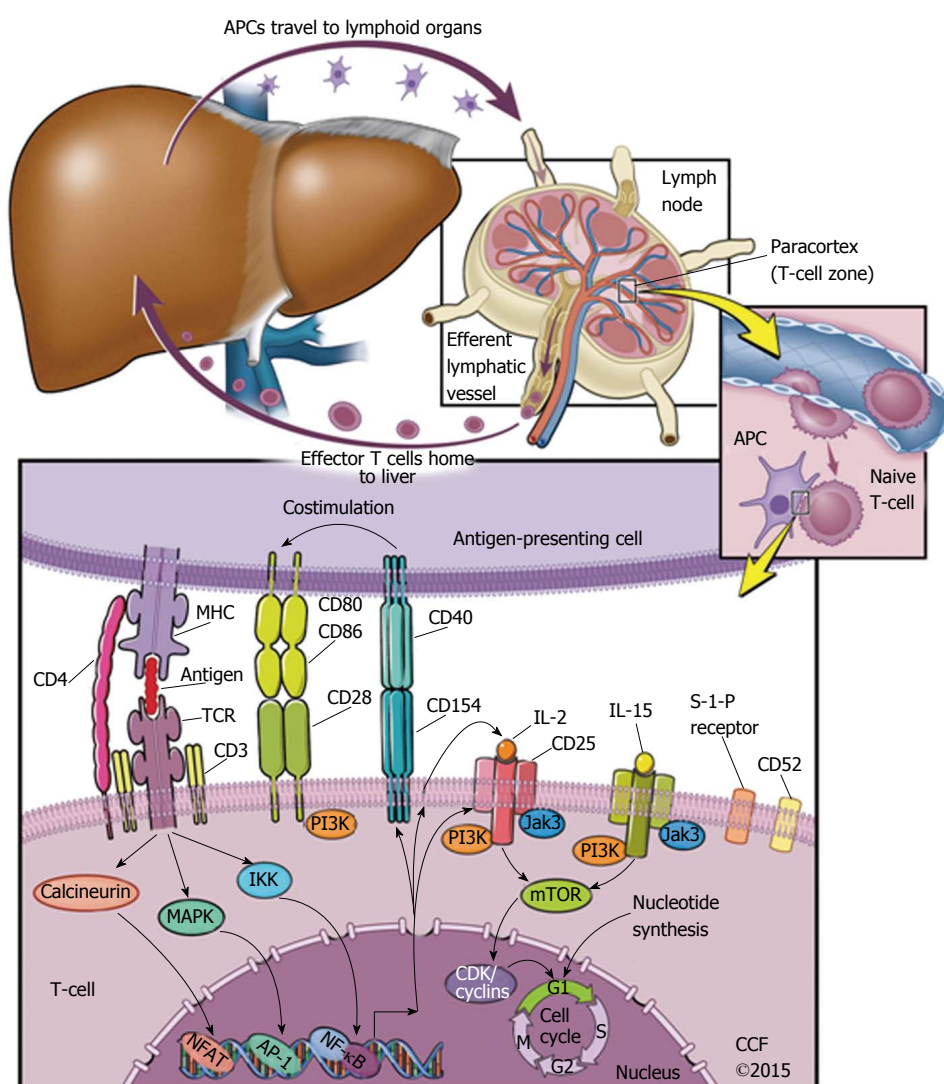
intracellular signal transduction protein that mediates response to antigenic peptides. CNIs include cyclosporin A (CsA) and tacrolimus (TAC). There is no direct reduction in T or B cell count, so these agents are considered non-depleting.

Second, there is the non-depleting immunosuppressant, mycophenolate mofetil (MMF). MMF is a prodrug of mycophenolic acid (MPA), which inhibits inosine monophosphate dehydrogenase (IMPDH). Because IMPDH catalyzes the rate-limiting step of *de novo* guanosine synthesis, both genetic replication and transcription are inhibited. Further, MPA is five times more effective at inhibiting the type II isoform of IMPDH - the isoform expressed in activated lymphocytes<sup>[31]</sup>. Because it is specific to the lymphocyte isoform of IMPDH, MPA prevents lymphocyte proliferation and transcription of activation-associated genes. As far as small molecules go, MMF is one of the more specific immunosuppressant agents. Side effects of MMF include nonimmune issues such as diarrhea and anemia<sup>[27]</sup>, but, more importantly, exacerbation of cytomegalovirus infection<sup>[32]</sup>.

Finally, the third major class of immunosuppressive drugs are called mTOR inhibitors. Found in soil from an Easter Island bacteria called *Streptomyces Hygroscopicus*, this class blocks IL-2-mediated autocrine leukocyte proliferation *via* inhibition of an intracellular signal transduction mechanism - thus, mTOR inhibitors are considered non-depleting agents<sup>[33,34]</sup>. Everolimus (EVR) and sirolimus (SIR) are the two best-known mTOR inhibitors.

Besides their use as immunosuppressants, another interesting property of mTOR inhibitors is their effect on longevity and age-related diseases. It is well-established that one way to increase longevity is through dietary restriction, and this effect is partially mediated by the mTOR pathway. Inhibition of mTORC1 has been associated with protection against neurodegenerative disease, heart disease, metabolic diseases, and a host of other age-related diseases<sup>[35]</sup>. Rapamycin administration, specifically, has repeatedly been shown to increase both mean and median lifespan in genetically heterogenous mice<sup>[36]</sup>.

Immunosuppressive steroids such as methylprednisone are considered essential to graft tolerance induction, yet their use is highly associated with metabolic complications<sup>[37]</sup>. Several studies have examined outcomes of steroid-free or reduced-steroid immunosuppressive maintenance regimens<sup>[38,39]</sup>. In a prospective, randomized, placebo-controlled, double-blinded study, Lerut *et al.*<sup>[40]</sup> (2014) report that in a cohort of 156 patients, patients treated with minimal or steroid-free immunosuppression displayed excellent outcomes over a period of 5 years. They report 5-year biopsy results, finding that histology presents similarly across both groups. In support of steroid withdrawal, other prospective studies of prednisone withdrawal post-OLT have demonstrated no difference in 2-year and 1-year survival of patients treated with or without



**Figure 1 Signaling pathways targeted by modern immunosuppressive agents.** Starting from the top left, an antigen-presenting cell (APC) migrates from local tissue to lymphoid organs. In the paracortex of the lymphoid organ, the APC meets a naive T-cell. If the naive T-cell has a T-cell receptor (TCR) that binds the antigen as it is presented by a Major Histocompatibility Complex on the APC, a set of other T-cell-APC interactions are likely to follow. This includes T-cell CD4 binding to the MHC on an APC, as well as costimulatory signals via extracellular receptors CD28 or CD40. After this set of T-cell-APC interactions begins, a set of intracellular signals follow towards the nucleus of the T-cell. The naive T-cell is then activated, and begins to replicate. This replication is called clonal expansion, and produces a population of T-cells that eventually migrate back to the tissue that contains antigen. AP-1: Activator protein 1; CD: Cluster of differentiation; CDK: Cyclin-dependent kinase; IKK: Inhibitor of kappa-B kinase; IL: Interleukin; Jak3: Janus-associated kinase 3; PI3K: Phosphatidylinositol 3-kinase; MAPK: Mitogen-activated protein kinase; mTOR: Mechanistic target of rapamycin; NFAT: Nuclear factor of activated T-cells; NF- $\kappa$ B: Nuclear factor kappa-light-chain-enhancer of activated B cells; TCR: T-cell receptor; MHC: Major histocompatibility complex.

prednisone<sup>[41,42]</sup>. Today, the use of steroids is generally limited to tolerance induction and treatment of acute cellular rejection (ACR); many studies have further investigated the possibility of steroid-free transplantation<sup>[43-45]</sup>.

Depleting immunosuppressant agents are mostly antibody-based. The first monoclonal antibody to be approved for use in humans was OKT3, an anti-CD3 antibody that modulates T-cell activation<sup>[46,47]</sup>. There are currently a host of biologics directed against T-cell proliferation, these are specific enough that their metabolic impact is significantly less than that of CNIs<sup>[48,49]</sup>.

Issues associated with biological immunosuppression are unlike those of smaller molecules because they act extracellularly and are specific to their antigen<sup>[50]</sup>, in

contrast to small molecules such as tacrolimus that affect intracellular processes across a wider variety of cells. For example, one OKT3 side effect is called cytokine release syndrome, where widespread T-cell activation outweighs the antibody-mediated T-cell destruction that follows it<sup>[51]</sup>. This complication can become more severe if it leads to a positive-feedback loop, potentially causing a "cytokine storm"<sup>[52]</sup>.

Besides OKT3, biologic agents have shown varying degrees of success and outcomes. For example, basiliximab is an IL-2 receptor antagonist that reduces rates of ACR at the expense of increased disease progression in HCV liver transplant patients<sup>[53]</sup>. Basiliximab, though, has been found to successfully treat graft-vs-host disease<sup>[54]</sup>. For those patients with

metabolic dysfunction, avoidance of steroid immunosuppression may be enough of a concern to warrant use of basiliximab.

Another biologic used to treat multiple sclerosis and Crohn's disease, natalizumab, is associated with significant liver injury as a side effect<sup>[55]</sup>. While the medical field is ripe for biologic development, implementation of biologics requires close evaluation.

On the other hand, some biologics open doors that might otherwise stay shut. Rituximab is an anti-CD20 monoclonal antibody that has been successfully used as immunological prophylaxis for ABO-incompatible (ABOi) liver transplantation<sup>[56]</sup>. Further, Yoshizawa *et al.*<sup>[57]</sup> (2005) report that ABOi living-donor liver transplantation is possible without humoral rejection. Their protocol involves hepatic artery infusion and prophylactic use of rituximab, but, unlike previous attempts, does not involve splenectomy. Tanabe *et al.*<sup>[58]</sup> (2010) later explain that ABOi has progressed to the point that it is as effective as ABO compatible transplantation, in part due to rituximab prophylaxis. Perfection of ABOi OLT will hopefully lead to other organ allocation advancements, such as humanized livers grown in non-human primates<sup>[59]</sup>. Immunosuppression in this context may become a hot topic in coming years.

## ACR: DIAGNOSIS AND TREATMENT

ACR is most likely to occur within the first 6 wk of transplant, and is a very common event; in a study of 762 patients, the incidence of ACR post-transplantation is 64%. Several factors are associated with the occurrence of ACR, such as cold ischemic time of the organ, lower age of recipient, presence of edema, and HLA-DR mismatch<sup>[2]</sup>.

There are three distinguishing features of ACR, each visible on histological examination. The first feature is portal triad inflammation, indicated by mixed inflammatory infiltrate; the second feature is damage to the bile ducts, specifically nonsuppurative cholangitis involving interlobular ductal epithelia; third, venous endotheliitis<sup>[60]</sup>. Venous endotheliitis is the most reliable diagnostic sign of ACR, but it is worth noting that phasic increase and decrease in lymphocyte aggregation may affect biopsy results<sup>[2]</sup>.

In the clinic, ACR is suspected upon elevated liver function tests preceding jaundice and fever. Unfortunately, blood tests are neither sensitive nor specific for ACR diagnosis<sup>[61,62]</sup>. It follows, then, that liver biopsy is the gold standard of liver tissue evaluation<sup>[63]</sup>. To provide a level of standardization to biopsy evaluation, the Banff rejection activity index (RAI) assigns a score between zero and three to each characteristic of ACR<sup>[64]</sup>.

Treatment for ACR includes high-dose steroids, optionally tapering off steroids and/or using biological immunosuppression<sup>[65]</sup>. Response to steroids is favorable; however, incidence of steroid-resistant rejection has been found to reach up to 14%<sup>[66]</sup>.

## ACR MORBIDITY AND MORTALITY

Timing of rejection is of major clinical significance in evaluating the potential impact of ACR<sup>[67,68]</sup>. While different studies have used different definitions, one commonly accepted cutoff defines early and late ACR as occurring within and after 90 d of transplantation, respectively. Several studies have found that early ACR is common and of lesser significance than late ACR<sup>[69]</sup>. For example, in a retrospective review of 231 histologically-confirmed cases of early ACR, Höroldt *et al.*<sup>[64]</sup> (2006) report that neither total RAI score nor any of its components were correlated to steroid treatment response or graft survival. Indeed, Thurairajah *et al.*<sup>[70]</sup> (2013), in a retrospective review of 970 patients, confirms that early acute rejection cases yield the best 10-year graft survival rates, at 85%.

In contrast to the minimal impact of early ACR, it appears that late ACR is associated with decreased graft survival. Uemura *et al.*<sup>[69]</sup> (2008) found that of 1604 patients, 19.0% developed ACR later than 6 mo after the transplant; the only predictor of late ACR here was post-transplant lymphoproliferative disease. Thurairajah *et al.*<sup>[70]</sup> (2013) found that 11% of patients developed late ACR, and that the highest rates of late ACR were found in patients with seronegative hepatitis, primary biliary sclerosis, and primary sclerosing cholangitis. Other studies have found that post-transplant lymphoproliferative disorder, decreased age, and increased medication level variability index are associated with and can predict late ACR<sup>[71]</sup>.

Epidemiological evaluation of ACR is masked by its frequently subclinical character. Bartlett *et al.*<sup>[72]</sup> (2002) explain that in a retrospective review examining 15 studies with a total of 1566 patients, 32% of standard protocol post-OLT biopsy samples showed evidence of ACR without any biochemical dysfunction. Since ACR is defined according to biopsy but not serum biomarkers, incidence of ACR may be higher than previously accepted. Another study, Tisone *et al.*<sup>[42]</sup> (1999), explains that 80% of acute rejection episodes in their 45-patient cohort resolved spontaneously. The chances of clinically significant acute rejection, then, must be balanced against the risks of liver biopsy. In the future, metabolomic and other noninvasive studies could shed significant light on incidence of ACR<sup>[73-75]</sup>.

Reduction of immunosuppression in light of ACR is not a new subject: Volpin *et al.*<sup>[76]</sup> (2002), in a controlled study, evaluated two methylprednisolone regimens in the treatment of acute cellular rejection. They find that a 6-d taper regimen is safer than the higher-dose standard because ACR impact on graft rejection is minor, and the toxic effects of methylprednisolone outweigh the potential benefit of ACR suppression. Goddard *et al.*<sup>[77]</sup> (2002) reviewed the Volpin study, concluding that immunosuppression therapies should be tailored to the individual patient after careful consideration of the interaction between past medical history and



immunosuppression side effects.

More recently, Rodríguez-Perálvarez *et al.*<sup>[78]</sup> (2013) report that standard TAC trough concentrations are set too high (at 10–15 ng/mL), and that target TAC levels between 7 and 10 ng/mL are associated with longer graft survival while maintaining safety against rejection.

In contrast to the safety of simply reducing TAC, a randomized prospective trial of SIR monotherapy conversion regimen efficacy<sup>[79]</sup> found that liver transplant patients have no demonstrable benefit at 12 mo. Cumulative rates of graft loss or death were not significantly different, at 6.6% for the SIR group vs 5.6% for the CNI group. However, rates of acute rejection and discontinuation due to side effects were higher for patients treated with SRL. Then, one must consider the characteristics of the individual patient when designing an immunosuppressive regimen. For patients who are at great risk of end-stage renal disease (ESRD), the risk of acute rejection that is posed by conversion to SRL may be outweighed by the nephrotoxicity associated with the use of CNIs. For patients who are more concerned about acute rejection than nephrotoxicity, it makes sense to use CNI therapy.

## MALIGNANT COMPLICATIONS POST-OLT

Recurrent and *de novo* malignancies are the top nonhepatic causes of late death in liver transplant patients, often listed alongside CVD. Some of the increased incidence in *de novo* malignancies in liver transplant recipients compared to the general population can be attributed to the use of exogenous immunosuppression<sup>[80,81]</sup>.

The greatest incidence of post-transplant malignancies is associated with chronic viral infection. Specifically, Epstein-Barr virus-associated post-transplant lymphoproliferative disease, skin cancers, squamous cell carcinoma, and Kaposi's sarcoma are associated with status post-OLT<sup>[82]</sup>. Baccarani *et al.*<sup>[82]</sup> (2009) find that 42 (12.8%) of patients undergoing OLT, out of 330, developed *de novo* cancers. Further, these patients had a lower 10-year survival rate than those who did not develop *de novo* cancer.

In hopes of reducing malignancy, current immunosuppression strategies focus on minimizing TAC with optional use of mTOR inhibitors or MMF. mTOR inhibitors are known for their antineoplastic activity<sup>[83]</sup>, and CNI use can be associated with increased development of malignancy<sup>[84]</sup>, making CNI reduction and replacement with mTOR inhibitors particularly favorable for patients at risk for malignancy.

## MS

MS constitutes a number of symptoms that, when occurring simultaneously, indicate a primary clinical outcome of CVD. The criteria for MS is that a patient meet three of five components: Abdominal obesity

and visceral fat, increased triglyceride (TG), decreased serum high-density lipoprotein, high blood pressure (HTN), insulin resistance and/or glucose intolerance<sup>[85]</sup>. Despite the wide range of systemic effects, each of these symptoms converges towards provoking CVD<sup>[86]</sup>.

The Framingham study<sup>[87]</sup> found that 25% of all new-onset CVD could be predicted by presence of MS. More recently, Watt *et al.*<sup>[4]</sup> (2010) report that causes of death more than 1 year after OLT have the following etiologies: 28% hepatic, 22% malignancy, 11% cardiovascular, 9% infection, and 6% renal failure. They conclude that modifiable risk factors such as diabetes, hypertension, and renal insufficiency can be used to improve long-term outcomes. Table 1 describes some of the interplay between MS components and immunosuppression. Because immunosuppressive strategies can sometimes be altered, relative risks and benefits should be weighed on a case-by-case basis.

### Obesity: The boss of MS

Abdominal obesity is a prevalent characteristic of MS, and adipocyte dysfunction is hypothesized to underlie many metabolic disorders. Some explanations of adipocyte metabolism focus on the location of fat as a determinant of metabolic properties, such that visceral or abdominal fat might contribute more to MS than subcutaneous fat<sup>[88]</sup>; other explanations emphasize immunological modulation of adipocyte metabolism<sup>[89]</sup>. Whatever the underlying cause, obesity is a major public health issue<sup>[90]</sup>, one that may be an environmental hit to a genetic predisposition.

Stegall *et al.*<sup>[91]</sup> (1995) report that the incidence of obesity for adult liver transplant recipients 1 year after transplant was 41.9% for women, and 39.3% in men. In a more recent article, Richards *et al.*<sup>[92]</sup> (2005) found that by one and 3 years after liver transplant, 24% and 31% of patients met criteria for obesity as body mass index (BMI) > 30 kg/m<sup>2</sup>.

Despite clear evidence that obesity reduces graft and patient survival<sup>[93,94]</sup>, there are studies that dispute its independent predictive power. Leonard *et al.*<sup>[95]</sup> (2008) found that in a cohort of 1313 patients, obesity does not independently correlate to risk of graft or patient survival. Perhaps a measure of obesity is not enough, and there are more specific characteristics of excess adipose tissue that lead to it being a risk factor.

Després *et al.*<sup>[96]</sup> (2006) explain that the deposition of visceral fat, as opposed to normal subcutaneous fat, can lead to adipose tissue overflow and hormonal imbalance. The net effect of these factors is an increase in ectopic fat to muscle, liver, and epicardial tissue. Compounding this issue, visceral adipocytes are less responsive to insulin, and thus not subject to the antilipolytic effects of insulin<sup>[97]</sup>.

While the anatomical location of an adipocyte may be illuminating, physiologic factors also demonstrate the heterogeneity of metabolic dysfunction: Abnormal fat can accumulate as a result of defects in nuclear receptor genes involved in lipid sensing, synthesis, and

**Table 1 Summary of immunosuppressant effects on metabolic syndrome**

	Calcineurin inhibitors	Mycophenolate mofetil	mTOR inhibitors	Steroids
Body mass	Increase <sup>[92,103]</sup>	No change <sup>[105]</sup>	Less weight gain than CNIs <sup>[37]</sup>	Increase <sup>[144,145]</sup>
Dyslipidemia	Increase <sup>[104,121]</sup>	Less than CNIs <sup>[115]</sup>	Increase, but anti-atherosclerotic <sup>[144]</sup>	Increase <sup>[145]</sup>
Hypertension	Increase <sup>[107]</sup>	Less than CNIs <sup>[115,144]</sup>	No difference from CNI <sup>[146]</sup>	Increase <sup>[145]</sup>
Insulin resistance	Increase <sup>[128]</sup>	Potential benefit <sup>[145,146]</sup>	Potential benefit <sup>[149,150]</sup>	Increase <sup>[145]</sup>
Renal damage	Increase <sup>[112,113]</sup>	Less than both CNIs and mTOR inhibitors <sup>[147,151]</sup>	Decrease compared to CNIs <sup>[152]</sup>	Not significant
Note	Neoplastic <sup>[84]</sup>	Leukopenic <sup>[147,153,154]</sup>	Antineoplastic <sup>[155]</sup>	

CNIs: Calcineurin inhibitors; mTOR: Mechanistic target of rapamycin.

oxidation<sup>[98]</sup>. Supporting the ectopic fat hypothesis, Porter *et al.*<sup>[99]</sup> (2009) report that analysis of 3000 Framingham study participants indicated that abdominal subcutaneous fat had no corresponding linear increase of obesity-associated risk factors.

Further complicating the issue is what type of lipids are most abundant in a dysfunctional metabolic state. In the context of non-alcoholic steatohepatitis/nonalcoholic fatty liver diseases, it has been suggested that hepatocyte TG accumulation may be protective against free fatty acid (FFA)-induced oxidative lipotoxicity<sup>[100]</sup>. As far as dietary fats go, there is evidence that unsaturated fats contribute to this lipotoxicity, whereas saturated fats are actually hepatoprotective<sup>[101]</sup>.

Each of these issues - lipid distribution and lipid metabolism - is a qualitative issue that is outside the measure of BMI. Then, a more specific measure of adipose metabolism is necessary to further individualize treatment options for post-OLT patients. Given the heterogeneity of adipose metabolism, some authors suggest adipocyte transplantation as a treatment for metabolic issues such as diabetes, atherosclerosis, and nonalcoholic steatohepatitis<sup>[102]</sup>.

Not only is obesity incident to the population of liver transplant recipients, it is exacerbated by the effects of immunosuppression<sup>[92]</sup>. A mainstay of immunosuppression, steroids, are well-known for effects on weight gain<sup>[103]</sup>. CNIs are also associated with weight gain: Ersoy *et al.*<sup>[103]</sup> (2008) report that weight gain at 12 mo for renal transplant recipients prescribed TAC and CsA was 3.5 and 8.0 kg, respectively. As far as changing immunosuppressive regimens, it appears that using the mTOR inhibitor EVR in combination with a reduced dose of TAC can cause less weight gain than full-dose TAC immunosuppression<sup>[104]</sup>. If mTOR inhibitors are inappropriate for the specific patient, MMF is a different potential substitute that is not associated with post-transplant weight gain<sup>[105]</sup>.

### Hypertension and renal insufficiency

HTN and subsequent renal insufficiency are major concerns for post-OLT patients. Increased blood pressure and systemic vascular resistance is pathological, and can lead to hepatorenal syndrome. The use of immunosuppression, specifically CNIs, compounds the metabolic issues already present in liver transplant recipients<sup>[106,107]</sup>.

The incidence of severe renal dysfunction can

reach up to 18.1% at a mean of 13 years post-OLT<sup>[108]</sup>. Longenecker *et al.*<sup>[109]</sup> (2015) recently reviewed renal function before and after OLT, finding that the overall rate of progression to ESRD is strongly associated with estimated GFR (eGFR) less than 60 mL/min × 1.73 m<sup>2</sup> and diabetes, but find that eGFR at OLT is not associated with 12-mo mortality.

For patients presenting with proteinuria as a result of HTN due to renal-insufficiency, treatment includes standard approaches such as angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers<sup>[86,110]</sup>.

Aside from the standard of care for MS, the specific treatment of post-OLT patients with renal insufficiency should include reduction or withdrawal of CNI therapy as soon as possible because of the nephrotoxicity associated with CNIs<sup>[111,112]</sup>. Masetti *et al.*<sup>[113]</sup> (2010) evaluated whether early withdrawal of CsA followed by initiation of EVR monotherapy preserves kidney function compared to their standard CsA regimen. The study found that incidence of stage 3 chronic kidney disease (< 60 mL/min GFR) at 1 year was significantly higher in the standard CsA group (55%) than in the group treated with EVR monotherapy (15.4%). Further, there was no difference in patient survival between the two groups.

Saliba *et al.*<sup>[104]</sup> (2013) found that TAC reduction with addition of EVR were associated with increased estimated GFR, demonstrating a significant benefit to renal function. Tsai *et al.*<sup>[114]</sup> (2009) confirm that, for renal allograft recipients, CNI minimization with the introduction of SRL reduces acute rejection and improves renal function and survival.

CNI withdrawal and replacement with MMF is another promising approach to post-OLT immunosuppressive management in patients who have renal insufficiency. Orlando *et al.*<sup>[115]</sup> (2007) report success with MMF monotherapy as a means of reducing the toxic effects of CNIs. Of 42 post-OLT individuals who were weaned off of CNI therapy and placed on subsequent MMF monotherapy, renal function improved in 89% and arterial hypertension decreased in 80% of cases. In a separate study examining post-OLT patients with severe side effects from CNI therapy, Dharancy *et al.*<sup>[116]</sup> (2009) found that a switch to MMF monotherapy could lead to increased eGFR without significant increase in rejection. Several studies have concluded that MMF is

less nephrotoxic, indicating that MMF could be used preferentially in patients with renal dysfunction<sup>[117,118]</sup>.

### Dyslipidemia

Incidence of dyslipidemia exceeds 70% and 40% for patients with and without pre-transplant MS, respectively<sup>[119]</sup>. Because MS affects a such great proportion of OLT patients<sup>[7]</sup>, methods of preventing or reducing dyslipidemia could benefit a preponderance of patients.

In an evaluation of the metabolic impact of OLT, Luzi *et al.*<sup>[120]</sup> (1996) reported that liver transplant recipients had abnormal FFA levels at 5 mo post-OLT. A follow-up at 26 mo found reduction in abnormal lipid and protein metabolism - in fact, plasma free fatty acids were reduced for transplant recipients with respect to the control group.

CNI therapy is associated with dyslipidemia post-OLT; but the level of dyslipidemia might be reduced upon use of mTOR inhibitors in combination with TAC<sup>[121]</sup>. Saliba *et al.*<sup>[104]</sup> (2013) report that hyperlipidemia was more frequent in patients on EVR + reduced dose TAC compared to patients on only full dose TAC. In spite of an increase in dyslipidemia, mTOR inhibitors do seem to decrease arteriovascular plaque formation<sup>[122]</sup>.

Orlando *et al.*<sup>[115]</sup> (2007) found that CNI withdrawal and subsequent replacement with MMF improved dyslipidemia. Out of 41 patients, blood cholesterol decreased in 76% and blood TG decreased in 89%. Further supporting the use of MMF in patients who are at-risk for complications of atherosclerosis, Romero *et al.*<sup>[123]</sup> (2000) also reported that MMF specifically reduces atherosclerosis in rabbits.

For patients with hyperlipidemia who are resistant to lifestyle changes, hydroxymethylglutaryl-CoA reductase inhibitors (statins) should be considered. Even with the potential for hepatotoxicity, the use of statins to counter immunosuppressive side effects can benefit patients<sup>[124]</sup>. Martin *et al.*<sup>[125]</sup> (2007), in a retrospective review of 69 liver transplant patients, explain that there is a general tolerability and low incidence of adverse events in patients treated with lipid-lowering agents. Indeed, they report that there is no change in liver function tests.

### Diabetes

Post-transplant diabetes mellitus (PTDM) is a well-recognized issue, and minimization of immunosuppression is currently the best treatment option for PTDM patients. The diabetogenic properties of immunosuppressive therapies seem to be intimately related to the signaling processes that are shared between pancreatic islets and leukocytes. Moreover, in contrast to the physiological proliferative signaling mechanisms used by white blood cells, renal calcineurin mediates glomerular hypertrophy and extracellular matrix accumulation<sup>[126]</sup>.

Immunosuppressive regimens play a major role in new-onset diabetes, affecting patient and graft survival<sup>[127]</sup>. Rostambeigi *et al.*<sup>[128]</sup> (2011) explain that beta cells exposed to TAC and CsA decreased insulin

secretion and reduced mitochondrial density without affecting apoptosis rates, and posit that maybe there is a mitochondria-mediated dysfunction imposed by CNIs. Notably, the tacrolimus-exposed beta cells fared marginally better than their CsA counterparts. This is a reflection of the diabetogenic properties of TAC compared to CsA. On the other hand, some research finds no major metabolic differences between TAC and CsA post-OLT low-dose maintenance therapies<sup>[129,130]</sup>.

## HCV: FROM INVARIABLE TO INCONSEQUENTIAL

HCV recurrence after liver transplantation is nearly universal, immediate, and has an accelerated natural history<sup>[131]</sup>. However, the discovery of directly acting antiviral (DAA) protease inhibitors has dramatically reduced the impact of HCV. With SVR rates exceeding 90%<sup>[132]</sup>, high safety<sup>[133]</sup>, and a well-tolerated side-effects profile<sup>[134]</sup>, Hepatitis C treatment will hopefully become a non-issue.

Still, there are OLT patients who experience HCV recurrence, and these patients deserve special consideration. Notably, in contrast to patients who do not have HCV, an episode of early ACR in post-OLT HCV patients is associated with a higher risk for mortality<sup>[135]</sup>. Despite the emphatic importance of treating ACR in this population, there has been a lack of consensus on the impact of using steroids - the front line of ACR treatment - in post-OLT HCV patients<sup>[136,137]</sup>.

At the Cleveland Clinic Foundation, ACR treatment protocol first considers RAI of the HCV-positive post-OLT patient. For patients with RAI less than six, there is an increase in CNI dose and further monitoring for rejection before a bolus of steroids is administered. In contrast, patients who have HCV with a RAI greater than or equal to six are treated the same as patients who do not have HCV: 1 g of methylprednisone is administered daily for 3 d followed by steroid taper. Antibody therapy is used for steroid-resistant rejection.

Besides treatment of ACR, maintenance therapy for HCV-positive post-OLT patients can be nuanced. For example, use of the monoclonal antibody OKT3 is associated with early and severe post-OLT HCV recurrence, and must be approached with caution<sup>[138]</sup>. On the other hand, treatment with MMF and a 24-mo CNI taper appears to benefit liver function tests and presentation on histology for the hepatitis C patient<sup>[139]</sup>.

With the introduction of DAAs, however, focus has shifted from accommodating immunosuppression to early HCV treatment for potential liver transplant recipients. Achieving SVR before the time of transplantation is ideal, and helps reduce risk of HCV recurrence post-OLT<sup>[140-142]</sup>. Still, the efficacy of recently-developed protease inhibitors must be evaluated specifically for post-OLT patients<sup>[143]</sup>.

## CONCLUSION

While the landscape of immunosuppressive medications

remains steadfast, temperamental clinical weather demands that clinicians stay up to date on best practices. The good news is that HCV is nearly subdued as a post-transplant complication, increasing graft survival, perhaps even decreasing allocation of organs to retransplantation for HCV. The bad news is that MS is increasingly harming patients in ways that are exacerbated by immunosuppression - an issue in sore need of revolution like that in HCV treatment. Finally, given the confluence of MS and immunosuppressive side effects, treatment of early ACR could be excessive and must be reevaluated in light of today's average patient.

## REFERENCES

- 1 **Alberti KG**, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**: 1640-1645 [PMID: 19805654 DOI: 10.1161/CIRCULATIONAHA.109.192644]
- 2 **Wiesner RH**, Demetris AJ, Belle SH, Seaberg EC, Lake JR, Zetterman RK, Everhart J, Detre KM. Acute hepatic allograft rejection: incidence, risk factors, and impact on outcome. *Hepatology* 1998; **28**: 638-645 [PMID: 9731552 DOI: 10.1002/hep.510280306]
- 3 **Banff Working Group on Liver Allograft Pathology**. Importance of liver biopsy findings in immunosuppression management: biopsy monitoring and working criteria for patients with operational tolerance. *Liver Transpl* 2012; **18**: 1154-1170 [PMID: 22645090 DOI: 10.1002/lt.23481]
- 4 **Watt KD**, Pedersen RA, Kremers WK, Heimbach JK, Charlton MR. Evolution of causes and risk factors for mortality post-liver transplant: results of the NIDDK long-term follow-up study. *Am J Transplant* 2010; **10**: 1420-1427 [PMID: 20486907 DOI: 10.1111/j.1600-6143.2010.03126.x]
- 5 **Pruthi J**, Medkiff KA, Esrason KT, Donovan JA, Yoshida EM, Erb SR, Steinbrecher UP, Fong TL. Analysis of causes of death in liver transplant recipients who survived more than 3 years. *Liver Transpl* 2001; **7**: 811-815 [PMID: 11552217 DOI: 10.1053/jlts.2001.27084]
- 6 **Madhwal S**, Atreja A, Albeldawi M, Lopez R, Post A, Costa MA. Is liver transplantation a risk factor for cardiovascular disease? A meta-analysis of observational studies. *Liver Transpl* 2012; **18**: 1140-1146 [PMID: 22821899 DOI: 10.1002/lt.23508]
- 7 **Pagadala M**, Dasarathy S, Egtesad B, McCullough AJ. Posttransplant metabolic syndrome: an epidemic waiting to happen. *Liver Transpl* 2009; **15**: 1662-1670 [PMID: 19938136 DOI: 10.1002/lt.21952]
- 8 **Alberti A**, Piovesan S. The evolution of the therapeutic strategy in hepatitis C: features of sofosbuvir and indications. *Dig Liver Dis* 2014; **46** Suppl 5: S174-S178 [PMID: 25458777 DOI: 10.1016/j.dld.2014.09.028]
- 9 **Lawitz E**, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naïve and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; **383**: 515-523 [PMID: 24209977 DOI: 10.1016/S0140-6736(13)62121-2]
- 10 **Lang KS**, Georgiev P, Recher M, Navarini AA, Bergthaler A, Heikenwalder M, Harris NL, Junt T, Odermatt B, Clavien PA, Pircher H, Akira S, Hengartner H, Zinkernagel RM. Immuno-privileged status of the liver is controlled by Toll-like receptor 3 signaling. *J Clin Invest* 2006; **116**: 2456-2463 [PMID: 16955143 DOI: 10.1172/JCI28349]
- 11 **Demirkiran A**, Kok A, Kwekkeboom J, Kusters JG, Metselaar HJ, Tilanus HW, van der Laan LJ. Low circulating regulatory T-cell levels after acute rejection in liver transplantation. *Liver Transpl* 2006; **12**: 277-284 [PMID: 16447185 DOI: 10.1002/lt.20612]
- 12 **O'Leary JG**, Demetris AJ, Friedman LS, Gebel HM, Halloran PF, Kirk AD, Knechtle SJ, McDiarmid SV, Shaked A, Terasaki PI, Tincam KJ, Tomlanovich SJ, Wood KJ, Woodle ES, Zachary AA, Klintmalm GB. The role of donor-specific HLA alloantibodies in liver transplantation. *Am J Transplant* 2014; **14**: 779-787 [PMID: 24580828 DOI: 10.1111/ajt.12667]
- 13 **Kaneku H**, O'Leary JG, Banuelos N, Jennings LW, Susskind BM, Klintmalm GB, Terasaki PI. De novo donor-specific HLA antibodies decrease patient and graft survival in liver transplant recipients. *Am J Transplant* 2013; **13**: 1541-1548 [PMID: 23721554 DOI: 10.1111/ajt.12212]
- 14 **Musat AI**, Agni RM, Wai PY, Pirsch JD, Lorentzen DF, Powell A, Levenson GE, Bellingham JM, Fernandez LA, Foley DP, Mezrich JD, D'Alessandro AM, Lucey MR. The significance of donor-specific HLA antibodies in rejection and ductopenia development in ABO compatible liver transplantation. *Am J Transplant* 2011; **11**: 500-510 [PMID: 21342448 DOI: 10.1111/j.1600-6143.2010.03414.x]
- 15 **Schindl MJ**, Millar AM, Redhead DN, Fearon KC, Ross JA, Dejong CH, Garden OJ, Wigmore SJ. The adaptive response of the reticuloendothelial system to major liver resection in humans. *Ann Surg* 2006; **243**: 507-514 [PMID: 16552202 DOI: 10.1097/01.sla.0000205826.62911.a7]
- 16 **Gao B**, Jeong WI, Tian Z. Liver: An organ with predominant innate immunity. *Hepatology* 2008; **47**: 729-736 [PMID: 18167066 DOI: 10.1002/hep.22034]
- 17 **Wick MJ**, Leithäuser F, Reimann J. The hepatic immune system. *Crit Rev Immunol* 2002; **22**: 47-103 [PMID: 12186188 DOI: 10.1615/CritRevImmunol.v22.i1.30]
- 18 **Racanelli V**, Rehmann B. The liver as an immunological organ. *Hepatology* 2006; **43**: S54-S62 [PMID: 16447271 DOI: 10.1002/hep.21060]
- 19 **Starzl TE**. The mystique of organ transplantation. *J Am Coll Surg* 2005; **201**: 160-170 [PMID: 16038811 DOI: 10.1016/j.jamcollsurg.2005.03.023]
- 20 **Ramos HC**, Reyes J, Abu-Elmagd K, Zevevi A, Reinsmoen N, Tzakis A, Demetris AJ, Fung JJ, Flynn B, McMichael J. Weaning of immunosuppression in long-term liver transplant recipients. *Transplantation* 1995; **59**: 212-217 [PMID: 7839442 DOI: 10.1097/00007890-199501000-00010]
- 21 **Simpson N**, Cho YW, Cicciarella JC, Selby RR, Fong TL. Comparison of renal allograft outcomes in combined liver-kidney transplantation versus subsequent kidney transplantation in liver transplant recipients: Analysis of UNOS Database. *Transplantation* 2006; **82**: 1298-1303 [PMID: 17130778 DOI: 10.1097/01.tp.0000241104.58576.e6]
- 22 **Katznelson S**, Cecka JM. The liver neither protects the kidney from rejection nor improves kidney graft survival after combined liver and kidney transplantation from the same donor. *Transplantation* 1996; **61**: 1403-1405 [PMID: 8629305 DOI: 10.1097/0007890-199605150-00021]
- 23 **Castillo-Rama M**, Castro MJ, Bernardo I, Meneu-Diaz JC, Elola-Olaso AM, Calleja-Antolin SM, Romo E, Morales P, Moreno E, Paz-Artal E. Preformed antibodies detected by cytotoxic assay or multibead array decrease liver allograft survival: role of human leukocyte antigen compatibility. *Liver Transpl* 2008; **14**: 554-562 [PMID: 18383092 DOI: 10.1002/lt.21408]
- 24 **Jakab SS**, Navarro VJ, Colombe BW, Daskalakis C, Herrine SK, Rossi S. Human leukocyte antigen and adult living-donor liver transplantation outcomes: an analysis of the organ procurement and transplantation network database. *Liver Transpl* 2007; **13**: 1405-1413 [PMID: 17902126 DOI: 10.1002/lt.21264]
- 25 **Neumann UP**, Guckelberger O, Langrehr JM, Lang M, Schmitz V,



- Theruvath T, Schonemann C, Menzel S, Klupp J, Neuhaus P. Impact of human leukocyte antigen matching in liver transplantation. *Transplantation* 2003; **75**: 132-137 [PMID: 12544885 DOI: 10.1097/00007890-200301150-00024]
- 26 **Knechtle SJ**, Kwun J. Unique aspects of rejection and tolerance in liver transplantation. *Semin Liver Dis* 2009; **29**: 91-101 [PMID: 19235662 DOI: 10.1055/s-0029-1192058]
  - 27 **Halloran PF**. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004; **351**: 2715-2729 [PMID: 15616206 DOI: 10.1056/NEJMra033540]
  - 28 **Swiatecka-Urban A**. Anti-interleukin-2 receptor antibodies for the prevention of rejection in pediatric renal transplant patients: current status. *Paediatr Drugs* 2003; **5**: 699-716 [PMID: 14510627 DOI: 10.2165/00148581-200305100-00005]
  - 29 **Waki K**. UNOS Liver Registry: ten year survivals. *Clin Transpl* 2006; 29-39 [PMID: 18368704]
  - 30 **Hariharan S**, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med* 2000; **342**: 605-612 [PMID: 10699159 DOI: 10.1056/NEJM200003023420901]
  - 31 **Allison AC**, Eugui EM. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology* 2000; **47**: 85-118 [PMID: 10878285 DOI: 10.1016/S0162-3109(00)00188-0]
  - 32 **Basic-Jukic N**, Kes P, Bubic-Filipi LJ, Puretic Z, Brunetta B, Pasini J. Does mycophenolate mofetil increase the incidence of cytomegalovirus disease compared with azathioprine after cadaveric kidney transplantation? *Transplant Proc* 2005; **37**: 850-851 [PMID: 15848553 DOI: 10.1016/j.transproceed.2004.12.228]
  - 33 **Guertin DA**, Sabatini DM. The pharmacology of mTOR inhibition. *Sci Signal* 2009; **2**: pe24 [PMID: 19383975 DOI: 10.1126/scisignal.267pe24]
  - 34 **Sehgal SN**. Sirolimus: its discovery, biological properties, and mechanism of action. *Transplant Proc* 2003; **35**: 7S-14S [PMID: 12742462 DOI: 10.1016/S0041-1345(03)00211-2]
  - 35 **Johnson SC**, Rabinovitch PS, Kaeblerlein M. mTOR is a key modulator of ageing and age-related disease. *Nature* 2013; **493**: 338-345 [PMID: 23325216 DOI: 10.1038/nature11861]
  - 36 **Lamming DW**, Ye L, Sabatini DM, Baur JA. Rapalogs and mTOR inhibitors as anti-aging therapeutics. *J Clin Invest* 2013; **123**: 980-989 [PMID: 23454761 DOI: 10.1172/JCI64099]
  - 37 **Araki M**, Flechner SM, Ismail HR, Flechner LM, Zhou L, Derweesh IH, Goldfarb D, Modlin C, Novick AC, Faiman C. Posttransplant diabetes mellitus in kidney transplant recipients receiving calcineurin or mTOR inhibitor drugs. *Transplantation* 2006; **81**: 335-341 [PMID: 16477217 DOI: 10.1097/01.tp.0000195770.31960.18]
  - 38 **Gómez R**, Moreno E, Colina F, Loinaz C, Gonzalez-Pinto I, Lumbreras C, Perez-Cerdá F, Castellón C, García I. Steroid withdrawal is safe and beneficial in stable cyclosporine-treated liver transplant patients. *J Hepatol* 1998; **28**: 150-156 [PMID: 9537852 DOI: 10.1016/S0168-8278(98)80214-6]
  - 39 **Klintmalm GB**, Washburn WK, Rudich SM, Heffron TG, Teperman LW, Fasola C, Eckhoff DE, Netto GJ, Katz E. Corticosteroid-free immunosuppression with daclizumab in HCV(+) liver transplant recipients: 1-year interim results of the HCV-3 study. *Liver Transpl* 2007; **13**: 1521-1531 [PMID: 17969201 DOI: 10.1002/lt.21182]
  - 40 **Lerut JP**, Pinheiro RS, Lai Q, Stouffs V, Orlando G, Juri JM, Ciccarelli O, Sempoux C, Roggen FM, De Reyck C, Latinne D, Gianello P. Is minimal, [almost] steroid-free immunosuppression a safe approach in adult liver transplantation? Long-term outcome of a prospective, double blind, placebo-controlled, randomized, investigator-driven study. *Ann Surg* 2014; **260**: 886-891; discussion 891-892 [PMID: 25379858 DOI: 10.1097/SLA.0000000000000969]
  - 41 **Tisone G**, Angelico M, Palmieri G, Pisani F, Baiocchi L, Vennarecci G, Anselmo A, Orlando G, Negrini S, Casciani CU. Immunosuppression without prednisone after liver transplantation is safe and associated with normal early graft function: preliminary results of a randomized study. *Transpl Int* 1998; **11** Suppl 1: S267-S269 [PMID: 9664993 DOI: 10.1007/s001470050475]
  - 42 **Tisone G**, Angelico M, Palmieri G, Pisani F, Anselmo A, Baiocchi L, Negrini S, Orlando G, Vennarecci G, Casciani CU. A pilot study on the safety and effectiveness of immunosuppression without prednisone after liver transplantation. *Transplantation* 1999; **67**: 1308-1313 [PMID: 10360582 DOI: 10.1097/00007890-199905270-00003]
  - 43 **Reding R**. Steroid withdrawal in liver transplantation: benefits, risks, and unanswered questions. *Transplantation* 2000; **70**: 405-410 [PMID: 10949177 DOI: 10.1097/00007890-200008150-00001]
  - 44 **Kim JM**, Joh JW, Kim SJ, Kwon CH, Song S, Shin M, Hong SH, Lee SK. Steroid withdrawal in adult liver transplantation: occurrence at a single center. *Transplant Proc* 2010; **42**: 4132-4136 [PMID: 21168644 DOI: 10.1016/j.transproceed.2010.10.018]
  - 45 **Eason JD**, Loss GE, Blazek J, Nair S, Mason AL. Steroid-free liver transplantation using rabbit antithymocyte globulin induction: results of a prospective randomized trial. *Liver Transpl* 2001; **7**: 693-697 [PMID: 11510013 DOI: 10.1053/jlts.2001.26353]
  - 46 **Bonnefoy-Berard N**, Revillard JP. Mechanisms of immunosuppression induced by antithymocyte globulins and OKT3. *J Heart Lung Transplant* 1996; **15**: 435-442 [PMID: 8771497]
  - 47 **Bolt S**, Routledge E, Lloyd I, Chateaufort L, Pope H, Gorman SD, Clark M, Waldmann H. The generation of a humanized, non-mitogenic CD3 monoclonal antibody which retains in vitro immunosuppressive properties. *Eur J Immunol* 1993; **23**: 403-411 [PMID: 8436176 DOI: 10.1002/eji.1830230216]
  - 48 **Shiheid H**, Aoyama T, Takahashi H, Hanaoka K, Abe T, Nishida E, Chen C, Koga O, Hikida M, Shibagaki Y, Morita A, Nikawa T, Hattori S, Watanabe T, Shimizu J. Novel CD3-specific antibody induces immunosuppression via impaired phosphorylation of LAT and PLC $\gamma$ 1 following T-cell stimulation. *Eur J Immunol* 2014; **44**: 1770-1780 [PMID: 24595757 DOI: 10.1002/eji.201344146]
  - 49 **Webber A**, Hirose R, Vincenti F. Novel strategies in immunosuppression: issues in perspective. *Transplantation* 2011; **91**: 1057-1064 [PMID: 21412186 DOI: 10.1097/TP.0b013e3182145306]
  - 50 **Page EK**, Dar WA, Knechtle SJ. Biologics in organ transplantation. *Transpl Int* 2012; **25**: 707-719 [PMID: 22420711 DOI: 10.1111/j.1432-2277.2012.01456.x]
  - 51 **Chateaufort L**, Ferran C, Legendre C, Thouard I, Merite S, Reuter A, Gevaert Y, Kreis H, Franchimont P, Bach JF. In vivo cell activation following OKT3 administration. Systemic cytokine release and modulation by corticosteroids. *Transplantation* 1990; **49**: 697-702 [PMID: 2109379 DOI: 10.1097/00007890-199004000-00009]
  - 52 **Frigault MJ**, June CH. Predicting cytokine storms: it's about density. *Blood* 2011; **118**: 6724-6726 [PMID: 22194391 DOI: 10.1182/blood-2011-10-382598]
  - 53 **Hanounch IA**, Zein NN, Lopez R, Yerian L, Fung J, Eghtesad B. IL-2 Receptor Antagonist (Basiliximab) Is Associated with Rapid Fibrosis Progression in Patients with Recurrent Hepatitis C after Liver Transplantation Using Serial Biopsy Specimens. *Int J Organ Transplant Med* 2010; **1**: 7-14 [PMID: 25013557 DOI: 10.1016/s0016-5085(08)63573-8]
  - 54 **Perri R**, Assi M, Talwalkar J, Heimbach J, Hogan W, Moore SB, Rosen CB. Graft vs. host disease after liver transplantation: a new approach is needed. *Liver Transpl* 2007; **13**: 1092-1099 [PMID: 17663410 DOI: 10.1002/lt.21203]
  - 55 **Bezabeh S**, Flowers CM, Kortepeter C, Avigan M. Clinically significant liver injury in patients treated with natalizumab. *Aliment Pharmacol Ther* 2010; **31**: 1028-1035 [PMID: 20163378 DOI: 10.1111/j.1365-2036.2010.04262.x]
  - 56 **Usuda M**, Fujimori K, Koyamada N, Fukumori T, Sekiguchi S, Kawagishi N, Akamatsu Y, Enomoto Y, Satoh K, Satoh A, Ishida K, Moriya T, Satomi S. Successful use of anti-CD20 monoclonal antibody (rituximab) for ABO-incompatible living-related liver transplantation. *Transplantation* 2005; **79**: 12-16 [PMID: 15714163 DOI: 10.1097/01.tp.0000149337.40911.e4]
  - 57 **Yoshizawa A**, Sakamoto S, Ogawa K, Kasahara M, Uryuhara K, Oike F, Ueda M, Takada Y, Egawa H, Tanaka K. New protocol of immunosuppression for liver transplantation across ABO barrier: the use of Rituximab, hepatic arterial infusion, and preservation of

- spleen. *Transplant Proc* 2005; **37**: 1718-1719 [PMID: 15919443 DOI: 10.1016/j.transproceed.2005.03.148]
- 58 **Tanabe M**, Kawachi S, Obara H, Shinoda M, Hibi T, Kitagawa Y, Wakabayashi G, Shimazu M, Kitajima M. Current progress in ABO-incompatible liver transplantation. *Eur J Clin Invest* 2010; **40**: 943-949 [PMID: 20636381 DOI: 10.1111/j.1365-2362.2010.02339.x]
- 59 **Sanal MG**. Future of liver transplantation: non-human primates for patient-specific organs from induced pluripotent stem cells. *World J Gastroenterol* 2011; **17**: 3684-3690 [PMID: 21990949 DOI: 10.3748/wjg.v17.i32.3684]
- 60 Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997; **25**: 658-663 [PMID: 9049215 DOI: 10.1002/hep.510250328]
- 61 **Dickson RC**, Lauwers GY, Rosen CB, Cantwell R, Nelson DR, Lau JY. The utility of noninvasive serologic markers in the management of early allograft rejection in liver transplantation recipients. *Transplantation* 1999; **68**: 247-253 [PMID: 10440396 DOI: 10.1097/00007890-199907270-00015]
- 62 **Abraham SC**, Furth EE. Receiver operating characteristic analysis of serum chemical parameters as tests of liver transplant rejection and correlation with histology. *Transplantation* 1995; **59**: 740-746 [PMID: 7886803 DOI: 10.1097/00007890-199503150-00018]
- 63 **Rodríguez-Perálvarez M**, García-Caparrós C, Tsochatzis E, Germani G, Hogan B, Poyato-González A, O'Beirne J, Senzolo M, Guerrero-Misas M, Montero-Álvarez JL, Patch D, Barrera P, Briceño J, Dhillon AP, Burra P, Burroughs AK, De la Mata M. Lack of agreement for defining 'clinical suspicion of rejection' in liver transplantation: a model to select candidates for liver biopsy. *Transpl Int* 2015; **28**: 455-464 [PMID: 25557691 DOI: 10.1111/tri.12514]
- 64 **Höroldt BS**, Burattin M, Gunson BK, Bramhall SR, Nightingale P, Hübscher SG, Neuberger JM. Does the Banff rejection activity index predict outcome in patients with early acute cellular rejection following liver transplantation? *Liver Transpl* 2006; **12**: 1144-1151 [PMID: 16799959 DOI: 10.1002/lt.20779]
- 65 **Shaked A**, Ghobrial RM, Merion RM, Shearon TH, Emond JC, Fair JH, Fisher RA, Kulik LM, Pruett TL, Terrault NA. Incidence and severity of acute cellular rejection in recipients undergoing adult living donor or deceased donor liver transplantation. *Am J Transplant* 2009; **9**: 301-308 [PMID: 19120082 DOI: 10.1111/j.1600-6143.2008.02487.x]
- 66 **Aydogan C**, Sevmis S, Aktas S, Karakayali H, Demirhan B, Haberal M. Steroid-resistant acute rejections after liver transplant. *Exp Clin Transplant* 2010; **8**: 172-177 [PMID: 20565375 DOI: 10.1097/01.tp.0000331525.53169.6e]
- 67 **Fisher LR**, Henley KS, Lucey MR. Acute cellular rejection after liver transplantation: variability, morbidity, and mortality. *Liver Transpl Surg* 1995; **1**: 10-15 [PMID: 9346535 DOI: 10.1002/lt.500010104]
- 68 **Seiler CA**, Renner EL, Czerniak A, Didonna D, Büchler MW, Reichen J. Early acute cellular rejection: no effect on late hepatic allograft function in man. *Transpl Int* 1999; **12**: 195-201 [PMID: 10429957 DOI: 10.1007/s001470050210]
- 69 **Uemura T**, Ikegami T, Sanchez EQ, Jennings LW, Narasimhan G, McKenna GJ, Randall HB, Chinnakotla S, Levy MF, Goldstein RM, Klintmalm GB. Late acute rejection after liver transplantation impacts patient survival. *Clin Transplant* 2008; **22**: 316-323 [PMID: 18190550 DOI: 10.1111/j.1399-0012.2007.00788.x]
- 70 **Thurairajah PH**, Carbone M, Bridgestock H, Thomas P, Hebbard S, Gunson BK, Shah T, Neuberger J. Late acute liver allograft rejection; a study of its natural history and graft survival in the current era. *Transplantation* 2013; **95**: 955-959 [PMID: 23442806 DOI: 10.1097/TP.0b013e3182845f6c]
- 71 **Christina S**, Annunziato RA, Schiano TD, Anand R, Vaidya S, Chuang K, Zack Y, Florman S, Shneider BL, Shemesh E. Medication level variability index predicts rejection, possibly due to nonadherence, in adult liver transplant recipients. *Liver Transpl* 2014; **20**: 1168-1177 [PMID: 24931127 DOI: 10.1002/lt.23930]
- 72 **Bartlett AS**, Ramadas R, Furness S, Gane E, McCall JL. The natural history of acute histologic rejection without biochemical graft dysfunction in orthotopic liver transplantation: a systematic review. *Liver Transpl* 2002; **8**: 1147-1153 [PMID: 12474154 DOI: 10.1053/jlts.2002.36240]
- 73 **Wishart DS**. Metabolomics: the principles and potential applications to transplantation. *Am J Transplant* 2005; **5**: 2814-2820 [PMID: 16302993 DOI: 10.1111/j.1600-6143.2005.01119.x]
- 74 **Hrydziusko O**, Silva MA, Perera MT, Richards DA, Murphy N, Mirza D, Viant MR. Application of metabolomics to investigate the process of human orthotopic liver transplantation: a proof-of-principle study. *OMICS* 2010; **14**: 143-150 [PMID: 20210660 DOI: 10.1089/omi.2009.0139]
- 75 **Adolf J**, Martin WG, Müller DF, Beckurts KT, Schneider-Eicke J, Wittekind C, Heidecke CD. [The effect of acute cellular rejection on liver function following orthotopic liver transplantation. Quantitative functional studies with the <sup>14</sup>C-aminopyrine breath test]. *Dtsch Med Wochenschr* 1992; **117**: 1823-1828 [PMID: 1451647 DOI: 10.1055/s-2008-1062516]
- 76 **Volpin R**, Angeli P, Galioto A, Fasolato S, Neri D, Barbazza F, Merenda R, Del Piccolo F, Strazzabosco M, Casagrande F, Feltracco P, Sticca A, Merkel C, Gerunda G, Gatta A. Comparison between two high-dose methylprednisolone schedules in the treatment of acute hepatic cellular rejection in liver transplant recipients: a controlled clinical trial. *Liver Transpl* 2002; **8**: 527-534 [PMID: 12037783 DOI: 10.1053/jlts.2002.33456]
- 77 **Goddard S**, Adams DH. Methylprednisolone therapy for acute rejection: too much of a good thing? *Liver Transpl* 2002; **8**: 535-536 [PMID: 12037784 DOI: 10.1053/jlts.2002.33486]
- 78 **Rodríguez-Perálvarez M**, Germani G, Papastergiou V, Tsochatzis E, Thalassinou E, Luong TV, Rolando N, Dhillon AP, Patch D, O'Beirne J, Thorburn D, Burroughs AK. Early tacrolimus exposure after liver transplantation: relationship with moderate/severe acute rejection and long-term outcome. *J Hepatol* 2013; **58**: 262-270 [PMID: 23023010 DOI: 10.1016/j.jhep.2012.09.019]
- 79 **Abdelmalek MF**, Humar A, Stickel F, Andreone P, Pascher A, Barroso E, Neff GW, Ranjan D, Toselli LT, Gane EJ, Scarola J, Alberts RG, Maller ES, Lo CM. Sirolimus conversion regimen versus continued calcineurin inhibitors in liver allograft recipients: a randomized trial. *Am J Transplant* 2012; **12**: 694-705 [PMID: 22233522 DOI: 10.1111/j.1600-6143.2011.03919.x]
- 80 **Fung JJ**, Jain A, Kwak EJ, Kusne S, Dvorchik I, Eghtesad B. De novo malignancies after liver transplantation: a major cause of late death. *Liver Transpl* 2001; **7**: S109-S118 [PMID: 11689783 DOI: 10.1053/jlts.2001.28645]
- 81 **Jiménez-Romero C**, Justo-Alonso I, Cambra-Molero F, Calvo-Pulido J, García-Sesma Á, Abradelo-Usera M, Caso-Maestro O, Manrique-Municio A. Incidence, risk factors and outcome of de novo tumors in liver transplant recipients focusing on alcoholic cirrhosis. *World J Hepatol* 2015; **7**: 942-953 [PMID: 25954477 DOI: 10.4254/wjh.v7.i7.942]
- 82 **Baccarani U**, Adani GL, Serraino D, Lorenzin D, Gambato M, Buda A, Zanusi G, Vitale A, Piselli P, De Paoli A, Bresadola V, Risaliti A, Toniutto P, Cillo U, Bresadola F, Burra P. De novo tumors are a major cause of late mortality after orthotopic liver transplantation. *Transplant Proc* 2009; **41**: 1303-1305 [PMID: 19460546 DOI: 10.1016/j.transproceed.2009.03.079]
- 83 **Yamanaka K**, Petrucci M, Lin S, Gao C, Galli U, Richter S, Winkler S, Houben P, Schultze D, Hatano E, Schemmer P. Therapeutic potential and adverse events of everolimus for treatment of hepatocellular carcinoma - systematic review and meta-analysis. *Cancer Med* 2013; **2**: 862-871 [PMID: 24403259 DOI: 10.1002/cam4.150]
- 84 **Rodríguez-Perálvarez M**, De la Mata M, Burroughs AK. Liver transplantation: immunosuppression and oncology. *Curr Opin Organ Transplant* 2014; **19**: 253-260 [PMID: 24685671 DOI: 10.1097/MOT.0000000000000069]
- 85 **Grundy SM**, Brewer HB, Cleeman JI, Smith SC, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Arterioscler Thromb*

- Vasc Biol* 2004; **24**: e13-e18 [PMID: 14766739 DOI: 10.1161/01.ATV.0000111245.75752.C6]
- 86 **Bonora BM**, Marescotti M, Marcuzzo G, Avogaro A, Fadini GP. Synergistic interactions among metabolic syndrome components and homeostasis model assessment of insulin resistance in a middle-aged general population over time. *Metab Syndr Relat Disord* 2015; **13**: 171-178 [PMID: 25734622 DOI: 10.1089/met.2014.0163]
  - 87 **Wannamethee SG**, Shaper AG, Lennon L, Morris RW. Metabolic syndrome vs Framingham Risk Score for prediction of coronary heart disease, stroke, and type 2 diabetes mellitus. *Arch Intern Med* 2005; **165**: 2644-2650 [PMID: 16344423 DOI: 10.1001/archinte.165.22.2644]
  - 88 **Björntorp P**. The regulation of adipose tissue distribution in humans. *Int J Obes Relat Metab Disord* 1996; **20**: 291-302 [PMID: 8680455]
  - 89 **Hotamisligil GS**. Inflammation and metabolic disorders. *Nature* 2006; **444**: 860-867 [PMID: 17167474 DOI: 10.1038/nature05485]
  - 90 **Ogden CL**, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity and trends in body mass index among US children and adolescents, 1999-2010. *JAMA* 2012; **307**: 483-490 [PMID: 22253364 DOI: 10.1001/jama.2012.40]
  - 91 **Stegall MD**, Everson G, Schroter G, Bilir B, Karrer F, Kam I. Metabolic complications after liver transplantation. Diabetes, hypercholesterolemia, hypertension, and obesity. *Transplantation* 1995; **60**: 1057-1060 [PMID: 7491685]
  - 92 **Richards J**, Gunson B, Johnson J, Neuberger J. Weight gain and obesity after liver transplantation. *Transpl Int* 2005; **18**: 461-466 [PMID: 15773968 DOI: 10.1111/j.1432-2277.2004.00067.x]
  - 93 **Nair S**, Verma S, Thuluvath PJ. Obesity and its effect on survival in patients undergoing orthotopic liver transplantation in the United States. *Hepatology* 2002; **35**: 105-109 [PMID: 11786965 DOI: 10.1053/jhep.2002.30318]
  - 94 **Ducloux D**, Kazory A, Simula-Faivre D, Chalopin JM. One-year post-transplant weight gain is a risk factor for graft loss. *Am J Transplant* 2005; **5**: 2922-2928 [PMID: 16303006 DOI: 10.1111/j.1600-6143.2005.01104.x]
  - 95 **Leonard J**, Heimbach JK, Malinchoc M, Watt K, Charlton M. The impact of obesity on long-term outcomes in liver transplant recipients-results of the NIDDK liver transplant database. *Am J Transplant* 2008; **8**: 667-672 [PMID: 18294163 DOI: 10.1111/j.1600-6143.2007.02100.x]
  - 96 **Després JP**, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006; **444**: 881-887 [PMID: 17167477 DOI: 10.1038/nature05488]
  - 97 **Björntorp P**. Metabolic implications of body fat distribution. *Diabetes Care* 1991; **14**: 1132-1143 [PMID: 1773700 DOI: 10.2337/diacare.14.12.1132]
  - 98 **Sanal MG**. The blind men 'see' the elephant-the many faces of fatty liver disease. *World J Gastroenterol* 2008; **14**: 831-844 [PMID: 18240340 DOI: 10.3748/wjg.14.831]
  - 99 **Porter SA**, Massaro JM, Hoffmann U, Vasan RS, O'Donnel CJ, Fox CS. Abdominal subcutaneous adipose tissue: a protective fat depot? *Diabetes Care* 2009; **32**: 1068-1075 [PMID: 19244087 DOI: 10.2337/dc08-2280]
  - 100 **Ono M**, Okamoto N, Saibara T. The latest idea in NAFLD/NASH pathogenesis. *Clin J Gastroenterol* 2010; **3**: 263-270 [PMID: 26190482 DOI: 10.1007/s12328-010-0182-9]
  - 101 **Purohit V**, Russo D, Coates PM. Role of fatty liver, dietary fatty acid supplements, and obesity in the progression of alcoholic liver disease: introduction and summary of the symposium. *Alcohol* 2004; **34**: 3-8 [PMID: 15670659 DOI: 10.1016/j.alcohol.2004.06.008]
  - 102 **Sanal MG**. Adipose tissue transplantation may be a potential treatment for diabetes, atherosclerosis and nonalcoholic steatohepatitis. *Med Hypotheses* 2009; **72**: 247-249 [PMID: 19046821 DOI: 10.1016/j.mehy.2008.10.009]
  - 103 **Ersoy A**, Baran B, Ersoy C, Kahvecioglu S, Akdag I. Calcineurin inhibitors and post-transplant weight gain. *Nephrology (Carlton)* 2008; **13**: 433-439 [PMID: 18331443 DOI: 10.1111/j.1440-1797.2008.00916.x]
  - 104 **Saliba F**, De Simone P, Nevens F, De Carlis L, Metselaar HJ, Beckebaum S, Jonas S, Sudan D, Fischer L, Duvoux C, Chavin KD, Koneru B, Huang MA, Chapman WC, Foltys D, Dong G, Lopez PM, Fung J, Junge G. Renal function at two years in liver transplant patients receiving everolimus: results of a randomized, multicenter study. *Am J Transplant* 2013; **13**: 1734-1745 [PMID: 23714399 DOI: 10.1111/ajt.12280]
  - 105 **Schlitt HJ**, Barkmann A, Böker KH, Schmidt HH, Emmanouilidis N, Rosenau J, Bahr MJ, Tusch G, Manns MP, Nashan B, Klempnauer J. Replacement of calcineurin inhibitors with mycophenolate mofetil in liver-transplant patients with renal dysfunction: a randomised controlled study. *Lancet* 2001; **357**: 587-591 [PMID: 11558484 DOI: 10.1016/S0140-6736(00)04055-1]
  - 106 A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. The U.S. Multicenter FK506 Liver Study Group. *N Engl J Med* 1994; **331**: 1110-1115 [PMID: 7523946 DOI: 10.1056/nejm199410273311702]
  - 107 **Hoorn EJ**, Walsh SB, McCormick JA, Fürstenberg A, Yang CL, Roeschel T, Paliege A, Howie AJ, Conley J, Bachmann S, Unwin RJ, Ellison DH. The calcineurin inhibitor tacrolimus activates the renal sodium chloride cotransporter to cause hypertension. *Nat Med* 2011; **17**: 1304-1309 [PMID: 21963515 DOI: 10.1038/nm.2497]
  - 108 **Gonwa TA**, Mai ML, Melton LB, Hays SR, Goldstein RM, Levy MF, Klintmalm GB. End-stage renal disease (ESRD) after orthotopic liver transplantation (OLT) using calcineurin-based immunotherapy: risk of development and treatment. *Transplantation* 2001; **72**: 1934-1939 [PMID: 11773892 DOI: 10.1097/00007890-200112270-00012]
  - 109 **Longenecker JC**, Estrella MM, Segev DL, Atta MG. Patterns of Kidney Function Before and After Orthotopic Liver Transplant: Associations With Length of Hospital Stay, Progression to End-Stage Renal Disease, and Mortality. *Transplantation* 2015; **99**: 2556-2564 [PMID: 25989501 DOI: 10.1097/TP.0000000000000767]
  - 110 **Praga M**, Hernández E, Herrero JC, Morales E, Revilla Y, Díaz-González R, Rodicio JL. Influence of obesity on the appearance of proteinuria and renal insufficiency after unilateral nephrectomy. *Kidney Int* 2000; **58**: 2111-2118 [PMID: 11044232 DOI: 10.1111/j.1523-1755.2000.00384.x]
  - 111 **Naesens M**, Kuypers DR, Sarwal M. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol* 2009; **4**: 481-508 [PMID: 19218475 DOI: 10.2215/CJN.04800908]
  - 112 **Morales JM**, Andres A, Rengel M, Rodicio JL. Influence of cyclosporin, tacrolimus and rapamycin on renal function and arterial hypertension after renal transplantation. *Nephrol Dial Transplant* 2001; **16** Suppl 1: 121-124 [PMID: 11369839 DOI: 10.1093/ndt/16.suppl\_1.121]
  - 113 **Masetti M**, Montalti R, Rompianesi G, Codeluppi M, Gerring R, Romano A, Begliomini B, Di Benedetto F, Gerunda GE. Early withdrawal of calcineurin inhibitors and everolimus monotherapy in de novo liver transplant recipients preserves renal function. *Am J Transplant* 2010; **10**: 2252-2262 [PMID: 20486905 DOI: 10.1111/j.1600-6143.2010.03128.x]
  - 114 **Tsai MK**, Wu FL, Lai IR, Lee CY, Hu RH, Lee PH. Decreased acute rejection and improved renal allograft survival using sirolimus and low-dose calcineurin inhibitors without induction therapy. *Int J Artif Organs* 2009; **32**: 371-380 [PMID: 19670189]
  - 115 **Orlando G**, Baiocchi L, Cardillo A, Iaria G, De Liguori Carino N, De Luca L, Ielpo B, Taricotti L, Angelico M, Tisone G. Switch to 1.5 grams MMF monotherapy for CNi-related toxicity in liver transplantation is safe and improves renal function, dyslipidemia, and hypertension. *Liver Transpl* 2007; **13**: 46-54 [PMID: 17154392 DOI: 10.1002/lt.20926]
  - 116 **Dharancy S**, Iannelli A, Hulin A, Declerck N, Schneck AS, Mathurin P, Boleslawski E, Gugenheim J, Pruvot FR. Mycophenolate mofetil monotherapy for severe side effects of calcineurin inhibitors following liver transplantation. *Am J Transplant* 2009; **9**: 610-613 [PMID: 19260838 DOI: 10.1111/j.1600-6143.2008.02529.x]
  - 117 **Wu YG**, Lin H, Qi XM, Wu GZ, Qian H, Zhao M, Shen JJ, Lin ST. Prevention of early renal injury by mycophenolate mofetil and its



- mechanism in experimental diabetes. *Int Immunopharmacol* 2006; **6**: 445-453 [PMID: 16428080 DOI: 10.1016/j.intimp.2005.09.006]
- 118 **Rodríguez-Iturbe B**, Quiroz Y, Shahkarami A, Li Z, Vaziri ND. Mycophenolate mofetil ameliorates nephropathy in the obese Zucker rat. *Kidney Int* 2005; **68**: 1041-1047 [PMID: 16105034 DOI: 10.1111/j.1523-1755.2005.00496.x]
- 119 **Laryea M**, Watt KD, Molinari M, Walsh MJ, McAlister VC, Marotta PJ, Nashan B, Peltekian KM. Metabolic syndrome in liver transplant recipients: prevalence and association with major vascular events. *Liver Transpl* 2007; **13**: 1109-1114 [PMID: 17663411 DOI: 10.1002/lt.21126]
- 120 **Luzi L**, Perseghin G, Regalia E, Sereni LP, Battezzati A, Baratti D, Bianchi E, Terruzzi I, Hilden H, Groop LC, Pulvirenti A, Taskinen MR, Gennari L, Mazzaferro V. Metabolic effects of liver transplantation in cirrhotic patients. *J Clin Invest* 1997; **99**: 692-700 [PMID: 9045872 DOI: 10.1172/jci119213]
- 121 **Trotter JF**, Wachs ME, Trouillot TE, Bak T, Kugelmas M, Kam I, Everson G. Dyslipidemia during sirolimus therapy in liver transplant recipients occurs with concomitant cyclosporine but not tacrolimus. *Liver Transpl* 2001; **7**: 401-408 [PMID: 11349259 DOI: 10.1053/jlts.2001.23916]
- 122 **Martinet W**, De Loof H, De Meyer GR. mTOR inhibition: a promising strategy for stabilization of atherosclerotic plaques. *Atherosclerosis* 2014; **233**: 601-607 [PMID: 24534455 DOI: 10.1016/j.atherosclerosis.2014.01.040]
- 123 **Romero F**, Rodríguez-Iturbe B, Pons H, Parra G, Quiroz Y, Rincón J, González L. Mycophenolate mofetil treatment reduces cholesterol-induced atherosclerosis in the rabbit. *Atherosclerosis* 2000; **152**: 127-133 [PMID: 10996347 DOI: 10.1016/S0021-9150(99)00458-X]
- 124 **Anfossi G**, Massucco P, Bonomo K, Trovati M. Prescription of statins to dyslipidemic patients affected by liver diseases: a subtle balance between risks and benefits. *Nutr Metab Cardiovasc Dis* 2004; **14**: 215-224 [PMID: 15553600 DOI: 10.1016/S0939-4753(04)80008-5]
- 125 **Martin JE**, Cavanaugh TM, Trumbull L, Bass M, Weber F, Aranda-Michel J, Hanaway M, Rudich S. Incidence of adverse events with HMG-CoA reductase inhibitors in liver transplant patients. *Clin Transplant* 2008; **22**: 113-119 [PMID: 18217912 DOI: 10.1111/j.1399-0012.2007.00780.x]
- 126 **Gooch JL**, Barnes JL, Garcia S, Abboud HE. Calcineurin is activated in diabetes and is required for glomerular hypertrophy and ECM accumulation. *Am J Physiol Renal Physiol* 2003; **284**: F144-F154 [PMID: 12388427 DOI: 10.1152/ajprenal.00158.2002]
- 127 **Van Laecke S**, Van Biesen W, Verbeke F, De Bacquer D, Peeters P, Vanholder R. Posttransplantation hypomagnesemia and its relation with immunosuppression as predictors of new-onset diabetes after transplantation. *Am J Transplant* 2009; **9**: 2140-2149 [PMID: 19624560 DOI: 10.1111/j.1600-6143.2009.02752.x]
- 128 **Rostambeigi N**, Lanza IR, Dzeja PP, Deeds MC, Irving BA, Reddi HV, Madde P, Zhang S, Asmann YW, Anderson JM, Schimke JM, Nair KS, Eberhardt NL, Kudva YC. Unique cellular and mitochondrial defects mediate FK506-induced islet  $\beta$ -cell dysfunction. *Transplantation* 2011; **91**: 615-623 [PMID: 21200364 DOI: 10.1097/TP.0b013e3182094a33]
- 129 **Fernandez LA**, Lehmann R, Luzi L, Battezzati A, Angelico MC, Ricordi C, Tzakis A, Alejandro R. The effects of maintenance doses of FK506 versus cyclosporin A on glucose and lipid metabolism after orthotopic liver transplantation. *Transplantation* 1999; **68**: 1532-1541 [PMID: 10589951 DOI: 10.1097/00007890-199911270-00017]
- 130 **Konrad T**, Steinmüller T, Vicini P, Toffolo G, Grewerus D, Schüller A, Bechstein WO, Usadel KH, Cobelli C, Neuhaus P. Regulation of glucose tolerance in patients after liver transplantation: impact of cyclosporin versus tacrolimus therapy. *Transplantation* 2000; **69**: 2072-2078 [PMID: 10852599 DOI: 10.1097/00007890-200005270-00017]
- 131 **Gane E**. The natural history and outcome of liver transplantation in hepatitis C virus-infected recipients. *Liver Transpl* 2003; **9**: S28-S34 [PMID: 14586892 DOI: 10.1053/jlts.2003.50248]
- 132 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]
- 133 **Bansal S**, Singal AK, McGuire BM, Anand BS. Impact of all oral anti-hepatitis C virus therapy: A meta-analysis. *World J Hepatol* 2015; **7**: 806-813 [PMID: 25914781 DOI: 10.4254/wjh.v7.i5.806]
- 134 **Fazel Y**, Lam B, Golabi P, Younossi Z. Safety analysis of sofosbuvir and ledipasvir for treating hepatitis C. *Expert Opin Drug Saf* 2015; **14**: 1317-1326 [PMID: 26043900 DOI: 10.1517/14740338.2015.1053868]
- 135 **Charlton M**, Seaberg E. Impact of immunosuppression and acute rejection on recurrence of hepatitis C: results of the National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. *Liver Transpl Surg* 1999; **5**: S107-S114 [PMID: 10431024]
- 136 **Kato T**, Gaynor JJ, Yoshida H, Montalvano M, Takahashi H, Pyrsopoulos N, Nishida S, Moon J, Selvaggi G, Levi D, Ruiz P, Schiff E, Tzakis A. Randomized trial of steroid-free induction versus corticosteroid maintenance among orthotopic liver transplant recipients with hepatitis C virus: impact on hepatic fibrosis progression at one year. *Transplantation* 2007; **84**: 829-835 [PMID: 17984834 DOI: 10.1097/01.tp.0000282914.20578.7b]
- 137 **Brillanti S**, Vivarelli M, De Ruvo N, Aden AA, Camaggi V, D'Errico A, Furlini G, Bellusci R, Roda E, Cavallari A. Slowly tapering off steroids protects the graft against hepatitis C recurrence after liver transplantation. *Liver Transpl* 2002; **8**: 884-888 [PMID: 12360428 DOI: 10.1053/jlts.2002.34640]
- 138 **Rosen HR**, Shackleton CR, Higa L, Gralnek IM, Farmer DA, McDiarmid SV, Holt C, Lewin KJ, Busuttil RW, Martin P. Use of OKT3 is associated with early and severe recurrence of hepatitis C after liver transplantation. *Am J Gastroenterol* 1997; **92**: 1453-1457 [PMID: 9317061]
- 139 **Bahra M**, Neumann UI, Jacob D, Puhl G, Klupp J, Langrehr JM, Berg T, Neuhaus P. MMF and calcineurin taper in recurrent hepatitis C after liver transplantation: impact on histological course. *Am J Transplant* 2005; **5**: 406-411 [PMID: 15644002 DOI: 10.1111/j.1600-6143.2004.00706.x]
- 140 **Aghemo A**, Donato MF. Sofosbuvir treatment in the pre and post liver transplantation phase: the sooner, the better. *Gastroenterology* 2015; **148**: 13-16 [PMID: 25451651 DOI: 10.1053/j.gastro.2014.11.025]
- 141 **Forns X**, García-Retortillo M, Serrano T, Feliu A, Suarez F, de la Mata M, García-Valdecasas JC, Navasa M, Rimola A, Rodés J. Antiviral therapy of patients with decompensated cirrhosis to prevent recurrence of hepatitis C after liver transplantation. *J Hepatol* 2003; **39**: 389-396 [PMID: 12927925 DOI: 10.1016/S0168-8278(03)00310-6]
- 142 **Everson GT**, Terrault NA, Lok AS, Rodrigo del R, Brown RS, Saab S, Shiffman ML, Al-Osaimi AM, Kulik LM, Gillespie BW, Everhart JE. A randomized controlled trial of pretransplant antiviral therapy to prevent recurrence of hepatitis C after liver transplantation. *Hepatology* 2013; **57**: 1752-1762 [PMID: 22821361 DOI: 10.1002/hep.25976]
- 143 **Price JC**, Terrault NA. Treatment of hepatitis C in liver transplant patients: interferon out, direct antiviral combos in. *Liver Transpl* 2015; **21**: 423-434 [PMID: 25604355 DOI: 10.1002/lt.24080]
- 144 **Siddiqui MS**, Sterling RK. Posttransplant metabolic syndrome. *Int J Hepatol* 2012; **2012**: 891516 [PMID: 23227347 DOI: 10.1155/2012/891516]
- 145 **Rike AH**, Mogilishetty G, Alloway RR, Succop P, Roy-Chaudhury P, Cardi M, Kaiser TE, Thomas M, Woodle ES. Cardiovascular risk, cardiovascular events, and metabolic syndrome in renal transplantation: comparison of early steroid withdrawal and chronic steroids. *Clin Transplant* 2008; **22**: 229-235 [PMID: 18339144 DOI: 10.1111/j.1399-0012.2007.00779.x]
- 146 **Morrisett JD**, Abdel-Fattah G, Kahan BD. Sirolimus changes



- lipid concentrations and lipoprotein metabolism in kidney transplant recipients. *Transplant Proc* 2003; **35**: 143S-150S [PMID: 12742487 DOI: 10.1016/S0041-1345(03)00233-1]
- 147 **Manzia TM**, De Liguori Carino N, Orlando G, Toti L, De Luca L, D'Andria D, Cardillo A, Anselmo A, Casciani CU, Tisone G. Use of mycophenolate mofetil in liver transplantation: a literature review. *Transplant Proc* 2005; **37**: 2616-2617 [PMID: 16182764 DOI: 10.1016/j.transproceed.2005.06.073]
  - 148 **Peddi VR**, Wiseman A, Chavin K, Slakey D. Review of combination therapy with mTOR inhibitors and tacrolimus minimization after transplantation. *Transplant Rev (Orlando)* 2013; **27**: 97-107 [PMID: 23932018 DOI: 10.1016/j.trre.2013.06.001]
  - 149 **Mathis AS**, Egloff G, Ghin HL. Calcineurin inhibitor sparing strategies in renal transplantation, part one: Late sparing strategies. *World J Transplant* 2014; **4**: 57-80 [PMID: 25032096 DOI: 10.5500/wjt.v4.i2.57]
  - 150 **Vodenik B**, Rovira J, Campistol JM. Mammalian target of rapamycin and diabetes: what does the current evidence tell us? *Transplant Proc* 2009; **41**: S31-S38 [PMID: 19651294 DOI: 10.1016/j.transproceed.2009.06.159]
  - 151 **Xiao X**, Wang J, Chang X, Zhen J, Zhou G, Hu Z. Mycophenolate mofetil ameliorates diabetic nephropathy through epithelial mesenchymal transition in rats. *Mol Med Rep* 2015; **12**: 4043-4050 [PMID: 26080907 DOI: 10.3892/mmr.2015.3934]
  - 152 **Pérez T**, Segovia R, Castro L, Roblero JP, Estela R. Conversion to everolimus in liver transplant patients with renal dysfunction. *Transplant Proc* 2011; **43**: 2307-2310 [PMID: 21839260 DOI: 10.1016/j.transproceed.2011.06.009]
  - 153 **Gonwa T**, Mendez R, Yang HC, Weinstein S, Jensik S, Steinberg S. Randomized trial of tacrolimus in combination with sirolimus or mycophenolate mofetil in kidney transplantation: results at 6 months. *Transplantation* 2003; **75**: 1213-1220 [PMID: 12717205 DOI: 10.1097/01.TP.0000062837.99400.60]
  - 154 **Knoll GA**, MacDonald I, Khan A, Van Walraven C. Mycophenolate mofetil dose reduction and the risk of acute rejection after renal transplantation. *J Am Soc Nephrol* 2003; **14**: 2381-2386 [PMID: 12937317 DOI: 10.1097/01.ASN.0000079616.71891.F5]
  - 155 **Faivre S**, Kroemer G, Raymond E. Current development of mTOR inhibitors as anticancer agents. *Nat Rev Drug Discov* 2006; **5**: 671-688 [PMID: 16883305 DOI: 10.1038/nrd2062]

**P- Reviewer:** Sanal MG **S- Editor:** Kong JX  
**L- Editor:** A **E- Editor:** Liu SQ



## Innate immunity and hepatocarcinoma: Can toll-like receptors open the door to oncogenesis?

Jorge André Gomes Lopes, Marta Borges-Canha, Pedro Pimentel-Nunes

Jorge André Gomes Lopes, Marta Borges-Canha, Pedro Pimentel-Nunes, Department of Physiology and Cardiothoracic Surgery, Cardiovascular Research and Development Unit, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

Pedro Pimentel-Nunes, Gastroenterology Department, Portuguese Oncology Institute, 4200-072 Porto, Portugal

Pedro Pimentel-Nunes, CINTESIS/Department of Biostatistics and Medical Informatics, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

**Author contributions:** Pimentel-Nunes P and Borges-Canha M designed the methodology followed; Lopes JAG performed the research and analysed the data; Lopes JAG and Borges-Canha M wrote the paper; Borges-Canha M and Pimentel-Nunes P made critical revisions to the paper.

**Conflict-of-interest statement:** All the authors certify that they have no conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Pedro Pimentel-Nunes, Professor, Department of Physiology and Cardiothoracic Surgery, Cardiovascular Research and Development Unit, Faculty of Medicine, University of Porto, Al. Prof. Hernâni Monteiro, 4200-319 Porto, Portugal. [pedronunesml@gmail.com](mailto:pedronunesml@gmail.com)  
 Telephone: +351-96-7340096  
 Fax: +351-22-5513601

Received: June 28, 2015

Peer-review started: July 11, 2015

First decision: August 16, 2015

Revised: November 15, 2015

Accepted: December 4, 2015

Article in press: December 8, 2015

Published online: January 28, 2016

### Abstract

Hepatocarcinoma (HCC) is a highly prevalent cancer worldwide and its inflammatory background was established long ago. Recent studies have shown that innate immunity is closely related to the HCC carcinogenesis. An effective innate immunity response relies on the toll-like receptors (TLR) found in several different liver cells which, through different ligands and many signaling pathways can elicit, not only a pro-inflammatory but also an oncogenic or anti-oncogenic response. Our aim was to study the role of TLRs in the liver oncogenesis and as a consequence their value as potential therapeutic targets. We performed a systematic review of PubMed searching for original articles studying the relationship between HCC and TLRs until March 2015. TLR2 appears to be a fundamental stress-sensor as its absence reveals an augmented tendency to accumulate DNA-damages and to cell survival. However, pathways are still not fully understood as TLR2 up-regulation was also associated to enhanced tumorigenesis. TLR3 has a well-known protective role influencing crucial processes like angiogenesis, cell growth or proliferation. TLR4 works as an interesting epithelial-mesenchymal transition's inducer and a promoter of cell survival probably inducing HCC carcinogenesis even though an anti-cancer role has already been observed. TLR9's influence on carcinogenesis is also controversial and despite a potential anti-cancer capacity, a pro-tumorigenic role is more likely. Genetic polymorphisms in some TLRs have been found and its influence on the risk of HCC has been reported. As therapeutic targets, TLRs are already in use and have a great potential. In conclusion, TLRs have been shown to be an interesting influence on the HCC's micro-environment, with TLR3 clearly determining an anti-tumour influence. TLR4 and TLR9 are considered to have a positive relationship with tumour development even though, in each of them anti-tumorigenic signals have

been described. TLR2 presents a more ambiguous role, possibly depending on the stage of the inflammation-HCC axis.

**Key words:** Hepatocarcinoma; Carcinogenesis; Toll-like receptor; Innate immunity; Chronic inflammation

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The importance of hepatocarcinoma (HCC) is undeniable in the current medical perspective. However, a lot still remains to be understood in this context. Therefore, this review aims to present the significance of innate immunity in HCC through toll-like receptors as they have already shown interesting effects on tumour's microenvironment, influencing its progression or regression. As a result we also render some therapeutic usages of the established knowledge in this area.

Lopes JAG, Borges-Canha M, Pimentel-Nunes P. Innate immunity and hepatocarcinoma: Can toll-like receptors open the door to oncogenesis? *World J Hepatol* 2016; 8(3): 162-182 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i3/162.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i3.162>

## INTRODUCTION

Liver cancer is one of the most common cancers worldwide, with hepatocarcinoma (HCC) being, by far, the most frequent type<sup>[1,2]</sup>.

Due to its close contact with gut, *via* portal vein, liver faces a continuous exposure to gut-derived bacterial products, toxics and many other agents<sup>[3]</sup>. In the presence of such pathogens or irritants and associated molecules our body is able to respond in a manner that aims to prevent injury and combat infection. This protection system is called inflammation which, despite its tremendous defensive and antiviral/antibacterial importance in the short term, starts to become deleterious when prolonged or exaggerated - chronic inflammation - possibly leading to fibrosis, cirrhosis and, ultimately, HCC<sup>[4]</sup>.

Therefore, the idea that hepatic carcinogenesis arouses from an inflammatory basis is not new. Several studies already focused on the development of HCC and possibilities like the c-Myc elevation or the deregulated SRY and SGF29 pathways have been proposed<sup>[5]</sup>. However, just in the last few years we have become aware of the critical role of innate immunity in chronic liver diseases, including HCC<sup>[6,7]</sup>.

Toll-like receptors (TLRs) are a family of pattern-recognition receptors (PRRs) that can be activated by either pathogen-associated molecular patterns (PAMPs) or danger/damage-associated molecular patterns (DAMPs), with their own importance in eliciting innate immunity, regulation of inflammation and tissue

regeneration. To date, 11 human TLRs have been identified<sup>[8]</sup>. In recent years, activation of several TLRs have been associated with viral hepatitis, steatohepatitis (alcoholic or non-alcoholic) and to the progression of the inflammation-fibrosis-HCC axis<sup>[9-11]</sup>. However, data is somewhat contradictory and no clear conclusions have been made.

In this line of thoughts, this review aims to present an overview of the expression of TLRs in the liver, its influence on the development of liver carcinogenesis as chronic inflammatory inducers or potential oncogenes as well as possible therapeutic targets.

## RESEARCH

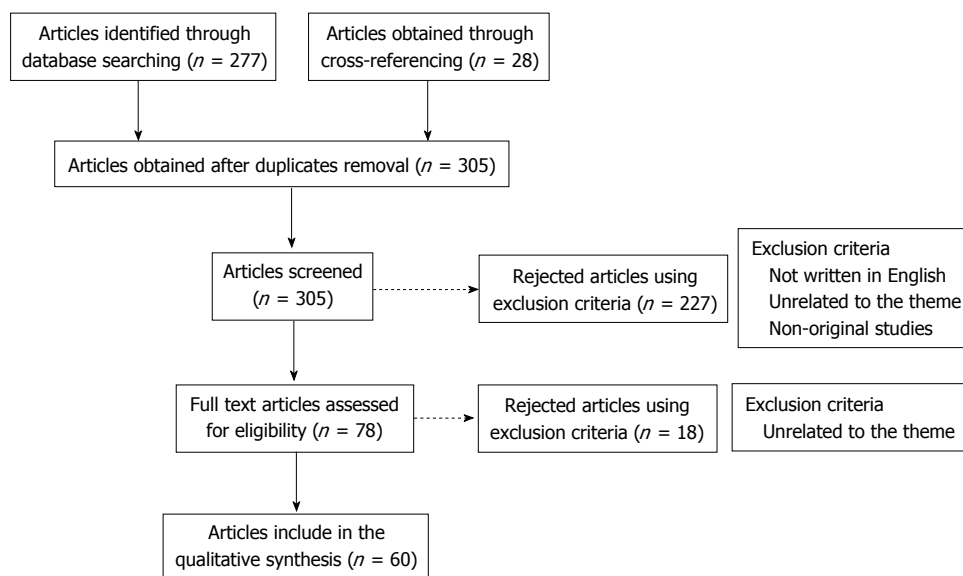
Specific criteria were defined in order to guide this systematic review. Firstly, a query to obtain the articles related to the theme on PubMed was built: [(Hepatocarcinoma) OR (Hepatocarcinogenesis) OR (hepatic cancer) OR (hepatocellular carcinoma) OR (liver cancer)] AND [(toll like receptors) OR (toll like receptor)]. With this query we intended to embrace a wide range of articles until March 2015, which then would be carefully selected.

A total amount of 277 articles were obtained through the referred search. After discarding the duplicates and adding 28 articles obtained through cross-referencing, 305 articles were available to be screened. The following inclusion criteria were used: (1) studies that were published until the end March 2015; (2) the article should be written in English; and (3) studies relevant to the theme (presenting original data). As exclusion criteria we defined: (1) studies considered by the authors as unrelated to the theme; and (2) non-original studies. These criteria were applied by reading the title and abstract resulting in 227 articles excluded. After this step, the remaining 78 studies were selected for full-text reading. On a second level of eligibility, 18 more studies were excluded and 60 studies were selected, analysed and included in this revision (Figure 1).

Data about the defined topics were obtained from each article (Table 1) and the information was then summed up and organized in the present systematic review according to: The TLRs' expression in each liver cell; separately role of TLR2, TLR3, TLR4 and TLR9 in inflammatory-driven hepatocarcinoma; known TLRs' polymorphisms/genetic variations that influence the risk of hepatocarcinoma and lastly, TLRs modulators possibly used in hepatocarcinoma's therapeutics.

## TLR EXPRESSION IN HEPATIC CELL POPULATION

The liver is a very special organ when it comes to dealing with pathogens. Due to its vascular links, contact with gut-derived bacteria is constant and, therefore, mechanisms not only to defend the organism from these pathogens but also to tolerate them, had to be



**Figure 1 Methods' flowchart.** A total amount of 277 articles were obtained, on PubMed, through the query [(hepatocarcinoma) OR (hepatocarcinogenesis) OR (hepatic cancer) OR (hepatocellular carcinoma) OR (liver cancer)] AND [(toll like receptors) OR (toll like receptor)]. After discarding the duplicates and adding 28 articles obtained through cross-referencing, 305 articles were available to be screened. The following inclusion criteria were used: (1) studies that were published until the end March 2015; (2) the article should be written in English; and (3) studies relevant to the theme (presenting original data). As exclusion criteria we defined: (1) studies considered by the authors as unrelated to the theme; and (2) non-original studies. These criteria were applied by reading the title and abstract resulting in 227 articles excluded. After this step, the remaining 78 studies were selected for full-text reading. On a second level of eligibility, 18 more studies were excluded and 60 studies were selected, analysed and included in this revision.

developed<sup>[8]</sup>. In this duality, TLRs play an interesting role as it is known that, in a healthy liver, mRNA levels of TLRs like TLR1, 2, 4, 6, 7, 8, 9 and 10 are decreased when compared to other organs<sup>[9]</sup>.

In the liver, hepatocytes represent 60%-80% of the total cell population. Here, it can be found mRNA from all TLRs; however, only a response from TLR2 and TLR4, to their ligands, can be obtained<sup>[12]</sup>. Interestingly, only the response of TLR2 is up-regulated under inflammatory conditions<sup>[13]</sup>.

Besides hepatocytes, it is possible to find, in the liver, non-parenchymal cells which consist of Kupffer cells (KCs), dendritic cells (DCs), Lymphocytes, hepatic stellate cells (HSCs) and liver sinusoidal endothelial cells (LSECs).

KCs not only express lipopolysaccharide (LPS)-responsive TLR4 but also TLR2, TLR3 and TLR9 that respond to their ligands<sup>[14]</sup>. These cells develop an inflammatory response to high levels of LPS but produce an anti-inflammatory cytokine (IL-10) in response to continuous low levels of LPS, known as LPS tolerance<sup>[15]</sup>. DCs represent a small population (< 1%). In humans, the plasmacytoid DCs subset expresses TLR1, TLR7 and TLR9 while other subsets carry all TLRs with the exception of TLR9<sup>[16]</sup>. When it comes to Lymphocytes population and TLRs relationship it is important to notice that differences can be found from one subpopulation to another. Natural killer (NK) cells contain TLR1, TLR2, TLR3, TLR4, TLR6, TLR7, TLR8 and TLR9<sup>[1,17]</sup> but T cells are only activated through TLR2 while B cells rely on TLR2, TLR4, TLR7 and TLR9<sup>[18]</sup>. HSCs, also in small proportion (< 1%), when activated are able to express TLR4 responsive to LPS which, in turn, enables

inflammatory cytokines' secretion<sup>[19]</sup>. LSECs express mRNA from TLR1 to TLR9 despite not being able to respond to TLR5 ligands<sup>[20]</sup>.

## IMPORTANCE OF TLRs IN INFLAMMATORY-INDUCED HCC

HCC has long been considered a chronic-inflammation driven cancer independently of the possible risk factors; virus induced hepatitis, smoke, alcohol or metabolic diseases. Despite that, the liver is an organ with several mechanisms readily available to defend against carcinogenesis. Among these, we found PRRs, with special attention to TLRs which were already shown to exhibit different roles in the regulation of tumorigenesis and tumour progression. To date several works have documented its influence not only in specific types of cancer - breast, ovarian, prostate and lung - but also in processes directly linked with cancer - resistance to apoptosis, increased invasiveness and metastasis. This is the reflection of their actions on metalloproteases and integrins, tumour cell immune escape, among others<sup>[21-23]</sup>. However, the cellular and molecular effectors mediating the interplay between TLRs and HCC are still largely unknown.

### TLR2

When the TLR2 signal is triggered, the downstream cascade initiates through a "Myd88 dependent pathway" with the activation of the apoptosis signal regulating kinase 1 (ASK1)/p38 mitogen-activated protein kinase (p38 MAPK)/nuclear factor kappa B (NF-κB), or through



Table 1 Table of original studies

Ref.	Year	Type of study	Methods	Limitations	Conclusions
Chew <i>et al</i> <sup>[1]</sup>	2012 December	Experimental	Natural killer cell activation and cytotoxicity were assessed <i>in vitro</i> after treatment with the TLR3 ligand poly (I:C). The effect of TLR in a spontaneous liver tumor mouse model and a transplanted tumor mouse model were determined by Immunohistochemistry and PCR	The effect of poly (I:C) on tumor growth was only analyzed in a transplanted, nonorthotopic model of HCC. The effect of poly (I:C) on human NK cells was assessed only with cells from healthy donors. Not all HCC cell lines undergo apoptosis after TLR3 triggering and the reason is not known	TLR3 is an important modulator of HCC progression and is a potential target for novel immunotherapy
Mohamed <i>et al</i> <sup>[2]</sup>	2015 March	Experimental	Tissue microarrays containing liver samples from patients with cirrhosis, viral hepatitis and HCC were examined for expression of TLR7 and TLR9. Proliferation of human HCC cell lines was studied following stimulation of TLR7 and TLR9 using agonists (imiquimod and CpG-ODN respectively) and inhibition with a specific antagonist (IRS-954) or chloroquine. The effect of these interventions was confirmed in a xenograft model and DEN/NMOR-induced model of HCC	Before translation to the clinical arena, it is important to further characterize the exact mechanisms through which TLR7 and TLR9 exert their actions and determine what effects their inhibition may have on the immune system	Inhibiting TLR7 and TLR9 with IRS-954 or chloroquine could potentially be used as a novel therapeutic approach for preventing HCC development and/or progression in susceptible patients
Dapito <i>et al</i> <sup>[3]</sup>	2012 April	Experimental	TLR2-deficient mice, TLR4-deficient mice, TNFR1-/IL-1R1-double deficient and C57Bl/6 mice were used. HCC was induced by intraperitoneal injection of DEN. Gut-sterilization was done using a combination of ampicillin (1 g/L), neomycin (1 g/L), metronidazole (1 g/L) and vancomycin (500 mg/L) in drinking water. Samples from patients with features of alcoholic hepatitis were used. Liver biopsies were obtained from mice and from cadaveric donors or resection of liver metastases	Clinically feasible methods of targeting the intestinal microbiota or TLR4 need to be established. The quadruple combination of antibiotics employed is not suitable for long-term treatment due to known side effects in patients with advanced liver disease	Gut sterilization restricted to late stages of hepatocarcinogenesis reduced HCC, suggesting that the intestinal microbiota and TLR4 represent therapeutic targets for HCC prevention in advanced liver disease
Eiró <i>et al</i> <sup>[8]</sup>	2014 July	Experimental	The expression levels of TLR3, TLR4 and TLR9 were analyzed from 30 patients with HCC and correlated with various clinicopathological findings and with overall survival	In the scoring system, after immunostaining analysis, when setting of the threshold for positive staining and the determination of the intensity different observers can set different thresholds and intensity levels	An association between TLR3, TLR4 and TLR9 expression and tumor aggressiveness and poor prognosis in HCC has been observed
Liu <i>et al</i> <sup>[12]</sup>	2002 July	Experimental	Cultures of primary mouse hepatocytes were incubated with LPS to assess its effects on the global gene expression, hepatic transcription factors, and MAP kinase activation	Using hepatocytes' cell lines loses the capacity to observe the importance of a direct response to LPS by hepatocytes	NF-κB activation was reduced in TLR4-mutant or -null hepatocytes compared to control hepatocytes
Matsumura <i>et al</i> <sup>[13]</sup>	2000 October	Experimental	PCR analysis of mice's hepatocytes and an murine hepatoma cell line Hepa 1-6	Murine hepatoma cell line Hepa 1-6 may have reached an overquantitative level after stimulation	LPS and proinflammatory cytokines differentially regulate gene expression of TLR2 and TLR4 in murine hepatocytes, which may lead to pathologic and host defense reactions in the liver
Thobe <i>et al</i> <sup>[14]</sup>	2007 March	Experimental	Western blotting and cytokine analysis in a cell culture. Evaluation of Kupfer cells response after a trauma-hemorrhage procedure	Does not explain if the increase in MAPK-activity is due to TLRs' overexpression	Kupffer cell TLR signaling employs different MAPK pathways in eliciting cytokine and chemokine responses following trauma-hemorrhage

Knolle <i>et al</i> <sup>[15]</sup>	1995 February	Experimental	Human Kupffer cells were isolated by collagenase perfusion followed by centrifugal elutriation and analyzed for cytokine secretion after 3 d in culture	Only IL-10 and IL-6 were analysed	The important role for IL-10 in the regulation of the local immune response in the liver sinusoid after Kupffer cells exposure to lipopolysaccharide
Edwards <i>et al</i> <sup>[16]</sup>	2003 April	Experimental	Splenocyte repopulations were enriched for D11c <sup>+</sup> and for Ly6C <sup>+</sup> cells using magnetic selection. Four populations were routinely isolated and TLR's mRNA was amplified by PCR	To analyze the functional significance of TLR mRNA expression in DCs subsets it was only used ligands for TLR7 and TLR9	mRNA for most TLRs is expressed at similar levels by murine splenic DC subtypes. TLR expression between plasmacytoid and non-plasmacytoid DC is not conserved between species
Sawaki <i>et al</i> <sup>[17]</sup>	2007 March	Experimental	Total RNA was extracted, and mRNA for TLR1, 2, 3, 4, 5, 6, 7, 9 and b-actin was determined by reverse transcription-PCR. Nuclear localization of NF- $\kappa$ B was determined and cytokines and chemokines were measured by a commercially available kit	It was not evaluated precise roles of NK cell responses <i>in vivo</i>	Upon microbial infection, macrophages produce IL-12 that renders NK cells highly responsive to TLR agonists to produce IFN- $\gamma$ and chemokines, which might in turn recruit and fully activate macrophages
Meyer-Bahlburg <i>et al</i> <sup>[18]</sup>	2007 December	Experimental	It was compared the TLR response profile of germinal center after immunization <i>vs</i> naive mature B cell subsets, using real time PCR, ELISA and Western Blotting to evaluate MyD88 pathway	TLRs' role in B-cells immune response was only accessed in splenic B cells from MyD88 WT, Het, or KO, being studied only the MyD88-dependent pathway	B cell-intrinsic TLR signals are not required for antibody production or maintenance
Paik <i>et al</i> <sup>[19]</sup>	2003 May	Experimental	LPS-associated signalling molecules in culture-activated HSCs and HSCs isolated from patients with hepatitis C virus-induced cirrhosis was evaluated by NF- $\kappa$ B-dependent luciferase reporter gene assays, electrophoretic mobility shift assays and <i>in vitro</i> kinase assays	It does not fully explain why only full activated HSCs respond to LPS. It was not evaluated the activation of TLR4 downstream molecules like MyD88	Human activated HSCs utilize components of TLR4 signal transduction cascade to stimulate NF- $\kappa$ B and JNK and up-regulate chemokines and adhesion molecules
Wu <i>et al</i> <sup>[20]</sup>	2010 March	Experimental	Isolated Kupffer cell and liver sinusoidal endothelial cells from wild-type C57BL/6 mice and examined their responses to TLR1 to TLR9 agonists. Characterization of cell surface protein expression was done by flow cytometry and quantification of mRNA was done by reverse transcription-polymerase chain reaction	The <i>in vitro</i> assay does not explore the organ-specific regulation of immune responses. For the identification of TLR-induced antiviral cytokine(s) only TLR3 and TLR4 were used	Non-parenchymal cells display a restricted TLR-mediated activation profile when compared with "classical" antigen-presenting cells which may, at least in part, explain their tolerogenic function in the liver
Huang <i>et al</i> <sup>[21]</sup>	2012 July	Experimental	TLR expression in BLE-7402 cells was assayed by RT-PCR, real-time PCR and FCM. To investigate the function of TLR2 in hepatocarcinoma growth, BLE-7402 cells were transfected with recombinant plasmids expressing one TLR2 siRNA	Only the effect on tumour volume is evaluated after tumour implantation in nude mice	TLR2 knockdown inhibit proliferation of cultured hepatocarcinoma cells and decrease the secretion of cytokines
Kim <i>et al</i> <sup>[22]</sup>	2009 January	Experimental	LLC cells were implanted in mice. Metastasis enhancing factors were identified on a QSTAR XL qTOF mass spectrometer. Gene and protein expression were monitored by Q-PCR and immunoblot analysis. Tumors were analyzed by immunohistochemistry and indirect immunofluorescence	It does not explain if the interaction between versican and TLR2 is direct or depends on a versican's ligand	By activating TLR2:TLR6 complexes and inducing TNF- $\alpha$ secretion by myeloid cells, versican strongly enhances lewis lung carcinoma metastatic growth
Lin <i>et al</i> <sup>[24]</sup>	2013 January	Experimental	A DEN injection was done in TLR2 <sup>-/-</sup> and WT mice. Then they were sham-treated or treated with interferon-gamma. TUNEL, heterochromatin and SA b-gal staining were performed	The mechanism by which TLR2 signaling participates in the regulation of cellular senescence to maintain growth arrest and promote programmed cell death remains inconclusive	Loss of immune networks may play a role in the failure of initiating and maintaining cellular senescence and autophagy flux in the TLR2-mutant liver tissue

Lin <i>et al</i> <sup>[25]</sup>	2013 October	Experimental	WT mice were pre-treated with anti-TLR2 antibody and a subset of TLR2 <sup>-/-</sup> mice were pre-treatment with NAC (antioxidant) or physiological saline. Both were submitted to DEN. Histology was submitted to western blotting, ROS assay, immunohistochemistry and immunofluorescence	It does not report any results about the effects on non-parenchymal cells like Kupffer cells. It does not reveal interactions that regulate the signal from TLR2 activation to suppression of oxidant and ER stressors in HCC	A TLR2 activity defends against hepatocarcinogenesis through diminishing the accumulation of ROS and alleviating ER stress and unfold protein response
Li <i>et al</i> <sup>[26]</sup>	2015 March	Experimental	WT and Tlr2 <sup>-/-</sup> mice were used. Flow cytometry, Histopathological analysis and Immunofluorescence, Western blot and ELISA were performed. MDSC induction <i>in vitro</i> and functional T cell suppression assay and knockdown of IL-18 and caspase-8 in hepatocytes with quantitative PCR were also done	The exact role of IL-18 in MDSC generation is still unknown. It does not reveal the levels of TLR2 that determine the possible use of IL-18 as a therapeutic target	TLR2 deficiency accelerates IL-18-mediated immunosuppression during liver carcinogenesis, providing new insights into immune control that may assist the design of effective immunotherapies to treat HCC
Soares <i>et al</i> <sup>[27]</sup>	2012 October	Analytic - cross sectional	It was used samples from patients with hepatitis, cirrhosis and hepatocarcinoma. mRNA isolation and quantification of TLR2, TLR4, NF-κB, TNF-α and COX-2 were performed. Immunohistochemical evaluation of TLR2 and TLR4 was also done	Most patients included in the reference group have evidence of NAFLD and it was demonstrated that NAFLD is associated with increased hepatic TLR2 and TLR4-mRNA expression. the hepatitis, cirrhosis and hepatocarcinoma groups included both patients with HBV infection or HCV infection. Included only patients with virus-induced chronic hepatitis. The method used for quantification of protein expression was semi-quantitative	Increased expression of TLR2 and TLR4 in hepatitis and cirrhosis and maintained expression in hepatocarcinoma. Up-regulation of TLR2, TLR4 and their pro-inflammatory mediators is associated with virus-induced hepatic IFC sequence
Dolado <i>et al</i> <sup>[31]</sup>	2007 February	Experimental	WT and p38a <sup>-/-</sup> were used. Growth in soft agar was evaluated. Intracellular ROS levels were determined, immunoblot Analysis was performed. To induce p38 MAPK activation, cells were treated with H <sub>2</sub> O <sub>2</sub> , sorbitol and cisplatin	The tumorigenesis enhanced by ROS is not evaluated on hepatocarcinoma	Oxidative stress sensing plays a key role in the inhibition of tumor initiation by p38alpha
Kang <i>et al</i> <sup>[32]</sup>	2011 November	Experimental	For transposon-mediated intra-hepatic gene transfer mice received a transposon- to transposase encoding vector (30 mg total DNA). DNA was administered by hydrodynamic tail vein injection. Immunohistochemical analyses were performed	It was not investigated if factors secreted from pre-malignant senescent hepatocytes also contribute to the oncogenic transformation of neighbouring cells	Indicates that senescence surveillance represents an important extrinsic component of the senescence anti-tumour barrier, and illustrates how the cellular senescence program is involved in tumour immune surveillance
Ogata <i>et al</i> <sup>[34]</sup>	2006 December	Experimental	Electron microscopic analysis was performed using neuroblastoma SK-N-SH cells exposed to ER stressors. GFP-LC3 fluorescence was used to monitor autophagy in cells transiently transfected with an expression vector for GFP-LC3. Then was performed an Amino acid uptake assay and autophagosome formation was evaluated	A signalling pathway other than the IRE1-JNK pathway may also play important roles in the activation of autophagy signalling after ER stress. The detailed signalling pathway for activation of the autophagy induced by ER stress is still unknown	Disturbance of autophagy rendered cells vulnerable to ER stress, suggesting that autophagy plays important roles in cell survival after ER stress
Pikarsky <i>et al</i> <sup>[36]</sup>	2004 September	Experimental	The possibility that NF-κB activation is involved in Mdr2-knockout hepatocarcinogenesis was investigated by RelA (p65) immunostaining. Hystological analysis was performed. To study the relationship between the	It does not explain how the inflammatory process in Mdr2-knockout mice is maintained in the double mutants as it is independent of hepatocyte NF-κB activity	NF-κB is essential for promoting inflammation-associated cancer, and is therefore a potential target for cancer prevention in chronic inflammatory diseases

Gong <i>et al</i> <sup>[37]</sup>	2013 September	Experimental	TNF- $\alpha$ -producing cells and NF- $\kappa$ B activation in the hepatocytes, liver sections were stained for both TNF- $\alpha$ and p65	It does not explain the mechanisms responsible by the NF- $\kappa$ B's phosphorylation in the first 30 min. It was observed only one of the pathways responsible for the involvement of HMGB1/RAGE in the NF- $\kappa$ B signaling	Activation of NF- $\kappa$ B was indispensable for the effect of HSP70. HSP70 induced a positive feedback loop involving Beclin-1/HMGB1 production, causing re-phosphorylation of NF- $\kappa$ B
			BALB/c mice were used and inoculated with H22 hepatocarcinoma cells into the hind thigh muscle. They were treated with TLR2/4 ligands, HSP70 and HMGB1. The main tumor nodules were measured and satellite tumor nodes counted. To downregulate HMGB1, RAGE or Beclin-1 in tumor cells, cells were transduced with short interfering RNA		
			Human hepatocellular carcinoma cell lines were used. Into the cell lines were transfected small-interfering-RNAs and at 48 h after transfection, the TLR2-siRNA-transfected group, scramble control group, and blank group were treated with recombinant-HMGB1. Evaluation included real time PCR, Western blot, MTT assay, Transwell assay and Flow cytometry assay		
			It was used mice and HCC cell lines. Eukaryotic expression vectors psTLR2 and psTLR4 were created. An adhesion assay, a tumor cell proliferation assay, a flow cytometric analysis, an apoptosis analysis, an analysis of gene expression by RT-PCR and a western blot analysis were performed		
			HCC cell lines and 74 HCC samples were used. Poly (I:C), cycloheximide and actinomycin were included in the study. Profiling analysis of TLRs recognized by viral components, flow cytometric analysis, immunohistochemical staining, Detection of TLR3 by immunofluorescence, Detection of cell viability and apoptosis assays, Detection of apoptosis-related proteins by immunoblotting, NF- $\kappa$ B activity assays and measurement of IFN- $\beta$ were also performed		
Shi <i>et al</i> <sup>[38]</sup>	2014 October	Experimental	Human hepatocellular carcinoma cell lines were used. Into the cell lines were transfected small-interfering-RNAs and at 48 h after transfection, the TLR2-siRNA-transfected group, scramble control group, and blank group were treated with recombinant-HMGB1. Evaluation included real time PCR, Western blot, MTT assay, Transwell assay and Flow cytometry assay	It does not explore the signaling pathway that regulates NF- $\kappa$ B through TLR2 inhibition or stimulation with recombinant-HMGB1	TLR2-siRNA could effectively inhibit the growth, migration, invasion, and expression of NF- $\kappa$ B/P65, and HMGB1 promoted HCC progression <i>via</i> TLR2
Wu <i>et al</i> <sup>[39]</sup>	2012 April	Experimental	It was used mice and HCC cell lines. Eukaryotic expression vectors psTLR2 and psTLR4 were created. An adhesion assay, a tumor cell proliferation assay, a flow cytometric analysis, an apoptosis analysis, an analysis of gene expression by RT-PCR and a western blot analysis were performed	More than one signaling pathways activated by HSPA1A might be required for the survival of tumor cells. The effect of eHSPA1A was only evaluated in one cell line. Injection of HSPA1A suppressed tumor growth in early stage of tumor development, but promoted tumor growth in later stage	Extracellular HSPA1A functions as endogenous ligand for TLR2 and TLR4 to facilitate tumor growth
Yoneda <i>et al</i> <sup>[41]</sup>	2008 November	Experimental	HCC cell lines and 74 HCC samples were used. Poly (I:C), cycloheximide and actinomycin were included in the study. Profiling analysis of TLRs recognized by viral components, flow cytometric analysis, immunohistochemical staining, Detection of TLR3 by immunofluorescence, Detection of cell viability and apoptosis assays, Detection of apoptosis-related proteins by immunoblotting, NF- $\kappa$ B activity assays and measurement of IFN- $\beta$ were also performed	Further evaluation of the possible roles and the type of regulation associated with TLR3 needs to be undertaken	Intracellular TLR3 signalling is involved in cell death, while in contrast, the cell surface TLR3 signalling is responsible for activation of NF- $\kappa$ B
Zorde-Khvalevsky <i>et al</i> <sup>[42]</sup>	2009 July	Experimental	It was used TLR3-WT mice and TLR3 <sup>-/-</sup> mice. Partial hepatectomy was done followed by immunohistochemistry stainings, plasma aminotransferase activity assay, measurements of serum cytokine levels, semi-quantitative reverse-transcription polymerase chain reaction, Western blotting, caspase-8 immunopurification and injection with poly (I:C) or saline solution	It is not explained what happens to the levels of ALT in mice's serum before the 10-h time point following 70% PHx. Cytokine evaluation only includes IL-6 and IL-22	TLR3 plays an inhibitory role in the priming of liver regeneration, thus reinforcing the role of the innate immune system in balancing tissue regeneration
Khvalevsky <i>et al</i> <sup>[44]</sup>	2007 April	Experimental	Various cell lines and plasmids pTLR7, pTLR8, and pTLR9, carrying the respective human <i>TLR</i> gene, were used. Transfection	The role of TLR3 signaling in normal hepatocytes requires further investigation <i>in vivo</i> . It is not specified the degree of	Preferential induction of the apoptotic pathway over the cytokine induction pathway by TLR3 signaling in



Chen <i>et al</i> <sup>[45]</sup>	2012 July	Experimental	assays, RNA quantification, immuno-staining and flow cytometry, were performed  The human HCC cell line HepG2.2.15 was used. After treating HepG2.2.15 with BM-06 or poly (I:C), NF-κB activity was checked by dual luciferase reporter gene kit. Then it was performed a nuclear and cytoplasmic extraction, Western blot analysis, a cell proliferation assay, cell invasion assays and flow-cytometry was used to determine the apoptotic rate	NF-κB activation obtained from the overexpression of TLR3 nor the degree of this overexpression that is needed  The role of TLR3 in the antiviral defense against HBV was not analyzed according to differences in the type of viruses, the type of cells that are infected, the viral load, its model of infection (endoplasmic <i>vs</i> cytoplasmic), and stage of infection	hepatocellular carcinoma cells with potential implications for therapeutic strategies  BM-06 inhibited the proliferation, invasion and secretion of HBV, and induced apoptosis in HepG2.2.15 cells. In addition, the antitumor effects of BM-06 were superior to poly (I:C)
Guo <i>et al</i> <sup>[46]</sup>	2012 February	Experimental	Cell cultures were used and submitted to BM-06 and poly (I:C) treatment. RNA isolation and one-step quantitative real-time PCR were performed. Analysis included detection of TLR3 by immunocytochemistry, luciferase reporter assays, Endothelial cell tube formation assay, rat aortic ring assay, annexin V/PI for cell apoptotic analysis and Cell migration assays	It does not evaluate the molecular mechanisms after TLR3 stimulation that lead to modulation of endothelial tube-forming activity of HUVECs and vascular sprouting or enhanced apoptosis	TLR3 agonists not only affect tumor microenvironment by suppressing angiogenesis but also directly induce tumor cell apoptosis and inhibit tumor cell migration
Bergé <i>et al</i> <sup>[47]</sup>	2010 December	Experimental	It was injected transgenic mice developing HCC with either control siRNAs or siRNA targeting neuropilin-1. The study used antibodies (goat anti-TLR3 and rabbit anti-tubulin antibody), Western Blotting, and Immunofluorescence Analysis. Real-time RT-PCR, ELISA, MTT assay and three-dimensional collagen assay were also performed	It is not known why INF-γ does not inhibit cells' functions in the <i>in vitro</i> study despite the high levels in HCC. <i>In vivo</i> evaluation was not performed	Synthetic siRNAs inhibit target-independently HCC growth and angiogenesis through the activation of the innate interferon response and by directly inhibiting endothelial cell function
Xu <i>et al</i> <sup>[48]</sup>	2013 October	Experimental	Thirty rats were used, all 30 were fed with 2-acetylaminofluorene to establish the HCC model. Two animal groups were treated, respectively, with the drug candidate (BM-06) and poly (I:C). It was performed a H and E staining, an Immunohistochemical staining, a Western blot analysis	It does not explore the pathway through which BM-06 and poly (I:C) are capable of inducing cell death. It is not evaluated TLR3's downstream molecules to explain the signalling pathway responsible for these results	Treatment with BM-06, showed a decrease in tumor growth and cell proliferation, and an increase in apoptosis compared with that in a phosphate-buffered saline control group
Wang <i>et al</i> <sup>[49]</sup>	2013 August	Experimental	Fifty-three HCC and ten normal liver specimens were analyzed by immunohistochemistry, and three cell lines were used for <i>in vitro</i> studies. Lipopolysaccharide was used to activate TLR4 signaling. Cell survival, proliferation and invasion were examined	Only a specific amount of LPS has shown to have an effect on the mRNA expression of IL-6, EGFR and HB-EGF. Opposing to HL-7702 cell line, PLC/PRF/5, with a moderate level of TLR4 expression, was not affected by inhibiting p38	Indicate that TLR4 signaling in cancer cells promotes cell survival and proliferation in HCC
Liu <i>et al</i> <sup>[50]</sup>	2015 March	Experimental	Two HCC cell lines and a splenic vein metastasis of the nude mouse model were used. A total of 88 clinical samples from HCC patients were used. A fluorescence activated cell sorting system and flow cytometry analysis were performed. Nude mouse splenic vein metastasis assay, immunohistochemistry analysis, real-time quantitative PCR, Western blot analysis, immunofluorescence and cell apoptosis assay were also done	More pathological specimens should be enrolled to verify the tendencies of association between TLR4 expression and malignant characteristics of HCC found in this study. A particular signaling pathway involved in the relationship between TLR4 expression and stem cell features remains elusive	There is a relationship between TLR4 expression and CSC's features, TLR4 may act as a CSC marker, prompting tumor invasion and migration, which contributes to the poor prognosis of HCC

Li <i>et al.</i> <sup>[52]</sup>	2014 October	Experimental	A HCC cell line was used where a Scratch assay was performed. Invasion assay, Western blot analysis, quantitative real-time reverse transcription PCR and siRNA knockdown of <i>TLR4</i> gene expression were also done	It does not reveal the time needed for induction of epithelial-mesenchymal transition after LPS stimulus. Does not explore influence of LPS on TLR2	TLR4/JNK/MAPK signaling is required for LPS-induced EMT, tumor cell invasion and metastasis, which provide molecular insights for LPS-related pathogenesis and a basis for developing new strategies against metastasis in HCC
Jing <i>et al.</i> <sup>[53]</sup>	2012 August	Experimental	Four HCC cell lines and a splenic vein metastasis of the nude mouse model were used and stable TLR4-expressed and knocked-down cell lines were generated. 106 clinical samples from HCC patients were also used. Quantitative real-time PCR, Western-blot analysis, Immunofluorescence, FACS Analysis and IHC analysis were performed	HCC development is a multifactorial and complicated process, which has a close association with various risk factors. Many gene alterations and cytokines also could induce EMT. HCC cells with low expression or even a lack of TLR4 are not susceptible to LPS, they might perform EMT induced by other TLR4-independent mechanisms	TLR4 signaling is required for LPS-induced EMT, tumor cell invasion and metastasis, which provide molecular insights for LPS-related pathogenesis and a basis for developing new strategies against metastasis in HCC
Xu <i>et al.</i> <sup>[54]</sup>	2014 October	Experimental	HCC and adjacent tissues were obtained from 84 patients. HCC cell lines were used and a PLV-PTPRO-GFP plasmid was constructed. Real-time PCR, immunofluorescence, Western blot analysis and cell proliferation assay were performed	It does not specify the doses of NF- $\kappa$ B specific inhibitor needed to result in a decreasing of PTPRO's levels in Huh7 cells stimulated with LPS	The effect of PTPRO on TLR4 signaling is dependent on NF- $\kappa$ B pathway, suggesting an interesting PTPRO/TLR4/NF- $\kappa$ B signaling feedback loop in HCC carcinogenesis and progression
Wang <i>et al.</i> <sup>[55]</sup>	2015 January	Experimental	It was used LPS-induced human hepatocellular carcinoma cell lines. Cell viability was assessed using the MTT assay. Double staining for annexin V-FITC and propidium iodide was performed. Inflammatory mediators were evaluated through a specific ELISA kit. Immunoprecipitation and Western blot analysis were also used	Only one type of cell line is used to observe the effect of CXC-195. It does not reveal the level (high or low) of TLR4 expression. It does not explore the influence of LPS in TLR2	Treatment with CXC195 could attenuate the TLR4-mediated proliferation and inflammatory response in LPS-induced HepG2 cells
Yu <i>et al.</i> <sup>[56]</sup>	2010 October	Experimental	Rats and mice were used, including TLR4-deficient mice. Immunohistochemical analysis and bone marrow transplantation were performed	It does not explore the effect of modulating gut flora. It does not evaluate the effect of different LPS' levels	Sustained LPS accumulation represents a pathological mediator of inflammation-associated HCC and manipulation of the gut flora to prevent pathogenic bacterial translocation
Lin <i>et al.</i> <sup>[58]</sup>	2012 September	Experimental	It was used wild-type and TLR4-deficient mice. A flow cytometry analysis and Isolation and Culture of CD4 <sup>+</sup> cells were performed	TLR4 knockout showed decreased liver injury induced by Con A, contrarily to what was expected. It is needed to determine whether the regimen with antiendotoxin effects will prove beneficial in preventing or delaying T cell-mediated hepatitis and hepatitis-induced HCC	Gut-derived LPS and TLR4 play important positive roles in Con A-induced hepatitis and modulation of the gut microbiota may represent a new avenue for therapeutic intervention
Chen <i>et al.</i> <sup>[60]</sup>	2013 July	Experimental	It used HCV Tg mouse models and patients with HCC functional cDNA. Then, functional cDNA screening for oncogenes was performed. <i>In vitro</i> and <i>in vivo</i> oncogenic activities were evaluated. It was also done a liver TIC engraftment <i>via</i> splenic injection	The degree of attenuation of TLR4 expression in TICs by Nanog, implying a feedback loop is not shown. Besides this, the underlying mechanisms are not known	TLR4/NANOG oncogenic pathway is linked to suppression of cytostatic TGF- $\beta$ signaling and could potentially serve as a therapeutic target for HCV-related HCC
French <i>et al.</i> <sup>[63]</sup>	2013 August	Experimental	Liver biopsies from patients diagnosed with alcoholic hepatitis, with or without cirrhosis were selected. Double Immunohistochemistry was performed	The antibody stain was only against TLR4	The Mallory-Denk-bodies forming cells expressed two additional progenitor cell markers. These markers were CD49f and TLR4

Machida <i>et al</i> <sup>[64]</sup>	2014 November	Experimental	An immunostaining of liver tumor sections from alcohol-fed Ns5a mice was performed along with TLR4 silencing with lentiviral short-hairpin RNA	LPS-independent mechanisms of TLR4 activation in TICs remain to be elucidated. The oncogenic role of TLR4 is explored only around the synergism alcohol-HCV	TLR4-dependent mechanisms of TIC generation actually contribute to or at least promote the initiation of HCC
Yan <i>et al</i> <sup>[65]</sup>	2012 June	Experimental	Human HCC liver samples and mice were used. Stable HMGB1-expressing cells and HMGB1 knockdown cells were established. immunoblotting analysis, RNA Interference by short interfering RNA, enzyme-linked immunosorbent assay, confocal microscopy exam, caspase-1 colorimetric assay, cell migration and invasion assays and metastatic potential exam were all performed	Mechanisms by which caspase-1 affects tumor cancer progression remain incompletely understood	In hypoxic HCC cells, HMGB1 activates TLR4- and RAGE-signalling pathways to induce caspase-1 activation which, in turn, promote cancer invasion and metastasis
Xu <i>et al</i> <sup>[67]</sup>	2008 February	Analytic - cross sectional	52 patients were studied. The protein and mRNA levels of TLR7 and TLR9 were evaluated using real-time PCR, Western blot analysis, and flow cytometry. We also detected the serum viral load of HBV in the patients and analyzed the correlation between HBV-DNA copies and the TLR expression	The statistical analysis indicated no difference in the TLR9 levels among the HCC and LC groups. If the sample size was enlarged, the results may be different. The expression of TLR7 was not different among the groups of patients, suggesting that TLR7 has no correlation with HCC	There are downregulations of TLR7 expression and TLR9 mRNA in PBMC of HBV-infected patients, but an increased TLR9 expression at the protein level
Tanaka <i>et al</i> <sup>[68]</sup>	2010 October	Experimental	HCC cell lines and 42 HCC tissues were used. The type C CpG oligonucleotide was used as TLR9 ligand. Flow cytometric analysis, Immunohistochemical staining, Cell proliferation assay, Immunoblotting, NF-κB activity assays and expression analysis of IRF-7, RNA extraction and oligonucleotide microarray and Microarray data analysis were all performed	Despite being present both intracellular or extracellular TLR9's intracellular function is not observed with TLR9 ligands and its function is not known	Functional cell surface expression of TLR9 in human HCC may play an important role in tumorigenesis and cancer progression
Liu <i>et al</i> <sup>[69]</sup>	2015 February	Experimental	C57BL/6 mice were injected with Hepa1-6 cancer cells. TLR9 and HMGB1 were inhibited using shRNA or direct antagonists. HuH7 and Hepa1-6 cancer cells were investigated <i>in vitro</i> to determine how the interaction of HMGB1 and mtDNA activates TLR9 signaling pathways	The contribution of TLR9 to cancer pathophysiology remains incompletely understood. The regulation of TLR9 signaling and the physiological ligands which may induce TLR9 mediated tumor growth remain poorly characterized	Reveals a novel mechanism by which the interactions of HMGB1 and mtDNA activate TLR9 signaling during hypoxia to induce tumor growth
Zhang <i>et al</i> <sup>[70]</sup>	2014 December	Experimental	It was used HCC cell lines to where was transfected CpG oligodeoxynucleotide and poly (I:C). Proliferation analyses, Detection of apoptosis with an Apoptosis Detection Kit, quantitative real-time PCR analysis, Western blot analysis and Fluorescence microscopy were also performed	The precise molecular interactions that likely occur between CpG ODNs and poly (I:C) to block poly (I:C) entry, remain to be established. Poly (I:C) may be influenced by many molecules in the microenvironment	When combining poly (I:C) and CpG ODN for cancer therapy, these agents should be used in an alternating rather than simultaneous manner to avoid the blocking effect of phosphorothioate-modified TLR9 ligands
Zhang <i>et al</i> <sup>[71]</sup>	2014 April	Experimental	Human hepatoma cell lines were used. Cells were transfected with CpG ODNs or small interfering RNAs targeting TLR9. Reverse transcriptase polymerase chain reaction assay, Proliferation measurements, cell cycle analysis, detection of apoptosis, quantitative real-time PCR analysis, Western blot analysis were all performed. An <i>in vivo</i> study was also done	Apoptosis induced by ODN M362 Ctrl and ODN M362 occurred independently of TLR9 stimulation. TLR9- and MyD88-independent mechanisms in ODN-stimulated immune cells, including B lymphocytes and neutrophils may exist	Phosphorothioate-modified TLR9 agonist ODN M362, and its control, elicit antitumor activity in HCC cells and may serve as a novel therapeutic target for HCC therapy

Bubici <i>et al</i> <sup>[74]</sup>	2004 December	Perspective			Induction of FHC and Mn-SOD represents an additional, indirect means by which NF- $\kappa$ B controls proapoptotic JNK signaling
Liu <i>et al</i> <sup>[75]</sup>	2009 April	Experimental	Cell cultures were used. Immunocytochemistry stain for TLR9, a Cell proliferation assay, reverse transcriptase PCR for TLR9 and real-time reverse transcriptase PCR for DNMT-1 and Bcl-2, NF- $\kappa$ B activation measurement and Cellular apoptosis analysis were all performed	L-02 cells were used to allow <i>in vitro</i> studies but cells may behave differently <i>in vivo</i> . Future <i>in vivo</i> models are needed	Identified a possible novel mechanism that indicates how CpG DNA of HBV DNA may contribute to the malignant transformation of benign liver cells
Nischalke <i>et al</i> <sup>[76]</sup>	2012 March	Analytic - cross sectional	A total of 197 patients with HCV-associated HCC, 192 HCV-infected patients without HCC and 347 healthy controls were included. HCV antibodies were detected for diagnosis. Determination of TLR2-196 to -174 del/ins polymorphism was performed by LightCycler real-time PCR. <i>In vitro</i> induction of TLR2 expression and IL-8 was performed	Analysis of the functional role of TLR2-196 to -174 del/ins alleles with respect to TLR2 expression was based on <i>in vitro</i> stimulation studies but it is not known if an <i>in vivo</i> analysis would have the same results	TLR2-196 to -174 del allele to affect HCV viral loads and to increase the risk for HCC in HCV genotype 1-infected patients
Junjie <i>et al</i> <sup>[77]</sup>	2012 February	Single center-based case-control	SNaPshot method was used to genotype sequence variants of TLR2 and TLR9 in 211 patients with HCC and 232 subjects as controls	Despite the SNP rs3804099 and rs3804100 were out of HWE ( $P = 0.01-0.02$ ), they were retained in the analyses	TLR2 rs3804099 C/T and rs3804100 C/T polymorphisms were closely associated with HCC. In addition, the haplotypes composed of these two TLR2 synonymous SNPs have stronger effects on the susceptibility of HCC
Jiang <i>et al</i> <sup>[79]</sup>	2014 December	Single center-based case-control study	426 HCC subjects and 438 cancer-free control subjects were used. SNP genotyping was performed. A Vector was constructed and luciferase reporter assays were done. TLR4 mRNA levels were evaluated and Western blotting was done	The hypothesis that the overexpression of TLR4 induced by the rs1057317 polymorphism miRNA-disrupting function may influence the development of hepatocellular carcinoma is possible but still not proved. More studies in this area are needed	The risk of hepatocellular carcinoma was associated with a functional variant at miR-34a binding site in <i>TLR4</i> gene. miR-34a/TLR4 axis may play an important role in the development of HCC
Minmin <i>et al</i> <sup>[80]</sup>	2011 April	Analytic-case-control	A systematic genetic analysis of sequence variants of TLR4 by evaluating ten single-nucleotide polymorphisms was performed from 216 hepatocellular carcinoma cases and 228 controls	The contribution of the SNPs in TLR4 to HCC is modest. More studies are needed to validate this finding in independent populations and to understand the mechanism by which TLR4 sequence variants affect the pathological role of TLR4 in the signaling pathways that control carcinogenesis	The risk of hepatocellular carcinoma was associated with TLR4 sequence variation. TLR4 single nucleotide polymorphisms may play an important protective role in the development of hepatocellular carcinoma
Kawamoto <i>et al</i> <sup>[82]</sup>	2008 April	Experimental	Mouse cells were used together with plasmids containing TLRs. Cells were submitted to LPS and TAK-242. Nitrite and TNF- $\alpha$ were measured. Reporter gene assay for ligand-dependent signaling by TLRs, Reporter gene assay for ligand-independent signaling by TLR4, CD4-TLR or adaptors and Western blot analysis were performed	Human studies are needed as the interacting affinity of TAK-242 with TLR4 may be affected by a subtle difference in the amino acid sequences of TIR between humans and mice	TAK-242 selectively suppresses TLR4-signaling mediated by the intracellular domain
Matsunaga <i>et al</i> <sup>[83]</sup>	2011 January	Experimental	293 cells of human embryonic kidney and murine resident peritoneal macrophages were used. They were subited to TAK-242 and LPS. Vectors for FLAG-TLR4 and FLAG-TLR2 were cloned. Measurement of nitrite and	To fully understand the physical basis whereby TAK-242 disturbs signaling complex formation and intracellular signal transduction, a crystal structure analysis of the TLR4-TAK-242 complex is needed	TAK-242 binds selectively to TLR4 and subsequently disrupts the interaction of TLR4 with adaptor molecules, thereby inhibiting TLR4 signal transduction and its downstream



			cytokine concentrations in culture supernatants, radiolabeling of the cells, immunoprecipitation, Western blot analysis and autoradiography, reporter gene assay and <i>in vitro</i> IL-1 receptor-associated kinase-1 kinase assay were all performed		signaling events
Xu <i>et al.</i> <sup>[84]</sup>	2013 November	Experimental	Four dsRNAs were designed and synthesized. The expression of proteins was compared. The migration, proliferation and apoptosis of HepG2.2.15 cells were evaluated in presence of BM-06, sorafenib alone or in combination of both. The similar treatments were also applied in an SD rat primary HCC model	Since synthetic siRNAs must be transfected into the target cells through a vector, such as Lipofectamine™ 2000 reagent, they always exhibit cytotoxicity, which may limit their use in clinic	dsRNA alone was capable of inhibiting the proliferation of HepG2.2.15 cells and tumor growth of orthotopic HCC SD rats, but the effect of combination of dsRNA with sorafenib was more prominent
Behm <i>et al.</i> <sup>[85]</sup>	2014 December	Experimental	Rabbits were randomised to receive RFA, CpG B, their combination or no therapy, further tested by rechallenging a separate group with intravenously injected VX2 tumour cells after 120 d. Animals were assessed for survival, tumour size and spread, and tumour and immune related histological markers after 120 d. Peripheral blood mononuclear cells were tested for tumour-specific T cell activation and cytotoxicity. Immune modulatory cytokines were measured in serum	Lack of antibody reagents for the VX2-tumour model in rabbits. It was not possible to elucidate in depth histopathological changes	The combination of TLR9 stimulation with RFA resulted in a potentiated antitumour T cell response and cytotoxicity in the VX2 tumour model. Only this combination prevented subsequent tumour spread and resulted in a significantly improved survival

TLR: Toll-like receptor; PCR: Polymerase chain reaction; HCC: Hepatocarcinoma; LPS: Lipopolysaccharides; TNF- $\alpha$ : Tumour necrosis factor  $\alpha$ ; DEN: Diethylnitrosamine; NAC: N-acetyl cysteine; MAPK: Mitogen-activated protein kinase; NF- $\kappa$ B: Nuclear transcription factor kappa B; ER: Endoplasmic reticulum; MDSC: Myeloid-derived suppressor cells; NAFLD: Non-alcoholic fatty liver disease; JNK: Junamino-terminal kinase; HMGB1: High mobility group box 1; INF- $\gamma$ : Interferon gamma; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HSCs: Hepatic stellate cells; ODN: Oligodeoxynucleotides; NMOR: N-nitrosomorpholine; TNFR1: Tumor necrosis factor receptor 1; IL: Interleukine; DC: Dendritic cells; NK: Natural killer; HSCs: Hematopoietic stem cells; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling; SA b-gal: Senescence-associated beta-galactosidase; ELISA: Enzyme-linked Immunosorbent Assay; COX: Ciclo-oxygenase; IFC: Inflammation-fibrosis-carcinoma; ROS: Reactive oxygen species; HMGB1: High mobility group box 1; RAGE: Receptor for advanced glycation endproducts; HSP: Heat shock protein; HUVECs: Human umbilical vein endothelial cells; CSC: Colony stem cells; EMT: Epithelial-mesenchymal transition; TICs: Tumor-initiating cells; IRF: Interferon regulatory transcription factor; SNPs: Single nucleotide polymorphisms; HWE: Hardy-Weinberg equilibrium.

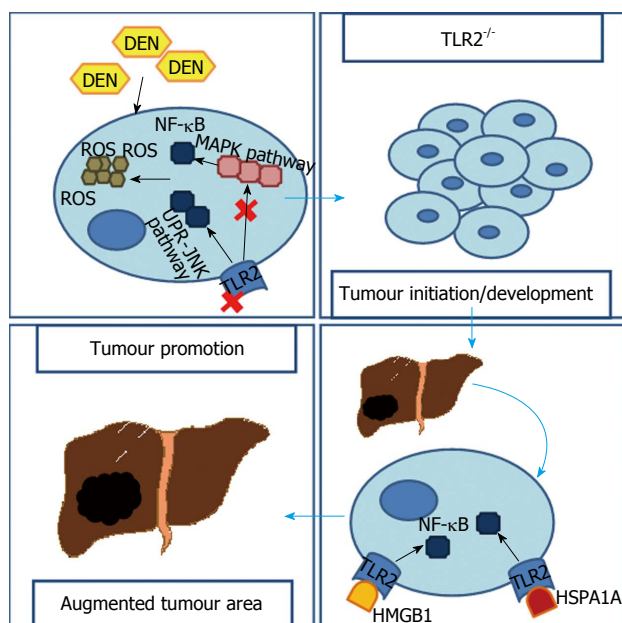
a "Myd88 independent manner/toll/interleukin-1 receptor domain-containing adaptor protein inducing interferon beta (TRIF) dependent" with the extracellular signal-regulated kinase (ERK)/Junamino-terminal kinase (JNK) and PI3K/Akt pathways<sup>[24]</sup>. Besides this, TLR2 signal is also involved in processes like autophagy and senescence in response to oxidative stress and DAMPS release<sup>[25]</sup>.

Diethylnitrosamine (DEN) is a chemical carcinogen capable of inducing HCC through accumulation of reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress. It was found that when a TLR2-deficient (TLR2<sup>-/-</sup>) mouse was submitted to DEN treatment the ROS and ER stress were abundantly accumulated, even though less apoptosis was observed<sup>[25]</sup>.

In fact, Lin *et al.*<sup>[24]</sup> demonstrated, in two separated works, that both TLR2<sup>-/-</sup> and wild-type (WT) mice developed HCC after being submitted to a DEN-treatment. However, the TLR2<sup>-/-</sup> mice revealed earlier tumours (every TLR2<sup>-/-</sup> mouse developed HCC at 6 mo after DEN treatment vs only 68% WTs)<sup>[24]</sup> that were, not only

significantly increased in number and in volume, but also less differentiated<sup>[24,25]</sup>. This increase reached the 3 fold (20.1%  $\pm$  4.5% vs 6.4%  $\pm$  1.0%,  $P < 0.01$ ) in the tumour area and 5 fold in visible tumour nodules (29.1%  $\pm$  2.8% vs 5.5%  $\pm$  0.9%,  $P < 0.001$ )<sup>[24]</sup>. Meanwhile, in the WT mice, is possible to attenuate HCC development if a TLR2 agonist is used<sup>[26]</sup>. Ultimately, TLR2<sup>-/-</sup> mice had shorter mean survival times with HCC than WTs<sup>[24]</sup>. Moreover, similar scenery was observed when a WT was pre-treated with an anti-TLR2 antibody<sup>[25]</sup>. Indeed, when observing liver samples from patients in different stages of liver diseases it is notorious that, in patients with HCC, not only the mRNA levels of TLR2 are lower but also TLR2 immunohistochemical expression grade and intensity are reduced, when compared to patients with hepatitis or cirrhosis<sup>[27]</sup>.

A ROS-generation reaction in cytochrome p450 2E1 is responsible for DEN metabolism. In spite of not finding any significant difference in cytochrome activity, TLR2<sup>-/-</sup> mice still revealed enhanced accumulation of ROS in their liver tissue<sup>[24]</sup>.



**Figure 2** Toll-like receptor 2's signalling pathways contributing to hepatocarcinoma. In the absence of TLR2, cells are incapable of responding to an increasing ROS when submitted to DEN. This is the result of an absence of MAPK/NF- $\kappa$ B pathway and an up-regulation of the UPR-JNK pathway. Consequently, cells containing higher ROS and DNA damages have more chances to survive and, HCC develops. In a second stage, where HCC is already established, HMGB1 and HSPA1A released by tumour's dying cells, through TLR2 stimulation, lead to an NF- $\kappa$ B up-regulation which, in this contest, seems to contribute to tumour's growth. HCC: Hepatocarcinoma; TLR2: Toll-like receptor 2; ROS: Reactive oxygen species; DEN: Diethylnitrosamine; MAPK: Mitogen-activated protein kinase; NF- $\kappa$ B: Nuclear transcription factor kappa B; UPR: Unfold protein response; JNK: Junamino-terminal kinase; HMGB1: High mobility group box 1; HSPA1A: Heat shock protein A1A.

Generation of ROS results in oxidative stress, which is often the source of DNA mutation or a direct link with chronic inflammation<sup>[28-30]</sup>. The ASK1/p38 MAPK/NF- $\kappa$ B pathway is one of the major sensors for ROS accumulation contributing to induced senescence cell death when risk of mutation is present<sup>[31]</sup>. However, in TLR2<sup>-/-</sup> mice submitted to DEN treatment, it is possible to assist to an attenuation of this major pathway<sup>[25]</sup> together with a suppression of biomarkers of autophagy-associated cell death and cellular senescence, like  $\beta$ -galactosidase<sup>[24]</sup>. Moreover, unlike the WT, TLR2<sup>-/-</sup> mice fail not only, to induce other important channels to premature cellular senescence like the p16-pRb/p21 pathway<sup>[24]</sup>, but also to activate DNA damage repair mechanisms<sup>[32]</sup>.

Furthermore, ER-stress is augmented after DEN-treatment in TLR2<sup>-/-</sup> mice as a result of ROS accumulation<sup>[25]</sup>. This leads to an enhanced unfold protein response (UPR) and activation of UPR-JNK pathway<sup>[33]</sup>, necessary for autophagy activation under ER-stress which, paradoxically, plays a dominant pro-survival role<sup>[34]</sup>. Lin *et al.*<sup>[25]</sup> noticed that, in livers from TLR2<sup>-/-</sup> mice there was an increased JNK activity.

Overall this data indicates that in the absence of TLR2, a down-regulation of common ROS neutralizing mechanisms, due to suppressed activation of ASK1/p38

MAPK/NF- $\kappa$ B, results in HCC cells containing higher ROS and DNA damages that, because of an up-regulated UPR-JNK pathway, have more chances to survive.

However, other pathways relating to TLR2 and hepatocarcinogenesis exist. Li *et al.*<sup>[26]</sup> focus their work on IL-18, which was found to be fundamental to carcinogenesis in TLR2<sup>-/-</sup> mice. In these mice, HCC developing after DEN treatment was capable of inducing IL-18 up-regulation in a caspase-8-dependent manner, therefore contributing to promotion of angiogenesis and suppression of NK cell arm of tumour immunosurveillance<sup>[26]</sup>.

Another perspective is related to the High mobility group box 1 (HMGB1), a nuclear protein released from dead/dying cells or even from cancer cells. It has the ability to bind to TLR2 and, with that, successfully activate NF- $\kappa$ B<sup>[35]</sup> which, in turn, can have an important role as a tumour promoter in inflammation-associated cancer<sup>[36]</sup>. Up-regulation of HMGB1 in an HCC cell line can result in increased matrix metalloprotease 9 and satellite tumour nodules in the liver, while blocking it suppresses tumour growth<sup>[37]</sup>. A recombinant HMGB1 (rHMGB1) was used by Shi *et al.*<sup>[38]</sup> in order to simulate TLR2 activation in an HCC cell line. Interestingly, rHMGB1 not only reduced cell apoptosis but also accelerated the tumour's growth and enhanced the ability of migration and invasion. Additionally, rHMGB1 activity significantly declined when HCC cells were pre-treated with a TLR2 inhibitor<sup>[38]</sup>.

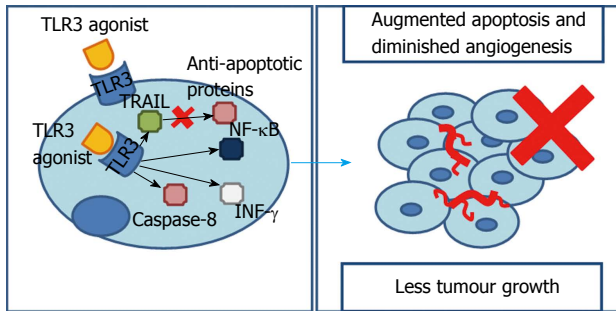
Similarly to HMGB1, HSPA1A - a member of the HSP70 family - is also a TLR2's ligand released by the tumour's necrotic cells. With a resembling pathway based on up-regulation of NF- $\kappa$ B, HSPA1A is capable of promoting the proliferation and survival of tumour cells<sup>[39]</sup>.

TLR2 clearly represents an important modulator of cells' response to stress situations. It has influence in mechanisms like autophagy, apoptosis or even DNA damage repair, possibly contributing to a protective role against HCC. However, it is, also, important to notice that these pathways may not be, already, clearly understood as studies reveal that TLR2's ligands like HMGB1 and HSPA1A, can result in tumour enhancement (Figure 2).

Taken altogether this data suggests that TLR2 activation may slow down initiation and development of HCC (anti-oncogenic potential) in the earlier phases of HCC carcinogenesis. However, at later stages its activation may influence the progression of inflammation and fibrosis (pro-oncogenic potential). Therefore, new studies are required in order to understand the exactly pathways through which this receptor is able to work and to conclude if its role in HCC carcinogenesis is different or not depending on the stage of the Inflammation-fibrosis-carcinoma axis.

### TLR3

Several studies have already shown that TLR3 is expressed in many cancer cells such as colonic adenocarcinoma, lung cancer, breast cancer and melanoma.



**Figure 3** How toll-like receptor 3 stimulus works against hepatocarcinoma. Stimulation of intracellular TLR3 is able to elicit cell apoptosis in a TRAIL-dependent manner that synergistically accompanies a down-regulation of anti-apoptotic proteins. Additionally, TLR3 stimulus can promote either an inflammatory or an apoptotic response. The first one pending on NF-κB, the second in caspase-8 activation and INF-γ release. As a result, TLR3 works as a protector against cancer which stimulation results in diminished tumour growth. TLR3: Toll-like receptor 3; TRAIL: Tumour necrosis factor-related apoptosis-inducing ligand; INF-γ: Interferon gamma; NF-κB: Nuclear transcription factor kappa B.

In HCC it was found that 17-fold longer median survival accompanied patients with higher intratumoral TLR3 expression<sup>[40]</sup>. However, Yoneda *et al.*<sup>[41]</sup> observed that 52.7% of the HCC tissues and 34.8% of the HCC metastasis studied expressed TLR3. Furthermore, the receptor was not only present in the cytoplasm, but also in the membrane, particularly in the exterior, suggesting a cell surface recognition mechanism for TLR3 agonists<sup>[41]</sup>.

Other works have already implied that the TRIF-dependent pathway of TLR3 signalling could have a special contribution to a tumours response. In fact, this adaptor molecule can promote either an inflammatory or an apoptotic response. The first one pending on NF-κB, the second in caspase-8 activation and interferon-γ (INF-γ) release<sup>[42]</sup>. Experiments showed that using synthetic TLR3 agonists resulted in a rise in NF-κB. In fact, as it was seen with TLR2 signalling, NF-κB is normally associated with augmented tumour necrosis factor α (TNF-α) responsible for cells' growth and proliferation<sup>[43]</sup>. However, here, an NF-κB rise is responsible for affecting the tumour microenvironment and driving HCC and endothelial cells to apoptosis<sup>[44]</sup>, accompanied by a significantly decreased tumour invasiveness and angiogenesis/vascular endothelial growth factor (VEGF) levels<sup>[45-47]</sup>. Thus, it seems that, whether NF-κB promotes or inhibits hepatocarcinogenesis depends on the presence of inflammation and the degree of NF-κB inhibition/promotion<sup>[3]</sup>.

Moreover, INF-γ - a potent inhibitor of endothelial cell proliferation/angiogenesis - and caspase-8/caspase-3 - inhibitors of hepatocytes proliferation - were found to be significantly increased in HCC cell lines pre-treated with TLR3 agonists<sup>[48]</sup>.

However, it is important to notice that, when stimulated through polyinosinicpolycytidylic acid, the surface TLR3 is only able to induce apoptosis if a protein synthesis inhibitor or a RNA synthesis inhibitor are

present<sup>[41]</sup>. This might indicate that, in an HCC cell line, endogenous suppressors of TLR3-mediated apoptosis are present. Curiously, stimulation of intracellular TLR3, even without protein or RNA synthesis' inhibitors, was able to elicit cell apoptosis in a tumour necrosis factor-related apoptosis-inducing ligand-dependent manner that synergistically accompanies a down-regulation of anti-apoptotic proteins<sup>[41]</sup>.

Notably, despite overall tumour growth could be reduced through TLR3 activation (from a 3-fold increase, when no TLR3's stimulus is present, to an only 1.9-fold increase after TLR3's agonists being used), the number of tumour nodules increases even after eliciting TLR3 signalling, leading to the conclusion that it does not affects the incidence but limits their growth<sup>[47]</sup>.

Interestingly, it appears that in HCC carcinogenesis TLR3 is a TLR that works as a protector against cancer. This is possible through molecules, downstream to TLR3, such as caspases, INF-γ or NF-κB, influencing crucial processes like angiogenesis, cell growth or proliferation (Figure 3).

#### TLR4

It is known that, despite being present in multiple liver cells, TLR4 expression is relatively low in this organ<sup>[49]</sup>. However, following liver damage and inflammation it is possible to assist to an up-regulation of this receptor<sup>[50]</sup>. Emerging evidence associates TLR4 to several types of tumours, enlightening its role in carcinogenesis, metastasis and cancer progression<sup>[51]</sup>. Observation of human's livers detected a high expression of TLR4 in cancer cells of HCC patients<sup>[49]</sup>.

Bacterial LPS is capable of initiating TLR4 signalling and subsequently activating NF-κB and MAPK signalling pathways - p38, ERK, JNK. In fact, in a HCC cell line incubated with bacterial LPS both TLR4 expression<sup>[52-54]</sup> and MAPK signalling pathways are significantly augmented<sup>[52]</sup>. However, it was found that, in contrast with a normal hepatocytes cell line, in a HCC cell line, the cellular growth was augmented and the cytotoxicity induced by LPS was decreased and dependent on TLR4 expression (higher expression is equal to less cytotoxicity)<sup>[49]</sup>. Additionally, these effects are reduced after inhibiting TLR4 signalling<sup>[55]</sup>.

The explanation of these results is based on two perspectives. One based on the fact that, in TLR4-overexpressing cells, ERK and JNK's activity is promoted<sup>[49]</sup> contributing to cell survival and proliferation. Nonetheless, loss of TLR4 results in a substantial decrease in proliferating hepatocytes as well as in a reduced duration of JNK and ERK mitogenic signals<sup>[56]</sup>. This pro-survival effect, when facing LPS, can also be blocked by down-regulating this TLR4-downstream molecules - ERK and JNK<sup>[49]</sup>.

A second and slightly opposing situation relies on p38 - capable of inducing cell cycle arrest and apoptosis - and NF-κB - capable of stimulating pro-inflammatory cytokines (IL-1, -6, -10, TNF-α)<sup>[57]</sup> - that were inhibited by LPS, in a TLR4-overexpressing HCC cell line, allowing

cell proliferation<sup>[49]</sup>. In fact, after stimulating TLR4, either blocking<sup>[49]</sup> or augmenting<sup>[39,55]</sup> NF- $\kappa$ B have been reported to promote tumour's survival. Once more we face an ambiguity in interpreting NF- $\kappa$ B values. However, in this situation, the explanation can rely on the degree of the stimuli/block and the underlying inflammation<sup>[3]</sup>.

Consequently, we are able to conclude that increased expression of TLR4 may protect HCC cells from LPS-induced cytotoxicity and promote cell HCC survival and proliferation.

This pro-tumorigenic effect of TLR4 is confirmed by the fact that, in TLR4<sup>-/-</sup> mice subjected to DEN, tumour incidence is 25% lower and diameters are smaller accompanied by less inflammation, proliferation as well as enhanced apoptosis<sup>[56]</sup>. Moreover, using antibiotics to reduce the LPS levels results in diminished activation of T helper 1 cells<sup>[58]</sup> and consequently less liver damage, and lower cell proliferation in tumour mass<sup>[56]</sup>.

However, Xu *et al.*<sup>[54]</sup> presented a different vision when reported increased expression of protein tyrosine phosphatase receptor type O (PTPRO) in TLR4-over-expressing HCC cell lines after LPS treatment. Here, cell proliferation was inhibited and apoptosis was augmented as a result of the tumour suppressor capability of PTPRO<sup>[54]</sup>. To that end, it was found that, contrarily to the effects on LPS-induced cytotoxicity, TLR4-overexpression might also have a protective role through PTPRO and thus, worth being subjected to new studies.

Li *et al.*<sup>[52]</sup> also observed that, with TLR4 over-expression, came a gradual disappearance of epithelial cell markers and increased mesenchymal ones, suggesting an epithelial-mesenchymal transition (EMT). This EMT is considered to be the molecular basis of tumour cell infiltration and metastasis<sup>[59]</sup> and can, actually, be induced by two possible pathways related to TLR4 and LPS stimulus. On one hand, the TLR4 - MAPK/JNK pathway, confirmed by the fact that, blocking directly MAPK/JNK or indirectly through TLR4, lead to inhibition of LPS-induced EMT<sup>[52]</sup>. On the other hand, Snail, a transcription factor handled by NF- $\kappa$ B and a major inducer of EMT<sup>[53]</sup>.

For this reason, LPS, *via* activation of TLR4 signalling pathway and consequently MAPK/JNK pathway activation or NF- $\kappa$ B up-regulation, can significantly induce EMT.

This EMT phenotype is conveyed by cancer stem cells<sup>[60]</sup> which, in turn, are thought to be involved in processes like formation and progression of cancer, being, inclusively, responsible for chemotherapy resistance, metastasis and postoperative recurrence<sup>[61,62]</sup>. Recent studies revealed that TLR4 positive cells exhibit a series of stem cells characteristics<sup>[50,60]</sup>. These cells not only display a higher invasive ability, when compared to TLR4 negatives, but also express many stem cell markers (CD133 increase 85% when TLR4 is overexpressed<sup>[60]</sup>) as well as a stronger colony forming ability and increased chemotherapy/apoptosis resistance<sup>[50]</sup>.

In agreement with these results, Chen *et al.*<sup>[60]</sup> proposed that TLR4 could work as a proto-oncogene which aberrant expression/activation leads to induction

of pluripotency genes and genesis of tumour-initiating stem-like cells (TICs). This process is possible through activation of a TLR4/NANOG pathway<sup>[60,62-64]</sup> and consequent inhibition of the transforming growth factor  $\beta$  (TGF- $\beta$ )<sup>[60,62,64]</sup>.

NANOG is *per se* a core transcription factor found in pluripotent stem cells<sup>[62]</sup>. TGF- $\beta$  is an effective proliferation inhibitor and an apoptosis promoter that, when down-regulated, is able to initiate tumorigenesis *via* stemness gene induction in an epithelial tissue such as liver<sup>[60]</sup>. In fact, knockdown of TLR4 attenuated the induction of stem cell genes as well as DNA synthesis of TICs in 50% to 80% and blocking NANOG, results in a tumour growth reduction of 60% to 75%<sup>[60]</sup>.

However, some cancer cells grow efficiently *in vitro* without addition of LPS but this growth is still reduced by TLR4 knockdown, suggesting LPS-independent mechanisms of TLR4 activation in these cells<sup>[64]</sup>. One possibility includes non-LPS ligands influencing tumorigenesis through TLR4 signalling.

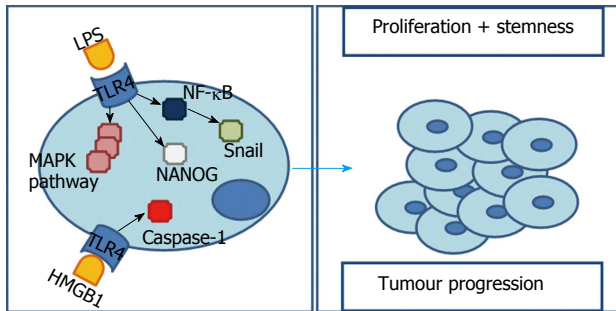
Yan *et al.*<sup>[65]</sup> observed that hypoxia was also responsible for TLR4 up-regulation in an HCC cell line. Hypoxia is a hallmark of several solid tumours, including HCC, and an important factor in tumour progression<sup>[66]</sup>. A possible explanation of this relationship may involve hypoxia-induced HMGB1 release, capable of activating TLR4 signalling and consequently augment caspase-1. This one is, in turn, related with maturation of pro-inflammatory cytokines and consequent tumorigenesis and tumour progression. After TLR4 blockage, caspase-1 expression diminished significantly<sup>[65]</sup>. Additionally, caspase-1 blocking was capable of decreasing HCC cell invasiveness<sup>[65]</sup>. This suggests that hypoxia-induced caspase-1 activation, as well as caspase-1-mediated tumour progression, can depend on TLR4 signalling.

In spite of several evidences attributing a pro-tumorigenic role to TLR4, the pathways to that end are many and still not fully understood. Diminished apoptotic-response to LPS, EMT-induction or caspase-1 up-regulation through TLR4 were already proposed but, opposing effects mediated by tumour suppressors like PTPRO were also found (Figure 4). According to these results new studies are suggested to clarify not only how each work, but also how they are related. However, contrarily to TLR2 most data suggests that TLR4 activation not only has an important role in inflammation and fibrosis but also in HCC initiation and progression.

## TLR9

A possible relationship between TLR9 and carcinogenesis came to light when its high expression levels of TLR9 were found in samples of lung and breast cancer cell lines<sup>[67]</sup>. HCC cells exhibit a broad repertoire of TLRs, also including TLR9. This receptor plays a crucial role in cell survival as it recognises several bacterial and viral components, including unmethylated CpG-DNA. Different works revealed that there is an augmented TLR9 positivity in human HCC cells<sup>[8,68,69]</sup> with Eiró *et al.*<sup>[8]</sup>



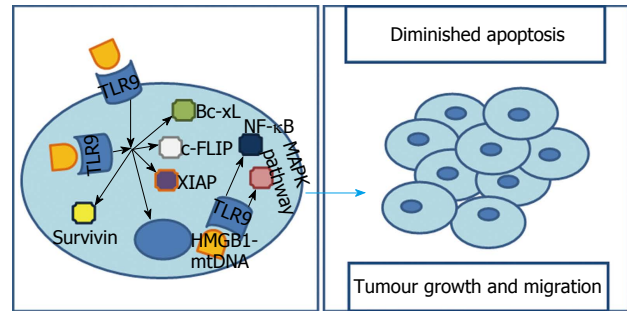


**Figure 4** Toll-like receptor 4's signalling pathways influencing hepatocarcinogenesis. Bacterial LPS is capable of initiate TLR4 signalling and subsequently activate NF- $\kappa$ B and MAPK signalling pathways. In one hand, in a HCC cell line incubated with bacterial LPS both TLR4 expression and MAPK signalling pathways are significantly augmented, contributing to cell survival and proliferation. On the other hand, TLR4 over-expression contributes to an EMT through MAPKs pathway and Snail. Additionally, NANOG induces TICs' formation. Both are considered to be the molecular basis of tumour cell infiltration and metastasis. HMGB1's stimulation of TLR4 with caspase-1 activation is related with maturation of pro-inflammatory cytokines and consequent tumorigenesis and tumour progression. TLR4: Toll-like receptor 4; LPS: Lipopolysaccharides; HCC: Hepatocarcinoma; MAPK: Mitogen-activated protein kinase; EMT: Epithelial-mesenchymal transition; TICs: Tumour-initiating stem-like cells; NF- $\kappa$ B: Nuclear transcription factor kappa B; HMGB1: High mobility group box 1.

showing a TLR9's prevalence of 60% (in a population of 30 cases) and Tanaka *et al.*<sup>[68]</sup> reaching the 85.7% (in a population of 42 cases) in their works with human samples of HCC. Moreover, in the later, 7 of 8 cases of HCC metastasis presented TLR9 positivity<sup>[68]</sup>. Additionally, it was found that, in both HCC cell line or HCC human samples, TLR9 was present not only in the cytoplasm but also on cells' membrane<sup>[68]</sup>. However, is important to notice that, possibly, only the stimulation of membrane receptors could result in increased cell viability as transfecting a TLR9 agonist, CpG-oligodesoxynucleotide (CpG-ODN), which stimulates intracellular TLR9 receptors, may not affect proliferation and survival<sup>[68]</sup>. The explanation for this tumour-promoter role of TLR9 comes from the fact that, after TLR9 stimulation, a HCC cell line is able to, not only up-regulate apoptosis inhibitors such as survivin, Bcl-xL, XIAP and cFLIP, but also, to closely modulate oncogenic genes with a major contribution in tumorigenesis and cancer progression<sup>[68]</sup> (Figure 5).

Although, this data is not that linear, and, somehow, different from what Zhang *et al.*<sup>[70,71]</sup> stated in their studies. Here, transfecting a TLR9 agonist into a HCC cell line lead to a marked increase in IFN- $\alpha$ , IFN- $\beta$ , TNF- $\alpha$ , IL-6 and IL-8 without activating NF- $\alpha$ B. As a result a cell-proliferation's inhibition rate was increased approximately 50% and apoptosis was augmented<sup>[70,71]</sup>.

The contradictory findings about the influence on tumour's environment of intracellular TLR9 agonists could be explained by the fact that the phosphorothioate-modified backbone of CpG-ODN are able to form a complex with or cause conformational changes in other compounds, like Poly (I:C) that, normally would result in enhanced apoptosis but, when together with CpG-ODN, are unable to act<sup>[70]</sup>. Moreover is important to look at the



**Figure 5** Toll-like receptor 9's signalling pathways influencing hepatocarcinoma. TLR9 is present not only in the cytoplasm but also on cells' membrane. However, it is still controversial whether cytoplasmatic stimulation results in increased cell viability. Independently, membrane receptors' stimulation results, not only, in up-regulation of apoptosis inhibitors such as survivin, Bcl-xL, XIAP and c-FLIP, but also, in a modulation of oncogenic genes with a major contribution in tumorigenesis and cancer progression. Additionally, a cytoplasmatic HMGB1-mtDNA interaction was proved to be capable of activating TLR9 and MAPK pathway as well as NF- $\kappa$ B leading to augmented survival, growth, proliferation, differentiation and migration of cancer cells. TLR9: Toll-like receptor 9; XIAP: X-linked inhibitor of apoptosis protein; c-FLIP: Cellular FLICE-Like inhibitory protein; MAPK: Mitogen-activated protein kinase; NF- $\kappa$ B: Nuclear transcription factor kappa B; HMGB1: High mobility group box 1.

protocols used, as CpG-ODN induces HCC cell apoptosis in a dose-dependent manner, at concentrations below 0.5  $\mu$ g/mL. In contrast, high concentrations of this agonist (e.g., 5  $\mu$ g/mL) had no effect on HCC cells<sup>[71]</sup>.

Additionally, this pathway from TLR9 signalling to carcinogenesis is supported by HMGB1. We have already seen that HMGB1 and hypoxia could influence tumorigenesis through different TLRs. Interestingly, they are, also, both involved with TLR9. It was seen that along with TLR9 overexpression, hypoxic cancer cells accumulate structurally and functionally abnormal mitochondria, which release mitochondrial DNA (mtDNA) to the cytosol, and induce translocation of HMGB1 from nucleus to cytoplasm<sup>[69]</sup>. The role of HMGB1 as a promoter of invasion, metastasis and angiogenesis when its location is extracellular is not new<sup>[72]</sup>. However, Liu *et al.*<sup>[69]</sup> revealed that, on top of this, an cytoplasmatic HMGB1-mtDNA interaction is required for complete activation of TLR9 signalling cascade and therefore essential for HCC cells to proliferate under hypoxic conditions. The underlying mechanism in this pro-tumorigenic pathway lies in MAPKs activation - fundamental in growth, proliferation, differentiation and migration<sup>[73]</sup> - and also in NF- $\kappa$ B signalling - capable of suppressing apoptosis in response to stress<sup>[74]</sup> - after the interaction between HMGB1/mtDNA and TLR9<sup>[69,75]</sup> (Figure 5).

## TLRS GENETIC POLYMORPHISMS AND VARIANTS AND HCC SUSCEPTIBILITY

Several authors have already focused their studies on the relationship between TLRs' genetics and carcinogenesis, approaching different cancers such as non-Hodgkin lymphoma, endometrial cancer, cervical cancer, non-cardiac gastric cancer, among others.

Genetic studies on the *TLR2* gene have shown a number of polymorphisms capable of interfering with host defenses and disease progression<sup>[76]</sup>. In fact, it was already seen that inherited variation in *TLR2* influence the risk of HCC. Genetic *TLR2* analysis revealed that two single nucleotide polymorphisms (SNP), rs3804099 and rs3804100, had a significantly different distribution between HCC patients and the healthy controls<sup>[77]</sup>. Interestingly, in what is concerned to these SNPs, Junjie *et al.*<sup>[77]</sup> suggested that, *TLR2* gene variation could play an important protective role in HCC as the heterozygous genotype comprise lesser HCC risk (OR from 0.331 to 0.759,  $P < 0.001$ ) when compared to wild-type homozygous genotype. In fact, individuals carrying the TT haplotype had a significantly decreased risk of HCC [odds ratio (OR) = 0.524, 95%CI: 0.394-0.697,  $P < 0.001$ ]. Contrarily, the CC haplotype had greater risk (OR = 2.743, 95%CI: 1.915-3.930,  $P < 0.001$ ). Unfortunately, the authors do not reveal the real influence of the referred SNPs on the *TLR2*'s activity and more studies are suggested to clarify this information.

Moreover, the frequency of a -196 to -174 deletion allele was, also, significantly higher in HCC patients than in healthy controls (22.5% vs 15.3%) and HCV-infected patients without HCC (22.5% vs 15.6%)<sup>[76]</sup>. Nischalke *et al.*<sup>[76]</sup> observations indicate that the -196 to -174 deletion allele possibly augment the risk of HCV-induced HCC, probably as a result of diminished *TLR2* signalling and thus increased viral loads. This -196 to -174 deletion not only had greater viral loads than -196 to -174 ins/ins but also, contribute to a 3-fold increase in HCC risk relatively to this -196 to -174 ins/ins when both are compared to healthy controls or a 1.5 fold increase when both are compared to hepatitis C patients without HCC<sup>[76]</sup>.

Researchers have already studied the possible presence of polymorphisms in the area of *TLR3*. It was found that, at least in the chinese population, a +1234CT polymorphism is present which might contribute to increased susceptibility to HCC (specially 1234CT and TT genotypes)<sup>[78]</sup>. The presence of this SNP is responsible for a markedly diminished *TLR3* function, which may result in up-regulated vasculature remodelling and tumour growth and, in that way, contributing to HCC<sup>[78]</sup>.

The *TLR4* is probably the more extensively studied TLR and therefore, not an exception when it comes to having polymorphisms or variants capable of influence carcinogenesis. Growing evidence has shown that *TLR4* polymorphisms are related to chronic inflammation and inflammatory-related cancer. As a matter of fact, a polymorphism in microRNA-34a binding site in *TLR4* (rs1057317) was significantly associated with higher HCC risk, especially in HBsAg (+) patients and in the AA homozygous genotypes<sup>[79]</sup>. MicroRNA-34a is capable of inducing apoptosis, G1 arrest and senescence explaining why its down-regulation may be associated with malignancy. However, there are not only polymorphisms related to augmented risk. Indeed, some mutations

of *TLR4* gene - four SNPs in 5'-UTR (rs10759930, rs2737190, rs10116253 and rs1927914) and one intron polymorphism (rs1927911) - may allow a two-fold decrease in HCC risk, especially in heterozygous genotypes when compared to wild-type homozygous<sup>[80]</sup>. The justification can rely on the fact that 5'-UTR is involved in regulation of proteins concerned with growth and differentiation in normal tissues and these SNPs may, therefore, exert regulator effects in these proteins<sup>[80]</sup>. Therefore, according to the *TLR4*'s polymorphism observed, an augmented or diminished risk of HCC is possible, even though its magnitude is small.

## TLRS AS THERAPEUTIC TARGETS FOR HCC

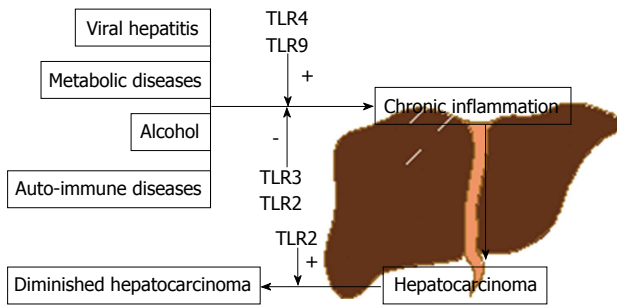
So far we have seen that different TLRs could work as specific modulators of HCC. Therefore it is logical to think that its use as therapeutic targets could open the door to new promising strategies in the fighting against HCC. In fact, the modulation of TLRs' signalling, by targeting either the TLRs or their adaptors or downstream signalling molecules, is not new and they have already proved to be useful in ovarian, colorectal or head and neck cancer<sup>[81]</sup>.

*TLR4* modulators seem to be important chemotherapy adjuvants that enhance chemotherapy efficacy and prolong survival<sup>[81]</sup>. TAK-242 is a *TLR4* ligand capable of selectively suppress both ligand-dependent and independent signalling *via* the intracellular domain of *TLR4*, disrupting the TRAM and TIRAP interactions with *TLR4*<sup>[82]</sup>. This small molecule is, therefore, able to down-regulate NF- $\kappa$ B and consequently diminish inflammatory mediators such as nitric oxide, TNF- $\alpha$ , IL-1, -6 and with that, reduce the proliferation/invasion activity induced by LPS in the liver cancer cell lines<sup>[57,82,83]</sup>. Furthermore, TAK-242 might also show an efficacy against inflammation mediated by excessive expression of *TLR4*, what, in fact, has been shown to happen in HCC<sup>[82]</sup>. Independently, new studies are still required for better evaluating effects, doses and other characteristics of TAK-242.

To date, we still lack an effective systemic curative therapy for advanced cases of HCC and, in most cases the only alternative is palliative treatment.

Even though, sorafenib, a multi-kinase inhibitor, represents an important chemotherapeutic drug in the treatment of this type of cancer. Xu *et al.*<sup>[84]</sup> found that a combination of sorafenib with a *TLR3*-synergist (BM-06) results in a superior inhibition of tumour growth in HCC cell lines or rat models when compared to the two different agents alone. Their results were based on a significantly reduced proliferative capacity, invasion ability, tumour volume and an increased apoptotic rate<sup>[84]</sup>. Therefore, BM-06 emerges as a possible adjuvant agent in the therapeutic against HCC.

In the *TLR9* domain several studies were already conducted. It was reported that using *TLR9* antagonists



**Figure 6 Toll-like receptors influence on the pathway to hepatocarcinoma.** Several factors are known to contribute to the carcinogenic process including viral hepatitis, alcohol, auto-immune or metabolic diseases, among others and the link between all this factors is chronic inflammation which, in turn, is an important hepatocarcinoma's precursor. Moreover, innate immunity represents an important player in this equation with TLRs such as 4 and 9 having, mainly, a positive contribution to hepatocarcinogenesis and TLR3, essentially, a negative/protective one. TLR2 still presents an ambiguous role, possibly depending on liver's stage in the inflammation-cirrhosis-carcinoma axis to exert its pro-tumorigenic or anti-tumorigenic capacity. TLR: Toll-like receptor.

like chloroquine could be useful in several autoimmune diseases<sup>[2]</sup>. In fact, a markedly reduced proliferation is seen in a HCC cell line when TLR9 is inhibited by chloroquine<sup>[2]</sup>. This antimalaric agent works as a direct TLR9 antagonist, being proposed that its activity on HCC cells may be brought about *via* its effects on the protein kinase AKT, tumour-associated angiogenesis factor VEGF as well as NF- $\kappa$ B. Moreover, the same tumour growth restriction, followed by smaller volume and reduction in tumour's markers of aggressiveness was seen when this treatment was used in HCC cell lines intrahepatic implanted in mice<sup>[2]</sup>.

However, as it was said, sometimes, the only option is the palliation and radiofrequency ablation (RFA) which has already established an important role in this setting. Behm *et al.*<sup>[85]</sup> successfully demonstrated that, in a rabbit model, TLR9 agonists could work together with RFA in an anti-tumour response through a strong cytotoxic immune response mediated by increased tumour-specific lymphocytes. In fact, it was not only a good predictor of containment of tumour growth and spread but also of prolonged survival<sup>[85]</sup>.

Moreover, some studies focus on the use of TLRs as vaccine's adjuvants against HCV or HBV mediated hepatocarcinogenesis. There are also some proposals for using TLR4's antagonists in patients with septic shock<sup>[86]</sup>. Besides this, the use of TLR4's antagonists is being investigated in the prevention of alcoholic liver injury and Non Alcoholic Steato-Hepatitis<sup>[9]</sup>.

Despite the good results, when it comes to using TLRs as a novel HCC therapeutic it still has a long run before every mechanism is understood. TLRs' signalling pathways are too many and effects remain controversial but a lot is to be expected from these innate immunity receptors.

## CONCLUSION

HCC occupies the third place when it comes to mortality

in cancer<sup>[1]</sup>. Several factors are known to contribute to the carcinogenic process including viral hepatitis, alcohol, auto-immune or metabolic diseases, among others. The link between all these factors is inflammation or, more precisely, chronic inflammation.

However, despite all this malignant potential or this knowledge around the inflammatory causality, most of the pathways of carcinogenesis are still unknown or, at least, not entirely known.

TLRs' role in the tumour formation is part of a more recent concept that involves innate immunity but, despite all the advances, a lot is still waiting to be studied. The reasons for this lack of information include the range of responses that can be obtained from a single TLR signalling. Humans dispose of 11 TLRs capable of initiating a signal cascade from only five molecular adaptors. Consequently, some can be used by more than one receptor. Moreover, each of this activated adaptor molecules, depending on the initial TLR, elicit a response based on the production of several effectors, pro-inflammatory or anti-inflammatory cytokines, interferons and many others with countless results.

To understand the role of TLRs we must remember that HCC comes from an inflammatory background where a complex and progressive process appears with fibrosis and cirrhosis until the last stage, HCC, is reached. This review tried to show that, the path taken can be closely influenced by innate immunity/TLRs (Figure 6). TLR2 was shown to be an important stress manager so that, in its absence, an attenuated ASK1/p38 MAPK/NF- $\kappa$ B pathway and an increased JNK activity result in a larger and less differentiated HCC. Contrarily, TLR2's stimulation through HMGB1 and HSPA1A also indicates a tumour-promoter role. TLR3 may be responsible for driving HCC and endothelial cells to apoptosis and decreasing invasiveness and angiogenesis by mediating NF- $\kappa$ B, caspase-8 and INF- $\gamma$  up-regulation. TLR4 is closely related to LPS cytotoxic, which is diminished in TLR4-overexpressing HCC due to promoted ERK's and JNK's activity and limited NF- $\kappa$ B and p38 activation. Moreover, this receptor is tightly involved in EMT and progression of cancer based on non-LPS ligands like NANOG and HMGB1. TLR9 activity is different whether the membrane or the intracellular receptor is activated. The first promotes HCC through apoptosis inhibitors and oncogenic genes. The second augments apoptosis by increasing Interferons and interleukines. Consequently, initial findings attribute a pro-tumorigenic role to TLR4 and TLR9 and a protective capacity to TLR3. When it comes to TLR2, the available data suggests that its influence may go both ways (pro-tumorigenic and protective) depending on the liver's stage in the inflammation-cirrhosis-carcinoma axis. However this is not that simple or linear as a closer look easily reveals studies with interesting but opposing conclusions from the ones before.

Independently of this lack of knowledge, one thing is certain; TLRs can have a determining influence on the cancer's progression. Therefore, the usage of TLRs



as therapeutic targets has already been established, especially as adjuvants to other agents currently in use. However, the possibilities are many and with a deeper insight over the mechanisms involved new ways of dealing with HCC are expected to emerge.

In conclusion, we cannot say that TLRs came to facilitate our understanding of HCC mechanisms. Instead they came to open the door to a new reality and, with that, to possible new approaches, perhaps in a closer future than we might know.

## REFERENCES

- 1 **Chew V**, Tow C, Huang C, Bard-Chapeau E, Copeland NG, Jenkins NA, Weber A, Lim KH, Toh HC, Heikenwalder M, Ng IO, Nardin A, Abastado JP. Toll-like receptor 3 expressing tumor parenchyma and infiltrating natural killer cells in hepatocellular carcinoma patients. *J Natl Cancer Inst* 2012; **104**: 1796-1807 [PMID: 23197495 DOI: 10.1093/jnci/djs436]
- 2 **Mohamed FE**, Al-Jehani RM, Minogue SS, Andreola F, Winstanley A, Olde Damink SW, Habtesion A, Malagó M, Davies N, Luong TV, Dhillon AP, Mookerjee RP, Dhar DK, Jalan R. Effect of toll-like receptor 7 and 9 targeted therapy to prevent the development of hepatocellular carcinoma. *Liver Int* 2015; **35**: 1063-1076 [PMID: 24990399 DOI: 10.1111/liv.12626]
- 3 **Dapito DH**, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, Caviglia JM, Khiabanian H, Adeyemi A, Bataller R, Lefkowitz JH, Bower M, Friedman R, Sartor RB, Rabadan R, Schwabe RF. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 2012; **21**: 504-516 [PMID: 22516259 DOI: 10.1016/j.ccr.2012.02.007]
- 4 **Kipanyula MJ**, Seke Etet PF, Vecchio L, Farahna M, Nukenine EN, Nwabo Kamdje AH. Signaling pathways bridging microbial-triggered inflammation and cancer. *Cell Signal* 2013; **25**: 403-416 [PMID: 23123499 DOI: 10.1016/j.cellsig.2012.10.014]
- 5 **Kurabe N**, Murakami S, Tashiro F. SGF29 and Sry pathway in hepatocarcinogenesis. *World J Biol Chem* 2015; **6**: 139-147 [PMID: 26322172 DOI: 10.4331/wjbc.v6.i3.139]
- 6 **Nakamoto N**, Kanai T. Role of toll-like receptors in immune activation and tolerance in the liver. *Front Immunol* 2014; **5**: 221 [PMID: 24904576 DOI: 10.3389/fimmu.2014.00221]
- 7 **Soares JB**, Pimentel-Nunes P, Roncon-Albuquerque R, Leite-Moreira A. The role of lipopolysaccharide/toll-like receptor 4 signaling in chronic liver diseases. *Hepatol Int* 2010; **4**: 659-672 [PMID: 21286336 DOI: 10.1007/s12072-010-9219-x]
- 8 **Eiró N**, Altadill A, Juárez LM, Rodríguez M, González LO, Atienza S, Bermúdez S, Fernandez-Garcia B, Fresno-Forcelledo MF, Rodrigo L, Vizoso FJ. Toll-like receptors 3, 4 and 9 in hepatocellular carcinoma: Relationship with clinicopathological characteristics and prognosis. *Hepatol Res* 2014; **44**: 769-778 [PMID: 23742263 DOI: 10.1111/hepr.12180]
- 9 **Mencin A**, Kluwe J, Schwabe RF. Toll-like receptors as targets in chronic liver diseases. *Gut* 2009; **58**: 704-720 [PMID: 19359436 DOI: 10.1136/gut.2008.156307]
- 10 **Roh YS**, Seki E. Toll-like receptors in alcoholic liver disease, non-alcoholic steatohepatitis and carcinogenesis. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 38-42 [PMID: 23855294 DOI: 10.1111/jgh.12019]
- 11 **Aravalli RN**. Role of innate immunity in the development of hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 7500-7514 [PMID: 24282342 DOI: 10.3748/wjg.v19.i43.7500]
- 12 **Liu S**, Gallo DJ, Green AM, Williams DL, Gong X, Shapiro RA, Gambotto AA, Humphris EL, Vodovotz Y, Billiar TR. Role of toll-like receptors in changes in gene expression and NF-kappa B activation in mouse hepatocytes stimulated with lipopolysaccharide. *Infect Immun* 2002; **70**: 3433-3442 [PMID: 12065483 DOI: 10.1128/IAI.70.7.3433-3442.2002]
- 13 **Matsumura T**, Ito A, Takii T, Hayashi H, Onozaki K. Endotoxin and cytokine regulation of toll-like receptor (TLR) 2 and TLR4 gene expression in murine liver and hepatocytes. *J Interferon Cytokine Res* 2000; **20**: 915-921 [PMID: 11054280 DOI: 10.1089/10799900050163299]
- 14 **Thobe BM**, Frink M, Hildebrand F, Schwacha MG, Hubbard WJ, Choudhry MA, Chaudry IH. The role of MAPK in Kupffer cell toll-like receptor (TLR) 2-, TLR4-, and TLR9-mediated signaling following trauma-hemorrhage. *J Cell Physiol* 2007; **210**: 667-675 [PMID: 17117477 DOI: 10.1002/jcp.20860]
- 15 **Knolle P**, Schlaak J, Uhrig A, Kempf P, Meyer zum Büschenfelde KH, Gerken G. Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. *J Hepatol* 1995; **22**: 226-229 [PMID: 7790711]
- 16 **Edwards AD**, Diebold SS, Slack EM, Tomizawa H, Hemmi H, Kaisho T, Akira S, Reis e Sousa C. Toll-like receptor expression in murine DC subsets: lack of TLR7 expression by CD8 alpha+ DC correlates with unresponsiveness to imidazoquinolines. *Eur J Immunol* 2003; **33**: 827-833 [PMID: 12672047 DOI: 10.1002/eji.200323797]
- 17 **Sawaki J**, Tsutsui H, Hayashi N, Yasuda K, Akira S, Tanizawa T, Nakanishi K. Type 1 cytokine/chemokine production by mouse NK cells following activation of their TLR/MyD88-mediated pathways. *Int Immunol* 2007; **19**: 311-320 [PMID: 17289654 DOI: 10.1093/intimm/dxl148]
- 18 **Meyer-Bahlburg A**, Khim S, Rawlings DJ. B cell intrinsic TLR signals amplify but are not required for humoral immunity. *J Exp Med* 2007; **204**: 3095-3101 [PMID: 18039950 DOI: 10.1084/jem.20071250]
- 19 **Paik YH**, Schwabe RF, Bataller R, Russo MP, Jobin C, Brenner DA. Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells. *Hepatology* 2003; **37**: 1043-1055 [PMID: 12717385 DOI: 10.1053/jhep.2003.50182]
- 20 **Wu J**, Meng Z, Jiang M, Zhang E, Trippier M, Broering R, Bucchi A, Krux F, Dittmer U, Yang D, Roggendorf M, Gerken G, Lu M, Schlaak JF. Toll-like receptor-induced innate immune responses in non-parenchymal liver cells are cell type-specific. *Immunology* 2010; **129**: 363-374 [PMID: 19922426 DOI: 10.1111/j.1365-2567.2009.03179.x]
- 21 **Huang Y**, Cai B, Xu M, Qiu Z, Tao Y, Zhang Y, Wang J, Xu Y, Zhou Y, Yang J, Han X, Gao Q. Gene silencing of Toll-like receptor 2 inhibits proliferation of human liver cancer cells and secretion of inflammatory cytokines. *PLoS One* 2012; **7**: e38890 [PMID: 22815694 DOI: 10.1371/journal.pone.0038890]
- 22 **Kim S**, Takahashi H, Lin WW, Descargues P, Grivennikov S, Kim Y, Luo JL, Karin M. Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. *Nature* 2009; **457**: 102-106 [PMID: 19122641 DOI: 10.1038/nature07623]
- 23 **Wang W**, Xu GL, Jia WD, Ma JL, Li JS, Ge YS, Ren WH, Yu JH, Liu WB. Ligation of TLR2 by versican: a link between inflammation and metastasis. *Arch Med Res* 2009; **40**: 321-323 [PMID: 19608024 DOI: 10.1016/j.arcmed.2009.04.005]
- 24 **Lin H**, Yan J, Wang Z, Hua F, Yu J, Sun W, Li K, Liu H, Yang H, Lv Q, Xue J, Hu ZW. Loss of immunity-supported senescence enhances susceptibility to hepatocellular carcinogenesis and progression in Toll-like receptor 2-deficient mice. *Hepatology* 2013; **57**: 171-182 [PMID: 22859216 DOI: 10.1002/hep.25991]
- 25 **Lin H**, Liu XB, Yu JJ, Hua F, Hu ZW. Antioxidant N-acetylcysteine attenuates hepatocarcinogenesis by inhibiting ROS/ER stress in TLR2 deficient mouse. *PLoS One* 2013; **8**: e74130 [PMID: 24098333 DOI: 10.1371/journal.pone.0074130]
- 26 **Li S**, Sun R, Chen Y, Wei H, Tian Z. TLR2 limits development of hepatocellular carcinoma by reducing IL18-mediated immunosuppression. *Cancer Res* 2015; **75**: 986-995 [PMID: 25600646 DOI: 10.1158/0008-5472.CAN-14-2371]
- 27 **Soares JB**, Pimentel-Nunes P, Afonso L, Rolanda C, Lopes P, Roncon-Albuquerque R, Gonçalves N, Boal-Carvalho I, Pardal F, Lopes S, Macedo G, Lara-Santos L, Henrique R, Moreira-Dias L, Gonçalves R, Dinis-Ribeiro M, Leite-Moreira AF. Increased hepatic expression of TLR2 and TLR4 in the hepatic inflammation-fibrosis-carcinoma sequence. *Innate Immun* 2012; **18**: 700-708



- [PMID: 22330637 DOI: 10.1177/1753425912436762]
- 28 **Marra M**, Sordelli IM, Lombardi A, Lamberti M, Tarantino L, Giudice A, Stiuso P, Abbruzzese A, Sperlongano R, Accardo M, Agresti M, Caraglia M, Sperlongano P. Molecular targets and oxidative stress biomarkers in hepatocellular carcinoma: an overview. *J Transl Med* 2011; **9**: 171 [PMID: 21985599 DOI: 10.1186/1479-5876-9-171]
  - 29 **Malhi H**, Kaufman RJ. Endoplasmic reticulum stress in liver disease. *J Hepatol* 2011; **54**: 795-809 [PMID: 21145844 DOI: 10.1016/j.jhep.2010.11.005]
  - 30 **Lin H**, Hua F, Hu ZW. Autophagic flux, supported by toll-like receptor 2 activity, defends against the carcinogenesis of hepatocellular carcinoma. *Autophagy* 2012; **8**: 1859-1861 [PMID: 22996042 DOI: 10.4161/auto.22094]
  - 31 **Dolado I**, Swat A, Ajenjo N, De Vita G, Cuadrado A, Nebreda AR. p38alpha MAP kinase as a sensor of reactive oxygen species in tumorigenesis. *Cancer Cell* 2007; **11**: 191-205 [PMID: 17292829 DOI: 10.1016/j.ccr.2006.12.013]
  - 32 **Kang TW**, Yevsa T, Woller N, Hoenicke L, Wuestefeld T, Dauch D, Hohmeyer A, Gereke M, Rudalska R, Potapova A, Iken M, Vucur M, Weiss S, Heikenwalder M, Khan S, Gil J, Bruder D, Manns M, Schirmacher P, Tacke F, Ott M, Luedde T, Longerich T, Kubicka S, Zender L. Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature* 2011; **479**: 547-551 [PMID: 22080947 DOI: 10.1038/nature10599]
  - 33 **Clarke R**, Cook KL, Hu R, Facey CO, Tavassoly I, Schwartz JL, Baumann WT, Tyson JJ, Xuan J, Wang Y, Warri A, Shajahan AN. Endoplasmic reticulum stress, the unfolded protein response, autophagy, and the integrated regulation of breast cancer cell fate. *Cancer Res* 2012; **72**: 1321-1331 [PMID: 22422988 DOI: 10.1158/0008-5472.CAN.11-3213]
  - 34 **Ogata M**, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, Murakami T, Taniguchi M, Tani I, Yoshinaga K, Shiosaka S, Hammarback JA, Urano F, Imaizumi K. Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol* 2006; **26**: 9220-9231 [PMID: 17030611 DOI: 10.1128/MCB.01453-06]
  - 35 **van Beijnum JR**, Buurman WA, Griffioen AW. Convergence and amplification of toll-like receptor (TLR) and receptor for advanced glycation end products (RAGE) signaling pathways via high mobility group B1 (HMGB1). *Angiogenesis* 2008; **11**: 91-99 [PMID: 18264787 DOI: 10.1007/s10456-008-9093-5]
  - 36 **Pikarsky E**, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature* 2004; **431**: 461-466 [PMID: 15329734 DOI: 10.1038/nature02924]
  - 37 **Gong W**, Wang ZY, Chen GX, Liu YQ, Gu XY, Liu WW. Invasion potential of H22 hepatocarcinoma cells is increased by HMGB1-induced tumor NF-kB signaling via initiation of HSP70. *Oncol Rep* 2013; **30**: 1249-1256 [PMID: 23836405 DOI: 10.3892/or.2013.2595]
  - 38 **Shi W**, Su L, Li Q, Sun L, Lv J, Li J, Cheng B. Suppression of toll-like receptor 2 expression inhibits the bioactivity of human hepatocellular carcinoma. *Tumour Biol* 2014; **35**: 9627-9637 [PMID: 24964964 DOI: 10.1007/s13277-014-2268-3]
  - 39 **Wu FH**, Yuan Y, Li D, Liao SJ, Yan B, Wei JJ, Zhou YH, Zhu JH, Zhang GM, Feng ZH. Extracellular HSPA1A promotes the growth of hepatocarcinoma by augmenting tumor cell proliferation and apoptosis-resistance. *Cancer Lett* 2012; **317**: 157-164 [PMID: 22115967 DOI: 10.1016/j.canlet.2011.11.020]
  - 40 **Chew V**, Abastado JP. Immunomodulation of the tumor micro-environment by Toll-like receptor-3 (TLR3) ligands. *Oncimmunology* 2013; **2**: e23493 [PMID: 23734310 DOI: 10.4161/onci.23493]
  - 41 **Yoneda K**, Sugimoto K, Shiraki K, Tanaka J, Beppu T, Fuke H, Yamamoto N, Masuya M, Horie R, Uchida K, Takei Y. Dual topology of functional Toll-like receptor 3 expression in human hepatocellular carcinoma: differential signaling mechanisms of TLR3-induced NF-kappaB activation and apoptosis. *Int J Oncol* 2008; **33**: 929-936 [PMID: 18949355 DOI: 10.3892/ijo.00000080]
  - 42 **Zorde-Khvaleyevsky E**, Abramovitch R, Barash H, Spivak-Pohis I, Rivkin L, Rachmilewitz J, Galun E, Giladi H. Toll-like receptor 3 signaling attenuates liver regeneration. *Hepatology* 2009; **50**: 198-206 [PMID: 19441101 DOI: 10.1002/hep.22973]
  - 43 **Maeda S**. NF-kB, JNK, and TLR Signaling Pathways in Hepatocarcinogenesis. *Gastroenterol Res Pract* 2010; **2010**: 367694 [PMID: 21151655 DOI: 10.1155/2010/367694]
  - 44 **Khvaleyevsky E**, Rivkin L, Rachmilewitz J, Galun E, Giladi H. TLR3 signaling in a hepatoma cell line is skewed towards apoptosis. *J Cell Biochem* 2007; **100**: 1301-1312 [PMID: 17243100 DOI: 10.1002/jcb.21119]
  - 45 **Chen L**, Xu YY, Zhou JM, Wu YY, E Q, Zhu YY. TLR3 dsRNA agonist inhibits growth and invasion of HepG2.2.15 HCC cells. *Oncol Rep* 2012; **28**: 200-206 [PMID: 22552584 DOI: 10.3892/or.2012.1791]
  - 46 **Guo Z**, Chen L, Zhu Y, Zhang Y, He S, Qin J, Tang X, Zhou J, Wei Y. Double-stranded RNA-induced TLR3 activation inhibits angiogenesis and triggers apoptosis of human hepatocellular carcinoma cells. *Oncol Rep* 2012; **27**: 396-402 [PMID: 22075935 DOI: 10.3892/or.2011.1538]
  - 47 **Bergé M**, Bonnin P, Sulpice E, Vilar J, Allan D, Silvestre JS, Lévy BI, Tucker GC, Tobelem G, Merkulova-Rainon T. Small interfering RNAs induce target-independent inhibition of tumor growth and vasculature remodeling in a mouse model of hepatocellular carcinoma. *Am J Pathol* 2010; **177**: 3192-3201 [PMID: 20971743 DOI: 10.2353/ajpath.2010.100157]
  - 48 **Xu YY**, Chen L, Zhou JM, Wu YY, Zhu YY. Inhibitory effect of dsRNA TLR3 agonist in a rat hepatocellular carcinoma model. *Mol Med Rep* 2013; **8**: 1037-1042 [PMID: 23970360 DOI: 10.3892/mmr.2013.1646]
  - 49 **Wang L**, Zhu R, Huang Z, Li H, Zhu H. Lipopolysaccharide-induced toll-like receptor 4 signaling in cancer cells promotes cell survival and proliferation in hepatocellular carcinoma. *Dig Dis Sci* 2013; **58**: 2223-2236 [PMID: 23828139 DOI: 10.1007/s10620-013-2745-3]
  - 50 **Liu WT**, Jing YY, Yu GF, Han ZP, Yu DD, Fan QM, Ye F, Li R, Gao L, Zhao QD, Wu MC, Wei LX. Toll like receptor 4 facilitates invasion and migration as a cancer stem cell marker in hepatocellular carcinoma. *Cancer Lett* 2015; **358**: 136-143 [PMID: 25511737 DOI: 10.1016/j.canlet.2014.12.019]
  - 51 **Chen R**, Alvero AB, Silasi DA, Steffensen KD, Mor G. Cancers take their Toll--the function and regulation of Toll-like receptors in cancer cells. *Oncogene* 2008; **27**: 225-233 [PMID: 18176604 DOI: 10.1038/sj.onc.1210907]
  - 52 **Li H**, Li Y, Liu D, Liu J. LPS promotes epithelial-mesenchymal transition and activation of TLR4/JNK signaling. *Tumour Biol* 2014; **35**: 10429-10435 [PMID: 25053598 DOI: 10.1007/s13277-014-2347-5]
  - 53 **Jing YY**, Han ZP, Sun K, Zhang SS, Hou J, Liu Y, Li R, Gao L, Zhao X, Zhao QD, Wu MC, Wei LX. Toll-like receptor 4 signaling promotes epithelial-mesenchymal transition in human hepatocellular carcinoma induced by lipopolysaccharide. *BMC Med* 2012; **10**: 98 [PMID: 22938142 DOI: 10.1186/1741-7015-10-98]
  - 54 **Xu D**, Wang X, Yan S, Yin Y, Hou J, Wang X, Sun B. Interaction of PTPRO and TLR4 signaling in hepatocellular carcinoma. *Tumour Biol* 2014; **35**: 10267-10273 [PMID: 25034527 DOI: 10.1007/s13277-014-2302-5]
  - 55 **Wang Y**, Tu Q, Yan W, Xiao D, Zeng Z, Ouyang Y, Huang L, Cai J, Zeng X, Chen YJ, Liu A. CXCL19 suppresses proliferation and inflammatory response in LPS-induced human hepatocellular carcinoma cells via regulating TLR4-Myd88-TAK1-mediated NF-kB and MAPK pathway. *Biochem Biophys Res Commun* 2015; **456**: 373-379 [PMID: 25475726 DOI: 10.1016/j.bbrc.2014.11.090]
  - 56 **Yu LX**, Yan HX, Liu Q, Yang W, Wu HP, Dong W, Tang L, Lin Y, He YQ, Zou SS, Wang C, Zhang HL, Cao GW, Wu MC, Wang HY. Endotoxin accumulation prevents carcinogen-induced apoptosis and promotes liver tumorigenesis in rodents. *Hepatology* 2010; **52**: 1322-1333 [PMID: 20803560 DOI: 10.1002/hep.23845]
  - 57 **Yu P**, Cheng X, Du Y, Huang L, Dong R. TAK-242 can be the potential agents for preventing invasion and metastasis of

- hepatocellular carcinoma. *Med Hypotheses* 2013; **81**: 653-655 [PMID: 23910073 DOI: 10.1016/j.mehy.2013.06.034]
- 58 **Lin Y**, Yu LX, Yan HX, Yang W, Tang L, Zhang HL, Liu Q, Zou SS, He YQ, Wang C, Wu MC, Wang HY. Gut-derived lipopolysaccharide promotes T-cell-mediated hepatitis in mice through Toll-like receptor 4. *Cancer Prev Res (Phila)* 2012; **5**: 1090-1102 [PMID: 22617167 DOI: 10.1158/1940-6207.CAPR-11-0364]
- 59 **Thiery JP**, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009; **139**: 871-890 [PMID: 19945376 DOI: 10.1016/j.cell.2009.11.007]
- 60 **Chen CL**, Tsukamoto H, Liu JC, Kashiwabara C, Feldman D, Sher L, Dooley S, French SW, Mishra L, Petrovic L, Jeong JH, Machida K. Reciprocal regulation by TLR4 and TGF- $\beta$  in tumor-initiating stem-like cells. *J Clin Invest* 2013; **123**: 2832-2849 [PMID: 23921128 DOI: 10.1172/JCI65859]
- 61 **Magee JA**, Piskounova E, Morrison SJ. Cancer stem cells: impact, heterogeneity, and uncertainty. *Cancer Cell* 2012; **21**: 283-296 [PMID: 22439924 DOI: 10.1016/j.ccr.2012.03.003]
- 62 **Machida K**, Chen CL, Liu JC, Kashiwabara C, Feldman D, French SW, Sher L, Hyeonnam JJ, Tsukamoto H. Cancer stem cells generated by alcohol, diabetes, and hepatitis C virus. *J Gastroenterol Hepatol* 2012; **27** Suppl 2: 19-22 [PMID: 22320911 DOI: 10.1111/j.1440-1746.2011.07010.x]
- 63 **French SW**, Vitocruz E, French BA. Balloon liver cells forming Mallory-Denk-bodies are progenitor cells. *Exp Mol Pathol* 2013; **95**: 117-120 [PMID: 23773849 DOI: 10.1016/j.yexmp.2013.06.001]
- 64 **Machida K**, Feldman DE, Tsukamoto H. TLR4-dependent tumor-initiating stem cell-like cells (TICs) in alcohol-associated hepatocellular carcinogenesis. *Adv Exp Med Biol* 2015; **815**: 131-144 [PMID: 25427905 DOI: 10.1007/978-3-319-09614-8\_8]
- 65 **Yan W**, Chang Y, Liang X, Cardinal JS, Huang H, Thorne SH, Monga SP, Geller DA, Lotze MT, Tsung A. High-mobility group box 1 activates caspase-1 and promotes hepatocellular carcinoma invasiveness and metastases. *Hepatology* 2012; **55**: 1863-1875 [PMID: 22234969 DOI: 10.1002/hep.25572]
- 66 **Finger EC**, Giaccia AJ. Hypoxia, inflammation, and the tumor microenvironment in metastatic disease. *Cancer Metastasis Rev* 2010; **29**: 285-293 [PMID: 20393783 DOI: 10.1007/s10555-010-9224-5]
- 67 **Xu N**, Yao HP, Sun Z, Chen Z. Toll-like receptor 7 and 9 expression in peripheral blood mononuclear cells from patients with chronic hepatitis B and related hepatocellular carcinoma. *Acta Pharmacol Sin* 2008; **29**: 239-244 [PMID: 18215354 DOI: 10.1111/j.1745-7254.2008.00711.x]
- 68 **Tanaka J**, Sugimoto K, Shiraki K, Tameda M, Kusagawa S, Nojiri K, Beppu T, Yoneda K, Yamamoto N, Uchida K, Kojima T, Takei Y. Functional cell surface expression of toll-like receptor 9 promotes cell proliferation and survival in human hepatocellular carcinomas. *Int J Oncol* 2010; **37**: 805-814 [PMID: 20811701 DOI: 10.3892/ijo.00000730]
- 69 **Liu Y**, Yan W, Tohme S, Chen M, Fu Y, Tian D, Lotze M, Tang D, Tsung A. Hypoxia induced HMGB1 and mitochondrial DNA interactions mediate tumor growth in hepatocellular carcinoma through Toll-like receptor 9. *J Hepatol* 2015; **63**: 114-121 [PMID: 25681553 DOI: 10.1016/j.jhep.2015.02.009]
- 70 **Zhang Y**, Lin A, Sui Q, Zhang C, Tian Z, Zhang J. Phosphorothioate modification of the TLR9 ligand CpG ODN inhibits poly(I: C)-induced apoptosis of hepatocellular carcinoma by entry blockade. *Cancer Lett* 2014; **355**: 76-84 [PMID: 25224571 DOI: 10.1016/j.canlet.2014.09.013]
- 71 **Zhang Y**, Lin A, Zhang C, Tian Z, Zhang J. Phosphorothioate-modified CpG oligodeoxynucleotide (CpG ODN) induces apoptosis of human hepatocellular carcinoma cells independent of TLR9. *Cancer Immunol Immunother* 2014; **63**: 357-367 [PMID: 24452201 DOI: 10.1007/s00262-014-1518-y]
- 72 **Kang R**, Zhang Q, Zeh HJ, Lotze MT, Tang D. HMGB1 in cancer: good, bad, or both? *Clin Cancer Res* 2013; **19**: 4046-4057 [PMID: 23723299 DOI: 10.1158/1078-0432.CCR-13-0495]
- 73 **Dhillon AS**, Hagan S, Rath O, Kolch W. MAP kinase signalling pathways in cancer. *Oncogene* 2007; **26**: 3279-3290 [PMID: 17496922 DOI: 10.1038/sj.onc.1210421]
- 74 **Bubici C**, Papa S, Pham CG, Zazzeroni F, Franzoso G. NF-kappaB and JNK: an intricate affair. *Cell Cycle* 2004; **3**: 1524-1529 [PMID: 15611622 DOI: 10.4161/cc.3.12.1321]
- 75 **Liu X**, Xu Q, Chen W, Cao H, Zheng R, Li G. Hepatitis B virus DNA-induced carcinogenesis of human normal liver cells by virtue of nonmethylated CpG DNA. *Oncol Rep* 2009; **21**: 941-947 [PMID: 19287992 DOI: 10.3892/or.00000307]
- 76 **Nischalke HD**, Coenen M, Berger C, Aldenhoff K, Müller T, Berg T, Krämer B, Körner C, Odenthal M, Schulze F, Grünhage F, Nattermann J, Sauerbruch T, Spengler U. The toll-like receptor 2 (TLR2) -196 to -174 del/ins polymorphism affects viral loads and susceptibility to hepatocellular carcinoma in chronic hepatitis C. *Int J Cancer* 2012; **130**: 1470-1475 [PMID: 21500195 DOI: 10.1002/ijc.26143]
- 77 **Junjie X**, Songyao J, Minmin S, Yanyan S, Baiyong S, Xiaxing D, Jiabin J, Xi Z, Hao C. The association between Toll-like receptor 2 single-nucleotide polymorphisms and hepatocellular carcinoma susceptibility. *BMC Cancer* 2012; **12**: 57 [PMID: 22309608 DOI: 10.1186/1471-2407-12-57]
- 78 **Li G**, Zheng Z. Toll-like receptor 3 genetic variants and susceptibility to hepatocellular carcinoma and HBV-related hepatocellular carcinoma. *Tumour Biol* 2013; **34**: 1589-1594 [PMID: 23404408 DOI: 10.1007/s13277-013-0689-z]
- 79 **Jiang ZC**, Tang XM, Zhao YR, Zheng L. A functional variant at miR-34a binding site in toll-like receptor 4 gene alters susceptibility to hepatocellular carcinoma in a Chinese Han population. *Tumour Biol* 2014; **35**: 12345-12352 [PMID: 25179842 DOI: 10.1007/s13277-014-2547-z]
- 80 **Minmin S**, Xiaoqian X, Hao C, Baiyong S, Xiaxing D, Junjie X, Xi Z, Jianquan Z, Songyao J. Single nucleotide polymorphisms of Toll-like receptor 4 decrease the risk of development of hepatocellular carcinoma. *PLoS One* 2011; **6**: e19466 [PMID: 21559380 DOI: 10.1371/journal.pone.0019466]
- 81 **Garay RP**, Viens P, Bauer J, Normier G, Bardou M, Jeannin JF, Chiavaroli C. Cancer relapse under chemotherapy: why TLR2/4 receptor agonists can help. *Eur J Pharmacol* 2007; **563**: 1-17 [PMID: 17383632 DOI: 10.1016/j.ejphar.2007.02.018]
- 82 **Kawamoto T**, Ii M, Kitazaki T, Iizawa Y, Kimura H. TAK-242 selectively suppresses Toll-like receptor 4-signaling mediated by the intracellular domain. *Eur J Pharmacol* 2008; **584**: 40-48 [PMID: 18299127 DOI: 10.1016/j.ejphar.2008.01.026]
- 83 **Matsunaga N**, Tsuchimori N, Matsumoto T, Ii M. TAK-242 (resatorvid), a small-molecule inhibitor of Toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. *Mol Pharmacol* 2011; **79**: 34-41 [PMID: 20881006 DOI: 10.1124/mol.110.068064]
- 84 **Xu YY**, Chen L, Wang GL, Zhou JM, Zhang YX, Wei YZ, Zhu YY, Qin J. A synthetic dsRNA, as a TLR3 pathwaysynergist, combined with sorafenib suppresses HCC in vitro and in vivo. *BMC Cancer* 2013; **13**: 527 [PMID: 24195809 DOI: 10.1186/1471-2407-13-527]
- 85 **Behm B**, Di Fazio P, Michl P, Neureiter D, Kemmerling R, Hahn EG, Strobel D, Gress T, Schuppan D, Witsniewski TT. Additive antitumour response to the rabbit VX2 hepatoma by combined radio frequency ablation and toll like receptor 9 stimulation. *Gut* 2016; **65**: 134-143 [PMID: 25524262 DOI: 10.1136/gutjnl-2014-308286]
- 86 **Chen Y**, Sun R. Toll-like receptors in acute liver injury and regeneration. *Int Immunopharmacol* 2011; **11**: 1433-1441 [PMID: 21601014 DOI: 10.1016/j.intimp.2011.04.023]

P- Reviewer: Dang SS, Ding MX, Sugimura H

S- Editor: Kong JX L- Editor: A E- Editor: Liu SQ



## Sofosbuvir treatment and hepatitis C virus infection

Masato Nakamura, Tatsuo Kanda, Yuki Haga, Reina Sasaki, Shuang Wu, Shingo Nakamoto, Shin Yasui, Makoto Arai, Fumio Imazeki, Osamu Yokosuka

Masato Nakamura, Tatsuo Kanda, Yuki Haga, Reina Sasaki, Shuang Wu, Shingo Nakamoto, Shin Yasui, Makoto Arai, Fumio Imazeki, Osamu Yokosuka, Department of Gastroenterology and Nephrology, Chiba University, Graduate School of Medicine, Chiba 260-8670, Japan

Shingo Nakamoto, Department of Molecular Virology, Chiba University, Graduate School of Medicine, Chiba 260-8677, Japan

Fumio Imazeki, Safety and Health Organization, Chiba University, Chiba 263-8522, Japan

**Author contributions:** All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

**Conflict-of-interest statement:** Kanda T reports receiving lecture fees from Chugai Pharmaceutical, MSD, Tanabe-Mitsubishi, Ajinomoto, Bristol-Myers Squibb, Daiichi-Sankyo, Janssen Pharmaceutical and GlaxoSmithKline; Yokosuka O reports receiving grant support from Chugai Pharmaceutical, Bayer, MSD, Daiichi-Sankyo, Tanabe-Mitsubishi, Bristol-Myers Squibb, Gilead Sciences and Taiho Pharmaceutical. None of the other authors have any conflicts of interest or financial support to declare.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Tatsuo Kanda, MD, PhD, Associate Professor, Department of Gastroenterology and Nephrology, Chiba University, Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. [kandat-cib@umin.ac.jp](mailto:kandat-cib@umin.ac.jp)  
 Telephone: +81-43-2262086  
 Fax: +81-43-2262088

Received: October 15, 2015  
 Peer-review started: October 16, 2015

First decision: November 24, 2015  
 Revised: November 27, 2015  
 Accepted: January 5, 2016  
 Article in press: January 7, 2016  
 Published online: January 28, 2016

### Abstract

Hepatitis C virus (HCV) infection is a serious problem worldwide. The use of interferon-based therapy has made HCV eradication challenging. The recent appearance of direct-acting antiviral agents (DAAs) has changed HCV therapy. Combining the use of DAAs with peginterferon and ribavirin has improved treatment efficacy. Furthermore, the combination of different orally administered DAAs has enabled interferon-free therapy with much higher efficacy and safety. In particular, sofosbuvir, a nucleotide-based NS5B inhibitor, prevents HCV RNA synthesis by acting as a "chain terminator". Treatment with sofosbuvir has attained an extremely high rate of sustained virologic response. The current review summarizes the efficacy and safety of sofosbuvir therapy.

**Key words:** Hepatitis C virus; Interferon; Interferon-free; Genotype; Sofosbuvir

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Sofosbuvir, a nucleotide-based NS5B inhibitor, is an effective treatment against pangenotypic strains of hepatitis C virus (HCV). Sofosbuvir-containing regimens have attained extremely high rates of sustained virologic response. Because regimens including sofosbuvir result in fewer adverse events than interferon-based regimens, sofosbuvir has taken a central role in HCV treatment.

Nakamura M, Kanda T, Haga Y, Sasaki R, Wu S, Nakamoto S, Yasui S, Arai M, Imazeki F, Yokosuka O. Sofosbuvir treatment and



hepatitis C virus infection. *World J Hepatol* 2016; 8(3): 183-190  
Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i3/183.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i3.183>

## INTRODUCTION

Hepatitis C virus (HCV) infection is a global public health problem. Approximately 130 to 170 million people experience chronic HCV infection, which has a global prevalence of 2%-3%<sup>[1,2]</sup>. In 2002, worldwide, 27% of 783000 deaths from cirrhosis and 25% of 619000 deaths from hepatocellular carcinoma were attributed to HCV infection<sup>[3]</sup>.

The use of interferon-based therapy has made HCV eradication challenging, especially for patients infected with HCV genotype 1. Treatment with peginterferon plus ribavirin induces only about 50% of patients infected with HCV genotype 1 at high viral loads to achieve sustained virologic response (SVR)<sup>[4]</sup>, while about 80% of patients infected with HCV genotypes 2 and 3 achieve SVR<sup>[5]</sup>.

The appearance of direct-acting antiviral agents (DAAs), which specifically target HCV proteins, has provided insights into the current situation. The use of protease inhibitors, such as telaprevir, boceprevir, simeprevir, faldaprevir and vaniprevir, in combination with peginterferon and ribavirin has improved treatment efficacy in treatment-naïve patients (70% to 80% achieve SVR) and in patients infected with HCV genotype 1 who have relapsed post-treatment<sup>[6-10]</sup>. However, SVR rates in patients who exhibited no responses to previous treatments remain low<sup>[11,12]</sup>. Furthermore, patients who are ineligible for or intolerant to treatment with peginterferon plus ribavirin are contra-indicated from receiving the above treatment.

The use of combinations of different orally administered DAAs has enabled the realization of interferon-free therapy. DAA-based therapies generally have higher treatment efficacy and lower risk for adverse effects compared to regimens using interferon. In particular, sofosbuvir, a pyrimidine nucleoside analog inhibitor of HCV pangenotype NS5B polymerase, has produced extremely high rates of SVR when used in combination with other DAAs. In the current review, we discuss the use of treatment regimens that include sofosbuvir to combat HCV.

## STRUCTURE OF HCV GENOME AND DAAS

HCV is an enveloped, positive-stranded RNA virus. Its genome spans 9600 nucleotides in length and contains a 5' non-translated region (5' NTR), a single open reading frame and a 3' NTR. A single polyprotein is translated from HCV genome and is then cleaved into structural (core, E1, E2 and p7) and non-structural (NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins<sup>[4]</sup>. The

serine protease NS3 plays a key role in processing the HCV polyprotein. The phosphoprotein NS5A and RNA-dependent RNA polymerase NS5B are essential to the replication of HCV RNA. DAAs against HCV target these proteins and strongly inhibit HCV replication.

The first-generation NS3/4A protease inhibitors telaprevir and boceprevir are typically used in combination with peginterferon and ribavirin. This triple regimen has improved SVR rates in HCV genotype 1-infected patients<sup>[6,7]</sup> but often produces serious adverse events<sup>[13]</sup>. The second-generation NS3/4A protease inhibitors simeprevir, faldaprevir and vaniprevir have further improved treatment efficacy while resulting in fewer adverse effects<sup>[8-10]</sup>.

In Japan, daclatasvir, a first-in-class NS5A inhibitor, enabled effective interferon-free therapy for the first time. Interferon-free dual oral therapy with daclatasvir and asunaprevir, a NS3/4A protease inhibitor, led to high SVR rates (87.4% in interferon-ineligible or -intolerant patients, 80.5% in non-responders, 89.1% in treatment-naïve patients, and 95.5% in relapsers)<sup>[14,15]</sup>. However, the evolution of drug-resistant HCV variants has created new problems. For example, Y93H variants of the NS5A protein have markedly decreased SVR rates to as low as 43.3%<sup>[14]</sup>.

### Sofosbuvir

Sofosbuvir (formerly known as GS-7977; Gilead Sciences, Foster City, CA, United States) is a nucleotide NS5B inhibitor<sup>[16]</sup>. Sofosbuvir is converted into a pharmacologically active form (GS-461203) within hepatocytes<sup>[17]</sup>. GS-461203 inhibits RNA-dependent RNA polymerase activity by competing with uridine and prevents HCV RNA synthesis by acting as "chain terminator". Because the catalytic site of the NS5B protein is highly conserved, sofosbuvir is believed to have pangenotypic activity<sup>[18]</sup>.

Another favorable characteristic of sofosbuvir is its high genetic barrier. In an HCV replicon study, a S282T substitution in NS5B was reported to impart drug resistance<sup>[19]</sup>. However, in clinical trials, this NS5B variant was detected in only one patient after treatment with sofosbuvir monotherapy<sup>[16,20-22]</sup>. Foster *et al.*<sup>[23]</sup> reported that sofosbuvir treatment led to the emergence of drug-resistant variants in 9/78 (12%) of patients. An L159F variant was present both at baseline and at the time of virologic failure in 1 patient and at the time of virologic failure in 7 additional patients. The V321A variant emerged at the time of virologic failure in 2 patients.

Sofosbuvir also possess a noteworthy safety profile and high tolerability. In phase 3 trials of sofosbuvir, the frequency of serious adverse events ranged from 1% to 8% and the rate of treatment discontinuation because of adverse events range from 0% to 4.4%<sup>[24,25]</sup>.

## CLINICAL EFFICACY OF TREATMENT REGIMEN CONTAINING SOFOSBUVIR

More than 3000 patients have been assessed in clinical



**Table 1 Summary of phase 3 study for hepatitis C virus genotype 1-infected patients**

Study name	Population	Treatment	Duration (wk)	n	LC (%)	SVR12 (%)		
						All	Non-LC	LC
NEUTRINO	Naïve	SOF/PEG-IFN/RBV	12	291	-	89	-	81
ION-1	Naïve	SOF/LDV	12	210	16	99	100	97
		SOF/LDV/RBV	12	216	15	97	97	100
		SOF/LDV	24	214	15	98	99	97
		SOF/LDV/RBV	24	214	17	99	99	100
ION-3	Naïve	SOF/LDV	8	214	0	94	94	-
		SOF/LDV/RBV	8	216	0	93	93	-
		SOF/LDV	12	216	0	95	95	-
Japanese study	Naïve	SOF/LDV	12	83	16	100	100	100
		SOF/LDV/RBV	12	83	14	96	97	92
ION-2	Experienced	SOF/LDV	12	20	94	95	86	20
		SOF/LDV/RBV	12	20	96	100	82	20
		SOF/LDV	24	20	99	99	100	20
		SOF/LDV/RBV	24	20	99	99	100	20
Japanese study	Experienced	SOF/LDV	12	32	100	100	100	32
		SOF/LDV/RBV	12	25	100	100	100	25

LC: Liver cirrhosis; SVR12: Sustained virologic response at 12 wk; Naïve: Treatment-naïve; Experienced: Treatment-experienced; SOF: Sofosbuvir; LDV: Ledipasvir; PEG-IFN: Peginterferon; RBV: Ribavirin.

studies of treatment regimens containing sofosbuvir. Below, we describe the efficacy of a sofosbuvir-containing regimen.

#### **HCV genotype 1-specific virologic response of sofosbuvir**

The results from phase 3 clinical trials evaluating the use of treatment regimens containing sofosbuvir against HCV genotype 1 are shown in Table 1. The NEUTRINO study was the first phase 3 trial to evaluate sofosbuvir-containing therapy<sup>[26]</sup>. In this single-group, open-label study, 291 treatment-naïve patients infected with HCV genotype 1 were treated with sofosbuvir plus peginterferon and ribavirin for 12 wk. The patients attained a high rate of SVR at 12 wk after completion of therapy (SVR12) (89%) and had a low rate of treatment discontinuation (2%) compared with historical controls. This study demonstrated the efficacy and safety of combining sofosbuvir with peginterferon and ribavirin; however, patients contraindicated for peginterferon or ribavirin were excluded.

Based on high rates of SVR in phase 2 studies, phase 3 ION studies (ION-1, ION-2 and ION-3 studies) were conducted to assess a fixed-dose combination of sofosbuvir and ledipasvir<sup>[27-30]</sup>. In the ION-1 trial, 865 treatment-naïve patients infected with HCV genotype 1 were randomly divided into four groups and received either 12 or 24 wk of sofosbuvir and ledipasvir with or without ribavirin. High SVR12 rates were attained in all groups (range: 97%-99%), and no patients in either 12-wk group discontinued therapy because of adverse events<sup>[28]</sup>.

The ION-2 trial was conducted to evaluate the combination of sofosbuvir and ledipasvir with or without ribavirin in treatment-experienced patients. In this trial, 440 patients were divided into groups and treated with either 12 or 24 wk of sofosbuvir and ledipasvir with

or without ribavirin. As in the ION-1 trial, high SVR12 rates were attained in all treatment groups (range: 94%-99%). No cases of treatment discontinuation owing to adverse events were reported<sup>[29]</sup>.

The ION-3 trial was conducted to evaluate the feasibility of shorter duration therapy in previously untreated patients without cirrhosis. The noninferiority of an 8-wk regimen was demonstrated by the similar SVR12 rates in all groups (range: 93%-95%)<sup>[30]</sup>. The results from the ION-3 trial also indicated that an 8-wk regimen of sofosbuvir plus ledipasvir is not generally equal to 12 wk of treatment; however, international guidelines recommend that an HCV RNA threshold of 6 million IU/mL is maintained.

Among Asian countries, Mizokami *et al.*<sup>[25]</sup> reported a phase 3 study conducted to evaluate Japanese patients. In this study, 166 treatment-naïve and 175 treatment-experienced patients received 12 wk of sofosbuvir and ledipasvir with or without ribavirin. High SVR12 rates (range: 96%-100%) were also attained in this study. This is noteworthy, as Japanese patients tend to be older, have more advanced fibrosis and are more frequently treated with previous therapy than patients in other countries. In the above studies, the inclusion of ribavirin in the treatment regimens produced no additional benefit. Furthermore, adverse events were more common in groups treated with ribavirin than in those without ribavirin.

The SOLAR-1 and SOLAR-2 studies showed the usefulness of sofosbuvir plus ledipasvir for decompensated cirrhosis<sup>[31,32]</sup>. In patients with cirrhosis and moderate or severe hepatic impairment who had not undergone liver transplantation, 86%-89% SVR12 rates were achieved<sup>[31]</sup>. In patients who had undergone liver transplantation, 96%-98%, 85%-88%, and 60%-75% SVR12 rates were achieved in patients without cirrhosis or with well-compensated cirrhosis, patients with

**Table 2 Summary of phase 3 study for hepatitis C virus genotype 2-infected patients**

Study name	Population	Treatment	Duration (wk)	n	LC (%)	SVR12 (%)		
						All	Non-LC	LC
FISSION	Naïve	SOF/RBV	12	70	-	97	-	-
POSITRON	IFN-ineligible/intolerant	SOF/RBV	12	109	15	93	92	94
VALENCE	Naïve	SOF/RBV	12	32	-	97	97	100
Japanese study	Naïve	SOF/RBV	12	90	9	98	97	100
FUSION	Experienced	SOF/RBV	12	36	28	86	96	60
			16	32	-	94	100	78
VALENCE	Experienced	SOF/RBV	12	41	-	90	91	88
Japanese study	Experienced	SOF/RBV	12	63	14	95	96	89

LC: Liver cirrhosis; SVR12: Sustained virologic response at 12 wk; Naïve: Treatment-naïve; Experienced: Treatment-experienced; SOF: Sofosbuvir; IFN: Interferon; RBV: Ribavirin.

moderate hepatic impairment, and patients with severe hepatic impairment, respectively<sup>[31]</sup>. All 6 patients with fibrosing cholestatic hepatitis achieved SVR12. Response rates in the 12- and 24-wk treatment groups were similar<sup>[31]</sup>. The use of sofosbuvir plus simeprevir for 12 and 8 wk in HCV genotype 1-infected patients without cirrhosis resulted in 83% and 97% SVR12 rates, respectively<sup>[33]</sup>. Likewise, the use of sofosbuvir plus simeprevir for 12 wk resulted in an 83% SVR12 rate in HCV genotype 1 - infected patients with cirrhosis<sup>[34]</sup>.

#### **HCV genotype 2-specific virologic response of sofosbuvir**

The results from phase 3 clinical trials evaluating the use of treatments containing sofosbuvir against HCV genotype 2-infected patients are shown in Table 2. Three phase 3 trials have evaluated previously untreated patients (the FISSION, POSITRON and VALENCE studies)<sup>[23,29-31]</sup>. In the FISSION study, 12 wk of sofosbuvir plus ribavirin treatment and 24 wk of Peg-interferon  $\alpha$ -2a (Peg-IFN $\alpha$ 2a) plus ribavirin treatment produced comparable results in treatment-naïve patients. The SVR12 rate was 97% in a group of 70 patients who received sofosbuvir plus ribavirin and 78% in a group of 67 patients who received Peg-IFN $\alpha$ 2a plus ribavirin. The noninferiority of the sofosbuvir plus ribavirin regimen relative to interferon-based therapy was shown. Furthermore, adverse events were less frequent with the sofosbuvir plus ribavirin regimen<sup>[26,35]</sup>.

In the POSITRON study, 109 patients for whom treatment with Peg-IFN was not an option received 12 wk of sofosbuvir plus ribavirin, and 78% of these patients achieved SVR12<sup>[36]</sup>. These results were confirmed by the VALENCE study, in which 97% of 32 treatment-naïve patients achieved SVR12<sup>[37]</sup>.

In addition, the FUSION study<sup>[36]</sup> and the other arm of the VALENCE study evaluated the use of a sofosbuvir plus ribavirin regimen in treatment-experienced patients. In the FUSION study, 103 patients received sofosbuvir plus ribavirin for 12 wk, and 98 patients received the treatment for 16 wk. The rates of SVR12 were 86% and 94% in the 12 and 16-wk groups, respectively<sup>[36]</sup>.

Omata *et al.*<sup>[38]</sup> reported the efficacy and safety of a 12-wk sofosbuvir plus ribavirin treatment in Japanese

patients. The rates of SVR12 were 98% and 95% in treatment-naïve and previously treated patients, respectively. According to these trials, the use of sofosbuvir plus ribavirin has become a standard of care for the treatment of HCV genotype 2-infected patients.

#### **HCV genotype 3-specific virologic response of sofosbuvir**

HCV genotype 3 has become the most difficult genotype to cure in the era of interferon-free therapy. Although sofosbuvir is considered to have pangenotypic inhibitory activities, its treatment efficacy against HCV genotype 3 is lower than against the other genotypes. The results from phase 3 clinical trials evaluating treatments containing sofosbuvir to combat HCV genotype 3 are shown in Table 3.

In the FISSION study, SVR12 occurred in 56% of patients who received 12 wk of sofosbuvir plus ribavirin and in 63% of patients who received 24 wk of peginterferon plus ribavirin. These results were poor, as 97% of patients infected with HCV genotype 2 achieved SVR12 following treatment with sofosbuvir plus ribavirin in the same study<sup>[26,35]</sup>.

The results of the POSITRON study were similar to those of the FISSION study. In the FISSION study, 61% patients infected with HCV genotype 3 achieved SVR12 compared to 93% of patients infected with HCV genotype 2. The rate of SVR12 in patients with cirrhosis was especially low in the HCV genotype 3 group (21%)<sup>[36]</sup>.

Although the above results are somewhat disappointing, extending the duration of treatment might improve sofosbuvir's efficacy against HCV genotype 3. In the FUSION study, 16 wk of sofosbuvir plus ribavirin therapy resulted in higher SVR12 rates than 12 wk therapy (37% vs 63% in patients without cirrhosis; 19% vs 61% in patients with cirrhosis)<sup>[36]</sup>.

In addition, the VALENCE study was conducted to assess the efficacy of 24 wk of sofosbuvir plus ribavirin treatment in 250 HCV genotype 3-infected patients<sup>[37]</sup>. The rates of SVR12 were 95% and 92% in treatment-naïve patients with and without cirrhosis, respectively, and 87% and 62% in previously treated patients with and without cirrhosis. A longer treatment duration

**Table 3 Summary of phase 3 study for hepatitis C virus genotype 3-infected patients**

Study name	Population	Treatment	Duration (wk)	n	LC (%)	SVR12 (%)		
						All	Non-LC	LC
FISSION	Naïve	SOF/RBV	12	183	-	56	-	-
POSITRON	IFN-ineligible/intolerant	SOF/RBV	12	98	15	61	68	21
VALENCE	Naïve	SOF/RBV	24	105	12	93	95	92
FUSION	Experienced	SOF/RBV	12	64	39	30	37	19
			16	63	-	62	63	61
VALENCE	Experienced	SOF/RBV	24	145	32	79	87	62

LC: Liver cirrhosis; SVR12: Sustained virologic response at 12 wk; Naïve: Treatment-naïve; Experienced: Treatment-experienced; SOF: Sofosbuvir; IFN: Interferon; RBV: Ribavirin.

**Table 4 Summary of phase 3 study for hepatitis C virus-human immunodeficiency virus co-infected patients**

Genotype	Study name	Population	Treatment	Duration (wk)	n	LC (%)	SVR12 (%)
1	PHOTON-1	Naïve	SOF/RBV	12	114	4.4	76
	PHOTON-2	Naïve	SOF/RBV	24	112	15	85
2	PHOTON-1	Naïve	SOF/RBV	12	26	-	88
		Experienced	SOF/RBV	24	24	-	92
	PHOTON-2	Naïve	SOF/RBV	12	19	5	89
		Experienced	SOF/RBV	24	6	33	83
3	PHOTON-1	Naïve	SOF/RBV	12	42	-	67
		Experienced	SOF/RBV	24	17	-	94
	PHOTON-2	Naïve	SOF/RBV	24	57	5	91
		Experienced	SOF/RBV	24	49	47	86
4	PHOTON-2	Naïve	SOF/RBV	24	31	26	84

LC: Liver cirrhosis; SVR12: Sustained virologic response at 12 wk; Naïve: Treatment-naïve; Experienced: Treatment-experienced; SOF: Sofosbuvir; RBV: Ribavirin.

was not associated with a higher frequency of adverse events or treatment discontinuation. However, further studies will be necessary to improve treatment efficacy in previously treated patients with cirrhosis.

Foster *et al.*<sup>[23]</sup> reported a 93% SVR12 rate following 12 wk of treatment with peginterferon, sofosbuvir and ribavirin vs an 84% SVR12 rate following 24 wk of treatment with sofosbuvir plus ribavirin vs an 88% SVR12 rate following 12 wk of treatment with peginterferon, sofosbuvir and ribavirin in cirrhotic patients. Treatment with daclatasvir plus sofosbuvir for 12 wk led to a 96% SVR12 rate in HCV genotype 3-infected patients without cirrhosis<sup>[39]</sup>.

#### Other HCV genotypes-specific virologic response of sofosbuvir

In the NEUTRINO study, 35 patients infected with HCV genotypes 4 through 6 were treated with sofosbuvir, ribavirin and peginterferon for 12 wk. The rates of SVR12 were 96% and 100% in patients infected with HCV genotypes 4 and 5-6, respectively<sup>[26]</sup>. A treatment regimen that contains sofosbuvir might therefore be efficient against these genotypes.

The use of a ribavirin- and interferon-free regimen of ledipasvir/sofosbuvir for 12 wk resulted in an SVR4 rate of 93% in genotype 4 and genotype 5 HCV-infected treatment-naïve and treatment-experienced patients with or without cirrhosis<sup>[40]</sup>.

#### HIV co-infection

The PHOTON-1 and -2 studies were conducted to evaluate the use of sofosbuvir plus ribavirin therapy to combat HCV-human immunodeficiency virus (HIV) co-infection<sup>[41,42]</sup>. The results of these trials are summarized in Table 4.

In the PHOTON-1 study, in patients infected with HCV genotype 1, 12 wk of sofosbuvir plus ribavirin led to a 78% SVR12 rate. Extending treatment to 24 wk tended to result in higher rates of SVR (85%)<sup>[41]</sup>.

The use of a longer treatment duration also appeared to be effective in treatment-naïve patients infected with HCV genotype 3. Similar to the results from the study against HCV genotype 3 infection alone, 12 wk of sofosbuvir plus ribavirin therapy attained low rates of SVR12 compared with other genotypes in the PHOTON-1 study (67%)<sup>[32]</sup>. Extending treatment from 12 to 24 wk improved the rate of SVR12 in the PHOTON-2 study (91%)<sup>[42]</sup>. Among patients infected with other HCV genotypes, high rates of SVR12 (83%-92%) were attained after 12 wk of sofosbuvir plus ribavirin treatment in treatment-naïve patients and 24 wk of the above treatment in previously treated patients<sup>[41,42]</sup>.

The ALLY-2 study examined the use of daclatasvir plus sofosbuvir in patients co-infected with HIV and HCV genotype 1. The results showed that SVR rates were 96.4% following 12 wk of treatment and 75.6% following 8 wk of treatment in treatment-naïve patients.

The SVR12 rate was 97.7% in treatment-experienced patients following 12 wk of treatment with the above regimen<sup>[43]</sup>.

The ION-4 study evaluated the use of ledipasvir plus sofosbuvir for 12 wk in HIV co-infected patients. The SVR12 rates were 96% in HCV genotype 1a-infected patients, 96% in HCV genotype 1b-infected patients, and 100% in HCV genotype 4-infected patients<sup>[44]</sup>.

## CONCLUSION

Sofosbuvir, a first-in-class NS5B inhibitor, has rapidly become the standard of care for the treatment of numerous HCV genotypes. However, its efficacy against HCV genotype 3, especially in patients with cirrhosis, has not been satisfactory. The optimal duration of treatment and use of novel combinations with other DAAs should be examined in the future.

Patients with severe renal impairment (estimated glomerular filtration rate < 30 mL/min per 1.73 m<sup>2</sup>) and on hemodialysis are contraindicated for sofosbuvir-containing regimens. This limitation of sofosbuvir should be recognized. Nevertheless, sofosbuvir is an important drug that possesses high efficacy and safety. Sofosbuvir-containing therapy has become a standard of care for the majority of patients with HCV infections.

## REFERENCES

- Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol* 2014; **61**: S45-S57 [PMID: 25086286 DOI: 10.1016/j.jhep.2014.07.027]
- Lavanchy D. The global burden of hepatitis C. *Liver Int* 2009; **29** Suppl 1: 74-81 [PMID: 19207969 DOI: 10.1111/j.1478-3231.2008.01934.x]
- Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; **45**: 529-538 [PMID: 16879891 DOI: 10.1016/j.jhep.2006.05.013]
- Kanda T, Imazeki F, Yokosuka O. New antiviral therapies for chronic hepatitis C. *Hepatol Int* 2010; **4**: 548-561 [PMID: 21063477 DOI: 10.1007/s12072-010-9193-3]
- Lagging M, Rembeck K, Rauning Buhl M, Christensen P, Dalgard O, Färkkilä M, Hellstrand K, Langeland N, Lindh M, Westin J, Norkrans G. Retreatment with peg-interferon and ribavirin in patients with chronic hepatitis C virus genotype 2 or 3 infection with prior relapse. *Scand J Gastroenterol* 2013; **48**: 839-847 [PMID: 23795661 DOI: 10.3109/00365521.2013.793389]
- Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]
- Poordad F, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]
- Fried MW, Buti M, Dore GJ, Flisiak R, Ferenci P, Jacobson I, Marcellin P, Manns M, Nikitin I, Poordad F, Sherman M, Zeuzem S, Scott J, Gilles L, Lenz O, Peeters M, Sekar V, De Smedt G, Beumont-Mauviel M. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: the randomized PILLAR study. *Hepatology* 2013; **58**: 1918-1929 [PMID: 23907700 DOI: 10.1002/hep.26641]
- Sulkowski MS, Asselah T, Lalezari J, Ferenci P, Fainboim H, Leggett B, Bessone F, Mauss S, Heo J, Datsenko Y, Stern JO, Kukolj G, Scherer J, Nehmiz G, Steinmann GG, Böcher WO. Faldaprevir combined with pegylated interferon alfa-2a and ribavirin in treatment-naïve patients with chronic genotype 1 HCV: SILEN-C1 trial. *Hepatology* 2013; **57**: 2143-2154 [PMID: 23359516 DOI: 10.1002/hep.26276]
- Rodriguez-Torres M, Stoehr A, Gane EJ, Serfaty L, Lawitz E, Zhou A, Bourque M, Bhanja S, Strizki J, Barnard RJ, Hwang PM, DiNubile MJ, Mobashery N. Combination of vaniprevir with peginterferon and ribavirin significantly increases the rate of SVR in treatment-experienced patients with chronic HCV genotype 1 infection and cirrhosis. *Clin Gastroenterol Hepatol* 2014; **12**: 1029-37.e5 [PMID: 24120953 DOI: 10.1016/j.cgh.2013.09.067]
- Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; **364**: 2417-2428 [PMID: 21696308 DOI: 10.1056/NEJMoa1013086]
- Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]
- D'Ambrosio R, Colombo M. Safety and direct antiviral agents in real life. *Dig Liver Dis* 2013; **45**: S363-S366 [PMID: 24091117 DOI: 10.1016/j.dld.2013.07.012]
- Kumada H, Suzuki Y, Ikeda K, Toyota J, Karino Y, Chayama K, Kawakami Y, Ido A, Yamamoto K, Takaguchi K, Izumi N, Koike K, Takehara T, Kawada N, Sata M, Miyagoshi H, Eley T, McPhee F, Damokosh A, Ishikawa H, Hughes E. Daclatasvir plus asunaprevir for chronic HCV genotype 1b infection. *Hepatology* 2014; **59**: 2083-2091 [PMID: 24604476 DOI: 10.1002/hep.27113]
- Kumada H, Suzuki F, Suzuki Y, Toyota J, Karino Y, Chayama K, Kawakami Y, Fujiyama S, Ito T, Itoh Y, Tamura E, Ueki T, Ishikawa H, Hu W, McPhee F, Linaberry M, Hughes E. Randomized comparison of daclatasvir + asunaprevir versus telaprevir + peginterferon/ribavirin in Japanese HCV patients. *J Gastroenterol Hepatol* 2015; Epub ahead of print [PMID: 26252875 DOI: 10.1111/jgh.13073]
- Gane EJ, Stedman CA, Hyland RH, Ding X, Svarovskaia E, Symonds WT, Hindes RG, Berrey MM. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. *N Engl J Med* 2013; **368**: 34-44 [PMID: 23281974 DOI: 10.1056/NEJMoa1208953]
- Murakami E, Tolstykh T, Bao H, Niu C, Steuer HM, Bao D, Chang W, Espiritu C, Bansal S, Lam AM, Otto MJ, Sofia MJ, Furman PA. Mechanism of activation of PSI-7851 and its diastereoisomer PSI-7977. *J Biol Chem* 2010; **285**: 34337-34347 [PMID: 20801890 DOI: 10.1074/jbc.M110.161802]
- Lam AM, Espiritu C, Bansal S, Micolochick Steuer HM, Niu C, Zennou V, Keilman M, Zhu Y, Lan S, Otto MJ, Furman PA. Genotype and subtype profiling of PSI-7977 as a nucleotide inhibitor of hepatitis C virus. *Antimicrob Agents Chemother* 2012; **56**: 3359-3368 [PMID: 22430955 DOI: 10.1128/AAC.00054-12]
- Kowdley KV, Hassanein T, Gane EJ. Sofosbuvir safety and tolerability in 741 patients treated for up to 24 weeks. *J Hepatol* 2013; **58**: s345
- Ludmerer SW, Graham DJ, Boots E, Murray EM, Simcoe A, Markel EJ, Grobler JA, Flores OA, Olsen DB, Hazuda DJ, LaFemina RL. Replication fitness and NS5B drug sensitivity of diverse hepatitis C virus isolates characterized by using a transient replication assay. *Antimicrob Agents Chemother* 2005; **49**: 2059-2069 [PMID: 15855532]
- Svarovskaia E, Dvory-Sobol H, Gontcharova V, Martin R,



- Hyland R, Symonds WT, Lalezari J, Miller MD, Mo H. No S282T mutation detected by deep sequencing in a large number of HCV patients who received GS-7977 with RBV and/or GS-0938: the QUANTUM study. *J Hepatol* 2013; **58**: S496
- 22 **Svarovskaia ES**, Dvory-Sobol H, Parkin N, Hebner C, Gontcharova V, Martin R, Ouyang W, Han B, Xu S, Ku K, Chiu S, Gane E, Jacobson IM, Nelson DR, Lawitz E, Wyles DL, Bekele N, Brainard D, Symonds WT, McHutchison JG, Miller MD, Mo H. Infrequent development of resistance in genotype 1-6 hepatitis C virus-infected subjects treated with sofosbuvir in phase 2 and 3 clinical trials. *Clin Infect Dis* 2014; **59**: 1666-1674 [PMID: 25266287 DOI: 10.1093/cid/ciu697]
  - 23 **Foster GR**, Pianko S, Cooper C, Brown A, Forton D, Nahass RG, George J, Barnes E, Brainard DM, Massetto B, Lin M, McHutchison JG, Subramanian GM, Agarwal K. Sofosbuvir peginterferon/ribavirin for 12 weeks vs sofosbuvir ribavirin for 16 or 24 weeks in genotype 3 HCV infected patients and treatment-experienced cirrhotic patients with genotype 2 HCV: The boson study. *J Hepatol* 2015; **62**: S259-S262 [DOI: 10.1016/S0168-8278(15)30151-3]
  - 24 **Liu X**, Wang Y, Zhang G, Li N, Zhu Q, Chang H, Han Q, Lv Y, Liu Z. Efficacy and safety of sofosbuvir-based therapy for the treatment of chronic hepatitis C in treatment-naïve and treatment-experienced patients. *Int J Antimicrob Agents* 2014; **44**: 145-151 [PMID: 25034873 DOI: 10.1016/j.ijantimicag.2014.04.018]
  - 25 **Mizokami M**, Yokosuka O, Takehara T, Sakamoto N, Korenaga M, Mochizuki H, Nakane K, Enomoto H, Ikeda F, Yanase M, Toyoda H, Genda T, Umemura T, Yatsushashi H, Ide T, Toda N, Nirei K, Ueno Y, Nishigaki Y, Betular J, Gao B, Ishizaki A, Omote M, Mo H, Garrison K, Pang PS, Knox SJ, Symonds WT, McHutchison JG, Izumi N, Omata M. Ledipasvir and sofosbuvir fixed-dose combination with and without ribavirin for 12 weeks in treatment-naïve and previously treated Japanese patients with genotype 1 hepatitis C: an open-label, randomised, phase 3 trial. *Lancet Infect Dis* 2015; **15**: 645-653 [PMID: 25863559 DOI: 10.1016/S1473-3099(15)70099-X]
  - 26 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
  - 27 **Lawitz E**, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naïve and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; **383**: 515-523 [PMID: 24209977 DOI: 10.1016/S0140-6736(13)62121-2]
  - 28 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]
  - 29 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]
  - 30 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Di Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]
  - 31 **Charlton M**, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS, Fried MW, Terrault NA, O'Leary JG, Vargas HE, Kuo A, Schiff E, Sulkowski MS, Gilroy R, Watt KD, Brown K, Kwo P, Pungpapong S, Korenblat KM, Muir AJ, Teperman L, Fontana RJ, Denning J, Arterburn S, Dvory-Sobol H, Brandt-Sariff T, Pang PS, McHutchison JG, Reddy KR, Afdhal N. Ledipasvir and sofosbuvir Plus Ribavirin for Treatment of HCV Infection in Patients With Advanced Liver Disease. *Gastroenterology* 2015; **149**: 649-659 [PMID: 25985734 DOI: 10.1053/j.gastro.2015.05.010]
  - 32 **Manns M**, Forns X, Samuel D, Denning J, Arterburn S, Brandt-Sariff T, Dvory-Sobol H, Pang PS, McHutchison JG, Gane E, Mutimer D. Ledipasvir/Sofosbuvir With Ribavirin is Safe and Efficacious in Decompensated and Post-Liver Transplantation Patients With HCV Infection: Preliminary Results of the SOLAR-2 Trial. *J Hepatol* 2015; **62**: S187-S188 [DOI: 10.1016/S0168-8278(15)30003-9]
  - 33 **Kwo P**, Gitlin N, Nahass R, Bernstein D, Rojter S, Schiff E, Davis M, Ruane PJ, Younes Z, Kalmeijer R, Peeters M, Lenz O, Fevery B, De La Rosa G, Scott J, Sinha R, Witek J. A Phase 3, Randomised, open-label study to evaluate the efficacy and safety of 12 and 8 weeks of simeprevir (smv) plus sofosbuvir (sof) in treatment-naïve and -experienced patients with chronic hcv genotype 1 infection without cirrhosis: optimist-1. [Accessed 2015 Oct 7]. Available from: URL: [http://www.viraled.com/modules/info/files/\\_555bae903b796.pdf](http://www.viraled.com/modules/info/files/_555bae903b796.pdf)
  - 34 **Lawitz E**, Matusow G, DeJesus E, Yoshida E, Felizarta F, Ghalib R, Godofsky E, Herring R, Poleyndard G, Sheikh A, Tobias H, Kugelmas M, Kalmeijer R, Peeters M, Lenz O, Fevery B, De La Rosa G, Scott J, Sinha R, Witek J. A phase 3, open-label, single-arm study to evaluate the efficacy and safety of 12 weeks of simeprevir (smv) plus sofosbuvir (sof) in treatment-naïve or -experienced patients with chronic hcv genotype 1 infection and cirrhosis: optimist-2. [Accessed 2015 Oct 7]. Available from: URL: <https://depts.washington.edu/hepstudy/presentations/uploads/157/simepreviroptimist2.pdf#search=CIRRHOSIS%3A+OPTIMIST2>
  - 35 **AASLD/IDSA HCV Guidance Panel**. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology* 2015; **62**: 932-954 [PMID: 26111063 DOI: 10.1002/hep.27950]
  - 36 **Jacobson IM**, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]
  - 37 **Zeuzem S**, Dusheiko G, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]
  - 38 **Omata M**, Nishiguchi S, Ueno Y, Mochizuki H, Izumi N, Ikeda F, Toyoda H, Yokosuka O, Nirei K, Genda T, Umemura T, Takehara T, Sakamoto N, Nishigaki Y, Nakane K, Toda N, Ide T, Yanase M, Hino K, Gao B, Garrison KL, Dvory-Sobol H, Ishizaki A, Omote M, Brainard D, Knox S, Symonds WT, McHutchison JG, Yatsushashi H, Mizokami M. Sofosbuvir plus ribavirin in Japanese patients with chronic genotype 2 HCV infection: an open-label, phase 3 trial. *J Viral Hepat* 2014; **21**: 762-768 [PMID: 25196837 DOI: 10.1111/jvh.12312]
  - 39 **Nelson DR**, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R, Oguchi G, Thuluvath PJ, Ortiz-Lasanta G, Rabinovitz M, Bernstein D, Bennett M, Hawkins T, Ravendhran N, Sheikh AM, Varunok P, Kowdley KV, Hennicken D, McPhee F, Rana K, Hughes EA. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology*

- 2015; **61**: 1127-1135 [PMID: 25614962 DOI: 10.1002/hep.27726]
- 40 **Abergel A**, Loustaud-Ratti V, Metivier S, Jiang D, Kersey K, Knox SJ, Pang PS, Samuel D, Asselah T. Ledipasvir/sofosbuvir treatment results in high SVR rates in patients with chronic genotype 4 and 5 HCV infection. *J Hepatol* 2015; **62**: S219-S220 [DOI: 10.1016/S0168-8278(15)30070-2]
- 41 **Sulkowski MS**, Naggie S, Lalezari J, Fessel WJ, Mounzer K, Shuhart M, Luetkemeyer AF, Asmuth D, Gaggar A, Ni L, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Rodriguez-Torres M, Dieterich D. Sofosbuvir and ribavirin for hepatitis C in patients with HIV coinfection. *JAMA* 2014; **312**: 353-361 [PMID: 25038354 DOI: 10.1001/jama.2014.7734]
- 42 **Molina JM**, Orkin C, Iser DM, Zamora FX, Nelson M, Stephan C, Massetto B, Gaggar A, Ni L, Svarovskaia E, Brainard D, Subramanian GM, McHutchison JG, Puoti M, Rockstroh JK; PHOTON-2 study team. Sofosbuvir plus ribavirin for treatment of hepatitis C virus in patients co-infected with HIV (PHOTON-2): a multicentre, open-label, non-randomised, phase 3 study. *Lancet* 2015; **385**: 1098-1106 [PMID: 25659285 DOI: 10.1016/S0140-6736(14)62483-1]
- 43 **Wyles DL**, Ruane PJ, Sulkowski MS, Dieterich D, Luetkemeyer A, Morgan TR, Sherman KE, Dretler R, Fishbein D, Gathe JC, Henn S, Hiestrosa F, Huynh C, McDonald C, Mills A, Overton ET, Ramgopal M, Rashbaum B, Ray G, Scarsella A, Yozviak J, McPhee F, Liu Z, Hughes E, Yin PD, Noviello S, Ackerman P. Daclatasvir plus Sofosbuvir for HCV in Patients Coinfected with HIV-1. *N Engl J Med* 2015; **373**: 714-725 [PMID: 26196502 DOI: 10.1056/NEJMoa1503153]
- 44 **Naggie S**, Cooper C, Saag M, Workowski K, Ruane P, Towner WJ, Marks K, Luetkemeyer A, Baden RP, Sax PE, Gane E, Santana-Bagur J, Stamm LM, Yang JC, German P, Dvory-Sobol H, Ni L, Pang PS, McHutchison JG, Stedman CA, Morales-Ramirez JO, Bräu N, Jayaweera D, Colson AE, Tebas P, Wong DK, Dieterich D, Sulkowski M. Ledipasvir and Sofosbuvir for HCV in Patients Coinfected with HIV-1. *N Engl J Med* 2015; **373**: 705-713 [PMID: 26196665 DOI: 10.1056/NEJMoa1501315]

**P- Reviewer:** da Silva NMO, Felmlee DJ, Parvez MK  
**S- Editor:** Qi Y **L- Editor:** A **E- Editor:** Liu SQ



## Ablation techniques for primary and metastatic liver tumors

Michael J Ryan, Jonathon Willatt, Bill S Majdalany, Ania Z Kielar, Suzanne Chong, Julie A Ruma, Amit Pandya

Michael J Ryan, Jonathon Willatt, Bill S Majdalany, Suzanne Chong, Julie A Ruma, Amit Pandya, Department of Radiology, University of Michigan, Ann Arbor, MI 48109, United States

Ania Z Kielar, Department of Radiology, University of Ottawa, Ontario K1H 8LZ, Canada

**Author contributions:** Ryan MJ was the primary author, producing the initial manuscripts; Majdalany BS reviewed and edited the section on microwave ablation; Kielar AZ reviewed and edited the section on RFA; Willatt J reviewed and edited the sections on cryoablation and IRE; Chong S and Ruma JA researched and collated the evidence for the efficacy of each ablation modality, and selected the references to support the conclusions; Pandya A reviewed and edited the section on choice of imaging modality and was responsible for the references.

**Conflict-of-interest statement:** The authors have no conflicts of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Jonathon Willatt, Department of Radiology, University of Michigan, 500 S State st, Ann Arbor, MI 48109, United States. [jwillatt@med.umich.edu](mailto:jwillatt@med.umich.edu)  
 Telephone: +1-734-8455650  
 Fax: +1-734-8453228

Received: November 3, 2015  
 Peer-review started: November 3, 2015  
 First decision: November 24, 2015  
 Revised: December 1, 2015  
 Accepted: January 5, 2016  
 Article in press: January 7, 2016  
 Published online: January 28, 2016

### Abstract

Ablative treatment methods have emerged as safe

and effective therapies for patients with primary and secondary liver tumors who are not surgical candidates at the time of diagnosis. This article reviews the current literature and describes the techniques, complications and results for radiofrequency ablation, microwave ablation, cryoablation, and irreversible electroporation.

**Key words:** Liver; Ablation; Hepatocellular carcinoma; Metastasis; Radiofrequency; Microwave; Cryoablation

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Innovative ablation techniques, including radiofrequency ablation, microwave ablation, cryoablation and irreversible electroporation have become accepted as treatment modalities for patients with early stage tumor or for single metastases. This review paper describes the available ablation techniques and summarizes the evidence supporting the use of each modality.

Ryan MJ, Willatt J, Majdalany BS, Kielar AZ, Chong S, Ruma JA, Pandya A. Ablation techniques for primary and metastatic liver tumors. *World J Hepatol* 2016; 8(3): 191-199 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i3/191.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i3.191>

### INTRODUCTION

The liver is a common site for both primary malignancy and metastatic disease. Hepatocellular carcinoma (HCC) remains the fifth most common malignancy in the world and its incidence is rising<sup>[1,2]</sup>. Traditionally, the first line therapy for hepatic tumors has been surgical resection or transplantation. However, many patients are not surgical candidates at the time of diagnosis<sup>[2]</sup>. For this reason interest in minimally invasive, ablative treatment methods has grown<sup>[3]</sup>. Percutaneous ablative techniques include radiofrequency ablation (RFA), microwave ablation, cryoablation, and irreversible electroporation (IRE).



**Figure 1** Sixty-eight-year-old male with hepatitis C and cirrhosis. A: Contrast enhanced CT shows a 16 mm HCC; B: RFA probe covering the lesion; C: Post contrast follow up CT shows capsular retraction at the site of the RFA and no residual tumor. HCC: Hepatocellular carcinoma; RFA: Radiofrequency ablation; CT: Computed tomography.

This review focuses on the use of percutaneous ablative techniques in the treatment of HCC, as well as of metastatic disease from colorectal, neuroendocrine, and breast carcinomas.

## TECHNIQUE AND COMPLICATIONS

### RFA

RFA is a low risk alternative treatment for HCC and liver metastases in patients who cannot undergo surgery or transplant<sup>[4]</sup>. Unlike other non-surgical strategies (TACE, Y90), the goal of RFA is curative<sup>[4]</sup>.

### Technique

RFA creates a closed loop circuit which results in an alternating electric field causing agitation of ions within the target tissue<sup>[5]</sup>. The circuit is created using an RF generator, an electrode, grounding pads, and the patient<sup>[3]</sup>. The resultant ionic agitation creates heat leading to cell death from coagulative necrosis<sup>[6]</sup>. In order to ensure tumor destruction, the mass needs to be treated to a temperature of 50 °C-100 °C for approximately 4-5 min<sup>[6]</sup>. Temperatures higher than 100 °C can cause gas formation, also known as carbonization, which can reduce ablation effectiveness, and char adjacent tissues<sup>[7]</sup>.

In order to achieve primary technical success, the entire tumor must be ablated as well as a sufficient margin around the tumor. Similar to surgical techniques, a 1 cm margin in all planes is needed to minimize the risk of residual disease or local recurrence<sup>[3]</sup>. Therefore, the planned target ablation diameter should be 2 cm larger than the tumor diameter<sup>[3]</sup>. If the tumor is small enough, this can be accomplished with one electrode (Figure 1). However, if the tumor is too large, multiple ablations can be performed<sup>[8]</sup>, although there is a risk of local recurrence due to inadequate tumor destruction from the error inherent in positioning electrodes<sup>[3]</sup>. Other causes of inadequate tumor ablation include heterogeneous tissue composition (*i.e.*, fibrosis, calcification) and adjacent blood flow, known as a "heat sink", which can cool the tissue and reduce the maximum achieved

temperature<sup>[9]</sup>.

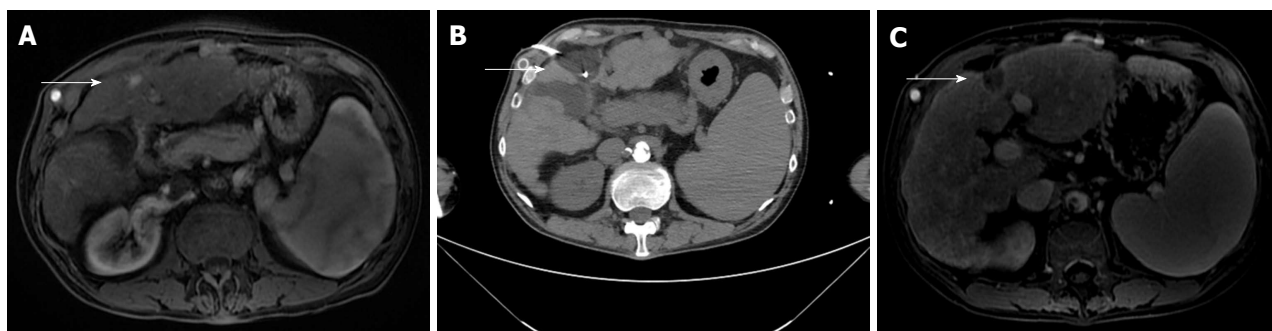
RFA can be performed with guidance by ultrasound (US), computed tomography (CT), or magnetic resonance imaging (MRI) depending on lesion visibility and operator experience. Patients typically receive either conscious sedation or general anesthesia to control pain and minimize patient movement during the procedure. The decision to administer prophylactic antibiotics is somewhat controversial and institution dependent. A longer course of antibiotics may be warranted in patients who are at increased risk of liver abscess, including patients with a history of biliary-enteric anastomosis, biliary stents, or sphincterotomy<sup>[6]</sup>. This is thought to be due to retrograde movement of bacteria into the ablation cavity as a result of altered anatomy<sup>[10]</sup>.

### Complications

RFA has a low rate of major complications. The largest study on RFA complications by Koda *et al.*<sup>[11]</sup> evaluated 13283 patients (16346 treated lesions) with a total of 579 complications (3.5%) and 5 deaths (0.04%). The rate of liver injury was 1.69% (276 patients) which included 75 (0.47%) hepatic infarcts, 32 (0.19%) liver abscesses, 110 (0.67%) bile duct injuries, and 37 (0.23%) bile leaks<sup>[11]</sup>. A more recent study from Lee *et al.*<sup>[1]</sup> reported a similar major complication rate of 3.1% in 169 treated lesions, including 2 bile duct injuries. The overall reported complication rate ranges from 2.2% to 9.5%<sup>[6]</sup>.

The rate of extrahepatic injury is also extremely rare. Koda *et al.*<sup>[11]</sup> reported a total of 113 (0.69%) extrahepatic complications including, in order of decreasing frequency, pleural effusions, skin burns, pneumothorax, gastrointestinal injury, diaphragmatic injury, gallbladder injury, and cardiac tamponade. The risk of extrahepatic injury can be reduced by a technique called "hydrodissection", which involves injecting D5W to create space between adjacent organs. Saline infusions are not used for hydrodissection due to the theoretical risk of conduction of the electrical current through this type of fluid. Another potential complication is seeding, either in the peritoneum or along the ablation track. The





**Figure 2** Sixty-one-year-old with a history of alcohol abuse and cirrhosis. A: MRI demonstrates a 13 mm HCC in the left lobe; B: Two cryoablation probes covering the lesion; C: Post contrast follow up MRI shows capsular retraction at the site of the cryoablation and no residual tumor. MRI: Magnetic resonance imaging; HCC: Hepatocellular carcinoma.

reported risk of tumor seeding ranges from 0.04%<sup>[11]</sup> to 0.6%<sup>[12]</sup>. The risk (0.95%) of tumor seeding has been described to be slightly increased when concomitant biopsy is performed<sup>[12]</sup>.

### Cryoablation

Cryoablation involves rapid cooling of a cryoprobe resulting in cell death<sup>[13]</sup>. Cryoablation has been historically used for both HCC (Figure 2) and hepatic metastases.

### Technique

Cryoablation works by passing high pressure argon gas through a probe resulting in cooling of the metallic. As the probe cools, surrounding tissues are also cooled by convection and conduction<sup>[14]</sup>. Helium gas is then forced through the probe causing warming of the probe and thawing of the adjacent tissues. The cooling and subsequent thawing of the probe results in cell death by a variety of methods. The initial cooling results in intracellular ice crystal formation leading to cell membrane damage and death<sup>[15]</sup>. Larger ice crystals also form during slow thawing, resulting in a shearing effect and additional cell death<sup>[16]</sup>. Lastly, ice crystals develop in the small blood vessels feeding a tumor, leading to ischemia<sup>[16]</sup>. Like the other ablative techniques, cryoablation can be performed percutaneously or laparoscopically. Percutaneous cryoablation can be performed with CT, MRI or US guidance.

Although cryoablation has many uses for tumor ablation, including renal and osseous lesions, its utility in the liver is somewhat limited. The disadvantages of cryoablation include variable ablation size (resulting in the need for multiple cryoprobes), reduced cooling effect due to a heat sink from hepatic vessels, and the risk of major complications. An advantage of cryoablation over other ablative techniques is that the ice ball can be visualized during the procedure under both CT and ultrasound guidance, allowing for better adjustment.

### Complications

The risk of complication within the liver is higher with cryoablation compared to RFA. Complications include hemorrhage, injury to adjacent organs, biliary injury, and

“cryoshock”. Hemorrhage results from ice ball formation within the liver leading to shearing injury to the liver parenchyma and nearby blood vessels. Shearing forces can also cause biliary injury which can lead to late hemorrhage or hepatic abscess formation. Cryoablation of lesions near the liver edge risks damage to adjacent organs, usually bowel, kidney or adrenal glands. A complication unique to cryoablation is “cryoshock” which occurs due to the release of cytokines, resulting in a systemic syndrome characterized by fever, tachycardia, and tachypnea. A retrospective study by Adam *et al.*<sup>[17]</sup> found increased complication rates among patients treated with cryoablation (29%) compared to those who underwent RFA (8%). Additional studies have found similar results including a study demonstrating a 41% complication rate for cryoablation patients compared to 3% in patients who underwent RFA<sup>[18]</sup>.

However, a large study by Yang *et al.*<sup>[19]</sup> found very low rates of major complications with cryoablation. In this study of 300 patients who underwent cryoablation, the major complication rate was 6.3%<sup>[19]</sup>. Major complications included cryoshock (6 patients), extensive hemorrhage (5 patients), gastric bleeding (4 patients), liver abscess (1 patient), intestinal fistula (1 patient), and liver failure (2 patients)<sup>[19]</sup>. The risk of minor complications is reported to be 48.6%<sup>[19]</sup>. These include fever, pain, skin frostbite, pleural effusion, and arterial-portal venous fistula. Pneumothorax is rarely reported in treated tumors located near the diaphragm<sup>[19]</sup>.

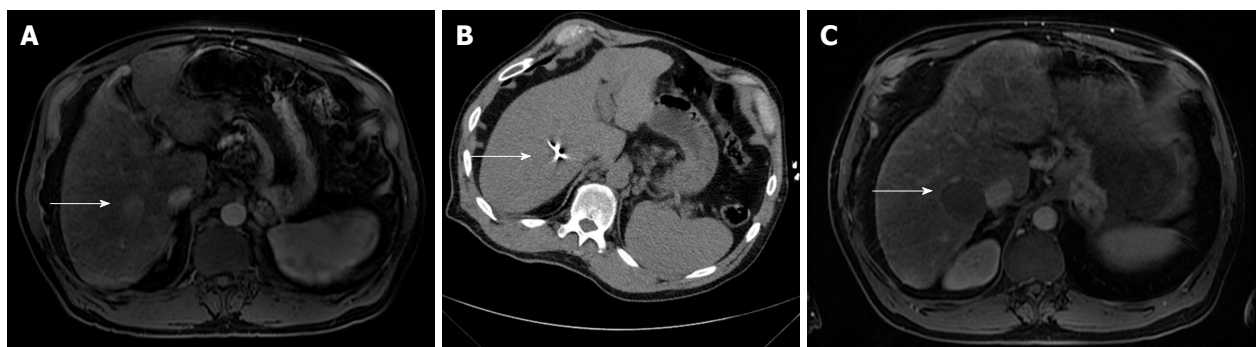
## MICROWAVE ABLATION

### Background/indications

Microwave ablation is an emerging technology with particular applicability in treating hepatic tumors in patients who are not surgical candidates. It has been used for larger tumors than those treated by RFA<sup>[20]</sup>.

### Technique

Microwave ablation utilizes an antenna to locally deliver a high frequency (915 MHz or 2.45 GHz) oscillating electromagnetic field to induce rapid realignment of polar molecules (typically water molecules) in a lesion



**Figure 3** Sixty-two-year-old with hepatitis C cirrhosis. A: MRI shows an arterial enhancing lesion consistent with hepatocellular carcinoma; B: CT guided microwave ablation of the right hepatic lobe lesion; C: MRI shows an ablation zone and no evidence of residual tumor. MRI: Magnetic resonance imaging; CT: Computed tomography.

(Figure 3). This results in markedly increased kinetic energy and subsequent tissue heating<sup>[21]</sup>. Tissues with a larger concentration of water, such as tumors, are particularly susceptible to microwave heating<sup>[21]</sup>.

Microwave ablation can be performed with one or multiple antenna probes. Multiple antenna probes in close proximity allow for electrical and thermal synergy. Multiple probes can also be powered simultaneously which is not possible with RF ablation. Recent developments in microwave technology have produced high-powered water cooled systems which allow for smaller applicators and increased power.

Compared to RF ablation, microwave has several advantages. Microwave is capable of producing very high temperatures (greater than 150 °C) much faster than RF. In addition, microwave is more effective in propagating heating through charred and desiccated tissues which allows for a large ablation zone. Microwave does not require grounding pads or other similar devices<sup>[15]</sup>. Microwave ablation is not as susceptible to heat sink phenomena as RF ablation. This is particularly useful in the liver, which has a rich vascular supply. A recent study demonstrated larger zones of ablation and faster heating with microwave compared to RFA<sup>[22]</sup>. Additional studies have demonstrated larger and more consistent ablation zones with microwave without significant influence from adjacent hepatic vessels<sup>[23,24]</sup>. Ablation time is often less than 10 min, typically averaging 2-5 min, which improves overall efficiency and reduces anesthesia time.

Although microwave ablation is promising, several disadvantages have limited its widespread adoption. Compared to RFA, microwave power is more difficult to generate safely, mostly due to larger cables which are prone to heating issues<sup>[21]</sup>. In addition there remains still uncertainty about the size and shape of ablation zones with microwave<sup>[21]</sup>.

Microwave ablation is typically performed under general anesthesia to reduce patient discomfort and for better control of patient breathing and motion. As with RF ablation, microwave can be performed under CT or ultrasound guidance. Ultrasound allows for real time monitoring of the ablation and shorter procedure time. CT guidance allows for localization of lesions which are

difficult to visualize, and for better evaluation of adjacent structures. Hydrodissection can be used to displace adjacent structures, typically bowel or diaphragm.

### Complications

A systematic review of the literature by Lahat *et al.*<sup>[25]</sup> evaluated the safety of ablative techniques including microwave ablation. In the review of 16 studies, they reported a major complication rate of 4.6% for microwave ablation compared to 4.1% for RFA. The pooled mortality rate for microwave was 0.23% compared to 0.15% for RFA. The most common major complication was hemorrhage requiring blood transfusion. Additional complications included portal vein thrombosis, bile leak/biloma, liver abscess, pleural effusion, and tumor seeding.

### IRE

IRE is a relatively new non-thermal ablative technique approved by the Food and Drug Administration in 2006 for soft tissue ablation<sup>[26]</sup>. It has been used for liver, pancreas, kidney and lung ablations. IRE has several advantages over current, more proven ablative techniques.

### Technique

IRE utilizes multiple electrodes to deliver high voltage (2-3 kV) direct current pulses lasting microseconds to milliseconds<sup>[27]</sup>. The repeated electrical pulses cause damage to the cell membranes<sup>[26]</sup>. Initially the cell membrane damage is reversible, but it becomes irreversible after a period of time leading to apoptosis<sup>[26]</sup>. Because of the extremely short ablation time, care must be taken to ensure proper electrode positioning as mid treatment adjustment is not possible. Most IRE devices require simulation planning with the use of multiple probes placed in parallel to achieve the desired ablation zone.

IRE results in a well-defined ablation zone with sharp margins and relatively little damage to nearby tissues<sup>[27]</sup>. Because IRE does not utilize thermal methods for ablation, adjacent tissue architecture is well preserved<sup>[28]</sup>. The combination of fast ablation times

and minimal damage to nearby tissues makes IRE well suited for treatment of lesions in sensitive locations, including those adjacent to blood vessels and bile ducts. In addition, this eliminates the problems with heat sink seen in other thermal ablative techniques. However, the use of multiple parallel probes results in a significant increase in procedural cost and complexity<sup>[29]</sup>. One potential drawback to IRE is that imaging changes related to the ablation zone may take several minutes to manifest by ultrasound<sup>[30]</sup>. IRE also requires general anesthesia with paralytic agents as the electrical current generated during the procedure can cause muscle spasms and arrhythmias<sup>[31]</sup>. To lessen this risk, the IRE generator is connected to an ECG triggering device and pulses are delivered to the target/treatment zone during the cardiac refractory period<sup>[27]</sup>.

### Complications

A recent large systematic review investigated the safety and efficacy of IRE in several organs. The reported overall complication rate was 16% in 129 treated patients<sup>[26]</sup>. The most common complications included pneumothorax, portal vein thrombosis, biliary occlusion, pleural effusion, and cholangitis<sup>[26]</sup>. There was no periprocedural mortality reported in treated liver lesions, although 3 patients died after pancreatic IRE<sup>[26]</sup>. Self-reported post-procedural pain scores were similar between patients treated with IRE and RFA. Arrhythmias were reported in 4% of cases<sup>[26]</sup>. Ventricular arrhythmias were seen without synchronized pulse delivery while only atrial arrhythmias were seen in patients who received synchronized pulses<sup>[26]</sup>. No uncontrolled muscle spasms were reported in any of the reviewed studies in patients who received paralytic agents<sup>[26]</sup>.

## RESULTS OF INNOVATIVE ABLATION TECHNIQUES

### HCC

**Radiofrequency ablation:** Numerous studies support the usage of RFA as a first line treatment for HCC in patients who are poor surgical candidates. One of the largest studies by Tateishi *et al*<sup>[32]</sup> evaluated RFA of 2140 nodules measuring less than 3 cm in 664 patients. Survival rates at 1-5 years post-treatment were similar for patients with first line RFA alone compared with those who underwent RFA as part of a combination therapy<sup>[32]</sup>. In addition, the rate of local progression of disease was similar for RFA alone when compared to ethanol treatment or hepatectomy<sup>[32]</sup>. A study by Lencioni *et al*<sup>[33]</sup> evaluated patients with early stage HCC (single lesion < 5 cm or up to 3 lesions < 3 cm each) who underwent RFA alone or palliative TACE or ethanol injection. Overall survival rates at 5 years were 48% with a median survival of 57 mo for the RFA group, which was not significantly different from the TACE or ethanol groups<sup>[33]</sup>. Histologic analysis of tumors which underwent RFA and subsequent transplantation found that 74% of ablated tumors were treated successfully

by histologic criteria<sup>[34]</sup>. For tumors measuring less than 3 cm, the percentage successfully treated rose to 83%<sup>[34]</sup>. Another large study of 1502 HCC tumors in 1305 patients over 12 years by Kim *et al*<sup>[35]</sup> found survival rates of 59.7% and 32.3% at 5 and 10 years respectively. Additional studies have demonstrated similar overall recurrence and survival rates for patients who were poor surgical candidates using RFA as first line treatment<sup>[36]</sup>.

Several recent studies have evaluated RFA as a first line treatment in tumors measuring more than 3 cm. A study by Lee *et al*<sup>[1]</sup> evaluated 162 patients who underwent RFA for up to three tumors with a maximum diameter of 5 cm. Overall 5 year survival and recurrence-free survival rates were 67.9% and 25.9% respectively<sup>[1]</sup>. The most significant predictors of poor survival were Child-Pugh class B, elevated serum  $\alpha$ -fetoprotein level, and presence of portal-systemic collaterals<sup>[1]</sup>. The rate of local tumor progression at 5 years was 14.5% with tumor size being the only significant predictive factor<sup>[1]</sup>. Local tumor progression did have a significant negative effect on median recurrence free survival (28.0 mo vs 12.0 mo) and resulted in over two times more interventional procedures<sup>[1]</sup>. A study by Livraghi *et al*<sup>[37]</sup> evaluated RFA of 126 HCCs larger than 3 cm in 114 patients. Complete necrosis on follow up CT scan was observed in 47.6% of patients and near complete necrosis (90%-99%) was observed in 31.7% of patients. The observed complication rate was similar to other studies<sup>[37]</sup>.

More recent studies have called into question the conclusion that RFA is equivalent to surgery in the treatment of HCC. A recent meta-analysis by Qi *et al*<sup>[38]</sup> evaluated 3 randomized control trials. Surgical resection was found to be superior to RFA with respect to overall survival (HR = 1.41) and recurrence free survival (HR = 1.41)<sup>[38]</sup>. However, surgical patients had a significantly higher incidence of complications and a significantly longer hospital stay than patients treated with RFA<sup>[38]</sup>. A more recent study by Miura *et al*<sup>[39]</sup> investigated 2804 patients who underwent ablation or surgical resection for a solitary HCC < 3 cm. Overall survival at 3 and 5 years was higher in the resection group (67%, 55%) than in the RFA group (52%, 36%)<sup>[39]</sup>. There were baseline differences between the two groups which somewhat limited the analysis. However, after propensity matching, the overall survival rate was still higher in the resection group (54%) vs RFA (37%)<sup>[39]</sup>. Surgical resection was also independently associated with improved survival (HR = 0.62)<sup>[39]</sup>.

**Cryoablation:** Multiple studies have evaluated the utility of cryoablation in the treatment of HCC. Chen *et al*<sup>[40]</sup> performed percutaneous cryoablation in 76 lesions of unresectable HCC and 76 lesions of recurrent HCC. 1 and 3 year survival rates in the unresectable group were 81.4% and 60.3% while the disease-free survival rates were 67.6% and 20.8%<sup>[40]</sup>. Survival rates in the recurrent HCC group were 70.2% and 28.8% at 1 and 3 years respectively, while the disease-free survival



rates were 53.8% and 7.7%. There was a low overall complication rate (12.1%) and there were no peri-procedural deaths<sup>[40]</sup>. A similar study by Wang *et al.*<sup>[41]</sup> evaluated cryoablation of 156 patients with HCC < 5 cm in diameter. The reported 1, 2 and 3 years overall survival rates were 92%, 82% and 64%<sup>[41]</sup>. Disease free survival rates were 72%, 56% and 43% at 1, 2 and 3 years<sup>[41]</sup>.

One of the largest studies evaluating cryoablation and HCC was performed by Yang *et al.*<sup>[19]</sup> and looked at 300 patients with unresectable HCC. A total of 223 tumors were incompletely ablated while 185 tumors were completely ablated<sup>[19]</sup>. The rate of local progression of disease at a median 36.7 mo follow up time was 31%<sup>[19]</sup>. The most significant risk factors for tumor recurrence were size and tumor location. The mean survival of patients after cryoablation was 45.7, 28.4 and 17.7 mo, in increasing order of tumor stage<sup>[19]</sup>. A study by Adam *et al.*<sup>[17]</sup> looked at cryoablation vs RFA for unresectable HCC. Despite similar initial post-treatment results, they found a significantly higher rate of local progression of disease in patients treated with cryoablation vs RFA (53% vs 18%)<sup>[17]</sup>.

**Microwave:** Many studies have demonstrated the safety and effectiveness of microwave ablation in the treatment of HCC. Dong *et al.*<sup>[42]</sup> studied 234 patients who underwent microwave ablation, showing 1, 2, 3, 4 and 5 years survival rates of 92.7%, 81.6%, 72.9%, 66.4% and 56.7%. The reported local recurrence rate was 7%<sup>[42]</sup>. A more recent study from Ziemlewicz *et al.*<sup>[43]</sup> of microwave ablation in 107 HCC lesions found an overall survival rate of 76.0% at median 14 mo follow up. The primary effectiveness was 93.7% for tumors 4 cm or smaller and 75.0% for tumors greater than 4 cm<sup>[43]</sup>, with an overall primary effectiveness of 91.6%. This illustrates the ability of microwave to effectively treat larger tumors measuring more than 4 cm in diameter. No major complications or mortality were reported<sup>[43]</sup>. A study of microwave ablation in 182 patients with a single HCC was performed by Sun *et al.*<sup>[44]</sup>. The complete ablation rate was 93%<sup>[44]</sup>. The overall survival rates were 89%, 74% and 60% at 1, 2 and 3 years respectively, while the recurrence-free survival rates were 51%, 36%, 27% at 1, 2 and 3 years respectively. Tumor recurrence was associated with increasing patient age and tumor size. The major complication rate was 2.7%<sup>[44]</sup>.

Microwave ablation also compares favorably to treatment with RFA. A study of 102 patients with HCC found similar complete ablation rates of 94.9% for microwave and 93.1% for RFA<sup>[45]</sup>. The local recurrence rate was better with microwave ablation (11.8%) when compared to RFA (20.9%)<sup>[45]</sup>. A similar study by Shibata *et al.*<sup>[46]</sup> reported complete ablation rates of 89% for microwave ablation compared to 96% for RFA. Overall complication rates were also similar.

**Irreversible electroporation:** There is less data on the efficacy of IRE in comparison with other ablative

techniques because the procedure is relatively new. However, several studies have demonstrated the efficacy of IRE in treating hepatocellular carcinoma. Cheung *et al.*<sup>[47]</sup> evaluated IRE of 18 HCC lesions in 11 patients with a size range of 1.0-6.1 cm and a mean follow up of 18 mo. In tumors measuring less than 3 cm, complete ablation was achieved in 93%, with an overall 73% complete ablation rate. Cannon *et al.*<sup>[48]</sup> reported a primary efficacy of 97% in 14 HCC lesions ranging in size from 1.1-5.0 cm. Thomson *et al.*<sup>[31]</sup> performed IRE in 18 patients with HCC, achieving complete tumor ablation in 15 patients.

### Metastatic disease

**Radiofrequency ablation:** Percutaneous RFA is also increasingly used to treat hepatic metastases, including metastases from colorectal carcinoma (CRCLM), neuroendocrine tumors, and breast cancer<sup>[4]</sup>. The requirements for surgical resection of metastases are similar to HCC and therefore only 10%-20% of patients are surgical candidates at the time of presentation<sup>[49]</sup>. The ideal candidate for RFA has biopsy proven hepatic metastases without underlying liver disease. A study of patients with colorectal metastases who were not surgical candidates and underwent RFA found survival rates of 86%-99%, 46%-68%, and 24%-44% at 1, 3 and 5 years respectively<sup>[9]</sup>. A study by Oshowo *et al.*<sup>[50]</sup> of patients with a solitary CRCLM reported a 3-year survival rate of 52% in patients who underwent RFA vs 55% in patients who underwent surgery. Kim *et al.*<sup>[51]</sup> found similar overall and disease free survival rates in patients who underwent resection vs RFA for a solitary CRCLM < 3 cm. The disease free survival rate was significantly lower in patients with metastases > 3 cm<sup>[51]</sup>. There is additional data supporting the role of RFA as an adjunctive therapy in palliative treatment of CRCLM vs chemotherapy alone. Berber *et al.*<sup>[52]</sup> evaluated RFA in 135 patients with colorectal metastases and found a median survival of 28.9 mo, compared to 11-14 mo in patients who underwent chemotherapy alone.

RFA has also been successfully used in treating hepatic metastases from neuroendocrine tumors. As with HCC and colorectal metastases, only 10% of patients with neuroendocrine metastases are surgical candidates at the time of presentation. Berber *et al.*<sup>[53]</sup> evaluated the role of RFA in treating patients with carcinoid syndrome, as well as other neuroendocrine metastases. Two hundred and thirty-four tumors in 34 patients were treated with RFA<sup>[53]</sup>. Symptoms were improved in 95% of patients with significant or complete symptom control seen in 80% of patients<sup>[53]</sup>. This was compared to a response rate of 90% with surgery and 50%-88% with somatostatin analogues<sup>[53]</sup>. The rate of local progression of disease was 26% during the follow up period (1.6 years) while 41% of patients had no evidence of disease progression during the same period<sup>[53]</sup>. Another study by Elvin *et al.*<sup>[54]</sup> of 109 RFA treatments of neuroendocrine metastases showed a local recurrence rate of 10% during follow up (mean 3.2



years) with CT evidence of successful treatment in 90% of patients.

**Cryoablation:** The data on using cryoablation for metastatic disease is limited compared to the data for RFA since few centers use cryoablation for treating liver lesions. An older study by Kerkar *et al.*<sup>[55]</sup> in 2004 evaluated 56 patients who underwent cryoablation for colorectal metastases. The 3 and 5 years overall survival rates in the colorectal metastases group was 43% and 22% with a median survival of 30 mo<sup>[55]</sup>. A more recent and larger study by Ng *et al.*<sup>[56]</sup> reported the results of cryoablation in 293 patients with unresectable colorectal metastases. 1-, 3-, 5- and 10-year survival rates were 87%, 41.8%, 24.2% and 13.3%<sup>[56]</sup>. Disease-free survival rates were 37.9%, 17.2%, 13.4% and 10.8% at 1, 3, 5 and 10 years<sup>[56]</sup>. "Recurrences" were reported elsewhere in the liver in 73%, at the cryoablation site in 23%, and at the edge of the ablation cavity in 14%<sup>[56]</sup>.

Seifert *et al.*<sup>[57]</sup> reported results of cryoablation in 13 patients with metastatic neuroendocrine tumors. Twelve patients (93%) had complete ablations without reported local progression of disease on follow up imaging. Of additional clinical importance, 7 patients who had preoperative hormone-related symptoms experienced helpful palliative results<sup>[57]</sup>.

Zhang *et al.*<sup>[58]</sup> reported recent results with cryoablation of breast cancer metastases. They performed cryoablation of 39 liver metastases in 17 patients. Tumor response was 92% in the immediate post-op period and 87.1% at 1 mo. Local progression was seen in 6 lesions (15.4%) at 3 mo. The 1 year survival rate was 70.6%.

**Microwave ablation:** One of the first studies to evaluate microwave ablation in the treatment of metastatic disease was by Shibata *et al.*<sup>[59]</sup>. They compared microwave ablation to surgical resection in patients with metastatic colorectal cancer and found similar 1, 2 and 3 years survival rates (71%, 57% and 14% for microwave and 69%, 56% and 23% for resection), and mean survival rates (27 mo for microwave vs 25 mo for resection)<sup>[59]</sup>. A study by Tanaka *et al.*<sup>[60]</sup> also found similar survival and recurrence rates in patients who underwent microwave alone compared to microwave and eventual resection for colorectal metastases. Another study reported identical five-year survival rates (24%) for patients with colorectal metastases treated with microwave ablation vs microwave and surgery<sup>[61]</sup>.

**Irreversible electroporation:** Silk *et al.*<sup>[62]</sup> reported results of IRE in 9 patients with a total of 19 metastatic colorectal cancer lesions ranging from 1.0-4.7 cm. They reported an efficacy of 55% with local tumor recurrence in 5 of 9 patients at 9 mo<sup>[62]</sup>. Thomson *et al.*<sup>[63]</sup> reported a primary efficacy of 67% in a total of 45 metastatic lesions (including colorectal, breast, and neuroendocrine cancers) treated with IRE. Kingham *et al.*<sup>[63]</sup> evaluated IRE of 28 metastatic lesions including metastatic colorectal and neuroendocrine cancers. They reported

a total local failure rate of 7.5% with time to recurrence ranging from 66-230 d.

## ABLATION MODALITY

The choice of ablation modality is important to potential treatment success. While each case is unique and modality choice is often driven by local expertise and operator experience, several general concepts prevail. RFA is very safe and effective in smaller hepatic tumors. However, RFA is less effective with larger tumors and tumors near blood vessels. In contrast, microwave ablation has been shown to be more effective with larger tumor sizes and is affected less by the heat sink effect. Although cryoablation has historically been avoided with hepatic tumors due to concerns about complications, it has been used very safely more recently following the development of smaller probes. Lastly, in limited studies, IRE has been shown to be safe and effective in the treatment of both HCC and metastatic disease especially near sensitive structures such as blood vessels and bile ducts, although continued research is needed to demonstrate long term efficacy.

## CONCLUSION

Percutaneous ablation has become widely accepted as a curative technique in the treatment of HCC and hepatic metastatic disease. Specifically, ablation is useful in the treatment of patients who are not surgical candidates but in whom curative treatment is desired. Percutaneous ablation is safe and effective. Although additional studies are needed, percutaneous ablation continues to evolve as an option in the treatment of HCC and metastatic disease.

## REFERENCES

- 1 Lee DH, Lee JM, Lee JY, Kim SH, Yoon JH, Kim YJ, Han JK, Choi BI. Radiofrequency ablation of hepatocellular carcinoma as first-line treatment: long-term results and prognostic factors in 162 patients with cirrhosis. *Radiology* 2014; **270**: 900-909 [PMID: 24475823 DOI: 10.1148/radiol.13130940]
- 2 Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. *J Hepatol* 2008; **48** Suppl 1: S20-S37 [PMID: 18304676 DOI: 10.1016/j.jhep.2008.01.022]
- 3 Rhim H, Goldberg SN, Dodd GD, Solbiati L, Lim HK, Tonolini M, Cho OK. Essential techniques for successful radio-frequency thermal ablation of malignant hepatic tumors. *Radiographics* 2001; **21** Spec No: S17-35; discussion S36-39 [PMID: 11598245 DOI: 10.1148/radiographics.21.suppl\_1.g01oc11s17]
- 4 McDermott S, Gervais DA. Radiofrequency ablation of liver tumors. *Semin Intervent Radiol* 2013; **30**: 49-55 [PMID: 24436517 DOI: 10.1055/s-0033-1333653]
- 5 Lencioni R, Crocetti L, Cioni D, Della Pina C, Bartolozzi C. Percutaneous radiofrequency ablation of hepatic colorectal metastases: technique, indications, results, and new promises. *Invest Radiol* 2004; **39**: 689-697 [PMID: 15486530]
- 6 Venkatesan AM, Gervais DA, Mueller PR. Percutaneous radiofrequency thermal ablation of primary and metastatic hepatic tumors: current concepts and review of the literature. *Semin Intervent Radiol* 2006; **23**: 73-84 [PMID: 21326722 DOI: 10.1055/s-2006-939843]

- 7 **Goldberg SN**, Gazelle GS, Mueller PR. Thermal ablation therapy for focal malignancy: a unified approach to underlying principles, techniques, and diagnostic imaging guidance. *AJR Am J Roentgenol* 2000; **174**: 323-331 [PMID: 10658699 DOI: 10.2214/ajr.174.2.1740323]
- 8 **Dodd GD**, Frank MS, Aribandi M, Chopra S, Chintapalli KN. Radiofrequency thermal ablation: computer analysis of the size of the thermal injury created by overlapping ablations. *AJR Am J Roentgenol* 2001; **177**: 777-782 [PMID: 11566672 DOI: 10.2214/ajr.177.4.1770777]
- 9 **Lencioni R**, Crocetti L. Radiofrequency ablation of liver cancer. *Tech Vasc Interv Radiol* 2007; **10**: 38-46 [PMID: 17980317 DOI: 10.1053/j.tvir.2007.08.006]
- 10 **Choi D**, Lim HK, Rhim H, Kim YS, Lee WJ, Paik SW, Koh KC, Lee JH, Choi MS, Yoo BC. Percutaneous radiofrequency ablation for early-stage hepatocellular carcinoma as a first-line treatment: long-term results and prognostic factors in a large single-institution series. *Eur Radiol* 2007; **17**: 684-692 [PMID: 17093964 DOI: 10.1007/s00330-006-0461-5]
- 11 **Koda M**, Murawaki Y, Hirooka Y, Kitamoto M, Ono M, Sakaeda H, Joko K, Sato S, Tamaki K, Yamasaki T, Shibata H, Shimoe T, Matsuda T, Toshikuni N, Fujioka S, Ohmoto K, Nakamura S, Kariyama K, Aikata H, Kobayashi Y, Tsutsui A. Complications of radiofrequency ablation for hepatocellular carcinoma in a multicenter study: An analysis of 16346 treated nodules in 13283 patients. *Hepatol Res* 2012; **42**: 1058-1064 [PMID: 22583706 DOI: 10.1111/j.1872-034X.2012.01025.x]
- 12 **Stigliano R**, Marelli L, Yu D, Davies N, Patch D, Burroughs AK. Seeding following percutaneous diagnostic and therapeutic approaches for hepatocellular carcinoma. What is the risk and the outcome? Seeding risk for percutaneous approach of HCC. *Cancer Treat Rev* 2007; **33**: 437-447 [PMID: 17512669 DOI: 10.1016/j.ctrv.2007.04.001]
- 13 **O'Rourke AP**, Haemmerich D, Prakash P, Converse MC, Mahvi DM, Webster JG. Current status of liver tumor ablation devices. *Expert Rev Med Devices* 2007; **4**: 523-537 [PMID: 17605688 DOI: 10.1586/17434440.4.4.523]
- 14 **Walker K**, Lindeque B. The application of cryoprobe therapy in orthopedic oncology. *Orthopedics* 2014; **37**: 536-540 [PMID: 25102496 DOI: 10.3928/01477447-20140728-06]
- 15 **Yu H**, Burke CT. Comparison of percutaneous ablation technologies in the treatment of malignant liver tumors. *Semin Intervent Radiol* 2014; **31**: 129-137 [PMID: 25071303 DOI: 10.1055/s-0034-1373788]
- 16 **Baust JG**, Gage AA. The molecular basis of cryosurgery. *BJU Int* 2005; **95**: 1187-1191 [PMID: 15892798 DOI: 10.1111/j.1464-410X.2005.05502.x]
- 17 **Adam R**, Hagopian EJ, Linhares M, Krissat J, Savier E, Azoulay D, Kunstlinger F, Castaing D, Bismuth H. A comparison of percutaneous cryosurgery and percutaneous radiofrequency for unresectable hepatic malignancies. *Arch Surg* 2002; **137**: 1332-1339; discussion 1340 [PMID: 12470093]
- 18 **Pearson AS**, Izzo F, Fleming RY, Ellis LM, Delrio P, Roh MS, Granchi J, Curley SA. Intraoperative radiofrequency ablation or cryoablation for hepatic malignancies. *Am J Surg* 1999; **178**: 592-599 [PMID: 10670879]
- 19 **Yang Y**, Wang C, Lu Y, Bai W, An L, Qu J, Gao X, Chen Y, Zhou L, Wu Y, Feng Y, Zhang M, Chang X, Lv J. Outcomes of ultrasound-guided percutaneous argon-helium cryoablation of hepatocellular carcinoma. *J Hepatobiliary Pancreat Sci* 2012; **19**: 674-684 [PMID: 22187145 DOI: 10.1007/s00534-011-0490-6]
- 20 **Liang HH**, Chen MS, Peng ZW, Zhang YJ, Zhang YQ, Li JQ, Lau WY. Percutaneous radiofrequency ablation versus repeat hepatectomy for recurrent hepatocellular carcinoma: a retrospective study. *Ann Surg Oncol* 2008; **15**: 3484-3493 [PMID: 18679754 DOI: 10.1245/s10434-008-0076-y]
- 21 **Lubner MG**, Brace CL, Ziemlewicz TJ, Hinshaw JL, Lee FT. Microwave ablation of hepatic malignancy. *Semin Intervent Radiol* 2013; **30**: 56-66 [PMID: 24436518 DOI: 10.1055/s-0033-1333654]
- 22 **Fan W**, Li X, Zhang L, Jiang H, Zhang J. Comparison of microwave ablation and multipolar radiofrequency ablation in vivo using two internally cooled probes. *AJR Am J Roentgenol* 2012; **198**: W46-W50 [PMID: 22194514 DOI: 10.2214/AJR.11.6707]
- 23 **Awad MM**, Devgan L, Kamel IR, Torbensen M, Choti MA. Microwave ablation in a hepatic porcine model: correlation of CT and histopathologic findings. *HPB (Oxford)* 2007; **9**: 357-362 [PMID: 18345319 DOI: 10.1080/13651820701646222]
- 24 **Brace CL**. Microwave ablation technology: what every user should know. *Curr Probl Diagn Radiol* 2009; **38**: 61-67 [PMID: 19179193 DOI: 10.1067/j.cpradiol.2007.08.011]
- 25 **Lahat E**, Eshkenazy R, Zendel A, Zakai BB, Maor M, Dreznik Y, Ariche A. Complications after percutaneous ablation of liver tumors: a systematic review. *Hepatobiliary Surg Nutr* 2014; **3**: 317-323 [PMID: 25392844 DOI: 10.3978/j.issn.2304-3881.2014.09.07]
- 26 **Scheffer HJ**, Nielsen K, de Jong MC, van Tilborg AA, Vieveen JM, Bouwman AR, Meijer S, van Kuijk C, van den Tol PM, Meijerink MR. Irreversible electroporation for nonthermal tumor ablation in the clinical setting: a systematic review of safety and efficacy. *J Vasc Interv Radiol* 2014; **25**: 997-1011; quiz 1011 [PMID: 24656178 DOI: 10.1016/j.jvir.2014.01.028]
- 27 **Knave EM**, Brace CL. Tumor ablation: common modalities and general practices. *Tech Vasc Interv Radiol* 2013; **16**: 192-200 [PMID: 24238374 DOI: 10.1053/j.tvir.2013.08.002]
- 28 **Lee EW**, Thai S, Kee ST. Irreversible electroporation: a novel image-guided cancer therapy. *Gut Liver* 2010; **4** Suppl 1: S99-S104 [PMID: 21103304 DOI: 10.5009/gnl.2010.4.S1.S99]
- 29 **Adeyanju OO**, Al-Angari HM, Sahakian AV. The optimization of needle electrode number and placement for irreversible electroporation of hepatocellular carcinoma. *Radiol Oncol* 2012; **46**: 126-135 [PMID: 23077449 DOI: 10.2478/v10019-012-0026-y]
- 30 **Schmidt CR**, Shires P, Mootoo M. Real-time ultrasound imaging of irreversible electroporation in a porcine liver model adequately characterizes the zone of cellular necrosis. *HPB (Oxford)* 2012; **14**: 98-102 [PMID: 22221570 DOI: 10.1111/j.1477-2574.2011.00409.x]
- 31 **Thomson KR**, Cheung W, Ellis SJ, Federman D, Kavnoudias H, Loader-Oliver D, Roberts S, Evans P, Ball C, Haydon A. Investigation of the safety of irreversible electroporation in humans. *J Vasc Interv Radiol* 2011; **22**: 611-621 [PMID: 21439847 DOI: 10.1016/j.jvir.2010.1012.1014]
- 32 **Tateishi R**, Shiina S, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, Yoshida H, Kawabe T, Omata M. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. *Cancer* 2005; **103**: 1201-1209 [PMID: 15690326 DOI: 10.1002/cncr.20892]
- 33 **Lencioni R**, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, Bartolozzi C. Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. *Radiology* 2005; **234**: 961-967 [PMID: 15665226 DOI: 10.1148/radiol.2343040350]
- 34 **Lu DS**, Yu NC, Raman SS, Limanond P, Lassman C, Murray K, Tong MJ, Amado RG, Busuttil RW. Radiofrequency ablation of hepatocellular carcinoma: treatment success as defined by histologic examination of the explanted liver. *Radiology* 2005; **234**: 954-960 [PMID: 15681691 DOI: 10.1148/radiol.2343040153]
- 35 **Kim YS**, Lim HK, Rhim H, Lee MW, Choi D, Lee WJ, Paik SW, Koh KC, Lee JH, Choi MS, Gwak GY, Yoo BC. Ten-year outcomes of percutaneous radiofrequency ablation as first-line therapy of early hepatocellular carcinoma: analysis of prognostic factors. *J Hepatol* 2013; **58**: 89-97 [PMID: 23023009 DOI: 10.1016/j.jhep.2012.09.020]
- 36 **N'Kontchou G**, Mahamoudi A, Aout M, Ganne-Carrié N, Grando V, Coderc E, Vicaute E, Trinchet JC, Sellier N, Beaugrand M, Seror O. Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognostic factors in 235 Western patients with cirrhosis. *Hepatology* 2009; **50**: 1475-1483 [PMID: 19731239 DOI: 10.1002/hep.23181]
- 37 **Livraghi T**, Goldberg SN, Lazzaroni S, Meloni F, Ierace T, Solbiati L, Gazelle GS. Hepatocellular carcinoma: radio-frequency ablation of medium and large lesions. *Radiology* 2000; **214**: 761-768 [PMID: 10801365 DOI: 10.1148/radiol.214.3.10801365]

- 10715043 DOI: 10.1148/radiology.214.3.r00mr02761]
- 38 **Qi X**, Tang Y, An D, Bai M, Shi X, Wang J, Han G, Fan D. Radio-frequency ablation versus hepatic resection for small hepatocellular carcinoma: a meta-analysis of randomized controlled trials. *J Clin Gastroenterol* 2014; **48**: 450-457 [PMID: 24172183 DOI: 10.1097/MCG.000000000000008]
  - 39 **Miura JT**, Johnston FM, Tsai S, Eastwood D, Banerjee A, Christians KK, Turaga KK, Gamblin TC. Surgical resection versus ablation for hepatocellular carcinoma  $\leq 3$  cm: a population-based analysis. *HPB* (Oxford) 2015; **17**: 896-901 [PMID: 26228076]
  - 40 **Chen HW**, Lai EC, Zhen ZJ, Cui WZ, Liao S, Lau WY. Ultrasound-guided percutaneous cryotherapy of hepatocellular carcinoma. *Int J Surg* 2011; **9**: 188-191 [PMID: 21093616 DOI: 10.1016/j.ijsu.2010.11.008]
  - 41 **Wang C**, Lu Y, Chen Y, Feng Y, An L, Wang X, Su S, Bai W, Zhou L, Yang Y, Xu D. Prognostic factors and recurrence of hepatitis B-related hepatocellular carcinoma after argon-helium cryoablation: a prospective study. *Clin Exp Metastasis* 2009; **26**: 839-848 [PMID: 19784786 DOI: 10.1007/s10585-009-9283-6]
  - 42 **Dong B**, Liang P, Yu X, Su L, Yu D, Cheng Z, Zhang J. Percutaneous sonographically guided microwave coagulation therapy for hepatocellular carcinoma: results in 234 patients. *AJR Am J Roentgenol* 2003; **180**: 1547-1555 [PMID: 12760916 DOI: 10.2214/ajr.180.6.1801547]
  - 43 **Ziemlewicz TJ**, Hinshaw JL, Lubner MG, Brace CL, Alexander ML, Agarwal P, Lee FT. Percutaneous microwave ablation of hepatocellular carcinoma with a gas-cooled system: initial clinical results with 107 tumors. *J Vasc Interv Radiol* 2015; **26**: 62-68 [PMID: 25446425 DOI: 10.1016/j.jvir.2014.09.012]
  - 44 **Sun AX**, Cheng ZL, Wu PP, Sheng YH, Qu XJ, Lu W, Zhao CG, Qian GJ. Clinical outcome of medium-sized hepatocellular carcinoma treated with microwave ablation. *World J Gastroenterol* 2015; **21**: 2997-3004 [PMID: 25780298]
  - 45 **Lu MD**, Xu HX, Xie XY, Yin XY, Chen JW, Kuang M, Xu ZF, Liu GJ, Zheng YL. Percutaneous microwave and radiofrequency ablation for hepatocellular carcinoma: a retrospective comparative study. *J Gastroenterol* 2005; **40**: 1054-1060 [PMID: 16322950 DOI: 10.1007/s00535-005-1671-3]
  - 46 **Shibata T**, Iimuro Y, Yamamoto Y, Maetani Y, Ametani F, Itoh K, Konishi J. Small hepatocellular carcinoma: comparison of radio-frequency ablation and percutaneous microwave coagulation therapy. *Radiology* 2002; **223**: 331-337 [PMID: 11997534 DOI: 10.1148/radiol.2232010775]
  - 47 **Cheung W**, Kavnoudias H, Roberts S, Szkandera B, Kemp W, Thomson KR. Irreversible electroporation for unresectable hepatocellular carcinoma: initial experience and review of safety and outcomes. *Technol Cancer Res Treat* 2013; **12**: 233-241 [PMID: 23369152 DOI: 10.7785/tert.2012.500317]
  - 48 **Cannon R**, Ellis S, Hayes D, Narayanan G, Martin RC. Safety and early efficacy of irreversible electroporation for hepatic tumors in proximity to vital structures. *J Surg Oncol* 2013; **107**: 544-549 [PMID: 23090720 DOI: 10.1002/jso.23280]
  - 49 **Solbiati L**, Livraghi T, Goldberg SN, Ierace T, Meloni F, Dellanoce M, Cova L, Halpern EF, Gazelle GS. Percutaneous radio-frequency ablation of hepatic metastases from colorectal cancer: long-term results in 117 patients. *Radiology* 2001; **221**: 159-166 [PMID: 11568334 DOI: 10.1148/radiol.2211001624]
  - 50 **Oshowo A**, Gillams A, Harrison E, Lees WR, Taylor I. Comparison of resection and radiofrequency ablation for treatment of solitary colorectal liver metastases. *Br J Surg* 2003; **90**: 1240-1243 [PMID: 14515293 DOI: 10.1002/bjs.4264]
  - 51 **Kim KH**, Yoon YS, Yu CS, Kim TW, Kim HJ, Kim PN, Ha HK, Kim JC. Comparative analysis of radiofrequency ablation and surgical resection for colorectal liver metastases. *J Korean Surg Soc* 2011; **81**: 25-34 [PMID: 22066097 DOI: 10.4174/jkss.2011.81.1.25]
  - 52 **Berber E**, Pelley R, Siperstein AE. Predictors of survival after radiofrequency thermal ablation of colorectal cancer metastases to the liver: a prospective study. *J Clin Oncol* 2005; **23**: 1358-1364 [PMID: 15684312 DOI: 10.1200/JCO.2005.12.039]
  - 53 **Berber E**, Flesher N, Siperstein AE. Laparoscopic radiofrequency ablation of neuroendocrine liver metastases. *World J Surg* 2002; **26**: 985-990 [PMID: 12016479 DOI: 10.1007/s00268-002-6629-5]
  - 54 **Elvin A**, Skogseid B, Hellman P. Radiofrequency ablation of neuroendocrine liver metastases. *Abdom Imaging* 2005; **30**: 427-434 [PMID: 15791486 DOI: 10.1007/s00261-004-0257-5]
  - 55 **Kerkar S**, Carlin AM, Sohn RL, Steffes C, Tyburski J, Litttrup P, Weaver D. Long-term follow up and prognostic factors for cryotherapy of malignant liver tumors. *Surgery* 2004; **136**: 770-779 [PMID: 15467661 DOI: 10.1016/j.surg.2004.07.001]
  - 56 **Ng KM**, Chua TC, Saxena A, Zhao J, Chu F, Morris DL. Two decades of experience with hepatic cryotherapy for advanced colorectal metastases. *Ann Surg Oncol* 2012; **19**: 1276-1283 [PMID: 21913018 DOI: 10.1245/s10434-011-2025-4]
  - 57 **Seifert JK**, Cozzi PJ, Morris DL. Cryotherapy for neuroendocrine liver metastases. *Semin Surg Oncol* 1998; **14**: 175-183 [PMID: 9492888 DOI: 10.1002/(sici)1098-2388(199803)14]
  - 58 **Zhang W**, Yu H, Guo Z, Li B, Si T, Yang X, Wang H. Percutaneous cryoablation of liver metastases from breast cancer: initial experience in 17 patients. *Clin Radiol* 2014; **69**: 231-238 [PMID: 24238876 DOI: 10.1016/j.crad.2013.09.014]
  - 59 **Shibata T**, Niinobu T, Ogata N, Takami M. Microwave coagulation therapy for multiple hepatic metastases from colorectal carcinoma. *Cancer* 2000; **89**: 276-284 [PMID: 10918156]
  - 60 **Tanaka K**, Shimada H, Nagano Y, Endo I, Sekido H, Togo S. Outcome after hepatic resection versus combined resection and microwave ablation for multiple bilobar colorectal metastases to the liver. *Surgery* 2006; **139**: 263-273 [PMID: 16455336 DOI: 10.1016/j.surg.2005.07.036]
  - 61 **Morita T**, Shibata T, Okuyama M, Ikeda K, Tsukahara Y, Kitada M, Nishikubo M, Ishida T, Shimano T. [Microwave coagulation therapy for liver metastases from colorectal cancer]. *Gan To Kagaku Ryoho* 2004; **31**: 695-699 [PMID: 15170975]
  - 62 **Silk MT**, Wimmer T, Lee KS, Srimathveeravalli G, Brown KT, Kingham PT, Fong Y, Durack JC, Sofocleous CT, Solomon SB. Percutaneous ablation of peribiliary tumors with irreversible electroporation. *J Vasc Interv Radiol* 2014; **25**: 112-118 [PMID: 24262034 DOI: 10.1016/j.jvir.2013.10.1012]
  - 63 **Kingham TP**, Karkar AM, D'Angelica MI, Allen PJ, Dematteo RP, Getrajdman GI, Sofocleous CT, Solomon SB, Jarnagin WR, Fong Y. Ablation of perivascular hepatic malignant tumors with irreversible electroporation. *J Am Coll Surg* 2012; **215**: 379-387 [PMID: 22704820 DOI: 10.1016/j.jamcollsurg.2012.10.04.1029]

**P-Reviewer:** He JY, Kaya M, Qin JM **S-Editor:** Qi Y  
**L-Editor:** A **E-Editor:** Liu SQ



Observational Study

## Cirrhotic cardiomyopathy: Isn't stress evaluation always required for the diagnosis?

Mara Barbosa, Joana Guardado, Carla Marinho, Bruno Rosa, Isabel Quelhas, António Lourenço, José Cotter

Mara Barbosa, Joana Guardado, Carla Marinho, Bruno Rosa, Isabel Quelhas, António Lourenço, José Cotter, Gastroenterology Department, Centro Hospitalar do Alto Ave, 4835 Guimarães, Portugal

**Author contributions:** All authors had made substantial contributions to the study; Barbosa M, Guardado J, Marinho C, Quelhas I and Cotter J participated in the study concept and design; Barbosa M, Guardado J, Rosa B and Quelhas I were involved in acquisition, analysis and interpretation of the data; Barbosa M, Guardado J and Rosa B performed statistical analysis; Barbosa M and Guardado J drafted the manuscript; Marinho C, Quelhas I, Lourenço A and Cotter J reviewed the manuscript; all authors read and approved the final manuscript.

**Institutional review board statement:** This study was approved by the Institutional Review Board of Centro Hospitalar do Alto Ave, Guimarães, Portugal.

**Informed consent statement:** Written informed consent was obtained from every patient included in the study.

**Conflict-of-interest statement:** Bruno Rosa is a consultant for Given Imagin<sup>®</sup>. Mara Barbosa, Joana Guardado, Carla Marinho, Isabel Quelhas, António Lourenço and José Cotter certify that they have NO conflict-of-interest.

**Data sharing statement:** Technical appendix, statistical code, dataset is available from the corresponding at [maraisabelbarbosa@net.sapo.pt](mailto:maraisabelbarbosa@net.sapo.pt). Consent was not obtained but the presented data are anonymized and risk of identification is low.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Mara Barbosa, MD, Gastroenterology Department, Centro Hospitalar do Alto Ave, Rua dos Cutileiros, Creixomil, 4835 Guimarães,

Portugal. [maraisabelbarbosa@net.sapo.pt](mailto:maraisabelbarbosa@net.sapo.pt)  
Telephone: +351-933-112632  
Fax: +351-253-513592

Received: October 12, 2015  
Peer-review started: October 14, 2015  
First decision: November 11, 2015  
Revised: December 2, 2015  
Accepted: December 17, 2015  
Article in press: December 18, 2015  
Published online: January 28, 2016

### Abstract

**AIM:** To describe the proportion of patients with cirrhotic cardiomyopathy (CCM) evaluated by stress echocardiography and investigating its association with the severity of liver disease.

**METHODS:** A cross-sectional study was conducted. Cirrhotic patients without risk factors for cardiovascular disease were included. Data regarding etiology and severity of liver disease (Child-Pugh score and model for end-stage liver disease), presence of ascites and gastro-esophageal varices, pro-brain natriuretic peptide (pro-BNP) and corrected QT (QTc) interval were collected. Dobutamine stress echocardiography (conventional and tissue Doppler imaging) was performed. CCM was considered present when diastolic and/or systolic dysfunction was diagnosed at rest or after pharmacological stress. Therapy interfering with cardiovascular system was suspended 24 h before the examination.

**RESULTS:** Twenty-six patients were analyzed, 17 (65.4%) Child-Pugh A, mean model for end-stage liver disease (MELD) score of 8.7. The global proportion of patients with CCM was 61.5%. At rest, only 2 (7.7%) patients had diastolic dysfunction and none of the patients had systolic dysfunction. Dobutamine stress echocardiography revealed the presence of diastolic dysfunction in more 6 (23.1%) patients and of systolic



dysfunction in 10 (38.5%) patients. QTc interval prolongation was observed in 68.8% of the patients and increased pro-BNP levels in 31.2% of them. There was no association between the presence of CCM and liver impairment assessed by Child-Pugh score or MELD ( $P = 0.775$ ,  $P = 0.532$ , respectively). Patients with QTc interval prolongation had a significant higher rate of gastroesophageal varices comparing with those without QTc interval prolongation (95.0% *vs* 50.0%,  $P = 0.028$ ).

**CONCLUSION:** CCM is a frequent complication of cirrhosis that is independent of liver impairment. Stress evaluation should always be performed, otherwise it will remain an underdiagnosed condition.

**Key words:** Dobutamine stress echocardiography; Cirrhotic cardiomyopathy; Cirrhosis; Corrected QT interval prolongation; Liver impairment

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Our study demonstrates that cirrhotic cardiomyopathy (CCM) is a frequent condition that is independent of the severity of liver disease. Furthermore, it shows that CCM is currently underdiagnosed, even after a comprehensive evaluation at rest. Consequently, a stress test should always be considered in the diagnostic approach to CCM, as it is here. Moreover, an association between corrected QT (QTc) interval prolongation and the presence of gastroesophageal varices was revealed, irrespective of the diagnosis of CCM. As such, the clinical significance of QTc interval prolongation is emphasized and it can be regarded as a marker of severe liver disease.

Barbosa M, Guardado J, Marinho C, Rosa B, Quelhas I, Lourenço A, Cotter J. Cirrhotic cardiomyopathy: Isn't stress evaluation always required for the diagnosis? *World J Hepatol* 2016; 8(3): 200-206 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i3/200.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i3.200>

## INTRODUCTION

The presence of cardiac dysfunction related to chronic liver disease was first hypothesized by Kowalski *et al*<sup>[1]</sup> in 1953. Subsequent studies revealed that cirrhosis is associated with a hyperdynamic circulation (increased cardiac output and diminished systemic vascular resistance)<sup>[2-6]</sup>, which corresponds to a high-output heart failure under resting conditions<sup>[7,8]</sup>. This clinical entity, formally named cirrhotic cardiomyopathy (CCM), is unrelated to the etiology of cirrhosis<sup>[3-5,8-10]</sup> and is different from alcoholic disease<sup>[9]</sup>. It has been defined as a cardiac dysfunction in patients with cirrhosis characterized by impaired contractile responsiveness to stress and/or altered diastolic relaxation with electrophysiological abnormalities in the absence of other

known cardiac disease<sup>[11]</sup>. Some decades ago, this cardiac dysfunction was attributed to the effects of alcohol toxicity on the heart; however, data from studies performed since 1980s showed that the dysfunction at rest and the blunted cardiac response to stress was associated with cirrhosis *per se* rather than being an adverse effect of alcohol, which justify the specific cardiac disease term - "CCM"<sup>[3,8,12]</sup>.

The pathophysiology of diastolic dysfunction is an increased stiffness of the myocardial wall, most likely because of a combination of mild myocardial hypertrophy, fibrosis and subendotelial edema<sup>[3,9]</sup>. Systolic dysfunction relates to the inability of the heart to maintain an adequate arterial blood pressure and output<sup>[5-7]</sup>. QT interval prolongation is the main electrophysiological abnormality in cirrhosis<sup>[2-8,13,14]</sup>. Several mechanisms have been implicated in the impaired contractile function of the cardiomyocyte: Down-regulation of  $\beta$ -adrenergic receptors and impaired  $\beta$ -adrenergic signalling, altered cardiomyocyte plasma membrane biophysical characteristics, increased activity of the endocannabinoids, nitric oxide and cytokines systems, as well as abnormal myofilaments<sup>[3-5,7-9]</sup>. Though almost always clinically silent at rest, due to diminished preload and afterload that occurs in liver cirrhosis, CCM has been described as a blunted ventricular contractile response usually unmasked by physiological, pharmacological and/or surgical stress<sup>[3-9]</sup>. Consequently, overt congestive heart failure might result<sup>[4,5,9]</sup>. CCM has been linked to sodium and water retention, ascites formation and hepatorenal syndrome development<sup>[7,11,15-17]</sup>, mainly following infections such as spontaneous bacterial peritonitis<sup>[2,3,17-19]</sup>. The prevalence and natural history of CCM is not accurately known and studies that better describe it are still needed<sup>[4,5,8]</sup>. It has also been proposed that this condition has prognosis significance<sup>[5-7]</sup>. There is no specific treatment for CCM, the goal being the management of congestive heart failure<sup>[2-5,7,8]</sup>.  $\beta$ -blockers therapy reduce the prolonged QT interval towards normal values<sup>[20,21]</sup> but its impact on survival is not clear<sup>[12,22]</sup>. Though liver transplantation may initially aggravate the CCM, it remains the ultimate therapy for cardiovascular complications of cirrhosis, being associated with normalization of cardiac function with improvements in cardiac hypertrophy, diastolic and systolic functions and QT interval, several months after transplantation<sup>[5,7,23-25]</sup>.

The aims of our study were: (1) to describe the proportion of patients with CCM evaluated by stress echocardiography in a population of cirrhotic patients, defining blunted ventricular contractile response and/or impaired diastolic relaxation as predictors of CCM; and (2) to investigate whether CCM is related to severity of liver disease.

## MATERIALS AND METHODS

### Study population

A cross-sectional study was conducted during 2011 and 2012. Cirrhotic outpatients followed at our department

were included. The diagnosis of cirrhosis was based on clinical, biochemical, echographic, endoscopic, and, when available, histological criteria. Exclusion criteria were: Age under 18, known or suspected risk factors for cardiovascular disease (diabetes, systemic hypertension, smoking and obesity defined as body mass index  $> 30 \text{ kg/m}^2$ ), pulmonary major illness, severe anemia ( $\text{Hg} < 7 \text{ g/dL}$ ), severe systemic disease, hyperthyroidism and hypothyroidism, pregnancy and baseline electrocardiographic or echocardiographic evidence of structural heart disease, such as bundle branch block, regional wall motion abnormalities or valvular heart disease. Inclusion criteria were as strict as dictated by the definition discussed at the 2005 World Congress of Gastroenterology in Montreal and presented thereafter, so the cardiac dysfunction can be attributed to the CCM *per se*; moreover, they are similar to that described in other reports<sup>[26,27]</sup>. All the therapy interfering with cardiovascular system was suspended 24 h before the electrocardiographic and echocardiographic examinations not to alter the examinations' results. The study protocol was approved by the Ethics Committee of our hospital. Written informed consent was obtained from every patient included in the study.

### **Clinical and analytical data**

Data regarding gender, age, etiology and severity of liver disease (Child-Pugh classification and model for end-stage liver disease (MELD), presence of ascites, gastroesophageal varices, history of overt encephalopathy, heart rate and systolic and diastolic blood pressure were retrospectively recorded. Compensated disease refers to Child-Pugh class A and decompensated disease includes Child-Pugh class B or C patients.

Laboratory parameters - hematological, biochemical [including pro-brain natriuretic peptide (pro-BNP)] and clotting profiles were measured in fasting venous blood samples.

### **Electrocardiographic examination**

Patients were submitted to a 12-lead electrocardiogram and corrected QT interval (QTc interval), adjusted for heart rate, was calculated, according to Bazett's formula [ $\text{QTc} = \text{QTmax}/(\text{RR})^{1/2}$  interval]. A QTc interval  $> 440 \text{ ms}$  was considered prolonged.

### **Echocardiographic examination**

Transthoracic echocardiographic examination (standard 2D-echocardiographic imaging, pulsed Doppler interrogation of mitral inflow and tissue Doppler imaging (TDI) of the annular region of the left ventricle) using a General Electric™ Vivid 7, was performed by an experienced cardiologist in the echocardiography laboratory. Diastolic and systolic functions were evaluated at rest and after pharmacological stress with intravenous infusion of dobutamine. An initial dose of dobutamine at  $5 \mu\text{g/kg}$  per minute was administered and increased to 10, 20, 30 and  $40 \mu\text{g/kg}$  per minute every 3 min, in

order to achieve, at least, 85% of maximum heart rate predicted for age ( $220 - \text{age}$ ). In case maximum heart rate was not reached, atropine (0.25 mg every minute up to maximum dose of 1 mg) was added to the  $40 \mu\text{g/kg}$  per minute dobutamine infusion. After the examination, intravenous metoprolol (2.5 mg every 5 min up to a maximum dose of 10 mg) was administered until basal heart rate was achieved. Off-line analysis of echocardiographic images was performed by two investigators under blind conditions and agreement between the two observers was required.

Echocardiographic systolic left ventricular function parameters [left ventricular end-diastolic volume (LV EDV), LV end-systolic volume (LV ESV) and LV ejection fraction (LV EF) using Simpson's rule] were evaluated at rest and after a dobutamine inotropic dose perfusion ( $20 \mu\text{g/kg}$  per minute), as recommended to assess LV contractile reserve (LV CR). LV systolic dysfunction was considered present at rest when LV EF was below 50%. A reduced left ventricular contractile reserve was defined as an increase in LV EF  $< 10\%$  after dobutamine infusion.

To assess left ventricular diastolic function, peak early filling (E-wave) velocity, late diastolic atrial filling (A-wave) velocity and E-wave deceleration time (DT) were measured by pulsed Doppler examination and E/A ratio (early diastolic/atrial filling ratio) was calculated. Average early diastolic myocardial velocity (e'-septal and lateral sides of the mitral annulus average) was obtained by TDI. E/e' average ratio was calculated combining the parameter E from the pulsed wave Doppler and the parameter e' from the TDI. Left ventricular diastolic dysfunction at rest was diagnosed if septal e' velocity was  $< 8 \text{ cm/s}$  and lateral e' velocity was  $< 10 \text{ cm/s}$ , according to the American Society of Echocardiography recommendations<sup>[28]</sup> (patients whose e' velocities values were within normal limits for age were considered as not having diastolic dysfunction). Three categories of increasing severity of diastolic dysfunction were defined according to the following parameters: Grade I - E/e' average ratio  $\leq 8$ , E/A ratio  $< 0.8$  and DT  $> 200 \text{ ms}$ ; grade II - E/e' average ratio between 9 and 12, E/A between 0.8 and 1.5 and DT between 160 and 200 ms; and grade III - E/e' average ratio  $\geq 13$ , E/A  $\geq 1.5$ . E/e' average ratio at rest and after stress (using dobutamine maximum dose perfusion) has been applied in the diastolic stress test. If myocardial relaxation is normal, E and e' velocities increase proportionally, and the E/e' ratio remains unchanged or is reduced; in patients with impaired myocardial relaxation, the increase in e' with stress is much less than that of mitral E velocity and the E/e' ratio increases.

### **CCM**

CCM was diagnosed when LV diastolic dysfunction and/or systolic dysfunction was present, irrespective of the presence of other supportive criteria [electrophysiological abnormalities as QTc interval prolongation or increased

**Table 1** Baseline characteristics of cirrhotic patients

	<i>n</i> = 26 patients
Gender (male), <i>n</i> (%)	22 (84.6)
Age (yr)	54.6 ± 10.4
Cirrhosis etiology	
Alcoholic, <i>n</i> (%)	20 (77.0)
Viral, <i>n</i> (%)	3 (11.5)
Mixed, <i>n</i> (%)	3 (11.5)
Child-Pugh score (units)	6.2 ± 1.3
Child-Pugh class	
A, <i>n</i> (%)	17 (65.4)
B, <i>n</i> (%)	8 (30.8)
C, <i>n</i> (%)	1 (3.8)
MELD score (units)	8.7 ± 5.3
Medical therapy	
Propranolol, <i>n</i> (%)	15 (57.7)
Diuretics, <i>n</i> (%)	12 (46.1)
Ascites, <i>n</i> (%)	5 (19.2)
Mild/moderate (diuretic responsive) ascites, <i>n</i> (%)	3 (11.5)
Severe (diuretic refractory) ascites, <i>n</i> (%)	2 (7.7)
Gastroesophageal varices, <i>n</i> (%)	22 (84.6)
Decompensation by ascites, <i>n</i> (%)	12 (46.1)
Decompensation by variceal bleeding, <i>n</i> (%)	12 (46.2)
Decompensation by overt encephalopathy, <i>n</i> (%)	3 (11.5)
≥ 2 Decompensations by ascites, <i>n</i> (%)	5 (19.2)
≥ 2 Decompensations by variceal bleeding, <i>n</i> (%)	4 (15.4)
≥ 2 Decompensations by overt encephalopathy, <i>n</i> (%)	2 (7.7)
Heart rate (bpm)	67.8 ± 13.0
Systolic pressure (mmHg)	115.7 ± 11.6
Diastolic pressure (mmHg)	62.1 ± 21.6
Sodium (mEq/L)	140.6 ± 2.7
Creatinine (mg/dL)	0.9 ± 0.2
Albumin (g/dL)	3.6 ± 0.4

MELD: Model for end-stage liver disease.

cardiac biomarkers (pro-BNP)].

### Statistical analysis

Statistical analysis was performed using SPSS 16.0 for Windows (SPSS INC. Chicago, IL, United States). Variables with normal distribution were expressed as mean ± SD and variables with non-normal distribution as median and range. Student's *t*-test, Fisher's Exact Test and Pearson's correlation were used when appropriate. *P* values < 0.05 were considered as significant. The statistical review of the study was performed by the author Mara Barbosa.

## RESULTS

### Clinical and analytical data

Seventy-three cirrhotic patients were evaluated and only 26 fulfilled the inclusion criteria. The main reasons for exclusion were: Diabetes mellitus (51%), hypertension (26%), ischemic cardiac disease (9%) and arrhythmia (6%). Regarding included patients, 22 (85%) were men, with mean age 55 ± 10 years. The etiology of cirrhosis was predominantly alcoholic (77%). The majority of patients (65%) were compensated (Child-Pugh class A). Mean MELD score was 8.7 ± 5.3. Five (19.2%) patients had ascites and the vast majority (84.6%) had gastroesophageal varices. Baseline characteristics of

patients are listed in Table 1. Non selective β-blockers (propranolol) were suspended in 15 patients and diuretics (furosemide and/or spironolactone) in 12 patients.

Pro-BNP levels were 110.8 ± 110.6 μg/mL and were elevated in 8 (30.8%) patients (normal levels < 125 μg/mL). Pro-BNP values were not significantly different between alcoholic and non-alcoholic patients (*P* = 0.757). Pro-BNP levels were similar in Child-Pugh class B/C and Child-Pugh class A patients (*P* = 0.651). There was no correlation between MELD score and pro-BNP values (*P* = 0.950). The presence of ascites, gastroesophageal varices and history of overt encephalopathy was not significantly associated with more elevated pro-BNP levels (*P* = 0.525; *P* = 0.615 and *P* = 0.186, respectively).

### Electrocardiographic characteristics

QTc interval duration was 460 ± 23 ms. Prolongation of the QTc interval was found in 77% of the patients. The existence of a prolonged QTc interval or its duration was unrelated to the etiology of cirrhosis (alcoholic vs non-alcoholic) (*P* = 0.562 and *P* = 0.696, respectively). Regarding severity of disease, there was a significant correlation between QTc interval duration and MELD score (*P* = 0.453, *P* = 0.020) but not between QTc interval duration and Child-Pugh score (*P* = 0.322, *P* = 0.108); QTc interval prolongation was not more frequent in patients with decompensated (Child-Pugh class B/C) vs compensated cirrhotic patients (*P* = 0.380); however, patients with QTc interval prolongation tended to have higher MELD score (9.8 ± 3.7 vs 5.0 ± 8.2, *P* = 0.053). Patients with QTc interval prolongation had a statistically significant higher rate of gastroesophageal varices comparing with those without QTc interval prolongation (95.0% vs 50.0%, *P* = 0.028). However, the presence of QTc prolongation was not associated with the presence of ascites or history of overt encephalopathy (*P* = 0.678 and *P* = 0.438, respectively). Regarding QTc interval duration, there was no relation with ascites, gastroesophageal varices or history of encephalopathy. Although more elevated, pro-BNP levels were not significantly increased in patients with a prolonged QTc interval (*P* = 0.483). Furthermore, there was no correlation between pro-BNP levels and QTc interval duration (*P* = 0.125).

### Echocardiographic characteristics

Echocardiographic examinations were performed as planned, maximum heart rate predicted for age was achieved in all patients and no adverse events were recorded.

**Left ventricular systolic function:** At rest, none of the patients had LV EF below 50% (69.1% ± 8.1%) and LV EDV and LV ESV were within normal limits (94.2 ± 29.7 mL and 28.4 ± 9.5 mL, respectively). A reduced LV CR was observed in 10 (38.5%) patients with LV EF mean increment of 0.4% ± 7.6% vs 20.7% ± 10.4% in the other 16 patients with normal LV CR. Comparing the two groups, at rest, LV EF and LV ESV were similar (73.0% ± 7.1% vs 66.7% ± 7.9%, *P* = 0.051 and 30.1 ± 12.3

**Table 2** The characteristics of patients with and without cirrhotic cardiomyopathy

	CCM ( <i>n</i> = 16)	Non-CCM ( <i>n</i> = 10)	<i>P</i> value
Gender (male), <i>n</i> (%)	14 (87.5%)	8 (80.0%)	0.625
Age (yr)	52.8 ± 9.9	57.4 ± 11.2	0.284
Etiology (alcoholic/non-alcoholic), <i>n</i> (%)	14 (87.5)/2 (12.5)	9 (90.0)/1 (10.0)	0.677
Child-Pugh score (units)	6.3 ± 1.3	6.1 ± 1.2	0.775
Child-Pugh class (A/B + C), <i>n</i> (%)	9 (56.2)/7 (43.8)	8 (80.0)/2 (20.0)	0.399
MELD score (units)	8.1 ± 5.8	9.5 ± 4.6	0.532
Ascites, <i>n</i> (%)	4 (25.0)	1 (10.0)	0.617
Gastroesophageal varices, <i>n</i> (%)	14 (87.5)	8 (80.0)	0.625
History of overt encephalopathy, <i>n</i> (%)	1 (6.3)	2 (20.0)	0.538
Sodium (mEq/L)	140.7 ± 2.9	140.5 ± 2.3	0.865
Creatinine (mg/dL)	0.8 ± 0.2	0.9 ± 0.1	0.343
Albumin (g/dL)	3.6 ± 0.3	3.5 ± 0.4	0.446

CCM: Cirrhotic cardiomyopathy; MELD: Model for end-stage liver disease.

mL vs  $27.3 \pm 7.5$  mL,  $P = 0.466$ , respectively), but LV EDV was significantly increased in the group of reduced LV CR ( $111.4 \pm 32.8$  mL vs  $83.5 \pm 22.4$  mL,  $P = 0.016$ ). After pharmacological stress, LV ESV mean reduction was significantly inferior in the group of reduced LV CR ( $6.1 \pm 12.6$  mL vs  $44.0 \pm 21.8$  mL,  $P = 0.000$ ) and LV EDV mean reduction was similar in the two groups ( $0.53 \pm 11.6$  mL vs  $5.2 \pm 18.5$  mL,  $P = 0.481$ ).

**Left ventricular diastolic function:** At rest, 2 patients were diagnosed with diastolic dysfunction (grade I). In 8 (30.8%) patients, the E/e' average ratio increased from  $6.9 \pm 2.0$  to  $9.1 \pm 2.7$ ; in the others, the E/e' reduced from  $8.9 \pm 1.9$  to  $6.8 \pm 2.3$ .

### CCM

Sixteen (61.5%) patients were diagnosed with CCM: 10 patients had systolic dysfunction, 8 patients had diastolic dysfunction and 2 presented with both cardiac systolic and diastolic dysfunction. Among those patients with CCM, QTc interval prolongation was observed in 11 (68.8%) patients and increased pro-BNP levels were measured in 5 (31.2%) patients. QTc prolongation and elevation of pro-BNP were simultaneously present in 5 (31.2%) cases and none of the alterations was observed in 5 (31.2%) patients. The characteristics of patients with and without CCM are listed in Table 2. Of note, the presence of CCM was unrelated to the etiology and severity of cirrhosis and presence of ascites, gastroesophageal varices and history of overt encephalopathy. Moreover, sodium, creatinine and albumin values were similar between patients with and without CCM.

## DISCUSSION

In order to describe CCM prevalence, natural history and prognosis accurately, an effort to define diagnostic criteria has been made. However, universal consensus is still lacking and important points remain to be elucidated, such as: The minimum number of criteria required to make the diagnosis, the need to always

performing a stress test to unmask CCM and the most adequate stress test to use, as the disease is usually latent and revealed by stress. Moreover, recent studies have used tissue Doppler parameters to diagnose CCM<sup>[28,29]</sup>, as they are more sensitive and less dependent on loading conditions<sup>[30,31]</sup>, comparing with mitral inflow velocity variables. Consequently, there is a considerable heterogeneity in the results published in the literature.

Diastolic dysfunction relates to impaired myocardial relaxation<sup>[28]</sup> and elevated left ventricular filling pressures is the main physiological consequence of it<sup>[32]</sup>. During stress, left ventricular filling pressures change minimally in healthy subjects. However, if cardiac dysfunction is present, a rise in filling pressures is observed in order to maintain left ventricular filling and stroke volume<sup>[28]</sup>. The E/e' ratio was shown to relate significantly to left ventricular filling pressures during stress<sup>[33]</sup>. Diastolic dysfunction was present in only 2 cases at rest and in 8 after stress. In fact, stress dobutamine echocardiography could identify patients with diastolic dysfunction (average E/e' ratio increase) not recognized at rest. The observation of an impaired myocardial relaxation during stress provides a possible explanation for the frequent development of cardiovascular complications (such as pulmonary edema) after transjugular intrahepatic portosystemic shunt (TIPS) insertion and liver transplantation, as these interventions promote a sudden increase in the preload and, consequently, a rise in left ventricular filling pressures.

None of the patients was diagnosed with systolic dysfunction at rest, which is consistent with the data reported in the literature, when LV EF is used as diagnostic criteria. The pharmacological stimuli revealed the existence of a systolic dysfunction in a considerable number of patients (38.5%). Due to the hyperdynamic state, with central hypovolemia and diminished preload and afterload, it remains an underdiagnosed condition even after a careful echocardiographic evaluation at rest.

The proportion of patients with CCM in our population was 61.5%. A significant number of patients (68.8%) had concomitant QTc interval prolongation and a smaller fraction (31.2%) had increased levels of pro-BNP.



CCM was independent of the etiology of cirrhosis, as has already been described in previous studies<sup>[3-5,8-10]</sup>. Controversy exists regarding the relation between the CCM and the severity of the disease. Some studies suggest that CCM can be more severe in decompensated liver disease while others report CCM is not directly related to disease severity<sup>[7,26,29,33,34]</sup>. In our study, we did not find any relation between CCM and Child-Pugh classification or MELD. Furthermore, clinical markers of higher liver impairment (ascites, gastroesophageal varices and history of overt encephalopathy) did not predict the presence of CCM.

QTc interval prolongation was a very common finding in this population of cirrhotic patients. This result is in agreement with the data reported in the literature. It is already established that QTc interval prolongation is significantly related to the severity of the underlying liver disease<sup>[13,35,36]</sup>. In our study, QTc interval duration was positively correlated with the degree of liver dysfunction assessed by MELD score, but not by Child-Pugh classification, probably because of small sample size. Interestingly, patients who were diagnosed with QTc interval prolongation had more commonly gastroesophageal varices, the last being an established surrogate of more severe liver disease and increased risk. However, this could not be demonstrated in patients with ascites. Recently, Trevisani *et al.*<sup>[37]</sup> reported further QTc interval prolongation in the setting of acute gastrointestinal bleeding in cirrhotic patients. Although the clinical significance of QTc interval prolongation is not completely clarified<sup>[2,4-7,13,22]</sup>, it may increase the risk of cardiac events and be associated with a poorer survival<sup>[13]</sup>. Therefore, close monitoring during stressful events is advised<sup>[6]</sup>.

In summary, our study demonstrated that CCM is a frequent condition that is independent of the severity of liver disease. Furthermore, it showed that CCM is currently underdiagnosed, even after a comprehensive evaluation at rest. Consequently, a stress test should always be considered in the diagnostic approach to CCM, as it is highlighted in the current study. Moreover, an association between QTc interval prolongation and the presence of gastroesophageal varices was revealed, irrespective of the diagnosis of CCM. As such, the clinical significance of QTc interval prolongation is emphasized and it can be regarded as a marker of severe liver disease. A limitation of our study is its small sample size.

Hepatologists should be aware of this silent entity and actively search for it because it is of major importance in the management of the cirrhotic patient as it contributes to the high cardiovascular morbidity and mortality related to TIPS insertion and liver transplantation. It remains of the utmost importance to better define CCM diagnostic criteria, to suggest specific stress test protocols and to update echocardiographic criteria for the diagnosis, probably including TDI parameters which have already been used in several studies besides ours, in order to achieve more reproducible results.

Also, the performance of strain evaluation by speckle tracking analysis, a new sophisticated echocardiographic technique, might be a promising method to diagnose CCM in patients with advanced liver disease as it can detect subtle systo-diastolic dysfunction before left ventricular ejection fraction becomes impaired<sup>[12,29]</sup>. Data regarding the impact of CCM in the natural history of cirrhosis is also needed.

## COMMENTS

### Background

Cirrhotic cardiomyopathy (CCM) relates to a cardiac dysfunction in patients with cirrhosis characterized by impaired contractile responsiveness to stress and/or altered diastolic relaxation with electrophysiological abnormalities in the absence of other known cardiac disease. It is independent of the etiology of cirrhosis and is different from alcoholic disease. Although almost always clinically silent at rest, CCM is usually unmasked by physiological, pharmacological and/or surgical stress, such as transjugular intrahepatic portosystemic shunt or liver transplant. In this study, the authors aimed at describing the proportion of patients with CCM evaluated by stress echocardiography in a population of cirrhotic patients, and at investigating whether CCM is related to severity of liver disease.

### Research frontiers

Very few prior reports address the question of diagnostic evaluation of CCM in cirrhotic patients using accurate criteria and stress testing. The results of the authors' study contribute to the diagnostic approach to CCM in these patients.

### Innovations and breakthroughs

This study demonstrated that CCM is a frequent condition that is independent of the severity of liver disease. Also, it revealed that CCM is currently underdiagnosed at rest, even after a comprehensive electrocardiographic and echocardiographic evaluation. A substantial number of patients were diagnosed as having CCM only after the stress echocardiographic evaluation. Furthermore, an association between corrected QT (QTc) interval prolongation and the presence of gastroesophageal varices was revealed, irrespective of the presence of CCM. As such, QTc interval prolongation can be regarded as a surrogate of severe liver disease.

### Applications

This study suggests that a stress test should always be considered in the diagnostic approach to CCM, otherwise it will remain an underdiagnosed entity.

### Terminology

This study demonstrated that stress echocardiography was useful at revealing CCM. As such, it can identify patients at risk of cardiac decompensation and can be used as diagnostic tool of CCM in cirrhotic patients in clinical practice.

### Peer-review

This is an interesting manuscript, and especially interesting for the general gastroenterologists, hepatologists, cardiologists and the internist.

## REFERENCES

- 1 Kowalski HJ, Abelman WH. The cardiac output at rest in Laennec's cirrhosis. *J Clin Invest* 1953; **32**: 1025-1033 [PMID: 13096569]
- 2 Al Hamoudi W, Lee SS. Cirrhotic cardiomyopathy. *Ann Hepatol* 2006; **5**: 132-139 [PMID: 17060868]
- 3 Lee RF, Glenn TK, Lee SS. Cardiac dysfunction in cirrhosis. *Best Pract Res Clin Gastroenterol* 2007; **21**: 125-140 [PMID: 17223501]
- 4 Baik SK, Fouad TR, Lee SS. Cirrhotic cardiomyopathy. *Orphanet J Rare Dis* 2007; **2**: 15 [PMID: 17389039]

- 5 **Møller S**, Henriksen JH. Cirrhotic cardiomyopathy. *J Hepatol* 2010; **53**: 179-190 [PMID: 20462649 DOI: 10.1016/j.jhep.2010.02.023]
- 6 **Møller S**, Bernardi M. Interactions of the heart and the liver. *Eur Heart J* 2013; **34**: 2804-2811 [PMID: 23853073 DOI: 10.1093/eurheartj/ehd246]
- 7 **Møller S**, Hove JD, Dixel U, Bendtsen F. New insights into cirrhotic cardiomyopathy. *Int J Cardiol* 2013; **167**: 1101-1108 [PMID: 23041091 DOI: 10.1016/j.ijcard.2012.09.089]
- 8 **Zardi EM**, Abbate A, Zardi DM, Dobrina A, Margiotta D, Van Tassell BW, Afeltra A, Sanyal AJ. Cirrhotic cardiomyopathy. *J Am Coll Cardiol* 2010; **56**: 539-549 [PMID: 20688208 DOI: 10.1016/j.jacc.2009]
- 9 **Ma Z**, Lee SS. Cirrhotic cardiomyopathy: getting to the heart of the matter. *Hepatology* 1996; **24**: 451-459 [PMID: 8690419]
- 10 **Pozzi M**, Carugo S, Boari G, Pecci V, de Ceglia S, Maggiolini S, Bolla GB, Roffi L, Failla M, Grassi G, Giannattasio C, Mancina G. Evidence of functional and structural cardiac abnormalities in cirrhotic patients with and without ascites. *Hepatology* 1997; **26**: 1131-1137 [PMID: 9362352]
- 11 **Møller S**, Henriksen JH. Cardiovascular complications of cirrhosis. *Gut* 2008; **57**: 268-278 [PMID: 18192456 DOI: 10.1136/gut.2006]
- 12 **Ruiz-Del-Arbol L**, Serradilla R. Cirrhotic cardiomyopathy. *World J Gastroenterol* 2015; **21**: 11502-11521 [PMID: 26556983 DOI: 10.3748/wjg.v21.i41.11502]
- 13 **Bernardi M**, Calandra S, Colantoni A, Trevisani F, Raimondo ML, Sica G, Schepis F, Mandini M, Simoni P, Contin M, Raimondo G. Q-T interval prolongation in cirrhosis: prevalence, relationship with severity, and etiology of the disease and possible pathogenetic factors. *Hepatology* 1998; **27**: 28-34 [PMID: 9425913]
- 14 **Zambruni A**, Trevisani F, Caraceni P, Bernardi M. Cardiac electrophysiological abnormalities in patients with cirrhosis. *J Hepatol* 2006; **44**: 994-1002 [PMID: 16510203]
- 15 **Ruiz-del-Arbol L**, Monescillo A, Arocena C, Valer P, Ginès P, Moreira V, Milicua JM, Jiménez W, Arroyo V. Circulatory function and hepatorenal syndrome in cirrhosis. *Hepatology* 2005; **42**: 439-447 [PMID: 15977202]
- 16 **Gaskari SA**, Honar H, Lee SS. Therapy insight: Cirrhotic cardiomyopathy. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 329-337 [PMID: 16741552]
- 17 **Krag A**, Bendtsen F, Burroughs AK, Møller S. The cardiorenal link in advanced cirrhosis. *Med Hypotheses* 2012; **79**: 53-55 [PMID: 22537409 DOI: 10.1016/j.mehy.2012.03.032]
- 18 **Lee SS**. Cardiac dysfunction in spontaneous bacterial peritonitis: a manifestation of cirrhotic cardiomyopathy? *Hepatology* 2003; **38**: 1089-1091 [PMID: 14578846]
- 19 **Ruiz-del-Arbol L**, Urman J, Fernández J, González M, Navasa M, Monescillo A, Albillos A, Jiménez W, Arroyo V. Systemic, renal, and hepatic hemodynamic derangement in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 2003; **38**: 1210-1218 [PMID: 14578859]
- 20 **Henriksen JH**, Bendtsen F, Hansen EF, Møller S. Acute non-selective beta-adrenergic blockade reduces prolonged frequency-adjusted Q-T interval (QTc) in patients with cirrhosis. *J Hepatol* 2004; **40**: 239-246 [PMID: 14739094]
- 21 **Zambruni A**, Trevisani F, Di Micoli A, Savelli F, Berzigotti A, Bracci E, Caraceni P, Domenicali M, Feline P, Zoli M, Bernardi M. Effect of chronic beta-blockade on QT interval in patients with liver cirrhosis. *J Hepatol* 2008; **48**: 415-421 [PMID: 18194821 DOI: 10.1016/j.jhep.2007.11.012]
- 22 **Bal JS**, Thuluvath PJ. Prolongation of QTc interval: relationship with etiology and severity of liver disease, mortality and liver transplantation. *Liver Int* 2003; **23**: 243-248 [PMID: 12895263]
- 23 **Torregrosa M**, Aguadé S, Dos L, Segura R, González A, Evangelista A, Castell J, Margarit C, Esteban R, Guardia J, Genescà J. Cardiac alterations in cirrhosis: reversibility after liver transplantation. *J Hepatol* 2005; **42**: 68-74 [PMID: 15629509]
- 24 **Liu H**, Lee SS. What happens to cirrhotic cardiomyopathy after liver transplantation? *Hepatology* 2005; **42**: 1203-1205 [PMID: 16250041]
- 25 **Adigun AQ**, Pinto AG, Flockhart DA, Gorski JC, Li L, Hall SD, Chalasani N. Effect of cirrhosis and liver transplantation on the gender difference in QT interval. *Am J Cardiol* 2005; **95**: 691-694 [PMID: 15721125]
- 26 **Karagiannakis DS**, Vlachogiannakos J, Anastasiadis G, Vafiadis-Zouboulis I, Ladas SD. Frequency and severity of cirrhotic cardiomyopathy and its possible relationship with bacterial endotoxemia. *Dig Dis Sci* 2013; **58**: 3029-3036 [PMID: 23907333 DOI: 10.1007/s10620-013-2693-y]
- 27 **Alexopoulou A**, Papatheodoridis G, Pouriki S, Chrysoshoou C, Raftopoulos L, Stefanadis C, Pectasides D. Diastolic myocardial dysfunction does not affect survival in patients with cirrhosis. *Transpl Int* 2012; **25**: 1174-1181 [PMID: 22909305 DOI: 10.1111/j.1432-2277.2012.01547.x]
- 28 **Nagueh SF**, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, Waggoner AD, Flachskampf FA, Pellicka PA, Evangelista A. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *J Am Soc Echocardiogr* 2009; **22**: 107-133 [PMID: 19187853 DOI: 10.1016/j.echo.2008]
- 29 **Merli M**, Calicchia A, Ruffa A, Pellicori P, Riggio O, Giusto M, Gaudio C, Torromeo C. Cardiac dysfunction in cirrhosis is not associated with the severity of liver disease. *Eur J Intern Med* 2013; **24**: 172-176 [PMID: 22958907 DOI: 10.1016/j.ejim.2012.08.007]
- 30 **Kazankov K**, Holland-Fischer P, Andersen NH, Torp P, Sloth E, Aagaard NK, Vilstrup H. Resting myocardial dysfunction in cirrhosis quantified by tissue Doppler imaging. *Liver Int* 2011; **31**: 534-540 [PMID: 21382164 DOI: 10.1111/j.1478-3231.2011.02468.x]
- 31 **Mahadevan G**, Dwivedi G, Williams L, Steeds RP, Frenneaux M. Epidemiology and diagnosis of heart failure with preserved left ventricular ejection fraction: rationale and design of the study. *Eur J Heart Fail* 2012; **14**: 106-112 [PMID: 22120964 DOI: 10.1093/eurjhf/hfr153]
- 32 **Brutsaert DL**, Sys SU, Gillebert TC. Diastolic failure: pathophysiology and therapeutic implications. *J Am Coll Cardiol* 1993; **22**: 318-325 [PMID: 8509558]
- 33 **Burgess MI**, Jenkins C, Sharman JE, Marwick TH. Diastolic stress echocardiography: hemodynamic validation and clinical significance of estimation of ventricular filling pressure with exercise. *J Am Coll Cardiol* 2006; **47**: 1891-1900 [PMID: 16682317]
- 34 **Enache I**, Oswald-Mammoser M, Woehl-Jaegle ML, Habersetzer F, Di Marco P, Charloux A, Doutreleau S. Cirrhotic cardiomyopathy and hepatopulmonary syndrome: prevalence and prognosis in a series of patients. *Respir Med* 2013; **107**: 1030-1036 [PMID: 23615223 DOI: 10.1016/j.rmed.2013.03.010]
- 35 **Kempler P**, Szalay F, Váradi A, Keresztes K, Kádár E, Tanczos E, Petrik J. Prolongation of the QTc-interval reflects the severity of autonomic neuropathy in primary biliary cirrhosis and in other non-alcoholic liver diseases. *Z Gastroenterol* 1993; **31** Suppl 2: 96-98 [PMID: 7483730]
- 36 **Trevisani F**, Merli M, Savelli F, Valeriano V, Zambruni A, Riggio O, Caraceni P, Domenicali M, Bernardi M. QT interval in patients with non-cirrhotic portal hypertension and in cirrhotic patients treated with transjugular intrahepatic porto-systemic shunt. *J Hepatol* 2003; **38**: 461-467 [PMID: 12663238]
- 37 **Trevisani F**, Di Micoli A, Zambruni A, Biselli M, Santi V, Erroi V, Lenzi B, Caraceni P, Domenicali M, Cavazza M, Bernardi M. QT interval prolongation by acute gastrointestinal bleeding in patients with cirrhosis. *Liver Int* 2012; **32**: 1510-1515 [PMID: 22776742 DOI: 10.1111/j.1478-3231.2012.02847.x]

P- Reviewer: Baffy G, Hoff DAL, La Mura V, Maruyama H

S- Editor: Ji FF L- Editor: A E- Editor: Liu SQ





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

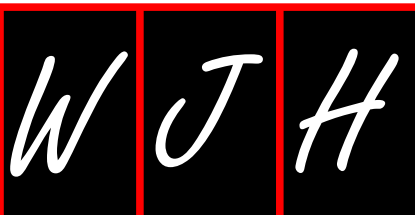


# World Journal of *Hepatology*

*World J Hepatol* 2016 February 8; 8(4): 207-264







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 469 members, representing a team of worldwide experts in hepatology. They are from 53 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (1), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (98), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (19), Greece (11), Hungary (5), India (15), Indonesia (2), Iran (4), Israel (1), Italy (52), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (11), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (17), and United States (56).

### EDITORS-IN-CHIEF

Clara Balsano, Rome  
Wan-Long Chuang, Kaohsiung

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, Kaohsiung  
Tai-An Chiang, Tainan  
Chi-Tan Hu, Hualien  
Sen-Yung Hsieh, Taoyuan  
Wenya Huang, Tainan  
Liang-Yi Hung, Tainan  
Jih RU Hwu, Hsinchu  
Jing-Yi Lee, Taipei  
Mei-Hsuan Lee, Taipei  
Chih-Wen Lin, Kaohsiung  
Chun-Che Lin, Taichung  
Wan-Yu Lin, Taichung  
Tai-Long Pan, Tao-Yuan  
Suh-Ching Yang, Taipei  
Chun-Yan Yeung, Taipei

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, Batna



**Argentina**

Fernando O Bessone, Rosario  
Maria C Carrillo, Rosario  
Melisa M Dirchwolf, Buenos Aires  
Bernardo Frider, Buenos Aires

Jorge Quarleri, Buenos Aires  
Adriana M Torres, Rosario



**Armenia**

Narina Sargsyants, Yerevan



**Australia**

Mark D Gorrell, Sydney



**Austria**

Harald Hofer, Vienna  
Gustav Paumgartner, Vienna  
Matthias Pinter, Vienna  
Thomas Reiberger, Vienna



**Bangladesh**

Shahinul Alam, Dhaka  
Mamun Al Mahtab, Dhaka



**Belgium**

Nicolas Lanthier, Brussels  
Philip Meuleman, Ghent  
Luisa Vonghia, Antwerp



**Botswana**

Francesca Cainelli, Gaborone

Sandro Vento, Gaborone



**Brazil**

Edson Abdala, Sao Paulo  
Ilka FSF Boin, Campinas  
Niels OS Camara, Sao Paulo  
Ana Carolina FN Cardoso, Rio de Janeiro  
Roberto J Carvalho-Filho, Sao Paulo  
Julio CU Coelho, Curitiba  
Flavio Henrique Ferreira Galvao, São Paulo  
Janaina L Narciso-Schiavon, Florianopolis  
Sílvia HC Sales-Peres, Bauru  
Leonardo L Schiavon, Florianópolis  
Luciana D Silva, Belo Horizonte  
Vanessa Souza-Mello, Rio de Janeiro  
Jaques Waisberg, Santo André



**Bulgaria**

Mariana P Penkova-Radicheva, Stara Zagora  
Marieta Simonova, Sofia



**Canada**

Runjan Chetty, Toronto  
Michele Molinari, Halifax  
Giada Sebastiani, Montreal



**Chile**

Luis A Videla, Santiago



## China

Guang-Wen Cao, Shanghai  
 En-Qiang Chen, Chengdu  
 Gong-Ying Chen, Hangzhou  
 Jin-lian Chen, Shanghai  
 Jun Chen, Changsha  
 Alfred Cheng, Hong Kong  
 Chun-Ping Cui, Beijing  
 Shuang-Suo Dang, Xi'an  
 Ming-Xing Ding, Jinhua  
 Zhi-Jun Duang, Dalian  
 He-Bin Fan, Wuhan  
 Xiao-Ming Fan, Shanghai  
 James Yan Yue Fung, Hong Kong  
 Yi Gao, Guangzhou  
 Zuo-Jiong Gong, Wuhan  
 Zhi-Yong Guo, Guangzhou  
 Shao-Liang Han, Wenzhou  
 Tao Han, Tianjin  
 Jin-Yang He, Guangzhou  
 Ming-Liang He, Hong Kong  
 Can-Hua Huang, Chengdu  
 Bo Jin, Beijing  
 Shan Jin, Hohhot  
 Hui-Qing Jiang, Shijiazhuang  
 Wan-Yee Joseph Lau, Hong Kong  
 Guo-Lin Li, Changsha  
 Jin-Jun Li, Shanghai  
 Qiang Li, Jinan  
 Sheng Li, Jinan  
 Zong-Fang Li, Xi'an  
 Xu Li, Guangzhou  
 Xue-Song Liang, Shanghai  
 En-Qi Liu, Xi'an  
 Pei Liu, Shenyang  
 Zhong-Hui Liu, Changchun  
 Guang-Hua Luo, Changzhou  
 Yi Lv, Xi'an  
 Guang-Dong Pan, Liuzhou  
 Wen-Sheng Pan, Hangzhou  
 Jian-Min Qin, Shanghai  
 Wai-Kay Seto, Hong Kong  
 Hong Shen, Changsha  
 Xiao Su, Shanghai  
 Li-Ping Sun, Beijing  
 Wei-Hao Sun, Nanjing  
 Xue-Ying Sun, Harbin  
 Hua Tang, Tianjin  
 Ling Tian, Shanghai  
 Eric Tse, Hong Kong  
 Guo-Ying Wang, Changzhou  
 Yue Wang, Beijing  
 Shu-Qiang Wang, Chengdu  
 Mary MY Wayne, Hong Kong  
 Hong-Shan Wei, Beijing  
 Danny Ka-Ho Wong, Hong Kong  
 Grace Lai-Hung Wong, Hong Kong  
 Bang-Fu Wu, Dongguan  
 Feng Wu, Chongqing  
 Xiong-Zhi Wu, Tianjin  
 Chun-Fang Xu, Suzhou  
 Rui-An Xu, Quanzhou  
 Rui-Yun Xu, Guangzhou  
 Wei-Li Xu, Shijiazhuang  
 Shi-Ying Xuan, Qingdao  
 Ming-Xian Yan, Jinan  
 Lv-Nan Yan, Chengdu  
 Jin Yang, Hangzhou  
 Ji-Hong Yao, Dalian  
 Winnie Yeo, Hong Kong

Zheng Zeng, Beijing  
 Qi Zhang, Hangzhou  
 Shi-Jun Zhang, Guangzhou  
 Xiao-Lan Zhang, Shijiazhuang  
 Xiao-Yong Zhang, Guangzhou  
 Xin-Chen Zhang, Harbin  
 Yong Zhang, Xi'an  
 Hong-Chuan Zhao, Hefei  
 Ming-Hua Zheng, Wenzhou  
 Yu-Bao Zheng, Guangzhou  
 Ren-Qian Zhong, Shanghai  
 Fan Zhu, Wuhan  
 Xiao Zhu, Dongguan



## Czech Republic

Kamil Vyslouzil, Olomouc



## Denmark

Henning Gronbaek, Aarhus  
 Christian Mortensen, Hvidovre



## Egypt

Ihab T Abdel-Raheem, Damanhour  
 NGB G Bader EL Din, Cairo  
 Hatem Elalfy, Mansoura  
 Mahmoud M El-Bendary, Mansoura  
 Mona El SH El-Raziky, Cairo  
 Mohammad El-Sayed, Cairo  
 Yasser M Fouad, Minia  
 Mohamed AA Metwally, Benha  
 Hany Shehab, Cairo  
 Mostafa M Sira, Shebin El-koom  
 Ashraf Taye, Minia  
 MA Ali Wahab, Mansoura



## France

Laurent Alric, Toulouse  
 Sophie Conchon, Nantes  
 Daniel J Felmlee, Strasbourg  
 Herve Lerat, Creteil  
 Dominique Salmon, Paris  
 Jean-Pierre Vartanian, Paris



## Germany

Laura E Buitrago-Molina, Hannover  
 Enrico N De Toni, Munich  
 Oliver Ebert, Muenchen  
 Rolf Gebhardt, Leipzig  
 Janine V Hartl, Regensburg  
 Sebastian Hinz, Kiel  
 Benjamin Juntermanns, Essen  
 Roland Kaufmann, Jena  
 Viola Knop, Frankfurt  
 Veronika Lukacs-Kornek, Homburg  
 Benjamin Maasoumy, Hannover  
 Jochen Mattner, Erlangen  
 Nadja M Meindl-Beinker, Mannheim  
 Ulf P Neumann, Aachen  
 Margarete Odenthal, Cologne  
 Yoshiaki Sunami, Munich

Christoph Roderburg, Aachen  
 Frank Tacke, Aachen  
 Yuchen Xia, Munich



## Greece

Alex P Betrosian, Athens  
 George N Dalekos, Larissa  
 Ioanna K Delladetsima, Athens  
 Nikolaos K Gatselis, Larissa  
 Stavros Gourgiotis, Athens  
 Christos G Savopoulos, Thessaloniki  
 Tania Siahaniidou, Athens  
 Emmanouil Sinakos, Thessaloniki  
 Nikolaos G Symeonidi, Thessaloniki  
 Konstantinos C Thomopoulos, Larissa  
 Konstantinos Tziomalos, Thessaloniki



## Hungary

Gabor Banhegyi, Budapest  
 Peter L Lakatos, Budapest  
 Maria Papp, Debrecen  
 Ferenc Sipos, Budapest  
 Zsolt J Tulassay, Budapest



## India

Deepak N Amarapurkar, Mumbai  
 Girish M Bhopale, Pune  
 Sibnarayan Datta, Tezpur  
 Nutan D Desai, Mumbai  
 Sorabh Kapoor, Mumbai  
 Jaswinder S Maras, New Delhi  
 Nabeen C Nayak, New Delhi  
 C Ganesh Pai, Manipal  
 Amit Pal, Chandigarh  
 K Rajeshwari, New Delhi  
 Anup Ramachandran, Vellore  
 D Nageshwar Reddy, Hyderabad  
 Shivaram P Singh, Cuttack  
 Ajith TA, Thrissur  
 Balasubramaniyan Vairappan, Pondicherry



## Indonesia

Cosmas RA Lesmana, Jakarta  
 Neneng Ratnasari, Yogyakarta



## Iran

Seyed M Jazayeri, Tehran  
 Sedigheh Kafi-Abad, Tehran  
 Iradj Maleki, Sari  
 Fakhraddin Naghibalhossaini, Shiraz



## Israel

Stephen DH Malnick, Rehovot



## Italy

Francesco Angelico, Rome

Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Andrea Galli, *Florence*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Salvatore Gruttadauria, *Palermo*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*



**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*  
 Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*

Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhito Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*



**Jordan**

Kamal E Bani-Hani, *Zarqa*



**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*



**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*



**Moldova**

Angela Peltec, *Chishinev*



**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*



**Nigeria**

CA Asabamaka Onyekwere, *Lagos*



**Pakistan**

Bikha Ram Devrajani, *Jamshoro*



**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*



**Poland**

Jacek Zielinski, *Gdansk*



**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*



**Qatar**

Reem Al Olaby, *Doha*



**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*



**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*



**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*



**Singapore**

Ser Yee Lee, *Singapore*



**South Korea**

Young-Hwa Chung, *Seoul*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonju*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*



**Spain**

Ivan G Marina, *Madrid*  
 Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*

José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodríguez-Frias, *Córdoba*  
 Manuel L Rodríguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Traperó-Marugán, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpaboon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*  
 Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*

Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



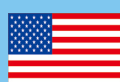
#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*



#### **United States**

Naim Alkhouri, *Cleveland*  
 Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*

Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Grigoriy E Gurvits, *New York*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Kusum K Kharbanda, *Omaha*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*





## Contents

Three issues per month Volume 8 Number 4 February 8, 2016

### EDITORIAL

- 207 Inflammasome activation in decompensated liver cirrhosis  
*González-Navajas JM*

### ORIGINAL ARTICLE

#### Basic Study

- 211 Lack of hepcidin expression attenuates steatosis and causes fibrosis in the liver  
*Lu S, Bennett RG, Kharbanda KK, Harrison-Findik DD*

#### Retrospective Study

- 226 Total hepatectomy and liver transplantation as a two-stage procedure for fulminant hepatic failure: A safe procedure in exceptional circumstances  
*Sanabria Mateos R, Hogan NM, Dorcaratto D, Heneghan H, Udupa V, Maguire D, Geoghegan J, Hoti E*

### SYSTEMATIC REVIEWS

- 231 Portal hypertensive gastropathy: A systematic review of the pathophysiology, clinical presentation, natural history and therapy  
*Gjeorgjievski M, Cappell MS*

### LETTERS TO THE EDITOR

- 263 Non-invasive evaluation of liver fibrosis by acoustic radiation force impulse and aminotransferase:platelet ratio index in chronic hepatitis C  
*Karagoz E, Ozturker C, Sivrioglu AK*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Christos G Savopoulos, MD, PhD, Associate Professor, 1<sup>st</sup> Medical Propedeutic Department of Internal Medicine, AHEPA Hospital, Aristotle University of Thessaloniki, 54636 Thessaloniki, Greece

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: editorialoffice@wjnet.com  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjnet.com](mailto:bpgoffice@wjnet.com)  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

**PUBLICATION DATE**  
February 8, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

Full instructions are available online at [http://www.wjnet.com/1948-5182/g\\_info\\_20100316080002.htm](http://www.wjnet.com/1948-5182/g_info_20100316080002.htm)

**ONLINE SUBMISSION**

<http://www.wjnet.com/esps/>

## Inflammasome activation in decompensated liver cirrhosis

José M González-Navajas

José M González-Navajas, FISABIO Biomedical Research Foundation, Hospital General Universitario de Alicante, 03010 Alicante, Spain

José M González-Navajas, Networked Biomedical Research Center for Hepatic and Digestive Disease (CIBERehd), Instituto de Salud Carlos III, 28029 Madrid, Spain

**Author contributions:** González-Navajas JM solely contributed to this article.

**Supported by** Grant PI13/00315 from the Instituto de Salud Carlos III (co-financed by FEDER funds); and grants UGP-14-123 and UGP-14-248 from FISABIO Research Foundation.

**Conflict-of-interest statement:** The author declares no conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** José M González-Navajas, PhD, FISABIO Biomedical Research Foundation, Hospital General Universitario de Alicante, Av. Pintor Baeza, 12, 03010 Alicante, Spain. [gonzalez\\_josnav@gva.es](mailto:gonzalez_josnav@gva.es)  
Telephone: +34-965-913928  
Fax: +34-965-913922

Received: January 19, 2015

Peer-review started: January 20, 2015

First decision: March 6, 2015

Revised: November 2, 2015

Accepted: January 16, 2016

Article in press: January 19, 2016

Published online: February 8, 2016

### Abstract

Inflammation participates in the pathogenesis of

many liver diseases, including liver cirrhosis. Certain inflammatory cytokines, such as interleukin (IL)-1 $\beta$  and IL-18, are produced after the activation of a multiprotein complex known as the inflammasome. Activation of the inflammasome has been documented in several liver diseases, but its role in the development and progression of liver cirrhosis or the complications associated with this disease is still largely unknown. We have recently studied the impact of the inflammasome in the sterile inflammatory response that takes place in the ascitic fluid of patients with decompensated cirrhosis, providing evidence that activation of the absent in melanoma 2 (AIM2) inflammasome is an important response in these patients. Ascitic fluid-derived macrophages were able to mount a very robust AIM2-mediated response even in the absence of a priming signal, which is usually required for the full activation of all the inflammasomes. In addition, high level of inflammasome activation in these patients was associated with a higher degree of liver disease and an increased incidence of spontaneous bacterial peritonitis. These results may help explain the exacerbated inflammatory response that usually occurs in patients with decompensated cirrhosis in the absence of detectable infections. Thus, inflammasomes should be considered as possible therapeutic targets in sterile inflammatory complications in patients with cirrhosis.

**Key words:** Cirrhosis; Ascites; Inflammasome; Absent in melanoma 2; Interleukin-1 $\beta$

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In this Editorial I discuss the involvement of the inflammasome in the inflammatory reactions that occur in patients with liver cirrhosis and ascites. I focus on a recent work in which we observed that the absent in melanoma 2 inflammasome is highly activated in the ascitic fluid of patients with advanced cirrhosis and that its activation is linked to the severity of liver disease. These findings are important for the understanding of the sterile inflammatory reactions in these patients, and could have important therapeutic implications.

González-Navajas JM. Inflammasome activation in decompensated liver cirrhosis. *World J Hepatol* 2016; 8(4): 207-210 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i4/207.htm> DOI: <http://dx.doi.org/10.4254/wjgh.v8.i4.207>

Liver cirrhosis is the result of a long pathologic process initiated by chronic infection with hepatitis B virus or hepatitis C virus (HCV), excessive alcohol consumption, accumulation of fat in liver cells, and other metabolic alterations. The most important complications of liver cirrhosis include intestinal bleeding, encephalopathy, and ascites, and the development of any of these complications is clinically known as decompensated cirrhosis. Ascites is the most common cause of hepatic decompensation, and usually precedes the others. Decompensation of cirrhosis is usually associated with a systemic inflammatory response characterized by activation of innate immune cells and elevated expression of pro-inflammatory cytokines [tumor necrosis factor  $\alpha$ , interleukin (IL)-1 $\beta$ , IL-6] in the ascitic fluid. This inflammatory response is usually the result of bacterial translocation from the intestinal lumen to extra-intestinal sites, such as mesenteric lymph nodes and ascitic fluid. Bacterial translocation does not necessarily mean bacterial infection, since the translocating bacteria is often killed by the innate immune system. However, the sole presence of molecules of microbial origin [such as lipopolysaccharide (LPS) or bacterial DNA] is sufficient to mount a sterile inflammatory response in the ascitic fluid in the absence of active infection<sup>[1-3]</sup>.

Since its first description in 2002 by Martinon *et al*<sup>[4]</sup>, the inflammasome has been a key subject of research in multiple inflammatory diseases. Very comprehensive reviews of the expression, activation, and function of the inflammasomes have been published elsewhere<sup>[5-7]</sup>, and therefore these topics are discussed here only briefly. The inflammasome is a cytosolic multiprotein complex that controls the activation of the enzyme caspase-1<sup>[4,6]</sup>. Once activated, caspase-1 mediates the maturation and release of pro-inflammatory cytokines such as IL-1 $\beta$  and IL-18. Caspase-1 activity can also result in a highly inflammatory form of cell death called "pyroptosis" in some cells<sup>[8]</sup>, which occurs most frequently upon infection with intracellular pathogens<sup>[9]</sup>. Inflammasomes are assembled upon recognition of pathogen-associated molecular patterns (PAMPs), as well as host-derived signals known as damage-associated molecular patterns (DAMPs) that are released as a result of tissue damage or cellular stress. Several members of the NLR family (nucleotide-binding and oligomerization domain and leucine-rich-repeat-containing proteins) have been reported to exhibit inflammasome activity, including NLRP1, NLRP3, NLRP6 or NLRC4. In addition to NLRs, the HIN-200 domain-containing protein absent in melanoma 2 (AIM2) has also the ability to induce inflammasome activation. Full activation of the inflammasome requires two different signals. The first

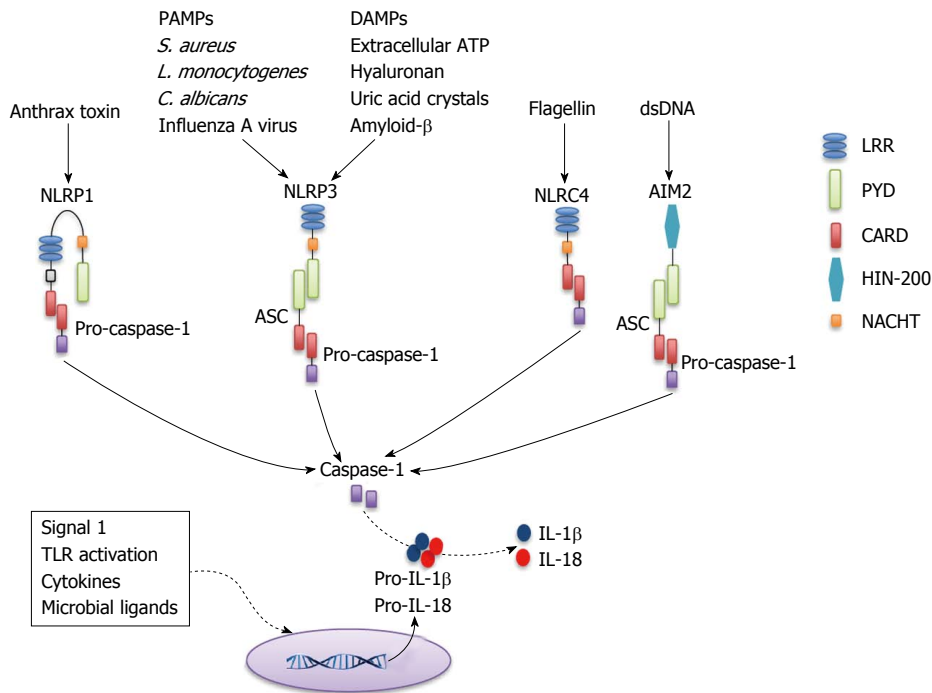
signal is provided by the activation of pattern recognition receptors, such as Toll-like receptors, resulting in the accumulation of inactive pro-IL-1 $\beta$  and pro-IL-18 inside the cell. The second signal is then provided by the activation of NLRPs or AIM2 by different danger signals<sup>[5-7]</sup>. For example, NLRP3 is activated by a wide range of PAMPs and DAMPs (e.g., toxins, uric acid, ATP), whereas AIM2 is activated only by double-stranded DNA (dsDNA) of any origin<sup>[10,11]</sup> (Figure 1).

Recent studies have suggested that the inflammasome also plays an important role in chronic liver disease<sup>[12]</sup>. For example, the inflammasome is activated in response to HCV infection<sup>[13]</sup>, in drug-induced liver injury<sup>[14]</sup>, or in the pathogenesis of non-alcoholic steatohepatitis<sup>[15,16]</sup>. However, the inflammasome-mediated response in decompensated cirrhosis was unexplored until publication of our recent study by Lozano-Ruiz *et al*<sup>[17]</sup>. In this study we show that activation of the inflammasome is an important response in the ascitic fluid of cirrhotic patients. Macrophages from ascitic fluid showed high levels of pro-IL-1 $\beta$  and pro-IL-18 mRNA, constitutive activation of caspase-1 and enhanced expression of AIM2 protein and mRNA when compared to blood-derived macrophages from the same patients. Moreover, contrary to blood macrophages, activation of the AIM2 inflammasome did not require a priming signal in these cells, demonstrating the pre-activated state of the inflammasome in the ascitic fluid. This pre-activated state of the AIM2 inflammasome was associated with the presence of bacterial DNA fragments in the ascitic fluid of these patients, suggesting that translocation of bacteria and their products could be responsible for this priming<sup>[17]</sup>.

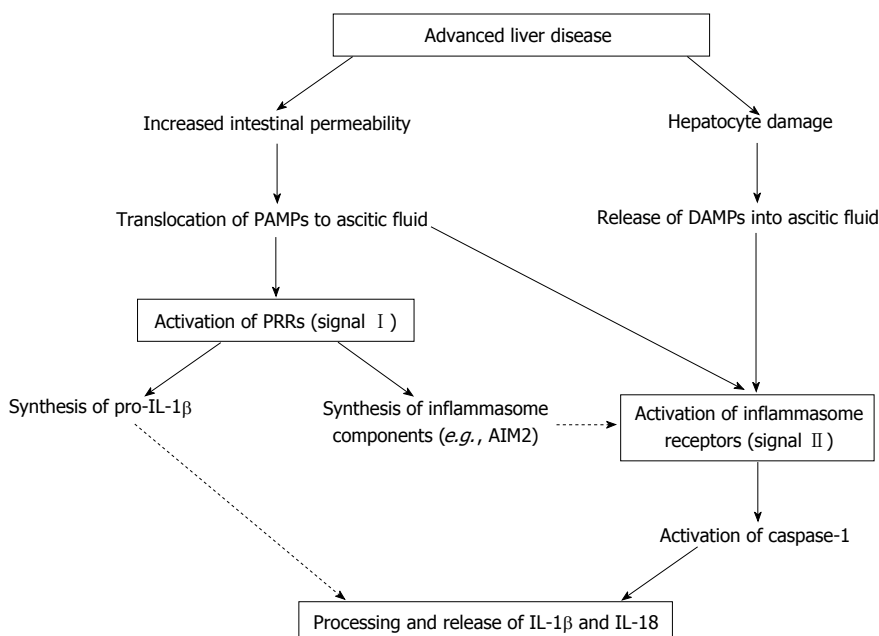
It was previously shown that bacterial translocation and inflammation increases depending on the degree of liver damage and the clinical stage of the disease<sup>[18]</sup>. Thus, it was conceivable that the severity of liver disease could affect the extent of inflammasome activation in cirrhosis. Indeed, activation of caspase-1 and AIM2-mediated production of IL-1 $\beta$  and IL-18 were increased in patients with Child-Pugh C score, compared to those with Child-Pugh B. Additionally, high level of IL-18 in ascitic fluid showed a significant association with the occurrence of spontaneous bacterial peritonitis (SBP) in these patients independently of the Child-Pugh score, suggesting that increased inflammasome activation might be a marker of increased risk of SBP.

In summary, these findings are important for the understanding of the sterile inflammatory reactions in patients with advanced cirrhosis. In these patients, complications associated with high mortality are normally accompanied by excessive inflammation, and therefore our results could have important translational implications. We propose that a two-hit process could explain the exacerbated inflammasome activation in advanced cirrhosis (Figure 2). First, bacterial translocation would lead to an abnormal influx of exogenous PAMPs (e.g., LPS or bacterial DNA) that induce a pre-activation state of the inflammasome in ascitic fluid cells.





**Figure 1 Basic representation of inflammasome activation.** Inflammasomes are formed after NLR or PYHIN family members recognize signals associated with tissue damage or infection. Receptors that have a CARD domain can recruit pro-caspase-1 directly (e.g., NLRC4), whereas those that contain a PYD domain (e.g., NLRP3 and AIM2) recruit pro-caspase-1 through the accessory protein ASC (which contains a PYD and a CARD). NLRP1 contains a CARD and can bypass the requirement for ASC, but also contains a PYD and its interaction with ASC enhances the activity of the NLRP1 inflammasome. CARD: Caspase-1 recruitment domain; PYD: Pyrin domain; ASC: Apoptosis-associated speck-like protein containing a CARD; LRR: Leucine rich repeat; HIN-200: Hematopoietic interferon-inducible nuclear antigen with 200 amino-acid repeat; PAMPs: Pathogen-associated molecular patterns; DAMPs: Damage-associated molecular patterns; AIM2: Absent in melanoma 2; dsDNA: Double-stranded DNA; IL: Interleukin; *S. aureus*: *Staphylococcus aureus*; *L. monocytogenes*: *Listeria monocytogenes*; *C. albicans*: *Candida albicans*; TLR: Toll-like receptor.



**Figure 2 Theoretical mechanism of inflammasome activation in ascitic fluid.** Advanced cirrhosis is typically associated with overgrowth of intestinal bacteria and increased intestinal permeability, which results in the translocation of bacterial products (e.g., DNA or LPS) to the ascitic fluid. The presence of these PAMPs activates PRRs in innate immune cells of the ascitic fluid, inducing the synthesis of IL-1 $\beta$  and IL-18 precursors and inflammasome components (signal I). At the same time, continuous liver damage (e.g., by virus or alcohol) would result in hepatocyte death and release of DAMPs (e.g., host dsDNA). These DAMPs (and probably new translocation events of PAMPs from the intestinal lumen) would activate inflammasome-forming receptors such as AIM2 (providing signal II), which in turn results in the activation of caspase-1 and the maturation and release of IL-1 $\beta$  and IL-18 into the ascitic fluid. IL: Interleukin; PAMPs: Pathogen-associated molecular patterns; DAMPs: Damage-associated molecular patterns; AIM2: Absent in melanoma 2; dsDNA: Double-stranded DNA; PRRs: Pattern recognition receptors; LPS: Lipopolysaccharide.

Second, endogenous DAMPs released from damaged liver cells (e.g., host dsDNA) would provide the second signal for the activation of the AIM2 inflammasome and the promotion of inflammation in the absence of active infection<sup>[17]</sup>. However, some questions remain that need to be further clarified. For example, it is not clear whether the inflammasome contributes to, or is a consequence of, cirrhosis progression. In addition, the use of the inflammasome as a therapeutic target in cirrhosis needs to be carefully addressed. Several IL-1 $\beta$  blocking agents are currently approved and used in patients suffering from different inflammatory diseases<sup>[19]</sup>, but the increased risk of infections would argue against using these immunosuppressive drugs in certain situations, such as in SBP. Therefore, more studies are needed to determine the exact role of the inflammasome in the pathogenesis of advanced cirrhosis and its potential use as a therapeutic target for the treatment or prevention of inflammatory complications.

## REFERENCES

- 1 **Francés R**, Zapater P, González-Navajas JM, Muñoz C, Caño R, Moreu R, Pascual S, Bellot P, Pérez-Mateo M, Such J. Bacterial DNA in patients with cirrhosis and noninfected ascites mimics the soluble immune response established in patients with spontaneous bacterial peritonitis. *Hepatology* 2008; **47**: 978-985 [PMID: 18306221 DOI: 10.1002/hep.22083]
- 2 **González-Navajas JM**, Bellot P, Francés R, Zapater P, Muñoz C, García-Pagán JC, Pascual S, Pérez-Mateo M, Bosch J, Such J. Presence of bacterial-DNA in cirrhosis identifies a subgroup of patients with marked inflammatory response not related to endotoxin. *J Hepatol* 2008; **48**: 61-67 [PMID: 17998145 DOI: 10.1016/j.jhep.2007.08.012]
- 3 **Zapater P**, Francés R, González-Navajas JM, de la Hoz MA, Moreu R, Pascual S, Monfort D, Montoliu S, Vila C, Escudero A, Torras X, Cirera I, Llanos L, Guarner-Argente C, Palazón JM, Carnicer F, Bellot P, Guarner C, Planas R, Solá R, Serra MA, Muñoz C, Pérez-Mateo M, Such J. Serum and ascitic fluid bacterial DNA: a new independent prognostic factor in noninfected patients with cirrhosis. *Hepatology* 2008; **48**: 1924-1931 [PMID: 19003911 DOI: 10.1002/hep.22564]
- 4 **Martinon F**, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell* 2002; **10**: 417-426 [PMID: 12191486 DOI: 10.1016/S1097-2765(02)00599-3]
- 5 **Schroder K**, Tschopp J. The inflammasomes. *Cell* 2010; **140**: 821-832 [PMID: 20303873 DOI: 10.1016/j.cell.2010.01.040]
- 6 **Rathinam VA**, Vanaja SK, Fitzgerald KA. Regulation of inflammasome signaling. *Nat Immunol* 2012; **13**: 333-342 [PMID: 22430786 DOI: 10.1038/ni.2237]
- 7 **Strowig T**, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. *Nature* 2012; **481**: 278-286 [PMID: 22258606 DOI: 10.1038/nature10759]
- 8 **Bergsbaken T**, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. *Nat Rev Microbiol* 2009; **7**: 99-109 [PMID: 19148178 DOI: 10.1038/nrmicro2070]
- 9 **Case CL**, Shin S, Roy CR. Asc and Ipaf Inflammasomes direct distinct pathways for caspase-1 activation in response to Legionella pneumophila. *Infect Immun* 2009; **77**: 1981-1991 [PMID: 19237518 DOI: 10.1128/IAI.01382-08]
- 10 **Fernandes-Alnemri T**, Yu JW, Datta P, Wu J, Alnemri ES. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature* 2009; **458**: 509-513 [PMID: 19158676 DOI: 10.1038/nature07710]
- 11 **Hornung V**, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, Latz E, Fitzgerald KA. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* 2009; **458**: 514-518 [PMID: 19158675 DOI: 10.1038/nature07725]
- 12 **Szabo G**, Csak T. Inflammasomes in liver diseases. *J Hepatol* 2012; **57**: 642-654 [PMID: 22634126 DOI: 10.1016/j.jhep.2012.03.035]
- 13 **Burdette D**, Haskett A, Presser L, McRae S, Iqbal J, Waris G. Hepatitis C virus activates interleukin-1 $\beta$  via caspase-1-inflammasome complex. *J Gen Virol* 2012; **93**: 235-246 [PMID: 21994322 DOI: 10.1099/vir.0.034033-0]
- 14 **Imaeda AB**, Watanabe A, Sohail MA, Mahmood S, Mohamadnejad M, Sutterwala FS, Flavell RA, Mehal WZ. Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome. *J Clin Invest* 2009; **119**: 305-314 [PMID: 19164858 DOI: 10.1172/JCI35958]
- 15 **Kamari Y**, Shaish A, Vax E, Shemesh S, Kandel-Kfir M, Arbel Y, Olteanu S, Barshack I, Dotan S, Voronov E, Dinarello CA, Apte RN, Harats D. Lack of interleukin-1 $\alpha$  or interleukin-1 $\beta$  inhibits transformation of steatosis to steatohepatitis and liver fibrosis in hypercholesterolemic mice. *J Hepatol* 2011; **55**: 1086-1094 [PMID: 21354232 DOI: 10.1016/j.jhep.2011.01.048]
- 16 **Henao-Mejia J**, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaïss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JL, Hoffman HM, Flavell RA. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012; **482**: 179-185 [PMID: 22297845 DOI: 10.1038/nature10809]
- 17 **Lozano-Ruiz B**, Bachiller V, García-Martínez I, Zapater P, Gómez-Hurtado I, Moratalla A, Giménez P, Bellot P, Francés R, Such J, González-Navajas JM. Absent in melanoma 2 triggers a heightened inflammasome response in ascitic fluid macrophages of patients with cirrhosis. *J Hepatol* 2015; **62**: 64-71 [PMID: 25173967 DOI: 10.1016/j.jhep.2014.08.027]
- 18 **Cirera I**, Bauer TM, Navasa M, Vila J, Grande L, Taurá P, Fuster J, García-Valdecasas JC, Lacy A, Suárez MJ, Rimola A, Rodés J. Bacterial translocation of enteric organisms in patients with cirrhosis. *J Hepatol* 2001; **34**: 32-37 [PMID: 11211904 DOI: 10.1016/S0168-8278(00)00013-1]
- 19 **Hoffman HM**, Wanderer AA. Inflammasome and IL-1 $\beta$ -mediated disorders. *Curr Allergy Asthma Rep* 2010; **10**: 229-235 [PMID: 20425006 DOI: 10.1007/s11882-010-0109-z]

**P- Reviewer:** Liu ZH, Tsuchiya A, Uchiyama H  
**S- Editor:** Gong XM **L- Editor:** A **E- Editor:** Liu SQ



Basic Study

## Lack of hepcidin expression attenuates steatosis and causes fibrosis in the liver

Sizhao Lu, Robert G Bennett, Kusum K Kharbanda, Duygu Dee Harrison-Findik

Sizhao Lu, Robert G Bennett, Department of Biochemistry, University of Nebraska Medical Center, Omaha, NE 68198-5870, United States

Robert G Bennett, Division of Endocrinology, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198-4130, United States

Robert G Bennett, Kusum K Kharbanda, Nebraska-Western Iowa VA Health Care System, Omaha, NE 68105, United States

Kusum K Kharbanda, Duygu Dee Harrison-Findik, Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198-2000, United States

**Author contributions:** Lu S contributed to study design, data acquisition and drafting of the manuscript; Harrison-Findik DD obtained funding, contributed to study concept and supervision, and critical revision of the manuscript; Bennett RG and Kharbanda K helped with technical support and critical reading of the manuscript.

**Supported by** NIH grant No. R01AA017738 (to Harrison-Findik DD); and University of Nebraska Medical Center Graduate Assistantship/Fellowship (to Lu S).

**Institutional animal care and use committee statement:** All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of University of Nebraska Medical Center (IACUC protocol No. 03-075-10-FC).

**Conflict-of-interest statement:** The authors declare no conflict of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

[licenses/by-nc/4.0/](http://creativecommons.org/licenses/by-nc/4.0/)

**Correspondence to:** Duygu Dee Harrison-Findik, DVM, PhD, Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of Nebraska Medical Center, 92000 UNMC, Omaha, NE 68198-2000, United States. [dufindik@gmail.com](mailto:dufindik@gmail.com)  
Telephone: +1-402-5596209  
Fax: +1-402-5599004

Received: August 2, 2015

Peer-review started: August 3, 2015

First decision: September 29, 2015

Revised: October 14, 2015

Accepted: November 13, 2015

Article in press: November 13, 2015

Published online: February 8, 2016

### Abstract

**AIM:** To investigate the role of key iron-regulatory protein, hepcidin in non-alcoholic fatty liver disease (NAFLD).

**METHODS:** Hepcidin (*Hamp1*) knockout and floxed control mice were administered a high fat and high sucrose (HFS) or a regular control diet for 3 or 7 mo. Steatosis, triglycerides, fibrosis, protein and gene expression in mice livers were determined by histological and biochemical techniques, western blotting and real-time polymerase chain reaction.

**RESULTS:** Knockout mice exhibited hepatic iron accumulation. Despite similar weight gains, HFS feeding induced hepatomegaly in floxed, but not knockout, mice. The livers of floxed mice exhibited higher levels of steatosis, triglycerides and c-Jun N-terminal kinase (JNK) phosphorylation than knockout mice. In contrast, a significant increase in fibrosis was observed in knockout mice livers within 3 mo of HFS administration. The hepatic gene expression levels of sterol regulatory

element-binding protein-1c and fat-specific protein-27, but not peroxisome proliferator-activated receptor- $\alpha$  or microsomal triglyceride transfer protein, were attenuated in HFS-fed knockout mice. Knockout mice fed with regular diet displayed increased carnitine palmitoyltransferase-1 $\alpha$  and phosphoenolpyruvate carboxykinase-1 but decreased glucose-6-phosphatase expression in the liver. In summary, attenuated steatosis correlated with decreased expression of lipogenic and lipid storage genes, and JNK phosphorylation. Deletion of *Hamp1* alleles *per se* modulated hepatic expression of beta-oxidation and gluconeogenic genes.

**CONCLUSION:** Lack of hepcidin expression inhibits hepatic lipid accumulation and induces early development of fibrosis following high fat intake. Hepcidin and iron may play a role in the regulation of metabolic pathways in the liver, which has implications for NAFLD pathogenesis.

**Key words:** *Hamp*; Iron; Non-alcoholic steatohepatitis; Metabolic genes; Steatosis; Non-alcoholic fatty liver disease; Steatohepatitis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Due to obesity epidemic the incidence of non-alcoholic fatty liver disease (NAFLD) is on the rise. Iron contributes to disease severity and the expression of key iron regulatory hormone, hepcidin is modulated in NAFLD patients. The underlying mechanisms are unknown. We have generated hepcidin knockout mice with iron overload phenotype. This study investigates the role of hepcidin in NAFLD by using high fat and high sucrose-fed knockout mice as an experimental model of NAFLD. Our findings showed attenuated steatosis and early fibrosis development suggesting a role for hepcidin in the regulation of metabolic processes in the liver, and in NAFLD.

Lu S, Bennett RG, Kharbanda KK, Harrison-Findik DD. Lack of hepcidin expression attenuates steatosis and causes fibrosis in the liver. *World J Hepatol* 2016; 8(4): 211-225 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i4/211.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i4.211>

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver disease ranging from simple benign steatosis to non-alcoholic steatohepatitis (NASH). NASH, a more aggressive form of disease, is characterized by the presence of lobular inflammation, fibrosis, hepatocellular ballooning and Mallory-Denk bodies<sup>[1,2]</sup>. NASH with progressive fibrosis can progress to cirrhosis and end stage liver disease<sup>[1,3,4]</sup>.

The precise mechanisms of NASH development

are not well understood. Although a so-called “two-hit hypothesis”<sup>[5]</sup> has been widely adopted<sup>[6,7]</sup>, NASH can also develop in the absence of insulin resistance and simple benign steatosis (*i.e.*, initial hit)<sup>[8]</sup>. The potential candidates regarded as the “second hit” include oxidative stress, inflammation and changes in mitochondrial function<sup>[7,9-12]</sup>. Iron is also considered as a “second hit” in liver injury<sup>[13]</sup> and a role for iron has been reported in NASH pathogenesis. Patients with NAFLD/ NASH frequently display elevated serum iron indices and hepatic iron content<sup>[14,15]</sup>. A strong correlation between hepatic iron content and the level of liver fibrosis in NAFLD/NASH patients has been shown<sup>[16-18]</sup>. Phlebotomy has also been suggested to alleviate insulin resistance in NAFLD patients<sup>[19]</sup>.

The mechanisms by which iron contributes to NAFLD/ NASH pathogenesis have mainly been attributed to oxidative stress, which can induce lipid peroxidation<sup>[20]</sup> and ultimately the activation of fibrotic signaling<sup>[21]</sup>. Studies with genetic haemochromatosis (GH) patients have shown the association of primary iron overload with fibrogenesis<sup>[22]</sup>. By using dietary experimental models, some studies have also suggested a reverse connection between iron and steatosis in rat livers<sup>[23,24]</sup>. In contrast, another study with a mouse dietary model of iron and high fat failed to show any significant effect of iron on steatosis<sup>[25]</sup>. The consequences of altered iron homeostasis for lipid metabolism in the liver are therefore unclear.

In this study, we employed hepcidin knockout mice with iron overload phenotype as an experimental model to further study the role of iron metabolism in NAFLD/NASH. Hepcidin is the central regulator of iron homeostasis, which is primarily synthesized in hepatocytes as a circulatory protein<sup>[26]</sup>. Unlike humans, which express only one hepcidin gene, *HAMP*, mice express two hepcidin genes, hepcidin (*Hamp1*) and *Hamp2*<sup>[27]</sup>. *Hamp1*, the human equivalent of mouse hepcidin gene, is by itself sufficient to regulate iron metabolism<sup>[28,29]</sup>. Hepcidin controls iron homeostasis by decreasing iron absorption from the absorptive enterocytes in the duodenum and the release of iron from the macrophages<sup>[30]</sup>. The lack of hepcidin expression in knockout mice and in human iron disorders results in iron accumulation both in the liver and other organs<sup>[30-32]</sup>. GH patients also display impaired hepcidin expression<sup>[33]</sup>. Although changes in both serum and liver hepcidin expression levels have been reported in NAFLD/NASH patients<sup>[14,34-38]</sup>, the significance of hepcidin in disease pathogenesis is unknown. Our findings in this study with *Hamp1* knockout mice administered a high fat diet for different time points suggest a role for hepcidin in NAFLD/NASH pathogenesis. This mouse model may also serve as a novel experimental model of NAFLD/NASH.

## MATERIALS AND METHODS

### Animal studies

Animal experiments were approved by the Institutional



**Table 1 SYBR green real-time quantitative polymerase chain reaction primer sequences of mouse genes**

Mouse genes	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Mtbp</i>	CTCTGGCAGTGCTTTTCTCT	GAGCTTGATAGCCGCTCATT
<i>Cpt1a</i>	CTCCGCTGAGCCATGAAG	CACCAGTGATGATGCCATTCT
<i>Fsp27</i>	ATGAAGTCTCTCAGCCTCTG	AAGCTGTGAGCCATGATGC
<i>G6pc</i>	CGACTCGTATCTCCAAGTGA	GTGAACCAAGTCTCCGACCA
<i>Pck1</i>	CTGCATAACGGTCTGGACTTC	CAGCAACTGCCCGTACTCC
<i>Ppara</i>	AGAGCCCCATCTGTCTCTC	ACTGGTAGTCTGAAAACCAAA
<i>Srebp-1c</i>	GCAGCCACCATCTAGCCTG	CAGCAGTGAGTCTGCCTTGAT
<i>Gapdh</i>	GTGGAGATTGTGCCATCAACGA	CCCATTCTCGGCTTGACTGT

Animal Care and Use Committee at the University of Nebraska Medical Center. *Hamp1* floxed mice and ubiquitous *Hamp1* knockout mice, lacking hepcidin expression in all the organs, were generated, as published previously<sup>[29]</sup>. All mice are on C57BL/6J genetic background. *Hamp* floxed mice have been donated to the Jackson Laboratory (Catalog No. 026872, 026873).

Male mice (4-6-wk-old) were randomly separated into groups to feed with custom-made regular control (17.2% kcal from fat, 100 g/kg sucrose) or high fat and high sucrose (HFS) [42% kcal from fat (54% saturated, 9.7% trans-fat), 0.4% cholesterol, 340 g/kg sucrose] diets for 3 or 7 mo (Harlan Laboratories; TD.97184; TD.120654). Water was given ad libitum, and contained sucrose (40 g/L) with HFS-fed groups to imitate the western diet with fat and soda consumption.

### Liver histology

Formalin-fixed, paraffin-embedded liver tissues were sectioned and stained with hematoxylin and eosin at UNMC Histology Core Facility. To determine fibrosis, sections were stained with Picrosirius Red, as published previously<sup>[39]</sup> and histomorphometric analyses were performed using ImageJ ROI manager software.

### Quantification of liver triglycerides

Triglycerides were isolated, as described<sup>[40]</sup> and quantified using a commercial kit (Thermo Scientific DMA kit 2750) according to manufacturer's instructions.

### Real-time polymerase chain reaction

cDNA was synthesized from liver tissue RNA with Superscript II reverse transcriptase (Invitrogen), as described<sup>[41]</sup>. Real-time polymerase chain reaction (PCR) reactions were performed using iTaq Universal SYBR Green Supermix (Bio-Rad) with a StepOnePlus instrument (Life Technologies). Glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) gene was used as the endogenous control and gene amplification was calculated using comparative Ct method, as described<sup>[41]</sup>. Primer sequences are shown in Table 1.

### Western blotting

Western blots using whole liver tissue lysate proteins were performed, as published previously<sup>[41]</sup>. Antibodies

were obtained commercially (Cell Signaling, Sigma) and immune-reactive bands were detected by the ImmunStar™ kits (Bio-Rad).

### Statistical analysis

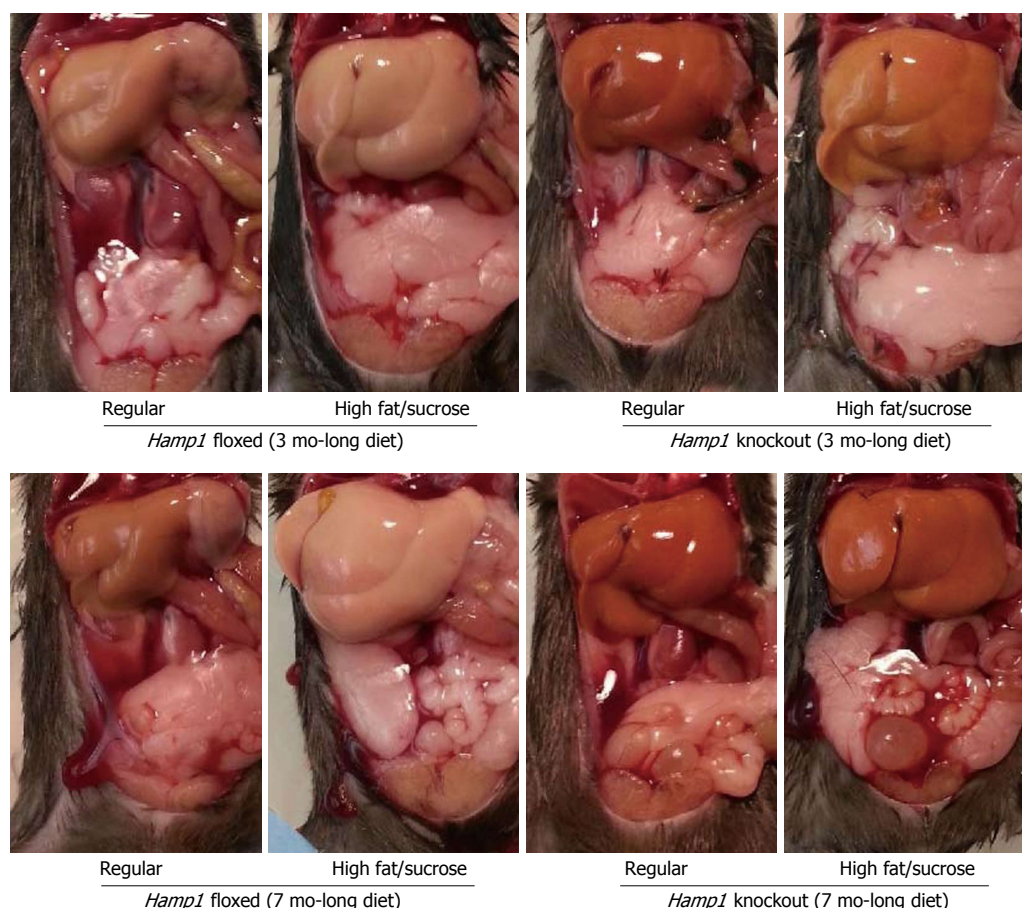
The significance of differences between groups was determined by Student's *t*-test or one-way ANOVA by using SPSS software. A value of *P* < 0.05 was accepted as statistically significant.

## RESULTS

To study the interaction of hepcidin-induced iron overload and lipid metabolism, ubiquitous *Hamp1* knockout and floxed control mice were administered either high fat and HFS or regular (control) diets, as described in Material and Methods. Since NAFLD/NASH progression can occur over a long period of time, mice were fed up to 7 mo. We have previously shown that the deletion of both *Hamp1* alleles induces significant iron overload in the livers of *Hamp1* knockout mice by using inductively coupled mass spectrometry (ICP-MS)<sup>[29]</sup>. ICP-MS analysis did not detect any significant level of iron in the livers of homozygous *Hamp1* floxed control mice. Gradual iron deposition was also indicated macroscopically by the darker color of knockout mice livers compared to those of floxed control mice (Figure 1).

Macroscopic analyses have confirmed that HFS intake induced hepatomegaly and more pronounced visceral fat accumulation in floxed control mice compared to knockout mice (Figure 1). In agreement, the liver weights of floxed mice were significantly higher ( $3.5 \pm 0.46$  g) than those of knockout mice ( $2.42 \pm 0.54$  g) particularly following 7 mo of HFS administration (Figure 2A and B). However, HFS intake induced similar increases in body weights in both floxed (Figure 2C) and knockout (Figure 2D) mice after either 3 or 7 mo-long feeding, as compared to respective controls fed with regular diet.

To further understand these discrepancies between floxed and knockout mice, histological analysis were performed. Hematoxylin and eosin staining of livers showed significantly higher levels of steatosis in floxed than in knockout mice both after 3 and 7 mo-long HFS feeding (Figure 3). The quantification of hepatic triglycerides further confirmed that HFS intake signifi-



**Figure 1** Macroscopic changes in *Hamp1* floxed and knockout mice fed with either a high fat and high sucrose or a regular control diet for 3 or 7 mo. Representative images showing the abdominal cavity of mice were obtained with a digital camera (Nikon).

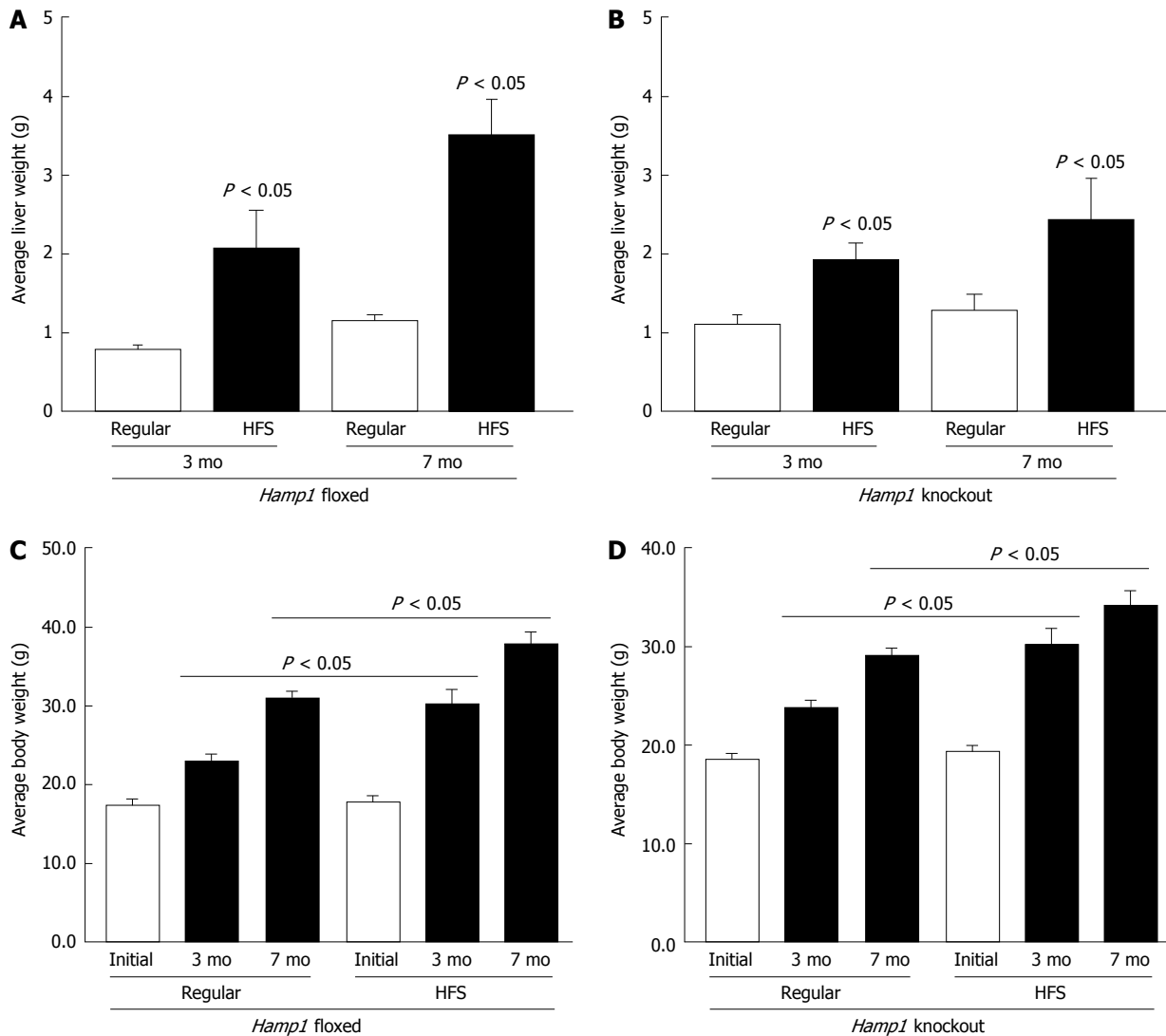
cantly increased hepatic triglyceride content to different extents in floxed and knockout mice (Figure 4). At the end of 3 mo-long high fat intake, the level of hepatic triglyceride accumulation was 2.85-fold higher in floxed mice compared to knockout mice ( $1876.64 \pm 370.84$  and  $657.98 \pm 186.89$   $\mu\text{mol/L}$  per 100 g BW) (Figure 4A). Seven mo-long feeding yielded 2.07-fold higher hepatic triglyceride content in floxed than in knockout mice ( $1837.71 \pm 118.12$  and  $886.91 \pm 89.51$   $\mu\text{mol/L}$  per 100 g BW) (Figure 4B).

Sirius Red staining of liver sections showed that knockout, but not floxed, mice developed fibrosis within 3 mo of high fat intake (Figure 5A). The deletion of both *Hamp1* alleles per se has also caused weaker but significant level of fibrosis in the livers of knockout mice (Figure 5A). Quantification by ImageJ analysis has shown a 2.56-fold higher level of fibrosis in the livers of high fat-fed knockout than regular diet-fed knockout mice at 3 mo (Figure 5B). In contrast to 3 mo, 7 mo of high fat intake induced fibrosis in the livers of floxed mice (Figure 6A). Compared to 3 mo, regular diet feeding for 7 mo slightly increased the level of fibrosis in knockout mice livers (Figure 6A). Knockout mice with 7 mo-long high fat intake developed the highest level of fibrosis, as shown by Image J quantification (Figure 6B). The hepatic expression patterns of alpha smooth

muscle actin ( $\alpha\text{SMA}$ ) protein, a marker for hepatic stellate cell activation, were in agreement with our histological analysis. Three months-long HFS feeding elevated liver  $\alpha\text{SMA}$  expression in knockout, but not floxed, mice, as shown by Western blotting (Figure 7A). The deletion of *Hamp1* alleles by itself increased hepatic  $\alpha\text{SMA}$  expression (Figure 7A). In contrast to 3 mo, 7 mo-long high fat intake increased  $\alpha\text{SMA}$  expression in the livers of both floxed and knockout mice (Figure 7A).

Studies with JNK knockout mice fed with methionine-choline-deficient diet (MCD) diets have indicated a role for c-Jun N-terminal kinase (JNK) in steatosis<sup>[42]</sup>. JNK is activated by phosphorylation on serine residues<sup>[43]</sup>. The expression levels of phosphorylated JNK protein in the livers of *Hamp1* transgenic mice were therefore determined by western blotting using specific anti-phospho JNK antibodies (Figure 7B). Three-month-long high fat intake significantly stimulated JNK phosphorylation in the livers of floxed, but not knockout, mice (Figure 7B). In contrast, the effect of high fat intake on JNK phosphorylation in the liver was weakened by 7 mo-long feeding (Figure 7B).

To further investigate the underlying mechanisms of attenuated fat accumulation in the livers of knockout mice with high fat intake, mRNA expression levels of genes, which are known to be involved in lipid meta-



**Figure 2** Liver and body weights of *Hamp1* floxed and knockout mice fed with high fat or regular diets. Average liver (A and B) and body (C and D) weights of floxed (A and C) and knockout (B and D) mice prior to (initial) and after feeding with high fat and sucrose (HFS) or regular control diets for 3 or 7 mo are shown as gram weight.

bolism, were examined by real-time PCR (Figure 8). The transcription factor, sterol regulatory element-binding protein-1c (*Srebp-1c*) is involved in *de novo* lipogenesis and its expression is also regulated at the transcriptional level<sup>[44,45]</sup>. The deletion of *Hamp1* alleles did not significantly alter basal hepatic expression levels of *Srebp-1c* (Figure 8A and B). Three months of high fat intake stimulated *Srebp-1c* expression by 13.39-fold in floxed and 7.40-fold knockout mice compared to controls (Figure 8A). In contrast, 7 mo of high fat intake elevated *Srebp-1c* expression only by 3.72-fold in floxed mice (Figure 8B). Furthermore, 7 mo-long high fat feeding did not significantly alter liver *Srebp-1c* expression in knockout mice (Figure 8B).

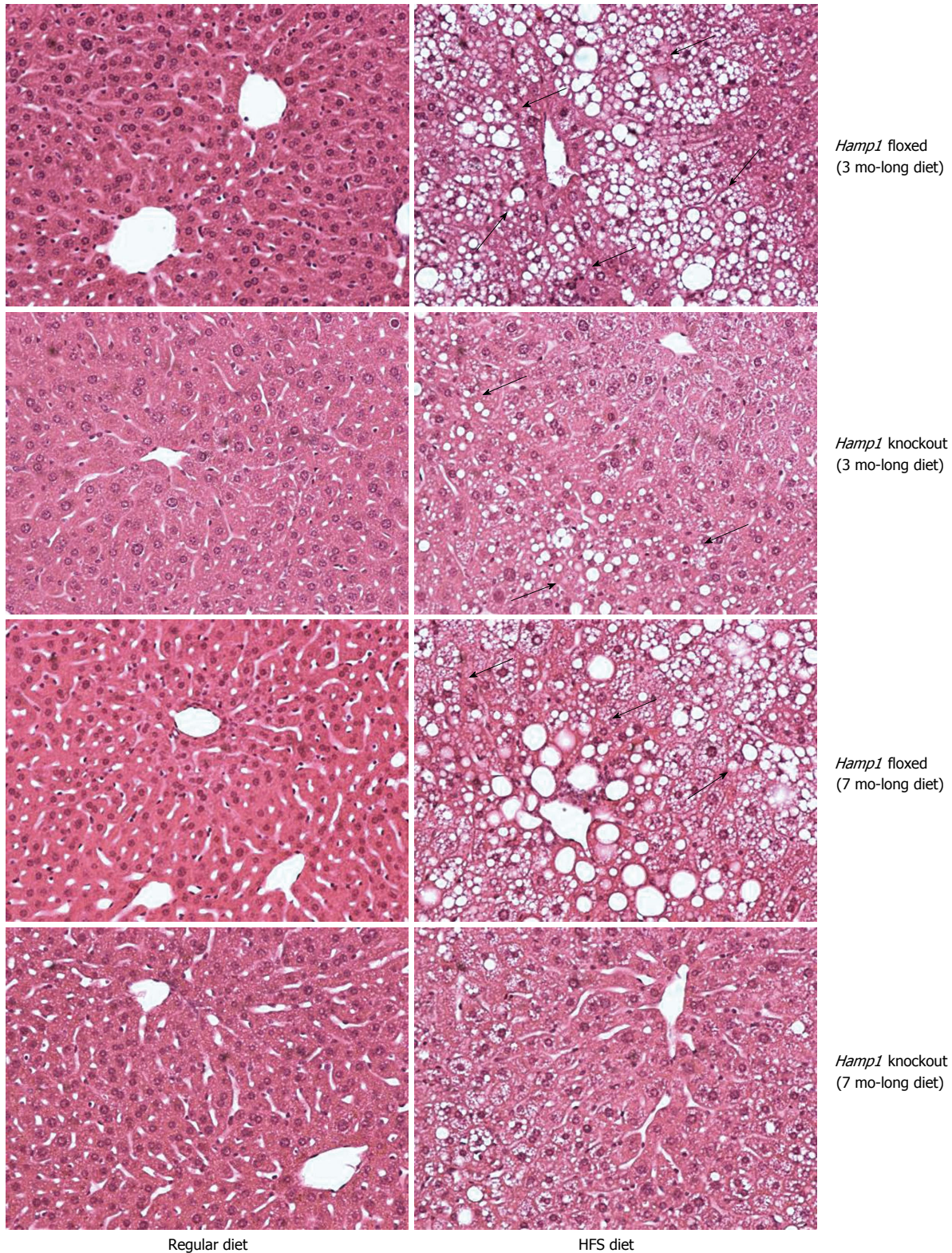
Fat-specific protein-27 (*Fsp27*) protein is involved in lipid droplet formation<sup>[46]</sup>. HFS feeding for 3 and 7 mo significantly induced *Fsp27* expression in the livers of floxed mice by 3.83- and 5.36-fold, respectively compared to regular diet-fed floxed mice (Figure 8C

and D). The livers of knockout mice fed with HFS for 3 or 7 mo displayed significantly lower induction of *Fsp27* expression than floxed mice, which was more prominent at 7 mo (Figure 8C and D). Liver *Fsp27* expression was not significantly altered in knockout mice fed with regular diets for 3 or 7 mo compared to respective floxed controls (Figure 8C and D).

Microsomal triglyceride transfer protein (*Mttp*) protein is responsible for the production and secretion of VLDL particles<sup>[47]</sup>. The mRNA expression level of *Mttp* in the liver was not significantly altered in floxed and knockout mice after 3 mo of high fat intake (Figure 8E). However, high fat exposure for 7 mo significantly suppressed *Mttp* expression in the livers of both floxed and knockout mice (Figure 8F).

Changes in fatty acid oxidation in the liver play an important role in NAFLD pathogenesis. Peroxisome proliferator-activated receptor- $\alpha$  (*Ppara*) activates the transcription of genes involved in the regulation of



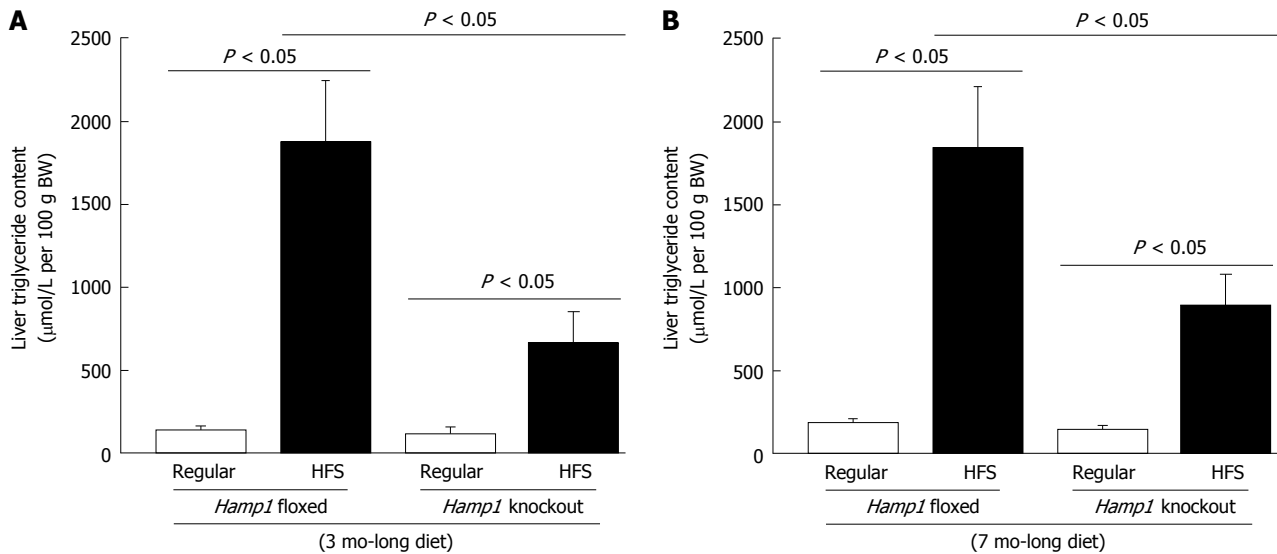


**Figure 3** Liver histology in *Hamp1* floxed and knockout mice fed high fat or regular diets. Liver sections from floxed and knockout mice fed with high fat and sucrose (HFS) or regular diets for 3 and 7 mo were stained with hemotoxylin and eosin. Representative images obtained with a Nikon Eclipse E400 light microscope are shown (20 ×). Arrows indicate steatosis.

fatty acid  $\beta$ -oxidation<sup>[48]</sup>. The mRNA expression levels of Ppar $\alpha$  were up-regulated at similar levels in the livers of both floxed and knockout mice within 3 mo of high fat feeding (Figure 9A). In contrast, the livers of floxed

and knockout mice with 7 mo of high fat exposure displayed significantly inhibited Ppar $\alpha$  expression (Figure 9B). Carnitine palmitoyltransferase-1 (Cpt1) is the rate-limiting enzyme in mitochondrial  $\beta$ -oxidation pathway<sup>[49]</sup>.





**Figure 4** Liver triglyceride content in *Hamp1* floxed and knockout mice fed high fat or regular diets. Hepatic triglyceride content in floxed and knockout mice fed with regular or high fat sucrose (HFS) diets for 3 (A) or 7 (B) mo was quantified using 50 mg of wet liver tissue. Liver triglyceride amount was expressed as  $\mu\text{mol}$  per liver per 100 g body weight ( $\mu\text{mol/L}$  per 100 g BW).

Three month-long high fat administration did not exert a significant effect on hepatic *Cpt1a* expression in floxed and knockout mice (Figure 9C). On the other hand, the livers of knockout mice fed with regular diet for 7 mo expressed higher *Cpt1a* levels compared to floxed mice fed under similar conditions, suggesting a role for gradual iron deposition (Figure 9D). Seven month-long high fat intake did not alter hepatic *Cpt1a* expression in floxed mice (Figure 9D). In contrast, long-term high fat exposure significantly suppressed *Cpt1a* expression in the livers of knockout mice compared to knockout controls (Figure 9D).

Both phosphoenolpyruvate carboxykinase-1 (*Pck1*) and glucose-6-phosphatase (*G6pc*) are involved in gluconeogenesis. Similar to *Cpt1a*, the deletion of *Hamp1* alleles significantly up-regulated basal *Pck1* mRNA expression in the liver. In contrast, the absence of hepcidin expression suppressed basal hepatic *G6pc* mRNA expression (Figure 9E-H). Both 3 and 7 mo-long high fat exposure significantly inhibited *Pck1* and *G6pc* mRNA expression in the livers of both floxed and knockout mice (Figure 9E-H).

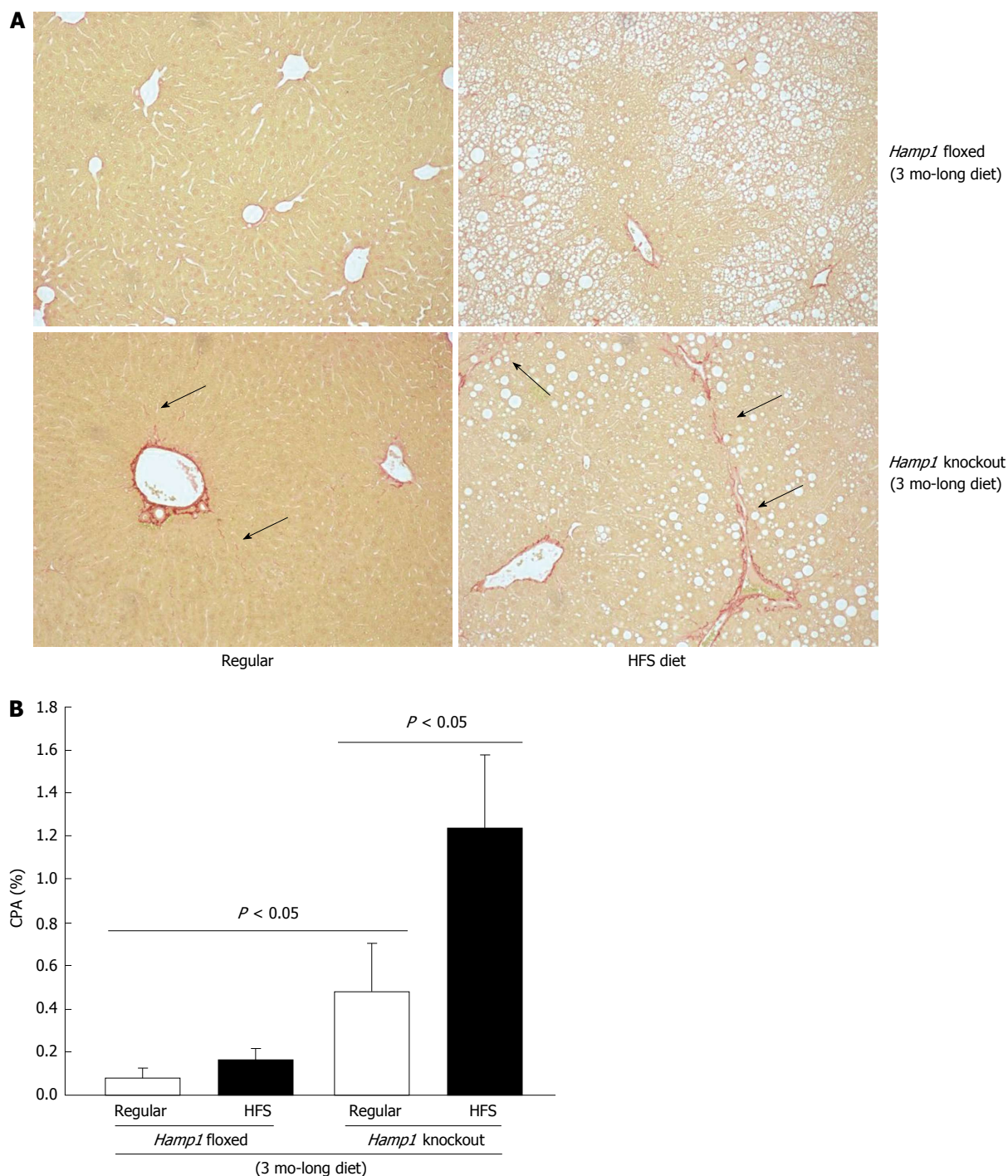
## DISCUSSION

Changes in iron metabolism contribute to liver injury<sup>[22,50]</sup>. The deposition of iron in the liver correlates with disease severity in NAFLD patients<sup>[15]</sup>. The mechanisms by which excess iron contribute to NAFLD pathogenesis is unclear. Although inconclusive, some studies suggested a role for iron in the regulation of lipid metabolism<sup>[23-25]</sup>. Since hepcidin is the central regulator of iron metabolism, we investigated its role in fatty liver disease. We and others showed iron accumulation in *Hamp1* knockout mice<sup>[29,31,51]</sup>. *Hamp1* knockout mice were administered high fat diets for different time periods to generate

pathological features in the liver, which are representative of NAFLD/NASH<sup>[2]</sup>. Collectively, our findings showed a strong correlation between hepcidin and lipid metabolism, and fibrosis in the liver.

The absence of hepcidin expression in *Hamp1* knockout mice exerted an inhibitory effect on hepatic lipid accumulation. This effect was not due to altered rates of diet consumption or weight gain and suggests the involvement of regulatory mechanisms. Previous studies showed a converse relationship between iron and lipid metabolism<sup>[22,23]</sup>. Since lack of hepcidin expression causes iron overload, elevated hepatic iron content may have interfered with fat accumulation in HFS-fed knockout mice. Furthermore, our findings suggest a role for JNK in this process. Namely, we showed a direct correlation between JNK phosphorylation and steatosis levels in floxed mice livers. In contrast, the livers of *Hamp1* knockout mice did not display significant JNK phosphorylation. Of note, the deletion of JNK1 reverses steatosis<sup>[52,53]</sup> and JNK is activated by phosphorylation<sup>[43]</sup>. Hepcidin-mediated changes in JNK activation may therefore be associated with attenuated steatosis in *Hamp1* knockout mice, particularly in early stages of high fat exposure.

Besides iron and JNK, altered metabolic gene expression in high fat-fed knockout mice may play a role in the inhibition of lipid accumulation. This is supported by our findings, which showed that the hepatic expression level of genes involved in lipogenesis and lipid storage do not adequately respond to high fat intake in *Hamp1* knockout mice. Namely, *Srebp-1c* and *Fsp27* expression were blunted in the livers of HFS-fed knockout, but not floxed, mice. These findings are significant because *Srebp-1c* and *Fsp27* expression are regulated at mRNA level<sup>[54]</sup>. Furthermore, the deletion of *Hamp1* alleles did not alter their basic expression levels.

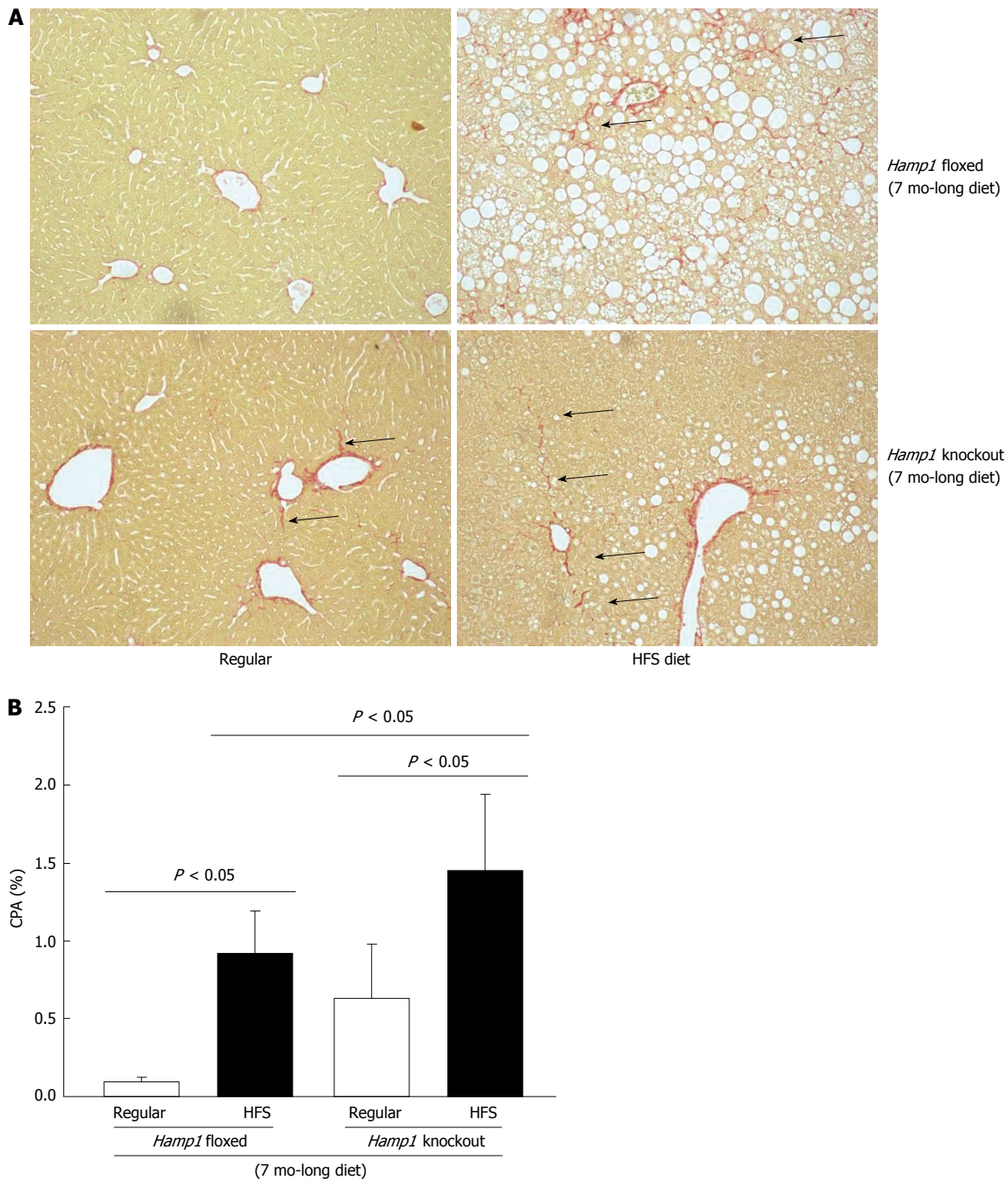


**Figure 5** Fibrosis in *Hamp1* floxed or knockout mice fed high fat or regular diets for 3 mo. A: Fibrosis in the livers of floxed and knockout mice fed on regular or high fat sucrose (HFS) diets for 3 mo was detected by Sirius Red staining of tissue sections. Representative images obtained with Nikon Eclipse E400 light microscope are shown; B: 10 independent images (10 x) taken from each group were quantified using ImageJ ROI manager software. The collagen proportional area (CPA) was determined by calculating the percentage of collagen-occupied pixels against the total pixel values.

Iron-deficient rodents have been reported to display elevated lipogenic gene expression, which indirectly supports our findings<sup>[55-57]</sup>. Hepatic lipid homeostasis is also regulated by lipid export *via* VLDL secretion. The hepatic expression levels of *Mttp*, which is important in this process, were comparable between control and knockout mice. Our findings therefore suggest that decreased lipogenesis and lipid storage, but not

increased lipid secretion, might lead to attenuated steatosis in high fat-fed *Hamp1* knockout mice.

Increased mitochondrial  $\beta$ -oxidation alleviates extra-hepatic fat burden in NAFLD by disposing of excess lipids<sup>[58]</sup>. Ppar $\alpha$ , which induces the transcription of genes involved in  $\beta$ -oxidation, is itself regulated at the transcriptional level<sup>[59,60]</sup>. However, Ppar $\alpha$  is not expected to contribute to liver pathology in *Hamp1*

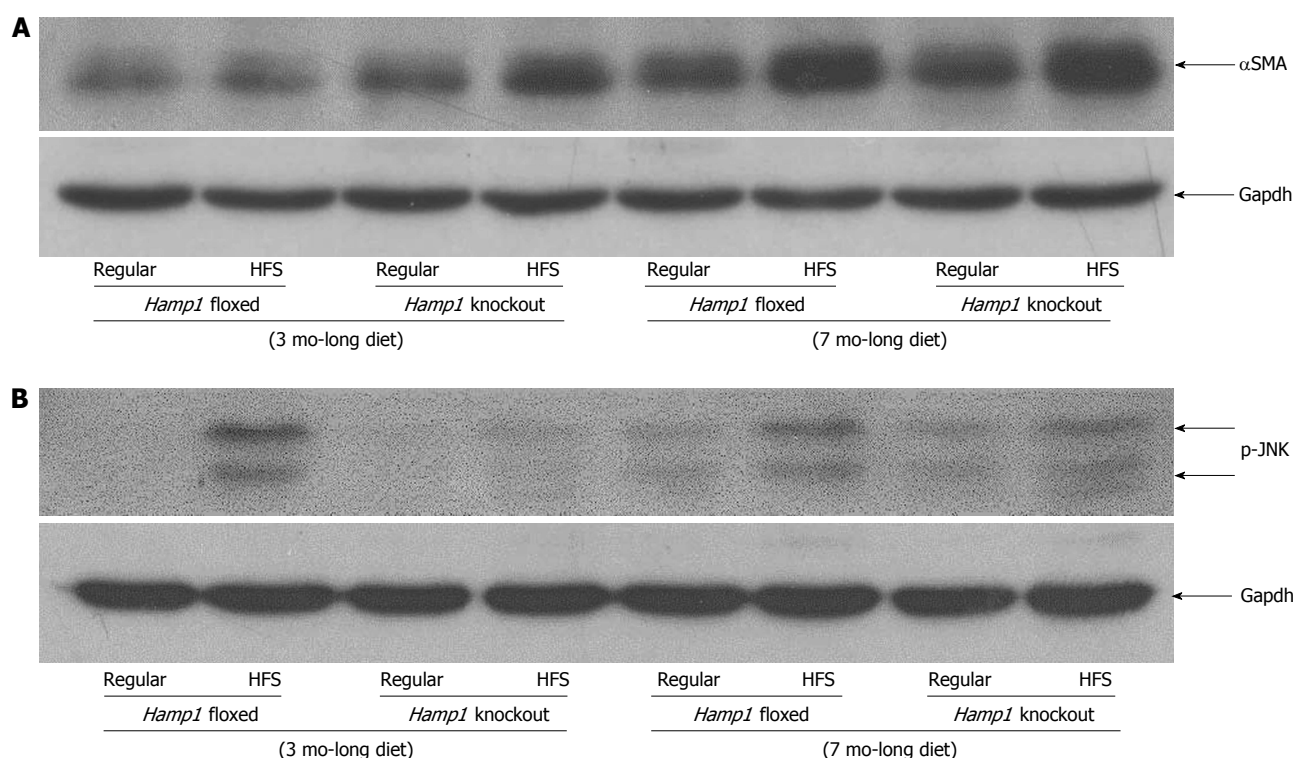


**Figure 6** Fibrosis in *Hamp1* floxed or knockout mice fed high fat or regular diets for 7 mo. Liver fibrosis in floxed and knockout mice fed on regular or high fat sucrose (HFS) diets for 7 mo was detected (A) and quantified (B), as described above. CPA: Collagen proportional area.

knockout mice because HFS-fed floxed and knockout mice livers displayed similar levels of *Ppar $\alpha$*  expression. *Cpt1* is the rate-limiting enzyme in  $\beta$ -oxidation. Long-term high fat intake significantly suppressed *Cpt1a* expression only in knockout mice livers suggesting a role for it in attenuated steatosis in *Hamp1* knockout mice. Interestingly, *Hamp1* deletion by itself elevated hepatic *Cpt1a* expression. Besides  $\beta$ -oxidation, mitochondria is also important for iron metabolism<sup>[61]</sup>. It is feasible that iron accumulation caused by *Hamp1* deletion modulates

metabolic gene expression in mitochondria. Of note, mitochondrial changes contribute to NAFLD/NASH pathology<sup>[11]</sup>. *Hamp1* deletion also altered the expression of gluconeogenic genes, *Pck1* and *G6pc*. Hepcidin serves as a gluconeogenic sensor in mice during starvation<sup>[62]</sup>. The reasons for the differential regulation of *Pck1* and *G6pc* expression in knockout mice livers are unclear. *Pck1* and *G6pc* are however regulated by various transcription factors including *Foxo1*<sup>[54]</sup> and iron regulates *Foxo1* in adipocytes<sup>[63]</sup>. The net effect of hepcidin and





**Figure 7** Protein expression levels of phosphorylated Jun N-terminal kinase and alpha smooth muscle actin in *Hamp1* floxed and knockout mice fed with high fat or regular diets for 3 or 7 mo. The expression levels of alpha smooth muscle actin ( $\alpha$ SMA) (A) and phosphorylated Jun N-terminal kinase (p-JNK) (B) proteins in the livers of floxed and knockout mice fed with regular or high fat sucrose (HFS) diets for 3 or 7 mo was determined by western blotting, as described in Material and Methods. An anti-gapdh antibody was used as control to determine equal protein loading; Gapdh: Glyceraldehyde 3-phosphate dehydrogenase.

iron on metabolic processes in the liver requires further investigation.

Despite amelioration of steatosis, high fat administration caused injury in the livers of *Hamp1* knockout mice. In fact, knockout mice displayed an earlier and more pronounced development of fibrosis compared to control mice. Previous studies using MCD experimental models have shown that iron supplementation attenuates steatosis and triggers fibrosis<sup>[24,64]</sup>. Of note, MCD diet does not reproduce the metabolic changes observed in NAFLD/NASH patients and induces weight loss<sup>[65,66]</sup>. On the other hand, most high fat diet models induce metabolic changes but not fibrosis<sup>[66,67]</sup>. Furthermore, introduction of iron in the diet can create secondary effects by up-regulating liver hepcidin synthesis and thereby inhibiting the expression of iron exporter, ferroportin<sup>[68-70]</sup>. This will then lead to sequestration of iron in Kupffer cells and trigger inflammation. These artefacts are avoided in our experimental system because iron accumulation is directly caused by the lack of hepcidin expression. Our high fat-fed *Hamp1* knockout mice, which develop early fibrosis, may therefore be an advantageous NAFLD/NASH model.

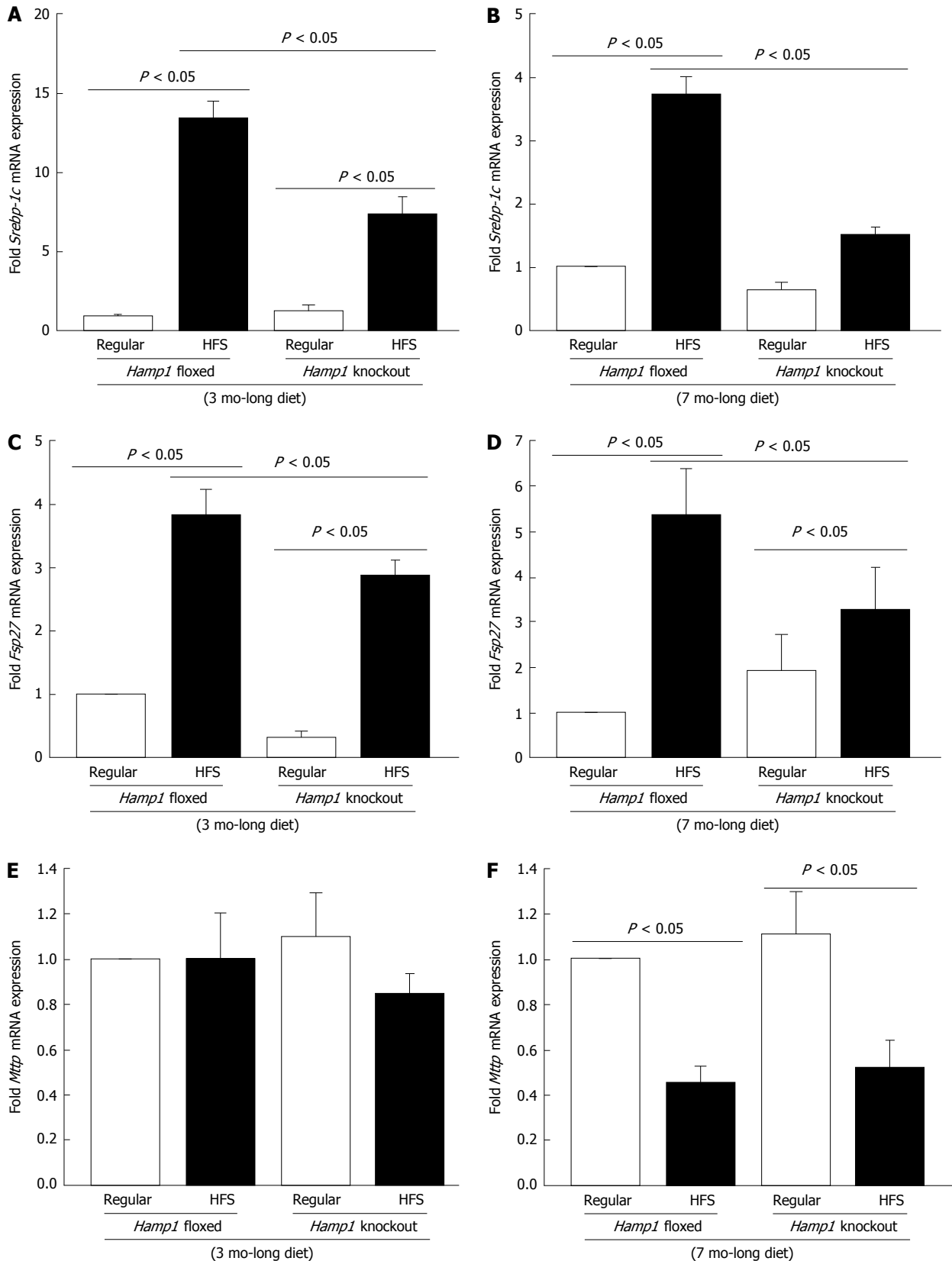
Simple steatosis is considered to be a benign condition in NAFLD patients. *In vivo* and *in vitro* studies have also shown this to be a beneficial process because triglycerides synthesis protects the liver from lipotoxicity induced by free fatty acid accumulation<sup>[64,71]</sup>. The de-

creased level of steatosis in synergy with iron might be responsible for early fibrosis development in the livers of HFS-fed *Hamp1* knockout mice.

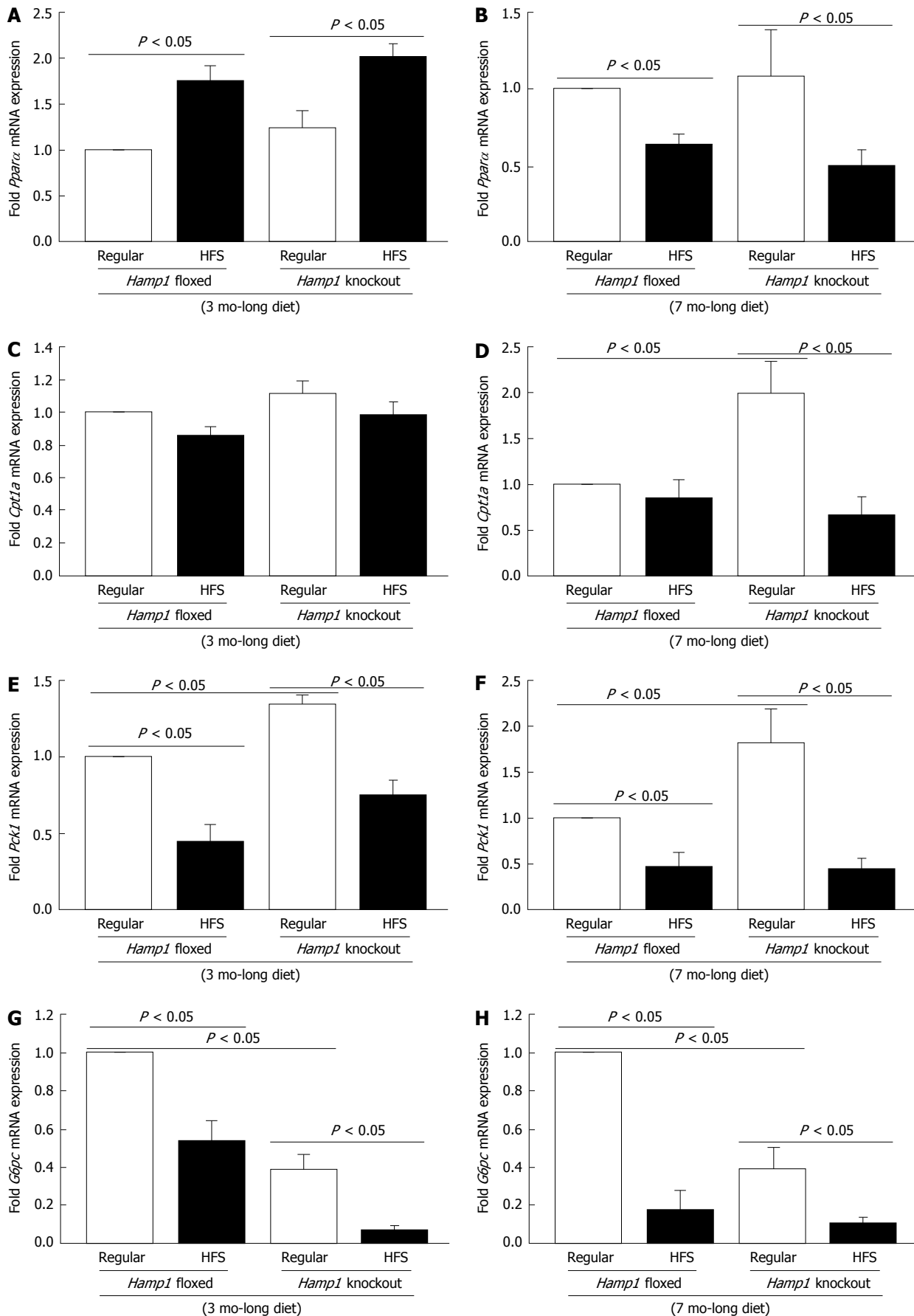
In summary, our findings strongly suggest a role for hepcidin in the regulation of hepatic lipid and carbohydrate metabolism. There are currently a limited number of NASH experimental models<sup>[66]</sup>. *Hamp1* knockout mice will therefore be useful to investigate the molecular mechanisms of metabolic processes and fibrosis in NASH pathogenesis.

Lack of hepcidin expression due to the deletion of *Hamp1* alleles inhibited lipid accumulation in the liver following a high fat and high sucrose diet administration. Lack of c-jun kinase phosphorylation and the changes in the expression of metabolic genes, which are involved in lipogenesis and lipid storage, played a role in attenuated steatosis observed in hepcidin knockout mice. Knockout mice developed fibrosis within 3 mo of high fat exposure, which was more prominent at 7 mo. Deletion of *Hamp1* alleles by itself modulated hepatic expression of genes involved in mitochondrial fatty acid oxidation and gluconeogenesis. In summary, hepcidin is associated with the regulation of metabolic processes in the liver and the lack of hepcidin expression triggers early fibrosis development. High fat-fed hepcidin knockout mice may therefore serve as a useful animal model to study different aspects of fatty liver disease pathogenesis.





**Figure 8** Expression of genes involved in lipogenesis, lipid storage and secretion. The mRNA expression levels of *Srebp-1c* (A and B), *Fsp27* (C and D), and *Mttp* (E and F) in the livers of floxed and knockout mice fed with regular and high fat sucrose (HFS) diets, was determined by real-time polymerase chain reaction. Gene expression in high fat-fed floxed or knockout and regular diet-fed knockout mice for 3 (A, C and E) or 7 mo (B, D and F) was expressed as fold change of that in floxed mice fed with a regular diet for the same time period.



**Figure 9 Expression of genes involved in  $\beta$ -oxidation and gluconeogenesis.** The mRNA expression levels of *Pparα* (A and B), *Cpt1a* (C and D), *Pck1* (E and F) and *G6pc* (G and H), in the livers of *Hamp1* floxed and knockout mice fed with regular and high fat sucrose (HFS) diets, was determined by real-time polymerase chain reaction. Gene expression in high fat-fed floxed or knockout and regular diet-fed knockout mice for 3 (A, C, E and G) or 7 mo (B, D, F and H) was expressed as fold change of that in floxed mice fed with a regular diet for the same time period.

## COMMENTS

## Background

Obesity-related metabolic syndrome and its hepatic manifestation, non-alcoholic fatty liver disease (NAFLD) are important public health problems. Hepcidin, synthesized primarily by the liver, is the key iron-regulatory hormone. The authors have previously shown a role for hepcidin in alcoholic liver disease. Hepcidin expression is modulated in NAFLD patients but its significance is unknown. Furthermore, there are only a few animal models of NAFLD, which resemble human disease pathology. The authors are one of the few laboratories with hepcidin transgenic mice models, which were employed in this study to investigate NAFLD pathogenesis.

## Research frontiers

NAFLD is a wide spectrum of disease ranging from simple benign fat accumulation (steatosis) to non-alcoholic steatohepatitis (NASH), which is characterized by inflammation (steatohepatitis) and fibrosis in the liver. A correlation between hepatic iron levels and disease severity in NAFLD/NASH patients has been clearly demonstrated. Since hepcidin is the central iron regulator, it is essential to understand its role in NAFLD/NASH.

## Innovations and breakthroughs

The previously published studies with hepcidin knockout mice generated in the laboratory have demonstrated significant iron accumulation in the liver. To establish a novel NAFLD/NASH experimental model, hepcidin knockout mice were fed with a high fat diet for different time periods. By showing that hepcidin is directly involved in lipid storage and fibrogenesis in the liver following high fat intake, the authors underlined the importance of hepcidin and iron homeostasis in NAFLD/NASH pathogenesis.

## Applications

This study indicated a role for hepcidin in the regulation of metabolic processes and early fibrosis development in the liver. These findings will further understanding of the mechanisms involved in NAFLD/NASH progression and liver fibrosis. Furthermore, the high fat-fed hepcidin knockout mice, as a novel experimental NAFLD/NASH model, can be useful in the search for functional biomarkers and therapeutics for NAFLD/NASH.

## Terminology

Hepcidin is essential for systemic iron homeostasis. Chronic high fat intake and obesity ultimately lead to metabolic syndrome, which is characterized by dyslipidemia and insulin resistance. Obesity also impairs metabolic functions and histology of the liver causing fat accumulation (steatosis), inflammation (steatohepatitis) and scar tissue formation (fibrogenesis), as observed in patients with NAFLD/NASH.

## Peer-review

This manuscript investigated the role of key iron-regulatory protein, hepcidin in non-alcoholic fatty liver disease in hepcidin (*Hamp1*) knockout and floxed control mice administered a high fat and high sucrose or a regular control diet for 3 or 7 mo. The authors suggest that *Hamp1* and iron may play a role in the regulation of metabolic pathways in the liver, which has implications for NAFLD pathogenesis. This manuscript was well designed *in vivo* experiments and well written with all the results obtained.

## REFERENCES

- 1 Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 330-344 [PMID: 23507799 DOI: 10.1038/nrgastro.2013.41]
- 2 Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]
- 3 Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]
- 4 Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. *Annu Rev Pathol* 2011; **6**: 425-456 [PMID: 21073339 DOI: 10.1146/annurev-pathol-011110-130246]
- 5 Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/S0016-5085(98)70599-2]
- 6 Wree A, Broderick L, Canbay A, Hoffman HM, Feldstein AE. From NAFLD to NASH to cirrhosis-new insights into disease mechanisms. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 627-636 [PMID: 23958599 DOI: 10.1038/nrgastro.2013.149]
- 7 Basaranoglu M, Basaranoglu G, Sentürk H. From fatty liver to fibrosis: a tale of "second hit". *World J Gastroenterol* 2013; **19**: 1158-1165 [PMID: 23483818 DOI: 10.3748/wjg.v19.i8.1158]
- 8 Tiniakos DG, Vos MB, Brunt EM. Nonalcoholic fatty liver disease: pathology and pathogenesis. *Annu Rev Pathol* 2010; **5**: 145-171 [PMID: 20078219 DOI: 10.1146/annurev-pathol-121808-102132]
- 9 Malaguarnera M, Di Rosa M, Nicoletti F, Malaguarnera L. Molecular mechanisms involved in NAFLD progression. *J Mol Med (Berl)* 2009; **87**: 679-695 [PMID: 19352614 DOI: 10.1007/s00109-009-0464-1]
- 10 Takaki A, Kawai D, Yamamoto K. Multiple hits, including oxidative stress, as pathogenesis and treatment target in non-alcoholic steatohepatitis (NASH). *Int J Mol Sci* 2013; **14**: 20704-20728 [PMID: 24132155 DOI: 10.3390/ijms141020704]
- 11 Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. *QJM* 2010; **103**: 71-83 [PMID: 19914930 DOI: 10.1093/qjmed/hcp158]
- 12 Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 2010; **51**: 679-689 [PMID: 20041406 DOI: 10.1002/hep.23280]
- 13 O'Brien J, Powell LW. Non-alcoholic fatty liver disease: is iron relevant? *Hepatol Int* 2012; **6**: 332-341 [PMID: 22020821 DOI: 10.1007/s12072-011-9304-9]
- 14 Martinelli N, Traglia M, Campostrini N, Biino G, Corbella M, Sala C, Busti F, Masciullo C, Manna D, Previtali S, Castagna A, Pistis G, Olivieri O, Toniolo D, Camaschella C, Girelli D. Increased serum hepcidin levels in subjects with the metabolic syndrome: a population study. *PLoS One* 2012; **7**: e48250 [PMID: 23144745 DOI: 10.1371/journal.pone.0048250]
- 15 Aigner E, Weiss G, Datz C. Dysregulation of iron and copper homeostasis in nonalcoholic fatty liver. *World J Hepatol* 2015; **7**: 177-188 [PMID: 25729473 DOI: 10.4254/wjh.v7.i2.177]
- 16 Valenti L, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, Canavesi E, Lattuada E, Roviato G, Marchesini G, Fargion S. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2010; **138**: 905-912 [PMID: 19931264 DOI: 10.1053/j.gastro.2009.11.013]
- 17 Nelson JE, Wilson L, Brunt EM, Yeh MM, Kleiner DE, Unalp-Arida A, Kowdley KV. Relationship between the pattern of hepatic iron deposition and histological severity in nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 448-457 [PMID: 21274866 DOI: 10.1002/hep.24038]
- 18 Nelson JE, Brunt EM, Kowdley KV. Nonalcoholic Steatohepatitis Clinical Research Network. Lower serum hepcidin and greater parenchymal iron in nonalcoholic fatty liver disease patients with C282Y HFE mutations. *Hepatology* 2012; **56**: 1730-1740 [PMID: 22611049 DOI: 10.1002/hep.25856]
- 19 Valenti L, Fracanzani AL, Dongiovanni P, Bugianesi E, Marchesini G, Manzini P, Vanni E, Fargion S. Iron depletion by phlebotomy improves insulin resistance in patients with nonalcoholic fatty liver disease and hyperferritinemia: evidence from a case-control study. *Am J Gastroenterol* 2007; **102**: 1251-1258 [PMID: 17391316 DOI: 10.1111/j.1572-0241.2007.01192.x]
- 20 Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest* 2004; **114**: 147-152 [PMID: 15254578 DOI: 10.1172/JCI22422]

- 21 **Ahmed U**, Latham PS, Oates PS. Interactions between hepatic iron and lipid metabolism with possible relevance to steatohepatitis. *World J Gastroenterol* 2012; **18**: 4651-4658 [PMID: 23002334 DOI: 10.3748/wjg.v18.i34.4651]
- 22 **Ramm GA**, Crawford DH, Powell LW, Walker NI, Fletcher LM, Halliday JW. Hepatic stellate cell activation in genetic haemochromatosis. Lobular distribution, effect of increasing hepatic iron and response to phlebotomy. *J Hepatol* 1997; **26**: 584-592 [PMID: 9075666 DOI: 10.1016/S0168-8278(97)80424-2]
- 23 **Cunnane SC**, McAdoo KR. Iron intake influences essential fatty acid and lipid composition of rat plasma and erythrocytes. *J Nutr* 1987; **117**: 1514-1519 [PMID: 3116180]
- 24 **Kirsch R**, Sijtsma HP, Tlali M, Marais AD, Hall Pde L. Effects of iron overload in a rat nutritional model of non-alcoholic fatty liver disease. *Liver Int* 2006; **26**: 1258-1267 [PMID: 17105592 DOI: 10.1111/j.1478-3231.2006.01329.x]
- 25 **Choi JS**, Koh IU, Lee HJ, Kim WH, Song J. Effects of excess dietary iron and fat on glucose and lipid metabolism. *J Nutr Biochem* 2013; **24**: 1634-1644 [PMID: 23643521 DOI: 10.1016/j.jnutbio.2013.02.004]
- 26 **Ganz T**, Nemeth E. Hepcidin and iron homeostasis. *Biochim Biophys Acta* 2012; **1823**: 1434-1443 [PMID: 22306005 DOI: 10.1016/j.bbamcr.2012.01.014]
- 27 **Pigeon C**, Ilyin G, Courselaud B, Leroyer P, Turlin B, Brissot P, Loréal O. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is over-expressed during iron overload. *J Biol Chem* 2001; **276**: 7811-7819 [PMID: 11113132 DOI: 10.1074/jbc.M008923200]
- 28 **Lou DQ**, Nicolas G, Lesbordes JC, Viatte L, Grimber G, Szajnert MF, Kahn A, Vaulont S. Functional differences between hepcidin 1 and 2 in transgenic mice. *Blood* 2004; **103**: 2816-2821 [PMID: 14604961 DOI: 10.1182/blood-2003-07-2524]
- 29 **Lu S**, Seravalli J, Harrison-Findik D. Inductively coupled mass spectrometry analysis of biometals in conditional Hamp1 and Hamp2 transgenic mouse models. *Transgenic Res* 2015; **24**: 765-773 [PMID: 25904410 DOI: 10.1007/s11248-015-9879-3]
- 30 **Ganz T**. Systemic iron homeostasis. *Physiol Rev* 2013; **93**: 1721-1741 [PMID: 24137020 DOI: 10.1152/physrev.00008.2013]
- 31 **Lesbordes-Brion JC**, Viatte L, Bennoun M, Lou DQ, Ramey G, Houbbron C, Hamard G, Kahn A, Vaulont S. Targeted disruption of the hepcidin 1 gene results in severe hemochromatosis. *Blood* 2006; **108**: 1402-1405 [PMID: 16574947 DOI: 10.1182/blood-2006-02-003376]
- 32 **Nemeth E**, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004; **306**: 2090-2093 [PMID: 15514116 DOI: 10.1126/science.1104742]
- 33 **van Dijk BA**, Laarakkers CM, Klaver SM, Jacobs EM, van Tits LJ, Janssen MC, Swinkels DW. Serum hepcidin levels are innately low in HFE-related haemochromatosis but differ between C282Y-homozygotes with elevated and normal ferritin levels. *Br J Haematol* 2008; **142**: 979-985 [PMID: 18557745 DOI: 10.1111/j.1365-2141.2008.07273.x]
- 34 **Bekri S**, Gual P, Anty R, Luciani N, Dahman M, Ramesh B, Iannelli A, Staccini-Myx A, Casanova D, Ben Amor I, Saint-Paul MC, Huet PM, Sadoul JL, Gugenheim J, Srai SK, Tran A, Le Marchand-Brustel Y. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. *Gastroenterology* 2006; **131**: 788-796 [PMID: 16952548 DOI: 10.1053/j.gastro.2006.07.007]
- 35 **Aigner E**, Theurl I, Theurl M, Lederer D, Haufe H, Dietze O, Strasser M, Datz C, Weiss G. Pathways underlying iron accumulation in human nonalcoholic fatty liver disease. *Am J Clin Nutr* 2008; **87**: 1374-1383 [PMID: 18469261]
- 36 **Senates E**, Yilmaz Y, Colak Y, Ozturk O, Altunoz ME, Kurt R, Ozkara S, Aksaray S, Tuncer I, Ovunc AO. Serum levels of hepcidin in patients with biopsy-proven nonalcoholic fatty liver disease. *Metab Syndr Relat Disord* 2011; **9**: 287-290 [PMID: 21417913 DOI: 10.1089/met.2010.0121]
- 37 **Hamza RT**, Hamed AI, Kharshoum RR. Iron homeostasis and serum hepcidin-25 levels in obese children and adolescents: relation to body mass index. *Horm Res Paediatr* 2013; **80**: 11-17 [PMID: 23817203 DOI: 10.1159/000351941]
- 38 **Sam AH**, Busbridge M, Amin A, Webber L, White D, Franks S, Martin NM, Sleeth M, Ismail NA, Daud NM, Papamargaritis D, Le Roux CW, Chapman RS, Frost G, Bloom SR, Murphy KG. Hepcidin levels in diabetes mellitus and polycystic ovary syndrome. *Diabet Med* 2013; **30**: 1495-1499 [PMID: 23796160 DOI: 10.1111/dme.12262]
- 39 **Junqueira LC**, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J* 1979; **11**: 447-455 [PMID: 91593]
- 40 **Folch J**, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957; **226**: 497-509 [PMID: 13428781]
- 41 **Harrison-Findik DD**, Klein E, Crist C, Evans J, Timchenko N, Gollan J. Iron-mediated regulation of liver hepcidin expression in rats and mice is abolished by alcohol. *Hepatology* 2007; **46**: 1979-1985 [PMID: 17763462 DOI: 10.1002/hep.21895]
- 42 **Czaja MJ**. JNK regulation of hepatic manifestations of the metabolic syndrome. *Trends Endocrinol Metab* 2010; **21**: 707-713 [PMID: 20888782 DOI: 10.1016/j.tem.2010.08.010]
- 43 **Ip YT**, Davis RJ. Signal transduction by the c-Jun N-terminal kinase (JNK)--from inflammation to development. *Curr Opin Cell Biol* 1998; **10**: 205-219 [PMID: 9561845 DOI: 10.1016/S0955-0674(98)80143-9]
- 44 **Chen G**, Liang G, Ou J, Goldstein JL, Brown MS. Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. *Proc Natl Acad Sci USA* 2004; **101**: 11245-11250 [PMID: 15266058 DOI: 10.1073/pnas.0404297101]
- 45 **Amemiya-Kudo M**, Shimano H, Yoshikawa T, Yahagi N, Hasty AH, Okazaki H, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Sato R, Kimura S, Ishibashi S, Yamada N. Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. *J Biol Chem* 2000; **275**: 31078-31085 [PMID: 10918064 DOI: 10.1074/jbc.M005353200]
- 46 **Gong J**, Sun Z, Li P. CIDE proteins and metabolic disorders. *Curr Opin Lipidol* 2009; **20**: 121-126 [PMID: 19276890 DOI: 10.1097/MOL.0b013e328328d0bb]
- 47 **Hussain MM**, Nijstad N, Franceschini L. Regulation of microsomal triglyceride transfer protein. *Clin Lipidol* 2011; **6**: 293-303 [PMID: 21808658 DOI: 10.2217/clp.11.21]
- 48 **Berger J**, Moller DE. The mechanisms of action of PPARs. *Annu Rev Med* 2002; **53**: 409-435 [PMID: 11818483 DOI: 10.1146/annurev.med.53.082901.104018]
- 49 **Bartlett K**, Eaton S. Mitochondrial beta-oxidation. *Eur J Biochem* 2004; **271**: 462-469 [PMID: 14728673 DOI: 10.1046/j.1432-1033.2003.03947.x]
- 50 **Lunova M**, Goehring C, Kuscuoglu D, Mueller K, Chen Y, Walther P, Deschemin JC, Vaulont S, Haybaeck J, Lackner C, Trautwein C, Strnad P. Hepcidin knockout mice fed with iron-rich diet develop chronic liver injury and liver fibrosis due to lysosomal iron overload. *J Hepatol* 2014; **61**: 633-641 [PMID: 24816174 DOI: 10.1016/j.jhep.2014.04.034]
- 51 **Nicolas G**, Bennoun M, Devaux I, Beaumont C, Grandchamp B, Kahn A, Vaulont S. Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. *Proc Natl Acad Sci USA* 2001; **98**: 8780-8785 [PMID: 11447267 DOI: 10.1073/pnas.151179498]
- 52 **Schattenberg JM**, Singh R, Wang Y, Lefkowitz JH, Rigoli RM, Scherer PE, Czaja MJ. JNK1 but not JNK2 promotes the development of steatohepatitis in mice. *Hepatology* 2006; **43**: 163-172 [PMID: 16374858 DOI: 10.1002/hep.20999]
- 53 **Singh R**, Wang Y, Xiang Y, Tanaka KE, Gaarde WA, Czaja MJ. Differential effects of JNK1 and JNK2 inhibition on murine steatohepatitis and insulin resistance. *Hepatology* 2009; **49**: 87-96 [PMID: 19053047 DOI: 10.1002/hep.22578]



- 54 **Rui L.** Energy metabolism in the liver. *Compr Physiol* 2014; **4**: 177-197 [PMID: 24692138 DOI: 10.1002/cphy.c130024]
- 55 **Sherman AR.** Lipogenesis in iron-deficient adult rats. *Lipids* 1978; **13**: 473-478 [PMID: 692295]
- 56 **Sherman AR,** Guthrie HA, Wolinsky I, Zulak IM. Iron deficiency hyperlipidemia in 18-day-old rat pups: effects of milk lipids, lipoprotein lipase, and triglyceride synthesis. *J Nutr* 1978; **108**: 152-162 [PMID: 619036]
- 57 **Davis MR,** Rendina E, Peterson SK, Lucas EA, Smith BJ, Clarke SL. Enhanced expression of lipogenic genes may contribute to hyperglycemia and alterations in plasma lipids in response to dietary iron deficiency. *Genes Nutr* 2012; **7**: 415-425 [PMID: 22228222 DOI: 10.1007/s12263-011-0278-y]
- 58 **Begriche K,** Igoudjil A, Pessayre D, Fromenty B. Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it. *Mitochondrion* 2006; **6**: 1-28 [PMID: 16406828 DOI: 10.1016/j.mito.2005.10.004]
- 59 **Pawlak M,** Lefebvre P, Staels B. Molecular mechanism of PPAR $\alpha$  action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *J Hepatol* 2015; **62**: 720-733 [PMID: 25450203 DOI: 10.1016/j.jhep.2014.10.039]
- 60 **Pineda Torra I,** Jamshidi Y, Flavell DM, Fruchart JC, Staels B. Characterization of the human PPAR $\alpha$  promoter: identification of a functional nuclear receptor response element. *Mol Endocrinol* 2002; **16**: 1013-1028 [PMID: 11981036 DOI: 10.1210/mend.16.5.0833]
- 61 **Richardson DR,** Lane DJ, Becker EM, Huang ML, Whitnall M, Suryo Rahmanto Y, Sheftel AD, Ponka P. Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion and cytosol. *Proc Natl Acad Sci USA* 2010; **107**: 10775-10782 [PMID: 20495089 DOI: 10.1073/pnas.0912925107]
- 62 **Vecchi C,** Montosi G, Garuti C, Corradini E, Sabelli M, Canali S, Pietrangelo A. Gluconeogenic signals regulate iron homeostasis via hepcidin in mice. *Gastroenterology* 2014; **146**: 1060-1069 [PMID: 24361124 DOI: 10.1053/j.gastro.2013.12.016]
- 63 **Gabrielsen JS,** Gao Y, Simcox JA, Huang J, Thorup D, Jones D, Cooksey RC, Gabrielsen D, Adams TD, Hunt SC, Hopkins PN, Cefalu WT, McClain DA. Adipocyte iron regulates adiponectin and insulin sensitivity. *J Clin Invest* 2012; **122**: 3529-3540 [PMID: 22996660 DOI: 10.1172/JCI44421]
- 64 **Yamaguchi K,** Yang L, McCall S, Huang J, Yu XX, Pandey SK, Bhanot S, Monia BP, Li YX, Diehl AM. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology* 2007; **45**: 1366-1374 [PMID: 17476695 DOI: 10.1002/hep.21655]
- 65 **Larter CZ,** Yeh MM. Animal models of NASH: getting both pathology and metabolic context right. *J Gastroenterol Hepatol* 2008; **23**: 1635-1648 [PMID: 18752564 DOI: 10.1111/j.1440-1746.2008.05543.x]
- 66 **Schattenberg JM,** Galle PR. Animal models of non-alcoholic steatohepatitis: of mice and man. *Dig Dis* 2010; **28**: 247-254 [PMID: 20460919 DOI: 10.1159/000282097]
- 67 **Imajo K,** Yoneda M, Kessoku T, Ogawa Y, Maeda S, Sumida Y, Hyogo H, Eguchi Y, Wada K, Nakajima A. Rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Int J Mol Sci* 2013; **14**: 21833-21857 [PMID: 24192824 DOI: 10.3390/ijms141121833]
- 68 **Ramos E,** Kautz L, Rodriguez R, Hansen M, Gabayan V, Ginzburg Y, Roth MP, Nemeth E, Ganz T. Evidence for distinct pathways of hepcidin regulation by acute and chronic iron loading in mice. *Hepatology* 2011; **53**: 1333-1341 [PMID: 21480335 DOI: 10.1002/hep.24178]
- 69 **Feng Q,** Migas MC, Waheed A, Britton RS, Fleming RE. Ferritin upregulates hepatic expression of bone morphogenetic protein 6 and hepcidin in mice. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G1397-G1404 [PMID: 22517766 DOI: 10.1152/ajpgi.00020.2012]
- 70 **Corradini E,** Meynard D, Wu Q, Chen S, Ventura P, Pietrangelo A, Babitt JL. Serum and liver iron differently regulate the bone morphogenetic protein 6 (BMP6)-SMAD signaling pathway in mice. *Hepatology* 2011; **54**: 273-284 [PMID: 21488083 DOI: 10.1002/hep.24359]
- 71 **Listenberger LL,** Han X, Lewis SE, Cases S, Farese RV, Ory DS, Schaffer JE. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci USA* 2003; **100**: 3077-3082 [PMID: 12629214 DOI: 10.1073/pnas.0630588100]

P- Reviewer: Yu DY S- Editor: Ma YJ

L- Editor: A E- Editor: Liu SQ



Retrospective Study

# Total hepatectomy and liver transplantation as a two-stage procedure for fulminant hepatic failure: A safe procedure in exceptional circumstances

Rebeca Sanabria Mateos, Niamh M Hogan, Dimitri Dorcaratto, Helen Heneghan, Venkatesh Udupa, Donal Maguire, Justin Geoghegan, Emir Hoti

Rebeca Sanabria Mateos, Niamh M Hogan, Dimitri Dorcaratto, Helen Heneghan, Venkatesh Udupa, Donal Maguire, Justin Geoghegan, Emir Hoti, Hepatobiliary and Liver Transplant Surgical Unit, St. Vincent's University Hospital, Dublin 4, Ireland

**Author contributions:** Sanabria Mateos R designed and performed the research and wrote the paper; Hogan NM supervised the report; Dorcaratto D designed the research and contributed to the analysis; Heneghan H and Udupa V supervised the report; Maguire D, Geoghegan J and Hoti E provided clinical advice and supervised the report.

**Institutional review board statement:** This study was reviewed and approved by St. Vincent's University Hospital Institutional Review Board.

**Informed consent statement:** Patients were not required to give informed consent because the analyses use anonymous clinical data.

**Conflict-of-interest statement:** We have no financial relationships to disclose.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Rebeca Sanabria Mateos, Hepatobiliary and Liver Transplant Unit, St. Vincent's University Hospital, Elm Park, Dublin 4, Ireland. [rebecasanabria@gmail.com](mailto:rebecasanabria@gmail.com)  
Telephone: +353-1-2214000

Received: November 3, 2015

Peer-review started: November 3, 2015  
First decision: December 4, 2015  
Revised: December 17, 2015  
Accepted: January 16, 2016  
Article in press: January 19, 2016  
Published online: February 8, 2016

## Abstract

**AIM:** To evaluate the outcomes of two-stage liver transplant at a single institution, between 1993 and March 2015.

**METHODS:** We reviewed our institutional experience with emergency hepatectomy followed by transplantation for fulminant liver failure over a twenty-year period. A retrospective review of a prospectively maintained liver transplant database was undertaken at a national liver transplant centre. Demographic data, clinical presentation, preoperative investigations, cardio-circulatory parameters, operative and postoperative data were recorded.

**RESULTS:** In the study period, six two-stage liver transplants were undertaken. Indications for transplantation included acute paracetamol poisoning ( $n = 3$ ), fulminant hepatitis A ( $n = 1$ ), trauma ( $n = 1$ ) and exertional heat stroke ( $n = 1$ ). Anhepatic time ranged from 330 to 2640 min. All patients demonstrated systemic inflammatory response syndrome in the first post-operative week and the incidence of sepsis was high at 50%. There was one mortality, secondary to cardiac arrest 12 h following re-perfusion. Two patients required re-transplantation secondary to arterial thrombosis. At a median follow-up of 112 mo, 5 of 6 patients are alive and without evidence of graft dysfunction.

**CONCLUSION:** Two-stage liver transplantation represents a safe and potentially life-saving treatment for carefully selected exceptional cases of fulminant hepatic failure.

**Key words:** Two-stage liver transplantation; Fulminant hepatic failure; Liver transplant; Survival

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We share our experience with selected cases of emergency total hepatectomy followed by liver transplantation for fulminant hepatic failure. This involves initial haemodynamic stabilization by recipient hepatectomy, creating a temporary porto-caval shunt to permit venous drainage during a variable anhepatic phase, then orthotopic transplantation once a suitable donor graft is available.

Sanabria Mateos R, Hogan NM, Dorcaratto D, Heneghan H, Udupa V, Maguire D, Geoghegan J, Hoti E. Total hepatectomy and liver transplantation as a two-stage procedure for fulminant hepatic failure: A safe procedure in exceptional circumstances. *World J Hepatol* 2016; 8(4): 226-230 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i4/226.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i4.226>

## INTRODUCTION

Liver transplantation is the treatment of choice for acute or chronic end-stage liver disease. In cases of acute liver failure, often the only life-saving intervention is a super-urgent liver transplantation. However, immediate allocation of a donor organ is not always achievable. Without urgent hepatectomy, some patients with fulminant hepatic failure develop a toxic hepatic syndrome with potentially catastrophic haemorrhage<sup>[1]</sup>. Toxic liver syndrome is defined as complete liver necrosis associated with critical multi-organ dysfunction<sup>[2]</sup>. In this grave circumstance, these critically-ill patients may benefit from a two-stage approach to transplantation; with urgent explantation of the toxic liver and creation of a temporary portocaval shunt, followed by transplantation as soon as a donor organ becomes available<sup>[2]</sup>. First reported in 1988 by Ringe *et al*<sup>[2]</sup> for a patient with primary graft failure causing multi-organ dysfunction, the goal of the first stage of this technique is haemodynamic and metabolic stabilisation. Subsequent to Ringe's description of this novel approach to retransplantation for primary graft failure, a wider variety of indications for this technique have been sporadically reported including liver trauma, spontaneous hepatic rupture, haemolysis elevated liver enzymes and low platelet syndrome associated with preeclampsia, and acute deterioration of chronic liver disease<sup>[2-11]</sup>. During the first stage of these two-stage transplantations, the inferior vena cava is

retained and a porto-caval anastomosis allows systemic and portal venous drainage during the subsequent anhepatic period<sup>[2-11]</sup>. Once an allograft becomes available, the second stage involves orthotopic liver transplantation using a modified piggyback technique without venovenous bypass<sup>[2-11]</sup>.

## MATERIALS AND METHODS

A retrospective review of a prospectively maintained database was undertaken to identify all patients who underwent two-stage liver transplantation at a single institution (a National Liver Transplant Unit) between January 1993 and March 2015. Demographic data, clinical presentation, preoperative investigations, operative details, postoperative course, and histopathological results were recorded. Data collection and analyses were performed with Statistical Package for the Social Sciences (version 16.0) (SPSS, Chicago, IL, United States). Descriptive statistics were computed for all variables. The Kolmogorov-Smirnov test was used to determine the variables' distribution. For nonparametric data, continuous variables are presented as median values (and range) and the Mann-Whitney *U* test was used for any two sample comparisons. Dichotomous variables were compared using the  $\chi^2$  test. All tests were two tailed and results with a *P*-value of < 0.05 were considered statistical significant.

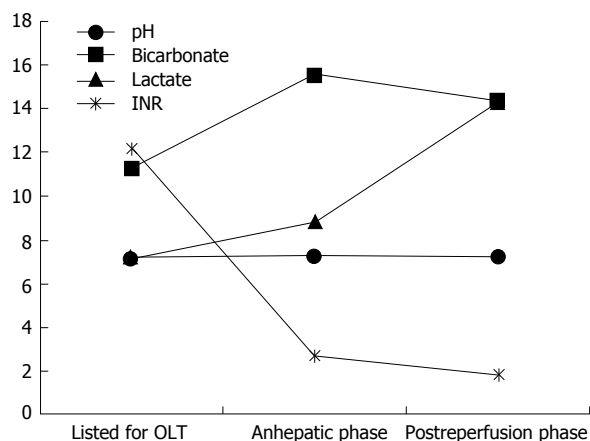
## RESULTS

During the study period, six cases of two-stage liver transplantation were undertaken in our centre. There was a male preponderance (4 males, 2 females). Median age at presentation was 28 years (range 20-47). Two patients had a past medical history of depression, no other co-morbidities were present. The most common indication for super-urgent transplantation was extensive liver necrosis secondary to paracetamol overdose (*n* = 3). One patient developed fulminant liver failure secondary to Hepatitis A infection. One patient had extensive haemorrhage secondary to liver trauma, and one patient developed exertional heat stroke causing ischemic hepatitis while running the final stages of an ultra-marathon. Five of six patients had evidence of toxic liver syndrome, as previously defined. The median model end stage liver disease was 39.50 (range 28-40). All patients had hypoglycemia and metabolic acidosis. In 50% of these cases, haemofiltration was initiated prior to hepatectomy, and all patients were intubated and mechanically ventilated before the procedure. The liver trauma case was the only patient who did not demonstrate signs of encephalopathy. Features of cerebral edema were present in 50% of cases. The anhepatic time ranged from 330 and 2640 min. In two patients, the hepatectomy was performed when a donor graft had been accepted for harvest but was not yet available. In one case, the hepatectomy was

**Table 1** Transplant details and patient survival

	Sex, age	Indication	MELD	Toxic liver syndrome	CVVH	Hptc before graft availability	Inotrope requirements	Anhepatic phase (h)	Survival (mo)
1	F, 26	Paracetamol overdose	39	Yes	No	Yes	Yes	7	116
2	M, 41	Heat stroke	40	Yes	Yes	Yes	Yes	16	22
3	M, 47	Fulminant hepatitis A	40	Yes	Yes	No	Yes	5.5	12 h
4	M, 20	Paracetamol overdose	40	Yes	No	No	No	5.8	107
5	F, 30	Paracetamol overdose	36	Yes	Yes	Yes	Yes	44	108
6	M, 21	Liver trauma	28	No	No	Yes	No	15	106

MELD: Model end stage liver disease; CVVH: Continuous venovenous hemodialysis; Hptc: Hepatectomy.



**Figure 1** Evolution of coagulation and gasometry parameters at all three stages of the procedure. INR: International normalized ratio; pH: Potential of hydrogen; OLT: Orthotopic liver transplant.

undertaken prior to acceptance of a donor organ due to uncontrollable haemorrhage in the recipient and the patient was listed as “super-urgent” for transplantation. For the remaining three patients the hepatectomy was performed at the time of listing the patient for superurgent transplantation (Table 1).

All patients underwent total hepatectomy and end-to side portocaval anastomosis with temporary abdominal closure as the first of a two-stage transplantation. During the anhepatic phase, patients were managed in the intensive care unit and received haemofiltration with plasma separation treatment. Orthotopic liver transplantation was then performed as soon as an allograft became available. In all cases, histological evaluation of the native liver confirmed the indication for emergency liver transplantation (total liver necrosis regardless the etiology). The use of noradrenaline was required in 4 patients before total hepatectomy with a median of 67 µg/min (range 50-200). During the anhepatic period the inotropic requirements increased to 74 µg/min (10-120), however inotropic requirements decreased immediately after reperfusion of the donor grafts, with a median noradrenaline requirement of 26 µg/min (range 5-60).

Arterial blood gasometry parameters demonstrated increased levels of lactate during the anhepatic phase from a median of 7.2 mmol/L pre-hepatectomy to 8.8

mmol/L. However their acidosis improved during the anhepatic phase as reflected by an increase in pH from median of 7.14 to 7.25, and an increase in bicarbonate from 11.3 to 15.6 mmol/L (Figure 1). Coagulation parameters pre-hepatectomy, during the anhepatic phase, and post transplant are shown in Table 2. During graft implantation, median blood loss was 8.5 L (range 2.5-43 L). All patients received transfusion of blood products, pools of platelets (median of 4 pools), plasma products (median of 9.5 units) and packed red cells (median 8.5 units). During the anhepatic phase, three patients developed ventricular tachycardia which was treated with amiodarone infusion. One patient (liver trauma case) required repeated cardioversions during the anhepatic phase as well as after reperfusion of the donor graft.

All patients ( $n = 6$ ) fulfilled criteria for the diagnosis of systemic inflammatory response syndrome in the first post-transplant week, with 50% having a source of sepsis identified which required anti-microbial treatment with a single broad-spectrum agent. The median time to extubation was 7 d (range 5-15), haemodialysis duration was 20 d (range 6-28) and median hospital stay was 33 d (range 2-210). Two patients required re-transplantation secondary to arterial thrombosis (33%). One of these patients necessitated right hemicolectomy secondary to ileocolic arterial ischemia as well as the early hepatic artery thrombosis which required re-transplantation. There was a single mortality which was due to cardiac arrest and occurred 12 h after reperfusion of the graft, with a median follow-up of 112 mo.

## DISCUSSION

Acute liver failure is a rapidly devastating pathology due to its potential to precipitate multi-organ failure, sepsis and cerebral oedema. Despite advances in supportive care, liver transplantation remains the only potentially life-saving treatment. Although fulminant hepatic failure (FHF) is not a common indication for orthotopic liver transplantation, these patients nonetheless represent a significant challenge for transplant surgeons. Data suggests that the most important prognostic indicators for patients with FHF undergoing transplantation are the degree of encephalopathy, patient's age, the etiology of FHF, and the time to transplantation with the majority of



**Table 2** Biochemical data pre and post transplant

No. of patient		Dose NA	pH	Bicarbonate (mmol/L)	Lactate (mmol/L)	INR	Sodium (mmol/L)	Potassium (mmol/L)
1	Before Hptc	70	7.14	11.30	5.32	11.25	133	3.9
	Anhepatic	80	7.24	17.20	7.3	2.50	133	3.7
	Post reperfusion	60	7.27	16.40	6	1.85	135	5.4
2	Before Hptc	64	7.15	13.30	9.20	15.80	136	5.5
	Anhepatic	80	7.26	14.00	11	3.33	137	4.5
	Post reperfusion	20	7.20	14.60	10	2.27	145	3.9
3	Before Hptc	50	7.06	14.70	10.20	13	138	4.8
	Anhepatic	36	7.17	13.30	10	1.75	143	4.9
	Post reperfusion	48	7.12	13.80	10.5	1.75	146	5.0
4	Before Hptc	None	7.34	11.10	5.30	17	133	3.6
	Anhepatic	10	7.34	20.70	4.20	2.90	133	3.6
	Post reperfusion	5	7.32	18.60	3.7	1.64	136	3.8
5	Before Hptc	200	7.16	11.30	12.00	6.3	148	3.7
	Anhepatic	68	7.26	18.00	12.30	3.01	141	4.2
	Post reperfusion	32	7.19	14.00	6.7	2.47	144	5.20
6	Before Hptc	None	7.02	10.70	3.30	10.59	151	5.0
	Anhepatic	120	6.91	13.50	7.7	2.46	146	4.3
	Post reperfusion	10	7.01	12.70	7.7	1.61	144	3.8
Median	Before Hptc	67	7.14	11.3	7.26	12.12	137	4.35
	Anhepatic	(50-200)	(7.02-7.34)	(10.70-14.7)	(3.3-12)	(6.3-17)	(133-152)	(3.6-5.5)
	Post reperfusion	26	7.19	14.3	14.3	1.8	144	4.45
range	Before Hptc	(5-60)	(7.01-7.32)	(12.7-18.60)	(12.7-18.60)	(1.61-2.47)	(135-146)	(3.8-5.4)
	Anhepatic	74	7.25	15.6	8.85	2.7	139	4.3
	Post reperfusion	(10-120)	(6.91-7.34)	(13.30-20.70)	(4.2-12.3)	(1.75-3.33)	(133-146)	(3.6-4.9)

INR: International normalized ratio; pH: Potential of hydrogen; NA: Noradrenaline; Hptc: Hepatectomy.

authors concurring that transplantation within 48-72 h is critical to reduce mortality<sup>[1]</sup>.

The anhepatic state requires considerable expertise in critical care to manage these gravely ill patients. In addition to cardiorespiratory support, haemofiltration and plasma separation are essential to prevent the development of severe lactate acidosis. The longest anhepatic period compatible with life is reported to be 66 h, which was recorded in a child with liver graft non-function after transplantation<sup>[12]</sup>. Herein we report a maximum anhepatic time of 44 h, in a patient who survived despite requiring re-transplantation secondary to hepatic artery thrombosis.

The number of cases of two-stage transplantation reported in the literature is scant and therefore survival rates vary widely<sup>[2-11]</sup>. However, advances in surgical techniques and supportive care appear to have exerted a beneficial effect on survival over time. In his seminal work almost two decades ago, Ringe *et al*<sup>[2,3]</sup> reported 32 patients treated with two-stage transplantation with 24 mortalities (75% mortality). In 2001, Domínguez Fernández *et al*<sup>[7]</sup> reported their outcomes from a series of eight patients who underwent emergency hepatectomy for FHF. Two patients died before a donor liver became available. A further five of six patients who underwent transplantation after an anhepatic period died postoperatively secondary to primary nonfunction or sepsis causing multiorgan failure (87.5% mortality). Herein, we report a series of six patients with a single death (16.6% mortality).

In conclusion, we report a series of cases of two-stage liver transplantation, which is a potentially life-

saving procedure in carefully selected patients in exceptional clinical circumstances.

## COMMENTS

### Background

In the setting of fulminant liver failure, immediate donor graft allocation for life-saving transplant may not always be possible and a two-stage approach may be necessary. This involves initial haemodynamic stabilisation by recipient hepatectomy - creating a temporary porto-caval shunt to allow circulation during a variable anhepatic phase. Once an allograft becomes available, orthotopic transplantation is undertaken using the standard technique. In this study, the authors evaluated the outcomes of two-stage liver transplant at a single institution, between 1993 and March 2015.

### Research frontiers

In cases of acute liver failure, the only life-saving procedure is frequently an emergency liver transplantation. However, immediate allocation of a donor organ is not always possible, particularly in the acute setting. Without urgent removal of the native liver, patients with fulminant hepatic failure, regardless of aetiology, can develop a life-threatening toxic hepatic syndrome. The results of this study suggest that in carefully selected patients a two-stage approach to super-urgent liver transplantation has utility, and can salvage these patients from the multi-organ failure arising from a toxic liver.

### Innovations and breakthroughs

In this study, two-stage liver transplantation appears to be a valuable, albeit exceptional, approach to the management of fulminant liver failure with associated toxic liver syndrome. The authors report a series of six patients treated with emergency hepatectomy and temporary portocaval shunt, followed by urgent orthotopic liver transplantation once a suitable donor graft became available. There was a single perioperative mortality in this series. Although two of the surviving five patients subsequently required re-transplantation for hepatic artery thrombosis, all are alive and without evidence of current graft dysfunction at a median follow-up of 112 mo. The number of cases of two-stage transplantation reported in the literature is scant, therefore mortality and

morbidity rates are largely unknown. This report contributes such data to the transplant literature.

## Applications

This study suggests that two-stage liver transplantation is a potentially life-saving procedure in carefully selected patients and in exceptional clinical circumstances.

## Terminology

Toxic liver syndrome: A critical systemic inflammatory syndrome-like response defined as complete liver necrosis associated with critical multi-organ dysfunction. Two-stage liver transplantation: A procedure which involves emergency hepatectomy and end-to-side portocaval anastomosis in the first stage, followed by liver transplantation when a donor organ becomes available in the second stage.

## Peer-review

The authors of this paper evaluated the outcomes of two-stage liver transplantation as an exceptional procedure in carefully selected patients with fulminant hepatic failure. Further reports of such cases are necessary to better evaluate its safety and utility in the management of acute liver failure.

## REFERENCES

- 1 **Gotthardt D**, Riediger C, Weiss KH, Encke J, Schemmer P, Schmidt J, Sauer P. Fulminant hepatic failure: etiology and indications for liver transplantation. *Nephrol Dial Transplant* 2007; **22** Suppl 8: viii5-viii8 [PMID: 17890263 DOI: 10.1093/ndt/gfm650]
- 2 **Ringe B**, Pichlmayr R, L  bbe N, Bornscheuer A, Kuse E. Total hepatectomy as temporary approach to acute hepatic or primary graft failure. *Transplant Proc* 1988; **20**: 552-557 [PMID: 3279648]
- 3 **Ringe B**, L  bbe N, Kuse E, Frei U, Pichlmayr R. Total hepatectomy and liver transplantation as two-stage procedure. *Ann Surg* 1993; **218**: 3-9 [PMID: 8328827 DOI: 10.1097/00000658-199307000-00002]
- 4 **Rozga J**, Podesta L, LePage E, Hoffman A, Morsiani E, Sher L, Woolf GM, Makowka L, Demetriou AA. Control of cerebral

- oedema by total hepatectomy and extracorporeal liver support in fulminant hepatic failure. *Lancet* 1993; **342**: 898-899 [PMID: 8105168 DOI: 10.1016/0140-6736(93)91947-K]
- 5 **So SK**, Barteau JA, Perdrizet GA, Marsh JW. Successful retransplantation after a 48-hour anhepatic state. *Transplant Proc* 1993; **25**: 1962-1963 [PMID: 8385827]
- 6 **Oldhafer KJ**, Bornscheuer A, Fr  hauf NR, Frerker MK, Schlitt HJ, Ringe B, Raab R, Pichlmayr R. Rescue hepatectomy for initial graft non-function after liver transplantation. *Transplantation* 1999; **67**: 1024-1028 [PMID: 10221488]
- 7 **Dom  nguez Fern  ndez E**, Lange K, Lange R, Eigler FW. Relevance of two-stage total hepatectomy and liver transplantation in acute liver failure and severe liver trauma. *Transpl Int* 2001; **14**: 184-190 [PMID: 11499909 DOI: 10.1111/j.1432-2277.2001.tb00039.x]
- 8 **Erhard J**, Lange R, Niebel W, Scherer R, Kox WJ, Philipp T, Eigler FW. Acute liver necrosis in the HELLP syndrome: successful outcome after orthotopic liver transplantation. A case report. *Transpl Int* 1993; **6**: 179-181 [PMID: 8499073 DOI: 10.1111/j.1432-2277.1993.tb00643.x]
- 9 **Chiumello D**, Gatti S, Caspani L, Savioli M, Fassati R, Gattinoni L. A blunt complex abdominal trauma: total hepatectomy and liver transplantation. *Intensive Care Med* 2002; **28**: 89-91 [PMID: 11819007 DOI: 10.1007/s00134-001-1162-9]
- 10 **Heneghan HM**, Nazirawan F, Dorcaratto D, Fiore B, Boylan JF, Maguire D, Hoti E. Extreme heatstroke causing fulminant hepatic failure requiring liver transplantation: a case report. *Transplant Proc* 2014; **46**: 2430-2432 [PMID: 24998305 DOI: 10.1016/j.transproceed.2013.12.055]
- 11 **Guirl MJ**, Michael J, Weinstein JS, Goldstein RM, Levy MF, Klintmalm GB. "Two-stage total hepatectomy and liver transplantation for acute deterioration of chronic liver disease: A new bridge to transplantation. *Liver Transpl* 2004; **10**: 564-570 [PMID: 15048803 DOI: 10.1002/lt.20134]
- 12 **Hammer GB**, So SK, Al-Uzri A, Conley SB, Concepcion W, Cox KL, Berquist WE, Esquivel CO. Continuous venovenous hemofiltration with dialysis in combination with total hepatectomy and portocaval shunting. Bridge to liver transplantation. *Transplantation* 1996; **62**: 130-132 [PMID: 8693530 DOI: 10.1097/00007890-199607150-00026]

**P- Reviewer:** Ferraioli G, He ST, Savopoulos CG  
**S- Editor:** Qi Y **L- Editor:** A **E- Editor:** Liu SQ



# Portal hypertensive gastropathy: A systematic review of the pathophysiology, clinical presentation, natural history and therapy

Mihajlo Gjeorgjievski, Mitchell S Cappell

Mihajlo Gjeorgjievski, Mitchell S Cappell, Division of Gastroenterology and Hepatology, William Beaumont Hospital, Royal Oak, MI 48073, United States

Mitchell S Cappell, Oakland University William Beaumont School of Medicine, Royal Oak, MI 48073, United States

**Author contributions:** Gjeorgjievski M and Cappell MS are equal authors; they contributed equally to conceiving and planning this project, to performing the systematic review, to retrieving and analyzing the data from the literature, to writing the manuscript, and to editing of the final manuscript.

**Conflict-of-interest statement:** None for all authors. This paper does not discuss any confidential pharmaceutical industry data reviewed by Cappell MS as a consultant for the United States Food and Drug Administration (FDA) Advisory Committee on Gastrointestinal Drugs. Cappell MS is a member of the speaker's bureau for AstraZeneca. This paper does not discuss any drug manufactured or marketed by AstraZeneca.

**Data sharing statement:** The paper has no original data or original statistics presented. No additional data are, therefore, available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Mitchell S Cappell, MD, PhD, Chief, Division of Gastroenterology and Hepatology, William Beaumont Hospital, MOB #602, 3535 West Thirteen Mile Road, Royal Oak, MI 48073, United States. [mccappell@yahoo.com](mailto:mccappell@yahoo.com)  
Telephone: +1-248-5511227  
Fax: +1-248-551758

Received: October 2, 2015  
Peer-review started: October 4, 2015

First decision: November 4, 2015

Revised: November 30, 2015

Accepted: January 16, 2016

Article in press: January 19, 2016

Published online: February 8, 2016

## Abstract

**AIM:** To describe the pathophysiology, clinical presentation, natural history, and therapy of portal hypertensive gastropathy (PHG) based on a systematic literature review.

**METHODS:** Computerized search of the literature was performed *via* PubMed using the following medical subject headings or keywords: "portal" and "gastropathy"; or "portal" and "hypertensive"; or "congestive" and "gastropathy"; or "congestive" and "gastroenteropathy". The following criteria were applied for study inclusion: Publication in peer-reviewed journals, and publication since 1980. Articles were independently evaluated by each author and selected for inclusion by consensus after discussion based on the following criteria: Well-designed, prospective trials; recent studies; large study populations; and study emphasis on PHG.

**RESULTS:** PHG is diagnosed by characteristic endoscopic findings of small polygonal areas of variable erythema surrounded by a pale, reticular border in a mosaic pattern in the gastric fundus/body in a patient with cirrhotic or non-cirrhotic portal hypertension. Histologic findings include capillary and venule dilatation, congestion, and tortuosity, without vascular fibrin thrombi or inflammatory cells in gastric submucosa. PHG is differentiated from gastric antral vascular ectasia by a different endoscopic appearance. The etiology of PHG is inadequately understood. Portal hypertension is necessary but insufficient to develop PHG because many patients have portal hypertension without PHG.

PHG increases in frequency with more severe portal hypertension, advanced liver disease, longer liver disease duration, presence of esophageal varices, and endoscopic variceal obliteration. PHG pathogenesis is related to a hyperdynamic circulation, induced by portal hypertension, characterized by increased intrahepatic resistance to flow, increased splanchnic flow, increased total gastric flow, and most likely decreased gastric mucosal flow. Gastric mucosa in PHG shows increased susceptibility to gastrototoxic chemicals and poor wound healing. Nitrous oxide, free radicals, tumor necrosis factor- $\alpha$ , and glucagon may contribute to PHG development. Acute and chronic gastrointestinal bleeding are the only clinical complications. Bleeding is typically mild-to-moderate. Endoscopic therapy is rarely useful because the bleeding is typically diffuse. Acute bleeding is primarily treated with octreotide, often with concomitant proton pump inhibitor therapy, or secondarily treated with vasopressin or terlipressin. Nonselective  $\beta$ -adrenergic receptor antagonists, particularly propranolol, are used to prevent bleeding after an acute episode or for chronic bleeding. Iron deficiency anemia from chronic bleeding may require iron replacement therapy. Transjugular-intrahepatic-portosystemic-shunt and liver transplantation are highly successful ultimate therapies because they reduce the underlying portal hypertension.

**CONCLUSION:** PHG is important to recognize in patients with cirrhotic or non-cirrhotic portal hypertension because it can cause acute or chronic GI bleeding that often requires pharmacologic therapy.

**Key words:** Portal hypertensive gastropathy; Congestive gastropathy; Portal hypertension; Cirrhosis; Cirrhotic; Chronic liver disease; Nonvariceal upper gastrointestinal bleeding; Esophageal varices; Hepatic fibrosis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Portal hypertensive gastropathy (PHG) is diagnosed by characteristic endoscopic findings of variably erythematous, small, polygonal areas surrounded by a whitish, reticular border in a mosaic pattern in the gastric fundus/body in a patient with portal hypertension of any etiology. The pathophysiology of PHG is inadequately understood. Portal hypertension is a prerequisite to develop PHG. PHG increases in frequency with increasing portal hypertension, liver disease progression, duration of liver disease, presence of esophageal varices, and endoscopic variceal obliteration. Pathogenesis is related to a hyperdynamic circulation induced by portal hypertension. Gastric mucosa in PHG exhibits greater susceptibility to gastrototoxic chemicals and poor wound healing. Acute or chronic gastrointestinal bleeding are the only clinical complications. Bleeding is typically mild-to-moderate and rarely fatal. Endoscopic therapy is rarely useful. Pharmacotherapy for acute bleeding includes octreotide with concomitant proton-pump-inhibitor therapy, or alternatively vasopressin. Nonselective  $\beta$ -adrenergic receptor antagonists, particularly propranolol, are used

to prevent re-bleeding after acute bleeding or for chronic bleeding. Transjugular-intrahepatic-portosystemic-shunt and liver transplantation is ultimate therapies because they treat the underlying portal hypertension.

Gjeorgjievski M, Cappell MS. Portal hypertensive gastropathy: A systematic review of the pathophysiology, clinical presentation, natural history and therapy. *World J Hepatol* 2016; 8(4): 231-262 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i4/231.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i4.231>

## INTRODUCTION

Portal hypertensive gastropathy (PHG) is an important, but underappreciated, cause of morbidity in patients with cirrhotic or non-cirrhotic portal hypertension. Researchers have recently intensely focused on this inadequately understood disease. However, the research studies have been published in a wide spectrum of journals including basic or biomedical journals not readily accessible to clinicians and a review incorporating the recent basic and clinical advances in this rapidly evolving subject is needed. This work systematically reviews this entity including pathophysiology, clinical presentation, natural history, and established, evolving, or experimental therapy, with a focus on data relevant to clinicians and an emphasis on recent data. This work aims to describe what is known about the disease and to expose gaps, requiring further research, in our current understanding of this disease.

## MATERIALS AND METHODS

Computerized search of the literature was performed *via* PubMed using the following medical subject headings or keywords: "portal" and "gastropathy"; or "portal" and "hypertensive"; or "congestive" and "gastropathy"; or "congestive" and "gastroenteropathy". The following criteria were applied for study inclusion: Publication in peer-reviewed journals, and publication since 1980, except for publications from 1957-1980 of historical significance reviewed in the history section. Articles were independently evaluated by each author and selected for inclusion by consensus after a thorough discussion based on the following criteria: Well-designed, prospective trials; recent studies; large study populations; and study emphasis on PHG. However, data from retrospective series, reviews from internationally recognized authorities, and even case reports were included when prospective trials were unavailable.

## RESULTS

### History

Palmer<sup>[1]</sup>, in 1957, proposed that the pathogenesis of erosive gastritis in cirrhotic patients was different than that in non-cirrhotic patients and that erosive gastritis



**Table 1** Rates of portal hypertensive gastropathy in patients with portal hypertension

Ref.	Analyzed patients	Total number	No. (%) with PHG	No. (%) with mild PHG	No. (%) with severe PHG
McCormack <i>et al</i> <sup>[3]</sup>	Portal hypertension	127	65 (51%)	37 (29%)	28 (22%)
Sarin <i>et al</i> <sup>[5]</sup>	Portal hypertension	136	10 (7%)		
DeWeert <i>et al</i> <sup>[6]</sup>	Non-alcoholic liver disease	81	23 (28%)	Not reported	Not reported
McCormick <i>et al</i> <sup>[7]</sup>	Portal hypertension	93 endoscopies in 74 patients	85 endoscopies (91%)	6 (6%), moderate 61 (66%)	18 (19%)
Sarin <i>et al</i> <sup>[8]</sup>	Portal hypertension	107	4 (3.7%) (only cirrhotic)	Not reported	Not reported
Parikh <i>et al</i> <sup>[9]</sup>	Portal hypertension	118	71 (60%)	41 (58%)	30 (42%)
Sarin <i>et al</i> <sup>[10]</sup>	Portal hypertension with prior variceal bleeding	967	86 (9%)	56 (5.8%)	30 (3.1%)
Itha <i>et al</i> <sup>[11]</sup>	EHPVO in children	163	(12%)	Not reported	Not reported
Rana <i>et al</i> <sup>[12]</sup>	Portal hypertension	41	27 (66%)	19 (46%)	8 (20%)
El-Rifai <i>et al</i> <sup>[13]</sup>	Portal hypertension	24	14 (58%)	10 (42%) - moderate	4 (16%)
Sogaard <i>et al</i> <sup>[14]</sup>	Portal vein thrombosis	67	28 (42%)	Not reported	Not reported
Figueiredo <i>et al</i> <sup>[15]</sup>	Portal hypertension; cirrhosis	36	27 (75%)		5 (46%)
Erden <i>et al</i> <sup>[16]</sup>	Portal hypertension	57	15 (26.3%)	Not reported	Not reported
Duché <i>et al</i> <sup>[17]</sup>	Children, portal hypertension with biliary atresia	125	27 (21%)	Not reported	Not reported
Aydoğan <i>et al</i> <sup>[18]</sup>	Portal hypertension	51	30 (58%)	Not reported	Not reported
dos Santos <i>et al</i> <sup>[19]</sup>	Portal hypertension	43	22 (51%)	Not reported	Not reported
Pantham <i>et al</i> <sup>[20]</sup>	Esophageal varices undergoing TEE	24	12 (50%)	Not reported	Not reported
Abdollahi <i>et al</i> <sup>[21]</sup>	Autoimmune hepatitis	60	27 (45%)	Not reported	Not reported
de Alcantara <i>et al</i> <sup>[22]</sup>	Chronic liver disease <i>vs</i> EHPVO	35 <i>vs</i> 18	7 (20%) <i>vs</i> 8 (44.4%)	Not reported	Not reported
Aoyama <i>et al</i> <sup>[23]</sup>	Portal hypertension	119	35 (29%)	Not reported	Not reported

PHG: Portal hypertensive gastropathy; EHPVO: Extrahepatic portal vein obstruction; TEE: Transesophageal echocardiogram.

in cirrhotic patients resulted from mechanical venous back-pressure from portal hypertension, rather than a circulating, mucosal, or intraluminal toxic factor. This proposal was supported by successful reversal of erosive gastritis in cirrhotic patients with portal decompression by surgical shunts<sup>[1]</sup>. In 1984, Sarfeh *et al*<sup>[2]</sup> recognized a distinct form of gastric mucosal hemorrhage in patients who had portal hypertension, demonstrated by cirrhosis and gastroesophageal varices, which they called "portal hypertensive gastritis". They proposed that gastric mucosa in portal hypertension reacts differently from gastric mucosa without portal hypertension and these patients with portal hypertension may benefit from portal decompressive surgery. One year later, McCormack *et al*<sup>[3]</sup> reported that the gastritis in patients with portal hypertension differed from that in patients without portal hypertension in mucosal histology, nonresponse to standard therapy for conventional gastritis, and in occasionally having very similar histological changes in other gastrointestinal (GI) organs such as the colon. They called these gastritis-like changes in patients with portal hypertension "congestive gastropathy"<sup>[3]</sup>, and classified it as "mild" or "severe", using criteria described by Taor *et al*<sup>[4]</sup>.

### Epidemiology

PHG can present at any age, including pediatric or adult patients. The reported prevalence of PHG varies greatly from 20% to 75% in patients with portal hypertension (Table 1)<sup>[3,5-23]</sup>, and varies greatly from about 35% to 80% in patients with cirrhosis (Table 2)<sup>[21,23-68]</sup>. For

example, in a study of 373 cirrhotic patients, 299 (80.2%) had PHG<sup>[34]</sup>. In the HALT-C trial, 374 (37%) of 1011 patients with biopsy-proven cirrhosis or bridging fibrosis from hepatitis C had PHG<sup>[69]</sup>. This wide variability likely reflects variability in classification criteria, interpretation of endoscopic lesions, study populations, and natural history of PHG<sup>[10,70,71]</sup>.

PHG is usually mild as reported by McCormack *et al*<sup>[3]</sup> or in the NIEC study<sup>[32,70]</sup>. The prevalence of mild PHG in patients with portal hypertension ranges from 29%-57%, and of severe PHG ranges from 9%-46%<sup>[71]</sup>.

### Risk factors for PHG

The main predictors of PHG are portal hypertension and severe liver disease<sup>[72]</sup>.

**Portal hypertension:** Most studies show that the frequency and severity of PHG is strongly correlated with the severity of portal hypertension, as indicated by multiple parameters, including hepatic venous pressure gradient (HVPG)<sup>[36,57]</sup>, esophageal intravariceal pressure<sup>[29]</sup>, and presence or size of esophageal varices<sup>[34,42,57,62,73]</sup>. Merkel *et al*<sup>[36]</sup> reported that the severity of PHG was correlated with the severity of portal hypertension as determined by HVPG, but this correlation was significant only for severe PHG (HVPG = 20.5 ± 4.0 mmHg) *vs* no PHG (HVPG = 17.4 ± 5.2 mmHg, *P* = 0.0004), and not for mild PHG (HVPG = 16.1 ± 3.2 mmHg) *vs* no PHG [17.4 ± 5.2 mmHg, not significant (NS)]. In a prospective study of 331 cirrhotic patients, Kim *et al*<sup>[57]</sup> found that patients with severe

**Table 2** Rates of portal hypertensive gastropathy in patients with cirrhosis

Ref.	Patients	Total number	PHG	Mild	Severe
Sacchetti <i>et al</i> <sup>[24]</sup>	Cirrhosis	142	38 (27%)	28 (20%)	10 (7%)
D'Amico <i>et al</i> <sup>[25]</sup>	Cirrhosis	212	130 (61%)	110 (52%)	20 (9%)
Calès <i>et al</i> <sup>[26]</sup>	Cirrhosis	100	98 (98%)	57 (57%)	41 (41%)
Rabinovitz <i>et al</i> <sup>[27]</sup>	Cirrhosis	510	(43%)	Not reported	Not reported
Iwao <i>et al</i> <sup>[28]</sup>	Cirrhosis	47	32 (68%)	15 (32%)	17 (36%)
Taranto <i>et al</i> <sup>[29]</sup>	Cirrhosis	394	317 (80.5%)	Not reported	Not reported
Gupta <i>et al</i> <sup>[30]</sup>	Cirrhosis	230	(61%)	(52%)	(9%)
Iwao <i>et al</i> <sup>[31]</sup>	Cirrhosis	476	254 (53%)	208 (43%)	46 (9%)
Carpinelli <i>et al</i> <sup>[32]</sup>	Cirrhosis	566	362 (64%)	192 (34%)	170 (30%)
Zaman <i>et al</i> <sup>[33]</sup>	Cirrhosis	120	74 (62%)	47 (39%)	27 (23%)
Primignani <i>et al</i> <sup>[34]</sup>	Cirrhosis	373	299 (80%)	127 (34%)	172 (46%)
Chaves <i>et al</i> <sup>[35]</sup>	Cirrhosis vs schistosomiasis	43	18 (81%) vs 7 (33%)	Not reported	Not reported
Merkel <i>et al</i> <sup>[36]</sup>	Cirrhosis	62	49 (79%)	29 (46%)	20 (32%)
Merli <i>et al</i> <sup>[37]</sup>	Cirrhosis, with mild portal hypertension	222	48 (21%)	43 (19%)	5 (2%)
Ito <i>et al</i> <sup>[38]</sup>	Cirrhosis	47	13 (27%)	10 (21%)	3 (6%)
De Palma <i>et al</i> <sup>[39]</sup>	Cirrhosis	37	23 (62%)	Not reported	Not reported
Menchén <i>et al</i> <sup>[40]</sup>	Cirrhosis	549	353 (64%)	275 (50%)	77 (14%)
Yüksel <i>et al</i> <sup>[41]</sup>	Cirrhosis	114 total	76 (66%)	38 (33%)	38 (33%)
Fontana <i>et al</i> <sup>[42]</sup>	Cirrhosis or bridging fibrosis from hepatitis C	1016	374 (37%)	345 (34%)	29 (3%)
Bresci <i>et al</i> <sup>[43]</sup>	Cirrhosis	85	36 (42%)	Not reported	Not reported
Akatsu <i>et al</i> <sup>[44]</sup>	End stage liver disease	29	19 (65.5%)	18 (62.1%)	1 (3.4%)
Zardi <i>et al</i> <sup>[45]</sup>	Cirrhosis	266	84 (31%)	Not reported	Not reported
Barakat <i>et al</i> <sup>[46]</sup>	Cirrhosis with portal hypertensive duodenopathy	105	105 (100%)	17 (16.2%)	88 (83.8%)
Bellis <i>et al</i> <sup>[47]</sup>	Cirrhosis	59	44 (76%)	16 (27%)	28 (47%)
Gravante <i>et al</i> <sup>[48]</sup>	Liver transplant candidates with cirrhosis	80	41 (51.2%)	Not reported	Not reported
Canlas <i>et al</i> <sup>[49]</sup>	Cirrhosis	19	13 (68.4%)	Not reported	Not reported
Kim <i>et al</i> <sup>[50]</sup>	Cirrhosis	83	48 (57.8%)	Not reported	Not reported
Higaki <i>et al</i> <sup>[51]</sup>	Cirrhosis	21	8 (38%)	Not reported	Not reported
Frenette <i>et al</i> <sup>[52]</sup>	Cirrhosis	50	45 (90%)	28 (56%)	17 (34%) moderate
Tarantino <i>et al</i> <sup>[53]</sup>	Cirrhosis	153	88 (57.5%)	Not reported	Not reported
Curvelo <i>et al</i> <sup>[54]</sup>	Cirrhosis	46	43 (93.4%)	21 (45%)	22 (47%)
Anegawa <i>et al</i> <sup>[55]</sup>	Cirrhosis	70	49 (70%)	32 (46%)	17 (24%)
Kumar <i>et al</i> <sup>[56]</sup>	Cirrhosis	254	140 (55%)	Not reported	Not reported
Kim <i>et al</i> <sup>[57]</sup>	Cirrhosis	331	298 (90%)	Mild 84 (25.4%)	214 (64.7%)
De Lisi <i>et al</i> <sup>[58]</sup>	Cirrhosis	611	448 (73.3%)	37.3%	36%
Abbasi <i>et al</i> <sup>[59]</sup>	Cirrhosis	102	87 (85%)	Not reported	Not reported
Ahmed <i>et al</i> <sup>[60]</sup>	Cirrhosis from hepatitis B or hepatitis C	360	300 (83%)	229 (64%)	71 (20%)
Garcia-Saenz-de-Sicilia <i>et al</i> <sup>[61]</sup>	Cirrhosis	105	72 (68.6%)	Not reported	Not reported
Abbasi <i>et al</i> <sup>[62]</sup>	Cirrhosis	217	172 (79.3%)	56 (25.8%)	116 (53.5%)
Aoyama <i>et al</i> <sup>[63]</sup>	Cirrhosis	60	13 (22%)	Not reported	Not reported
Laleman <i>et al</i> <sup>[64]</sup>	Cirrhosis with refractory chronic hepatic encephalopathy	36	13 (36%)	9 (25%)	4 (11%)
Giannini <i>et al</i> <sup>[65]</sup>	Cirrhosis and undergoing surgery for hepatocellular carcinoma	152	23 (15.1%)	Not reported	Not reported
Abdollahi <i>et al</i> <sup>[21]</sup>	Autoimmune hepatitis	60	27 (45%)	Not reported	Not reported
Aoyama <i>et al</i> <sup>[23]</sup>	Portal hypertension	119	35 (29%)	Not reported	Not reported
Aoyama <i>et al</i> <sup>[66]</sup>	Cirrhosis	134	42 (31%)	Not reported	Not reported
Zardi <i>et al</i> <sup>[67]</sup>	Cirrhosis without gastroesophageal varices	145	75 (51%)	45 (31%)	30 (20%)
Wu <i>et al</i> <sup>[68]</sup>	Cirrhosis	700	449 (64%)	Mild 208 (29.7%), moderate 160 (22.9%)	Severe 81 (11.6%)

PHG: Portal hypertensive gastropathy.

PHG had significantly higher HVP (15.6 ± 4.6 mmHg) than patients with mild PHG (10.7 ± 4.1 mmHg) or no PHG (4.9 ± 1.7 mmHg) ( $P < 0.001$ ). Merkel *et al*<sup>[36]</sup> similarly reported in a small study that HVP was significantly higher in patients with severe PHG as compared to mild or no PHG.

Primignani *et al*<sup>[34]</sup> confirmed the correlation of PHG with severity of portal hypertension, by correlating PHG with presence and size of esophageal varices. The rate of PHG was significantly higher in patients with esophageal varices [80 of 104 patients (76.9%)]

than in patients without esophageal varices [51 of 84 (60.7%),  $P < 0.007$ ]. The rate of PHG also significantly increased with increasing variceal size ( $\chi^2 = 13.2$ ;  $df = 1$ ,  $P = 0.0003$ ). Abbasi *et al*<sup>[62]</sup> reported a significantly positive correlation between esophageal variceal size and rate of PHG ( $r = 0.46$ ;  $P < 0.001$ ). Taranto *et al*<sup>[29]</sup> reported more severe PHG in cirrhotic patients with more severe portal hypertension, as measured by esophageal intravariceal pressure. Iwao *et al*<sup>[28]</sup> reported that patients with severe PHG had elevated HVP, high hepatic sinusoidal resistance, and low hepatic blood

flow, all markers of severe portal hypertension. For example, patients without PHG had hepatic sinusoidal resistance of  $1218 \pm 528 \text{ dyne} \times \text{s}^{-1} \times \text{cm}^{-5}$ , patients with mild PHG had resistance of  $1968 \pm 944 \text{ dyne} \times \text{s}^{-1} \times \text{cm}^{-5}$  ( $P < 0.05$ ), and patients with severe PHG had resistance of  $2082 \pm 672 \text{ dyne} \times \text{s}^{-1} \times \text{cm}^{-5}$  ( $P < 0.01$ ). Presence of PHG was independent of patient age, sex, or cirrhosis etiology<sup>[28]</sup>.

As discussed below, other data supporting an association between PHG and portal hypertension include resolution of PHG after intervention to decrease portal hypertension, including pharmacotherapy<sup>[74-78]</sup>, transjugular intrahepatic portosystemic shunt (TIPS), or liver transplantation<sup>[74]</sup>.

Contrariwise, a decided minority of studies showed no significant association between severity of portal hypertension and rate of PHG<sup>[8,9,16,26,28,30,31,47,54,67]</sup>. Curvêlo *et al*<sup>[54]</sup> found no significant difference in HVP in cirrhotic patients with vs without PHG. Bellis *et al*<sup>[47]</sup> demonstrated similar findings. Among patients with portal hypertension from cirrhosis without esophageal varices, Zardi *et al*<sup>[67]</sup> reported that patients with PHG vs patients without PHG had similar mean portal vein diameter, splenic vein diameter, and portal flow volume, all markers of severity of portal hypertension. Erden *et al*<sup>[16]</sup> showed that the mean diameters of the left gastric, paraesophageal, and azygos veins, which are markers of portal hypertension, were not significantly different between patients with vs without PHG. The preponderance of data strongly suggest that the severity of portal hypertension is associated with the severity or frequency of PHG.

#### **Cirrhotic vs non-cirrhotic portal hypertension:**

Primary liver disease usually occurs in PHG, but is not a prerequisite for PHG provided another cause of portal hypertension exists. PHG can occur among patients with non-cirrhotic portal fibrosis (NCPF), extrahepatic portal vein obstruction (EHPVO), hepatic veno-occlusive disease, and schistosomiasis<sup>[8,14,35,75,79]</sup>.

The frequency of PHG appears to be higher in portal hypertension with cirrhosis than in portal hypertension without cirrhosis. Sarin *et al*<sup>[8]</sup> reported that patients with cirrhosis had a significantly higher frequency of PHG (37.1%) than that in patients with NCPF (16.7%;  $P < 0.05$ ), or non-cirrhotic EHPVO (8.7%;  $P < 0.01$ ) and had a more aggressive course of PHG with progression to more severe PHG with time. These phenomena are attributed to the worse liver function in patients with cirrhosis as compared to patients with NCPF or EHPVO<sup>[8]</sup>. Chaves *et al*<sup>[35]</sup> similarly reported a higher incidence of PHG in patients with cirrhosis vs patients with portal hypertension from etiologies including schistosomiasis or postsinusoidal hypertension. Chaves *et al*<sup>[35]</sup> reported that PHG occurred in 18 (81.8%) of 22 patients with cirrhosis vs only 7 (33.3%) of 21 patients with portal hypertension from schistosomiasis ( $P < 0.05$ ). Parikh *et al*<sup>[9]</sup> reported a non-significant trend of more frequent PHG in patients with cirrhosis [64 of 102 patients (63%)]

vs NCPF [7 of 16 patients (44%)], but the lack of statistical significance may have resulted from the small number of patients with NCPF.

Chaves *et al*<sup>[35]</sup> reported that the mosaic pattern was significantly more prevalent in patients with cirrhosis [12 of 22 patients (54.5%)] than in patients with schistosomiasis [2 of 21 patients (9.5%);  $P < 0.05$ ]. Sarin *et al*<sup>[6]</sup> in a study of 50 patients with portal hypertension from various etiologies undergoing endoscopy, reported 6 (16.6%) of 36 patients with underlying cirrhosis had a mosaic pattern of PHG, whereas only 1 (8.5%) of 12 patients with EHPVO had a mosaic pattern of PHG (NS).

**Cirrhosis etiology:** Several research groups reported that the underlying etiology of cirrhosis did not affect PHG frequency or severity<sup>[13,71,80]</sup>. For example, Abbasi *et al*<sup>[62]</sup> reported among 217 patients with cirrhosis that PHG was unassociated with cirrhosis etiology ( $r = 0.056$ ;  $P = 0.414$ ), among 144 patients with hepatitis C, 36 patients with hepatitis B, 21 patients with cryptogenic cirrhosis, 15 patients with hepatitis C and hepatitis B coinfection, and 1 patient with hepatitis B and hepatitis D coinfection. Kim *et al*<sup>[57]</sup> similarly did not find a correlation between cirrhosis etiology and severity of PHG in a prospective study of 331 patients with cirrhosis, including cirrhosis etiologies of alcohol in 250, hepatitis B in 68, hepatitis C in 15, and cryptogenic cirrhosis in 8. Gupta *et al*<sup>[30]</sup> in a study of 230 patients with cirrhosis and esophageal varices found no significant difference in the rate of PHG between patients with cirrhosis from alcohol [32 of 52 patients (62%)] vs cirrhosis from other causes [110 of 178 patients (62%),  $P = \text{NS}$ ]. Iwao *et al*<sup>[31]</sup> in an endoscopic study of 47 patients with histologically-proven cirrhosis reported no significant differences in etiology of cirrhosis between patients without PHG vs patients with mild or severe PHG.

Iwao *et al*<sup>[31]</sup> reported no association between etiology of cirrhosis and PHG severity. The etiologies of cirrhosis in this study included 7 from alcoholism vs 8 from chronic hepatitis in patients without PHG, 5 from alcoholism vs 10 from chronic hepatitis in patients with mild PHG, and 8 from alcoholism vs 9 from chronic hepatitis in patients with severe PHG (NS).

**Liver disease duration:** Generally, duration of liver disease positively correlates with development of PHG<sup>[5]</sup>. Merli *et al*<sup>[37]</sup> reported a cumulative incidence of 3% at 1 year, 10% at 2 years, and 24% at 3 years. Most cases were mild, with only 10% of cases reported as severe PHG in cirrhotic patients undergoing esophagogastroduodenoscopy (EGD) to screen for esophageal varices. Primignani *et al*<sup>[34]</sup> reported that the prevalence of PHG was only 56% in patients with newly diagnosed cirrhosis, rose to 75% in patients with previously diagnosed cirrhosis and no prior variceal bleeding, and rose further to 91% in patients with previously diagnosed cirrhosis and prior variceal bleeding treated with sclerotherapy ( $\chi^2 = 34.25$ ;  $df = 1$ ;

$P < 0.0001$ ). The frequency of PHG increased by 46% after 5 years of follow-up in patients with cirrhosis<sup>[34]</sup>. In 30%-60% of cases, preexistent PHG remained stable with time<sup>[72]</sup>, but it can fluctuate in severity with time, with progression in 30%, and regression in 20% of cases<sup>[25,34,37]</sup>. Child-Pugh stage C cirrhosis was associated with faster progression of PHG<sup>[34]</sup>.

**Liver disease severity:** Numerous studies reported PHG is correlated with liver disease severity, as measured by Child-Pugh stage<sup>[8,9,29,35,37,55,57,67]</sup>. The reported strength of this correlation is variable. Some studies showed correlation between all stages of cirrhosis and PHG, whereas other studies showed correlation only for specific stages of cirrhosis. Sarin *et al.*<sup>[8]</sup> reported an 87% prevalence of PHG in patients with Child-Pugh stage C, vs only 13% prevalence in patients with Child-Pugh stage A. Another study reported that only Child-Pugh stage C was independently associated with PHG (OR = 2.68; 95%CI: 1.16-6.20,  $P = 0.021$ )<sup>[56]</sup>. Merli *et al.*<sup>[37]</sup> reported, in a study of 48 patients with PHG among 222 patients with cirrhosis, that Child-Pugh stage B or C, and presence of esophageal varices were independent risk factors for developing PHG. De Lisi *et al.*<sup>[58]</sup> reported a significantly higher prevalence of PHG in Child-Pugh stages B or C, as compared to stage A. Zardi *et al.*<sup>[67]</sup> reported that cirrhotic patients without esophageal varices with severe PHG had significantly more frequently Child-Pugh stage C than patients with mild PHG. In another study, the MELD (model for end-stage liver disease) score was significantly correlated with PHG severity (mean MELD score in patients without PHG =  $7.6 \pm 1.7$ , in patients with mild PHG =  $10.2 \pm 4.0$ , and in patients with severe PHG =  $11.3 \pm 3.5$ ;  $P < 0.001$ )<sup>[57]</sup>. In the HALT-C trial, hypoalbuminemia and hyperbilirubinemia, biochemical markers of advanced liver disease, were independent predictors of PHG in a logistic regression model (OR = 0.53, 95%CI: 0.37-0.76 for hypoalbuminemia; OR = 1.77, 95%CI: 1.25-2.51, for hyperbilirubinemia). Markers of portal hypertension (thrombocytopenia) and of insulin resistance (hyperglycemia) were also significant independent predictors of PHG.

Contrariwise, a minority of studies found no correlation between liver disease severity, as determined by Child-Pugh stage, and presence or severity of PHG<sup>[34,36,40,47,54,62,70,79]</sup>. For example, Primignani *et al.*<sup>[34]</sup> reported the prevalence of severe PHG was lowest in Child-Pugh stage C. In the NIEC study, patients with Child-Pugh stage B had a higher prevalence of PHG than patients with stages A or C. Zardi *et al.*<sup>[67]</sup> reported no significant differences in Child-Pugh stage or in MELD score among cirrhotic patients with vs without PHG. The preponderance of the data, however, suggest that severity of cirrhosis, as measured by Child-Pugh score, is correlated with frequency of PHG.

**Correlation with varices:** Many studies report a correlation between the presence and size of esopha-

geal varices and severity of PHG. For example, among the 188 of 373 patients with cirrhosis not undergoing variceal sclerotherapy in the NIEC study, the prevalence of PHG was significantly higher in patients with esophageal varices [80 of 104 patients (77%)] than in patients without esophageal varices [51 of 84 patients (61%);  $P = 0.007$ ]; and the prevalence of PHG significantly increased with increasing variceal size ( $\chi^2 = 13.2$ ;  $P < 0.0003$ )<sup>[34]</sup>. Numerous other studies also demonstrated significant correlation between presence of esophageal varices and PHG, and several studies also demonstrated significant correlations between variceal size and PHG<sup>[9,13,29,42,56,57,62]</sup>. For example, Abbasi *et al.*<sup>[62]</sup> reported that esophageal variceal size was significantly correlated with PHG frequency among 217 cirrhotic patients ( $r = 0.46$ ;  $P < 0.001$ ).

However, a few studies showed no correlation between presence or size of varices and PHG<sup>[26,28,30,47]</sup>. All these negative studies but one were relatively small. Gupta *et al.*<sup>[30]</sup> reported no significant association between frequency of PHG and size of esophageal varices among 230 cirrhotic patients. Similarly, in a study of 59 patients with cirrhosis, Bellis *et al.*<sup>[47]</sup> showed a non-significant trend towards more severe PHG in patients with large vs small varices. For example, three (50%) of 6 patients without esophageal varices had PHG, 6 of 10 patients (60%) with small varices had PHG, 19 of 25 patients (76%) with medium-sized varices had PHG, and 16 of 18 patients (89%) with large varices had PHG (NS). Iwao *et al.*<sup>[28]</sup> further reported that the frequency of PHG was not correlated with esophageal variceal size. The mean grade of gastroesophageal varices was  $1.4 \pm 0.9$  for no PHG,  $2.0 \pm 0.9$  for mild PHG, and  $1.9 \pm 1.0$  for severe PHG (all NS), and the mean grade of gastric varices was  $0.5 \pm 0.8$  for no PHG,  $1.3 \pm 1.3$  for mild PHG, and  $0.9 \pm 1.2$  for severe PHG (all NS).

**Location of varices:** Regarding variceal location, Sarin *et al.*<sup>[8]</sup> reported in a study of 107 patients with cirrhosis, NCPF or EHPVO, that PHG was significantly more common in patients with coexistent gastric and esophageal varices as compared to solely esophageal varices. PHG occurred in 15 (42%) of 36 patients with concomitant esophageal and gastric varices, but occurred in only 8 (11%) of 71 patients with solely esophageal varices ( $P < 0.01$ ). Likewise, Gupta *et al.*<sup>[30]</sup> reported a significantly higher prevalence of PHG in patients with esophageal and gastric varices [74 of 107 patients (69%)] compared to solely esophageal varices [68 of 123 patients (55%),  $P < 0.05$ ].

Iwao *et al.*<sup>[31]</sup> reported a significantly higher incidence of PHG in cirrhotic patients with esophageal varices as compared to fundal gastric varices. Merkel *et al.*<sup>[36]</sup> reported that patients with severe PHG localized to the gastric body or fundus had significantly higher HVPG than patients with severe PHG localized to the gastric antrum.

Portal hypertension is usually associated with porto-



**Table 3** Effects of variceal ligation on frequency of portal hypertensive gastropathy

Ref.	No. of patients and etiology	Study type	PHG rate before variceal ligation	PHG aggravation after variceal ligation	P value of pre vs post EVL
Hou <i>et al</i> <sup>[73]</sup>	90 patients with cirrhosis and recent variceal bleeding, 46 patients underwent EVL	Randomized, controlled trial	No PHG-4, mild PHG-33, severe-PHG-9	At eradication: 17/37; 17/37 (45.9%) in EVL; at 3 mo: 17/30 (56.7%); at 6 mo 18/29 (62.1%)	$P > 0.05$
Elnaser <i>et al</i> <sup>[80]</sup>	125 patients with upper GI bleeding undergoing variceal ligation, followed for mean of 31 mo	Retrospective study	22/125 (17.6%)	50/125 (50%)	$P < 0.05$
Yüksel <i>et al</i> <sup>[41]</sup>	114 patients with cirrhosis and portal hypertension undergoing EVL in 85 patients	Retrospective study	27/85 (31.8%) none; 28/85 (32.9%) mild; 30/85 (35.3%) severe	14/85 (16.5%) none; 30/85 (35.3%) mild; 41/85 (48.2%) severe	$P < 0.05$
Lo <i>et al</i> <sup>[81]</sup>	77 patients with bleeding from EV underwent variceal ligation and were randomized to receive propranolol (37/77) or control (40/77); patients with severe PHG prior to treatment excluded from the study	Prospective, randomized, controlled trial	Control group: 7/40 (17%); propranolol group: 8/37 (22%)	At variceal ligation: Control group: 67% (does not state number); Propranolol group: 31% (number not stated); 6 mo after treatment: Control group: 85% (number not stated) propranolol group: 48% (number not stated)	Pre vs post ligation, both groups; $P < 0.05$ ; frequency of PHG significantly higher in control group post ligation when compared to propranolol group; $P = 0.002$
de la Peña <i>et al</i> <sup>[82]</sup>	93 patients with history of variceal hemorrhage and cirrhosis, randomized to receive either EVS (46/88) or EVL (42/88); 5 patients excluded due to diagnosis of hepatoma, non-cirrhotic portal hypertension or portal vein thrombosis	Randomized, prospective study	Not reported	PHG significantly worsened in 23 patients, including 17 patients undergoing EVL	$P < 0.01$

PHG: Portal hypertensive gastropathy; EVL: Endoscopic variceal ligation; EVS: Endoscopic variceal sclerotherapy.

systemic collateral circulation, commonly including esophageal varices, gastric varices, and abdominal or umbilical or hemorrhoidal vein dilatation; and uncommonly including splenorenal, gastric, renal, retroperitoneal, or cardiac angle venous shunts. Wu *et al*<sup>[68]</sup> reported that the rate of moderate or severe PHG was higher in patients with common collaterals [296 of 439 patients (67.4%)] vs uncommon collaterals [70 of 118 patients (59.3%)], but this difference was not statistically significant.

In 2007, Zardi *et al*<sup>[45]</sup> proposed that PHG is promoted by minimal collateral circulation because a significant collateral circulation would otherwise reduce portal pressure and gastric mucosal congestion. They found that the portal vein diameter in cirrhotic patients was larger in patients with PHG and no esophageal varices ( $13.0 \pm 2.6$  mm) than in patients with F1 esophageal varices ( $12.6 \pm 2.3$  mm) or F2 esophageal varices ( $12.9 \pm 2.0$  mm) (NS). They further supported this concept by finding that patients with portal vein diameter  $< 12$  mm have a significantly higher prevalence of F1 and F2 esophageal varices than patients with a portal diameter between 12–13 mm, and argued that the absence of hepatofugal collateral circulation created by flow inversion, in patients without esophageal varices, left the entire pressure gradient over the portal vein<sup>[45]</sup>.

**Esophageal variceal eradication:** Numerous studies demonstrated that PHG increased in incidence and that preexistent PHG increased in severity after eradication of esophageal varices by either endoscopic variceal ligation

(Table 3)<sup>[41,73,80–82]</sup> or endoscopic variceal sclerotherapy (Table 4)<sup>[8,10,11,25,30,41,73,81–83]</sup> in cirrhotic patients with portal hypertension. Both phenomena also occurred after endoscopic variceal eradication in patients with non-cirrhotic portal hypertension, as shown in two studies in pediatric patients with EHPVO. For example, Poddar *et al*<sup>[83,84]</sup> reported in a prospective study of 274 children undergoing surveillance EGD after endoscopic sclerotherapy for EHPVO that the number of patients with PHG increased from 46 (24.7%) at baseline to 95 (51.6%) after sclerotherapy among 186 patients completing the study ( $P < 0.001$ ). Likewise, Itha *et al*<sup>[11]</sup> reported that the rate of PHG increased from 12% to 41% after endoscopic sclerotherapy ( $P < 0.001$ ) in a prospective study of 163 children undergoing surveillance EGD at 3 and 6 mo after endoscopic sclerotherapy. In the study by Sarin *et al*<sup>[10]</sup>, 86 (9%) of 967 patients with prior variceal bleeding treated with endoscopic sclerotherapy, had PHG at EGD, of whom 22 (26%) had PHG before variceal eradication and 64 (74%) developed PHG after variceal eradication.

PHG also increases in frequency after angiographic variceal obliteration. Duan *et al*<sup>[85]</sup> reported *de novo* PHG developed in 13 of 34 patients (38%) after percutaneous transhepatic variceal embolization for massive esophago-gastric variceal hemorrhage.

These phenomena are attributed to increased portal pressure and flow after eradication of esophageal varices because of redistribution of residual blood flow that had passed through the previously patent varices<sup>[5,41,45,81,86–90]</sup>. Itha *et al*<sup>[11]</sup> concluded that the significant increase in

**Table 4** Effects of variceal sclerotherapy on frequency of portal hypertensive gastropathy

Ref.	No. of patients and etiology	Study type	PHG before procedure	PHG aggravation after procedure	P value
Hou <i>et al</i> <sup>[73]</sup>	90 cirrhotic patients with recent variceal bleeding; EVS 44, EVL 46	Randomized, controlled trial	Pre EVS group: 6 none/24 mild/14 severe; pre EVL group: 4 none/33 mild/9 severe; total: 10 none/57 mild/23 severe	At eradication: 14/29 (48.3%) in EVS; 17/37 (45.9%) in EVL; at 3 mo: 15/26 (57.7%) in EVS; 17/30 (56.7%) in EVL; at 6 mo 15/25 (60%) in EVS; 18/29 (62.1%) in EVL	Non-significant difference in PHG aggravation between EVS and EVL; $P > 0.05$
Itha <i>et al</i> <sup>[11]</sup>	163 children with extrahepatic portal vein obstruction presenting with variceal bleeding underwent endoscopic injection sclerotherapy	Not reported	12% overall PHG (actual number not stated), 1 patient with severe PHG	41% overall PHG (actual number not stated), 12 patients with severe PHG	$P < 0.001$ for overall PHG; $P < 0.001$ for severe PHG
Poddar <i>et al</i> <sup>[83]</sup>	186 children with extrahepatic portal vein obstruction presenting with variceal bleeding undergoing endoscopic sclerotherapy, and mean follow up of $38 \pm 30$ mo	Retrospective study	PHG: 46/186 (24.7%), severe PHG: 6/186 (3.2%)	PHG: 96/186 (51.6%), severe PHG: 29/186 (15.6%)	$P < 0.001$ for overall PHG; $P < 0.05$ for severe PHG
Yüksel <i>et al</i> <sup>[41]</sup>	114 patients with cirrhosis and portal hypertension undergoing EVS (29/114) or EVL (85/114)	Retrospective study	Pre EVS group: 11/29 (37.9%) none; 10/29 (24.5%) mild; 8/29 (27.6%) severe; pre EVL group: 27/85 (31.8%) none; 28/85 (32.9%) mild; 30/85 (35.3%) severe	Post EVS group: 4/29 (13.8%) none; 8/29 (27.6%) mild; 17/29 (58.6%) severe; post EVL group: 14/85 (16.5%) none; 30/85 (35.3%) mild; 41/85 (48.2%) severe	Pre EVS <i>vs</i> post EVS; $P < 0.05$ ; pre EVL <i>vs</i> post EVL; $P < 0.05$ ; pre EVS <i>vs</i> pre EVL; $P > 0.05$ ; post EVS <i>vs</i> post EVL; $P > 0.05$
Sarin <i>et al</i> <sup>[10]</sup>	967 patients with variceal bleeding underwent endoscopic sclerotherapy; out of whom 88 patients fulfilled the inclusion criteria (including presence of endoscopic lesions consistent with PHG or GAVE, before or within 4 wk after obliteration) were prospectively followed (out of whom 2 had only GAVE)	Prospective study	22 patients had PHG prior to EVS; 2/22 transient (9%); 17/22 persistent (77%); 3/22 progressive (14%)	Additional development in 64 patients post procedure, 28/64 transient (44%), 31/64 persistent (48%), 5/64 progressive (8%)	Only statistically significant difference was the transient PHG that disappeared in 28 (44%) of patients in the group that developed PHG post procedure; $P < 0.05$
Gupta <i>et al</i> <sup>[30]</sup>	230 patients with liver cirrhosis; of which 44 underwent variceal eradication with sclerotherapy	Prospective study	24/44 (54%)	33/44 (75%)	$P < 0.05$
Sarin <i>et al</i> <sup>[8]</sup>	107 patients with portal hypertension presenting with variceal bleeding that underwent sclerotherapy with mean follow-up of $23.2 \pm 3.4$ mo	Prospective study	4/107 (3.7%)	21 additional patients, 25/107 (23%)	Does not state if this was statistically significant
de la Peña <i>et al</i> <sup>[82]</sup>	93 patients with history of variceal hemorrhage and cirrhosis, randomized to receive either EVS (46/88) or EVL (42/88); 5 patients were excluded due to diagnosis of hematoma, non-cirrhotic portal hypertension or portal vein thrombosis	Prospective study	Not reported	PHG worsened in 23 patients total; statistically significantly more in EVL group than EVS group (17 <i>vs</i> 6 patients respectively)	$P < 0.01$
D'Amico <i>et al</i> <sup>[25]</sup>	212 cirrhotic patients of which 75 had an episode of variceal bleeding and were treated with sclerotherapy; 137 without bleeding were not treated with sclerotherapy	Prospective study	No EVS group at admission: 104/137 (75%) none; 28/137 (20%) mild; 5/137 (4%) severe; EVS group at admission: 50/75 (66%) none; 17/75 (22%) mild; 8/75 (11%) severe	No EVS group at end of study: 69/137 (50%) none; 61/137 (45%) mild; 7/137 (5%) severe; EVS group at end of study: 13/75 (17%) none; 49/75 (65%) mild; 13/75 (17%) severe	The conclusion was that sclerotherapy is a significant indicator of the risk of PHG in a multivariate analysis ( $P = 0.00032$ )

PHG: Portal hypertensive gastropathy; EVL: Endoscopic variceal ligation; EVS: Endoscopic variceal sclerotherapy.

frequency and severity of PHG after variceal eradication resulted from decreasing collateral blood flow through esophageal varices causing increasing PHG from gastric mucosal congestion. This mechanism is supported by

finding that gastric mucosal blood flow increases after variceal ligation<sup>[91]</sup>. Another theory is that delayed gastric emptying after sclerotherapy from extravasation of sclerosant, may cause development of PHG<sup>[69]</sup>. No direct

**Table 5** Well-established, important risk factors for portal hypertensive gastropathy

Parameters	Ref.
Portal hypertension	
Non-cirrhotic portal hypertension	[8,14]
Cirrhotic portal hypertension	[8,9,34]
Cirrhosis	
Longer duration of cirrhosis	[34,71]
Greater severity of cirrhosis	[55,67]
Greater size of esophageal varices	[34,62]
Eradication of esophageal varices	
Endoscopic therapies	
Endoscopic variceal ligation	[11,41]
Endoscopic sclerotherapy	[11,83]
Angiographic	
Percutaneous transhepatic variceal embolization	[85]

evidence exists for delayed gastric emptying in PHG<sup>[71]</sup>.

Data on which technique of endoscopic variceal eradication leads to quantitatively more *de novo* PHG is contradictory. Most studies showed no differences in frequency or severity of PHG after variceal ligation vs sclerotherapy<sup>[19,34,41,73,92,93]</sup>, but some studies showed worse outcomes after variceal ligation<sup>[82,93,94]</sup>, while some other studies showed worse outcomes after sclerotherapy<sup>[95]</sup>.

*De novo* PHG after variceal obliteration is often transitory and less severe than PHG that predated the variceal obliteration<sup>[10,73]</sup>. For example, Sarin *et al.*<sup>[10]</sup> reported in a study of 84 patients followed for a mean of  $25 \pm 14$  mo that PHG resolved in 28 (44%) of 64 patients who developed PHG after sclerotherapy, but resolved in only 2 (9%) of 22 patients who had PHG present before sclerotherapy ( $P < 0.05$ ). Hou *et al.*<sup>[73]</sup> similarly reported that the increased severity of PHG after variceal obliteration was generally transitory and returned to baseline status. The return to baseline severity of PHG was significantly faster after variceal ligation than after sclerotherapy ( $P = 0.03$ ), attributed to ligation achieving subtotal variceal obliteration and permitting faster redistribution of blood flow<sup>[10]</sup>.

Some investigators believe the higher rate of PHG in patients undergoing endoscopic variceal sclerotherapy merely reflects a longer duration of portal hypertension, more advanced liver disease, or more severe portal hypertension in patients selected to undergo variceal sclerotherapy compared to controls rather than the performance of sclerotherapy *per se*<sup>[34,78,96]</sup>. Primignani *et al.*<sup>[34,97]</sup> demonstrated an almost identical increase in frequency of PHG with time in patients undergoing vs not undergoing sclerotherapy, and suggested that PHG evolved identically with time regardless of performance vs nonperformance of sclerotherapy.

**Additional risk factors:** PHG severity is significantly associated with thrombocytopenia or splenomegaly<sup>[42,57,58]</sup>. In a prospective study of 331 cirrhotic patients performed in South Korea, PHG severity was correlated with splenic diameter: Splenic diameter with

severe PHG =  $13.1 \pm 2.4$  cm, diameter with mild PHG =  $12.2 \pm 2.5$  cm, and diameter with no PHG =  $10.7 \pm 2.9$  cm,  $P < 0.001$ <sup>[57]</sup>. In this study, PHG severity was inversely correlated with platelet count: count with no PHG =  $174600 \pm 109400$  platelets/mm<sup>3</sup>, count with mild PHG =  $132000 \pm 100700$  platelets/mm<sup>3</sup>, count with severe PHG =  $102800 \pm 68800$  platelets/mm<sup>3</sup> ( $P < 0.001$ ). Among 1016 patients with bridging fibrosis or compensated cirrhosis undergoing EGD in the HALT-C trial, including 374 (37%) with PHG, PHG was negatively correlated with platelet count in a logistic regression model (negative estimate: -0.00407, OR = 0.99, 95%CI: 0.99-0.998;  $P = 0.0007$ )<sup>[42]</sup>.

In one study, PHG in patients with chronic liver disease was correlated with increasing thickness of the lesser omentum, and presence of a splenorenal shunt<sup>[22]</sup>. This study found that PHG frequency was not associated with severity of hypersplenism<sup>[62]</sup>. The HALT-C trial showed no association between prevalence or severity of PHG and lifetime alcohol consumption, nonsteroidal anti-inflammatory drugs (NSAIDs) use, COX- (cyclooxygenase-) 2 inhibitor use, or smoking<sup>[42]</sup>. The lack of association with alcoholism may reflect the need for near abstinence from alcohol to have enrolled in the clinical trial; at study enrollment 86% of patients reported abstinence and 14% reported minimal drinking of alcohol. Table 5<sup>[8,9,11,14,34,41,55,62,67,71,83,85]</sup> lists well-established risk factors for PHG; Table 6<sup>[11,41,44,55,75-77,83,85,98-107]</sup> lists therapies that affect the severity of PHG or the risk of bleeding from PHG, and Table 7<sup>[8,14,28,30,35,42,84,108-110]</sup> lists the factors that do not affect the risk of PHG.

### Pathogenesis

**Hemodynamic changes:** The pathogenesis of PHG is inadequately understood<sup>[96]</sup>. Hemodynamic changes, especially increased portal pressure, are the suspected underlying cause because PHG develops only with established portal hypertension<sup>[72]</sup>. However, portal hypertension cannot be the sole factor because many patients with portal hypertension do not develop PHG<sup>[36,111]</sup>. Hemodynamic changes in patients with portal hypertension lead to hyperdynamic congestion with a change in gastric mucosal blood flow<sup>[112]</sup>, that leads to activation of cytokines, growth factors, and hormones that perpetuate this hyperdynamic gastric circulation<sup>[113]</sup>. Vascular congestion in PHG alters the gastric microcirculation, but the nature and extent of this alteration is somewhat controversial. The hyperdynamic circulation of portal hypertension is characterized by increased intrahepatic vascular resistance, generalized splanchnic vasodilatation, decreased mean arterial pressure, decreased systemic vascular resistance, increased gastric blood flow, and most likely decreased gastric mucosal flow<sup>[110,114,115]</sup>. Hashizume *et al.*<sup>[116]</sup> reported that cirrhotic patients have dilated small gastric blood vessels, including arterioles, precapillaries, capillaries, submucosal veins, and subserosal veins, with decreased arteriovenous resistance and straightening of arterioles.

**Table 6** Therapies affecting the severity or the risk of bleeding from portal hypertensive gastropathy

Therapies reducing severity of PHG	Ref.
TIPS	[76,77,98]
Transcatheter splenic arterial embolization	[99]
Surgical shunt	
Portocaval shunt	[100]
Central splenorenal shunt	[101]
Laparoscopic splenectomy (in patients with hypersplenism)	[55]
Liver transplantation	[44]
Therapies reducing risk of bleeding from PHG	
TIPS	[75,98,102]
Surgical shunt (portocaval or splenorenal)	[100,101]
Nonselective $\beta$ -adrenergic receptor antagonists ( <i>e.g.</i> , propranolol)	[103 (in rats),104]
Somatostatin family of drugs	
Somatostatin	[105]
Octreotide	[106]
Vasopressin family of drugs	
Vasopressin	[106]
Terlipressin	[107]
Therapies that increase incidence or risk of bleeding from PHG	
Endoscopic therapies for varices	
Variceal ligation	[11,41]
Variceal sclerotherapy	[11,83]
Interventional angiography	
Percutaneous transhepatic variceal embolization	[85]

TIPS: Transjugular intrahepatic portosystemic shunt; PHG: Portal hypertensive gastropathy.

This hyperdynamic circulation impairs gastric mucosal defense mechanisms, causes release of proinflammatory mediators, and inhibits growth factors which render gastric mucosa more susceptible to injury<sup>[67,117]</sup> and impair mucosal healing<sup>[113,114,118,119]</sup>. This vulnerable mucosa becomes predisposed to bleeding<sup>[117,120]</sup>. Decreased gastric mucosal perfusion may explain the increased rate of erosions, ulcers, and bleeding in PHG<sup>[118]</sup>. Abnormal regulation of the gastric microcirculation in PHG may render gastric mucosa more vulnerable to hypoxia<sup>[112,122]</sup>, and more susceptible to noxious gastric factors, such as aspirin and ethanol<sup>[123-125]</sup>.

Misra *et al.*<sup>[126]</sup> showed that gastric mucosal capillaries, obtained by endoscopic mucosal biopsies, have a much thicker wall in patients with cirrhosis than in healthy volunteers. Ichikawa *et al.*<sup>[127]</sup> reported a narrower diameter in gastric mucosal capillaries and less capillary angiogenesis, measured as percentage of buds in microvessels, after exposure to ethanol in individuals with PHG as compared to healthy controls. Tarnawski *et al.*<sup>[121]</sup> reported prominent cytoplasm in endothelial cells of mucosal microvessels, that narrowed the capillary lumina, in rats with PHG. This finding was confirmed by electron microscopy which showed significantly larger cytoplasmic and pinocytic vesicular areas and increased capillary basement membrane thickness. Additionally, there was arterIALIZATION of submucosal veins and thickening of arterioles in the muscularis mucosae and submucosa<sup>[121]</sup>.

**Table 7** Factors not affecting risk of portal hypertensive gastropathy

Factors not affecting risk of portal hypertensive gastropathy	Ref.
Etiology of cirrhosis	[8,28,30]
Etiology of non-cirrhotic portal hypertension	[8,14,35,83,108]
Alcoholism	[30,42]
NSAID use	[42]
Use of COX-2 inhibitors	[42]
Smoking tobacco	[42]
Gastric infection with <i>Helicobacter pylori</i>	[109,110]

NSAID: Nonsteroidal anti-inflammatory drug; COX-2: Cyclooxygenase-2.

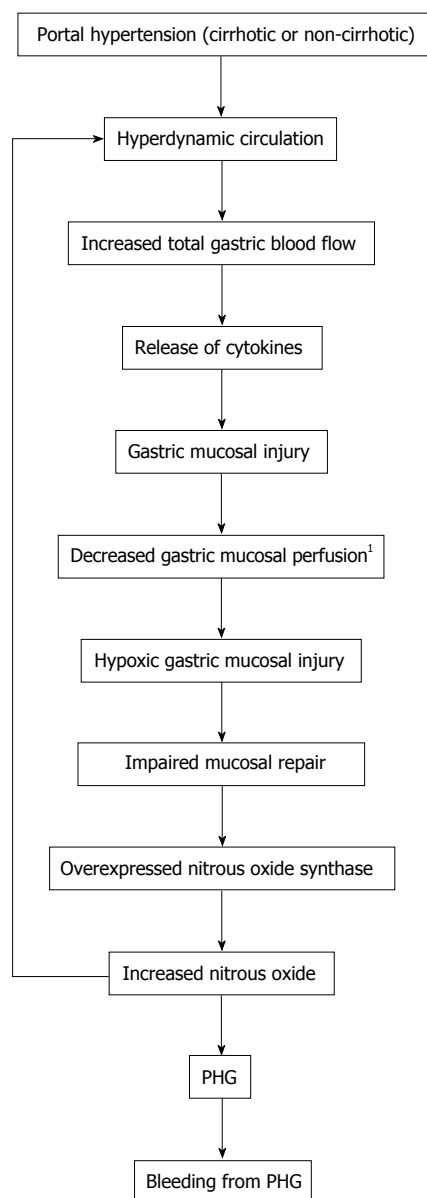
The level of gastric mucosal blood flow in PHG is controversial. Most studies reported decreased mucosal blood flow in patients with PHG<sup>[76,128-131]</sup>, whereas several studies reported increased gastric mucosal blood flow in experimental animals and in humans with PHG<sup>[132-136]</sup>. Makhija *et al.*<sup>[118]</sup> described in PHG a decrease in gastric mucosal blood flow, an increase in the submucosal and muscular layer blood flow, and a net increase in total gastric blood flow. Mezawa *et al.*<sup>[76]</sup> using a laser Doppler flowmeter to measure gastric mucosal blood flow and near-infrared endoscopy to measure total gastric blood flow, reported decreased mucosal blood flow and increased total blood flow in patients with PHG. These results reversed after undergoing TIPS, with an increase in mucosal blood flow and a decrease in total blood flow. These findings support the hypothesis of decreased mucosal blood flow in patients with PHG. Ohta *et al.*<sup>[113]</sup> reported a decrease in superficial mucosal blood flow rendering mucosa more susceptible to injury, but noted a net increase in total gastric blood flow. Variability in study results arise from study biases including chronic anemia in some patients, variable measurement techniques, different techniques of applying endoscopic probes in laser-Doppler flowmetry, and differences in gastric mucosal angioarchitecture<sup>[113]</sup>. Laser-Doppler flowmetry, moreover, has limited utility in clinical practice<sup>[137,138]</sup>.

Portal hypertension increases the splenic circulation<sup>[139]</sup>. Pan *et al.*<sup>[79]</sup> reported that PHG severity was strongly correlated with hypersplenism ( $P = 0.003$ ). However, Abbasi *et al.*<sup>[62]</sup> did not show this correlation. The difference between these studies may reflect use of different classifications for PHG. Figure 1 describes the hypothesized pathophysiology of PHG. This current mechanism is currently sketchy and likely incomplete.

Patients with secondary polycythemia A have decreased blood flow and oxygen carrying capacity because of sluggish movement of viscous blood; this phenomenon produced endoscopic and histopathologic findings of congestive gastropathy similar to those in PHG that reversed after the patient underwent serial phlebotomies to reverse the polycythemia<sup>[140]</sup>.

**Molecular mechanisms:** Numerous molecular and cellular mechanisms have been investigated regarding





**Figure 1** Hypothesized mechanism of portal hypertensive gastropathy.

<sup>1</sup>The finding of decreased gastric mucosal perfusion in PHG is somewhat controversial (see text). PHG: Portal hypertensive gastropathy.

the pathogenesis of PHG.

**Apoptosis:** Wu *et al.*<sup>[141]</sup> showed that rats with PHG had increased gastric mucosal apoptosis and decreased mucosal proliferation. Recently, a p53-upregulated modulator of apoptosis (PUMA) was reported markedly induced in gastric mucosa in patients or mouse models of PHG. PUMA is modulated by endoplasmic reticulum - stress-induced mucosal epithelial apoptosis in PHG<sup>[142]</sup>. This effect could promote mucosal injury in PHG.

**Free radicals and antioxidants:** Kaur *et al.*<sup>[143]</sup> showed elevated levels of injurious free radicals and lysosomal enzymes and decreased levels of protective antioxidant enzymes in gastric mucosal homogenates from rats with portal hypertension. Kawanaka *et al.*<sup>[144]</sup> showed impaired endoplasmic reticulum serine/

threonine kinase-2 (ERK2) activation after oxidative stress in rat gastric mucosa; ERK2 normally protects against cellular stress by inducing cell proliferation in gastric mucosa. Kinjo *et al.*<sup>[145]</sup> showed that enhanced nitration of ERK by peroxynitrite is involved in impaired MAPK (ERK) signaling in PHG, which impairs mucosal healing and promotes mucosal injury. The levels of lipid peroxide and nitrotyrosine that tend to promote gastric injury increased significantly in rats with PHG as compared to controls.

**Mucin:** Wang *et al.*<sup>[146]</sup> reported significantly reduced expression of mucin mRNA in rat models of portal hypertension induced by partial portal vein ligation. Decreased mucin production may impair gastric mucosal protection. Rats with portal hypertension had significantly greater injury to gastric mucosa than healthy controls after exposure to gastrototoxic compounds. Tomikawa *et al.*<sup>[135]</sup> reported decreased mucosal gel layer thickness, surface epithelial cell intracellular pH, and oxygenation of gastric mucosal surface in rats with PHG.

**Angiogenesis:** As aforementioned, the number of angiogenic buds decreased after injury to PHG mucosa. This phenomenon may decrease the reparative capacity of PHG mucosa<sup>[127]</sup>. However, Tsugawa *et al.*<sup>[136]</sup> reported humans with PHG had increased vascular endothelial growth factor (VEGF), a potent angiogenic factor. Additionally, rats with PHG had a significant decrease in the SaO<sub>2</sub> and PaO<sub>2</sub> of the arterial blood gas, and increased levels of VEGF, proliferating cell nuclear antigen (PCNA) expression, and gastric mucosal blood flow in gastric mucosa. They proposed that gastric mucosal hypoxia in portal hypertension and elevation of VEGF and PCNA levels might accelerate mucosal angiogenesis and increase blood flow<sup>[147]</sup>.

**Tumor necrosis factor alpha:** Tumor necrosis factor alpha (TNF- $\alpha$ ) may directly contribute to the hyperdynamic circulation in PHG. Patients and animal models with portal hypertension had an elevated TNF- $\alpha$  level which stimulated release of nitric oxide (NO) and prostacyclin, important mediators of a hyperdynamic circulation<sup>[148]</sup>. For example, in one study, 96 healthy rats were injected with either anti-TNF- $\alpha$  polyclonal antibodies or placebo before surgically creating portal vein stenosis (PVS) to induce portal hypertension and 4 d after in the short-term inhibition group and 1, 4, 7 and 10 d after PVS in the long term-inhibition group. Anti-TNF- $\alpha$  treated PVS rats exhibited lower serum levels of TNF- $\alpha$ , which normally stimulates the synthesis of NO and prostacyclin, and exhibited lower serum levels of nitrates and nitrites and of 6-keto-PGF<sub>1- $\alpha$</sub>  (6-keto-PGF<sub>1 $\alpha$</sub> ), used to monitor NO and prostacyclin release, respectively. The combined nitrate and nitrite level was significantly reduced from 68  $\pm$  9 nmol/mL in controls to 42  $\pm$  8 nmol/mL in the short-term inhibition group ( $P < 0.05$ ), and from 66  $\pm$  6 nmol/mL in controls to 44  $\pm$  4 nmol/mL in the long-term inhibition group ( $P$

< 0.05). Similarly the 6-keto-PGF<sub>1α</sub> was significantly reduced from 484 ± 92 pg/mL in the controls to 174 ± 12 pg/mL in the short-term inhibition group ( $P < 0.05$ ), and from 522 ± 98 pg/mL in the controls to 169 ± 18 (SD) pg/mL in the long-term inhibition group ( $P < 0.05$ ). Kaviani *et al.*<sup>[149]</sup> reported that TNF-α increased by 50% and inducible nitric oxide synthase (iNOS) mRNA levels increased by 300% in gastric strips after ligating the portal vein in rats ( $P < 0.01$  for both). These data are consistent with TNF-α playing a role in the hyperdynamic circulation in PHG via NO and prostacyclin.

Baseline constitutional NOS (cNOS) mRNA expression increased by 75% in the PHG group as compared to placebo ( $P < 0.01$ )<sup>[149]</sup>. NOS was significantly reduced after injecting a TNF-α neutralizing antibody during incubation of mucosal strips from portal hypertensive rats; the expression of inducible NOS mRNA levels was incrementally decreased by 40%, 70% and 80% after 1, 2, and 6 h of incubation, respectively ( $P < 0.05$ )<sup>[149]</sup>. Ohta *et al.*<sup>[150]</sup> similarly successfully used TNF-α antibody to normalize gastric mucosal blood flow in rats with PHG and to significantly reverse overexpression of gastric NOS isoform 3. In PHG rats, treatment with TNF-α antibody significantly reduced the elevated NOS isoform 3 mRNA expression by 48% ( $P < 0.01$ ). Moreover, administration of thalidomide, which enhances TNF-α mRNA degradation, decreased levels of TNF-α and NOS in animals with portal hypertension produced by partial portal vein ligation<sup>[114]</sup>.

**Nitric oxide:** Patients with portal hypertension and PHG have increased serum levels of NO, a potent vasodilator released by endothelial cells. Ohta *et al.*<sup>[151]</sup> demonstrated gastric cNOS significantly increased, by 67%, in portal hypertensive rats, experimentally produced by portal vein and splenic vein occlusion, as compared to sham-operated rats at 14 d after surgery ( $P < 0.05$ ). In portal hypertensive rats, cNOS fluorescence intensity was significantly higher in endothelia of submucosal veins [ $96.2 \pm 5.9$  (SD) U] as compared to endothelia of mucosal collecting veins [ $69.5 \pm 1.7$  (SD) U,  $P < 0.01$ ], or endothelia of veins of muscularis mucosae [ $55.7 \pm 10.0$  U,  $P < 0.01$ ]. The average fluorescence area in submucosal vein endothelia was significantly higher in portal hypertensive rats than in normal controls [ $1038.5 \pm 459.5$  (SD)  $\mu\text{m}^2$  vs  $372.4 \pm 180.3$  (SD)  $\mu\text{m}^2$ ,  $P < 0.01$ ]. This finding may provide a molecular mechanism for submucosal vascular dilation in the hyperdynamic circulation in PHG. In another study, gastric mucosal cNOS levels were significantly higher in patients with cirrhosis and severe PHG compared to healthy controls [ $125.4 \pm 4.3$  (SD) pmol/mg protein/minute vs  $88 \pm 8.6$  (SD) pmol/mg protein/minute,  $P < 0.002$ ]. Likewise, gastric mucosal iNOS levels were significantly higher in patients with cirrhosis and severe PHG than in healthy controls [ $259.7 \pm 5.5$  (SD) pmol/mg protein/min vs  $130.8 \pm 6.6$  (SD) pmol/mg protein/min,  $P < 0.0001$ ]<sup>[152]</sup>. Serum nitrate/nitrite levels were  $30.1 \pm 3.2$  nmol/mL in the first group vs  $15.5 \pm 0.09$  (SD) nmol/mL in the

second group ( $P < 0.001$ )<sup>[152]</sup>. In another study, iNOS and cNOS levels were also higher in gastric mucosa of patients with PHG than in controls<sup>[153]</sup>, and were significantly higher in patients with severe PHG as compared to patients with mild or no PHG<sup>[154]</sup>. Nitrous oxide may underlie the gastric vascular dilation<sup>[152]</sup>, and hyperdynamic circulation in PHG<sup>[148]</sup>.

However, Lee *et al.*<sup>[155]</sup> reported administration of aminoguanidine, an iNOS inhibitor, successfully corrected the hyperdynamic circulation without affecting PHG, suggesting that iNOS and NO are important in the hyperdynamic circulation in portal hypertension, but play a limited role in PHG development. They argued that PHG should be treated by reducing portal pressure rather than reversing the hyperdynamic circulation<sup>[155,156]</sup>.

**Glucagon:** Glucagon levels are elevated in patients with portal hypertension<sup>[118]</sup>. Curvêlo *et al.*<sup>[54]</sup> found in 43 patients with PHG from portal hypertension with cirrhosis, that the mean serum glucagon level after an overnight fast was significantly higher than the level in healthy controls. Serum glucagon levels were significantly correlated with high systemic vascular resistance index ( $r = -0.523$ ;  $P = 0$ ) and HVP (G) ( $r = 0.34$ ;  $P = 0.019$ ). Glucagon significantly increases portal pressure<sup>[157-159]</sup>, and causes splanchnic vasodilation<sup>[148]</sup>. Geraghty *et al.*<sup>[158]</sup> found a strong correlation between portal pressure and glucagon levels ( $r = 0.85$ ). Tsui *et al.*<sup>[160]</sup> reported that glucagon significantly increased portal pressure in rats with portal vein ligation, but did not alter portal pressure in sham-operated rats. The effect of glucagon occurred only in rats with preexisting portal hypertension. Exogenous glucagon rendered gastric mucosa more susceptible to injury from toxins, such as ethanol, which was attenuated by somatostatin<sup>[160]</sup>. For example, the lesion area was significantly higher at > 60% of gastric mucosa after glucagon administration, compared to somatostatin or glucagon and somatostatin administration ( $P < 0.05$ , ANOVA)<sup>[160]</sup>.

**Prostaglandins:** Studies in patients or animal models with portal hypertension failed to show significant differences in prostaglandin E2 (PGE2) levels as compared to healthy controls<sup>[125,141,161-163]</sup>. Low prostaglandin levels significantly decreased gastric perfusion velocity in cirrhotic rats, whereas misoprostol, a PGE2 analogue, significantly increased gastric perfusion in cirrhotic rats as compared to controls<sup>[125]</sup>. For example, Beck *et al.*<sup>[125]</sup> found that administration of indomethacin did not affect gastric perfusion velocity in healthy control rats, despite reducing gastric PGE2 synthesis by > 95%, but reduced gastric perfusion velocity by 30% within 10 min in cirrhotic rats achieved by ligating the common bile duct ( $P < 0.05$ ). The hyperemic response to application of ethanol was significantly reduced in cirrhotic rats compared to healthy rats ( $56.3\% \pm 21.7\%$  (SD) vs  $66.1\% \pm 17.1\%$  (SD) increase,  $P < 0.05$ ). Misoprostol applied to gastric mucosa caused

concentration-dependent increase in perfusion velocity, with a significantly greater increase in perfusion velocity in cirrhotic rats with concentrations of misoprostol  $> 0.8$  mcg/mL ( $P < 0.05$ ).

Beck *et al.*<sup>[164]</sup> further reported that administration of misoprostol to cirrhotic rats for 1 mo restored the hyperemia in response to ethanol that sham-operated, non-cirrhotic rats showed, whereas placebo-treated cirrhotic rats failed to increase gastric blood flow in response to ethanol. PGE2-treated cirrhotic rats exhibited significantly less spontaneous gastric mucosal damage [ $0.2\% \pm 0.07\%$  (SD)] than placebo-treated cirrhotic rats [ $3.0\% \pm 0.8\%$  (SD);  $P < 0.05$ ]. The mean microscopic gastric injury score was significantly less in PGE2-treated cirrhotic rats [ $0.7 \pm 0.3$  (SD)] than in placebo-treated cirrhotic rats [ $2.1 \pm 0.4$  (SD);  $P < 0.05$ ].

Rats with PHG exhibited suppression of gastric mucosal COX-1 levels, but exhibited normal COX-2 levels compared to healthy controls<sup>[141]</sup>. Nonselective COX inhibitors, such as aspirin, decrease PGE2 levels resulting in more apoptosis of cells. Payen *et al.*<sup>[123]</sup> reported that gastric mucosal potential difference, an index of mucosal integrity, decreased with increasing severity of PHG, suggesting greater vulnerability of gastric mucosa in patients with PHG. Payen *et al.*<sup>[123]</sup> further reported a significantly greater decline of potential difference after aspirin administration of  $11.1 \pm 3.6$  (SD) mV in 9 patients with severe PHG, vs  $9.2 \pm 3.6$  mV in 21 patients with moderate PHG ( $P < 0.05$ ), and vs  $6.4 \pm 1.9$  (SD) mV in 10 healthy controls ( $P < 0.05$ ). Also, PGE2 administration suppressed the increased apoptosis which occurred in rats with PHG.

**Prostacyclin:** Prostacyclin, a vasodilator that inhibits gastric acid secretion, has been proposed as a mediator of the hyperdynamic circulation in PHG from portal hypertension<sup>[148,163]</sup>. Ohta *et al.*<sup>[161]</sup> found significantly elevated serum levels of 6-keto-PGF<sub>1 $\alpha$</sub> , a metabolite of prostacyclin, in cirrhotic patients with PHG. They also reported that these patients had significantly elevated levels of 6-keto-PGF<sub>1 $\alpha$</sub>  in the mucosa of the gastric fundus.

**Other cytokines and growth factors:** Several studies have analyzed the roles of endothelin-1, VEGF, and other cytokines in PHG, but further research is required<sup>[165]</sup>. The gastric concentrations of epidermal growth factor were comparable between patients with and without PHG, and its significance in PHG remains unclear<sup>[166]</sup>. A high serum level of autotaxin, involved in liver fibrosis, was associated with advanced stage of cirrhosis, presence of esophageal varices, and PHG<sup>[167]</sup>.

**Helicobacter pylori:** Numerous studies demonstrated that *Helicobacter pylori* (*H. pylori*) infection is not associated with PHG<sup>[7,9,25,79,109,110,154,168-172]</sup>. Indeed, several studies reported that patients with PHG less frequently have *H. pylori* infection than controls. *H. pylori* does not appear to play a pathogenic role in

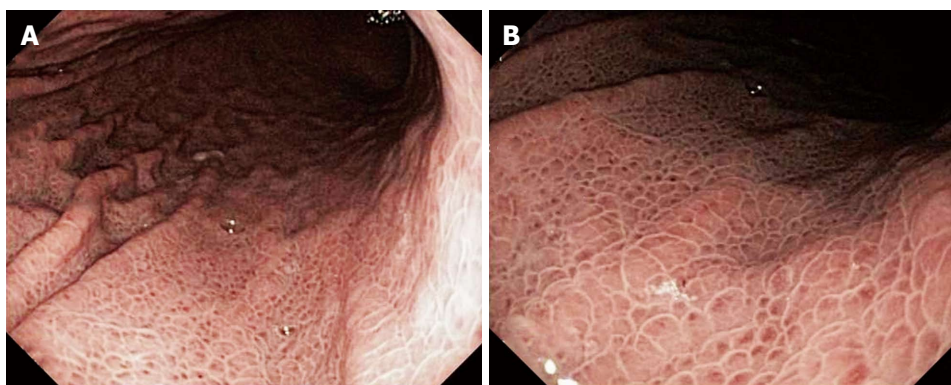
ulcers associated with PHG<sup>[173]</sup>. Contrariwise, Sathar *et al.*<sup>[174]</sup> reported an association between *H. pylori* infection and PHG in cirrhotic patients, but this study apparently had limitations, including low specificity and low sensitivity of *H. pylori* serology in cirrhotic patients, potential selection bias, and underreporting of *H. pylori* seroprevalence<sup>[174-178]</sup>.

## Diagnosis

**Endoscopy:** PHG is diagnosed by EGD<sup>[72]</sup>. The characteristic endoscopic appearance is a mosaic-like pattern or a diffuse, erythematous and reticular cobblestone pattern of gastric mucosa consisting of small polygonal areas, with superimposed red punctate lesions,  $> 2$  mm in diameter and a depressed white border<sup>[78,96,177]</sup>. The red lesions vary in size and in color depending on PHG severity. The lesions range from pink speckled lesions within a mosaic or snakeskin pattern in mild cases, to localized small areas of intense erythema, resembling a scarlatina rash, in severe cases<sup>[70,71,112,139]</sup>. These findings occur predominantly in the gastric body and fundus, and rarely in the antrum<sup>[71,72,139]</sup>. Figure 2 illustrates a patient with classic endoscopic findings of portal hypertensive gastropathy. Toyonaga *et al.*<sup>[178]</sup> reported in a meta-analysis of 6 studies that the mosaic-like pattern had high specificity at 98% (range: 93%-100%), but low sensitivity at 38% (range: 7%-94%) for PHG, with an accuracy of 78% (range: 63%-98%). In severe PHG numerous petechiae and bleeding spots present as a diffuse hemorrhagic gastropathy<sup>[112,139]</sup>.

**Endoscopic classification:** Endoscopic classification of PHG severity is clinically important because severity is correlated with bleeding risk<sup>[31,71,72,179]</sup>. PHG can be simply categorized as mild with a mosaic-like pattern without red spots, or as severe, with superimposed red lesions present<sup>[78,96]</sup>. Multiple formal endoscopic classifications exist (Table 8<sup>[3,7,8,70,180-183]</sup>), with no consensus as to which classification is the best<sup>[5,182-184]</sup>. The following classifications are commonly used: Classifications by McCormack *et al.*<sup>[3]</sup>, Tanoue *et al.*<sup>[180]</sup>, the New Italian Endoscopic Classification (NIEC)<sup>[70]</sup>, and the Baveno scoring system<sup>[181]</sup>.

In 1985, McCormack *et al.*<sup>[3]</sup> classified PHG according to presence of red spots into mild and severe disease. In 1991, McCormick *et al.*<sup>[7]</sup> divided the prior single category of mild PHG into mild and moderate PHG based on absence vs presence of erythema, respectively. This moderate category is infrequently used<sup>[7]</sup>. In 1992 Tanoue *et al.*<sup>[180]</sup> and Parker *et al.*<sup>[185]</sup> also expanded the scoring system by providing a grade between mild and severe. This classification is rendered cumbersome by a lack of sharply defined differences between the added intermediate category and the original categories<sup>[71]</sup>. This system is, however, simple and can help predict bleeding risk<sup>[71,182]</sup>. Iwao *et al.*<sup>[186]</sup> found McCormack's classification was accurate for fine pink speckling in 54%, for snakeskin pattern in 76%, and for cherry-red spots in 64%. The NIEC produced a better definition in



**Figure 2** A 60-year-old man presented for routine endoscopic screening for esophageal varices due to a history of Child-Pugh class B cirrhosis, with a model for end-stage liver disease score = 18, from hepatitis C secondary to former intravenous drug use. The patient denied a history of gastrointestinal bleeding. The hematocrit was 40.1%. Esophagogastroduodenoscopy revealed the classic findings of portal hypertensive gastropathy, including a pale white reticular (mosaic) pattern surrounding small polygonal areas of mucosa, with variable erythema, in the entire stomach, but most prominently in the gastric fundus and body. B is a relatively close-up view of the lesions seen in A.

**Table 8** Different classification systems for portal hypertensive gastropathy

Ref.	Mild	Moderate	Severe
McCormack <i>et al</i> <sup>[3]</sup>	Fine pink speckling (scarlatina type rash) Superficial reddening, especially on rugal surface (striped appearance) Fine white reticular pattern separating areas of raised edematous mucosa (snake skin)		Discrete red spots (analogous to cherry red spots in esophagus)  Diffuse hemorrhagic gastritis
McCormick <i>et al</i> <sup>[7]</sup> Tanoue <i>et al</i> <sup>[180]</sup>	Mosaic or snake skin appearance Mild reddening, congestive mucosa, no mosaic-like pattern	Presence of erythema Severe redness and a fine reticular pattern separating the areas of raised edematous mucosa (mosaic-like pattern) or a fine speckling	Presence of erosions or hemorrhagic gastritis Grade III (severe) Point bleeding + grade II (moderate)
Spina <i>et al</i> <sup>[70]</sup> (NIEC)	Mosaic-pattern: Presence of small, polygonal areas surrounded by a whitish-yellow depressed border		Red point lesions (1 mm in diameter, flat) Cherry-red spots (2 mm, slight protrusion) Black-brown spots (irregularly shaped, persistently present after washing)
Sarin <i>et al</i> <sup>[8]</sup>	Discrete cherry red spots, with or without mosaic pattern		Presence of confluent red spots, diffusely distributed in a large portion of the stomach
Sarin <i>et al</i> <sup>[181]</sup> (Baveno II Consensus Workshop) <sup>[181]</sup>	Mild $\leq 3$ points <sup>1</sup>		Severe $\geq 4$ points <sup>1</sup> Gastric antral ectasia Absent (0) Present (2)
Yoo <i>et al</i> <sup>[182]</sup> 2-category classification	Fine pink speckling (scarlatina type rash) Superficial reddening Mosaic pattern		Discrete red spots Diffuse hemorrhagic lesion
Yoo <i>et al</i> <sup>[182]</sup> 3-category classification	Mild reddening Congestive mucosa Diffuse pink areola	Flat red spot in center of a pink areola Severe redness and a fine reticular pattern	Diffusely red areola Pinpoint bleeding Discrete or confluent red mark lesion

<sup>1</sup>Points assigned for Baveno II consensus according to the following: Mild mucosal mosaic pattern = 1 point, severe mucosal mosaic pattern = 2 points; isolated red markings = 1 point, confluent red markings = 2 points; gastric antral ectasia present = 2 points.

1992 of mild and severe HPG<sup>[32,34,70]</sup>. Elementary lesions of PHG according to the NIEC classification include: (1) mosaic-like pattern defined as small, polygonal areas surrounded by a whitish-yellow, depressed border. This mosaic pattern is mild when the areola is uniformly pink, moderate if the center is red, and severe if the areola is uniformly red; (2) red-point lesions defined as small, flat, 1-mm-wide, punctate, red lesions; (3) cherry-red spots defined as red, 2-mm-wide, round lesions which protrude slightly into the gastric lumen;

and (4) black-brown spots defined as irregularly shaped flat black or brown spots from intramucosal hemorrhage that remain after endoscopic irrigation. PHG is defined as mild when only a mosaic-like pattern of any degree was present, and severe when red-point lesions, cherry red spots, or black-brown spots were present. Due to variable data on the classification systems, Hashizume *et al*<sup>[184]</sup> proposed a simplified classification that divides PHG into three stages by presence of: Non-specific redness, a mosaic pattern, and red spots.



Yoo *et al.*<sup>[182]</sup> demonstrated substantial limitations in intra-observer and inter-observer reproducibility in the most common 2-scoring and 3-scoring systems. Nevertheless, the 2-scoring system by McCormack *et al.*<sup>[3,7]</sup> produced better and more reproducible results than the 3-scoring system by Tanoue *et al.*<sup>[180]</sup>. The mean inter-observer kappa value was 32% higher and mean intra-observer kappa value was 15% higher for the 2-scoring system compared with the 3-scoring system. However, both inter-observer and intra-observer kappa values in both classification systems were below the desirable value of  $> 0.75$ <sup>[187]</sup>. Kappa values represent the degree of agreement as compared with that expected by chance alone, with one being perfect agreement, and zero being no greater agreement than expected by chance alone<sup>[182]</sup>.

The Baveno scoring system uses point calculations to define PHG as mild ( $\leq 3$  points) vs severe ( $\geq 4$  points)<sup>[181]</sup>. This system adds gastric antral vascular ectasia (GAVE) into the classification<sup>[179]</sup>. Stewart *et al.*<sup>[179]</sup> showed that this scoring system was reproducible and accurately reflected the risk of PHG-related bleeding in cirrhotic patients. Kappa values for mucosal mosaic pattern, red marks, and GAVE were  $> 0.75$ , indicating good reproducibility. Kappa values for lesion severity were lower, attributed to loss of details in endoscopic photographs.

de Macedo *et al.*<sup>[187]</sup> proposed analyzing binary criteria such as presence vs absence of mosaic-like pattern, red punctate lesions, and cherry-red spots, without subdivisions or classification systems. These binary criteria were associated with high inter-observer reliability and accuracy (94%, 81% and 83% respectively). Mosaic-like pattern was associated with high sensitivity (100%). The previously used classifications and subdivisions showed unsatisfactory reliability and low inter-observer agreement.

Current classification systems are suboptimal. The ideal classification system should be simple, clinically useful, accurate, and reproducible with high levels of intra-observer and inter-observer agreement<sup>[183]</sup>. The 2-scoring system is most commonly used due to relative simplicity and reasonable reproducibility<sup>[71]</sup>.

**Capsule endoscopy:** Several studies evaluated the diagnostic accuracy of capsule endoscopy. In a study using the PillCam ESO capsule, capsule endoscopy had an overall concordance with EGD of 90.6% for PHG<sup>[188]</sup>. This study included only 32 patients of whom 19 had PHG, and a large trial is underway to confirm these findings. In another study, PHG was identified by capsule endoscopy in 13 (68.4%) of 19 patients with cirrhosis, portal hypertension, and chronic anemia, but the 19 patients did not undergo EGD to determine capsule endoscopy test sensitivity and specificity<sup>[49]</sup>. In a study of 50 patients with cirrhosis undergoing both EGD and capsule endoscopy for screening or surveillance of esophageal varices, capsule endoscopy had an accuracy of 57%, sensitivity of 96%, and specificity of

17% compared to EGD. Inter-observer reliability was 0.61. The researchers concluded that more data are required to assess accuracy of capsule endoscopy for diagnosis and staging of PHG<sup>[52]</sup>. In another study of 50 patients with portal hypertension undergoing EGD and capsule endoscopy, only 24 of 35 patients with PHG diagnosed by EGD had PHG detected by capsule endoscopy (sensitivity = 69%)<sup>[23]</sup>. Capsule endoscopy was somewhat more sensitive at detecting severe than mild PHG (82% vs 63%), but this difference was not significant ( $P = 0.44$ ). The accuracy was significantly higher in diagnosing PHG in the gastric body (100%) than the fundus (48%) ( $P = 0.0009$ ).

**Dynamic CT:** Kim *et al.*<sup>[150]</sup> proposed using dynamic CT to diagnose PHG by demonstrating the transient perfusion defect sign, defined as the presence of transient segmental or subsegmental hypo-attenuating mucosa in the gastric fundus or body during hepatic arterial imaging that returns to normal attenuation on portal venous or equilibrium-phase imaging. This sign had a sensitivity of 75%, specificity of 88.6%, positive predictive value of 90%, and negative predictive value of 72.1% for diagnosing PHG in patients with cirrhosis. Further prospective trials are required to validate this diagnostic modality.

Screening for PHG is currently not recommended in patients with liver disease<sup>[189]</sup>. To identify predictors of PHG and varices noninvasively in patients with chronic liver disease to increase the cost-benefits of EGD, Min *et al.*<sup>[190]</sup> combined three independent parameters in a multivariate analysis into a "Varices and PHG" (VAP) score. The score = platelets/ $\text{mm}^3 \times$  albumin in g/dL/multidimensional index for spleen volume (M-Index) in  $\text{cm}^3$ . The M-Index is calculated from spleen length, width, and thickness, as determined by helical computerized tomography, which is designed to reflect splenomegaly as a predictor of esophageal varices and PHG. A VAP cut-off value of 861 had a sensitivity of 85%, positive likelihood ratio of 3.17, and negative predictive value of 86%. This scoring system requires prospective validation<sup>[190]</sup>.

**Differentiation from GAVE:** Differentiation of PHG from GAVE is important because they have distinct pathologic, clinical, and endoscopic characteristics, and different therapies (Table 9)<sup>[34,37,71,72,75,77,103,106,191-213]</sup>. Treatments that reduce portal pressure are effective for PHG but ineffective for GAVE<sup>[193]</sup>. PHG and GAVE also affect different gastric locations. PHG generally affects the proximal stomach, whereas GAVE generally affects the distal stomach<sup>[139]</sup>. A mosaic-like pattern surrounding polygonal areas of erythema is typical for PHG, but GAVE has erythema most commonly arranged linearly along folds in the antrum, less commonly arranged as diffuse erythema in the antrum, and least commonly arranged as diffuse gastric erythema<sup>[75,78,96]</sup>.

PHG is usually diagnosed by endoscopic criteria. When endoscopic features are uncertain, histologic

**Table 9** Differences between portal hypertensive gastropathy and gastric antral vascular ectasia

Parameter	Portal hypertensive gastropathy	Gastric antral vascular ectasia	Ref.
Associated conditions	Conditions associated with portal hypertension: cirrhotic or non-cirrhotic portal hypertension	Cirrhosis, autoimmune disorders, and connective tissue diseases (scleroderma, pernicious anemia, hypothyroidism)	[72]
Association with portal hypertension	Strong association	Only 30% of cases	[191,192]
Sex	Mildly more common in males (alcoholic cirrhosis more common in males than females)	Much more common in females (80%)	[193,194]
Age	Can occur at any age in patients with portal hypertension or cirrhosis	Typically elderly (average age > 70 years old)	
Location	Proximal stomach: Fundus, body	Distal stomach: Antrum	[72,192]
Diagnosis	Endoscopy (endoscopic biopsy sometimes useful). Radiologic imaging usually not helpful	Endoscopy (endoscopic biopsy sometimes useful)	[72,195]
Appearance at endoscopy	Mosaic/snakeskin mucosa with red or brown spots	Tortuous columns of ectatic vessels in "watermelon" or diffuse pattern; erythematous or hemorrhagic	[191]
Histology	Ectatic capillaries, mildly dilated mucosal and submucosal veins; no vascular inflammation, no vascular thrombi	Marked dilation of capillaries and venules in gastric mucosa and submucosa with areas of intimal thickening, fibrin thrombi, fibromuscular hyperplasia and spindle cell proliferation	[72,191,196,197]
Clinical presentation/ complications	Gastrointestinal bleeding: Usually chronic, but sometimes acute	Almost exclusively chronic gastrointestinal bleeding with guaiac positive stools	[37,193]
Primary prophylaxis	Not indicated	Not indicated (unless associated with large varices)	[198]
Medical therapy	Non-selective $\beta$ -adrenergic receptor antagonists (propranolol), octreotide (for acute bleeding)	No benefit of $\beta$ -adrenergic receptor antagonists Oral contraceptive pills to temporarily control bleeding Questionable benefit of octreotide	[103,106,198-201]
Endoscopic therapy	Occasionally helpful (for focal bleeding) Argon plasma coagulation Local hemostasis with hemostatic spray	Very helpful at reducing risk of bleeding: Argon plasma coagulation; EBL; Radiofrequency ablation; YAG laser therapy	[202-207]
TIPS	Significantly reduces severity and risk of bleeding by reducing portal hypertension. Option for very severe bleeding from PHG or for moderate PHG in patients with variceal bleeding	Not recommended. Does not affect severity of GAVE or risk of bleeding	[75,77]
Liver transplantation	Resolves. Ultimate therapy mostly reserved for patients with end-stage liver disease	Improves or resolves with liver transplantation	[75,200,208-210]
Other surgery	Usually resolves with shunt surgery that lowers portal pressure. Partial gastrectomy not recommended	Limited surgical resection (partial gastrectomy) recommended for refractory cases. Shunt surgery not recommended	[75,200,211-213]
Prognosis from bleeding	Bleeding rarely severe and very rarely fatal	Bleeding occasionally severe	[34,71,72]

YAG: Yttrium aluminum garnet; TIPS: Transjugular intrahepatic portosystemic shunt; PHG: Portal hypertensive gastropathy; GAVE: Gastric antral vascular ectasia; EBL: Endoscopic band ligation.

analysis of gastric biopsies is useful to differentiate PHG from GAVE<sup>[86,183,197,212]</sup>. Superficial mucosal biopsies are frequently falsely negative because the lesions of PHG are generally submucosal<sup>[214,215]</sup>. Endoscopists are reluctant to perform deep biopsies in patients with known portal hypertension or suspected PHG because of increased risks of bleeding because of a coagulopathy from underlying cirrhosis or a bleeding diathesis from underlying portal hypertension<sup>[137,183]</sup>. However, deep biopsies may be necessary for the histologic diagnosis of PHG.

Characteristic histologic findings of PHG include capillary and venule dilatation, and markedly congested and tortuous submucosal venules<sup>[137]</sup>. Stromal fibrosis and edema of lamina propria can occur<sup>[137]</sup>. Inflammatory cells and fibrin thrombi are generally absent<sup>[3,139]</sup>. Characteristic histologic features of GAVE include presence of fibrin thrombi in dilated capillaries and fibromuscular proliferation within the lamina propria<sup>[96,216]</sup>.

#### Differentiating GI bleeding from varices vs PHG:

PHG may occasionally resemble gastric varices at EGD. PHG can be prominent on gastric rugae in the gastric body and fundus. The intraluminal linear projections of gastric rugae might superficially resemble that of gastric varices. However, gastric varices tend to be more serpiginous than linear and tend to be grayish due to the presence of deoxygenated venous blood within varices, whereas the lesions of PHG on gastric rugae tend to be erythematous and surrounded by a prominent mosaic pattern. It is also important to distinguish between GI bleeding from esophageal varices vs PHG in patients having both lesions. Table 10 outlines differences in GI bleeding from PHG vs esophageal varices.

#### Clinical presentation

**Acute GI bleeding:** GI bleeding is the only known clinically relevant complication of PHG. PHG is responsible for < 1% of upper GI bleeding in the general population, and for about 8% of non-variceal upper GI bleeding in patients with liver disease<sup>[217]</sup>. The reported frequency of acute upper GI bleeding in patients with

**Table 10** Differences in gastrointestinal bleeding from portal hypertensive gastropathy *vs* esophageal varices

Parameter	Portal hypertensive gastropathy	Esophageal varices
Etiology	Portal hypertension: Cirrhotic or non-cirrhotic	Portal hypertension: Cirrhotic or non-cirrhotic
Concurrence	Frequently occur simultaneously with esophageal varices because the two diseases share common risk factors	Frequently occurs simultaneously with PHG because the two diseases have common risk factors
Location	Stomach: Predominantly fundus and body	Distal esophagus: Also can have gastric varices or ectopic varices in other gastrointestinal regions, particularly duodenum
Diagnosis	Esophagogastroduodenoscopy	Esophagogastroduodenoscopy
Endoscopic appearance	Erythematous small polygonal areas of mucosa surrounded by a fine, whitish, reticular or mosaic/snakeskin mucosa with red or brown spots	Serpiginous mucosal greyish luminal projections in distal esophagus
Clinical presentation	Mild acute or chronic bleeding	Acute gastrointestinal bleeding-typically massive
Severity of bleeding	Typically mild and not life-threatening	Typically severe and life-threatening
Histology		Not biopsied at endoscopy
Endoscopic therapy	Limited role	Variceal ligation recommended as initial therapy. Sclerotherapy an alternative therapy
Medical therapy	Octreotide Propranolol Vasopressin or vasopressin analogues-infrequently recommended any more	Octreotide Propranolol Vasopressin or vasopressin analogues-infrequently recommended any more
Blakemore tube	Not recommended	Sometimes used for refractory bleeding especially as a temporizing measure before performing more definitive therapy
Angiographic therapy	TIPS used as a last resort	TIPS recommended if endoscopic therapy fails
Transfusion of packed erythrocytes	Transfuse only to hematocrit of about 28. Over-transfusion may increase portal pressure and induce greater bleeding	Transfuse only to hematocrit of about 28. Over-transfusion may increase portal pressure and induce greater bleeding
Liver transplantation	Improves or resolves with liver transplantation	Improves or resolves with liver transplantation
Prognosis	Rarely fatal	Frequently fatal

TIPS: Transjugular intrahepatic portosystemic shunt; PHG: Portal hypertensive gastropathy.

PHG ranges in incidence from 2%-20% (Table 11<sup>[3,8,25,34,37,103,217,218]</sup>). Primignani *et al.*<sup>[34]</sup> reported acute GI bleeding in 2.7% of patients with PHG, whereas Stewart *et al.*<sup>[179]</sup> reported a 20% incidence of acute bleeding in patients with PHG. McCormack *et al.*<sup>[3]</sup> reported that 29 (44.6%) of 65 patients with PHG bled from this lesion. In this study, PHG was the second most common cause of GI bleeding, after esophageal varices<sup>[3]</sup>. The reported variability is partly due to inaccuracies in the endoscopic diagnosis of PHG and in the endoscopic diagnosis of PHG as the cause of bleeding<sup>[71]</sup>. Diagnosis of PHG as the cause of bleeding can be challenging if a bleeding point is not visualized at EGD<sup>[71]</sup>.

Major risk factors for bleeding from PHG are increasing PHG duration, extent, and severity<sup>[72,179]</sup>. For example, > 90% of acute bleeding occurs with severe PHG<sup>[10,35,71,72]</sup>, and < 10% of acute bleeding occurs with mild PHG<sup>[112]</sup>. Other risk factors for bleeding from PHG include advanced cirrhosis, and prior endoscopic eradication of esophageal varices<sup>[3,5,10,81,179,180,219]</sup>. Unlike bleeding from GAVE, acute bleeding from PHG is rarely severe, very rarely fatal, and typically requires transfusion of only one-to-two units of packed erythrocytes or less<sup>[34,71,72,139]</sup>.

**Chronic bleeding:** The frequency of chronic bleeding ranges from 3%-26%<sup>[25,34,37]</sup>. Stewart *et al.*<sup>[179]</sup> reported a 6% incidence of chronic bleeding from PHG, whereas Primignani *et al.*<sup>[34]</sup> reported chronic bleeding in 11% of patients with PHG (Table 11<sup>[3,8,25,34,37,103,217,218]</sup>).

The incidence of chronic gastrointestinal bleeding from PHG is difficult to determine precisely because of variable definitions of chronic GI bleeding<sup>[67,68]</sup>. Common definitions include: (1) > 2 g/dL decrease in hemoglobin level during > 6 mo in patients without acute GI bleeding and not receiving NSAID therapy; (2) presence of anemia in patients with cirrhosis; and (3) positive fecal occult blood (Baveno II)<sup>[181]</sup>. Moreover, chronic GI bleeding can be overestimated if hemoglobin decline is solely used for the diagnosis. Patients with chronic liver disease frequently have anemia without GI bleeding, from causes including alcoholism, chronic kidney disease, hypersplenism, or bone marrow suppression. No studies have objectively quantified chronic blood loss from PHG.

Chronic bleeding from PHG is usually mild to moderate but occasionally severe<sup>[37,139]</sup>. Patients after endoscopic variceal obliteration have a higher incidence of chronic GI bleeding from PHG<sup>[10,25,72]</sup>. Chronic GI bleeding from PHG can cause iron deficiency anemia<sup>[220,221]</sup>.

### Pharmacotherapy for PHG

Current pharmacologic therapies aim to reduce portal pressure to decrease bleeding from PHG<sup>[191,214]</sup>.

**$\beta$ -adrenergic receptor antagonists:** Nonselective  $\beta$ -adrenergic receptor antagonists reduce portal pressure and gastric mucosal blood flow, and thereby reduce bleeding from PHG<sup>[103,104,132,191,222-224]</sup>. Several studies evaluated the efficacy of propranolol, a nonselective  $\beta$ -adrenergic receptor antagonist in primary and secon-

**Table 11 Rates of gastrointestinal bleeding from portal hypertensive gastropathy**

Ref.	Year published	Population	Bleeding from PHG	Transfusions required
McCormack <i>et al</i> <sup>[3]</sup>	1985	127 patients with portal hypertension of various etiologies	29 patients out of 65 with PHG, representing 25% of the total number of bleeds from all sources; 9 episodes presenting with bleeding; 71 episodes of subsequent bleeding	2-15 units required for 60 bleeds
D'Amico <i>et al</i> <sup>[25]</sup>	1990	212 patients with cirrhosis; 75 being treated with sclerotherapy		
Sarin <i>et al</i> <sup>[8]</sup>	1992	107 patients with portal hypertension presenting with variceal bleeding, undergoing sclerotherapy	No bleeding before sclerotherapy from PHG (4/107 had PHG); 2/13 post-sclerotherapy patients who developed PHG	Average of 4 units per patient with range of 2-8 units
Pérez-Ayuso <i>et al</i> <sup>[103]</sup>	1991	54 cirrhotic patients with PHG, in a RCT to look for rebleeding; propranolol 26 <i>vs</i> control 28	First hemorrhage: Acute/chronic bleeding in propranolol group 12/14; in control 12/16, rebleeding: Acute/chronic; in propranolol 6/6; in control 10/12	
Gostout <i>et al</i> <sup>[217]</sup>	1993	Patients admitted for GI bleeding (1496)	12 patients (0.8%), representing 8% of nonvariceal bleeding in patients with liver disease	
Primignani <i>et al</i> <sup>[34]</sup>	2000	373 patients with cirrhosis; PHG in 299 patients (80.1%)	8 PHG patients with acute bleeding; chronic bleeding in 34 patients	
Merli <i>et al</i> <sup>[37]</sup>	2004	222 cirrhotic patients with portal hypertension; 48 patients with PHG on enrollment	During follow up for 47 ± 28 (SD) mo, acute bleeding 9, chronic bleeding 7 from PHG	
Kimura <i>et al</i> <sup>[218]</sup>	2014	297 patients with living donor liver transplantation; retrospective analysis	2 patients bled from PHG within 3 mo after transplantation	

PHG: Portal hypertensive gastropathy; RCT: Randomized controlled trial; GI: Gastrointestinal.

dary prevention of bleeding from PHG<sup>[104,225]</sup>. Pérez-Ayuso *et al*<sup>[103]</sup> reported in a multi-center, randomized, controlled trial of 57 patients with acute or chronic bleeding from severe PHG with cirrhosis that the 26 patients administered propranolol at 20-160 mg twice daily rebled significantly less frequently than the 31 controls receiving only iron therapy as needed at 12 mo (38% *vs* 65%;  $P < 0.05$ ), and at 30 mo follow-up (7% *vs* 52%,  $P < 0.05$ ). Patients receiving propranolol were transfused less units of packed erythrocytes than the controls, but this difference was not statistically significant [ $0.10 \pm 0.06$  (SD) units/mo *vs*  $0.60 \pm 0.20$  (SD) units/mo,  $P = 0.08$ ].

Propranolol also reduced the risk of developing PHG after esophageal variceal eradication<sup>[81]</sup>. Lo *et al*<sup>[81]</sup> reported in a randomized, controlled trial that 40 patients receiving placebo had a significant increase in PHG severity after variceal ligation ( $P < 0.01$ , ANOVA), but the 37 patients receiving propranolol [mean dose =  $96 \pm 20$  (SD) mg/d] had no significant increase in PHG severity. The frequency of PHG 6 mo after variceal ligation was significantly less in patients receiving propranolol than in the controls (48% *vs* 85%,  $P = 0.002$ ). This difference gradually decreased over time and became not significant at 12 mo. Also, the mean PHG severity score was lower in patients receiving propranolol than in the controls at 6 mo after variceal ligation ( $P < 0.05$ ).

Propranolol at 240-480 mg/d has been used to arrest acute bleeding from PHG. Bleeding stopped within 3 d in 13 (93%) of 14 patients with portal hypertension administered propranolol in one study<sup>[101]</sup>. None of these patients rebled while receiving propranolol during a median of 23 mo of follow-up, but 4 out of 7 patients rebled after electively discontinuing propranolol therapy<sup>[104]</sup>. Nonresponse to  $\beta$ -adrenergic receptor antagonists, defined as continued bleeding despite

this therapy and transfusion-dependency despite iron replacement therapy, should prompt consideration of interventional therapies<sup>[214]</sup>.

Nonspecific  $\beta$ -adrenergic receptor antagonists are a first line therapy for secondary prophylaxis of PHG bleeding<sup>[191,208]</sup>. Nadolol alone was as effective as nadolol with isosorbide mononitrate in preventing the first episode of PHG bleeding<sup>[226]</sup>. No studies have analyzed the efficacy of carvedilol, another nonspecific  $\beta$ -adrenergic receptor antagonist, in controlling bleeding from PHG<sup>[227]</sup>.

**Somatostatin and octreotide:** Somatostatin and octreotide, a synthetic somatostatin analogue, cause splanchnic vasoconstriction, reduce portal pressure, reduce portal blood flow, and decrease gastric perfusion in animal models and in patients with PHG<sup>[105,106,160,228-231]</sup>. For example, Chan *et al*<sup>[228]</sup> found octreotide infusion, compared to placebo, significantly increased systemic vascular resistance [ $3.4 \pm 0.2$  (SD) mmHg/mL per minute per 100 g *vs*  $2.7 \pm 0.2$  (SD) mmHg/mL per minute per 100 g,  $P < 0.05$ ], and significantly decreased portal pressure [ $9.9 \pm 0.5$  (SD) mmHg *vs*  $12.5 \pm 1.2$  (SD) mmHg,  $P < 0.05$ ] in cirrhotic rats. Another study found that somatostatin only modestly reduced portal pressure in PHG<sup>[232]</sup>. Octreotide treatment also significantly reduced mean cross-sectional area of gastric mucosal vessels compared to placebo [ $1810 \pm 101$  (SD) microns *vs*  $2290 \pm 145$  (SD) microns,  $P < 0.05$ ], and significantly inhibited release of several vasoactive gastrointestinal polypeptides, gastric acid, and pepsinogen<sup>[106,233]</sup>. In the stomach, somatostatin activates  $K^+$ -ATP channels (adenosine triphosphate-dependent potassium channels) which normally protect against gastric mucosal injury in the presence of portal hypertension, and antagonizes



the portal hypertensive and injury-promoting effects of glucagon<sup>[160]</sup>.

Octreotide is a first-line treatment for acute bleeding from PHG. In a randomized controlled trial, octreotide, at 100 mcg bolus followed by infusion of 25 mcg/min for the first 24 h and then 20 mcg/min for the second 24 h, controlled bleeding from PHG in 20 (83%) of 24 patients at 24 h and in 24 (100%) of 24 patients at 48 h<sup>[106]</sup>. Octreotide significantly more frequently controlled the bleeding than vasopressin (64%,  $P < 0.005$ ), or omeprazole (59%,  $P < 0.005$ ), and tended to be more effective than both vasopressin and omeprazole (88%, NS)<sup>[106]</sup>. Octreotide also controlled the bleeding significantly faster and required significantly less transfusions of packed erythrocytes than vasopressin or omeprazole<sup>[106]</sup>. Octreotide even successfully stopped bleeding within 48 h in the patients with bleeding refractory to vasopressin and omeprazole therapy.

Kouroumalis *et al.*<sup>[105]</sup> reported that somatostatin or octreotide infusion for 3 d stopped severe bleeding from PHG in all 26 study patients. Only 3 patients experienced recurrent bleeding which stopped after retreatment with somatostatin, and only one patient required gastrectomy for refractory bleeding.

Somatostatin and octreotide only temporarily reduce portal pressure. Escorsell *et al.*<sup>[232]</sup> reported rapid desensitization to the effects of octreotide with prolonged administration in cirrhotic patients with portal hypertension. Therefore somatostatin and octreotide are useful for acute bleeding but not for preventing chronic bleeding from PHG. Octreotide did not significantly change the severity of PHG at endoscopy.

**Vasopressin and terlipressin:** Vasopressin, a systemic vasoconstrictor, reduces splanchnic blood flow, lowers portal pressure, and decreases gastric mucosal blood flow<sup>[106]</sup>. In a randomized, controlled trial, vasopressin, administered intravenously (IV) at a rate of 1 unit/min for the first 10 min, followed by continuous infusion at 0.1 unit/min for 48 h, arrested bleeding from PHG in 14 (64%) of 22 patients<sup>[106]</sup>. However, as aforementioned, this result was inferior to that achieved by octreotide, possibly because vasopressin does not inhibit release of peptides, including glucagon and vasoactive intestinal polypeptide, and does not inhibit gastric acid secretion<sup>[106]</sup>. Concomitant omeprazole therapy may modestly increase vasopressin efficacy<sup>[106]</sup>. Vasopressin is considered an alternative therapy to octreotide.

Vasopressin causes significantly more frequent side effects (41%) than octreotide, especially abdominal pain<sup>[106]</sup>. Vasopressin reduces oxygen saturation of gastric mucosa, but this reduction can be partly reversed by administering supplemental oxygen<sup>[234,235]</sup>. Two analogues of vasopressin have been studied. Glypressin showed similar reduction in gastric mucosal perfusion as vasopressin by laser-Doppler flowmetry, but less impairment in gastric mucosal oxygenation<sup>[235]</sup>. Terlipressin may be useful in controlling acute bleeding from PHG,

especially when used at a dose of 1 mg IV every 4 h for 5 d<sup>[103,107,192]</sup>.

**Antioxidants:** Antioxidants help in free radical scavenging and in reversing impairment of oxidative stress-induced ERK2 activation. They may decrease susceptibility of PHG gastric mucosa to alcoholic injury, as demonstrated by administration of vitamin E, an antioxidant<sup>[144]</sup>. Vitamin E reduced oxidative state, normalized MKP-1 expression, and reversed impairment of oxidative stress-induced ERK2 activation<sup>[144]</sup>. Vitamin E helped reduce gastric injury from alcohol exposure in rats with PHG<sup>[71]</sup>.

Vitamin E administration decreased mucosal lipid peroxidation, decreased lysosomal enzymes, and increased levels of antioxidants. Kaur *et al.*<sup>[143]</sup> administered vitamin E 240 mg subcutaneously to rats with common bile duct ligation vs sham surgery. Seven days after ligation or sham surgery, the level of thiobarbituric acid reactive substances in gastric mucosa was significantly higher in ligated rats not receiving vitamin E [ $0.78 \pm 0.22$  (SD) nmol of malondialdehyde formed/mg protein] as compared to sham surgery rats administered vitamin E [ $0.56 \pm 0.07$  (SD) nmol of malondialdehyde formed/mg protein,  $P < 0.01$ ]. These data suggest that vitamin E decreased mucosal lipid peroxidation. In the ligation group vitamin E administered preoperatively significantly reduced the levels of  $\beta$ -glucuronidase as compared to untreated or post-operatively-treated groups ( $P < 0.01$ ). Preoperative vitamin E therapy significantly lowered levels of acid phosphatase as compared to untreated or postoperatively treated groups ( $P < 0.01$ ). Preoperative administration of vitamin E in the ligation group led to significantly increased levels of the three major antioxidant enzymes, including superoxide dismutase ( $P < 0.005$ ), glutathione peroxidase ( $P < 0.01$ ), and catalase ( $P < 0.05$ ) when compared to the levels of these antioxidant enzymes in untreated groups or in groups treated postoperatively with vitamin E. These data provide a theoretical basis that vitamin E administration may potentially improve PHG.

Kawanaka *et al.*<sup>[144]</sup> administered vitamin E or saline for 13 d in rats with PHG vs sham-operated rats used as a control. Rats with PHG had a 2.3-fold increased area of gastric mucosal necrosis after gastric exposure to ethanol than sham-operated rats ( $P < 0.05$ ), but vitamin E treatment in rats with PHG almost completely reversed this increased necrosis compared to controls ( $P < 0.05$ ). Vitamin E treatment did not significantly change portal pressure or gastric mucosal blood flow in PHG gastric mucosa.

**Rebamipide:** Rebamipide, an antiulcer medication, protects against oxygen-derived production of free radicals by scavenging free radicals. Intragastric administration ameliorates oxidative stress, reduces nitration of tyrosine residues of ERK, and reverses delayed mucosal healing occurring in PHG. It reversed the increased susceptibility to ethanol-induced injury and

reversed the delayed healing after gastric injury in rats with PHG<sup>[145]</sup>. Kinjo *et al.*<sup>[145]</sup> reported that administration of rebamipide in rats with PHG, significantly decreased lipid peroxide and nitrotyrosine and nitration of ERK by peroxynitrite in PHG mucosa, therefore normalizing ERK activation and restoring normal gastric mucosal healing after ethanol injury. Rebamipide requires further study as a therapy for PHG<sup>[119]</sup>.

**Estrogen and progesterone:** Estrogen and progesterone therapy reduced gastric mucosal blood flow, portal pressure, and porto-collateral resistance in rats with surgically-induced portal hypertension<sup>[236]</sup>. Panés *et al.*<sup>[236]</sup> reported that treatment with estradiol, dihydroxyprogesterone, or low dose combination estradiol-dihydroxyprogesterone significantly decreased gastric mucosal blood flow in rats that underwent portal vein ligation as compared to placebo [ $56 \pm 3.5$  (SD) mL/min per 100 g for placebo;  $43 \pm 3.4$  (SD) mL/min per 100 g for estrogen;  $32 \pm 2.6$  mL/min per 100 g for dihydroxyprogesterone, and  $42 \pm 6.1$  (SD) mL/min per 100 g for low dose estrogen/dihydroxyprogesterone,  $P < 0.05$ ]. Estrogen and progesterone have not been studied in patients with PHG.

**Thalidomide:** Thalidomide blunts development of a hyperdynamic circulation and decreases portal pressure by reducing NO production<sup>[114]</sup>. Thalidomide, at a low dose of 100 mg daily, was successful as a last resort therapy in one case report of bleeding from PHG caused by neoplastic invasion of the portal vein<sup>[237]</sup>. Before thalidomide therapy, the patient had required transfusion of 30 units of packed erythrocytes during 35 d while treated with propranolol and terlipressin.

**Corticosteroids:** There is one case report of cessation of PHG bleeding with corticosteroid therapy, using prednisolone 20 mg/d, after being admitted for five times during 5 mo with severe iron deficiency anemia from chronic GI bleeding from PHG that was refractory to propranolol therapy. At 2 mo follow-up the hemoglobin was rising and at 4 mo follow-up repeat EGD showed an improved endoscopic appearance of PHG<sup>[238]</sup>. The patient was stable during 3-years of follow-up using 15 mg prednisolone every other day, with no recurrence of the anemia.

**Losartan:** Hepatic stellate cells help modulate sinusoidal resistance and the sinusoidal microcirculation. These cells are influenced by vasoconstrictors such as endothelin and angiotensin II. The angiotensin II receptor antagonist, losartan, lowers portal pressure by inhibiting stellate cell contraction and by reducing sinusoidal resistance<sup>[239]</sup>. Administration of losartan at 25 or 50 mg/d resulted in improvement of PHG in 9 (56.3%) of 16 patients during 4 wk of follow-up. The higher dose had greater efficacy. There was also evidence of decreased portal pressure. Further studies are needed to evaluate the effect of losartan on PHG<sup>[239]</sup>.

### Sucralfate and acid-suppressing medications:

Proton pump inhibitors, sucralfate<sup>[74]</sup>, and histamine-2 receptor antagonists are not very effective at reducing bleeding from PHG because most patients with PHG already have hypochlorhydria<sup>[79,106,240-242]</sup>. However, proton pump inhibitors may indirectly stop bleeding from the stomach by raising intraluminal gastric pH and thereby stabilizing blood clots<sup>[243,244]</sup>. Zhou *et al.*<sup>[106]</sup> reported that omeprazole at 40 mg IV bolus every 12 h for 48 h successfully stopped bleeding in 59% of patients with PHG bleeding. Additionally, patients whose bleeding was refractory to vasopressin, benefited from omeprazole co-administration and vice versa.

**Teprenone:** In a controlled clinical trial, teprenone (geranylgeranyl acetone) administered to 15 patients with PHG decreased VEGF and hexosamine content in the gastric antrum, and thereby significantly decreased the severity of PHG<sup>[136]</sup>. Among these 15 treated patients, one patient decreased from severe to moderate PHG and another patient decreased from moderate to mild PHG<sup>[180]</sup>. Contrariwise, all 15 patients receiving placebo experienced no change in PHG severity.

**Endoscopic therapies:** Endoscopic therapies play a minor role in PHG bleeding because the bleeding is typically diffuse and obscure. Little data exist on efficacy of endoscopic therapy for PHG bleeding<sup>[71,245]</sup>. No single predominant site of bleeding is identified that can be locally treated at endoscopy. The role of endoscopic therapy is limited to the rare circumstances in which a single active bleeding site identified that is amenable to point therapy such as cauterization or sclerotherapy.

Argon plasma coagulation (APC) or hemospray, a rapid hemostatic agent, are experimental endoscopic therapies for PHG. These therapies can treat a larger bleeding surface area than cauterization or sclerotherapy. Nine (81%) of 11 patients undergoing APC for PHG bleeding achieved hemostasis, with a significant rise in hematocrit from baseline values after a mean of  $2.2 \pm 2.0$  (SD) endoscopic therapy sessions. The other two treated patients required fewer blood transfusions after APC therapy<sup>[202]</sup>.

Hemospray has recently shown promise in halting active PHG bleeding while long-term therapy is being initiated<sup>[203,246]</sup>. Ibrahim *et al.*<sup>[246]</sup> reported complete cessation of diffuse bleeding from severe PHG after spraying hemospray TC-325 (a nanopowder hemostatic agent<sup>[247]</sup>) in a 41-year-old woman who presented with a hemoglobin of 8.8 g/dL. No active bleeding was seen on follow-up endoscopy performed 24 h later. In another study, however, one of four patients treated with hemospray for bleeding from PHG expired from GI perforation<sup>[203]</sup>.

Endoscopic cryotherapy has been used for PHG bleeding after all other modalities failed. In one patient salvage cryotherapy was successful after failed TIPS and APC, with normal hemoglobin levels maintained during 4 wk of follow-up<sup>[248]</sup>.

**TIPS:** TIPS increases gastric mucosal blood flow while decreasing total gastric blood flow due to decreasing portal hypertension<sup>[76]</sup>. TIPS reduces the frequency and severity of PHG because it lowers portal pressure<sup>[76]</sup>. PHG can sometimes resolve completely after TIPS<sup>[75-77,249-253]</sup>. Urata *et al.*<sup>[77]</sup> reported in a prospective study of 12 Japanese patients undergoing TIPS for portal hypertension that portal pressure declined from  $25.1 \pm 8.8$  (SD) mmHg before TIPS to  $17.1 \pm 6.2$  (SD) mmHg after TIPS ( $P < 0.005$ ). This decline in portal pressure was correlated with a significant decrease in PHG severity after TIPS ( $P < 0.01$ ), and a significant decrease in number of patients with PHG (10 before vs 4 after TIPS,  $P < 0.01$ ). In particular, PHG disappeared in 2 of 5 patients who had severe PHG before TIPS. Mezawa *et al.*<sup>[76]</sup>, likewise, reported that mean portal pressure declined from 23.4 to 14.0 mmHg in 16 cirrhotic patients after TIPS ( $P < 0.01$ ). All four patients with severe PHG before TIPS, had significant improvement in PHG severity after lowering portal pressure with TIPS, and five of 12 patients with mild PHG had resolution of PHG after TIPS. In contrast, GAVE does not resolve after lowering portal pressure with TIPS, and GAVE is, therefore, likely related to hepatic dysfunction rather than portal hypertension<sup>[78]</sup>.

TIPS also decreased the risk of PHG bleeding<sup>[75-77,100,249-253]</sup>. For example, Kamath *et al.*<sup>[75]</sup> reported TIPS led to improvement or resolution of PHG findings and decreased transfusion requirements from  $2.9 \pm 2.0$  (SD) units/mo of packed erythrocytes before TIPS to  $0.6 \pm 0.8$  units/mo after TIPS performed for severe PHG ( $P = 0.04$ ). Ashraf *et al.*<sup>[98]</sup> reported one patient with chronic liver disease from hepatitis C who presented with chronic bleeding from PHG that required transfusions of 28 units of packed erythrocytes during the prior 6 mo. The bleeding ceased after TIPS with marked improvement in the severity of PHG. Contrariwise, GAVE does not respond to TIPS or other measures that lower portal pressure.

**Shunt surgery:** Shunt surgery decreases portal hypertension, decreases PHG severity, decreases risk of PHG bleeding, and may sometimes completely resolve the endoscopic features of PHG<sup>[100]</sup>. Shunt surgery is rarely used today to control bleeding because TIPS is preferred because of less invasiveness<sup>[100,101,238,254]</sup>.

TIPS and shunt surgery are therapies of last resort for patients who fail other therapies for PHG because they entail more morbidity and mortality than pharmacologic therapy<sup>[71]</sup>. TIPS, however, is very useful for refractory bleeding from esophageal varices from portal hypertension<sup>[255]</sup>, and is a reasonable option in patients having recurrent severe bleeding from PHG despite administration of  $\beta$ -adrenergic receptor antagonists<sup>[102]</sup>.

**Other invasive therapies:** Liver transplantation is the ultimate therapy for PHG<sup>[118]</sup>. In a study of 29 patients undergoing living donor liver transplantation for end stage liver disease, PHG resolved in all 19 patients who

had PHG before transplantation<sup>[44]</sup>.

Kimura *et al.*<sup>[218]</sup> retrospectively examined the 19 patients experiencing gross GI bleeding, defined as gross melena or hematemesis, within 3 mo after liver transplantation among 297 patients undergoing living donor liver transplantation. The etiologies included PHG in 2 patients, varices in 1, anastomotic ulcer in 13, and other in 3.

Splenic embolization, by transcatheter splenic arterial embolization, significantly improves PHG in patients with hypersplenism as compared to controls<sup>[99,255]</sup>. Ohmagari *et al.*<sup>[99]</sup> evaluated 30 patients with hypersplenism who underwent transcatheter splenic arterial embolization in 17 vs no interventional therapy in 13 patients. Splenic embolization significantly reduced the frequency of PHG (reduction of 71% vs 8%,  $P < 0.05$ ). Partial splenic embolization also successfully controlled PHG bleeding in one case report<sup>[256]</sup>.

Laparoscopic splenectomy, for various indications, was reported to decrease PHG severity<sup>[55]</sup>. Anegawa *et al.*<sup>[55]</sup> prospectively analyzed the effect of laparoscopic splenectomy on preexistent PHG in 70 patients with liver cirrhosis from various etiologies. All patients underwent EGD before and 1 mo after splenectomy. Splenectomy was performed for indications of bleeding diathesis, interferon induction, hepatocellular cancer treatment, and sclerotherapy-resistant varices. Before splenectomy, 49 of 70 patients had PHG, including mild PHG in 32 and severe PHG in 17 patients. After splenectomy, PHG resolved completely in 7 patients with prior severe PHG, resolved completely in 12 patients with mild PHG, and was reduced from severe to mild PHG in 9 patients ( $P < 0.0001$ ).

### Summary of clinical treatment

**Acute bleeding:** Variceal bleeding must be excluded by performing EGD before initiating treatment for PHG<sup>[257,258]</sup>. General measures for patients presenting with acute bleeding from PHG include volume resuscitation and cautious transfusion of packed erythrocytes, as necessary, to maintain the hemoglobin level at 8 g/dL<sup>[192,259,260]</sup>. Over-transfusion to a higher hemoglobin level could promote bleeding from PHG by raising portal pressure, as reported for bleeding from esophageal varices<sup>[261,262]</sup>. However, patients with cardiopulmonary disease or severe other comorbidities may require a hemoglobin level of 9-10 g/dL<sup>[263]</sup>. Antibiotic prophylaxis is generally recommended for bleeding from PHG<sup>[214]</sup>, just as it is recommended for esophageal variceal bleeding in cirrhotic patients<sup>[189,264-266]</sup> because of an increased risk of systemic infections, particularly spontaneous bacterial peritonitis, in cirrhotic patients with GI bleeding<sup>[267]</sup>.

Bleeding from PHG may be exacerbated by a coagulopathy with an elevated international normalized ratio from advanced liver disease. This coagulopathy may require transfusion of fresh frozen plasma. Severe thrombocytopenia may occur from bone marrow suppression in alcoholic cirrhosis and from hypersplenism with portal hypertension in any cirrhotic patient.

**Table 12 Treatment of acute or chronic gastrointestinal bleeding from portal hypertensive gastropathy**

Acute bleeding
Patient stabilization
Treat severe coagulopathy with highly elevated INR associated with cirrhosis with fresh frozen plasma
Treat severe thrombocytopenia associated with hypersplenism and bone marrow suppression from alcoholism with platelet transfusions
Transfuse packed erythrocytes to main hemoglobin level at about 8 g/dL
Consider antibiotic prophylaxis in patient with cirrhosis
Endoscopic therapy from bleeding-rarely used
Consider argon plasma coagulation
Hemospray - an experimental therapy
Pharmacotherapy
Octreotide - first line therapy
Vasopressin or terlipressin - second line therapy
Proton pump inhibitor therapy - adjunct therapy
Propranolol - can be instituted after bleeding controlled and patient stabilized
Interventional therapy
TIPS - for uncontrolled hemorrhage or for bleeding from PHG associated with variceal bleeding
Liver transplantation - for advanced end stage liver disease
Chronic bleeding
Treatment of anemia
Transfusions of packed erythrocytes as necessary
Iron replacement therapy
Pharmacotherapy
Consider propranolol

TIPS: Transjugular intrahepatic portosystemic shunt; PHG: Portal hypertensive gastropathy; INR: International normalized ratio.

Platelet transfusion may be necessary for severe thrombocytopenia in the setting of active bleeding from PHG.

Somatostatin or octreotide are first line therapies. Vasopressin or terlipressin are second-line therapies for acute bleeding<sup>[106]</sup>. Once the acute bleeding is controlled and the patient is hemodynamically stable, a nonselective  $\beta$ -adrenergic receptor antagonist is instituted for secondary prevention of PHG bleeding<sup>[103,258,268]</sup>. Propranolol is the recommended drug in this class because it has been the most studied. Propranolol should be started at a dose of 20 mg twice daily and gradually escalated to 160 mg twice daily or the maximum tolerated dose, maintaining it as long as the portal hypertension is present<sup>[214]</sup>. The dose is titrated to slow the pulse to 60 beats/min. This class of drugs is not used in the acute setting because it can blunt the physiologic tachycardia to restore end-organ perfusion in response to hypovolemia and requires gradual dose titration to achieve an adequate response<sup>[214]</sup>. This class of drugs also prevents bleeding from concomitant esophageal varices.

**Treatment of chronic bleeding:** Scant data exist regarding management of chronic bleeding from PHG<sup>[72]</sup>. In patients with suspected chronic bleeding from PHG, after excluding other etiologies, iron replacement therapy should be initiated to avoid depleting iron reserves<sup>[260]</sup>. Propranolol is the primary therapy to reduce portal pressure and prevent chronic bleeding<sup>[72,214,260]</sup>. However, propranolol was not superior to placebo in one study, as determined by percentage of patients free from chronic GI bleeding during long term follow-up<sup>[103]</sup>.

Liver transplantation is an option in patients with decompensated cirrhosis and PHG bleeding. Table 12 summarizes the recommendations for the treatment of

acute and chronic bleeding from PHG.

### Prevention

The risk of bleeding from mild PHG is low. Primary prophylaxis is, therefore, not recommended for patients with mild PHG<sup>[72,191,269]</sup>. Propranolol can be used for primary prophylaxis for severe PHG, and can significantly reduce the risk of bleeding. However, scant evidence exists that this reduction will affect the risk of a primary bleeding episode in PHG patients<sup>[72,103,104,269,270]</sup>. Propranolol is recommended regardless of the severity or presence of PHG in patients with esophageal varices because it treats both entities by reducing portal pressure<sup>[105-107,198,271,272]</sup>.

Prophylaxis of bleeding from PHG is not recommended<sup>[260]</sup>. Current guidelines do not recommend endoscopic surveillance in patients with cirrhosis who have asymptomatic PHG, without evident esophageal varices, other than the standard surveillance for development of esophageal varices in these patients<sup>[189]</sup>.

**Mortality:** Limited data exist on mortality from bleeding from PHG, but this bleeding is rarely fatal<sup>[72]</sup>. It contributes little to overall morbidity and mortality from portal hypertension, especially in comparison to variceal bleeding<sup>[118]</sup>. It represents a minor cause (< 1%) of mortality in cirrhotic patients because the bleeding is typically mild<sup>[25,34,37,71]</sup>. Only one patient expired from PHG in one series of 38 deaths among 373 study patients with cirrhosis<sup>[34]</sup>. Bleeding-related mortality was much lower for PHG [1 of 8 patients (12.5%)] than that for esophageal varices [9 of 20 patients (45%)]<sup>[34]</sup>.

**Lesions resembling PHG in other gastrointestinal regions:** PHG-like lesions can occur in other parts of



the GI tract and are named according to the involved segment as portal hypertensive duodenopathy<sup>[40]</sup>, portal hypertensive biliopathy, small intestinal vasculopathy<sup>[139]</sup>, and portal hypertensive colopathy<sup>[96,139]</sup>. These uncommon extragastric lesions occur particularly in patients with extrahepatic portal hypertension<sup>[71]</sup>. Portal hypertensive duodenopathy has been defined as the endoscopic appearance of patchy or diffuse congestion of duodenal mucosa associated with portal hypertension<sup>[40]</sup>, but a consensus definition is not established<sup>[273]</sup>. Histologically, vascular changes predominate, including capillary angiogenesis, dilatation and congestion, as well as fibrous proliferation and apoptosis<sup>[46]</sup>. This duodenopathy is significantly more severe in patients having severe than mild PHG (56.8% vs 23.5%,  $P < 0.05$ )<sup>[46]</sup>. Portal congestive jejunosopathy is defined histologically by the presence of ectatic capillaries and venules in the villi, with an increase in the number of vessels to  $> 6/\text{villus}$ <sup>[274]</sup>. Portal hypertensive ileopathy<sup>[275]</sup>, and portal hypertensive colopathy<sup>[38,276]</sup> are also associated with portal hypertension. The colopathy histologically appears as dilatation of mucosal blood vessels and is classified into four different types by Ito *et al.*<sup>[38]</sup>, including solitary vascular ectasias, diffuse vascular ectasias, erythema, and blue vein.

## DISCUSSION

The pathophysiology of PHG is not well understood. Portal hypertension plays a central role in the pathogenesis, and liver disease a subsidiary role in the disease, but a hyperdynamic circulation likely also plays an important role. However, the precise nature and pathophysiology of the hyperdynamic circulation must be further elucidated. The pathophysiology of more severe gastric mucosal injury and blunted reparative response after exposure to toxic substances in PHG must be clarified in terms of molecular mediators and histopathology. In particular, the pathophysiologic basis of decreased superficial mucosal perfusion in PHG must be better characterized in terms of its molecular mechanisms.

The current pharmacotherapy for bleeding from PHG focuses on decreasing portal pressure because portal hypertension is a prerequisite for developing PHG. Understanding the molecular mechanisms of this disease may permit development of better targeted and more effective pharmacotherapies.

## COMMENTS

### Background

Portal hypertensive gastropathy (PHG) is characterized at endoscopy by characteristic lesions present in the proximal stomach; characterized pathophysiologically by a hyperdynamic circulation induced by portal hypertension by inadequately understood mechanisms; and characterized clinically by mild-to-moderate acute or chronic gastrointestinal bleeding from the endoscopically identified lesions. However, much about the pathophysiology and clinical therapy of PHG is inadequately understood. This work systematically reviews the literature on the pathophysiology, natural history and

therapy of PHG to report what is known and what is not known or controversial about PHG.

### Research frontiers

This work systematically reviews gaps or controversies in the current understanding of PHG. First, this work exposes gaps in the current understanding of the pathophysiology. Portal hypertension is necessary but insufficient to develop PHG because many patients have portal hypertension without PHG. The pathogenesis is related to a hyperdynamic circulation, induced by portal hypertension, characterized by increased intrahepatic resistance to flow, increased splanchnic flow, increased total gastric flow, and most likely decreased gastric mucosal flow. However, this review shows that the cellular and molecular mechanisms for this hyperdynamic circulation are inadequately characterized. Nitrous oxide, free radicals, tumor necrosis factor- $\alpha$ , and glucagon may be important mediators of PHG. Second, this work reports the inadequacies of the current recommended therapies for PHG and for bleeding from PHG based on the currently inadequate understanding of the pathophysiology. This work should be useful to clinicians, clinical researchers, and basic researchers by describing what is known, controversial, or unknown about the pathophysiology, natural history, and therapy of PHG. It is hoped that this work stimulates further research in this field by exposing gaps in the current understanding of PHG.

### Innovations and breakthroughs

This systematic review extensively reviews what is known and what is not known or controversial about PHG. This work is particularly helpful to clinicians in reporting the current recommended therapy for PHG, the clinical trials supporting the current recommendations, and the limitations of the current therapies. This is also helpful to clinical and basic researchers in systematically reviewing the current state of knowledge about its pathophysiology, including gaps, uncertainties, and controversies in the current understanding of the pathophysiology.

### Applications

This systematic review extensively reviews what is known and what is not known or controversial about PHG. First, this work exposes gaps in the understanding of the pathophysiology. Portal hypertension is necessary but insufficient to develop PHG because many patients have portal hypertension without PHG. The pathogenesis is related to a hyperdynamic circulation, induced by portal hypertension, characterized by increased intrahepatic resistance to flow, increased splanchnic flow, increased total gastric flow, and most likely decreased gastric mucosal flow. However, this review shows that the cellular and molecular mechanisms for this hyperdynamic circulation are inadequately characterized. Nitrous oxide, free radicals, tumor necrosis factor- $\alpha$ , and glucagon may be important mediators of PHG development. Second, this work describes the natural history of PHG. PHG increases in frequency with more severe portal hypertension, advanced liver disease, longer liver disease duration, presence of esophageal varices, and endoscopic variceal obliteration. Acute and chronic gastrointestinal bleeding are the only clinical complications. Bleeding is typically mild-to-moderate. Third, this work reports the current therapies for PHG and for bleeding from PHG and characterizes their inadequacies based on the currently inadequate understanding of the pathophysiology. In particular, this work reviews clinical trials of the therapeutic efficacy of octreotide; proton pump inhibitors; nonselective  $\beta$ -adrenergic receptor antagonists, particularly propranolol; and vasopressin or terlipressin. This work should be useful to clinicians, clinical researchers, and basic researchers by describing what is known, controversial, and uncertain about the pathophysiology, natural history, and therapy of PHG.

### Terminology

This work systematically reviews portal hypertensive gastropathy, characterized at endoscopy by characteristic lesions present in the proximal stomach; characterized pathophysiologically by a hyperdynamic circulation induced by portal hypertension by inadequately understood mechanisms, and characterized clinically by mild-to-moderate acute or chronic gastrointestinal bleeding from the endoscopically identified lesions.

### Peer-review

This review by Mihajlo Gjeorgjievski on portal hypertensive gastropathy is well

written and helpful to understand its pathophysiology, clinical presentation, natural history and therapy. This review is informative and helpful to readers who are interested in the topic or subtopics.

## REFERENCES

- 1 **Palmer ED.** Erosive gastritis in cirrhosis; influence of portal hypertension on the gastric mucosa. *Am J Dig Dis* 1957; **2**: 31-36 [PMID: 13394564 DOI: 10.1007/BF02232594]
- 2 **Sarfeh IJ,** Tarnawski A. Portal hypertensive gastritis. *Gastroenterology* 1984; **86**: 592 [PMID: 6607188]
- 3 **McCormack TT,** Sims J, Eyre-Brook I, Kennedy H, Goepel J, Johnson AG, Triger DR. Gastric lesions in portal hypertension: inflammatory gastritis or congestive gastropathy? *Gut* 1985; **26**: 1226-1232 [PMID: 3877665 DOI: 10.1136/gut.26.11.1226]
- 4 **Taor RE,** Fox B, Ware J, Johnson AG. Gastritis: gastroscopic and microscopic. *Endoscopy* 1975; **7**: 209-215 [DOI: 10.1055/s-0028-1098575]
- 5 **Sarin SK,** Misra SP, Singal A, Thorat V, Broor SL. Evaluation of the incidence and significance of the "mosaic pattern" in patients with cirrhosis, noncirrhotic portal fibrosis, and extrahepatic obstruction. *Am J Gastroenterol* 1988; **83**: 1235-1239 [PMID: 3263791]
- 6 **DeWeert TM,** Gostout CJ, Wiesner RH. Congestive gastropathy and other upper endoscopic findings in 81 consecutive patients undergoing orthotopic liver transplantation. *Am J Gastroenterol* 1990; **85**: 573-576 [PMID: 2186617]
- 7 **McCormick PA,** Sankey EA, Cardin F, Dhillon AP, McIntyre N, Burroughs AK. Congestive gastropathy and *Helicobacter pylori*: an endoscopic and morphometric study. *Gut* 1991; **32**: 351-354 [PMID: 2026332 DOI: 10.1136/gut.32.4.351]
- 8 **Sarin SK,** Sreenivas DV, Lahoti D, Saraya A. Factors influencing development of portal hypertensive gastropathy in patients with portal hypertension. *Gastroenterology* 1992; **102**: 994-999 [PMID: 1537536]
- 9 **Parikh SS,** Desai SB, Prabhu SR, Trivedi MH, Shankaran K, Bhukhanwala FA, Kalro RH, Desai HG. Congestive gastropathy: factors influencing development, endoscopic features, *Helicobacter pylori* infection, and microvessel changes. *Am J Gastroenterol* 1994; **89**: 1036-1042 [PMID: 8017362]
- 10 **Sarin SK,** Shahi HM, Jain M, Jain AK, Issar SK, Murthy NS. The natural history of portal hypertensive gastropathy: influence of variceal eradication. *Am J Gastroenterol* 2000; **95**: 2888-2893 [PMID: 11051364 DOI: 10.1111/j.1572-0241.2000.03200.x]
- 11 **Itha S,** Yachha SK. Endoscopic outcome beyond esophageal variceal eradication in children with extrahepatic portal venous obstruction. *J Pediatr Gastroenterol Nutr* 2006; **42**: 196-200 [PMID: 16456415 DOI: 10.1097/01.mpg.0000189351.55666.45]
- 12 **Rana SS,** Bhasin DK, Jahagirdar S, Raja K, Nada R, Kochhar R, Joshi K. Is there ileopathy in portal hypertension? *J Gastroenterol Hepatol* 2006; **21**: 392-397 [PMID: 16509864 DOI: 10.1111/j.1440-1746.2005.04037.x]
- 13 **El-Rifai N,** Mention K, Guimber D, Michaud L, Boman F, Turck D, Gottrand F. Gastropathy and gastritis in children with portal hypertension. *J Pediatr Gastroenterol Nutr* 2007; **45**: 137-140 [PMID: 17592382 DOI: 10.1097/MPG.0b013e318049cbe2]
- 14 **Sogaard KK,** Astrup LB, Vilstrup H, Gronbaek H. Portal vein thrombosis; risk factors, clinical presentation and treatment. *BMC Gastroenterol* 2007; **7**: 34 [PMID: 17697371 DOI: 10.1186/1471-230X-7-34]
- 15 **Figueiredo P,** Almeida N, Lérias C, Lopes S, Gouveia H, Leitão MC, Freitas D. Effect of portal hypertension in the small bowel: an endoscopic approach. *Dig Dis Sci* 2008; **53**: 2144-2150 [PMID: 18026837 DOI: 10.1007/s10620-007-0111-z]
- 16 **Erden A,** Idilman R, Erden I, Ozden A. Veins around the esophagus and the stomach: do their calibrations provide a diagnostic clue for portal hypertensive gastropathy? *Clin Imaging* 2009; **33**: 22-24 [PMID: 19135925 DOI: 10.1016/j.clinimag.2008.06.023]
- 17 **Duché M,** Ducot B, Tournay E, Fabre M, Cohen J, Jacquemin E, Bernard O. Prognostic value of endoscopy in children with biliary atresia at risk for early development of varices and bleeding. *Gastroenterology* 2010; **139**: 1952-1960 [PMID: 20637201 DOI: 10.1053/j.gastro.2010.07.004]
- 18 **Aydoğan A,** Güllüoğlu M, Onder SY, Gökçe S, Celtik C, Durmaz O. Portal gastropathy and duodenopathy in children with extrahepatic and intrahepatic portal hypertension: endoscopic diagnosis and histologic scoring. *J Pediatr Gastroenterol Nutr* 2011; **52**: 612-616 [PMID: 21464749 DOI: 10.1097/MPG.0b013e3182125e7c]
- 19 **dos Santos JM,** Ferreira AR, Fagundes ED, Ferreira AP, Ferreira LS, Magalhães MC, Bittencourt PF, Carvalho SD, Figueiredo Filho PP, Penna FJ. Endoscopic and pharmacological secondary prophylaxis in children and adolescents with esophageal varices. *J Pediatr Gastroenterol Nutr* 2013; **56**: 93-98 [PMID: 22785415 DOI: 10.1097/MPG.0b013e318267c334]
- 20 **Pantham G,** Waghay N, Einstadter D, Finkelhor RS, Mullen KD. Bleeding risk in patients with esophageal varices undergoing transesophageal echocardiography. *Echocardiography* 2013; **30**: 1152-1155 [PMID: 23742625 DOI: 10.1111/echo.12274]
- 21 **Abdollahi MR,** Somi MH, Faraji E. Role of international criteria in the diagnosis of autoimmune hepatitis. *World J Gastroenterol* 2013; **19**: 3629-3633 [PMID: 23801865 DOI: 10.3748/wjg.v19.i23.3629]
- 22 **de Alcántara RV,** Yamada RM, Cardoso SR, de Fátima M, Servidoni CP, Hessel G. Ultrasonographic predictors of esophageal varices. *J Pediatr Gastroenterol Nutr* 2013; **57**: 700-703 [PMID: 23941999 DOI: 10.1097/MPG.0b013e3182a7bc2e]
- 23 **Aoyama T,** Oka S, Aikata H, Nakano M, Watari I, Naeshiro N, Yoshida S, Tanaka S, Chayama K. Is small-bowel capsule endoscopy effective for diagnosis of esophagogastric lesions related to portal hypertension? *J Gastroenterol Hepatol* 2014; **29**: 511-516 [PMID: 23981241 DOI: 10.1111/jgh.12372]
- 24 **Sacchetti C,** Capello M, Rebecchi P, Roncucci L, Zanghieri G, Tripodi A, Ponz de Leon M. Frequency of upper gastrointestinal lesions in patients with liver cirrhosis. *Dig Dis Sci* 1988; **33**: 1218-1222 [PMID: 3168693 DOI: 10.1007/BF01536669]
- 25 **D'Amico G,** Montalbano L, Traina M, Pisa R, Menozzi M, Spanò C, Pagliaro L. Natural history of congestive gastropathy in cirrhosis. The Liver Study Group of V. Cervello Hospital. *Gastroenterology* 1990; **99**: 1558-1564 [PMID: 2227271]
- 26 **Calès P,** Zabotto B, Meskens C, Caucanas JP, Vinel JP, Desmorat H, Fermanian J, Pascal JP. Gastroesophageal endoscopic features in cirrhosis. Observer variability, interassociations, and relationship to hepatic dysfunction. *Gastroenterology* 1990; **98**: 156-162 [PMID: 2293575]
- 27 **Rabinovitz M,** Yoo YK, Schade RR, Dindzans VJ, Van Thiel DH, Gavalier JS. Prevalence of endoscopic findings in 510 consecutive individuals with cirrhosis evaluated prospectively. *Dig Dis Sci* 1990; **35**: 705-710 [PMID: 2344804 DOI: 10.1007/BF01540171]
- 28 **Iwao T,** Toyonaga A, Sumino M, Takagi K, Oho K, Nishizono M, Ohkubo K, Inoue R, Sasaki E, Tanikawa K. Portal hypertensive gastropathy in patients with cirrhosis. *Gastroenterology* 1992; **102**: 2060-2065 [PMID: 1587424]
- 29 **Taranto D,** Suozzo R, Romano M, di Sapio M, Caporaso N, Del Vecchio Blanco C, Coltorti M. Gastric endoscopic features in patients with liver cirrhosis: correlation with esophageal varices, intra-variceal pressure, and liver dysfunction. *Digestion* 1994; **55**: 115-120 [PMID: 8187974 DOI: 10.1159/000201135]
- 30 **Gupta R,** Saraswat VA, Kumar M, Naik SR, Pandey R. Frequency and factors influencing portal hypertensive gastropathy and duodenopathy in cirrhotic portal hypertension. *J Gastroenterol Hepatol* 1996; **11**: 728-733 [PMID: 8872769 DOI: 10.1111/j.1440-1746.1996.tb00322.x]
- 31 **Iwao T,** Toyonaga A, Oho K, Sakai T, Tayama C, Masumoto H, Sato M, Nakahara K, Tanikawa K. Portal-hypertensive gastropathy develops less in patients with cirrhosis and fundal varices. *J Hepatol* 1997; **26**: 1235-1241 [PMID: 9210609 DOI: 10.1016/S0168-8278(97)80457-6]
- 32 **Carpinelli L,** Primignani M, Preatoni P, Angeli P, Battaglia G, Beretta L, Bortoli A, Capria A, Cestari R, Cosentino F, Crotta S, Gerunda G, Lorenzini I, Maiolo P, Merighi A, Rossi A, Sangiovanni A, de Franchis R. Portal hypertensive gastropathy: reproducibility of a classification, prevalence of elementary lesions, sensitivity and specificity in the diagnosis of cirrhosis of the liver.

- A NIEC multicentre study. New Italian Endoscopic Club. *Ital J Gastroenterol Hepatol* 1997; **29**: 533-540 [PMID: 9513828]
- 33 **Zaman A**, Hapke R, Flora K, Rosen H, Benner K. Prevalence of upper and lower gastrointestinal tract findings in liver transplant candidates undergoing screening endoscopic evaluation. *Am J Gastroenterol* 1999; **94**: 895-899 [PMID: 10201453 DOI: 10.1111/j.1572-0241.1999.984.g.x]
  - 34 **Primignani M**, Carpinelli L, Preatoni P, Battaglia G, Carta A, Prada A, Cestari R, Angeli P, Gatta A, Rossi A, Spinzi G, De Franchis R. Natural history of portal hypertensive gastropathy in patients with liver cirrhosis. The New Italian Endoscopic Club for the study and treatment of esophageal varices (NIEC). *Gastroenterology* 2000; **119**: 181-187 [PMID: 10889167 DOI: 10.1053/gast.2000.8555]
  - 35 **Chaves DM**, Sakai P, Mucenic M, Iriya K, Iriya Y, Ishioka S. Comparative study of portal hypertensive gastropathy in schistosomiasis and hepatic cirrhosis. *Endoscopy* 2002; **34**: 199-202 [PMID: 11870569]
  - 36 **Merkel C**, Schipilliti M, Bighin R, Bellini B, Angeli P, Bolognesi M, Vescovi F, Gatta A. Portal hypertension and portal hypertensive gastropathy in patients with liver cirrhosis: a haemodynamic study. *Dig Liver Dis* 2003; **35**: 269-274 [PMID: 12801039 DOI: 10.1016/S1590-8658(03)00064-1]
  - 37 **Merli M**, Nicolini G, Angeloni S, Gentili F, Attili AF, Riggio O. The natural history of portal hypertensive gastropathy in patients with liver cirrhosis and mild portal hypertension. *Am J Gastroenterol* 2004; **99**: 1959-1965 [PMID: 15447756 DOI: 10.1111/j.1572-0241.2004.40246.x]
  - 38 **Ito K**, Shiraki K, Sakai T, Yoshimura H, Nakano T. Portal hypertensive colopathy in patients with liver cirrhosis. *World J Gastroenterol* 2005; **11**: 3127-3130 [PMID: 15918202 DOI: 10.3748/wjg.v11.i20.3127]
  - 39 **De Palma GD**, Rega M, Masone S, Persico F, Siciliano S, Patrone F, Matantuono L, Persico G. Mucosal abnormalities of the small bowel in patients with cirrhosis and portal hypertension: a capsule endoscopy study. *Gastrointest Endosc* 2005; **62**: 529-534 [PMID: 16185966 DOI: 10.1016/S0016-5107(05)01588-9]
  - 40 **Menchén L**, Ripoll C, Marín-Jiménez I, Colón A, Gómez-Camarero J, González-Asanza C, Menchén P, Cos E, Bañares R. Prevalence of portal hypertensive duodenopathy in cirrhosis: clinical and haemodynamic features. *Eur J Gastroenterol Hepatol* 2006; **18**: 649-653 [PMID: 16702855 DOI: 10.1097/00042737-200606000-00012]
  - 41 **Yüksel O**, Köklü S, Arhan M, Yolcu OF, Ertugrul I, Odemiş B, Altıparmak E, Sahin B. Effects of esophageal varice eradication on portal hypertensive gastropathy and fundal varices: a retrospective and comparative study. *Dig Dis Sci* 2006; **51**: 27-30 [PMID: 16416205 DOI: 10.1007/s10620-006-3078-2]
  - 42 **Fontana RJ**, Sanyal AJ, Mehta S, Doherty MC, Neuschwander-Tetri BA, Everson GT, Kahn JA, Malet PF, Sheikh MY, Chung RT, Ghany MG, Gretch DR. Portal hypertensive gastropathy in chronic hepatitis C patients with bridging fibrosis and compensated cirrhosis: results from the HALT-C trial. *Am J Gastroenterol* 2006; **101**: 983-992 [PMID: 16573786 DOI: 10.1111/j.1572-0241.2006.0461.x]
  - 43 **Bresci G**, Parisi G, Capria A. Clinical relevance of colonic lesions in cirrhotic patients with portal hypertension. *Endoscopy* 2006; **38**: 830-835 [PMID: 17001574 DOI: 10.1055/s-2006-944629]
  - 44 **Akatsu T**, Yoshida M, Kawachi S, Tanabe M, Shimazu M, Kumai K, Kitajima M. Consequences of living-donor liver transplantation for upper gastrointestinal lesions: high incidence of reflux esophagitis. *Dig Dis Sci* 2006; **51**: 2018-2022 [PMID: 17024572 DOI: 10.1007/s10620-006-9362-3]
  - 45 **Zardi EM**, Uwechie V, Gentilucci UV, Dobrina A, Petitti T, Laghi V, Picardi A, Afeltra A. Portal diameter in the diagnosis of esophageal varices in 266 cirrhotic patients: which role? *Ultrasound Med Biol* 2007; **33**: 506-511 [PMID: 17337112 DOI: 10.1016/j.ultrasmedbio.2006.10.002]
  - 46 **Barakat M**, Mostafa M, Mahran Z, Soliman AG. Portal hypertensive duodenopathy: clinical, endoscopic, and histopathologic profiles. *Am J Gastroenterol* 2007; **102**: 2793-2802 [PMID: 17900330 DOI: 10.1111/j.1572-0241.2007.01536.x]
  - 47 **Bellis L**, Nicodemo S, Galossi A, Guarisco R, Spilabotti L, Durola L, Dell'Unto O, Puoti C. Hepatic venous pressure gradient does not correlate with the presence and the severity of portal hypertensive gastropathy in patients with liver cirrhosis. *J Gastrointest Liver Dis* 2007; **16**: 273-277 [PMID: 17925921]
  - 48 **Gravante G**, Delogu D, Venditti D. Upper and lower gastrointestinal diseases in liver transplant candidates. *Int J Colorectal Dis* 2008; **23**: 201-206 [PMID: 17932680 DOI: 10.1007/s00384-007-0386-8]
  - 49 **Canlas KR**, Dobozi BM, Lin S, Smith AD, Rockey DC, Muir AJ, Agrawal NM, Poleski MH, Patel K, McHutchison JG. Using capsule endoscopy to identify GI tract lesions in cirrhotic patients with portal hypertension and chronic anemia. *J Clin Gastroenterol* 2008; **42**: 844-848 [PMID: 18277884 DOI: 10.1097/MCG.0b013e318038d312]
  - 50 **Kim TU**, Kim S, Woo SK, Lee JW, Lee TH, Jeong YJ, Heo J. Dynamic CT of portal hypertensive gastropathy: significance of transient gastric perfusion defect sign. *Clin Radiol* 2008; **63**: 783-790 [PMID: 18555036 DOI: 10.1016/j.crad.2008.02.003]
  - 51 **Higaki N**, Matsui H, Imaoka H, Ikeda Y, Murakami H, Hiasa Y, Matsuura B, Onji M. Characteristic endoscopic features of portal hypertensive enteropathy. *J Gastroenterol* 2008; **43**: 327-331 [PMID: 18592149 DOI: 10.1007/s00535-008-2166-9]
  - 52 **Frenette CT**, Kuldau JG, Hillebrand DJ, Lane J, Pockros PJ. Comparison of esophageal capsule endoscopy and esophago-gastroduodenoscopy for diagnosis of esophageal varices. *World J Gastroenterol* 2008; **14**: 4480-4485 [PMID: 18680226 DOI: 10.3748/wjg.14.4480]
  - 53 **Tarantino G**, Citro V, Esposito P, Giaquinto S, de Leone A, Milan G, Tripodi FS, Cirillo M, Lobello R. Blood ammonia levels in liver cirrhosis: a clue for the presence of portosystemic collateral veins. *BMC Gastroenterol* 2009; **9**: 21 [PMID: 19292923 DOI: 10.1186/1471-230X-9-21]
  - 54 **Curvêlo LA**, Brabosa W, Rhor R, Lanzoni V, Parise ER, Ferrari AP, Kondo M. Underlying mechanism of portal hypertensive gastropathy in cirrhosis: a hemodynamic and morphological approach. *J Gastroenterol Hepatol* 2009; **24**: 1541-1546 [PMID: 19743998 DOI: 10.1111/j.1440-1746.2009.05871.x]
  - 55 **Anegawa G**, Kawanaka H, Uehara H, Akahoshi T, Konishi K, Yoshida D, Kinjo N, Hashimoto N, Tomikawa M, Hashizume M, Maehara Y. Effect of laparoscopic splenectomy on portal hypertensive gastropathy in cirrhotic patients with portal hypertension. *J Gastroenterol Hepatol* 2009; **24**: 1554-1558 [PMID: 19743999 DOI: 10.1111/j.1440-1746.2009.05906.x]
  - 56 **Kumar A**, Mishra SR, Sharma P, Sharma BC, Sarin SK. Clinical, laboratory, and hemodynamic parameters in portal hypertensive gastropathy: a study of 254 cirrhotics. *J Clin Gastroenterol* 2010; **44**: 294-300 [PMID: 19730114 DOI: 10.1097/MCG.0b013e3181b37ea1]
  - 57 **Kim MY**, Choi H, Baik SK, Yea CJ, Won CS, Byun JW, Park SY, Kwon YH, Kim JW, Kim HS, Kwon SO, Kim YJ, Cha SH, Chang SJ. Portal hypertensive gastropathy: correlation with portal hypertension and prognosis in cirrhosis. *Dig Dis Sci* 2010; **55**: 3561-3567 [PMID: 20407828 DOI: 10.1007/s10620-010-1221-6]
  - 58 **De Lisi S**, Peralta S, Arini A, Simone F, Craxi A. Oesophago-gastroduodenoscopy in patients with cirrhosis: Extending the range of detection beyond portal hypertension. *Dig Liver Dis* 2011; **43**: 48-53 [PMID: 20471338 DOI: 10.1016/j.dld.2010.04.004]
  - 59 **Abbas A**, Butt N, Bhutto AR, Munir SM. Correlation of thrombocytopenia with grading of esophageal varices in chronic liver disease patients. *J Coll Physicians Surg Pak* 2010; **20**: 369-372 [PMID: 20642964]
  - 60 **Ahmed S**, Mumtaz K, Ahmed US, Shah HA, Abid S, Hamid S, Jafri W. Frequency and characteristic features of portal hypertensive gastropathy in patients with viral cirrhosis. *J Coll Physicians Surg Pak* 2010; **20**: 714-718 [PMID: 21078242]
  - 61 **Garcia-Saenz-de-Sicilia M**, Sanchez-Avila F, Chavez-Tapia NC, Lopez-Arce G, Garcia-Osogobio S, Ruiz-Cordero R, Tellez-



- Avila FI. PPIs are not associated with a lower incidence of portal-hypertension-related bleeding in cirrhosis. *World J Gastroenterol* 2010; **16**: 5869-5873 [PMID: 21155009 DOI: 10.3748/wjg.v16.i46.5869]
- 62 **Abbasi A**, Bhutto AR, Butt N, Munir SM, Dhillon AK. Frequency of portal hypertensive gastropathy and its relationship with biochemical, haematological and endoscopic features in cirrhosis. *J Coll Physicians Surg Pak* 2011; **21**: 723-726 [PMID: 22166690]
  - 63 **Aoyama T**, Oka S, Aikata H, Nakano M, Watari I, Naeshiro N, Yoshida S, Tanaka S, Chayama K. Small bowel abnormalities in patients with compensated liver cirrhosis. *Dig Dis Sci* 2013; **58**: 1390-1396 [PMID: 23247799 DOI: 10.1007/s10620-012-2502-z]
  - 64 **Laleman W**, Simon-Talero M, Maleux G, Perez M, Ameloot K, Soriano G, Villalba J, Garcia-Pagan JC, Barrufet M, Jalan R, Brookes J, Thalassinou E, Burroughs AK, Cordoba J, Nevens F. Embolization of large spontaneous portosystemic shunts for refractory hepatic encephalopathy: a multicenter survey on safety and efficacy. *Hepatology* 2013; **57**: 2448-2457 [PMID: 23401201 DOI: 10.1002/hep.26314]
  - 65 **Giannini EG**, Savarino V, Farinati F, Ciccarese F, Rapaccini G, Marco MD, Benvenuto L, Zoli M, Borzio F, Caturelli E, Chiaramonte M, Trevisani F. Influence of clinically significant portal hypertension on survival after hepatic resection for hepatocellular carcinoma in cirrhotic patients. *Liver Int* 2013; **33**: 1594-1600 [PMID: 23654354 DOI: 10.1111/liv.12199]
  - 66 **Aoyama T**, Oka S, Aikata H, Igawa A, Nakano M, Naeshiro N, Yoshida S, Tanaka S, Chayama K. Major predictors of portal hypertensive enteropathy in patients with liver cirrhosis. *J Gastroenterol Hepatol* 2015; **30**: 124-130 [PMID: 24988903 DOI: 10.1111/jgh.12658]
  - 67 **Zardi EM**, Ghittoni G, Margiotta D, Viera FT, Di Matteo F, Rossi S. Portal hypertensive gastropathy in cirrhotics without varices: a case-control study. *Eur J Gastroenterol Hepatol* 2015; **27**: 91-96 [PMID: 25386762 DOI: 10.1097/MEG.0000000000000234]
  - 68 **Wu Q**, Shen L, Chu J, Ma X, Jin B, Meng F, Chen J, Wang Y, Wu L, Han J, Zhang W, Ma W, Wang H, Li H. Characterization of uncommon portosystemic collateral circulations in patients with hepatic cirrhosis. *Oncol Lett* 2015; **9**: 347-350 [PMID: 25435990]
  - 69 **Fontana RJ**, Sanyal AJ, Ghany MG, Bonkovsky HL, Morgan TR, Litman HJ, Reid AE, Lee WM, Naishadham D. Development and progression of portal hypertensive gastropathy in patients with chronic hepatitis C. *Am J Gastroenterol* 2011; **106**: 884-893 [PMID: 21139575 DOI: 10.1038/ajg.2010.456]
  - 70 **Spina GP**, Arcidiacono R, Bosch J, Pagliaro L, Burroughs AK, Santambrogio R, Rossi A. Gastric endoscopic features in portal hypertension: final report of a consensus conference, Milan, Italy, September 19, 1992. *J Hepatol* 1994; **21**: 461-467 [PMID: 7836719 DOI: 10.1016/S0168-8278(05)80329-0]
  - 71 **Thuluvath PJ**, Yoo HY. Portal Hypertensive gastropathy. *Am J Gastroenterol* 2002; **97**: 2973-2978 [PMID: 12492178 DOI: 10.1111/j.1572-0241.2002.07094.x]
  - 72 **Cubillas R**, Rockey DC. Portal hypertensive gastropathy: a review. *Liver Int* 2010; **30**: 1094-1102 [PMID: 20536720 DOI: 10.1111/j.1478-3231.2010.02286.x]
  - 73 **Hou MC**, Lin HC, Chen CH, Kuo BI, Perng CL, Lee FY, Lee SD. Changes in portal hypertensive gastropathy after endoscopic variceal sclerotherapy or ligation: an endoscopic observation. *Gastrointest Endosc* 1995; **42**: 139-144 [PMID: 7590049 DOI: 10.1016/S0016-5107(95)70070-6]
  - 74 **Trevino HH**, Brady CE, Schenker S. Portal hypertensive gastropathy. *Dig Dis* 1996; **14**: 258-270 [PMID: 8843981 DOI: 10.1159/000171557]
  - 75 **Kamath PS**, Lacerda M, Ahlquist DA, McKusick MA, Andrews JC, Nagorney DA. Gastric mucosal responses to intrahepatic portosystemic shunting in patients with cirrhosis. *Gastroenterology* 2000; **118**: 905-911 [PMID: 10784589 DOI: 10.1016/S0016-5085(00)70176-4]
  - 76 **Mezawa S**, Homma H, Ohta H, Masuko E, Doi T, Miyanishi K, Takada K, Kukitsu T, Sato T, Niitsu Y. Effect of transjugular intrahepatic portosystemic shunt formation on portal hypertensive gastropathy and gastric circulation. *Am J Gastroenterol* 2001; **96**: 1155-1159 [PMID: 11316163 DOI: 10.1111/j.1572-0241.2001.03694.x]
  - 77 **Urata J**, Yamashita Y, Tsuchigame T, Hatanaka Y, Matsukawa T, Sumi S, Matsuno Y, Takahashi M. The effects of transjugular intrahepatic portosystemic shunt on portal hypertensive gastropathy. *J Gastroenterol Hepatol* 1998; **13**: 1061-1067 [PMID: 9835325 DOI: 10.1111/j.1440-1746.1998.tb00571.x]
  - 78 **Kamath PS**, Shah VH. Portal hypertension and bleeding esophageal varices. In: Boyer TD, Manns MP, Sanyal AJ, editors. In: Zakim and Boyer's Hepatology: A textbook of liver disease. 6th ed. Philadelphia: Elsevier Saunders, 2012: 296-326
  - 79 **Pan WD**, Xun RY, Chen YM. Correlations of portal hypertensive gastropathy of hepatitis B cirrhosis with other factors. *Hepatobiliary Pancreat Dis Int* 2002; **1**: 527-531 [PMID: 14607680]
  - 80 **Elnaser MS**, Elebiary S, Bastawi MB, El Shafei A, Elmagd IM, Hamza MM. The prevalence of portal hypertensive gastropathy and duodenopathy in some Egyptian cirrhotic patients. *J Egypt Soc Parasitol* 2004; **34**: 915-923 [PMID: 15587317]
  - 81 **Lo GH**, Lai KH, Cheng JS, Hsu PI, Chen TA, Wang EM, Lin CK, Chiang HT. The effects of endoscopic variceal ligation and propranolol on portal hypertensive gastropathy: a prospective, controlled trial. *Gastrointest Endosc* 2001; **53**: 579-584 [PMID: 11323582 DOI: 10.1067/mge.2001.114062]
  - 82 **de la Peña J**, Rivero M, Sanchez E, Fábrega E, Crespo J, Pons-Romero F. Variceal ligation compared with endoscopic sclerotherapy for variceal hemorrhage: prospective randomized trial. *Gastrointest Endosc* 1999; **49**: 417-423 [PMID: 10202052 DOI: 10.1016/S0016-5107(99)70036-2]
  - 83 **Poddar U**, Bhatnagar S, Yachha SK. Endoscopic band ligation followed by sclerotherapy: Is it superior to sclerotherapy in children with extrahepatic portal venous obstruction? *J Gastroenterol Hepatol* 2011; **26**: 255-259 [PMID: 21261713 DOI: 10.1111/j.1440-1746.2010.06397.x]
  - 84 **Poddar U**, Thapa BR, Singh K. Frequency of gastropathy and gastric varices in children with extrahepatic portal venous obstruction treated with sclerotherapy. *J Gastroenterol Hepatol* 2004; **19**: 1253-1256 [PMID: 15482531 DOI: 10.1111/j.1440-1746.2004.03470.x]
  - 85 **Duan X**, Zhang K, Han X, Ren J, Xu M, Huang G, Zhang M. Comparison of percutaneous transhepatic variceal embolization (PTVE) followed by partial splenic embolization versus PTVE alone for the treatment of acute esophagogastric variceal massive hemorrhage. *J Vasc Interv Radiol* 2014; **25**: 1858-1865 [PMID: 25311969 DOI: 10.1016/j.jvir.2014.08.019]
  - 86 **Lo GH**, Liang HL, Lai KH, Chang CF, Hwu JH, Chen SM, Lin CK, Chiang HT. The impact of endoscopic variceal ligation on the pressure of the portal venous system. *J Hepatol* 1996; **24**: 74-80 [PMID: 8834028 DOI: 10.1016/S0168-8278(96)80189-9]
  - 87 **Korula J**, Ralls P. The effects of chronic endoscopic variceal sclerotherapy on portal pressure in cirrhotics. *Gastroenterology* 1991; **101**: 800-805 [PMID: 1860642]
  - 88 **Kimura K**, Ohto M, Matsutani S, Furuse J, Hoshino K, Okuda K. Relative frequencies of portosystemic pathways and renal shunt formation through the "posterior" gastric vein: portographic study in 460 patients. *Hepatology* 1990; **12**: 725-728 [PMID: 2210674 DOI: 10.1002/hep.1840120417]
  - 89 **Smith-Laing G**, Camilo ME, Dick R, Sherlock S. Percutaneous transhepatic portography in the assessment of portal hypertension. Clinical correlations and comparison of radiographic techniques. *Gastroenterology* 1980; **78**: 197-205 [PMID: 6965281]
  - 90 **Watanabe K**, Kimura K, Matsutani S, Ohto M, Okuda K. Portal hemodynamics in patients with gastric varices. A study in 230 patients with esophageal and/or gastric varices using portal vein catheterization. *Gastroenterology* 1988; **95**: 434-440 [PMID: 3391371]
  - 91 **Yoshikawa I**, Murata I, Nakano S, Otsuki M. Effects of endoscopic variceal ligation on portal hypertensive gastropathy and gastric mucosal blood flow. *Am J Gastroenterol* 1998; **93**: 71-74 [PMID: 9448178 DOI: 10.1111/j.1572-0241.1998.071\_c.x]



- 92 **Balan KK**, Grime JS, Sutton R, Critchley M, Jenkins SA. Do alterations in the rate of gastric emptying after injection sclerotherapy for oesophageal varices play any role in the development of portal hypertensive gastropathy? *HPB Surg* 1999; **11**: 141-148; discussion 148-150 [PMID: 10371058]
- 93 **Sarwar S**, Khan AA, Alam A, Butt AK, Shafqat F, Malik K, Ahmad I, Niazi AK. Effect of band ligation on portal hypertensive gastropathy and development of fundal varices. *J Ayub Med Coll Abbottabad* 2006; **18**: 32-35 [PMID: 16773966]
- 94 **Lo GH**, Lai KH, Cheng JS, Hwu JH, Chang CF, Chen SM, Chiang HT. A prospective, randomized trial of sclerotherapy versus ligation in the management of bleeding esophageal varices. *Hepatology* 1995; **22**: 466-471 [PMID: 7635414 DOI: 10.1002/hep.1840220215]
- 95 **Sarin SK**, Govil A, Jain AK, Gupta RC, Issar SK, Jain M, Murthy NS. Prospective randomized trial of endoscopic sclerotherapy versus variceal band ligation for esophageal varices: influence on gastropathy, gastric varices and variceal recurrence. *J Hepatol* 1997; **26**: 826-832 [PMID: 9126795 DOI: 10.1016/S0168-8278(97)80248-6]
- 96 **Feldman M**, Lee EL. Gastritis. In: Feldman M, Friedman LS, Brandt LJ, editors. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease: Pathophysiology, Diagnosis, Management*. 10th ed. Philadelphia: Elsevier Saunders, 2010: 868-883
- 97 **Primignani M**, Materia M, Preatoni P, Carta A, Morbin T. Does endoscopic treatment of esophageal varices with sclerotherapy (ES) or ligation (EL) influence the evolution of portal hypertensive gastropathy (PHG) in cirrhotic patients? *Gastroenterology* 1998; **114**: A1324 [DOI: 10.1016/S0016-5085(98)85375-4]
- 98 **Ashraf P**, Shah GM, Shaikh H, Manan A, Kumar M. Transjugular intrahepatic portosystemic stenting in portal hypertensive gastropathy. *J Coll Physicians Surg Pak* 2009; **19**: 584-585 [PMID: 19728947]
- 99 **Ohmagari K**, Toyonaga A, Tanikawa K. Effects of transcatheter splenic arterial embolization on portal hypertensive gastric mucosa. *Am J Gastroenterol* 1993; **88**: 1837-1841 [PMID: 8237929]
- 100 **Orloff MJ**, Orloff MS, Orloff SL, Haynes KS. Treatment of bleeding from portal hypertensive gastropathy by portacaval shunt. *Hepatology* 1995; **21**: 1011-1017 [PMID: 7705773 DOI: 10.1016/0270-9139(95)90248-1]
- 101 **Soin AS**, Acharya SK, Mathur M, Sahni P, Nundy S. Portal hypertensive gastropathy in noncirrhotic patients. The effect of lienorenal shunts. *J Clin Gastroenterol* 1998; **26**: 64-67; discussion 68 [PMID: 9492868 DOI: 10.1097/00004836-199801000-00017]
- 102 **Boyer TD**, Haskal ZJ. The role of transjugular intrahepatic portosystemic shunt in the management of portal hypertension. *Hepatology* 2005; **41**: 386-400 [PMID: 15660434 DOI: 10.1002/hep.20559]
- 103 **Pérez-Ayuso RM**, Piqué JM, Bosch J, Panés J, González A, Pérez R, Rigau J, Quintero E, Valderrama R, Viver J. Propranolol in prevention of recurrent bleeding from severe portal hypertensive gastropathy in cirrhosis. *Lancet* 1991; **337**: 1431-1434 [PMID: 1675316 DOI: 10.1016/0140-6736(91)93125-S]
- 104 **Hosking SW**, Kennedy HJ, Seddon I, Triger DR. The role of propranolol in congestive gastropathy of portal hypertension. *Hepatology* 1997; **7**: 437-441 [PMID: 3552921 DOI: 10.1002/hep.1840070304]
- 105 **Kouroumalis EA**, Koutroubakis IE, Manousos ON. Somatostatin for acute severe bleeding from portal hypertensive gastropathy. *Eur J Gastroenterol Hepatol* 1998; **10**: 509-512 [PMID: 9855068 DOI: 10.1097/00042737-199806000-00013]
- 106 **Zhou Y**, Qiao L, Wu J, Hu H, Xu C. Comparison of the efficacy of octreotide, vasopressin, and omeprazole in the control of acute bleeding in patients with portal hypertensive gastropathy: a controlled study. *J Gastroenterol Hepatol* 2002; **17**: 973-979 [PMID: 12167118 DOI: 10.1046/j.1440-1746.2002.02775.x]
- 107 **Bruha R**, Marecek Z, Spicak J, Hulek P, Lata J, Petrtyl J, Urbanek P, Taimr P, Volfova M, Dite P. Double-blind randomized, comparative multicenter study of the effect of terlipressin in the treatment of acute esophageal variceal and/or hypertensive gastropathy bleeding. *Hepatogastroenterology* 2002; **49**: 1161-1166 [PMID: 12143227]
- 108 **Mori T**, Aisa Y, Yajima T, Shimizu T, Kato J, Nakazato T, Hibi T, Ikeda Y, Okamoto S. Esophageal varices and portal hypertensive gastropathy associated with hepatic veno-occlusive disease after allogeneic hematopoietic stem cell transplantation. *Int J Hematol* 2008; **87**: 231-232 [PMID: 18256788 DOI: 10.1007/s12185-008-0023-5]
- 109 **Urso G**, Interlandi D, Puglisi M, Abate G, Bertino G, Raciti C, Sciacca C, Bruno M, Panarello A, Di Prima P, La Rosa G. Role of *Helicobacter pylori* in patients with portal hypertensive gastropathy by liver cirrhosis hepatitis C virus-related. *Minerva Gastroenterol Dietol* 2006; **52**: 303-308 [PMID: 16971874]
- 110 **Al Mofleh IA**. Does *Helicobacter pylori* affect portal hypertensive gastropathy? *Saudi J Gastroenterol* 2007; **13**: 95-97 [PMID: 19858622 DOI: 10.4103/1319-3767.32186]
- 111 **Bayraktar Y**, Balkanci F, Uzunalioglu B, Gokoz A, Koseoglu T, Batman F, Gurakar A, Van Thiel DH, Kayhan B. Is portal hypertension due to liver cirrhosis a major factor in the development of portal hypertensive gastropathy? *Am J Gastroenterol* 1996; **91**: 554-558 [PMID: 8633508]
- 112 **Thuluvath PJ**. Management of upper gastrointestinal hemorrhage related to portal hypertension. In: Yamada T, Alpers DH, Kalloo AN, Kaplowitz N, Owyang C, Powell DW, editors. *Textbook of Gastroenterology*. 5th ed. Wiley-Blackwell, 2009: 2897-3017 [DOI: 10.1002/9781444303414.ch89]
- 113 **Ohta M**, Yamaguchi S, Gotoh N, Tomikawa M. Pathogenesis of portal hypertensive gastropathy: a clinical and experimental review. *Surgery* 2002; **131**: S165-S170 [PMID: 11821805 DOI: 10.1067/msy.2002.119499]
- 114 **Lopez-Talavera JC**, Cadelina G, Olchowski J, Merrill W, Groszmann RJ. Thalidomide inhibits tumor necrosis factor alpha, decreases nitric oxide synthesis, and ameliorates the hyperdynamic circulatory syndrome in portal-hypertensive rats. *Hepatology* 1996; **23**: 1616-1621 [PMID: 8675185 DOI: 10.1053/jhep.1996.v23.pm0008675185]
- 115 **Blendis L**, Wong F. The hyperdynamic circulation in cirrhosis: an overview. *Pharmacol Ther* 2001; **89**: 221-231 [PMID: 11516477 DOI: 10.1016/S0163-7258(01)00124-3]
- 116 **Hashizume M**, Tanaka K, Inokuchi K. Morphology of gastric microcirculation in cirrhosis. *Hepatology* 1993; **3**: 1008-1012 [PMID: 6629315 DOI: 10.1002/hep.1840030619]
- 117 **Perini RF**, Camara PR, Ferraz JG. Pathogenesis of portal hypertensive gastropathy: translating basic research into clinical practice. *Nat Clin Pract Gastroenterol Hepatol* 2009; **6**: 150-158 [PMID: 19190600 DOI: 10.1038/ncpgasthep1356]
- 118 **Makhija S**, Burak K, Beck PL. Portal hypertensive gastropathy and gastric antral vascular ectasia. In: Helmy A, editor. *Portal hypertension: pathogenesis and management*. New York: Nova Science Publishers Inc, 2006: 137-166
- 119 **Nayeb-Hashemi H**, Kaunitz JD. Gastrointestinal mucosal defense. *Curr Opin Gastroenterol* 2009; **25**: 537-543 [PMID: 19654540 DOI: 10.1097/MOG.0b013e328330da7b]
- 120 **Ferraz JG**, Wallace JL. Underlying mechanisms of portal hypertensive gastropathy. *J Clin Gastroenterol* 1997; **25** Suppl 1: S73-S78 [PMID: 9479629 DOI: 10.1097/00004836-199700001-00012]
- 121 **Tarnawski AS**, Sarfeh IJ, Stachura J, Hajduczek A, Bui HX, Dabros W, Gergely H. Microvascular abnormalities of the portal hypertensive gastric mucosa. *Hepatology* 1988; **8**: 1488-1494 [PMID: 3192161 DOI: 10.1002/hep.1840080604]
- 122 **Albillos A**, Colombato LA, Enriquez R, Ng OC, Sikuler E, Groszmann RJ. Sequence of morphological and hemodynamic changes of gastric microvessels in portal hypertension. *Gastroenterology* 1992; **102**: 2066-2070 [PMID: 1587425]
- 123 **Payen JL**, Cales P, Pienkowski P, Sozzani P, Kervran A, Frexinos J, Pascal JP. Weakness of mucosal barrier in portal hypertensive gastropathy of alcoholic cirrhosis. Effects of propranolol and enprostil. *J Hepatol* 1995; **23**: 689-696 [PMID: 8750168 DOI: 10.1016/0168-8278(95)80035-2]

- 124 **Beck PL**, Lee SS, McKnight GW, Wallace JL. Characterization of spontaneous and ethanol-induced gastric damage in cirrhotic rats. *Gastroenterology* 1992; **103**: 1048-1055 [PMID: 1499905]
- 125 **Beck PL**, McKnight W, Lee SS, Wallace JL. Prostaglandin modulation of the gastric vasculature and mucosal integrity in cirrhotic rats. *Am J Physiol* 1993; **265**: G453-G458 [PMID: 8214067]
- 126 **Misra V**, Misra SP, Dwivedi M. Thickened gastric mucosal capillary wall: a histological marker for portal hypertension. *Pathology* 1998; **30**: 10-13 [PMID: 9534201 DOI: 10.1080/00313029800169595]
- 127 **Ichikawa Y**, Tarnawski A, Sarfeh IJ, Ishikawa T, Shimada H. Distorted microangioarchitecture and impaired angiogenesis in gastric mucosa of portal hypertensive rats. *Gastroenterology* 1994; **106**: 702-708 [PMID: 7509763]
- 128 **Gupta R**, Sawant P, Parameshwar RV, Lele VR, Kulhalli PM, Mahajani SS. Gastric mucosal blood flow and hepatic perfusion index in patients with portal hypertensive gastropathy. *J Gastroenterol Hepatol* 1998; **13**: 921-926 [PMID: 9794191 DOI: 10.1111/j.1440-1746.1998.tb00762.x]
- 129 **Iwao T**, Toyonaga A, Ikegami M, Oho K, Sumino M, Harada H, Sakaki M, Shigemori H, Aoki T, Tanikawa K. Reduced gastric mucosal blood flow in patients with portal-hypertensive gastropathy. *Hepatology* 1993; **18**: 36-40 [PMID: 8325619 DOI: 10.1002/hep.1840180107]
- 130 **Kotzampassi K**, Eleftheriadis E, Aletas H. Gastric mucosal blood flow in portal hypertension patients—a laser Doppler flowmetry study. *Hepatogastroenterology* 1992; **39**: 39-42 [PMID: 1533200]
- 131 **Vyas K**, Gala B, Sawant P, Das HS, Kulhalli PM, Mahajan SS. Assessment of portal hemodynamics by ultrasound color Doppler and laser Doppler velocimetry in liver cirrhosis. *Indian J Gastroenterol* 2002; **21**: 176-178 [PMID: 12416745]
- 132 **Shigemori H**, Iwao T, Ikegami M, Toyonaga A, Tanikawa K. Effects of propranolol on gastric mucosal perfusion and serum gastrin level in cirrhotic patients with portal hypertensive gastropathy. *Dig Dis Sci* 1994; **39**: 2433-2438 [PMID: 7956612 DOI: 10.1007/BF02087662]
- 133 **Ohta M**, Hashizume M, Higashi H, Ueno K, Tomikawa M, Kishihara F, Kawanaka H, Tanoue K, Sugimachi K. Portal and gastric mucosal hemodynamics in cirrhotic patients with portal-hypertensive gastropathy. *Hepatology* 1994; **20**: 1432-1436 [PMID: 7982641 DOI: 10.1002/hep.1840200609]
- 134 **Chung RS**, Bruch D, Dearlove J. Endoscopic measurement of gastric mucosal blood flow by laser Doppler velocimetry: effect of chronic esophageal variceal sclerosis. *Am Surg* 1988; **54**: 116-120 [PMID: 2963569]
- 135 **Tomikawa M**, Akiba Y, Kaunitz JD, Kawanaka H, Sugimachi K, Sarfeh IJ, Tarnawski AS. New insights into impairment of mucosal defense in portal hypertensive gastric mucosa. *J Gastrointest Surg* 2000; **4**: 458-463 [PMID: 11077319 DOI: 10.1016/S1091-255X(00)80086-4]
- 136 **Tsugawa K**, Hashizume M, Migou S, Kishihara F, Kawanaka H, Tomikawa M, Sugimachi K. Role of vascular endothelial growth factor in portal hypertensive gastropathy. *Digestion* 2000; **61**: 98-106 [PMID: 10705173 DOI: 10.1159/000007741]
- 137 **De BK**, Das TK. Color Doppler and laser velocimetry studies in the assessment of portal hemodynamics and severity of chronic liver disease. *Indian J Gastroenterol* 2002; **21**: 173-175 [PMID: 12416744]
- 138 **Casadevall M**, Panés J, Piqué JM, Bosch J, Terés J, Rodés J. Limitations of laser-Doppler velocimetry and reflectance spectrophotometry in estimating gastric mucosal blood flow. *Am J Physiol* 1992; **263**: G810-G815 [PMID: 1443154]
- 139 **Bhattacharya B**. Non-neoplastic disorders of the stomach. In: Iacobuzio-Donahue CA, Montgomery E, Goldblum JR, editors. *Gastrointestinal and liver pathology*. 2nd ed. Philadelphia: Elsevier Saunders, 2012: 65-141 [DOI: 10.1016/b978-1-4377-0925-4.00012-2]
- 140 **Misra SP**, Dwivedi M, Misra V, Barthwal R. Portal-hypertensive-gastropathy-like changes in a patient with secondary polycythemia: reversal of endoscopic and histopathologic changes with phlebotomy. *Gastrointest Endosc* 2004; **59**: 916-919 [PMID: 15173815 DOI: 10.1016/S0016-5107(04)00338-4]
- 141 **Wu B**, Zeng L, Lin Y, Wen Z, Chen G, Iwakiri R, Fujimoto K. Downregulation of cyclooxygenase-1 is involved in gastric mucosal apoptosis via death signaling in portal hypertensive rats. *Cell Res* 2009; **19**: 1269-1278 [PMID: 19668263 DOI: 10.1038/cr.2009.97]
- 142 **Tan S**, Wei X, Song M, Tao J, Yang Y, Khatoon S, Liu H, Jiang J, Wu B. PUMA mediates ER stress-induced apoptosis in portal hypertensive gastropathy. *Cell Death Dis* 2014; **5**: e1128 [PMID: 24625987 DOI: 10.1038/cddis.2014.95]
- 143 **Kaur S**, Kaur U, Tandon C, Dhawan V, Ganguly NK, Majumdar S. Gastropathy and defense mechanisms in common bile duct ligated portal hypertensive rats. *Mol Cell Biochem* 2000; **203**: 79-85 [PMID: 10724335 DOI: 10.1023/A:1007090205886]
- 144 **Kawanaka H**, Tomikawa M, Jones MK, Szabo IL, Pai R, Baatar D, Tsugawa K, Sugimachi K, Sarfeh IJ, Tarnawski AS. Defective mitogen-activated protein kinase (ERK2) signaling in gastric mucosa of portal hypertensive rats: potential therapeutic implications. *Hepatology* 2001; **34**: 990-999 [PMID: 11679970 DOI: 10.1053/jhep.2001.28507]
- 145 **Kinjo N**, Kawanaka H, Akahoshi T, Yamaguchi S, Yoshida D, Anegawa G, Konishi K, Tomikawa M, Tanoue K, Tarnawski A, Hashizume M, Maehara Y. Significance of ERK nitration in portal hypertensive gastropathy and its therapeutic implications. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G1016-G1024 [PMID: 18787063 DOI: 10.1152/ajpgi.90329.2008]
- 146 **Wang JY**, Hsieh JS, Lin SR, Huang TJ. The effect of portal hypertension on expression of gastric mucin mRNA in rats. *Hepatogastroenterology* 2001; **48**: 667-671 [PMID: 11462898]
- 147 **Tsugawa K**, Hashizume M, Tomikawa M, Migou S, Kawanaka H, Shiraishi S, Sueishi K, Sugimachi K. Immunohistochemical localization of vascular endothelial growth factor in the rat portal hypertensive gastropathy. *J Gastroenterol Hepatol* 2001; **16**: 429-437 [PMID: 11354282 DOI: 10.1046/j.1440-1746.2001.02452.x]
- 148 **Muñoz J**, Albillos A, Pérez-Páramo M, Rossi I, Alvarez-Mon M. Factors mediating the hemodynamic effects of tumor necrosis factor- $\alpha$  in portal hypertensive rats. *Am J Physiol* 1999; **276**: G687-G693 [PMID: 10070045]
- 149 **Kaviani A**, Ohta M, Itani R, Sander F, Tarnawski AS, Sarfeh IJ. Tumor necrosis factor- $\alpha$  regulates inducible nitric oxide synthase gene expression in the portal hypertensive gastric mucosa of the rat. *J Gastrointest Surg* 1997; **1**: 371-376 [PMID: 9834372 DOI: 10.1016/S1091-255X(97)80059-5]
- 150 **Ohta M**, Tarnawski AS, Itani R, Pai R, Tomikawa M, Sugimachi K, Sarfeh IJ. Tumor necrosis factor  $\alpha$  regulates nitric oxide synthase expression in portal hypertensive gastric mucosa of rats. *Hepatology* 1998; **27**: 906-913 [PMID: 9537427 DOI: 10.1002/hep.510270403]
- 151 **Ohta M**, Tanoue K, Tarnawski AS, Pai R, Itani RM, Sander FC, Sugimachi K, Sarfeh IJ. Overexpressed nitric oxide synthase in portal-hypertensive stomach of rat: a key to increased susceptibility to damage? *Gastroenterology* 1997; **112**: 1920-1930 [PMID: 9178684 DOI: 10.1053/gast.1997.v112.pm9178684]
- 152 **El-Newihi HM**, Kanji VK, Mihas AA. Activity of gastric mucosal nitric oxide synthase in portal hypertensive gastropathy. *Am J Gastroenterol* 1996; **91**: 535-538 [PMID: 8633504]
- 153 **Hartleb M**, Michielsens PP, Dziurkowska-Marek A. The role of nitric oxide in portal hypertensive systemic and portal vascular pathology. *Acta Gastroenterol Belg* 1997; **60**: 222-232 [PMID: 9396180]
- 154 **Arafa UA**, Fujiwara Y, Higuchi K, Shiba M, Uchida T, Watanabe T, Tominaga K, Oshitani N, Matsumoto T, Arakawa T. No additive effect between *Helicobacter pylori* infection and portal hypertensive gastropathy on inducible nitric oxide synthase expression in gastric mucosa of cirrhotic patients. *Dig Dis Sci* 2003; **48**: 162-168 [PMID: 12645804 DOI: 10.1023/A:1021707103590]
- 155 **Lee FY**, Wang SS, Tsai YT, Lin HJ, Lin HC, Chu CJ, Wu SL, Tai CC, Lee SD. Aminoguanidine corrects hyperdynamic

- circulation without ameliorating portal hypertension and portal hypertensive gastropathy in anesthetized portal hypertensive rats. *J Hepatol* 1997; **26**: 687-693 [PMID: 9075678 DOI: 10.1016/S0168-8278(97)80436-9]
- 156 **Chu CJ**, Lee FY, Wang SS, Chang FY, Lin HC, Hou MC, Wu SL, Tai CC, Chan CC, Lee SD. Aminoguanidine ameliorates splanchnic hyposensitivity to glypressin in a haemorrhage-transfused rat model of portal hypertension. *Clin Sci (Lond)* 1998; **95**: 629-636 [PMID: 9791050 DOI: 10.1042/cs0950629]
  - 157 **Silva G**, Navasa M, Bosch J, Chesta J, Pilar Pizcueta M, Casamitjana R, Rivera F, Rodés J. Hemodynamic effects of glucagon in portal hypertension. *Hepatology* 1990; **11**: 668-673 [PMID: 2328958 DOI: 10.1002/hep.1840110421]
  - 158 **Geraghty JG**, Angerson WJ, Carter DC. Splanchnic haemodynamics and vasoactive agents in experimental cirrhosis. *HPB Surg* 1994; **8**: 83-87; discussion 87-88 [PMID: 7880777 DOI: 10.1155/1994/52975]
  - 159 **Benoit JN**, Zimmerman B, Premen AJ, Go VL, Granger DN. Role of glucagon in splanchnic hyperemia of chronic portal hypertension. *Am J Physiol* 1986; **251**: G674-G677 [PMID: 3777172]
  - 160 **Tsui CP**, Sung JJ, Leung FW. Role of acute elevation of portal venous pressure by exogenous glucagon on gastric mucosal injury in rats with portal hypertension. *Life Sci* 2003; **73**: 1115-1129 [PMID: 12818720 DOI: 10.1016/S0024-3205(03)00413-2]
  - 161 **Ohta M**, Kishihara F, Hashizume M, Kawanaka H, Tomikawa M, Higashi H, Tanoue K, Sugimachi K. Increased prostacyclin content in gastric mucosa of cirrhotic patients with portal hypertensive gastropathy. *Prostaglandins Leukot Essent Fatty Acids* 1995; **53**: 41-45 [PMID: 7675821 DOI: 10.1016/0952-3278(95)90081-0]
  - 162 **Weiler H**, Weiler C, Gerok W. Gastric mucosal prostaglandin E2 levels in cirrhosis and portal hypertension. *J Hepatol* 1990; **11**: 58-64 [PMID: 2398267 DOI: 10.1016/0168-8278(90)90272-S]
  - 163 **Arakawa T**, Satoh H, Fukuda T, Nakamura H, Kobayashi K. Endogenous prostaglandin E2 in gastric mucosa of patients with alcoholic cirrhosis and portal hypertension. *Gastroenterology* 1987; **93**: 135-140 [PMID: 3472989]
  - 164 **Beck PL**, McKnight GW, Kelly JK, Wallace JL, Lee SS. Hepatic and gastric cytoprotective effects of long-term prostaglandin E1 administration in cirrhotic rats. *Gastroenterology* 1993; **105**: 1483-1489 [PMID: 8224652 DOI: 10.1016/0016-5085(93)90155-6]
  - 165 **Migoh S**, Hashizume M, Tsugawa K, Tanoue K, Sugimachi K. Role of endothelin-1 in congestive gastropathy in portal hypertensive rats. *J Gastroenterol Hepatol* 2000; **15**: 142-147 [PMID: 10735537 DOI: 10.1046/j.1440-1746.2000.02061.x]
  - 166 **Romano M**, Meise KS, Suozzo R, Sessa G, Persico M, Coffey RJ. Regional distribution of transforming growth factor- $\alpha$  and epidermal growth factor in normal and portal hypertensive gastric mucosa in humans. *Dig Dis Sci* 1995; **40**: 263-267 [PMID: 7851187 DOI: 10.1007/BF02065407]
  - 167 **Pleli T**, Martin D, Kronenberger B, Brunner F, Köberle V, Grammatikos G, Farnik H, Martinez Y, Finkelmeier F, Labocha S, Ferreirós N, Zeuzem S, Piiper A, Waidmann O. Serum autotaxin is a parameter for the severity of liver cirrhosis and overall survival in patients with liver cirrhosis—a prospective cohort study. *PLoS One* 2014; **9**: e103532 [PMID: 25062038 DOI: 10.1371/journal.pone.0103532]
  - 168 **Misra SP**, Dwivedi M, Misra V, Agarwal SK, Gupta R, Gupta SC, Mital VP. Endoscopic and histologic appearance of the gastric mucosa in patients with portal hypertension. *Gastrointest Endosc* 1990; **36**: 575-579 [PMID: 2279646 DOI: 10.1016/S0016-5107(90)71167-4]
  - 169 **Dong L**, Zhang ZN, Fang P, Ma SY. Portal hypertensive gastropathy and its interrelated factors. *Hepatobiliary Pancreat Dis Int* 2003; **2**: 226-229 [PMID: 14599974]
  - 170 **Giofré MR**, Meduri G, Pallio S, Calandra S, Magnano A, Niceforo D, Cinquegrani M, di Leo V, Mazzon E, Sturniolo GC, Longo G, Fries W. Gastric permeability to sucrose is increased in portal hypertensive gastropathy. *Eur J Gastroenterol Hepatol* 2000; **12**: 529-533 [PMID: 10833096 DOI: 10.1097/00042737-200012050-00009]
  - 171 **Bahnacy A**, Kupcsulik P, Elés ZS, Járay B, Flautner L. Helicobacter pylori and congestive gastropathy. *Z Gastroenterol* 1997; **35**: 109-112 [PMID: 9066100]
  - 172 **Balan KK**, Jones AT, Roberts NB, Pearson JP, Critchley M, Jenkins SA. The effects of Helicobacter pylori colonization on gastric function and the incidence of portal hypertensive gastropathy in patients with cirrhosis of the liver. *Am J Gastroenterol* 1996; **91**: 1400-1406 [PMID: 8678003]
  - 173 **Auroux J**, Lamarque D, Roudot-Thoraval F, Deforges L, Chaumette MT, Richardet JP, Delchier JC. Gastroduodenal ulcer and erosions are related to portal hypertensive gastropathy and recent alcohol intake in cirrhotic patients. *Dig Dis Sci* 2003; **48**: 1118-1123 [PMID: 12822873 DOI: 10.1023/A:1023772930681]
  - 174 **Sathar SA**, Kunnathuparambil SG, Sreesh S, Narayanan P, Vinayakumar KR. Helicobacter pylori infection in patients with liver cirrhosis: prevalence and association with portal hypertensive gastropathy. *Ann Gastroenterol* 2014; **27**: 48-52 [PMID: 24714519]
  - 175 **Zullo A**, Hassan C, Ridola L, Francesco VD. Helicobacter pylori and portal hypertensive gastropathy: any new information? *Ann Gastroenterol* 2014; **27**: 91 [PMID: 24714507]
  - 176 **Abbas Z**, Yakoob J, Usman MW, Shakir T, Hamid S, Jafri W. Effect of Helicobacter pylori and its virulence factors on portal hypertensive gastropathy and interleukin (IL)-8, IL-10, and tumor necrosis factor- $\alpha$  levels. *Saudi J Gastroenterol* 2014; **20**: 120-127 [PMID: 24705150 DOI: 10.4103/1319-3767.129477]
  - 177 **Vigneri S**, Termini R, Piraino A, Scialabba A, Pisciotto G, Fontana N. The stomach in liver cirrhosis. Endoscopic, morphological, and clinical correlations. *Gastroenterology* 1991; **101**: 472-478 [PMID: 2065923]
  - 178 **Toyonaga A**, Iwao T. Portal-hypertensive gastropathy. *J Gastroenterol Hepatol* 1998; **13**: 865-877 [PMID: 9794183 DOI: 10.1111/j.1440-1746.1998.tb00754.x]
  - 179 **Stewart CA**, Sanyal AJ. Grading portal gastropathy: validation of a gastropathy scoring system. *Am J Gastroenterol* 2003; **98**: 1758-1765 [PMID: 12907330 DOI: 10.1111/j.1572-0241.2003.07595.x]
  - 180 **Tanoue K**, Hashizume M, Wada H, Ohta M, Kitano S, Sugimachi K. Effects of endoscopic injection sclerotherapy on portal hypertensive gastropathy: a prospective study. *Gastrointest Endosc* 1992; **38**: 582-585 [PMID: 1397916 DOI: 10.1016/S0016-5107(92)70522-7]
  - 181 **Sarin SK**. Diagnostic issues: Portal hypertensive gastropathy and gastric varices. In: DeFranchis R, editor. Portal hypertension II. Proceedings of the second Baveno international consensus workshop on definitions, methodology and therapeutic strategies. Oxford: Blackwell Science, 1996: 30-55
  - 182 **Yoo HY**, Eustace JA, Verma S, Zhang L, Harris M, Kantsevoy S, Lee LA, Kalloo AN, Ravich WJ, Thuluvath PJ. Accuracy and reliability of the endoscopic classification of portal hypertensive gastropathy. *Gastrointest Endosc* 2002; **56**: 675-680 [PMID: 12397275 DOI: 10.1016/S0016-5107(02)70116-8]
  - 183 **Burak KW**, Beck PL. Diagnosis of portal hypertensive gastropathy. *Curr Opin Gastroenterol* 2003; **19**: 477-482 [PMID: 15703593 DOI: 10.1097/00001574-200309000-00008]
  - 184 **Hashizume M**, Sugimachi K. Classification of gastric lesions associated with portal hypertension. *J Gastroenterol Hepatol* 1995; **10**: 339-343 [PMID: 7548815 DOI: 10.1111/j.1440-1746.1995.tb01105.x]
  - 185 **Parker DM**, Wilsoncroft WE, Olshansky T. The relationship between life change and relative autonomic balance. *J Clin Psychol* 1976; **32**: 149-153 [PMID: 1249217]
  - 186 **Iwao T**, Toyonaga A, Ikegami M, Shigemori H, Oho K, Sumino M, Sakaki M, Nakayama M, Nishiyama T, Minetoma T. McCormack's endoscopic signs for diagnosing portal hypertension: comparison with gastroesophageal varices. *Gastrointest Endosc* 1994; **40**: 470-473 [PMID: 7926538 DOI: 10.1016/S0016-5107(94)70212-8]
  - 187 **de Macedo GF**, Ferreira FG, Ribeiro MA, Szutan LA, Assef MS, Rossini LG. Reliability in endoscopic diagnosis of portal hypertensive gastropathy. *World J Gastrointest Endosc* 2013; **5**: 323-331 [PMID: 23858376 DOI: 10.4253/wjge.v5.i7.323]
  - 188 **Eisen GM**, Eliakim R, Zaman A, Schwartz J, Faigel D, Rondonotti



- E, Villa F, Weizman E, Yassin K, deFranchis R. The accuracy of PillCam ESO capsule endoscopy versus conventional upper endoscopy for the diagnosis of esophageal varices: a prospective three-center pilot study. *Endoscopy* 2006; **38**: 31-35 [PMID: 16429352 DOI: 10.1055/s-2005-921189]
- 189 **Garcia-Tsao G**, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology* 2007; **46**: 922-938 [PMID: 17879356 DOI: 10.1002/hep.21907]
  - 190 **Min YW**, Bae SY, Gwak GY, Paik YH, Choi MS, Lee JH, Paik SW, Yoo BC, Koh KC. A clinical predictor of varices and portal hypertensive gastropathy in patients with chronic liver disease. *Clin Mol Hepatol* 2012; **18**: 178-184 [PMID: 22893868 DOI: 10.3350/cmh.2012.18.2.178]
  - 191 **Patwardhan VR**, Cardenas A. Review article: the management of portal hypertensive gastropathy and gastric antral vascular ectasia in cirrhosis. *Aliment Pharmacol Ther* 2014; **40**: 354-362 [PMID: 24889902 DOI: 10.1111/apt.12824]
  - 192 **Ripoll C**, Garcia-Tsao G. Treatment of gastropathy and gastric antral vascular ectasia in patients with portal hypertension. *Curr Treat Options Gastroenterol* 2007; **10**: 483-494 [PMID: 18221609]
  - 193 **Burak KW**, Lee SS, Beck PL. Portal hypertensive gastropathy and gastric antral vascular ectasia (GAVE) syndrome. *Gut* 2001; **49**: 866-872 [PMID: 11709525]
  - 194 **Dulai GS**, Jensen DM, Kovacs TO, Gralnek IM, Jutabha R. Endoscopic treatment outcomes in watermelon stomach patients with and without portal hypertension. *Endoscopy* 2004; **36**: 68-72 [PMID: 14722858]
  - 195 **Urrunaga NH**, Rockey DC. Portal hypertensive gastropathy and colopathy. *Clin Liver Dis* 2014; **18**: 389-406 [PMID: 24679502 DOI: 10.1016/j.cld.2014.01.008]
  - 196 **Naidu H**, Huang Q, Mashimo H. Gastric antral vascular ectasia: the evolution of therapeutic modalities. *Endosc Int Open* 2014; **2**: E67-E73 [PMID: 26135263 DOI: 10.1055/s-0034-1365525]
  - 197 **Payen JL**, Calès P, Voigt JJ, Barbe S, Pilette C, Dubuisson L, Desmorat H, Vinel JP, Kervran A, Chayvialle JA. Severe portal hypertensive gastropathy and antral vascular ectasia are distinct entities in patients with cirrhosis. *Gastroenterology* 1995; **108**: 138-144 [PMID: 7806035]
  - 198 **Siramolpiwat S**. Transjugular intrahepatic portosystemic shunts and portal hypertension-related complications. *World J Gastroenterol* 2014; **20**: 16996-17010 [PMID: 25493012 DOI: 10.3748/wjg.v20.i45.16996]
  - 199 **Tran A**, Villeneuve JP, Bilodeau M, Willems B, Marleau D, Fenyves D, Parent R, Pomier-Layrargues G. Treatment of chronic bleeding from gastric antral vascular ectasia (GAVE) with estrogen-progesterone in cirrhotic patients: an open pilot study. *Am J Gastroenterol* 1999; **94**: 2909-2911 [PMID: 10520843]
  - 200 **Spahr L**, Villeneuve JP, Dufresne MP, Tassé D, Bui B, Willems B, Fenyves D, Pomier-Layrargues G. Gastric antral vascular ectasia in cirrhotic patients: absence of relation with portal hypertension. *Gut* 1999; **44**: 739-742 [PMID: 10205216]
  - 201 **Barbara G**, De Giorgio R, Salvioli B, Stanghellini V, Corinaldesi R. Unsuccessful octreotide treatment of the watermelon stomach. *J Clin Gastroenterol* 1998; **26**: 345-346 [PMID: 9649027]
  - 202 **Herrera S**, Bordas JM, Llach J, Ginès A, Pellisé M, Fernández-Esparrach G, Mondelo F, Mata A, Cárdenas A, Castells A. The beneficial effects of argon plasma coagulation in the management of different types of gastric vascular ectasia lesions in patients admitted for GI hemorrhage. *Gastrointest Endosc* 2008; **68**: 440-446 [PMID: 18423466 DOI: 10.1016/j.gie.2008.02.009]
  - 203 **Smith LA**, Morris AJ, Stanley AJ. The use of hemospay in portal hypertensive bleeding: a case series. *J Hepatol* 2014; **60**: 457-460 [PMID: 24140803 DOI: 10.1016/j.jhep.2013.10.008]
  - 204 **Probst A**, Scheubel R, Wienbeck M. Treatment of watermelon stomach (GAVE syndrome) by means of endoscopic argon plasma coagulation (APC): long-term outcome. *Z Gastroenterol* 2001; **39**: 447-452 [PMID: 11474999]
  - 205 **Ng I**, Lai KC, Ng M. Clinical and histological features of gastric antral vascular ectasia: successful treatment with endoscopic laser therapy. *J Gastroenterol Hepatol* 1996; **11**: 270-274 [PMID: 8742925]
  - 206 **Wells CD**, Harrison ME, Gurudu SR, Crowell MD, Byrne TJ, Depetris G, Sharma VK. Treatment of gastric antral vascular ectasia (watermelon stomach) with endoscopic band ligation. *Gastrointest Endosc* 2008; **68**: 231-236 [PMID: 18533150 DOI: 10.1016/j.gie.2008.02.021]
  - 207 **McGorisk T**, Krishnan K, Keefer L, Komanduri S. Radiofrequency ablation for refractory gastric antral vascular ectasia (with video). *Gastrointest Endosc* 2013; **78**: 584-588 [PMID: 23660565 DOI: 10.1016/j.gie.2013.04.173]
  - 208 **Turon F**, Casu S, Hernández-Gea V, García-Pagán JC. Variceal and other portal hypertension related bleeding. *Best Pract Res Clin Gastroenterol* 2013; **27**: 649-664 [PMID: 24160925 DOI: 10.1016/j.bpg.2013.08.004]
  - 209 **Vincent C**, Pomier-Layrargues G, Dagenais M, Lapointe R, Létourneau R, Roy A, Paré P, Huet PM. Cure of gastric antral vascular ectasia by liver transplantation despite persistent portal hypertension: a clue for pathogenesis. *Liver Transpl* 2002; **8**: 717-720 [PMID: 12149766]
  - 210 **Novitsky YW**, Kercher KW, Czerniach DR, Litwin DE. Watermelon stomach: pathophysiology, diagnosis, and management. *J Gastrointest Surg* 2003; **7**: 652-661 [PMID: 12850679]
  - 211 **Jin T**, Fei BY, Zheng WH, Wang YX. Successful treatment of refractory gastric antral vascular ectasia by distal gastrectomy: a case report. *World J Gastroenterol* 2014; **20**: 14073-14075 [PMID: 25320549 DOI: 10.3748/wjg.v20.i38.14073]
  - 212 **Fuccio L**, Mussetto A, Laterza L, Eusebi LH, Bazzoli F. Diagnosis and management of gastric antral vascular ectasia. *World J Gastrointest Endosc* 2013; **5**: 6-13 [PMID: 23330048 DOI: 10.4253/wjge.v5.i1.6]
  - 213 **Calès P**, Voigt JJ, Payen JL, Bloom E, Berg P, Vinel JP, Pradère B, Broussy P, Pascal JP. Diffuse vascular ectasia of the antrum, duodenum, and jejunum in a patient with nodular regenerative hyperplasia. Lack of response to portosystemic shunt or gastrectomy. *Gut* 1993; **34**: 558-561 [PMID: 8491407]
  - 214 **Ripoll C**, Garcia-Tsao G. Management of gastropathy and gastric vascular ectasia in portal hypertension. *Clin Liver Dis* 2010; **14**: 281-295 [PMID: 20682235 DOI: 10.1016/j.cld.2010.03.013]
  - 215 **Suit PF**, Petras RE, Bauer TW, Petrini JL. Gastric antral vascular ectasia. A histologic and morphometric study of «the watermelon stomach». *Am J Surg Pathol* 1987; **11**: 750-757 [PMID: 3499091]
  - 216 **Kar P**, Mitra S, Resnick JM, Torbey CF. Gastric antral vascular ectasia: case report and review of the literature. *Clin Med Res* 2013; **11**: 80-85 [PMID: 23262190 DOI: 10.3121/cmr.2012.1036]
  - 217 **Gostout CJ**, Viggiano TR, Balm RK. Acute gastrointestinal bleeding from portal hypertensive gastropathy: prevalence and clinical features. *Am J Gastroenterol* 1993; **88**: 2030-2033 [PMID: 8249969]
  - 218 **Kimura K**, Ikegami T, Bekki Y, Ninomiya M, Yamashita Y, Yoshizumi T, Yoshiya S, Soejima Y, Harada N, Shirabe K, Maehara Y. Clinical significance of gastrointestinal bleeding after living donor liver transplantation. *Transpl Int* 2014; **27**: 705-711 [PMID: 24673842 DOI: 10.1111/tri.12325]
  - 219 **Kotzampassi K**, Eleftheriadis E, Aletras H. The 'mosaic-like' pattern of portal hypertensive gastric mucosa after variceal eradication by sclerotherapy. *J Gastroenterol Hepatol* 1990; **5**: 659-663 [PMID: 2129836]
  - 220 **Qamar AA**, Grace ND, Groszmann RJ, Garcia-Tsao G, Bosch J, Burroughs AK, Ripoll C, Maurer R, Planas R, Escorsell A, Garcia-Pagan JC, Patch D, Matloff DS, Makuch R, Rendon G. Incidence, prevalence, and clinical significance of abnormal hematologic indices in compensated cirrhosis. *Clin Gastroenterol Hepatol* 2009; **7**: 689-695 [PMID: 19281860 DOI: 10.1016/j.cgh.2009.02.021]
  - 221 **Mathurin SA**, Agüero AP, Dascani NA, Prestera JA, Gianserra C, Londero E, Chiorra C. [Anemia in hospitalized patients with cirrhosis: prevalence, clinical relevance and predictive factors]. *Acta Gastroenterol Latinoam* 2009; **39**: 103-111 [PMID: 19663083]
  - 222 **Pagliaro L**, Lebrec D, Poynard T, Hillon P, Benhamou J-P. Propranolol for prevention of recurrent gastrointestinal bleeding



- in patients with cirrhosis. A controlled study [N Engl J Med 1981; 305: 1371-1374]. *J Hepatol* 2002; **36**: 148-150 [PMID: 11830324]
- 223 **Panés J**, Bordas JM, Piqué JM, García-Pagán JC, Feu F, Terés J, Bosch J, Rodés J. Effects of propranolol on gastric mucosal perfusion in cirrhotic patients with portal hypertensive gastropathy. *Hepatology* 1993; **17**: 213-218 [PMID: 8428718]
  - 224 **Triantos C**, Kalafateli M. Primary prevention of bleeding from esophageal varices in patients with liver cirrhosis. *World J Hepatol* 2014; **6**: 363-369 [PMID: 25018847 DOI: 10.4254/wjh.v6.i6.363]
  - 225 **Lebrech D**, Poynard T, Hillon P, Benhamou JP. Propranolol for prevention of recurrent gastrointestinal bleeding in patients with cirrhosis: a controlled study. *N Engl J Med* 1981; **305**: 1371-1374 [PMID: 7029276]
  - 226 **Merkel C**, Marin R, Sacerdoti D, Donada C, Cavallarin G, Torboli P, Amodio P, Sebastianelli G, Bolognesi M, Felder M, Mazzaro C, Gatta A. Long-term results of a clinical trial of nadolol with or without isosorbide mononitrate for primary prophylaxis of variceal bleeding in cirrhosis. *Hepatology* 2000; **31**: 324-329 [PMID: 10655253]
  - 227 **Abid S**, Ali S, Baig MA, Waheed AA. Is it time to replace propranolol with carvedilol for portal hypertension? *World J Gastrointest Endosc* 2015; **7**: 532-539 [PMID: 25992192 DOI: 10.4253/wjge.v7.i5.532]
  - 228 **Chan CC**, Lee FY, Wang SS, Chang FY, Lin HC, Lin HJ, Chu CJ, Wu SL, Tai CC, Lee SD. Chronic administration of octreotide ameliorates portal hypertension and portal hypertensive gastropathy in rats with cirrhosis. *Clin Sci (Lond)* 1998; **94**: 367-371 [PMID: 9640342]
  - 229 **Li MK**, Sung JJ, Woo KS, Sanderson J, Leung NW, Yu LM, Tsui CP, Chung SC, Leung FW. Somatostatin reduces gastric mucosal blood flow in patients with portal hypertensive gastropathy: a randomized, double-blind crossover study. *Dig Dis Sci* 1996; **41**: 2440-2446 [PMID: 9011455]
  - 230 **Sung JJ**, Tsui CP, Li MK, Leung FW. Somatostatin attenuates the hyperdynamic circulatory state in the gastric mucosa of rats with portal hypertension. *Scand J Gastroenterol* 1995; **30**: 921-926 [PMID: 8578194]
  - 231 **Panés J**, Piqué JM, Bordas JM, Casadevall M, Terés J, Bosch J, Rodés J. Effect of bolus injection and continuous infusion of somatostatin on gastric perfusion in cirrhotic patients with portal-hypertensive gastropathy. *Hepatology* 1994; **20**: 336-341 [PMID: 7913906]
  - 232 **Escorsell A**, Bandi JC, Andreu V, Moitinho E, García-Pagán JC, Bosch J, Rodés J. Desensitization to the effects of intravenous octreotide in cirrhotic patients with portal hypertension. *Gastroenterology* 2001; **120**: 161-169 [PMID: 11208725]
  - 233 **Law AW**, Gales MA. Octreotide or vasopressin for bleeding esophageal varices. *Ann Pharmacother* 1997; **31**: 237-238 [PMID: 9034426]
  - 234 **Iwao T**, Toyonaga A, Shigemori H, Oho K, Sumino M, Sato M, Tanikawa K. Vasopressin plus oxygen vs vasopressin alone in cirrhotic patients with portal-hypertensive gastropathy: effects on gastric mucosal haemodynamics and oxygenation. *J Gastroenterol Hepatol* 1996; **11**: 216-222 [PMID: 8742916]
  - 235 **Panés J**, Piqué JM, Bordas JM, Llach J, Bosch J, Terés J, Rodés J. Reduction of gastric hyperemia by glypressin and vasopressin administration in cirrhotic patients with portal hypertensive gastropathy. *Hepatology* 1994; **19**: 55-60 [PMID: 8276367]
  - 236 **Panés J**, Casadevall M, Fernández M, Piqué JM, Bosch J, Casamitjana R, Cirera I, Bombi JA, Terés J, Rodés J. Gastric micro-circulatory changes of portal-hypertensive rats can be attenuated by long-term estrogen-progestagen treatment. *Hepatology* 1994; **20**: 1261-1270 [PMID: 7927261]
  - 237 **Karajeh MA**, Hurlstone DP, Stephenson TJ, Ray-Chaudhuri D, Gleeson DC. Refractory bleeding from portal hypertensive gastropathy: a further novel role for thalidomide therapy? *Eur J Gastroenterol Hepatol* 2006; **18**: 545-548 [PMID: 16607153]
  - 238 **Cremers MI**, Oliveira AP, Alves AL, Freitas J. Portal hypertensive gastropathy: treatment with corticosteroids. *Endoscopy* 2002; **34**: 177 [PMID: 11822018]
  - 239 **Wagatsuma Y**, Naritaka Y, Shimakawa T, Kanako H, Keiichiro I, Shunichi S, Konno S, Katsube T, Ogawa K. Clinical usefulness of the angiotensin II receptor antagonist losartan in patients with portal hypertensive gastropathy. *Hepatogastroenterology* 2006; **53**: 171-174 [PMID: 16608017]
  - 240 **Smart HL**, Triger DR. Clinical features, pathophysiology and relevance of portal hypertensive gastropathy. *Endoscopy* 1991; **23**: 224-228 [PMID: 1915140]
  - 241 **Pique JM**, Leung FW, Kitahora T, Sarfeh IJ, Tarnawski A, Guth PH. Gastric mucosal blood flow and acid secretion in portal hypertensive rats. *Gastroenterology* 1988; **95**: 727-733 [PMID: 3165076]
  - 242 **Benoit JN**, Barrowman JA, Harper SL, Kvietys PR, Granger DN. Role of humoral factors in the intestinal hyperemia associated with chronic portal hypertension. *Am J Physiol* 1984; **247**: G486-G493 [PMID: 6496739]
  - 243 **Yen HH**, Yang CW, Su WW, Soon MS, Wu SS, Lin HJ. Oral versus intravenous proton pump inhibitors in preventing re-bleeding for patients with peptic ulcer bleeding after successful endoscopic therapy. *BMC Gastroenterol* 2012; **12**: 66 [PMID: 22681960 DOI: 10.1186/1471-230X-12-66]
  - 244 **Pang SH**, Graham DY. A clinical guide to using intravenous proton-pump inhibitors in reflux and peptic ulcers. *Therap Adv Gastroenterol* 2010; **3**: 11-22 [PMID: 21180586 DOI: 10.1177/1756283X09352095]
  - 245 **Gostout CJ**, Ahlquist DA, Radford CM, Viggiano TR, Bowyer BA, Balm RK. Endoscopic laser therapy for watermelon stomach. *Gastroenterology* 1989; **96**: 1462-1465 [PMID: 2785467]
  - 246 **Ibrahim M**, Degré D, Devière J. Active bleeding caused by portal hypertensive gastropathy. *Gastrointest Endosc* 2014; **80**: 724 [PMID: 24576479 DOI: 10.1016/j.gie.2014.01.020]
  - 247 **Giday SA**. Preliminary data on the nanopowder hemostatic agent TC-325 to control gastrointestinal bleeding. *Gastroenterol Hepatol (N Y)* 2011; **7**: 620-622 [PMID: 22299002]
  - 248 **Patel J**, Parra V, Kedia P, Sharaiha RZ, Kahaleh M. Salvage cryotherapy in portal hypertensive gastropathy. *Gastrointest Endosc* 2015; **81**: 1003 [PMID: 25028270 DOI: 10.1016/j.gie.2014.05.326]
  - 249 **Sezai S**, Ito M, Sakurai Y, Kamisaka K, Abe T, Ikegami F, Yamamoto Y, Hirano M. Effects on gastric circulation of treatment for portal hypertension in cirrhosis. *Dig Dis Sci* 1998; **43**: 1302-1306 [PMID: 9635622 DOI: 10.1023/A:1018872227652]
  - 250 **Hassoun Z**, Pomier-Layrargues G. The transjugular intrahepatic portosystemic shunt in the treatment of portal hypertension. *Eur J Gastroenterol Hepatol* 2004; **16**: 1-4 [PMID: 15095845 DOI: 10.1097/00042737-200401000-00001]
  - 251 **Bosch J**, Abraldes JG. Management of gastrointestinal bleeding in patients with cirrhosis of the liver. *Semin Hematol* 2004; **41**: 8-12 [PMID: 14872414 DOI: 10.1053/j.seminhematol.2003.11.003]
  - 252 **Rosado B**, Kamath PS. Transjugular intrahepatic portosystemic shunts: an update. *Liver Transpl* 2003; **9**: 207-217 [PMID: 12619016 DOI: 10.1053/jlts.2003.50045]
  - 253 **McCashland TM**. Current use of transjugular intrahepatic portosystemic shunts. *Curr Gastroenterol Rep* 2003; **5**: 31-38 [PMID: 12530946 DOI: 10.1007/s11894-003-0007-9]
  - 254 **Babb RR**, Mitchell RL. Persistent hemorrhagic gastritis in a patient with portal hypertension and esophagogastric varices: the role of portal decompressive surgery. *Am J Gastroenterol* 1988; **83**: 777-779 [PMID: 3260069]
  - 255 **Biecker E**. Portal hypertension and gastrointestinal bleeding: diagnosis, prevention and management. *World J Gastroenterol* 2013; **19**: 5035-5050 [PMID: 23964137 DOI: 10.3748/wjg.v19.i31.5035]
  - 256 **Yoshida H**, Mamada Y, Taniai N, Tajiri T. Partial splenic embolization. *Hepatol Res* 2008; **38**: 225-233 [PMID: 18034810]
  - 257 **Shimizu T**, Onda M, Tajiri T, Yoshida H, Mamada Y, Taniai N, Aramaki T, Kumazaki T. Bleeding portal-hypertensive gastropathy managed successfully by partial splenic embolization. *Hepatogastroenterology* 2002; **49**: 947-949 [PMID: 12143250]
  - 258 **Hosking SW**. Congestive gastropathy in portal hypertension: variations in prevalence. *Hepatology* 1989; **10**: 257-258 [PMID: 2785467]

- 2663698]
- 259 **Garcia-Tsao G**, Bosch J. Management of varices and variceal hemorrhage in cirrhosis. *N Engl J Med* 2010; **362**: 823-832 [PMID: 20200386]
  - 260 **Ripoll C**, Garcia-Tsao G. The management of portal hypertensive gastropathy and gastric antral vascular ectasia. *Dig Liver Dis* 2011; **43**: 345-351 [PMID: 21095166 DOI: 10.1016/j.dld.2010.10.006]
  - 261 **Kravetz D**, Sikuler E, Groszmann RJ. Splanchnic and systemic hemodynamics in portal hypertensive rats during hemorrhage and blood volume restitution. *Gastroenterology* 1986; **90**: 1232-1240 [PMID: 3956942]
  - 262 **Toubia N**, Sanyal AJ. Portal hypertension and variceal hemorrhage. *Med Clin North Am* 2008; **92**: 551-574, viii [PMID: 18387376 DOI: 10.1016/j.mcna.2007.12.003]
  - 263 **Hogue CW Jr**, Goodnough LT, Monk TG. Perioperative myocardial ischemic episodes are related to hematocrit level in patients undergoing radical prostatectomy. *Transfusion* 1998; **38**: 924-931 [PMID: 9767742 DOI: 10.1046/j.1537-2995.1998.381098440856.x]
  - 264 **Hwang JH**, Shergill AK, Acosta RD, Chandrasekhara V, Chathadi KV, Decker GA, Early DS, Evans JA, Fanelli RD, Fisher DA, Foley KQ, Fonkalsrud L, Jue T, Khashab MA, Lightdale JR, Muthusamy VR, Pasha SF, Saltzman JR, Sharaf R, Cash BD. The role of endoscopy in the management of variceal hemorrhage. *Gastrointest Endosc* 2014; **80**: 221-227 [PMID: 25034836 DOI: 10.1016/j.gie.2013.07.023]
  - 265 **Soares-Weiser K**, Brezis M, Tur-Kaspa R, Leibovici L. Antibiotic prophylaxis for cirrhotic patients with gastrointestinal bleeding. *Cochrane Database Syst Rev* 2002; (2): CD002907 [PMID: 12076458 DOI: 10.1002/14651858.cd002907]
  - 266 **Soares-Weiser K**, Brezis M, Tur-Kaspa R, Paul M, Yahav J, Leibovici L. Antibiotic prophylaxis of bacterial infections in cirrhotic inpatients: a meta-analysis of randomized controlled trials. *Scand J Gastroenterol* 2003; **38**: 193-200 [PMID: 12678337 DOI: 10.1080/00365520310000690]
  - 267 **Chavez-Tapia NC**, Soares-Weiser K, Brezis M, Leibovici L. Antibiotics for spontaneous bacterial peritonitis in cirrhotic patients. *Cochrane Database Syst Rev* 2009; (1): CD002232 [PMID: 19160207 DOI: 10.1002/14651858.CD002232.pub2]
  - 268 **de Franchis R**. Evolving consensus in portal hypertension. Report of the Baveno IV consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2005; **43**: 167-176 [PMID: 15925423]
  - 269 **Cremers I**, Ribeiro S. Management of variceal and nonvariceal upper gastrointestinal bleeding in patients with cirrhosis. *Therap Adv Gastroenterol* 2014; **7**: 206-216 [PMID: 25177367 DOI: 10.1177/1756283X14538688]
  - 270 **Snyder P**, Ali R, Poles M, Gross SA. Portal hypertensive gastropathy with a focus on management. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 1207-1216 [PMID: 26293979 DOI: 10.1586/17474124.2015.1059275]
  - 271 **Cirera I**, Feu F, Luca A, García-Pagán JC, Fernández M, Escorsell A, Bosch J, Rodés J. Effects of bolus injections and continuous infusions of somatostatin and placebo in patients with cirrhosis: a double-blind hemodynamic investigation. *Hepatology* 1995; **22**: 106-111 [PMID: 7601400 DOI: 10.1016/0270-9139(95)90360-7]
  - 272 **Villanueva C**, Planella M, Aracil C, López-Balaguer JM, González B, Miñana J, Balanzó J. Hemodynamic effects of terlipressin and high somatostatin dose during acute variceal bleeding in nonresponders to the usual somatostatin dose. *Am J Gastroenterol* 2005; **100**: 624-630 [PMID: 15743361 DOI: 10.1111/j.1572-0241.2004.40665.x]
  - 273 **Oluyemi A**, Amole A. Portal hypertensive duodenopathy manifesting as "kissing" duodenal ulcers in a nigerian with alcoholic cirrhosis: a case report and brief review of the literature. *Case Rep Med* 2012; **2012**: 618729 [PMID: 23118766 DOI: 10.1155/2012/618729]
  - 274 **Nagral AS**, Joshi AS, Bhatia SJ, Abraham P, Mistry FP, Vora IM. Congestive jejunopathy in portal hypertension. *Gut* 1993; **34**: 694-697 [PMID: 8504973 DOI: 10.1136/gut.34.5.694]
  - 275 **Misra SP**, Dwivedi M, Misra V, Gupta M. Ileal varices and portal hypertensive ileopathy in patients with cirrhosis and portal hypertension. *Gastrointest Endosc* 2004; **60**: 778-783 [PMID: 15557954 DOI: 10.1016/S0016-5107(04)02049-8]
  - 276 **Kozarek RA**, Botoman VA, Bredfeldt JE, Roach JM, Patterson DJ, Ball TJ. Portal colopathy: prospective study of colonoscopy in patients with portal hypertension. *Gastroenterology* 1991; **101**: 1192-1197 [PMID: 1936789]

**P- Reviewer:** Dang SS, He JY, Mihaila RG, Pan JJ, Umit A

**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Liu SQ



## Non-invasive evaluation of liver fibrosis by acoustic radiation force impulse and aminotransferase:platelet ratio index in chronic hepatitis C

Ergenekon Karagoz, Coskun Ozturker, Ali Kemal Sivrioglu

Ergenekon Karagoz, Department of Infectious Diseases, Van Military Hospital, 65040 Van, Turkey

Coskun Ozturker, Department of Radiology, Canakkale Military Hospital, 17100 Canakkale, Turkey

Ali Kemal Sivrioglu, Department of Radiology, Kasimpasa Military Hospital, 34440 Beyoglu Istanbul, Turkey

**Author contributions:** Karagoz E and Ozturker C designed the research and wrote the letter; Sivrioglu AK revised the letter.

**Conflict-of-interest statement:** The author(s) indicate no potential conflicts of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Coskun Ozturker, MD, Department of Radiology, Canakkale Military Hospital, Zubeyde Hanim Sk., Cevatpasa Mh., 17100 Canakkale, Turkey. [drozturker@gmail.com](mailto:drozturker@gmail.com)  
Telephone: +90-534-5414979

Received: November 26, 2015

Peer-review started: November 26, 2015

First decision: December 22, 2015

Revised: December 28, 2015

Accepted: January 16, 2016

Article in press: January 19, 2016

Published online: February 8, 2016

*terology*, we have read the article by Li *et al* with great interest. We would like to thank the authors for their comprehensive contribution. However, it is our wish to make minor criticism over the present study from the perspective of methodology.

**Key words:** Cirrhosis; Intercostal approach; Subcostal approach; Acoustic radiation force impulse; Liver fibrosis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatitis B virus infection is still one of the leading causes of cirrhosis and hepatocellular carcinoma. Liver biopsy is the gold standard method to assess the severity of liver fibrosis. However, there are several limitations of liver biopsy, including its invasive nature, small tissue sample size, and subjective grading system. Nowadays, noninvasive parameters have been utilized to evaluate liver histology. Additionally, ultrasound-based techniques, such as acoustic radiation force impulse have gained popularity in assessing liver fibrosis. Herein, we aimed to make a minor criticism regarding this study.

Karagoz E, Ozturker C, Sivrioglu AK. Non-invasive evaluation of liver fibrosis by acoustic radiation force impulse and aminotransferase:platelet ratio index in chronic hepatitis C. *World J Hepatol* 2016; 8(4): 263-264 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i4/263.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i4.263>

### TO THE EDITOR

In a previous issue of the *World Journal of Gastroenterology*, we have read the article by Li *et al*<sup>[1]</sup> with great interest. We would like to thank the authors for their

### Abstract

In a previous issue of the *World Journal of Gastroen-*

comprehensive contribution. However, it is our wish to make minor criticism over the present study from the perspective of methodology.

First, the measurements of Acoustic Radiation Force Impulse Imaging were performed in the right liver lobe through the intercostal space in present study<sup>[1]</sup>. Uslu *et al*<sup>[2]</sup> demonstrated that subcostal approach to the liver parenchyma was significantly superior to intercostal approach for the evaluation of liver stiffness in their study. As the pressure was transmitted better to liver parenchyma and the anterior abdominal wall, we are of the opinion that subcostal approach would give better results than intercostal approach in terms of determining the elasticity of the liver.

Second, it would have been better, if the authors had stated the length of the biopsy material and the number of the pieces of the portal tracts. Fibrosis is heterogeneously distributed throughout the liver, whereas a biopsy evaluates only 1/50000 of the total volume of the liver<sup>[3]</sup>. Additionally, if the biopsy material is not long enough, appropriate evaluation cannot be done. A length of at least 25 mm is required to assess

the fibrosis score accurately<sup>[3]</sup>. It would have been better, if the authors had mentioned these conditions as limitations.

Further studies are needed to indicate the role of acoustic radiation force impulse imaging method in the management of liver fibrosis and cirrhosis in patients with chronic hepatitis C.

## REFERENCES

- 1 **Li SM**, Li GX, Fu DM, Wang Y, Dang LQ. Liver fibrosis evaluation by ARFI and APRI in chronic hepatitis C. *World J Gastroenterol* 2014; **20**: 9528-9533 [PMID: 25071348 DOI: 10.3748/wjg.v20.i28.9528]
- 2 **Uslu A**, Batur A, Biyik M, Acikgozolu S. Non-Invasive Evaluation of Liver Fibrosis Using Real-Time Elastography and Comparison of Intercostal and Subcostal Approaches. *Eur J Gen Med* 2015; **12**: 109-113 [DOI: 10.15197/sabad.1.12.23]
- 3 **Frulio N**, Trillaud H, Perez P, Asselineau J, Vandenhende M, Hessamfar M, Bonnet F, Maire F, Delaune J, De Ledinghen V, Morlat P. Acoustic Radiation Force Impulse (ARFI) and Transient Elastography (TE) for evaluation of liver fibrosis in HIV-HCV co-infected patients. *BMC Infect Dis* 2014; **14**: 405 [PMID: 25041708 DOI: 10.1186/1471-2334-14-405]

**P- Reviewer:** Grassi A, Wang K **S- Editor:** Qi Y  
**L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 February 18; 8(5): 265-306





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 469 members, representing a team of worldwide experts in hepatology. They are from 53 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (1), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (98), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (19), Greece (11), Hungary (5), India (15), Indonesia (2), Iran (4), Israel (1), Italy (52), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (11), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (17), and United States (56).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*

Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*  
Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*

Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *São Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*



**Chile**

Luis A Videla, *Santiago*



## China

Guang-Wen Cao, Shanghai  
 En-Qiang Chen, Chengdu  
 Gong-Ying Chen, Hangzhou  
 Jin-lian Chen, Shanghai  
 Jun Chen, Changsha  
 Alfred Cheng, Hong Kong  
 Chun-Ping Cui, Beijing  
 Shuang-Suo Dang, Xi'an  
 Ming-Xing Ding, Jinhua  
 Zhi-Jun Duang, Dalian  
 He-Bin Fan, Wuhan  
 Xiao-Ming Fan, Shanghai  
 James Yan Yue Fung, Hong Kong  
 Yi Gao, Guangzhou  
 Zuo-Jiong Gong, Wuhan  
 Zhi-Yong Guo, Guangzhou  
 Shao-Liang Han, Wenzhou  
 Tao Han, Tianjin  
 Jin-Yang He, Guangzhou  
 Ming-Liang He, Hong Kong  
 Can-Hua Huang, Chengdu  
 Bo Jin, Beijing  
 Shan Jin, Hohhot  
 Hui-Qing Jiang, Shijiazhuang  
 Wan-Yee Joseph Lau, Hong Kong  
 Guo-Lin Li, Changsha  
 Jin-Jun Li, Shanghai  
 Qiang Li, Jinan  
 Sheng Li, Jinan  
 Zong-Fang Li, Xi'an  
 Xu Li, Guangzhou  
 Xue-Song Liang, Shanghai  
 En-Qi Liu, Xi'an  
 Pei Liu, Shenyang  
 Zhong-Hui Liu, Changchun  
 Guang-Hua Luo, Changzhou  
 Yi Lv, Xi'an  
 Guang-Dong Pan, Liuzhou  
 Wen-Sheng Pan, Hangzhou  
 Jian-Min Qin, Shanghai  
 Wai-Kay Seto, Hong Kong  
 Hong Shen, Changsha  
 Xiao Su, Shanghai  
 Li-Ping Sun, Beijing  
 Wei-Hao Sun, Nanjing  
 Xue-Ying Sun, Harbin  
 Hua Tang, Tianjin  
 Ling Tian, Shanghai  
 Eric Tse, Hong Kong  
 Guo-Ying Wang, Changzhou  
 Yue Wang, Beijing  
 Shu-Qiang Wang, Chengdu  
 Mary MY Wayne, Hong Kong  
 Hong-Shan Wei, Beijing  
 Danny Ka-Ho Wong, Hong Kong  
 Grace Lai-Hung Wong, Hong Kong  
 Bang-Fu Wu, Dongguan  
 Feng Wu, Chongqing  
 Xiong-Zhi Wu, Tianjin  
 Chun-Fang Xu, Suzhou  
 Rui-An Xu, Quanzhou  
 Rui-Yun Xu, Guangzhou  
 Wei-Li Xu, Shijiazhuang  
 Shi-Ying Xuan, Qingdao  
 Ming-Xian Yan, Jinan  
 Lv-Nan Yan, Chengdu  
 Jin Yang, Hangzhou  
 Ji-Hong Yao, Dalian  
 Winnie Yeo, Hong Kong

Zheng Zeng, Beijing  
 Qi Zhang, Hangzhou  
 Shi-Jun Zhang, Guangzhou  
 Xiao-Lan Zhang, Shijiazhuang  
 Xiao-Yong Zhang, Guangzhou  
 Xin-Chen Zhang, Harbin  
 Yong Zhang, Xi'an  
 Hong-Chuan Zhao, Hefei  
 Ming-Hua Zheng, Wenzhou  
 Yu-Bao Zheng, Guangzhou  
 Ren-Qian Zhong, Shanghai  
 Fan Zhu, Wuhan  
 Xiao Zhu, Dongguan



## Czech Republic

Kamil Vysloulzil, Olomouc



## Denmark

Henning Gronbaek, Aarhus  
 Christian Mortensen, Hvidovre



## Egypt

Ihab T Abdel-Raheem, Damanhour  
 NGB G Bader EL Din, Cairo  
 Hatem Elalfy, Mansoura  
 Mahmoud M El-Bendary, Mansoura  
 Mona El SH El-Raziky, Cairo  
 Mohammad El-Sayed, Cairo  
 Yasser M Fouad, Minia  
 Mohamed AA Metwally, Benha  
 Hany Shehab, Cairo  
 Mostafa M Sira, Shebin El-koom  
 Ashraf Taye, Minia  
 MA Ali Wahab, Mansoura



## France

Laurent Alric, Toulouse  
 Sophie Conchon, Nantes  
 Daniel J Felmlee, Strasbourg  
 Herve Lerat, Creteil  
 Dominique Salmon, Paris  
 Jean-Pierre Vartanian, Paris



## Germany

Laura E Buitrago-Molina, Hannover  
 Enrico N De Toni, Munich  
 Oliver Ebert, Muenchen  
 Rolf Gebhardt, Leipzig  
 Janine V Hartl, Regensburg  
 Sebastian Hinz, Kiel  
 Benjamin Juntermanns, Essen  
 Roland Kaufmann, Jena  
 Viola Knop, Frankfurt  
 Veronika Lukacs-Kornek, Homburg  
 Benjamin Maasoumy, Hannover  
 Jochen Mattner, Erlangen  
 Nadja M Meindl-Beinker, Mannheim  
 Ulf P Neumann, Aachen  
 Margarete Odenthal, Cologne  
 Yoshiaki Sunami, Munich

Christoph Roderburg, Aachen  
 Frank Tacke, Aachen  
 Yuchen Xia, Munich



## Greece

Alex P Betrosian, Athens  
 George N Dalekos, Larissa  
 Ioanna K Delladetsima, Athens  
 Nikolaos K Gatselis, Larissa  
 Stavros Gourgiotis, Athens  
 Christos G Savopoulos, Thessaloniki  
 Tania Siahaniidou, Athens  
 Emmanouil Sinakos, Thessaloniki  
 Nikolaos G Symeonidi, Thessaloniki  
 Konstantinos C Thomopoulos, Larissa  
 Konstantinos Tziomalos, Thessaloniki



## Hungary

Gabor Banhegyi, Budapest  
 Peter L Lakatos, Budapest  
 Maria Papp, Debrecen  
 Ferenc Sipos, Budapest  
 Zsolt J Tulassay, Budapest



## India

Deepak N Amarapurkar, Mumbai  
 Girish M Bhopale, Pune  
 Sibnarayan Datta, Tezpur  
 Nutan D Desai, Mumbai  
 Sorabh Kapoor, Mumbai  
 Jaswinder S Maras, New Delhi  
 Nabeen C Nayak, New Delhi  
 C Ganesh Pai, Manipal  
 Amit Pal, Chandigarh  
 K Rajeshwari, New Delhi  
 Anup Ramachandran, Vellore  
 D Nageshwar Reddy, Hyderabad  
 Shivaram P Singh, Cuttack  
 Ajith TA, Thrissur  
 Balasubramaniyan Vairappan, Pondicherry



## Indonesia

Cosmas RA Lesmana, Jakarta  
 Neneng Ratnasari, Yogyakarta



## Iran

Seyed M Jazayeri, Tehran  
 Sedigheh Kafi-Abad, Tehran  
 Iradj Maleki, Sari  
 Fakhraddin Naghibalhossaini, Shiraz



## Israel

Stephen DH Malnick, Rehovot



## Italy

Francesco Angelico, Rome



Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Andrea Galli, *Florence*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Salvatore Gruttadauria, *Palermo*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verducci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*



**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*  
 Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*

Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhito Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*



**Jordan**

Kamal E Bani-Hani, *Zarqa*



**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*



**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*



**Moldova**

Angela Peltec, *Chishinev*



**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*



**Nigeria**

CA Asabamaka Onyekwere, *Lagos*



**Pakistan**

Bikha Ram Devrajani, *Jamshoro*



**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*



**Poland**

Jacek Zielinski, *Gdansk*



**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*



**Qatar**

Reem Al Olaby, *Doha*



**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*



**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*



**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*



**Singapore**

Ser Yee Lee, *Singapore*



**South Korea**

Young-Hwa Chung, *Seoul*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonju*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*



**Spain**

Ivan G Marina, *Madrid*  
 Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*

José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodríguez-Frias, *Córdoba*  
 Manuel L Rodríguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Traperó-Marugán, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpaboon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*  
 Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*

Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*



#### **United States**

Naim Alkhouri, *Cleveland*  
 Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*

Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Grigoriy E Gurvits, *New York*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Kusum K Kharbanda, *Omaha*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**REVIEW**

- 265 Controversies in the management of primary sclerosing cholangitis  
*Nayagam JS, Pereira SP, Devlin J, Harrison PM, Joshi D*

**MINIREVIEWS**

- 273 Hepatitis B virus and hepatitis C virus infection in healthcare workers  
*Coppola N, De Pascalis S, Onorato L, Calò F, Sagnelli C, Sagnelli E*

**ORIGINAL ARTICLE****Basic Study**

- 282 Hepatitis C virus inhibitor synergism suggests multistep interactions between heat-shock protein 90 and hepatitis C virus replication  
*Kubota N, Nomoto M, Hwang GW, Watanabe T, Kohara M, Wakita T, Naganuma A, Kuge S*

**Case Control Study**

- 291 High level of serum cholesteryl ester transfer protein in active hepatitis C virus infection  
*Satoh K, Nagano T, Seki N, Tomita Y, Aida Y, Sugita T, Itagaki M, Sutoh S, Abe H, Aizawa Y*

**Prospective Study**

- 301 Blood DNA methylation markers in prospectively identified hepatocellular carcinoma cases and controls from Taiwan  
*Wu HC, Shen J, Yang HI, Tsai WY, Chen CJ, Santella RM*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Daniel J Felmlee, Research Lecturer, Hepatology Research Group, Plymouth University Peninsula School of Medicine and Dentistry, 67000 Strasbourg, France

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: editorialoffice@wjnet.com  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjnet.com](mailto:bpgoffice@wjnet.com)  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

**PUBLICATION DATE**  
February 18, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

Full instructions are available online at [http://www.wjnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjnet.com/bpg/g_info_20160116143427.htm)

**ONLINE SUBMISSION**

<http://www.wjnet.com/esps/>



## Controversies in the management of primary sclerosing cholangitis

Jeremy S Nayagam, Stephen P Pereira, John Devlin, Phillip M Harrison, Deepak Joshi

Jeremy S Nayagam, John Devlin, Phillip M Harrison, Deepak Joshi, Institute of Liver Studies, King's College Hospital, London SE5 9RS, United Kingdom

Stephen P Pereira, Institute of Liver and Digestive Health, University College London, London NW3 2PF, United Kingdom

**Author contributions:** All authors contributed equally to the paper with concept and design of the review, literature review, drafting, critical revision and editing, and approval of the final version.

**Conflict-of-interest statement:** No potential conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Deepak Joshi, Institute of Liver Studies, King's College Hospital, Denmark Hill, London SE5 9RS, United Kingdom. [d.joshi@nhs.net](mailto:d.joshi@nhs.net)  
Telephone: +44-203-2999000

Received: July 29, 2015  
Peer-review started: August 6, 2015  
First decision: September 16, 2015  
Revised: January 13, 2016  
Accepted: January 21, 2016  
Article in press: January 22, 2016  
Published online: February 18, 2016

### Abstract

Primary sclerosing cholangitis (PSC) remains a rare but significant disease, which affects mainly young males in association with inflammatory bowel disease. There have been few advances in the understanding of the

pathogenesis of the condition and no therapeutics with proven mortality benefit aside from liver transplantation. There remain areas of controversy in the management of PSC which include the differentiation from other cholangiopathies, in particular immunoglobulin G4 related sclerosing cholangitis, the management of dominant biliary strictures, and the role of ursodeoxycholic acid. In addition, the timing of liver transplantation in PSC remains difficult to predict with standard liver severity scores. In this review, we address these controversies and highlight the latest evidence base in the management of PSC.

**Key words:** Immunoglobulin G4 related sclerosing cholangitis; Cholangiocarcinoma; Primary sclerosing cholangitis; Liver transplantation; Dominant strictures

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** There have been few advances in therapeutics for primary sclerosing cholangitis (PSC) and there remain areas of controversy in the management of PSC. In this review, we address these controversies, which include the differentiation of PSC from other cholangiopathies, in particular immunoglobulin G4 related sclerosing cholangitis, the management of dominant biliary strictures, the role of ursodeoxycholic acid, and the timing of liver transplantation.

Nayagam JS, Pereira SP, Devlin J, Harrison PM, Joshi D. Controversies in the management of primary sclerosing cholangitis. *World J Hepatol* 2016; 8(5): 265-272 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i5/265.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i5.265>

### INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic cho-

**Table 1 Differential diagnosis for primary sclerosing cholangitis**

Vascular	Hepatic artery thrombosis
	Portal hypertension bilopathy
	Portal cavernoma associated cholangiopathy
	Intra-arterial chemotherapy
Trauma	Sickle cell disease related cholangiopathy
	Trauma post cholecystectomy
	Abdominal trauma
Infections	AIDS related cholangiopathy
	Recurrent pyogenic cholangitis
Benign	Intraductal stone disease
Malignancy	Cholangiocarcinoma
Autoimmune	Autoimmune sclerosing cholangitis
	IgG4 related sclerosing cholangitis
	Systemic vasculitis
Other	Recurrent pancreatitis
	Sclerosing cholangitis in critically ill patient
	TPN related cholangiopathy
	Histocytosis X

IgG4: Immunoglobulin G4; TPN: Total parenteral nutrition; AIDS: Acquired immune deficiency syndrome.

lestatic disorder characterised by inflammation and fibrosis of intrahepatic and extrahepatic bile ducts, resulting in multifocal biliary strictures<sup>[1]</sup>. The pathogenesis of PSC remains unclear but hypotheses include genetic factors<sup>[2]</sup>, lymphocyte recruitment and activation<sup>[3]</sup>, portal bacteraemia<sup>[4]</sup> and bile salt toxicity<sup>[5]</sup>.

PSC commonly affects males<sup>[6]</sup> with a median age at diagnosis of 35 years<sup>[6,7]</sup>. In addition, there is a significant association with inflammatory bowel disease (IBD)<sup>[8]</sup>, hepatobiliary malignancies<sup>[9]</sup> and colorectal cancer<sup>[10]</sup>. A key aspect in the management of PSC is surveillance for the development of these conditions. Patients commonly present with cholestatic liver enzymes and a normal bilirubin<sup>[8]</sup>. The demonstration of a cholangiopathy is essential for the diagnosis of PSC<sup>[11]</sup>. Characteristic ERCP findings include diffuse multifocal strictures and irregularities, with normal or minimally dilated segments in between giving rise to the characteristic beaded pattern<sup>[12]</sup>. The use of MRCP has been increasing, partially driven by the complication rate of 11% following ERCP<sup>[13]</sup>, and a comparable sensitivity and specificity to ERCP<sup>[14]</sup>.

PSC is strongly associated with IBD, with prevalence of 63%-81%<sup>[8,15]</sup>, most commonly ulcerative colitis (UC), in 86%-88%<sup>[15,16]</sup>. The temporal relationship of the 2 conditions can be variable, although IBD usually precedes the diagnosis of PSC. Patients with colitis typically have mild symptoms and are sometimes asymptomatic<sup>[17]</sup>. Endoscopic findings are very different between PSC and non-PSC groups, with more pancolitis, backwash ileitis, and rectal sparing in those with PSC<sup>[16]</sup>. Colonoscopy with biopsies is recommended as part of the diagnostic work-up in any new diagnosis of PSC<sup>[18]</sup>. In addition, patients with PSC and IBD have a significantly higher risk of colorectal cancer than those with IBD alone<sup>[10]</sup> (OR = 4.79, 95%CI: 3.58-6.41).

To date, certain aspects remain controversial in the management of PSC. They include the differentiation

of PSC and from other causes of sclerosing cholangitis in particular immunoglobulin G4 related sclerosing cholangitis (IgG4-SC), the optimal management of dominant biliary strictures at endoscopy, the role of ursodeoxycholic acid (UDCA), its optimal dose and likely benefit, and the timing of liver transplantation. In this review article we will address these controversies.

## SEARCH STRATEGY

We searched PubMed using the following terms: "primary sclerosing cholangitis", "secondary sclerosing cholangitis", "cholangiocarcinoma", "IgG4 related disease" and "liver transplantation". We included data from full-text articles, published in English. Further relevant articles were identified from the reference lists of review articles and guidelines from liver societies.

## THE DIFFERENTIAL DIAGNOSIS OF PSC

Secondary sclerosing cholangitis (SSC), includes a heterogeneous group of conditions where different insults (*i.e.*, infections, thrombosis, iatrogenic, trauma) can give rise similar clinical characteristics to PSC<sup>[19]</sup> (Table 1). A single-centre series of 31 patients with SSC, identified a shorter transplant free survival (median 72 mo) when compared to controls with PSC, and with no complicating cases of cholangiocarcinoma (CCA)<sup>[20]</sup>. A more recently described entity is sclerosing cholangitis in critically ill patients. This may be related to hepatic hypoperfusion and biliary cast formation<sup>[21]</sup>, and has a particularly aggressive clinical manifestation with a reported transplant free survival of approximately 1 year<sup>[22]</sup>.

IgG4 disease was first described in 1995 in patients with pancreatitis, raised serum IgG levels and a response to corticosteroids<sup>[23]</sup>, which was termed autoimmune pancreatitis (AIP). Extra-pancreatic biliary changes that were found on ERCP, in addition to lymphoplasmocytic infiltration and fibrosis on liver biopsy, suggested an extra-pancreatic biliary component to the disease<sup>[24]</sup>. This has now been termed IgG4-SC, and is the commonest extra-pancreatic manifestation of AIP<sup>[25]</sup>.

IgG4-SC is an important differential diagnosis for PSC, with a different natural history and treatment profile. There are some subtle differences and similarities between PSC and IgG4-SC (Table 2). Similar to PSC, IgG4-SC has a male preponderance, however it usually presents in older patients<sup>[26]</sup>. The clinical presentation in patients with IgG4-SC is more commonly acute onset of obstructive jaundice, than is seen in classical PSC<sup>[25]</sup>, and was evident in 75% in one series<sup>[27]</sup>. IgG4-SC can also be diagnosed in asymptomatic patients with AIP, either at initial diagnosis or during follow up. The data on rates of co-existent IBD are limited to small case series from Japan<sup>[27]</sup> and United Kingdom<sup>[28]</sup>, which show much lower detection of IBD when compared to patients with PSC. Serum IgG4 is elevated in 76% of patients with AIP, and total IgG is elevated in 42%<sup>[29]</sup>. An elevated

**Table 2 Primary sclerosing cholangitis compared to immunoglobulin G4 related sclerosing cholangitis (adapted from Joshi 2014)**

	PSC	IAC
Gender (M:F)	1.5:1	7:1
Age of onset	Young (< 40 yr)	Older (> 50 yr)
Presentation	Cholestatic liver enzymes	Obstructive jaundice
Cholangiogram	Beading, band-like strictures, peripheral pruning	Long smooth strictures, low CBD strictures
Cholangioscopy	Dilated and tortuous vessels	Scarring, pseudo diverticula
Raised serum IgG4 levels	< 20%	> 70%
Pancreatic involvement	< 5%	> 80%
Cholangiocarcinoma	9%	Rare
Association with IBD	80%	< 10%
Response to steroids	Rare (IgG4 + ve PSC)	97%

M: Male; F: Female; PSC: Primary sclerosing cholangitis; IgG4: Immunoglobulin G4; IBD: Inflammatory bowel disease.

serum IgG4 level however is not sufficient to diagnose IgG4-SC, especially as the optimal cut-off value has not been defined and may differ for subgroups of IgG4-SC<sup>[30]</sup>.

There are 4 cholangiographic patterns of disease which vary in the level of stricture location<sup>[31]</sup>. These are usually long smooth strictures as opposed to beading and band-like strictures in PSC. Depending on the pattern of disease, hepatobiliary malignancy may form part of the differential diagnosis, hence the role for ERCP to obtain samples from dominant strictures<sup>[26]</sup>. Brush cytology during ERCP is beneficial in detecting malignancy, however intra-ductal or ampullary biopsies are required to confirm the diagnosis of IgG4-SC. Histological features of IgG4 related disease are infiltration of plasma cells and IgG4 positive plasma cells, storiform fibrosis, and obliterative phlebitis<sup>[32,33]</sup>. Cholangioscopy in IgG4-SC patients has demonstrated dilated and tortuous vessels in 69%, a feature that was not observed in patients with PSC<sup>[34]</sup>. In addition, less scarring and pseudo diverticula were noted in IgG4-SC patients compared to the PSC patients. When a second procedure was carried out after steroids (32 to 93 d), a significant improvement in stenosis, dilated and tortuous vessels, and mass lesions were identified<sup>[34]</sup>.

The cholangiopathy of IgG4-SC is very responsive to corticosteroids and an improvement in bilirubin can be detected within 8 wk of therapy<sup>[28]</sup>. We recommend 30-40 mg prednisolone o.d. for 4 wk followed by a slow wean, blood test monitoring and imaging at 6 wk<sup>[26]</sup>. Regular clinical assessment in this period is required due to the potential risk of cholangitis and sepsis. A follow up study of IgG4 related disease patients, which included 84 with IgG4-SC, demonstrated a response to steroids in 97% but also a high relapse rate of 50%, which was not predicted by initial or on treatment serum IgG4 levels<sup>[35]</sup>. Five percent were diagnosed with cirrhosis (histological and clinical) and one patient required liver transplantation. It has been hypothesised that unlike PSC, which presents as a more indolent disease with established fibrosclerotic changes, the biliary strictures found in IgG4-SC are at an earlier more inflammatory stage of the disease process which is more responsive

to steroids<sup>[36]</sup>. IgG4-SC patients have not been identified to develop CCA<sup>[37]</sup>, which is in contrast to PSC which confers a significant risk of CCA<sup>[38]</sup>.

## MANAGEMENT OF DOMINANT BILIARY STRICTURE

A dominant stricture is defined as a narrow biliary stricture which impedes normal bile flow, with a diameter < 15 mm in the CBD/CHD or < 10 mm in the hepatic duct<sup>[39]</sup>. In a follow up study of 9.8 years, a new dominant stricture was found in 63% of patients<sup>[40]</sup>. Where liver biopsies were available, those with more advanced liver disease histologically were more likely to have dominant strictures<sup>[39,40]</sup>. The mean survival of patients with dominant strictures was significantly poorer than those without<sup>[40]</sup> (14 years vs 23 years,  $P = 0.01$ ). Data from long-term follow up studies (7.1 and 9.8 years), demonstrates that patients who developed CCA, almost all had pre-existing dominant strictures<sup>[40,41]</sup>.

The most concerning differential diagnosis of a dominant stricture is CCA. When a new dominant stricture is identified, a malignant aetiology needs to be excluded using the combination of axial imaging, biliary cytology and/or histology. Therapy for dominant strictures should be offered to all symptomatic patients, and current guidelines recommend endoscopic dilatation with or without stenting<sup>[40]</sup>. A prospective study of 52 patients with dominant strictures who underwent biliary intervention (stent or dilatation) identified a significantly better survival free of transplantation at 3, 5 and 7 years, when compared to that predicted by the Mayo Risk Score<sup>[39]</sup>.

Despite an improvement in prognosis with biliary intervention, the evidence for the ideal therapeutic strategy is not clear and guidelines suggest endoscopic dilatation, but do not give definitive guidance regarding stenting<sup>[40]</sup>. This decision is a balance between the likelihood of biochemical improvement and the risk of intervention. A large retrospective review of all biliary interventions performed for dominant strictures revealed a similar clinical and biochemical course independent

of modality of intervention, although a lower procedure related complication rate was evident in the balloon dilatation group compared to those who underwent stenting<sup>[42]</sup>. A trial of short term temporary biliary stents (mean 11 d) demonstrated an improvement in symptoms, with only 20% requiring further intervention in 1 year and 40% in 3 years<sup>[43]</sup>. However, within the same study a procedure related complication rate of 15% was reported<sup>[43]</sup>.

A prospective multicentre study of patients with compensated PSC without recent biliary intervention, comparing balloon dilatation to short-term plastic stenting (1-2 wk) is underway (www.clinicaltrials.gov, NCT01398917). The main endpoints of the study include re-intervention free survival time at 2 years, change in cholestatic symptoms and biochemistry at 3 mo, and adverse incidents. Data from this study may guide us towards the optimal management strategy for this group of patients.

Cholangioscopy allows for direct optical visualisation and guided biopsies of the biliary epithelium and biliary lesions. It is another diagnostic tool in the management of dominant strictures. In a multi-centre retrospective study of 52 patients with sclerosing cholangitis (48 PSC, 4 IgG4-SC) who underwent 54 procedures for suspicious biliary strictures, the sensitivity and specificity (50% and 100%, respectively) for diagnosing malignancy was comparable to a control group of patients investigated for a single biliary stricture<sup>[44]</sup>. Failure of cannulation rate was higher in the sclerosing cholangitis group (15%), and was related to difficulty cannulating the narrowed bile duct. The adverse events rate was 17%, with 11% developing cholangitis post procedure despite prophylactic antibiotics.

A further single centre prospective study of patients with PSC referred for cholangioscopy, reported their findings in 41 consecutive patients<sup>[45]</sup>. Cholangioscopy identified ductal stones in 56% of patients (of which 30% were not previously identified on cholangiography), and achieved complete or partial clearance in approximately three quarters of patients. One patient was diagnosed with CCA. Two of the 8 patients who proceeded to transplant were diagnosed with CCA on their explants, both of whom had undergone cholangioscopy directed biopsies which were negative. It appears that the diagnostic accuracy may be related to difficulties in deciding which parts of a stricture to biopsy, especially as it may contain both inflammation and cancer<sup>[44]</sup>. The addition of narrow-band imaging increases the biopsy rate but does not improve the dysplasia detection rate<sup>[46]</sup>.

The use of fluorescence *in situ* hybridisation (FISH) on ERCP brushing samples has been studied in patients with PSC and suspicion of CCA. A recent meta-analysis involving 828 patients from 8 studies, identified a pooled sensitivity and specificity of 68% and 70%, respectively, and 51% and 93% respectively for the 6 studies characterising FISH polysomy<sup>[47]</sup>. In a patient with PSC, a dominant stricture and FISH polysomy, there was a 88% specificity for CCA<sup>[48]</sup>, and where

there was serial FISH polysomy detected with no overt evidence of malignancy, 69% were diagnosed with CCA upto 2.5 years post initial test<sup>[49]</sup>. FISH may play a role in patients with high pre-test probability of CCA, or where CCA is suspected with no clear radiological or histological evidence. However it needs to be used with caution due to the low sensitivity, and importantly the risks of repeated invasive tests and the implications of delaying or excluding patients from liver transplantation.

## THE ROLE OF UDCA

UDCA is a hydrophilic bile acid which was studied in patients with PSC following the discovery of its efficacy in chronic cholestatic conditions, in particular primary biliary cirrhosis<sup>[50]</sup>.

Small prospective studies using UDCA at different doses in patients with PSC showed improvement in symptoms and liver biochemistry (8-16 mg/kg<sup>[51]</sup>, 10 mg/kg<sup>[52]</sup>), as well as histology (13-15 mg/kg<sup>[53]</sup>). Further studies at standard (13-15 mg/kg)<sup>[54]</sup> and higher doses (17-23 mg/kg)<sup>[55]</sup> did not demonstrate any survival advantage or prevention of CCA, although none were powered sufficiently to answer this hypothesis. A more recent randomised placebo controlled study at high dose (28-30 mg/kg) was terminated early due to an interim analysis demonstrating significantly higher rate of serious adverse events in the high dose UDCA group<sup>[56]</sup>. A subsequent meta-analysis on the use of UDCA in PSC concluded that there is no significant difference in mortality, histology or risk of CCA, at both standard and higher dose<sup>[57]</sup>, and guidelines advise against its use in PSC<sup>[18]</sup> (Table 3).

Patients with PSC and IBD have an increased risk of developing colorectal dysplasia compared to patients with IBD alone<sup>[10]</sup>. Although the true mechanisms for this increased risk remains unclear, one proposed hypothesis is the increased exposure of the colon to toxic bile acids<sup>[58]</sup>. Attention has therefore turned to UDCA with some data suggesting a possible chemo-preventative role for UDCA in reducing the incidence of colorectal cancer in PSC and UC patients<sup>[59]</sup>. A meta-analysis also suggested a benefit of using low-dose UDCA (8-13 mL/kg per day) but there was significant heterogeneity of the studies<sup>[60]</sup>. A double-blind, placebo controlled multicentre trial of 56 patients with PSC and UC given high dose UDCA (28-30 mg/kg) identified a significantly increased risk of colorectal cancer and dysplasia on both univariate, HR = 4.44 (1.30-20.1), and multivariate analysis, HR = 5.97 (1.39-41.44), in those receiving UDCA<sup>[61]</sup>. At present based on published guidelines, the use of UDCA for chemo-prevention for colorectal dysplasia is not recommended<sup>[18]</sup>.

## THE ROLE AND TIMING OF LIVER TRANSPLANTATION

Liver transplantation is an effective treatment for PSC,



**Table 3** Important studies involving ursodeoxycholic acid

Ref.	Dose and study design	Number UDCA (number placebo)	Follow up	Parameter	Outcome
Chazouillères <i>et al</i> <sup>[51]</sup>	8-16 mg/kg UDCA alone	15	6 mo	Liver enzymes	Improved
O'Brien <i>et al</i> <sup>[52]</sup>	10 mg/kg UDCA alone	12	37 mo	Liver enzymes	Improved
Beuers <i>et al</i> <sup>[53]</sup>	13-15 mg/kg UDCA <i>vs</i> placebo	6 (8)	12 mo	Liver enzymes Histology	Improved Improved
Lindor <i>et al</i> <sup>[54]</sup>	13-15 mg/kg UDCA <i>v</i> placebo	51 (51)	2.2 yr	Liver enzymes Time to treatment failure Time to liver transplant	Improved No change No change
Olsson <i>et al</i> <sup>[55]</sup>	17-23 mg/kg UDCA <i>vs</i> placebo	110 (109)	5 yr	Liver enzymes Transplant free survival	No change No change
Lindor <i>et al</i> <sup>[56]</sup>	28-30 mg/kg UDCA <i>vs</i> placebo	76 (74)	Terminated	Liver enzymes Primary end-point Serious adverse events	Improved Increased Increased

UDCA: Ursodeoxycholic acid.

**Table 4** Mayo risk score<sup>1</sup>

Parameter	Weighting
Age	+ 0.03 × absolute value
Bilirubin	+ 0.54 × log
Aspartate aminotransferase	+ 0.54 × log
Variceal bleeding	+ 1.24 × yes/no
Albumin	- 0.84 × absolute value

<sup>1</sup>The link is: <http://www.mayoclinic.org/medical-professionals/model-end-stage-liver-disease/revised-natural-history-model-for-primary-sclerosing-cholangitis>.

with survival post-transplant 93.7% at 1 year, 86.4% at 5 years, 69.8% at 10 years<sup>[62]</sup>. The indications for liver transplantation are similar to other aetiologies of chronic liver disease, but also include intractable pruritus and recurrent cholangitis. Organ allocation varies according to national policy. Recurrence of PSC following liver transplantation occurs in up to 20% of patients at 5 years<sup>[63,64]</sup>. The diagnosis of PSC recurrence post-transplant can be challenging due to the variety of causes of biliary strictures and cholangiopathy in the post-transplant setting. After exclusion of these, in combination with a concordant liver biopsy, a diagnosis of PSC recurrence can be made<sup>[65]</sup>. In patients who develop PSC recurrence, re-transplant free survival is 85% at 1 year and 45% at 5 years<sup>[66]</sup>.

Identifying which patients will benefit from liver transplantation, and the optimal timing for this is a challenge in the management of PSC, and many predictive models have been developed to optimise this. The Mayo Risk Score was initially devised following a single centre analysis of 174 patients with PSC<sup>[67]</sup>. Using multivariate analysis, age, log bilirubin, log haemoglobin, presence of IBD and liver biopsy stage, were identified as key parameters in predicting transplant-free survival. These parameters were incorporated into a statistical survival model which stratified patients into "low", "intermediate", and "high" risk<sup>[67]</sup>. The Mayo Risk Score for PSC has had numerous iterations over the last 20 years<sup>[68,69]</sup>. The latest version of the Mayo Risk Score incorporates age,

log bilirubin, log aspartate aminotransferase, variceal bleeding and albumin<sup>[69]</sup> (Table 4). This model predicts survival over 4 years and classifies patients as "low", "medium" and "high" risk.

Cholangiograms of 129 patients with PSC identified that high-grade and diffuse strictures of the intrahepatic ducts were markers of poor prognosis<sup>[70]</sup>. A retrospective review of 181 cholangiograms from 4 centres<sup>[71]</sup>, utilised the Amsterdam Cholangiographic Classification System for PSC<sup>[72]</sup>. The intrahepatic and extrahepatic ducts are scored based on severity of cholangiographic changes, and combined to calculate a prognostic index, where a higher score is associated with a poorer prognosis. If a patient however has a normal cholangiogram, a score of 0 is attributed and a score cannot be calculated, thereby making this model invalid for patients with small-duct PSC. Using this classification, a validation study was able to construct a model which could predict medium and long term prognosis in individual patients with PSC<sup>[73]</sup>.

The enhanced liver fibrosis (ELF) test has recently been assessed to predict clinical outcomes in a cohort of patients with large-duct PSC<sup>[74]</sup>. Median transplant-free survival differed significantly in the tertiles based on ELF score and a cut-off value was calculated to stratify patients into "low-score" and "high-score" groups, with a sensitivity of 67% and specificity of 83%. Patients with higher ELF score had a shorter survival, which was confirmed in a validation group of 138 patients<sup>[74]</sup>. In a multivariate Cox regression analysis, the ELF score was associated with transplant-free survival independent of the Mayo Risk Score.

CCA remains a contraindication to liver transplantation in the majority of liver transplant centres. A protocol was developed at the Mayo Clinic (inclusion criteria: < 3 cm lesion, no metastases, no prior abdominal radiation therapy, no transperitoneal biopsy of the tumour, no prior attempt at resection with violation of the bile ducts), which included pre-transplant neo-adjuvant chemo-irradiation, and a modified post-transplant immunosuppression regimen<sup>[75]</sup>. Using this protocol reported outcomes were comparable to other

indications for liver transplantation<sup>[76]</sup>. Of 215 patients who received neo-adjuvant chemotherapy, 136 patients proceeded to liver transplantation (87 with PSC), with 92% 1 year and 74% 5 year survival. Twenty one percent of those who underwent operative staging were excluded from transplantation due to metastatic disease, and there was a 21% tumour recurrence post transplantation<sup>[77]</sup>. Unfortunately these promising results have not been reproduced at other centres<sup>[78]</sup>. There are currently several studies in progress in order to further understand the role for liver transplantation for CCA, and this data is eagerly awaited<sup>[79]</sup>.

## CONCLUSION

The management of patients with PSC continues to pose a challenge to clinicians worldwide. Although guidelines are available, there are few proven therapeutic options, and there remain clinical scenarios which lack a robust evidence base with which to guide management. Many of the commonly used diagnostic tests, particularly for the detection of hepatobiliary malignancy, lack an appropriate sensitivity and specificity. Until further advances in the field take place, the mainstay of management should involve optimal biliary drainage, timely referral for liver transplantation and a low threshold for investigation for hepatobiliary or colorectal malignancy.

## REFERENCES

- 1 Chapman RW, Arborgh BA, Rhodes JM, Summerfield JA, Dick R, Scheuer PJ, Sherlock S. Primary sclerosing cholangitis: a review of its clinical features, cholangiography, and hepatic histology. *Gut* 1980; **21**: 870-877 [PMID: 7439807 DOI: 10.1136/gut.21.10.870]
- 2 Bergquist A, Montgomery SM, Bahmanyar S, Olsson R, Danielsson A, Lindgren S, Prytz H, Hultcrantz R, Lööf LA, Sandberg-Gertzén H, Almer S, Askling J, Ehlin A, Ekblom A. Increased risk of primary sclerosing cholangitis and ulcerative colitis in first-degree relatives of patients with primary sclerosing cholangitis. *Clin Gastroenterol Hepatol* 2008; **6**: 939-943 [PMID: 18674735 DOI: 10.1016/j.cgh.2008.03.016]
- 3 Adams DH, Eksteen B. Aberrant homing of mucosal T cells and extra-intestinal manifestations of inflammatory bowel disease. *Nat Rev Immunol* 2006; **6**: 244-251 [PMID: 16498453 DOI: 10.1038/nri1784]
- 4 Angulo P, Lindor KD. Primary sclerosing cholangitis. *Hepatology* 1999; **30**: 325-332 [PMID: 10385674 DOI: 10.1002/hep.510300101]
- 5 Palmer RH. Bile acids, liver injury, and liver disease. *Arch Intern Med* 1972; **130**: 606-617 [PMID: 4627840 DOI: 10.1001/archinte.1972.03650040130012]
- 6 Farrant JM, Hayllar KM, Wilkinson ML, Karani J, Portmann BC, Westaby D, Williams R. Natural history and prognostic variables in primary sclerosing cholangitis. *Gastroenterology* 1991; **100**: 1710-1717 [PMID: 1850376]
- 7 Schrumpf E, Abdelnoor M, Fausa O, Elgjo K, Jenssen E, Kolmannskog F. Risk factors in primary sclerosing cholangitis. *J Hepatol* 1994; **21**: 1061-1066 [PMID: 7699228 DOI: 10.1016/S0168-8278(05)80618-X]
- 8 Tischendorf JJ, Hecker H, Krüger M, Manns MP, Meier PN. Characterization, outcome, and prognosis in 273 patients with primary sclerosing cholangitis: A single center study. *Am J Gastroenterol* 2007; **102**: 107-114 [PMID: 17037993 DOI: 10.1111/j.1572-0241.2006.00872.x]
- 9 Bergquist A, Ekblom A, Olsson R, Kornfeldt D, Lööf L, Danielsson A, Hultcrantz R, Lindgren S, Prytz H, Sandberg-Gertzén H, Almer S, Granath F, Broomé U. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. *J Hepatol* 2002; **36**: 321-327 [PMID: 11867174 DOI: 10.1016/S0168-8278(01)00288-4]
- 10 Soetikno RM, Lin OS, Heidenreich PA, Young HS, Blackstone MO. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis: a meta-analysis. *Gastrointest Endosc* 2002; **56**: 48-54 [PMID: 12085034 DOI: 10.1067/mge.2002.125367]
- 11 Lee YM, Kaplan MM. Primary sclerosing cholangitis. *N Engl J Med* 1995; **332**: 924-933 [PMID: 7877651 DOI: 10.1056/NEJM199504063321406]
- 12 MacCarty RL, LaRusso NF, Wiesner RH, Ludwig J. Primary sclerosing cholangitis: findings on cholangiography and pancreatography. *Radiology* 1983; **149**: 39-44 [PMID: 6412283 DOI: 10.1148/radiology.149.1.6412283]
- 13 Bangarulingam SY, Gossard AA, Petersen BT, Ott BJ, Lindor KD. Complications of endoscopic retrograde cholangiopancreatography in primary sclerosing cholangitis. *Am J Gastroenterol* 2009; **104**: 855-860 [PMID: 19259076 DOI: 10.1038/ajg.2008.161]
- 14 Berstad AE, Aabakken L, Smith HJ, Aasen S, Boberg KM, Schrumpf E. Diagnostic accuracy of magnetic resonance and endoscopic retrograde cholangiography in primary sclerosing cholangitis. *Clin Gastroenterol Hepatol* 2006; **4**: 514-520 [PMID: 16616358 DOI: 10.1016/j.cgh.2005.10.007]
- 15 Broomé U, Olsson R, Lööf L, Bodemar G, Hultcrantz R, Danielsson A, Prytz H, Sandberg-Gertzén H, Wallerstedt S, Lindberg G. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. *Gut* 1996; **38**: 610-615 [PMID: 8707097 DOI: 10.1136/gut.38.4.610]
- 16 Loftus EV, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* 2005; **54**: 91-96 [PMID: 15591511 DOI: 10.1136/gut.2004.046615]
- 17 Schrumpf E, Elgjo K, Fausa O, Gjone E, Kolmannskog F, Ritland S. Sclerosing cholangitis in ulcerative colitis. *Scand J Gastroenterol* 1980; **15**: 689-697 [PMID: 7209379 DOI: 10.3109/00365528009181516]
- 18 Chapman R, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Schneider B, Gores GJ. Diagnosis and management of primary sclerosing cholangitis. *Hepatology* 2010; **51**: 660-678 [PMID: 20101749 DOI: 10.1002/hep.23294]
- 19 Abdalian R, Heathcote EJ. Sclerosing cholangitis: a focus on secondary causes. *Hepatology* 2006; **44**: 1063-1074 [PMID: 17058222 DOI: 10.1002/hep.21405]
- 20 Gossard AA, Angulo P, Lindor KD. Secondary sclerosing cholangitis: a comparison to primary sclerosing cholangitis. *Am J Gastroenterol* 2005; **100**: 1330-1333 [PMID: 15929765 DOI: 10.1111/j.1572-0241.2005.41526.x]
- 21 Rueemmele P, Hofstaedt F, Gelbmann CM. Secondary sclerosing cholangitis. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 287-295 [PMID: 19404269 DOI: 10.1038/nrgastro.2009.46]
- 22 Kulaksiz H, Heuberger D, Engler S, Stiehl A. Poor outcome in progressive sclerosing cholangitis after septic shock. *Endoscopy* 2008; **40**: 214-218 [PMID: 18264887 DOI: 10.1055/s-2007-967024]
- 23 Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. *Dig Dis Sci* 1995; **40**: 1561-1568 [PMID: 7628283 DOI: 10.1007/BF02285209]
- 24 Hamano H, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, Fukushima M, Nikaide T, Nakayama K, Usuda N, Kiyosawa K. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* 2001; **344**: 732-738 [PMID: 11236777 DOI: 10.1056/NEJM200103083441005]
- 25 Björnsson E, Chari ST, Smyrk TC, Lindor K. Immunoglobulin G4 associated cholangitis: description of an emerging clinical entity based on review of the literature. *Hepatology* 2007; **45**: 1547-1554 [PMID: 17538931 DOI: 10.1002/hep.21685]
- 26 Joshi D, Webster GJ. Biliary and hepatic involvement in IgG4-related disease. *Aliment Pharmacol Ther* 2014; **40**: 1251-1261

- [PMID: 25312536 DOI: 10.1111/apt.12988]
- 27 **Nakazawa T**, Ohara H, Sano H, Ando T, Aoki S, Kobayashi S, Okamoto T, Nomura T, Joh T, Itoh M. Clinical differences between primary sclerosing cholangitis and sclerosing cholangitis with autoimmune pancreatitis. *Pancreas* 2005; **30**: 20-25 [PMID: 15632695 DOI: 10.2958/suizo.20.134]
  - 28 **Church NI**, Pereira SP, Deheragoda MG, Sandanayake N, Amin Z, Lees WR, Gillams A, Rodriguez-Justo M, Novelli M, Seward EW, Hatfield AR, Webster GJ. Autoimmune pancreatitis: clinical and radiological features and objective response to steroid therapy in a UK series. *Am J Gastroenterol* 2007; **102**: 2417-2425 [PMID: 17894845 DOI: 10.1111/j.1572-0241.2007.01531.x]
  - 29 **Ghazale A**, Chari ST, Smyrk TC, Levy MJ, Topazian MD, Takahashi N, Clain JE, Pearson RK, Pelaez-Luna M, Petersen BT, Vege SS, Farnell MB. Value of serum IgG4 in the diagnosis of autoimmune pancreatitis and in distinguishing it from pancreatic cancer. *Am J Gastroenterol* 2007; **102**: 1646-1653 [PMID: 17555461 DOI: 10.1111/j.1572-0241.2007.01264.x]
  - 30 **Ohara H**, Nakazawa T, Kawa S, Kamisawa T, Shimosegawa T, Uchida K, Hirano K, Nishino T, Hamano H, Kanno A, Notohara K, Hasebe O, Muraki T, Ishida E, Naitoh I, Okazaki K. Establishment of a serum IgG4 cut-off value for the differential diagnosis of IgG4-related sclerosing cholangitis: a Japanese cohort. *J Gastroenterol Hepatol* 2013; **28**: 1247-1251 [PMID: 23621484 DOI: 10.1111/jgh.12248]
  - 31 **Nakazawa T**, Naitoh I, Hayashi K, Miyabe K, Simizu S, Joh T. Diagnosis of IgG4-related sclerosing cholangitis. *World J Gastroenterol* 2013; **19**: 7661-7670 [PMID: 24282356 DOI: 10.3748/wjg.v19.i43.7661]
  - 32 **Chari ST**, Smyrk TC, Levy MJ, Topazian MD, Takahashi N, Zhang L, Clain JE, Pearson RK, Petersen BT, Vege SS, Farnell MB. Diagnosis of autoimmune pancreatitis: the Mayo Clinic experience. *Clin Gastroenterol Hepatol* 2006; **4**: 1010-1016; quiz 934 [PMID: 16843735 DOI: 10.1016/j.cgh.2006.05.017]
  - 33 **Hirano K**, Fukushima N, Tada M, Isayama H, Mizuno S, Yamamoto K, Yashima Y, Yagioka H, Sasaki T, Kogure H, Nakai Y, Sasahira N, Tsujino T, Kawabe T, Fukayama M, Omata M. Diagnostic utility of biopsy specimens for autoimmune pancreatitis. *J Gastroenterol* 2009; **44**: 765-773 [PMID: 19430718 DOI: 10.1007/s00535-009-0052-8]
  - 34 **Itoi T**, Kamisawa T, Igarashi Y, Kawakami H, Yasuda I, Itokawa F, Kishimoto Y, Kuwatani M, Doi S, Hara S, Moriyasu F, Baron TH. The role of peroral video cholangioscopy in patients with IgG4-related sclerosing cholangitis. *J Gastroenterol* 2013; **48**: 504-514 [PMID: 22948487 DOI: 10.1007/s00535-012-0652-6]
  - 35 **Huggett MT**, Culver EL, Kumar M, Hurst JM, Rodriguez-Justo M, Chapman MH, Johnson GJ, Pereira SP, Chapman RW, Webster GJ, Barnes E. Type 1 autoimmune pancreatitis and IgG4-related sclerosing cholangitis is associated with extrapancreatic organ failure, malignancy, and mortality in a prospective UK cohort. *Am J Gastroenterol* 2014; **109**: 1675-1683 [PMID: 25155229 DOI: 10.1038/ajg.2014.223]
  - 36 **Webster GJ**, Pereira SP, Chapman RW. Autoimmune pancreatitis/IgG4-associated cholangitis and primary sclerosing cholangitis-overlapping or separate diseases? *J Hepatol* 2009; **51**: 398-402 [PMID: 19505739 DOI: 10.1016/j.jhep.2009.04.010]
  - 37 **Takikawa H**, Takamori Y, Tanaka A, Kurihara H, Nakanuma Y. Analysis of 388 cases of primary sclerosing cholangitis in Japan; Presence of a subgroup without pancreatic involvement in older patients. *Hepatol Res* 2004; **29**: 153-159 [PMID: 15203079 DOI: 10.1016/j.hepres.2004.03.006]
  - 38 **Claessen MM**, Vleggaar FP, Tytgat KM, Siersema PD, van Buuren HR. High lifetime risk of cancer in primary sclerosing cholangitis. *J Hepatol* 2009; **50**: 158-164 [PMID: 19012991 DOI: 10.1016/j.jhep.2008.08.013]
  - 39 **Stiehl A**, Rudolph G, Klöters-Plachky P, Sauer P, Walker S. Development of dominant bile duct stenoses in patients with primary sclerosing cholangitis treated with ursodeoxycholic acid: outcome after endoscopic treatment. *J Hepatol* 2002; **36**: 151-156 [PMID: 11830325 DOI: 10.1016/j.jhep.2008.08.013]
  - 40 **Chapman MH**, Webster GJ, Bannoo S, Johnson GJ, Wittmann J, Pereira SP. Cholangiocarcinoma and dominant strictures in patients with primary sclerosing cholangitis: a 25-year single-centre experience. *Eur J Gastroenterol Hepatol* 2012; **24**: 1051-1058 [PMID: 22653260 DOI: 10.1097/MEG.0b013e3283554bbf]
  - 41 **Gotthardt DN**, Rudolph G, Klöters-Plachky P, Kulaksiz H, Stiehl A. Endoscopic dilation of dominant stenoses in primary sclerosing cholangitis: outcome after long-term treatment. *Gastrointest Endosc* 2010; **71**: 527-534 [PMID: 20189511 DOI: 10.1016/j.gie.2009.10.041]
  - 42 **Kaya M**, Petersen BT, Angulo P, Baron TH, Andrews JC, Gostout CJ, Lindor KD. Balloon dilation compared to stenting of dominant strictures in primary sclerosing cholangitis. *Am J Gastroenterol* 2001; **96**: 1059-1066 [PMID: 11316147 DOI: 10.1111/j.1572-0241.2001.03690.x]
  - 43 **Ponsioen CY**, Lam K, van Milligen de Wit AW, Huibregtse K, Tytgat GN. Four years experience with short term stenting in primary sclerosing cholangitis. *Am J Gastroenterol* 1999; **94**: 2403-2407 [PMID: 10483999 DOI: 10.1111/j.1572-0241.1999.01364.x]
  - 44 **Kalaitzakis E**, Sturgess R, Kaltsidis H, Oppong K, Lekharaju V, Bergenzaun P, Vlavianos P, Sharma H, Westaby D, Webster GJ. Diagnostic utility of single-user peroral cholangioscopy in sclerosing cholangitis. *Scand J Gastroenterol* 2014; **49**: 1237-1244 [PMID: 25007715]
  - 45 **Awadallah NS**, Chen YK, Piraka C, Antillon MR, Shah RJ. Is there a role for cholangioscopy in patients with primary sclerosing cholangitis? *Am J Gastroenterol* 2006; **101**: 284-291 [PMID: 16454832 DOI: 10.1111/j.1572-0241.2006.00383.x]
  - 46 **Azeem N**, Gostout CJ, Knipschild M, Baron TH. Cholangioscopy with narrow-band imaging in patients with primary sclerosing cholangitis undergoing ERCP. *Gastrointest Endosc* 2014; **79**: 773-779.e2 [PMID: 24206748 DOI: 10.1016/j.gie.2013.09.017]
  - 47 **Navaneethan U**, Njei B, Venkatesh PG, Vargo JJ, Parsi MA. Fluorescence in situ hybridization for diagnosis of cholangiocarcinoma in primary sclerosing cholangitis: a systematic review and meta-analysis. *Gastrointest Endosc* 2014; **79**: 943-950.e3 [PMID: 24360654 DOI: 10.1016/j.gie.2013.11.001]
  - 48 **Bangarulingam SY**, Björnsson E, Enders F, Barr Fritcher EG, Gores G, Halling KC, Lindor KD. Long-term outcomes of positive fluorescence in situ hybridization tests in primary sclerosing cholangitis. *Hepatology* 2010; **51**: 174-180 [PMID: 19877179 DOI: 10.1002/hep.23277]
  - 49 **Barr Fritcher EG**, Kipp BR, Voss JS, Clayton AC, Lindor KD, Halling KC, Gores GJ. Primary sclerosing cholangitis patients with serial polysomy fluorescence in situ hybridization results are at increased risk of cholangiocarcinoma. *Am J Gastroenterol* 2011; **106**: 2023-2028 [PMID: 21844920 DOI: 10.1038/ajg.2011.272]
  - 50 **Poupon RE**, Balkau B, Eschwège E, Poupon R. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. UDCA-PBC Study Group. *N Engl J Med* 1991; **324**: 1548-1554 [PMID: 1674105 DOI: 10.1056/NEJM199105303242204]
  - 51 **Chazouillères O**, Poupon R, Capron JP, Metman EH, Dhumeaux D, Amouretti M, Couzigou P, Labayle D, Trinchet JC. Ursodeoxycholic acid for primary sclerosing cholangitis. *J Hepatol* 1990; **11**: 120-123 [PMID: 1975818 DOI: 10.1016/0168-8278(90)90281-U]
  - 52 **O'Brien CB**, Senior JR, Arora-Mirchandani R, Batta AK, Salen G. Ursodeoxycholic acid for the treatment of primary sclerosing cholangitis: a 30-month pilot study. *Hepatology* 1991; **14**: 838-847 [PMID: 1937390 DOI: 10.1002/hep.1840140516]
  - 53 **Beuers U**, Spengler U, Kruis W, Aydemir U, Wiebecke B, Heldwein W, Weinzierl M, Pape GR, Sauerbruch T, Paumgartner G. Ursodeoxycholic acid for treatment of primary sclerosing cholangitis: a placebo-controlled trial. *Hepatology* 1992; **16**: 707-714 [PMID: 1505913 DOI: 10.1002/hep.1840160315]
  - 54 **Lindor KD**. Ursodiol for primary sclerosing cholangitis. Mayo Primary Sclerosing Cholangitis-Ursodeoxycholic Acid Study Group. *N Engl J Med* 1997; **336**: 691-695 [PMID: 9041099 DOI: 10.1056/NEJM199703063361003]
  - 55 **Olsson R**, Boberg KM, de Muckadell OS, Lindgren S, Hultcrantz R, Folvik G, Bell H, Gangsøy-Kristiansen M, Matre J, Rydning A, Wikman O, Danielsson A, Sandberg-Gertzén H, Ung KA,



- Eriksson A, Lööf L, Prytz H, Marschall HU, Broomé U. High-dose ursodeoxycholic acid in primary sclerosing cholangitis: a 5-year multicenter, randomized, controlled study. *Gastroenterology* 2005; **129**: 1464-1472 [PMID: 16285948 DOI: 10.1053/j.gastro.2005.08.017]
- 56 **Lindor KD**, Kowdley KV, Luketic VA, Harrison ME, McCashland T, Befeler AS, Harnois D, Jorgensen R, Petz J, Keach J, Mooney J, Sargeant C, Braaten J, Bernard T, King D, Miceli E, Schmoll J, Hoskin T, Thapa P, Enders F. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. *Hepatology* 2009; **50**: 808-814 [PMID: 19585548 DOI: 10.1002/hep.23082]
- 57 **Triantos CK**, Koukias NM, Nikolopoulou VN, Burroughs AK. Meta-analysis: ursodeoxycholic acid for primary sclerosing cholangitis. *Aliment Pharmacol Ther* 2011; **34**: 901-910 [PMID: 21883323 DOI: 10.1111/j.1365-2036.2011.04822.x]
- 58 **Tsaitis C**, Semertzidou A, Sinakos E. Update on inflammatory bowel disease in patients with primary sclerosing cholangitis. *World J Hepatol* 2014; **6**: 178-187 [PMID: 24799986 DOI: 10.4254/wjh.v6.i4.178]
- 59 **Pardi DS**, Loftus EV, Kremers WK, Keach J, Lindor KD. Ursodeoxycholic acid as a chemopreventive agent in patients with ulcerative colitis and primary sclerosing cholangitis. *Gastroenterology* 2003; **124**: 889-893 [PMID: 12671884 DOI: 10.1053/gast.2003.50156]
- 60 **Hansen JD**, Kumar S, Lo WK, Poulsen DM, Halai UA, Tater KC. Ursodiol and colorectal cancer or dysplasia risk in primary sclerosing cholangitis and inflammatory bowel disease: a meta-analysis. *Dig Dis Sci* 2013; **58**: 3079-3087 [PMID: 23896754 DOI: 10.1007/s10620-013-2772-0]
- 61 **Eaton JE**, Silveira MG, Pardi DS, Sinakos E, Kowdley KV, Luketic VA, Harrison ME, McCashland T, Befeler AS, Harnois D, Jorgensen R, Petz J, Lindor KD. High-dose ursodeoxycholic acid is associated with the development of colorectal neoplasia in patients with ulcerative colitis and primary sclerosing cholangitis. *Am J Gastroenterol* 2011; **106**: 1638-1645 [PMID: 21556038 DOI: 10.1038/ajg.2011.156]
- 62 **Graziadei IW**, Wiesner RH, Marotta PJ, Porayko MK, Hay JE, Charlton MR, Poterucha JJ, Rosen CB, Gores GJ, LaRusso NF, Krom RA. Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis. *Hepatology* 1999; **30**: 1121-1127 [PMID: 10534330 DOI: 10.1002/hep.510300501]
- 63 **Gautam M**, Cheruvattath R, Balan V. Recurrence of autoimmune liver disease after liver transplantation: a systematic review. *Liver Transpl* 2006; **12**: 1813-1824 [PMID: 17031826 DOI: 10.1002/lt.20910]
- 64 **Alabraba E**, Nightingale P, Gunson B, Hubscher S, Olliff S, Mirza D, Neuberger J. A re-evaluation of the risk factors for the recurrence of primary sclerosing cholangitis in liver allografts. *Liver Transpl* 2009; **15**: 330-340 [PMID: 19243003 DOI: 10.1002/lt.21679]
- 65 **Gordon F**. Recurrent primary sclerosing cholangitis: Clinical diagnosis and long-term management issues. *Liver Transpl* 2006; **12**: S73-S75 [PMID: 17051565 DOI: 10.1002/lt.20948]
- 66 **Campsen J**, Zimmerman MA, Trotter JF, Wachs M, Bak T, Steinberg T, Kam I. Clinically recurrent primary sclerosing cholangitis following liver transplantation: a time course. *Liver Transpl* 2008; **14**: 181-185 [PMID: 18236392 DOI: 10.1002/lt.21313]
- 67 **Wiesner RH**, Grambsch PM, Dickson ER, Ludwig J, MacCarty RL, Hunter EB, Fleming TR, Fisher LD, Beaver SJ, LaRusso NF. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. *Hepatology* 1989; **10**: 430-436 [PMID: 2777204 DOI: 10.1002/hep.1840100406]
- 68 **Dickson ER**, Murtaugh PA, Wiesner RH, Grambsch PM, Fleming TR, Ludwig J, LaRusso NF, Malinchoc M, Chapman RW, Kaplan MM. Primary sclerosing cholangitis: refinement and validation of survival models. *Gastroenterology* 1992; **103**: 1893-1901 [PMID: 1451982]
- 69 **Kim WR**, Therneau TM, Wiesner RH, Poterucha JJ, Benson JT, Malinchoc M, LaRusso NF, Lindor KD, Dickson ER. A revised natural history model for primary sclerosing cholangitis. *Mayo Clin Proc* 2000; **75**: 688-694 [PMID: 10907383 DOI: 10.4065/75.7.688]
- 70 **Craig DA**, MacCarty RL, Wiesner RH, Grambsch PM, LaRusso NF. Primary sclerosing cholangitis: value of cholangiography in determining the prognosis. *AJR Am J Roentgenol* 1991; **157**: 959-964 [PMID: 1927817 DOI: 10.2214/ajr.157.5.1927817]
- 71 **Ponsioen CY**, Vrouenraets SM, Prawirodirdjo W, Rajaram R, Rauws EA, Mulder CJ, Reitsma JB, Heisterkamp SH, Tytgat GN. Natural history of primary sclerosing cholangitis and prognostic value of cholangiography in a Dutch population. *Gut* 2002; **51**: 562-566 [PMID: 12235081 DOI: 10.1136/gut.51.4.562]
- 72 **Majoie CB**, Reeders JW, Sanders JB, Huibregtse K, Jansen PL. Primary sclerosing cholangitis: a modified classification of cholangiographic findings. *AJR Am J Roentgenol* 1991; **157**: 495-497 [PMID: 1651643]
- 73 **Ponsioen CY**, Reitsma JB, Boberg KM, Aabakken L, Rauws EA, Schrupf E. Validation of a cholangiographic prognostic model in primary sclerosing cholangitis. *Endoscopy* 2010; **42**: 742-747 [PMID: 20623444 DOI: 10.1055/s-0030-1255527]
- 74 **Vesterhus M**, Hov JR, Holm A, Schrupf E, Nygård S, Godang K, Andersen IM, Naess S, Thorburn D, Saffioti F, Vatn M, Gilja OH, Lund-Johansen F, Syversveen T, Brabrand K, Parés A, Ponsioen CY, Pinzani M, Färkkilä M, Moum B, Ueland T, Røsjø H, Rosenberg W, Boberg KM, Karlsen TH. Enhanced liver fibrosis score predicts transplant-free survival in primary sclerosing cholangitis. *Hepatology* 2015; **62**: 188-197 [PMID: 25833813 DOI: 10.1002/hep.27825]
- 75 **De Vreede I**, Steers JL, Burch PA, Rosen CB, Gunderson LL, Haddock MG, Burgart L, Gores GJ. Prolonged disease-free survival after orthotopic liver transplantation plus adjuvant chemoradiation for cholangiocarcinoma. *Liver Transpl* 2000; **6**: 309-316 [PMID: 10827231 DOI: 10.1053/lt.2000.6143]
- 76 **Gores GJ**, Nagorney DM, Rosen CB. Cholangiocarcinoma: is transplantation an option? For whom? *J Hepatol* 2007; **47**: 455-459 [PMID: 17697722 DOI: 10.1016/j.jhep.2007.07.003]
- 77 **Rosen CB**, Darwish Murad S, Heimbach JK, Nyberg SL, Nagorney DM, Gores GJ. Neoadjuvant therapy and liver transplantation for hilar cholangiocarcinoma: is pretreatment pathological confirmation of diagnosis necessary? *J Am Coll Surg* 2012; **215**: 31-38; discussion 38-40 [PMID: 22621893 DOI: 10.1016/j.jamcollsurg.2012.03.014]
- 78 **Duignan S**, Maguire D, Ravichand CS, Geoghegan J, Hoti E, Fennelly D, Armstrong J, Rock K, Mohan H, Traynor O. Neoadjuvant chemoradiotherapy followed by liver transplantation for unresectable cholangiocarcinoma: a single-centre national experience. *HPB (Oxford)* 2014; **16**: 91-98 [PMID: 23600750 DOI: 10.1111/hpb.12082]
- 79 **Neumann UP**, Schmedding M. Role of surgery in cholangiocarcinoma: From resection to transplantation. *Best Pract Res Clin Gastroenterol* 2015; **29**: 295-308 [PMID: 25966429 DOI: 10.1016/j.bpg.2015.02.007]

P- Reviewer: Chetty R, Kaya M, Mudawi HMY, Zhu X  
S- Editor: Kong JX L- Editor: A E- Ed





## Hepatitis B virus and hepatitis C virus infection in healthcare workers

Nicola Coppola, Stefania De Pascalis, Lorenzo Onorato, Federica Calò, Caterina Sagnelli, Evangelista Sagnelli

Nicola Coppola, Stefania De Pascalis, Lorenzo Onorato, Federica Calò, Evangelista Sagnelli, Department of Mental Health and Public Medicine, Section of Infectious Diseases, Second University of Naples, 80131 Naples, Italy

Caterina Sagnelli, Department of Clinical and Experimental Medicine and Surgery "F. Magrassi e A. Lanzara", Second University of Naples, 80131 Naples, Italy

**Author contributions:** Coppola N, De Pascalis S, Onorato L, Calò F, Sagnelli C and Sagnelli E were based on: (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published.

**Conflict-of-interest statement:** All the authors of the manuscript declare that they have no conflict of interest in connection with this paper.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Nicola Coppola, Department of Mental Health and Public Medicine, Section of Infectious Diseases, Second University of Naples, Via: L. Armanni 5, 80131 Naples, Italy. [nicola.coppola@unina2.it](mailto:nicola.coppola@unina2.it)  
 Telephone: +39-081-5666719  
 Fax: +39-081-5666013

Received: July 26, 2015  
 Peer-review started: July 27, 2015  
 First decision: September 30, 2015  
 Revised: October 25, 2015  
 Accepted: January 8, 2016  
 Article in press: January 11, 2016  
 Published online: February 18, 2016

### Abstract

Approximately 3 million healthcare workers per year receive an injury with an occupational instrument, with around 2000000 exposures to hepatitis B virus (HBV) and 1000000 to hepatitis C virus (HCV). Although an effective HBV vaccine has been available since the early eighties, and despite the worldwide application of universal vaccination programs started in the early nineties, HBV still remains a prominent agent of morbidity and mortality. There is no vaccine to limit the diffusion of HCV infection, which progresses to chronicity in the majority of cases and is a major cause of morbidity and mortality worldwide due to a chronic liver disease. Healthcare workers are frequently exposed by a mucosal-cutaneous or percutaneous route to accidental contact with human blood and other potentially infectious biological materials while carrying out their occupational duties. Mucosal-cutaneous exposure occurs when the biological material of a potentially infected patient accidentally comes in contact with the mucous membranes of the eyes or mouth or with the skin of a healthcare worker. Percutaneous exposure occurs when an operator accidentally injures himself with a sharp contaminated object, like a needle, blade or other sharp medical instrument. About 75% of the total occupational exposure is percutaneous and 25% mucosal-cutaneous, the risk of infecting a healthcare worker being higher in percutaneous than in mucosal-cutaneous exposure. All healthcare workers should be considered for HBV vaccination and should meticulously apply the universal prophylactic measures to prevent exposure to HBV and HCV.

**Key words:** Hepatitis B virus infection; Hepatitis C virus infection; Needle-stick injury; Healthcare workers

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Preventing the transmission of hepatitis B virus

(HBV) or hepatitis C virus infection from source patients to healthcare workers is of vital importance in all healthcare settings worldwide, since these workers are exposed daily to these infections over a period of almost four decades. Needle pricks with contaminated needles, cuts from sharp instruments and blood splashes to the conjunctiva are the most frequent causes of exposure, injuries largely preventable by taking the standard universal precautions. HBV vaccination of anti-HBs-negative healthcare workers is recommended in all countries, but numerous healthcare workers remain exposed to infection because they have eluded HBV vaccination.

Coppola N, De Pascalis S, Onorato L, Calò F, Sagnelli C, Sagnelli E. Hepatitis B virus and hepatitis C virus infection in healthcare workers. *World J Hepatol* 2016; 8(5): 273-281 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i5/273.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i5.273>

## INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are responsible for the two most widespread forms of chronic hepatitis worldwide<sup>[1-3]</sup>. Healthcare workers are exposed to the risk of acquiring HBV and HCV infection through mucosal-cutaneous exposure (eyes or mouth mucosa or skin) to potentially infectious blood or blood products or through percutaneous exposure to contaminated sharp objects (needles, blades, *etc.*). Twenty-five percent of the total occupational exposure is mucosal-cutaneous and 75% percutaneous<sup>[4]</sup>. The risk of HBV or HCV infecting a healthcare worker is higher in percutaneous than in mucosal-cutaneous exposure. According to the data provided by the World Health Organization (WHO), there are approximately 36 million healthcare workers worldwide, of whom around 3 million per year receive an injury with a sharp instrument, thus resulting in 2000000 subjects contaminated with HBV and 1000000 with HCV<sup>[4]</sup>. Other studies estimated that the incidence of injuries to healthcare workers caused by sharp objects ranges from 1.4 to 9.5 per 100 healthcare workers per year, resulting in 0.42 HBV infections per 100 sharp-object injuries per year<sup>[5]</sup>.

This review article will focus on the risks of healthcare workers acquiring HBV and HCV chronic infections while carrying out their occupational duties.

## HBV INFECTION

HBV infection is a global health problem since 240 to 350 million<sup>[6-9]</sup> people worldwide are estimated to be chronically infected, of whom 312000 die annually of advanced cirrhosis and 341000 of liver cancer<sup>[7]</sup>.

Ten genotypes of HBV have been identified to date, from A to J, based on a genetic diversity of at least 8% in the viral genome<sup>[10,11]</sup>. HBV genotypes show a peculiar

geographical distribution<sup>[12]</sup>. Genotype A predominates in Northern Europe and North America, genotypes B and C in central Asia, genotype D in Mediterranean countries, genotype E in sub-Saharan Africa and in Madagascar, genotype F in South and Central America, genotypes G and H in Mexico and some countries in Central America<sup>[13,14]</sup> and genotypes I and J in Eastern Asia<sup>[15]</sup>.

The age at the time of infection modulates the progression to chronicity of HBV infection, which occurs in around 90% of babies born to hepatitis B e antigen (HBeAg)-positive mothers, a rate that progressively decreases with the increase in age up to 2%-5% in the adult population<sup>[16]</sup>.

The endemicity of HBV infection in a geographical area is classified according to the prevalence of subjects with hepatitis B surface antigen (HBsAg) positivity in the general population as, high (> 8%), intermediate (2.0%-7.9%) and low (< 2%)<sup>[11]</sup>. These categories reflect the predominant patterns of transmission and outcomes of HBV infection. In geographical areas with a high HBV endemicity, such as some countries in Eastern Asia or in sub-Saharan Africa, the majority of HBsAg-positive individuals acquired HBV infection at birth or in early childhood, when the risk of progression to chronicity is very high<sup>[7,15]</sup>. In geographical areas with an intermediate HBV endemicity, such as countries in Northern Africa, the Middle East and Southern Asia, and Eastern Europe, the majority of HBsAg-positive subjects acquired HBV infection in childhood or early adulthood, and family transmission plays an important role in the spread of HBV infection. Finally, in most countries in Western Europe, North, Central and South America and Australia the prevalence of HBsAg-positive individuals is below 2%<sup>[6,7,14]</sup>, the impact of vertical and horizontal transmission in childhood is low, and most HBsAg-positive individuals acquired HBV infection through sexual contact, intravenous drug use (IVDU) or other parenteral exposure to infected blood<sup>[17,18]</sup>.

## HCV INFECTION

HCV infection progresses to chronicity in 70% of cases, a condition that may lead to liver cirrhosis and hepatocellular carcinoma<sup>[19,20]</sup>. According to a WHO report<sup>[21]</sup>, 130-150 million people are chronically infected with HCV. HCV epidemiology shows considerable regional differences. In some geographical areas the endemicity is high, with more than 3.5% of the population having an HCV chronic infection, such as some countries in the Middle East and Central and Eastern Asia and Northern Africa. Several countries in Southern Asia, sub-Saharan Africa, the Andean territories, Central and South America, the Caribbean area, Oceania, Australasia and central and Eastern Europe have a moderate endemicity, with 1.5%-3.5% of subjects carrying HCV infection. The areas considered at low endemicity (< 1.5% of HCV chronic carriers) include countries in the Asian Pacific regions, tropical Latin America, North America and Western Europe<sup>[22]</sup>.

HCV strains have been classified to date into seven genotypes, namely from 1 to 7, on the basis of phylogenetic and sequence analyses of the whole viral genome<sup>[23]</sup>. The global geographical distribution of HCV genotypes is complex. HCV genotype 1 is the most prevalent worldwide, comprising 83.4 million cases (46.2% of all HCV chronic carriers), of whom approximately one-third live in Eastern Asia. HCV genotype 3 is the second most prevalent, and genotypes 2, 4 and 6 are responsible for the majority of the remaining cases worldwide. Eastern Asia has the largest number of carriers with genotype 2 and genotype 6, while Northern Africa and the Middle East have the largest number of carriers with genotype 4. HCV genotype 5 is responsible for around 1.4 million chronic infections (< 1% of all HCV cases worldwide), of which the vast majority in Southern and Eastern sub-Saharan Africa<sup>[22-26]</sup>. Worthy of note, HCV genotypes 1 and 3 generally predominate irrespective of the economic status, while the largest proportions of chronic carriers of HCV genotypes 4 and 5 live in low-income countries.

## RISK FACTORS FOR THE ACQUISITION OF HBV AND HCV INFECTION

HBV circulates in peripheral blood of infected subjects and any parenteral or mucosal exposure to potentially infected blood or blood contaminated material can be considered a risk for HBV transmission to non-immune/non-infected subjects<sup>[18,27]</sup>. In addition, the virus is present at infectious concentrations in semen and cervical secretions, and, consequently, HBV is frequently transmitted also by sexual and vertical routes<sup>[8,16]</sup>. The impact of the various routes of transmission varies significantly from one country to another<sup>[8,16]</sup>. In countries with a high endemicity level, HBV infection is prevalently acquired at birth from an HBeAg-positive mother, in which case it becomes chronic in around 90% of cases, or by horizontal transmission in early childhood through household contact, with a progression to chronicity from 10% to 40% of cases. Promiscuous unprotected sexual activity and IVDU are the major risk factors for acquiring HBV infection in areas with a low-prevalence, such as the United States<sup>[28]</sup>.

Worthy of note, the screening of blood donors for markers of HBV infection has dramatically reduced the risk of HBV transmission through the transfusions of blood and blood products. At present, this risk is estimated as 1-4 cases per million blood components transfused in low-prevalence areas<sup>[29]</sup> and around 1 case per 20000 blood transfusions in high-prevalence regions<sup>[30]</sup>.

The transfusion of infected blood and blood products was the most prominent route of transmission of HCV infection until 1989<sup>[1,31]</sup>. Routine mandatory screening of blood donors for circulating antibodies to HCV that started in the early 1990s has drastically reduced the risk of HCV transmission due to the transfusion of blood and

blood products, currently estimated between 1/500000 and 1/1000000 blood units<sup>[32]</sup>. Some concern for HCV transmission through blood transfusion remains only for some geographical areas with limited resources<sup>[33]</sup>. In developed countries, the sharing of injection equipment among IVDUs is one of the major risk factors for the acquisition of HCV infection, as demonstrated by the high anti-HCV seroprevalence found (70% or more in some studies) in this subset of subjects<sup>[34]</sup>. Conversely, in low-income countries, HCV transmission is frequently due to re-using equipment for injection and other inadequately sterilized therapeutic instruments<sup>[35]</sup>. HCV is rarely acquired through sexual intercourse<sup>[36]</sup>, but some outbreaks of acute HCV infections occurring in men having sex with men in the last decade have attracted the interest of the scientific community on this route of transmission, particularly in human immuno-deficiency virus (HIV)-infected individuals<sup>[37]</sup>. Perinatal transmission of HCV infection from HCV-monoinfected mothers occurs infrequently (around 3% of the cases), whereas it reaches 20% among HIV-coinfected mothers<sup>[38]</sup>. Other risk factors for the acquisition of HCV infection have been described, including acupuncture, tattooing, body piercing, some cosmetic procedures, sharing of shaving razors, nail scissors and other personal effects, and needle-stick injury for healthcare workers<sup>[28]</sup>.

## MODES OF EXPOSURE AND FACTORS ASSOCIATED WITH HBV AND HCV TRANSMISSION IN HEALTHCARE WORKERS

Healthcare workers are exposed to human blood and other potentially infectious biological material more frequently than the general population. Among the 60 or more agents responsible for blood-borne transmissible infectious diseases, HCV and HBV are those most frequently transmitted to healthcare workers. Contact with potentially infectious material occurs in most cases through mucosal-cutaneous or percutaneous exposure. In mucosal-cutaneous exposure, a patient's blood, blood derivative or other potentially infected biological material accidentally enters into contact with the mucous membranes of the eyes or mouth or with the skin, healthy or non-intact, of a healthcare worker<sup>[39]</sup>. Percutaneous exposure occurs when an operator receives an injury with a sharp contaminated object, such as a needle, blade or piece of glass. Around 75% of the total occupational exposure is percutaneous and the remaining 25% mucosal-cutaneous. The risk of HBV or HCV infecting a healthcare worker is higher in percutaneous than in mucosal-cutaneous exposure. The rate of transmission of HCV infection can be five times higher in percutaneous than in mucosal-cutaneous exposure, but the risk of acquiring these infections through conjunctival exposure is also high.

A prominent role in the transmission of an infection

is also played by the degree of infectiveness of the contaminated biological material to which the healthcare worker has been exposed<sup>[40-42]</sup>. The highest rates of transmission of HBV or HCV infection follow exposure to blood or its products, but transmission can also occur, generally at a lower rate, after exposure to ascites, cerebrospinal fluid or solutions from cell cultures. HBV and HCV transmissions have never been observed following exposure to feces, urine, sweat, vomitor tears.

Other main factors significantly affecting the likelihood of transmission of infecting agents are the extent and depth of the cutaneous or mucosal wound and the volume of blood transferred<sup>[40-43]</sup>. All punctures from contaminated needles and sharp objects may be responsible for the transmission of infections. However, the medical devices used to access the blood vessels directly cause most of the conversions to positivity of HCV and HBV serum markers worldwide. Such conversions are less frequent after the intramuscular or subcutaneous use of hollow needles or the use of lancets for capillary blood collection, due to the lesser amount of organic material present on their surface. Nurses generally perform these clinical practices and are the occupational group with the highest risk of needle-stick injuries<sup>[40-43]</sup>.

The risk of exposure is also related to the medical procedure performed. For example, of the 99 percutaneous injuries observed by Tokars *et al.*<sup>[44]</sup> during 1382 surgical operations in five different wards (general, orthopedic, gynecologic, traumatic and cardiac surgery), most (73%) were related with the suturing. Risk factors for percutaneous injuries included the performing an emergency procedure, a patient blood loss greater than 250 mL, and a duration of surgery procedure greater than 1 h<sup>[45]</sup>.

The HBV load in the source patient may influence the risk of transmission of HBV infection to non-immune healthcare workers. In these cases, the risk of HBV transmission is estimated at 19%-30%, if the source patient is HBeAg-positive or shows an HBV load > 10<sup>6</sup> IU/mL and at 5% if the source patient is HBeAg-negative or has a lower HBV load. The anti HBV vaccination of healthcare workers was introduced in the 1980s in most countries, but a substantial number of healthcare workers worldwide have eluded vaccination and, despite the excellent immunogenicity of the vaccine, about 20% of vaccinated subjects show anti-HBs titers lower than 10 IU/mL. It is common opinion, however, that HBV-vaccinated subjects with an anti-HBs titer below 10 IU/mL and those who have become anti-HBs-negative can be considered protected against HBV infection, since the immunological memory for HBsAg may persist even in these cases and ensure a rapid rise in protective antibodies in the case of an HBV assault<sup>[46-48]</sup>. Nevertheless, a highly infectious HBV inoculum might overpower a low antibody titers against HBsAg (anti-HBs) titer during the long professional life of a healthcare worker. In these cases, the administration of a booster dose of HBV vaccine could be considered<sup>[49]</sup>.

The transmission of HCV infection occurs in nearly 10% of the healthcare workers after parenteral exposure to the blood of an HCV-RNA-positive source patient, a rate that may vary according to the HCV load of the source patient<sup>[39,42]</sup>.

## PRE-EXPOSURE MANAGEMENT

The prevention of exposure remains the primary strategy for reducing occupational infections by blood-borne pathogens. The healthcare organizations should make available for their personnel a system that includes written protocols for prompt reporting, evaluation, counseling, treatment, and follow-up of occupational exposures<sup>[39]</sup>. Healthcare workers should be trained to adopt effective measures to prevent infections from occupational exposure to blood, *i.e.*, eliminating unnecessary injections, implementing universal precautions, eliminating needle recapping and disposing of the sharp into a sharps container immediately after use, use of safer devices such as needles that sheath or retract after use<sup>[50]</sup>.

Furthermore, healthcare workers should be aware that any person at risk of contact with blood, blood-contaminated body fluids, other body fluids, or sharps should be vaccinated against HBV<sup>[51]</sup>. The vaccination should happen early after the start of their career to avoid infection and development of carrier status. The healthcare workers should be vaccinated with a standard vaccination schedule and the serum anti-HBs should be assessed 1-2 mo after completion of a 3-dose vaccination series<sup>[52]</sup>. The HBV vaccination is, therefore, an essential part of prevention and control of HBV infection for healthcare workers and its use was one of the causes of drastic reduction of its prevalence in healthcare workers.

## POST-EXPOSURE MANAGEMENT

Although the primary prevention constitutes the first line of defense against the risk of occupational infections by blood-borne viruses, the adequate management of exposures with the post-exposure prophylaxis constitutes a key element in managing and limiting the transmission of these infections to staff exposed.

Regarding HBV infection, the risk of infection and the post-exposure management depends on the HBV status of the source and of the healthcare worker.

The risk of developing clinical hepatitis or serological evidence of HBV infection is high (22%-31% and 37%-62%, respectively) if the source is HBsAg and HBeAg positive, and low (1%-6% and 23%-37%, respectively) if it is HBsAg positive and HBeAg negative<sup>[28]</sup>. Moreover, it needs to evaluate the serological status of the healthcare worker: If HBsAg, anti-HBs (or titer less than 10 IU/mL) and HBV core antigen (anti-HBc) are negative, the healthcare worker is at risk to HBV infection. Precisely, in this case it should be taken in account the post exposure prophylaxis with a first dose of HBV vaccine and anti-HBV-specific immunoglobulin



**Table 1** Prevalence of hepatitis B virus infection in healthcare workers

Ref.	Year of enrollment	Country	No. of subjects	Type of study	HBsAg positive, <i>n</i> (%)	Anti-HBc positive, <i>n</i> (%)
Elzouki <i>et al</i> <sup>[54]</sup>	2008	Libya	601	Cross-sectional	11 (1.8)	51 (8.5)
Alqahtani <i>et al</i> <sup>[55]</sup>	NR	Saudi Arabia	300	Cross-sectional	1 (0.3)	25 (8)
Arguillas <i>et al</i> <sup>[56]</sup>	1990	Philippines	123	Case-control	8 (6.5)	81 (65.8)
Aziz <i>et al</i> <sup>[57]</sup>	NR	Pakistan	250	Cross-sectional	6 (2.4)	
Butsashvili <i>et al</i> <sup>[58]</sup>	2006	Georgia	1386	Cross-sectional	28 (2)	402 (29)
Fisker <i>et al</i> <sup>[59]</sup>	1998	Denmark	960	Cross-sectional	14 (1.5)	
Fritzsche <i>et al</i> <sup>[60]</sup>	2011	Cameroon	237	Cross-sectional	15 (6.3)	174 (73.4)
Kateera <i>et al</i> <sup>[61]</sup>	2013	Rwanda	378	Cross-sectional	11 (2.9)	
Kondili <i>et al</i> <sup>[62]</sup>	2004	Albania	480	Cross-sectional	39 (8.1)	338 (70)
Calleja-Panero <i>et al</i> <sup>[63]</sup>	2007-2010	Spain	4986	Cross sectional	36 (0.77)	
Ozsoy <i>et al</i> <sup>[64]</sup>	1998	Turkey	702	Case-control	21 (3)	
Petrosillo <i>et al</i> <sup>[65]</sup>	1985	Italy	5813	Cross-sectional	108 (1.8)	
Rehman <i>et al</i> <sup>[66]</sup>	1996	Pakistan	95	Case-control	5 (5)	27 (28)
Rybacki <i>et al</i> <sup>[67]</sup>	2009	Poland	520	Cross-sectional	6 (1.2)	99 (19)
Sarwar <i>et al</i> <sup>[68]</sup>	2006	Pakistan	125	Cross-sectional	3 (2.4)	
Slusarczyk <i>et al</i> <sup>[69]</sup>	2008	Poland	961	Cross-sectional	4 (0.4) <sup>1</sup>	151 (15.7)
Thomas <i>et al</i> <sup>[70]</sup>	1991	United States	943	Case-control	1 (0.1)	59 (6.2)
Ciorlia <i>et al</i> <sup>[71]</sup>	1994-1999	Brazil	1433	Cross-sectional	11 (0.8)	

<sup>1</sup>Hepatitis B virus-DNA positivity in anti-HBc-positive subjects. NR: Not reported; HBsAg: Hepatitis B surface antigen; Anti-HBc: Hepatitis B virus core antigen.

(HBIG), that should be initiated as soon as possible, preferably within 24 h of exposure and not more than 7 d. If the healthcare worker is vaccinated with protective antibody response ( $\geq 10$  IU/mL) or is anti-HBc positive, no treatment is needed.

Currently, there is no prophylaxis for HCV infection: In fact, immunoglobulin and antivirals are not recommended and only the observation is indicated. However, recently the available of the second and third wave direct antiviral agents enhanced the efficacy and tolerability of anti-HCV treatment<sup>[53]</sup>; consequently, the traditional management of HCV infection after exposure in healthcare workers probably should be revised.

## STUDIES ON HBV INFECTION IN HEALTHCARE WORKERS

The rates of HBsAg and anti-HBc positivity in healthcare workers reported in several studies published in the last three decades<sup>[54-70]</sup> range from 0.1% to 8.1% and from 6.2% to 73.4%, respectively, depending on the age of the subjects investigated, the spread of HBV infection in their country of origin and on the prevention strategies used by the healthcare workers (Table 1).

Of 5813 healthcare workers tested in Italy in 1985, 21.5% were found to be anti-HBc-positive and 1.8% HBsAg-positive<sup>[65]</sup>. A logistic regression analysis showed that the exposure to HBV infection was associated with male sex, an older age, history of blood transfusion, dental treatment, needle-stick injury and working in a healthcare setting (surgeons and nurses vs others). A similar rate of HBsAg positivity (1.5%) was observed in a study on 960 healthcare workers tested in Denmark in 1998<sup>[59]</sup>. A much higher HBsAg prevalence (8.1%) was detected in 480 healthcare workers investigated by Kondili *et al*<sup>[62]</sup> in 2004 in Albania, in accordance

with the widespread of HBV infection in this country. In this study, the highest rates of HBsAg positivity were found in the youngest age group (11.4% in the aged 20-30) and in the auxiliaries (12.6%), but a high HBsAg prevalence (7.2%-7.5%) was also found in the healthcare workers aged over 30. The anti-HBc seroprevalence was also extremely high (70%) in this study and was associated with an age over 40 (OR = 2.9; 95%CI: 1.9-4.6).

In a study performed in Libya in 2008 on 601 healthcare workers, the rate of HBsAg positivity was 1.8%, higher in nurses (2.3%) and lower in physicians (0.7%) and laboratory staff (0.8%)<sup>[54]</sup>. Of 237 healthcare workers tested in Cameroon, 6.3% were HBsAg-positive and 73.4% anti-HBc-positive, in accordance with the wide spread of HBV infection in this geographical area<sup>[60]</sup>. Seroprevalence studies conducted in Asia showed varying results. Aziz *et al*<sup>[57]</sup>, Rehman *et al*<sup>[66]</sup> and Sarwar *et al*<sup>[68]</sup>, in three different studies conducted in Pakistan, reported that 2.4%, 5% and 2.4% of the healthcare workers, respectively, were HBsAg-positive.

Low rates of HBsAg positivity were found in two seroprevalence studies conducted on healthcare workers in the United States (0.1%) and Brazil (0.8%)<sup>[63,70]</sup>.

Some case-control studies allowing a comparison of the HBsAg prevalence in the healthcare workers with that of the general population provided contrasting results. Rehman *et al*<sup>[66]</sup>, in a small case-control study performed in Pakistan, enrolled 95 healthcare workers and 91 volunteer blood donors as controls and observed higher rates of HBsAg (14% vs 5%) and anti-HBc (36% vs 28%) positivity in the control group. Instead, in a case-control study conducted in Turkey<sup>[64]</sup>, the rate of HBsAg positivity was similar in 702 healthcare workers and 5670 blood donors (3% vs 2.1%), and in the Philippines, Arguillas *et al*<sup>[56]</sup> found 6.5% of 123 healthcare workers and 2.2% of 382 blood donors to be

**Table 2** Prevalence of hepatitis C virus infection in healthcare workers

Ref.	Year of enrollment	Country	No. of patients	Type of study	Anti-HCV positive, <i>n</i> (%)
Elzouki <i>et al</i> <sup>[54]</sup>	2008	Libya	601	Cross-sectional	12 (2)
Alqahtani <i>et al</i> <sup>[55]</sup>	NR	Saudi Arabia	300	Cross-sectional	0
Arguillas <i>et al</i> <sup>[56]</sup>	1990	Philippines	123	Case-control	12 (9.7)
Aziz <i>et al</i> <sup>[57]</sup>	NR	Pakistan	250	Cross-sectional	14 (5.6)
Butsashvili <i>et al</i> <sup>[58]</sup>	2006	Georgia	1386	Cross-sectional	69 (5)
Fisker <i>et al</i> <sup>[59]</sup>	1998	Denmark	960	Cross-sectional	2 (0.14)
Fritzschke <i>et al</i> <sup>[60]</sup>	2011	Cameroon	237	Cross-sectional	4 (1.7)
Kateera <i>et al</i> <sup>[61]</sup>	2013	Rwanda	378	Cross-sectional	5 (1.3)
Kondili <i>et al</i> <sup>[62]</sup>	2004	Albania	480	Cross-sectional	3 (0.6)
Calleja-Panero <i>et al</i> <sup>[63]</sup>	2007-2010	Spain	4981	Cross sectional	31 (0.62)
Ozsoy <i>et al</i> <sup>[64]</sup>	1998	Turkey	702	Case-control	2 (0.3)
Petrosillo <i>et al</i> <sup>[65]</sup>	1985	Italy	5813	Cross-sectional	117 (2)
Rehman <i>et al</i> <sup>[66]</sup>	1996	Pakistan	95	Case-control	4 (4)
Rybacki <i>et al</i> <sup>[67]</sup>	2009	Poland	520	Cross-sectional	4 (0.8)
Sarwar <i>et al</i> <sup>[68]</sup>	2006	Pakistan	125	Cross-sectional	4 (3.2)
Slusarczyk <i>et al</i> <sup>[69]</sup>	2008	Poland	961	Cross-sectional	16 (1.7)
Thomas <i>et al</i> <sup>[70]</sup>	1991	United States	943	Case-control	7 (0.7)
Campello <i>et al</i> <sup>[72]</sup>	1990	Italy	407	Case-control	5 (1.2)
Zaaijer <i>et al</i> <sup>[73]</sup>	2000-2009	Denmark	729	Cross-sectional	1 (0.14)
Okasha <i>et al</i> <sup>[74]</sup>	2008	Egypt	1770	Cross-sectional	141 (8.0)

NR: Not reported; HCV: Hepatitis C virus.

HBsAg-positive. Finally, in the United States, Thomas *et al*<sup>[70]</sup> observed the same HBsAg-positivity rate (0.1%) in 943 healthcare workers and 104239 blood donors, whereas they found a higher rate of anti-HBc positivity in the healthcare workers, 6.2% vs 1.8%, indicating a greater exposure to HBV in these subjects, but exposure was not followed by a chronic infection, most probably because it occurred in adulthood.

## STUDIES ON HCV INFECTION IN HEALTHCARE WORKERS

The prevalence of anti-HCV positivity in healthcare workers<sup>[54-74]</sup> ranges from 0% to 9.7% in different studies worldwide: 9.7% in the Philippines, 8% in Egypt, 3.2%-5.6% in three studies in Pakistan, 5% in Georgia, 0.14% in Denmark, 0.8% in Poland, 0.7% in the United States, 0.6% in Albania and 0.3% in Turkey (Table 2).

The majority of 1386 healthcare workers investigated in Georgia in 2006<sup>[58]</sup> stated an episode of occupational exposure to HCV infection, including accidental needle-stick injuries in 45% of cases, cuts with contaminated instruments in 38% and blood splashes in 46%. For the healthcare workers who received a cut, this unfavorable event occurred during the reassembling of tools or when receiving tools from a colleague in the majority of the cases, and the highest proportion of needle-stick injuries occurred when recapping used needles, more frequently in nurses (39%) than in physicians (22%).

In a cross-sectional study performed on 1770 healthcare workers in Egypt in 2008<sup>[74]</sup>, the anti-HCV seroprevalence was 8.0%, the estimated incidence of HCV infection 7.3 per 1000 persons-year and the risk factors independently associated with HCV seropositivity were an older age, performing a manual job, having a history

of blood transfusion or a history of parenteral treatment for schistosomiasis.

Only five case-control studies compared the prevalence of HCV infection in healthcare workers and in the general population of the same country<sup>[56,64,66,70,72]</sup>. Four of these 5 studies showed similar prevalences of anti-HCV positivity in the two groups of subjects: 0.3% vs 0.4%, respectively, in a study from Turkey on 702 healthcare workers<sup>[64]</sup> and 5670 blood donors, 1.6% in 123 healthcare workers and 2.2% in 382 blood donors investigated in the Philippines<sup>[56]</sup>, 1.2% in 407 healthcare workers and 0.8% in pregnant women studied in Italy<sup>[72]</sup> and 0.7% in 943 healthcare workers and 0.4% in 104239 blood donors in the United States<sup>[70]</sup>. Unexpectedly, but similarly to what was observed for HBsAg by Rehman *et al*<sup>[66]</sup> in a study in Pakistan (see above), they observed that the 95 healthcare workers less frequently than the 91 blood donors were anti-HCV positive (4% vs 14%). The results of this study are of difficult interpretation.

## CONCLUSION

Mucosal-cutaneous and percutaneous exposure to human blood or bloodstained medical instruments occurs more frequently in healthcare workers than the general population. A major role in the transmission of HBV or HCV infection is played by the virus concentration on the infecting materials, high in blood and blood products, much lower in ascites and in cerebrospinal fluid and at non-infectious concentrations in feces, urine, sweat, vomit and tears.

The characteristics of the wound received by a healthcare worker and the volume of blood transferred are other main factors influencing a possible transmission of HBV or HCV infection. In fact, the devices

used to access the blood vessels directly, more frequently than cable needles used for intramuscular or subcutaneous treatments, are responsible for HBV and HCV transmission because of the higher amount of organic material carried on their surface.

The transmission of HBV infection from a source patient to a healthcare worker is also influenced by the natural or vaccine-induced immunological protection against HBV in the healthcare worker. Although HBV vaccination of anti-HBs-negative healthcare workers is highly recommended in all countries, some healthcare workers have eluded vaccination and some were low- or non-responders to the vaccine, indicating that a highly infectious HBV inoculum might overpower low immunological protection.

Since no anti-HCV vaccine is at present available to counteract HCV transmission, healthcare workers should protect themselves by meticulously applying all the universal prophylactic measures whenever potentially exposed.

## REFERENCES

- 1 **Sagnelli E**, Stroffolini T, Mele A, Almasio P, Coppola N, Ferrigno L, Scolastico C, Onofrio M, Imperato M, Filippini P. The importance of HCV on the burden of chronic liver disease in Italy: a multicenter prevalence study of 9,997 cases. *J Med Virol* 2005; **75**: 522-527 [PMID: 15714480 DOI: 10.1002/jmv.20313]
- 2 **Sagnelli E**, Stroffolini T, Mele A, Imperato M, Sagnelli C, Coppola N, Almasio PL. Impact of comorbidities on the severity of chronic hepatitis B at presentation. *World J Gastroenterol* 2012; **18**: 1616-1621 [PMID: 22529690 DOI: 10.3748/wjg.v18.i14.1616]
- 3 **Sagnelli E**, Stroffolini T, Mele A, Imperato M, Almasio PL. Chronic hepatitis B in Italy: new features of an old disease—approaching the universal prevalence of hepatitis B e antigen-negative cases and the eradication of hepatitis D infection. *Clin Infect Dis* 2008; **46**: 110-113 [PMID: 18171224 DOI: 10.1086/524074]
- 4 **Elseviers MM**, Arias-Guillén M, Gorke A, Arens HJ. Sharps injuries amongst healthcare workers: review of incidence, transmissions and costs. *J Ren Care* 2014; **40**: 150-156 [PMID: 24650088 DOI: 10.1111/jorc.12050]
- 5 Immunization of health-care workers: recommendations of the Advisory Committee on Immunization Practices (ACIP) and the Hospital Infection Control Practices Advisory Committee (HICPAC). *MMWR Recomm Rep* 1997; **46**: 1-42 [PMID: 9427216]
- 6 **Ott JJ**, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine* 2012; **30**: 2212-2219 [PMID: 22273662 DOI: 10.1016/j.vaccine.2011.12.116]
- 7 **Lavanchy D**. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004; **11**: 97-107 [PMID: 14996343]
- 8 **Sagnelli E**, Sagnelli C, Pisaturo M, Macera M, Coppola N. Epidemiology of acute and chronic hepatitis B and delta over the last 5 decades in Italy. *World J Gastroenterol* 2014; **20**: 7635-7643 [PMID: 24976701 DOI: 10.3748/wjg.v20.i24.7635]
- 9 **Lozano R**, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**: 2095-2128 [PMID: 23245604 DOI: 10.1016/S0140-6736(12)61728-0]
- 10 **Lin CL**, Kao JH. Hepatitis B virus genotypes and variants. *Cold Spring Harb Perspect Med* 2015; **5**: a021436 [PMID: 25934462 DOI: 10.1101/cshperspect.a021436]
- 11 **Sagnelli C**, Ciccozzi M, Pisaturo M, Lo Presti A, Cella E, Coppola N, Sagnelli E. The impact of viral molecular diversity on the clinical presentation and outcome of acute hepatitis B in Italy. *New Microbiol* 2015; **38**: 137-147 [PMID: 25915056]
- 12 **Previsani N**, Lavanchy D. Hepatitis B. World Health Organisation-Department of Communicable Diseases Surveillance and Response. Geneva, 2002. Available from: URL: [http://www.who.int/csr/disease/hepatitis/HepatitisB\\_whocdscsryo2002\\_2.pdf](http://www.who.int/csr/disease/hepatitis/HepatitisB_whocdscsryo2002_2.pdf)
- 13 **Roman S**, Panduro A. HBV endemicity in Mexico is associated with HBV genotypes H and G. *World J Gastroenterol* 2013; **19**: 5446-5453 [PMID: 24023487 DOI: 10.3748/wjg.v19.i33.5446]
- 14 **Panduro A**, Maldonado-Gonzalez M, Fierro NA, Roman S. Distribution of HBV genotypes F and H in Mexico and Central America. *Antivir Ther* 2013; **18**: 475-484 [PMID: 23792777 DOI: 10.3851/IMP2605]
- 15 **Kao JH**. Role of viral factors in the natural course and therapy of chronic hepatitis B. *Hepatol Int* 2007; **1**: 415-430 [PMID: 19669337 DOI: 10.1007/s12072-007-9033-2]
- 16 **Wasley A**, Grytdal S, Gallagher K. Surveillance for acute viral hepatitis—United States, 2006. *MMWR Surveill Summ* 2008; **57**: 1-24 [PMID: 18354374]
- 17 **Coppola N**, Sagnelli C, Pisaturo M, Minichini C, Messina V, Alessio L, Starace M, Signoriello G, Gentile I, Filippini P, Sagnelli E. Clinical and virological characteristics associated with severe acute hepatitis B. *Clin Microbiol Infect* 2014; **20**: 0991-0997 [PMID: 24930916 DOI: 10.1111/1469-0691.12720]
- 18 **Liaw YF**, Chu CM. Hepatitis B virus infection. *Lancet* 2009; **373**: 582-592 [PMID: 19217993 DOI: 10.1016/S0140-6736(09)60207-5]
- 19 **Sagnelli E**, Pisaturo M, Stanzione M, Messina V, Alessio L, Sagnelli C, Starace M, Pasquale G, Coppola N. Clinical presentation, outcome, and response to therapy among patients with acute exacerbation of chronic hepatitis C. *Clin Gastroenterol Hepatol* 2013; **11**: 1174-1180.e11 [PMID: 23591280]
- 20 **Petruzzello A**, Coppola N, Diodato AM, Iervolino V, Azzaro R, Di Costanzo G, Di Macchia CA, Di Meo T, Loquercio G, Pasquale G, Cacciapuotì C. Age and gender distribution of hepatitis C virus



- genotypes in the metropolitan area of Naples. *Intervirology* 2013; **56**: 206-212 [PMID: 23594735 DOI: 10.1159/000348506]
- 21 **WHO Guidelines Approved by the Guidelines Review Committee.** Guidelines for the screening, care and treatment of persons with hepatitis C infection. Geneva: World Health Organization, 2014. Available from: URL: <http://apps.who.int/medicinedocs/documents/s22180en/s22180en.pdf>
  - 22 **Shepard CW, Finelli L, Alter MJ.** Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; **5**: 558-567 [PMID: 16122679 DOI: 10.1016/S1473-3099(05)70216-4]
  - 23 **Simmonds P, Alberti A, Alter HJ, Bonino F, Bradley DW, Brechot C, Brouwer JT, Chan SW, Chayama K, Chen DS.** A proposed system for the nomenclature of hepatitis C viral genotypes. *Hepatology* 1994; **19**: 1321-1324 [PMID: 8175159]
  - 24 **Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E.** Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; **61**: 77-87 [PMID: 25069599 DOI: 10.1002/hep.27259]
  - 25 **Coppola N, Alessio L, Gualdieri L, Pisaturo M, Sagnelli C, Caprio N, Maffei R, Starace M, Angelillo IF, Pasquale G, Sagnelli E.** Hepatitis B virus, hepatitis C virus and human immunodeficiency virus infection in undocumented migrants and refugees in southern Italy, January 2012 to June 2013. *Euro Surveill* 2015; **20**: 30009 [PMID: 26530499 DOI: 10.2807/1560-7917.es.2015.20.35.30009]
  - 26 **Murphy DG, Willems B, Deschênes M, Hilzenrat N, Mousseau R, Sabbah S.** Use of sequence analysis of the NS5B region for routine genotyping of hepatitis C virus with reference to C/E1 and 5' untranslated region sequences. *J Clin Microbiol* 2007; **45**: 1102-1112 [PMID: 17287328]
  - 27 **Roman S, Jose-Abrego A, Fierro NA, Escobedo-Melendez G, Ojeda-Granados C, Martinez-Lopez E, Panduro A.** Hepatitis B virus infection in Latin America: a genomic medicine approach. *World J Gastroenterol* 2014; **20**: 7181-7196 [PMID: 24966588 DOI: 10.3748/wjg.v20.i23.7181]
  - 28 **Mauss S, Berg T, Rockstroh J, Sarrazin C, Wedemeyer H.** *Hepatology* 6th edition. FlyingPublisher, 2015
  - 29 **Polizzotto MN, Wood EM, Ingham H, Keller AJ.** Reducing the risk of transfusion-transmissible viral infection through blood donor selection: the Australian experience 2000 through 2006. *Transfusion* 2008; **48**: 55-63 [PMID: 17894794]
  - 30 **Vermeylen M, Dickens C, Lelie N, Walker E, Coleman C, Keyter M, Reddy R, Crookes R, Kramvis A.** Hepatitis B virus transmission by blood transfusion during 4 years of individual-donation nucleic acid testing in South Africa: estimated and observed window period risk. *Transfusion* 2012; **52**: 880-892 [PMID: 21981386 DOI: 10.1111/j.1537-2995.2011.03355.x]
  - 31 **Lee MH, Yang HI, Yuan Y, L'Italiani G, Chen CJ.** Epidemiology and natural history of hepatitis C virus infection. *World J Gastroenterol* 2014; **20**: 9270-9280 [PMID: 25071320 DOI: 10.3748/wjg.v20.i28.9270]
  - 32 **Pomper GJ, Wu Y, Snyder EL.** Risks of transfusion-transmitted infections: 2003. *Curr Opin Hematol* 2003; **10**: 412-418 [PMID: 14564170]
  - 33 **Tagny CT, Mbanya D, Tapko JB, Lefrère JJ.** Blood safety in Sub-Saharan Africa: a multi-factorial problem. *Transfusion* 2008; **48**: 1256-1261 [PMID: 18713111 DOI: 10.1111/j.1537-2995.2008.01697.x]
  - 34 **Sutton AJ, Hope VD, Mathei C, Mravcik V, Sebakova H, Vallejo F, Suligoi B, Brugal MT, Ncube F, Wiessing L, Kretzschmar M.** A comparison between the force of infection estimates for blood-borne viruses in injecting drug user populations across the European Union: a modelling study. *J Viral Hepat* 2008; **15**: 809-816 [PMID: 18761605 DOI: 10.1111/j.1365-2893.2008.01041.x]
  - 35 **Sun CA, Chen HC, Lu CF, You SL, Mau YC, Ho MS, Lin SH, Chen CJ.** Transmission of hepatitis C virus in Taiwan: prevalence and risk factors based on a nationwide survey. *J Med Virol* 1999; **59**: 290-296 [PMID: 10502258]
  - 36 **Vandelli C, Renzo F, Romanò L, Tisminetzky S, De Palma M, Stroffolini T, Ventura E, Zanetti A.** Lack of evidence of sexual transmission of hepatitis C among monogamous couples: results of a 10-year prospective follow-up study. *Am J Gastroenterol* 2004; **99**: 855-859 [PMID: 15128350]
  - 37 **Urbanus AT, van de Laar TJ, Stolte IG, Schinkel J, Heijman T, Coutinho RA, Prins M.** Hepatitis C virus infections among HIV-infected men who have sex with men: an expanding epidemic. *AIDS* 2009; **23**: F1-F7 [PMID: 19542864 DOI: 10.1097/QAD.0b013e32832e5631]
  - 38 **Yeung CY, Lee HC, Chan WT, Jiang CB, Chang SW, Chuang CK.** Vertical transmission of hepatitis C virus: Current knowledge and perspectives. *World J Hepatol* 2014; **6**: 643-651 [PMID: 25276280 DOI: 10.4254/wjh.v6.i9.643]
  - 39 **US Public Health Service.** Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis. *MMWR Recomm Rep* 2001; **50**: 1-52 [PMID: 11442229]
  - 40 **Deuffic-Burban S, Delarocque-Astagneau E, Abiteboul D, Bouvet E, Yazdanpanah Y.** Blood-borne viruses in health care workers: prevention and management. *J Clin Virol* 2011; **52**: 4-10 [PMID: 21680238 DOI: 10.1016/j.jcv.2011.05.016]
  - 41 **Ippolito G, Puro V, Petrosillo N, De Carli G.** Surveillance of occupational exposure to bloodborne pathogens in health care workers: the Italian national programme. *Euro Surveill* 1999; **4**: 33-36 [PMID: 12631910]
  - 42 **Jagger J, Puro V, De Carli G.** Occupational transmission of hepatitis C virus. *JAMA* 2002; **288**: 1469; author reply 1469-1471 [PMID: 12243628]
  - 43 **Gruppiodi Studio PHASE.** Rischio biologico e punture accidentali negli operatori sanitari: un approccio organizzativo e gestionale alla prevenzione in ambito sanitario-ospedaliero. Milano, Lauri ed., 2001
  - 44 **Tokars JI, Bell DM, Culver DH, Marcus R, Mendelson MH, Sloan EP, Farber BF, Fligner D, Chamberland ME, McKibben PS.** Percutaneous injuries during surgical procedures. *JAMA* 1992; **267**: 2899-2904 [PMID: 1583758]
  - 45 **Panlilio AL, Foy DR, Edwards JR, Bell DM, Welch BA, Parrish CM, Culver DH, Lowry PW, Jarvis WR, Perlino CA.** Blood contacts during surgical procedures. *JAMA* 1991; **265**: 1533-1537 [PMID: 1999903]
  - 46 **Zanetti AR, Mariano A, Romanò L, D'Amelio R, Chironna M, Coppola RC, Cuccia M, Mangione R, Marrone F, Negrone FS, Parlato A, Zamparo E, Zotti C, Stroffolini T, Mele A.** Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. *Lancet* 2005; **366**: 1379-1384 [PMID: 16226616]
  - 47 **Stroffolini T, Almasio PL, Sagnelli E, Mele A, Gaeta GB.** Evolving clinical landscape of chronic hepatitis B: A multicenter Italian study. *J Med Virol* 2009; **81**: 1999-2006 [PMID: 19856477 DOI: 10.1002/jmv.21643]
  - 48 **Gasparini R, Coppola R, Marensi R, Crovari P.** L'epatite virale nel comune di Genova. *Giorn Ig Med Prev* 1980; **21**: 161-190
  - 49 **Coppola N, Corvino AR, De Pascalis S, Signoriello G, Di Fiore E, Nienhaus A, Sagnelli E, Lamberti M.** The long-term immunogenicity of recombinant hepatitis B virus (HBV) vaccine: contribution of universal HBV vaccination in Italy. *BMC Infect Dis* 2015; **15**: 149 [PMID: 25884719 DOI: 10.1186/s12879-015-0874-3]
  - 50 **Wilburn SQ, Eijkemans G.** Preventing needlestick injuries among healthcare workers: a WHO-ICN collaboration. *Int J Occup Environ Health* 2004; **10**: 451-456 [PMID: 15702761]
  - 51 **Schillie S, Murphy TV, Sawyer M, Ly K, Hughes E, Jiles R, de Perio MA, Reilly M, Byrd K, Ward JW.** CDC guidance for evaluating health-care personnel for hepatitis B virus protection and for administering postexposure management. *MMWR Recomm Rep* 2013; **62**: 1-19 [PMID: 24352112]
  - 52 **Puro V, De Carli G, Cicalini S, Soldani F, Balslev U, Begovac J, Boaventura L, Campins Marti M, Hernández Navarrete MJ, Kammerlander R, Larsen C, Lot F, Lunding S, Marcus U, Payne L, Pereira AA, Thomas T, Ippolito G.** European recommendations for the management of healthcare workers occupationally exposed



- to hepatitis B virus and hepatitis C virus. *Euro Surveill* 2005; **10**: 260-264 [PMID: 16282641]
- 53 **Coppola N**, Pisaturo M, Zampino R, Macera M, Sagnelli C, Sagnelli E. Hepatitis C virus markers in infection by hepatitis C virus: In the era of directly acting antivirals. *World J Gastroenterol* 2015; **21**: 10749-10759 [PMID: 26478667 DOI: 10.3748/wjg.v21.i38.10749]
  - 54 **Elzouki AN**, Elgamay SM, Zorgani A, Elahmer O. Hepatitis B and C status among health care workers in the five main hospitals in eastern Libya. *J Infect Public Health* 2014; **7**: 534-541 [PMID: 25151657 DOI: 10.1016/j.jiph.2014.07.006]
  - 55 **Alqahtani JM**, Abu-Eshy SA, Mahfouz AA, El-Mekki AA, Asaad AM. Seroprevalence of hepatitis B and C virus infections among health students and health care workers in the Najran region, southwestern Saudi Arabia: the need for national guidelines for health students. *BMC Public Health* 2014; **14**: 577 [PMID: 24912684 DOI: 10.1186/1471-2458-14-577]
  - 56 **Arguillas MO**, Domingo EO, Tsuda F, Mayumi M, Suzuki H. Seroepidemiology of hepatitis C virus infection in the Philippines: a preliminary study and comparison with hepatitis B virus infection among blood donors, medical personnel, and patient groups in Davao, Philippines. *Gastroenterol Jpn* 1991; **26** Suppl 3: 170-175 [PMID: 1909261]
  - 57 **Aziz S**, Memon A, Tily HI, Rasheed K, Jehangir K, Quraishy MS. Prevalence of HIV, hepatitis B and C amongst health workers of Civil Hospital Karachi. *J Pak Med Assoc* 2002; **52**: 92-94 [PMID: 12071075]
  - 58 **Butsashvili M**, Kamkamidze G, Kajaia M, Morse DL, Triner W, Dehovitz J, McNutt LA. Occupational exposure to body fluids among health care workers in Georgia. *Occup Med (Lond)* 2012; **62**: 620-626 [PMID: 22869786 DOI: 10.1093/occmed/kqs121]
  - 59 **Fisker N**, Mygind LH, Krarup HB, Licht D, Georgsen J, Christensen PB. Blood borne viral infections among Danish health care workers--frequent blood exposure but low prevalence of infection. *Eur J Epidemiol* 2004; **19**: 61-67 [PMID: 15012024]
  - 60 **Fritzsche C**, Becker F, Hemmer CJ, Riebold D, Klammt S, Hufert F, Akam W, Kinge TN, Reisinger EC. Hepatitis B and C: neglected diseases among health care workers in Cameroon. *Trans R Soc Trop Med Hyg* 2013; **107**: 158-164 [PMID: 23303802 DOI: 10.1093/trstmh/trs087]
  - 61 **Kateera F**, Walker TD, Mutesa L, Mutabazi V, Musabeyesu E, Mukabatsinda C, Bihizimana P, Kyamanywa P, Karenzi B, Orikiiriza JT. Hepatitis B and C seroprevalence among health care workers in a tertiary hospital in Rwanda. *Trans R Soc Trop Med Hyg* 2015; **109**: 203-208 [PMID: 25636951 DOI: 10.1093/trstmh/trv004]
  - 62 **Kondili LA**, Ulqinaku D, Hajdini M, Basho M, Chionne P, Madonna E, Taliani G, Candido A, Dentico P, Bino S, Rapicetta M. Hepatitis B virus infection in health care workers in Albania: a country still highly endemic for HBV infection. *Infection* 2007; **35**: 94-97 [PMID: 17401713]
  - 63 **Calleja-Panero JL**, Llop-Herrera E, Ruiz-Moraga M, de-la-Revilla-Negro J, Calvo-Bonacho E, Pons-Renedo F, Martínez-Porras JL, Vallejo-Gutiérrez D, Arregui C, Abreu-García L. Prevalence of viral hepatitis (B and C) serological markers in healthy working population. *Rev Esp Enferm Dig* 2013; **105**: 249-254 [PMID: 23971655]
  - 64 **Ozsoy MF**, Oncul O, Cavuslu S, Erdemoglu A, Emekdas G, Pahsa A. Seroprevalences of hepatitis B and C among health care workers in Turkey. *J Viral Hepat* 2003; **10**: 150-156 [PMID: 12614472]
  - 65 **Petrosillo N**, Puro V, Ippolito G, Di Nardo V, Albertoni F, Chiaretti B, Rava' L, Sommella L, Ricci C, Zullo G. Hepatitis B virus, hepatitis C virus and human immunodeficiency virus infection in health care workers: a multiple regression analysis of risk factors. *J Hosp Infect* 1995; **30**: 273-281 [PMID: 7499808]
  - 66 **Rehman K**, Khan AA, Haider Z, Shahzad A, Iqbal J, Khan RU, Ahmad S, Siddiqui A, Syed SH. Prevalence of seromarkers of HBV and HCV in health care personnel and apparently healthy blood donors. *J Pak Med Assoc* 1996; **46**: 152-154 [PMID: 8993043]
  - 67 **Rybacki M**, Piekarska A, Wiszniewska M, Walusiak-Skorupa J. Hepatitis B and C infection: is it a problem in Polish healthcare workers? *Int J Occup Med Environ Health* 2013; **26**: 430-439 [PMID: 23817869 DOI: 10.2478/s13382-013-0088-0]
  - 68 **Sarwar J**, Gul N, Idris M, Anis-ur-Rehman J, Adeel MY. Seroprevalence of hepatitis B and hepatitis C in health care workers in Abbottabad. *J Ayub Med Coll Abbottabad* 2008; **20**: 27-29 [PMID: 19610509]
  - 69 **Slusarczyk J**, Małkowski P, Bobilewicz D, Juszczczyk G. Cross-sectional, anonymous screening for asymptomatic HCV infection, immunity to HBV, and occult HBV infection among health care workers in Warsaw, Poland. *Przegl Epidemiol* 2012; **66**: 445-451 [PMID: 23230715]
  - 70 **Thomas DL**, Factor SH, Kelen GD, Washington AS, Taylor E, Quinn TC. Viral hepatitis in health care personnel at The Johns Hopkins Hospital. The seroprevalence of and risk factors for hepatitis B virus and hepatitis C virus infection. *Arch Intern Med* 1993; **153**: 1705-1712 [PMID: 8333808]
  - 71 **Ciorlia LA**, Zanetta DM. Hepatitis B in healthcare workers: prevalence, vaccination and relation to occupational factors. *Braz J Infect Dis* 2005; **9**: 384-389 [PMID: 16410889]
  - 72 **Campello C**, Majori S, Poli A, Pacini P, Nicolardi L, Pini F. Prevalence of HCV antibodies in health-care workers from northern Italy. *Infection* 1992; **20**: 224-226 [PMID: 1521888]
  - 73 **Zaaijer HL**, Appelman P, Frijstein G. Hepatitis C virus infection among transmission-prone medical personnel. *Eur J Clin Microbiol Infect Dis* 2012; **31**: 1473-1477 [PMID: 22045049 DOI: 10.1007/s10096-011-1466-9]
  - 74 **Okasha O**, Munier A, Delarocque-Astagneau E, El Houssinie M, Rafik M, Bassim H, Hamid MA, Mohamed MK, Fontanet A. Hepatitis C virus infection and risk factors in health-care workers at Ain Shams University Hospitals, Cairo, Egypt. *East Mediterr Health J* 2015; **21**: 199-212 [PMID: 26074220]

**P- Reviewer:** Aghakhani A, D'Amelio R, Honge BL, Panduro A, Wang L

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Liu SQ



Basic Study

# Hepatitis C virus inhibitor synergism suggests multistep interactions between heat-shock protein 90 and hepatitis C virus replication

Naoko Kubota, Masataka Nomoto, Gi-Wook Hwang, Toshihiko Watanabe, Michinori Kohara, Takaji Wakita, Akira Naganuma, Shusuke Kuge

Naoko Kubota, Masataka Nomoto, Gi-Wook Hwang, Akira Naganuma, Shusuke Kuge, Laboratory of Molecular and Biochemical Toxicology, Graduate School of Pharmaceutical Sciences, Tohoku University, Miyagi 980-8578, Japan

Toshihiko Watanabe, Shusuke Kuge, Department of Microbiology, Tohoku Pharmaceutical University, Miyagi 981-8558, Japan

Michinori Kohara, Department of Microbiology and Cell Biology, Tokyo Metropolitan Institute of Medical Science, Tokyo 156-0057, Japan

Takaji Wakita, Department of Virology II, National Institute of Infectious Diseases, Tokyo 162-8640, Japan

**Author contributions:** Kubota N, Nomoto M and Watanabe T performed the experiments; Hwang GW, Kohara M, Naganuma A and Kuge S designed the research study; Wakita T provided the JFH1/HCVcc system; Kuge S wrote the report; all of the authors critically read and commented on the article.

**Institutional review board statement:** No Ethics Committee approval was required.

**Informed consent statement:** No Ethics Committee approval was required.

**Conflict-of-interest statement:** All of the authors have no conflict of interest to declare in relationship to this article.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

[licenses/by-nc/4.0/](http://creativecommons.org/licenses/by-nc/4.0/)

**Correspondence to:** Shusuke Kuge, Professor, Department of Microbiology, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai, Miyagi 981-8558, Japan. [skuge@tohoku-pharm.ac.jp](mailto:skuge@tohoku-pharm.ac.jp)  
**Telephone:** +81-22-7270129  
**Fax:** +81-22-7270129

**Received:** September 29, 2015

**Peer-review started:** October 1, 2015

**First decision:** November 4, 2015

**Revised:** December 3, 2015

**Accepted:** January 16, 2016

**Article in press:** January 19, 2016

**Published online:** February 18, 2016

## Abstract

**AIM:** To address the effect of heat-shock protein 90 (HSP90) inhibitors on the release of the hepatitis C virus (HCV), a cell culture-derived HCV (JFH1/HCVcc) from Huh-7 cells was examined.

**METHODS:** We quantified both the intracellular and extracellular (culture medium) levels of the components (RNA and core) of JFH-1/HCVcc. The intracellular HCV RNA and core levels were determined after the JFH1/HCVcc-infected Huh-7 cells were treated with radicicol for 36 h. The extracellular HCV RNA and core protein levels were determined from the medium of the last 24 h of radicicol treatment. To determine the possible role of the HSP90 inhibitor in HCV release, we examined the effect of a combined application of low doses of the HSP90 inhibitor radicicol and the RNA replication inhibitors cyclosporin A (CsA) or interferon. Finally, we statistically examined the combined effect of radicicol

and CsA using the combination index (CI) and graphical representation proposed by Chou and Talalay.

**RESULTS:** We found that the HSP90 inhibitors had greater inhibitory effects on the HCV RNA and core protein levels measured in the medium than inside the cells. This inhibitory effect was observed in the presence of a low level of a known RNA replication inhibitor (CsA or interferon- $\alpha$ ). Treating the cells with a combination of radicicol and cyclosporin A for 24 h resulted in significant synergy (CI < 1) that affected the release of both the viral RNA and the core protein.

**CONCLUSION:** In addition to having an inhibitory effect on RNA replication, HSP90 inhibitors may interfere with an HCV replication step that occurs after the synthesis of viral RNA, such as assembly and release.

**Key words:** Hepatitis C virus; Inhibition of hepatitis C virus release; Cell culture-derived hepatitis C virus; Heat-shock protein 90 inhibitors; Hepatitis C virus RNA replication

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatitis C virus (HCV) is a major causative agent of hepatocellular carcinoma. Several non-structural proteins of HCV physically and functionally interact with heat-shock protein 90 (HSP90). Although HSP90 inhibitors, which inhibit the chaperone function of HSP90, have been shown to inhibit HCV replication by several groups, a recent report using a reporter system for HCV RNA replication (replicon) suggests that the effect is nonspecific. Thus, the inhibitory mechanism of HSP90 inhibitors remains controversial. Here, we address the effect of HSP90 inhibitors on the release of JFH1/cell culture-derived HCV from Huh-7 cells, and suggested that, HSP90 inhibitors may also interfere with an HCV replication step that occurs after the synthesis of viral RNA, such as assembly and release.

Kubota N, Nomoto M, Hwang GW, Watanabe T, Kohara M, Wakita T, Naganuma A, Kuge S. Hepatitis C virus inhibitor synergism suggests multistep interactions between heat-shock protein 90 and hepatitis C virus replication. *World J Hepatol* 2016; 8(5): 282-290 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i5/282.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i5.282>

## INTRODUCTION

Chronic infection with hepatitis C virus (HCV) frequently causes liver cirrhosis and hepatocellular carcinoma<sup>[1]</sup>. Approximately 170 million individuals have been infected by HCV<sup>[2]</sup> and are at risk for developing liver disease<sup>[3]</sup>. HCV has a positive-sense single-stranded RNA genome that encodes a 3000 amino acid polyprotein and also

contains an internal entry site for translation and a non-coding region for genome replication at the 5'- and 3'- flanking region. Translation of the polyprotein is followed by cleavage by the host and viral proteases, which yields three structural proteins (core, E1 and E2) and seven nonstructural (NS) proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B)<sup>[4]</sup>. Establishment of the cell culture-derived HCV (HCVcc) system (*i.e.*, human hepatoma Huh-7 cells propagating the highly infectious clone of HCV genotype 2a, JFH-1<sup>[5]</sup>) provides a native infection-cycle system, which is suitable for determination of the precise function of HCV proteins and thus the mechanism for replication and secretion of viral particles. Accumulating evidence has indicated that NS3, NS4B and NS5A are required for not only genome RNA replication but also virus assembly<sup>[6]</sup>, whereas P7 and NS2 are dispensable for RNA replication but are required for virus production<sup>[7]</sup>.

Heat-shock protein 90 (HSP90) functions as a molecular chaperone for various client proteins and interacts with a cohort of co-chaperones that modulate the HSP90 ATPase cycle. The ATPase activity of HSP90 is inhibited by HSP90 inhibitors, which compete with ATP for binding and thereby eliminate HSP90 chaperone activity<sup>[8]</sup>. HSP90 clients include not only host proteins but also some virus proteins. Thus, some of the replication steps are required for HSP90 activity<sup>[9]</sup>. In fact, activities of multiple HCV proteins are affected by inhibition of HSP90 by specific inhibitors of HSP90, such as radicicol and geldanamycin and its derivatives. HSP90 inhibitors restrict the activity of NS2/3 protease<sup>[10]</sup>, the stability of NS3<sup>[11]</sup>, and the RNA replication of HCV in cells harboring the HCV replicon by inhibiting a complex consisting of HSP90, NS5A and a human FK506-binding protein (FKBP8)<sup>[12]</sup>. The host factors that are required for HCV replication are also affected by inhibition of HSP90<sup>[13,14]</sup>. Apart from these positive results, Beran *et al.*<sup>[15]</sup> have shown that an HSP90 inhibitor elicits effects similar to those of known cytostatic compounds, abrogating propagation of the mini-genome (subgenomic) replicon of HCV indirectly, through slowing cell growth. HCV subgenomic replicons are generally composed of a selection marker gene, such as G418 resistance, a reporter gene such as luciferase, and a whole HCV genome, except for the regions encoding the structural proteins and NS2 protease, which are deleted<sup>[16]</sup>. The replicons propagating in Huh-7 cells are widely used for studying RNA replication as well as for screening for anti-HCV drugs. However, it is possible that the replicon system does not fully represent the native function of HCV proteins, because mutations that accumulate in NS3 and NS5A in the replicon during enhancement of the RNA replication interfere with virus assembly when the mutation is introduced in the HCVcc construct<sup>[17]</sup>. Hence, the HCVcc system may be more suitable than the subgenomic replicon system to evaluate the anti-viral effect of HSP90 inhibitors. In addition, HSP90 is required for NS3 stability<sup>[11]</sup> and for formation of the complex composing NS4A<sup>[12]</sup>. Because NS3 and NS4A can act on both RNA

replication and HCV assembly, it is possible that HSP90 activity may contribute to post-RNA replication steps, such as virus assembly and release, which is represented by HCVcc but not the replicon system.

In this study, we used JFH1/HCVcc to demonstrate the effect of HSP90 inhibition on HCVcc release from infected Huh-7 cells. Our results showed that the HSP90 inhibitor radicicol preferentially reduced the levels of the core and the HCV RNA released from cells in the medium compared with those in the cells. The HSP90 inhibitor had a more potent effect on viral release in the medium than that of the inhibitor for RNA replication.

## MATERIALS AND METHODS

### Reagents

Radicicol (Sigma-Aldrich, St. Louis, MO, United States) was dissolved in methanol (1 mg/mL). Cyclosporin A [cyclosporin A (CsA); Wako Pure Chemical, Osaka, Japan], 17-AAG [17-(allylamino)-17-demethoxygeldanamycin, Sigma-Aldrich] and 17-DMAG [17-(dimethylaminoethylamino)-17-demethoxygeldanamycin; BIOMOL, Plymouth Meeting, PA, United States] were dissolved in ethanol (1 mg/mL). Interferon- $\alpha$  (IFN- $\alpha$ ; PeproTec EC, London, United Kingdom) was dissolved in water (0.1 mg/mL).

### Culture of human hepatoma Huh-7 cells and preparation of JFH1/HCVcc

Human hepatoma Huh-7 cells (purchased from Human Science Resources Bank, Osaka, Japan) were cultured in DMEM (Nissui Pharmaceuticals, Tokyo, Japan) supplemented with 10% fetal calf serum, 0.06% glutamine, 0.35% glucose and 1% penicillin-streptomycin (Life Technologies, Carlsbad, CA, United States). The cells were maintained at 37 °C in an atmosphere of 5% CO<sub>2</sub>. We synthesized the HCV genomic RNA of genotype 2a (JFH1) *in vitro* using a MEGAscript™ T7 kit (Ambion, Austin, TX, United States) and introduced the RNA into Huh-7 cells by electroporating the cells with the GenePulser II electroporation system (Bio-Rad, Hercules, CA, United States) as previously described<sup>[5]</sup>. The cytotoxic effects of the reagents were examined with Alamar Blue cell viability reagent (Serotec, Raleigh, NC, United States), which allows an estimation of the oxidation levels in the cellular electron-transport pathways with a fluorescent indicator. Alamar Blue was used as described by the manufacturer.

### Quantification of the HCV core protein and genomic RNA

We washed the JFH1/HCVcc cells with PBS and lysed them in lysis buffer (20 mmol/L Tris-Cl, pH 7.5, 0.1% SDS, 1% Triton X-100, 1% deoxycholate, 0.1 mmol/L EDTA, 0.1 mmol/L phenylmethanesulfonyl fluoride, 50  $\mu$ mol/L N-*p*-tosyl-L-phenylalanine chloromethyl ketone, 5  $\mu$ mol/L N- $\alpha$ -tosyl-L-lysine chloromethyl ketone hydrochloride, 5  $\mu$ g/mL aprotinin and 5  $\mu$ g/mL leupeptin). We quantified the level of core protein present in

the lysates and spent culture medium using the Ortho HCV antigen ELISA test (Ortho Clinical Diagnostics, Rochester, NY, United States). We used a QIAamp™ Viral RNA Mini kit (Qiagen, Hilden, Germany) to isolate the HCV genomic RNA from the medium. We used an RNeasy™ mini kit (Qiagen) to isolate total RNA from the HCV-infected cells. We quantified the HCV genomic RNA using TaqMan™ EZ RT-PCR Core Reagents (Applied Biosystems, Foster City, CA, United States) and the iCycler™ iQ real-time detection system (Bio-Rad) as previously described<sup>[18]</sup>.

### Effects of radicicol and CsA

The JFH1/HCVcc cells ( $5 \times 10^4$  cells in one well of a 24-well culture dish) were treated with radicicol, CsA and/or IFN- $\alpha$  for 12 h, at which point the medium was replaced by fresh medium that contained the same level(s) of drug(s). After culturing for another 24 h, the core and the HCV RNA in each cell lysate and culture medium were quantified as described above. The synergism between CsA and radicicol was evaluated by the combination index (CI) equation and the Chou and Talalay method<sup>[19,20]</sup> using CalcuSyn software (BIOSOFT, Cambridge, United Kingdom). The CI equation is based on the multiple drug-effect equation of Chou *et al.*<sup>[20]</sup>, which is derived from enzyme-kinetic models. Assuming that CsA and radicicol were mutually non-exclusive drugs that have totally independent modes of action, we used the following equation:

Equation 1 dictates that the combination of drug 1 (D)<sub>1</sub> and drug 2 (D)<sub>2</sub> inhibits a reaction (or phenomenon) by x% in an actual experiment. (D<sub>x</sub>)<sub>1</sub> and (D<sub>x</sub>)<sub>2</sub> are the doses of drug 1 and drug 2 alone that inhibit the same reaction by x%.

$$CI = [(D)_1 / (D_x)_1] + [(D)_2 / (D_x)_2] + \frac{[(D)_1 (D)_2 / (D_x)_1 (D_x)_2]}{[(D)_1 (D)_2 / (D_x)_1 (D_x)_2]} \quad (1)$$

### Statistical analysis

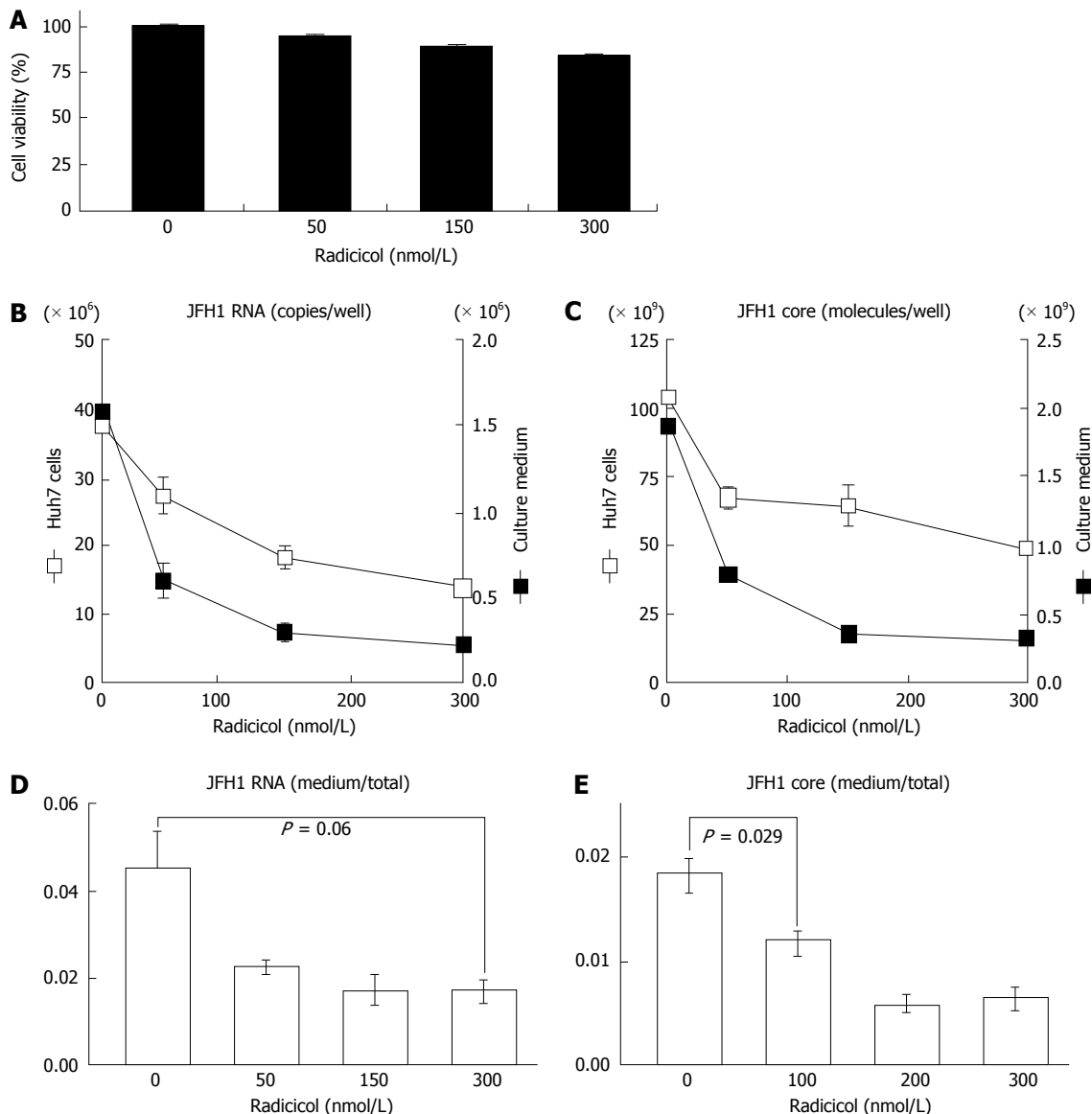
We used Student's *t*-test to examine statistical significance ( $P < 0.05$ ). All of the experiments were performed with multiple independent replicates, and all of the data are presented as the mean results of three independent experiments with the standard error of the mean. The statistical methods of this study were reviewed by professor Kotaro Tanahashi from Mathematics, Tohoku Pharmaceutical University.

## RESULTS

### HCV released into the medium is preferentially reduced by HSP90 inhibitors

To examine the effects of HSP90 inhibitor on the release of HCV, we quantified both the intracellular and extracellular (culture medium) levels of the components (RNA and core) of JFH-1/HCVcc. The intracellular HCV RNA and core levels were determined after the cells were treated with radicicol for 36 h. The extracellular HCV RNA and the core were determined from the medium of the



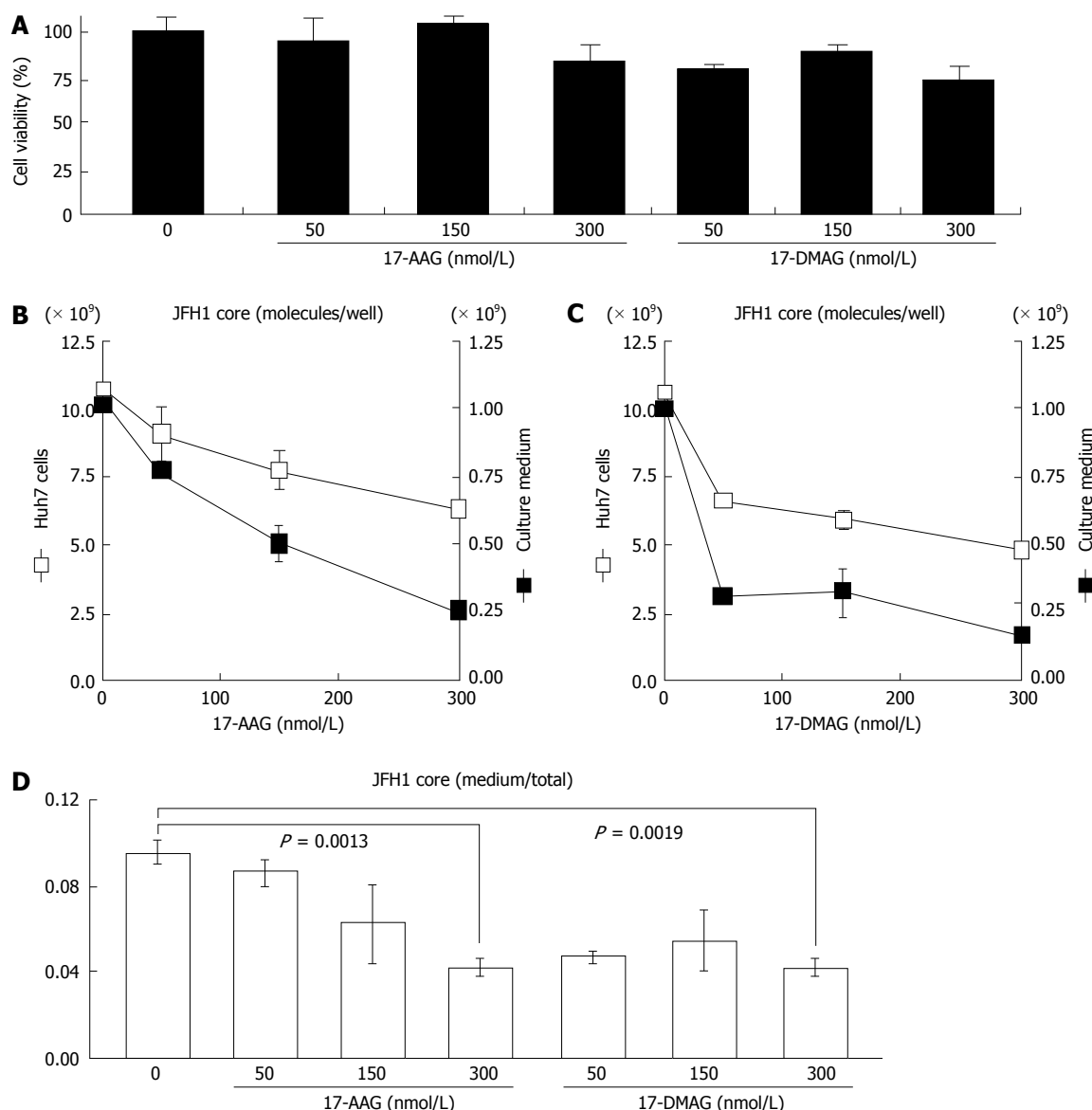


**Figure 1** Radicicol affects the relative level of hepatitis C virus (core and hepatitis C virus RNA) produced from the JFH1/cell culture-derived hepatitis C virus system of Huh-7 cells. A: After the cells were treated with radicicol (at final concentration of 0, 50, 150 and 300 nmol/L) for 12 h, the culture medium was replaced with fresh medium containing the same radicicol levels, and the HCV RNA and core levels released into the medium for the next 24 h and produced within cells were determined as described in the text. The cytotoxic effects of the treatment of radicicol on HCVcc Huh-7 cells were examined as described in the text; B and C: The levels of HCV RNA (B) and core (C) in the HCVcc Huh-7 cells (open squares) and the culture medium (filled squares) were quantified. The scales on the left sides (B and C) indicate the scales for the HCV RNA and the core in the Huh-7 cells. The scales on the right sides (B and C) indicate the scales for the HCV RNA and the core in the culture medium; D: The ratios of HCV RNA in the medium to the total HCV RNA (the sum of HCV RNA in the medium and in the cells) are shown; E: The ratios of the core in the medium to the total core (the sum of the core in the medium and in the cells) are shown. The data represent the mean values ( $\pm$  SEM) of the results from three independent experiments. HCV: Hepatitis C virus; HCVcc: Cell culture-derived HCV.

last 24 h of radicicol treatment. The radicicol treatment (50–300 nmol/L) exhibited no apparent cytotoxic effect (Figure 1A), reduced both the intracellular and extra-cellular (medium) levels of the HCV RNA (Figure 1B) and the core (Figure 1C) in a dose-dependent manner. Interestingly, the RNA level in the culture medium relative to the total RNA level was apparently reduced by radicicol even at a low concentration (50 nmol/L) (Figure 1D). Similarly, the core level in the medium relative to the total core level was also significantly decreased ( $P = 0.029$ ) in the presence of 50 nmol/L radicicol (Figure 1E). Furthermore, two derivatives of the geldanamycin HSP90 inhibitor, 17-AAG and 17-DMAG, also inhibited the

release of the HCV RNA and core more effectively than they decreased the intracellular HCV RNA and core levels (Figure 2).

We next examined whether the integrity of HCV was affected by the radicicol treatment during production of HCV from JFH1/HCVcc. The infectivity of the HCV that had been released into the medium in the presence of radicicol was compared to the infectivity of HCV released in the absence of radicicol. As shown in Figure 3, there was no significant difference ( $P > 0.3$ ) in the infectivity between the HCV produced in the presence and that produced in the absence of radicicol. These results suggested that even though radicicol preferentially reduced



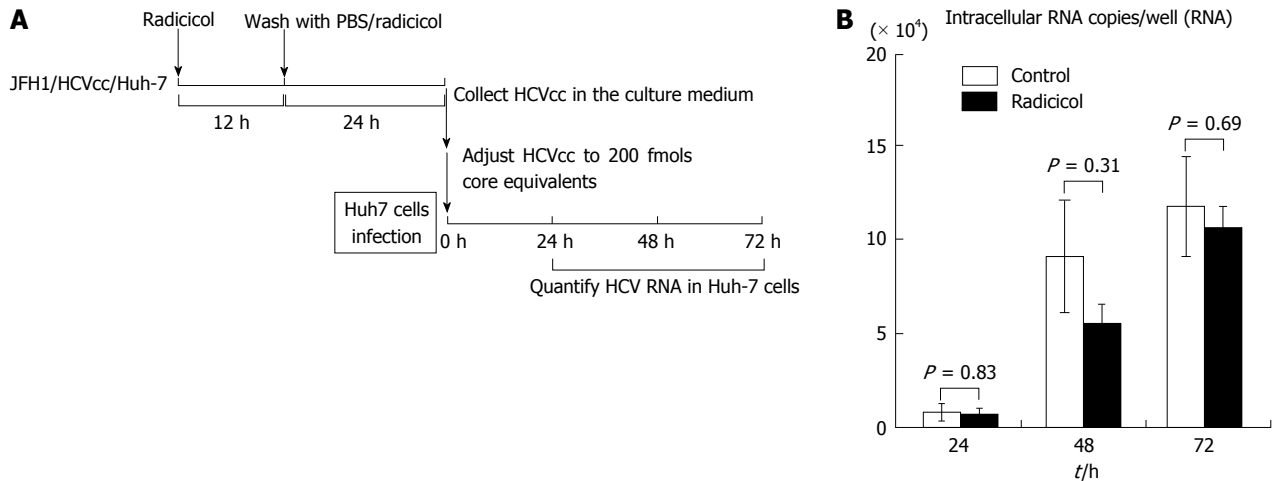
**Figure 2** Effects of the geldanamycin derivatives 17-allylamino-17-demethoxygeldanamycin and 17-dimethylaminoethylamino-17-demethoxygeldanamycin on the release of JFH1. A: The cytotoxic effects of 17-AAG and 17-DMAG on Huh-7 cells carrying JFH1/HCVcc were examined as described in the text; B and C: JFH1-infected Huh-7 cells were treated with 17-AAG (B) or 17-DMAG (C) for 24 h and then the protein samples were isolated and quantified. The levels of the HCV core present in the JFH1-infected Huh-7 cells (open squares) and the culture medium (filled squares) were examined as described in the text. The scales on the left (B and C) and right sides (B and C) indicate the scales for the HCV core in the Huh-7 cells and in the culture medium, respectively; D: The ratios of the core in the medium to the total core are shown. The concentrations of 17-AAG and 17-DMAG (0-300 nmol/L) are shown under the histograms. The data represent the mean values ( $\pm$  SEM) of the results from three independent experiments. HCV: Hepatitis C virus; 17-AGG: 17-allylamino-17-demethoxygeldanamycin; 17-DMAG: 17-dimethylaminoethylamino-17-demethoxygeldanamycin; HCVcc: Cell culture-derived HCV.

HCV release, radicicol did not affect its infectivity.

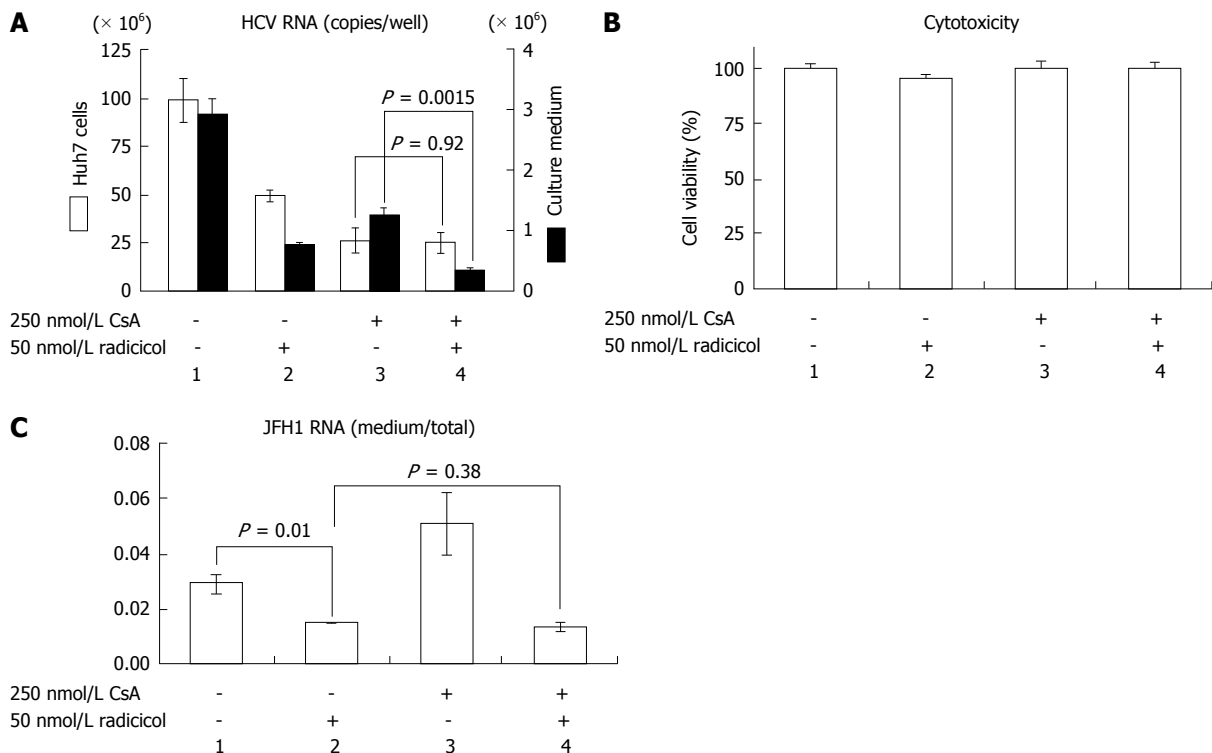
#### **Radicicol preferentially suppressed the HCV RNA release in the presence of CsA or interferon**

NS3 and NS5A, which are known targets of HSP90<sup>[11,12]</sup>, are required for both RNA replication and virus assembly<sup>[6,21]</sup>. Thus, it is difficult to distinguish whether the inhibitory step of the HSP90 inhibitor affects the RNA replication or the assembly. If the HSP90 inhibitor were to preferentially inhibit the post-RNA replication steps, the HSP90 inhibitor might enhance the RNA-replication inhibitor-dependent inhibitory effect on HCV release. CsA an immunosuppressant, inhibits interaction between a

CsA's target cyclophilin A and NS5A and the interaction of the cyclophilin A with the NS5B polymerase/RNA complex, thereby inhibiting RNA replication<sup>[22]</sup>. This inhibitory effect is distinct from CsA's immunosuppressive activity<sup>[23]</sup>. To determine the possible role of the HSP90 inhibitor on HCV release, we examined the effect of a combined application of low doses of radicicol and CsA. By studying the dose-dependent inhibition of HCV RNA production in Huh-7 cells (data not shown), we determined the doses showing the partial effect of CsA (250 nmol/L) and radicicol (50 nmol/L) on the viral RNA production in cells after 36 h of treatment (Figure 4A, column 1, 2 and 3, open bars). CsA alone, radicicol



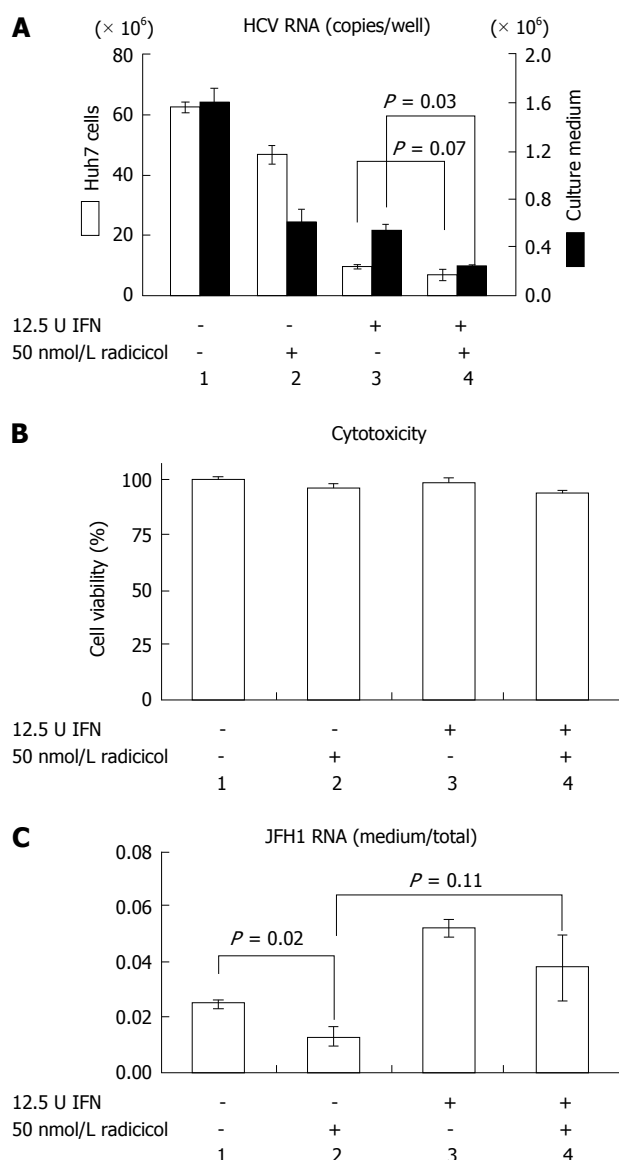
**Figure 3 Infectivity of hepatitis C virus produced from Huh-7 cells that were treated with radicicol.** A: Experimental design. To examine whether the infectivity of the JFH1/HCVcc released from the Huh-7 cells in the presence of radicicol was altered, we infected fresh Huh-7 cells with JFH1/HCVcc prepared in the presence of radicicol. To prepare a viral stock, the JFH1/HCVcc infected Huh-7 cells with 50 nmol/L radicicol were maintained for an additional 36 h. HCVcc released in the medium during the last 24 h was collected and used as the viral stock. We diluted the viral stock 59.7 times (None) and 28.6 times (Radicicol) to reduce the effects of radicicol carryover and to adjust the HCV levels (core protein level: 200 fmols). The final radicicol concentration was 1.75 nmol/L which did not affect HCV propagation. The HCV samples were added to fresh Huh-7 cells and cultured for 24, 48 and 72 h. The HCV RNA in the infected Huh-7 cells was quantified as described in the text. The multiplicities of infection for HCV were 0.2 copies/cell and 0.16 copies/cell for the HCV infection without and with radicicol, respectively; B: The copies of HCV RNA in the Huh-7 cells of each well after infection (24, 48 and 72 h) were determined as described in the text. The value of the RNA copy number in the Huh-7 cells [which were infected with the viral stock from radicicol-treated cells (values of the closed bars)] was adjusted by multiplying by a factor of 1.25. The data represent the mean values ( $\pm$  SE) of the results from three independent experiments. HCV: Hepatitis C virus; HCVcc: Cell culture-derived HCV.



**Figure 4 Radicicol and cyclosporin A have a synergistic effect on hepatitis C virus release into the medium.** A: JFH1/HCVcc-infected Huh-7 cells were treated with 250 nmol/L CsA alone, 50 nmol/L radicicol alone, or both for 36 h. The total RNA was prepared from the Huh-7 cells, and the HCV RNA was quantified by reverse transcription-polymerase chain reaction (open columns, the scale on the left of the panel indicates the copies/well). The medium was replaced by the fresh medium containing the same levels of the drugs after 12 h, and total RNA was prepared from the medium 24 h later. The copy numbers of the HCV RNA in the medium are indicated by the filled columns (scale on the right of the panel); B: The cytotoxic effects of the drugs used in (A) on the Huh-7 cells carrying JFH1/HCVcc; C: The ratios of the RNA in the medium to the total RNA (as described above) are shown. HCV: Hepatitis C virus; HCVcc: Cell culture-derived HCV; CsA: Cyclosporin A.

alone and simultaneous treatment with both drugs did not affect the cytotoxicity in JFH1-infected Huh-7 cells (Figure 4B). When the HCV-infected cells were treated

with the low concentration of both drugs simultaneously, the level of HCV RNA released in the medium after 24 h fell significantly (Figure 4A, comparison of the closed



**Figure 5 Radicicol and interferon- $\alpha$  have a synergistic effect on hepatitis C virus release into the medium.** A: JFH1/HCVcc-infected Huh-7 cells were treated with 12.5 U INF- $\alpha$  alone, 50 nmol/L radicicol alone, or both for 36 h. The total RNA was prepared from the Huh-7 cells, and the HCV RNA was quantified by reverse transcription-polymerase chain reaction (open columns, the scale on the left of the panel indicates the copies/well). The medium was replaced by fresh medium after 12 h, and the total RNA was prepared from the medium 24 h later. The copy numbers of the HCV RNA in the medium are indicated by the filled columns (scale on the right of the panel); B: The cytotoxic effects of the drugs used in (A) on the Huh-7 cells carrying JFH1/HCVcc; C: The ratios of RNA in the medium to RNA in the total RNA (as described above) are shown. HCV: Hepatitis C virus; HCVcc: Cell culture-derived HCV; INF- $\alpha$ : Interferon- $\alpha$ .

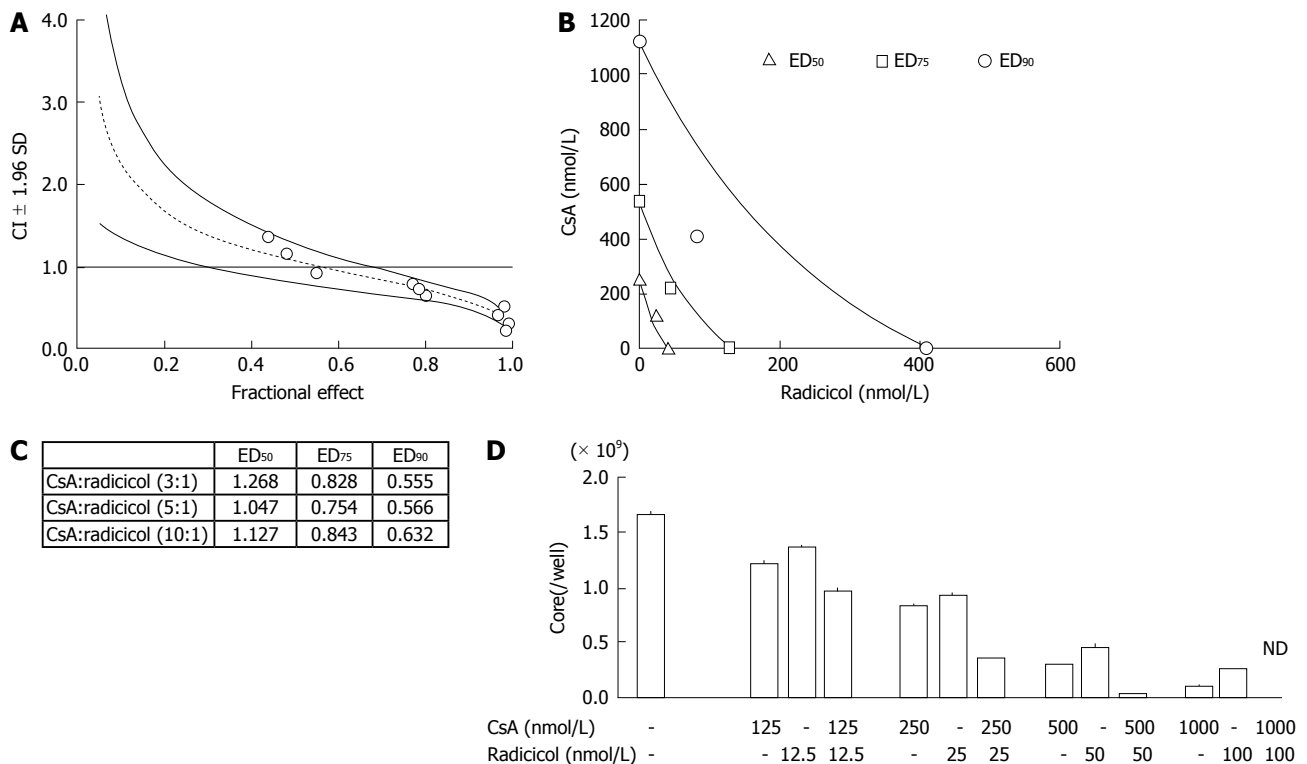
bars in columns 3 and 4,  $P = 0.0015$ ), whereas the level of HCV RNA in the cells was the same as that in the cells treated with CsA alone (Figure 4A, comparison of the open bars in columns 3 and 4). Intriguingly, the RNA level in the culture medium relative to the RNA levels in the infected cells fell in the presence of radicicol (Figure 4C, comparison of columns 1 and 2). However, this medium-to-total ratio was not affected by simultaneous treatment of CsA with radicicol (Figure 4C, column 2 and 4). Thus, radicicol could have more of an inhibitory effect on viral release from infected cells than CsA.

Previous results have indicated that the combined effect of IFN and CsA on RNA replication is mostly additive<sup>[23]</sup> and have suggested that both CsA and IFN target a similar point in HCV replication. However, Robida *et al.*<sup>[24]</sup> have indicated that CsA resistant mutants maintain their sensitivity to IFN- $\alpha$ . Thus, although both IFN- $\alpha$  and CsA inhibit RNA replication, the inhibitory effects of IFN- $\alpha$  and CsA seems to be different. As shown in Figure 5A, we observed that radicicol efficiently reduced the level of HCV in the medium even in the presence of IFN- $\alpha$ . Although the combined treatment of radicicol and IFN- $\alpha$  and the treatment of IFN- $\alpha$  alone exhibited a similar level of HCV RNA in cells (columns 3 and 4, open bars), HCV RNA in the medium was significantly reduced (columns 3 and 4, closed bars). Again, HCV RNA in the medium was significantly suppressed in the presence of radicicol (Figure 5C). All of these results suggested that HSP90 might be responsible for a post-replication step such as viral release. It should be noted that a similar synergism has been previously reported: A combined administration of an HSP90 inhibitor and polyethylene glycol-conjugated interferon (PEG-IFN) in HCV-infected chimeric mice with humanized livers was more effective at reducing the HCV genomic RNA levels in mouse serum than a single PEG-IFN treatment<sup>[25]</sup>.

#### Analysis of the synergistic effect between radicicol and CsA

Our results suggested that radicicol has a greater inhibitory effect on the release of HCVcc into the medium and that the point of inhibition of HCV production by the HSP90 inhibitor may be different from that of CsA. Thus, we statistically examined the combined effect of radicicol and CsA using the CI and the graphical representation proposed by Chou *et al.*<sup>[19,20]</sup>. We examined the effects of various CsA (125, 250, 750 and 1500 nmol/L) and radicicol (25, 50, 150 and 300 nmol/L) concentrations on the release of the core into the medium after 24 h (data not shown). Next, we examined the effects of a fixed molar ratio of these drugs (5:1 CsA to radicicol). The results (Figure 6A) indicated that CsA and radicicol had a synergistic effect ( $CI < 1$ ) above a fractional effect of 0.5 and close to a strong synergism ( $CI \leq 0.3$ ) at a fractional effect of 1. The combined effect was additive near a fractional effect of 0.5 and antagonistic at a fractional effect less than 0.4. Figure 6B shows the conservation isobologram ( $CI = 1$ ) for the different effective doses of the combination treatment that yielded 50% ( $ED_{50}$ ), 75% ( $ED_{75}$ ) and 90% ( $ED_{90}$ ) inhibition of the core release (constructed using actual experimental data). The combined effect at  $ED_{50}$  ( $CI = 1.05$ ) was additive, whereas the combined effects at  $ED_{75}$  ( $CI = 0.75$ ) and  $ED_{90}$  ( $CI = 0.57$ ) were synergistic (Figure 6). The combined use of 408 nmol/L CsA and 82 nmol/L radicicol yielded  $ED_{90}$  (Figure 6B; if a  $CI = 1$  was expected, an estimated 605 nmol/L CsA and 121 nmol/L radicicol would be required to yield  $ED_{90}$ ). We obtained similar results for the 3:1 and 10:1 molar ratios of CsA





**Figure 6 Analysis of the synergistic effect of cyclosporin A and radicicol.** A: The graphs were constructed using the Chou and Talalay method. The CI and the fractional effect were derived from the release of the HCV core protein into the culture medium of JFH1-infected Huh-7 cells that were treated with a combination of CsA and radicicol (5:1 molar ratio). The open circles indicate actual experimental data. The CI vs fractional effect plots were generated with CalcSyn software. The dotted line and solid lines represent the mean values and standard deviation (1.96), respectively, of three independent experiments. The CI < 1, CI = 1, and CI > 1 indicate synergy, an additive effect and antagonism, respectively; B: The conservation isobologram (CI = 1) depicting different effective doses that yielded 50% (ED<sub>50</sub>), 75% (ED<sub>75</sub>) and 90% (ED<sub>90</sub>) inhibition of viral release by the combination treatment was graphed with actual experimental data (ED<sub>50</sub>, open triangles; ED<sub>75</sub>, open squares; ED<sub>90</sub>, open circles). The data represent the mean values of the results from three independent experiments; C: The CI values of each ED<sub>50</sub>, ED<sub>75</sub> and ED<sub>90</sub> by the combined CsA and radicicol treatment at molar ratios of 3:1, 5:1 and 10:1, respectively; D: The combined effect of CsA and radicicol at a molar ratio of 10:1. The HCV core levels in the medium are shown. The mean values and standard deviation ( $\pm$  SD) of the amounts of the HCV core released into the medium during three independent experiments are shown. The concentrations used in each set of experiments are shown under the histogram. ND: Not detected; CsA: Cyclosporin A; HCV: Hepatitis C virus; CI: Combination index.

to radicicol (Figure 6C). One example, shown in Figure 6D, indicated that treating the cells with both 1000 nmol/L CsA and 100 nmol/L radicicol for 36 h caused the HCV core to be undetectable in the medium for the last 24 h of the combined treatment.

## DISCUSSION

Collectively, our results suggest that HSP90 inhibitors might affect both the RNA replication and the post-RNA replication stage of viral propagation. Previous reports have indicated that HSP90 is required for NS3 stability<sup>[11]</sup> and for the formation of a complex consisting of NS5A and FKBP8<sup>[12]</sup>. Thus, it is possible that HSP90 may affect the post RNA-replication step, such as assembly, through affecting the activity of NS3 and NS5A. Elucidating the precise mechanism for the HSP90 on HCV assembly may provide an alternative drug target for HCV clearance.

## COMMENTS

### Background

Although heat-shock protein 90 (HSP90) inhibitors, which inhibit the chaperone function of HSP90, have been shown to inhibit hepatitis C virus (HCV) repli-

cation by several groups, a recent report using a reporter system for HCV RNA replication (replicon) suggests that the effect is nonspecific. Thus, the inhibitory mechanism of HSP90 inhibitors remains controversial.

### Research frontiers

The authors found that the HSP90 inhibitors had greater inhibitory effects on the HCV RNA and core protein levels measured in the medium than inside the cells. This inhibitory effect was observed in the presence of a low level of a known RNA replication inhibitor [cyclosporin A (CsA) or interferon- $\alpha$ ].

### Innovations and breakthroughs

The authors' results suggested that, HSP90 inhibitors may also interfere with an HCV replication step that occurs after the synthesis of viral RNA, such as assembly and release.

### Applications

This study would benefit the effort to explore new targets for the treatment of HCV infection.

### Terminology

HSP90 inhibitors inhibit the chaperone function of HSP90. HSP90 clients include not only host proteins but also multiple HCV proteins including NS2/3 protease, NS3 and the RNA replication complex consisting of NS5A.

### Peer-review

The authors suggested that HSP90 inhibitors may interfere with an HCV replication step that occurs after the synthesis of viral RNA such as assembly

and release. The results of this study were quite interesting. The data were appropriately presented and interpreted. This manuscript also was well prepared.

## REFERENCES

- 1 Saito I, Miyamura T, Ohbayashi A, Harada H, Katayama T, Kikuchi S, Watanabe Y, Koi S, Onji M, Ohta Y. Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. *Proc Natl Acad Sci USA* 1990; **87**: 6547-6549 [PMID: 2168552 DOI: 10.1073/pnas.87.17.6547]
- 2 Alter MJ. Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007; **13**: 2436-2441 [PMID: 17552026 DOI: 10.3748/wjg.v13i17.2436]
- 3 Bosch FX, Ribes J, Borrás J. Epidemiology of primary liver cancer. *Semin Liver Dis* 1999; **19**: 271-285 [PMID: 10518307 DOI: 10.1055/s-2007-1007117]
- 4 Bartenschlager R, Frese M, Pietschmann T. Novel insights into hepatitis C virus replication and persistence. *Adv Virus Res* 2004; **63**: 71-180 [PMID: 15530561 DOI: 10.1016/S0065-3527(04)63002-8]
- 5 Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Kräusslich HG, Mizokami M, Bartenschlager R, Liang TJ. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 2005; **11**: 791-796 [PMID: 15951748 DOI: 10.1038/nm1268]
- 6 Jones DM, McLauchlan J. Hepatitis C virus: assembly and release of virus particles. *J Biol Chem* 2010; **285**: 22733-22739 [PMID: 20457608 DOI: 10.1074/jbc.R110.133017]
- 7 Jones CT, Murray CL, Eastman DK, Tassello J, Rice CM. Hepatitis C virus p7 and NS2 proteins are essential for production of infectious virus. *J Virol* 2007; **81**: 8374-8383 [PMID: 17537845]
- 8 Prodromou C, Pearl LH. Structure and functional relationships of Hsp90. *Curr Cancer Drug Targets* 2003; **3**: 301-323 [PMID: 14529383 DOI: 10.2174/1568009033481877]
- 9 Geller R, Tagawa S, Frydman J. Broad action of Hsp90 as a host chaperone required for viral replication. *Biochim Biophys Acta* 2012; **1823**: 698-706 [PMID: 22154817]
- 10 Waxman L, Whitney M, Pollok BA, Kuo LC, Darke PL. Host cell factor requirement for hepatitis C virus enzyme maturation. *Proc Natl Acad Sci USA* 2001; **98**: 13931-13935 [PMID: 11707594 DOI: 10.1073/pnas.241510898]
- 11 Ujino S, Yamaguchi S, Shimotohno K, Takaku H. Heat-shock protein 90 is essential for stabilization of the hepatitis C virus nonstructural protein NS3. *J Biol Chem* 2009; **284**: 6841-6846 [PMID: 19150985 DOI: 10.1074/jbc.M806452200]
- 12 Okamoto T, Nishimura Y, Ichimura T, Suzuki K, Miyamura T, Suzuki T, Moriishi K, Matsuura Y. Hepatitis C virus RNA replication is regulated by FKBP8 and Hsp90. *EMBO J* 2006; **25**: 5015-5025 [PMID: 17024179 DOI: 10.1038/sj.emboj.7601367]
- 13 Ujino S, Nishitsuji H, Sugiyama R, Suzuki H, Hishiki T, Sugiyama K, Shimotohno K, Takaku H. The interaction between human initiation factor eIF3 subunit c and heat-shock protein 90: a necessary factor for translation mediated by the hepatitis C virus internal ribosome entry site. *Virus Res* 2012; **163**: 390-395 [PMID: 22016036 DOI: 10.1016/j.virusres.2011.10.003]
- 14 Kim MG, Moon JS, Kim EJ, Lee SH, Oh JW. Destabilization of PDK1 by Hsp90 inactivation suppresses hepatitis C virus replication through inhibition of PRK2-mediated viral RNA polymerase phosphorylation. *Biochem Biophys Res Commun* 2012; **421**: 112-118 [PMID: 22490666 DOI: 10.1016/j.bbrc.2012.03.126]
- 15 Beran RK, Sharma R, Corsa AC, Tian Y, Golde J, Lundgaard G, Delaney WE, Zhong W, Greenstein AE. Cellular growth kinetics distinguish a cyclophilin inhibitor from an HSP90 inhibitor as a selective inhibitor of hepatitis C virus. *PLoS One* 2012; **7**: e30286 [PMID: 22347373 DOI: 10.1371/journal.pone.0030286]
- 16 Bartenschlager R, Lohmann V, Penin F. The molecular and structural basis of advanced antiviral therapy for hepatitis C virus infection. *Nat Rev Microbiol* 2013; **11**: 482-496 [PMID: 23748342 DOI: 10.1038/nrmicro3046]
- 17 Pietschmann T, Zayas M, Meuleman P, Long G, Appel N, Koutsoudakis G, Kallis S, Leroux-Roels G, Lohmann V, Bartenschlager R. Production of infectious genotype 1b virus particles in cell culture and impairment by replication enhancing mutations. *PLoS Pathog* 2009; **5**: e1000475 [PMID: 19521536 DOI: 10.1371/journal.ppat.1000475]
- 18 Takeuchi T, Katsume A, Tanaka T, Abe A, Inoue K, Tsukiyama-Kohara K, Kawaguchi R, Tanaka S, Kohara M. Real-time detection system for quantification of hepatitis C virus genome. *Gastroenterology* 1999; **116**: 636-642 [PMID: 10029622 DOI: 10.1016/S0016-5085(99)70185-X]
- 19 Chou TC, Talaly P. A simple generalized equation for the analysis of multiple inhibitions of Michaelis-Menten kinetic systems. *J Biol Chem* 1977; **252**: 6438-6442 [PMID: 893418]
- 20 Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul* 1984; **22**: 27-55 [PMID: 6382953 DOI: 10.1016/0065-2571(84)90007-4]
- 21 Paul D, Madan V, Bartenschlager R. Hepatitis C virus RNA replication and assembly: living on the fat of the land. *Cell Host Microbe* 2014; **16**: 569-579 [PMID: 25525790 DOI: 10.1016/j.chom.2014.10.008]
- 22 Yang F, Robotham JM, Nelson HB, Irsigler A, Kenworthy R, Tang H. Cyclophilin A is an essential cofactor for hepatitis C virus infection and the principal mediator of cyclosporine resistance in vitro. *J Virol* 2008; **82**: 5269-5278 [PMID: 18385230 DOI: 10.1128/JVI.02614-07]
- 23 Goto K, Watashi K, Murata T, Hishiki T, Hijikata M, Shimotohno K. Evaluation of the anti-hepatitis C virus effects of cyclophilin inhibitors, cyclosporin A, and NIM811. *Biochem Biophys Res Commun* 2006; **343**: 879-884 [PMID: 16564500 DOI: 10.1016/j.bbrc.2006.03.059]
- 24 Robida JM, Nelson HB, Liu Z, Tang H. Characterization of hepatitis C virus subgenomic replicon resistance to cyclosporine in vitro. *J Virol* 2007; **81**: 5829-5840 [PMID: 17376913 DOI: 10.1128/JVI.02524-06]
- 25 Nakagawa S, Umehara T, Matsuda C, Kuge S, Sudoh M, Kohara M. Hsp90 inhibitors suppress HCV replication in replicon cells and humanized liver mice. *Biochem Biophys Res Commun* 2007; **353**: 882-888 [PMID: 17196931 DOI: 10.1016/j.bbrc.2006.12.117]

P- Reviewer: Chuang WL, Jin B S- Editor: Qiu S

L- Editor: A E- Editor: Liu SQ



Case Control Study

## High level of serum cholesteryl ester transfer protein in active hepatitis C virus infection

Kenichi Satoh, Tomohisa Nagano, Nobuyoshi Seki, Yoichi Tomita, Yuta Aida, Tomonori Sugita, Munenori Itagaki, Satoshi Sutoh, Hiroshi Abe, Yoshio Aizawa

Kenichi Satoh, Tomohisa Nagano, Nobuyoshi Seki, Yoichi Tomita, Yuta Aida, Tomonori Sugita, Munenori Itagaki, Satoshi Sutoh, Hiroshi Abe, Yoshio Aizawa, Department of Gastroenterology and Hepatology, Internal Medicine of Jikei University Katsushika Medical Center, Katsushikaku, Tokyo 125-8506, Japan

**Author contributions:** Seki N and Aizawa Y designed research; Satoh K, Nagano T, Seki N, Tomita Y, Aida Y, Sugita T, Itagaki M, Sutoh S, Abe H and Aizawa Y treated patients and collected materials and clinical data; Satoh K, Nagano T, and Aizawa Y analyzed data; Satoh K and Aizawa Y wrote the paper.

**Institutional review board statement:** The study was approved by the ethics committee of Jikei University School of Medicine (Tokyo, Japan).

**Informed consent statement:** All patients gave informed consent.

**Conflict-of-interest statement:** There were no conflict-of-interests which must be declared.

**Data sharing statement:** The technical appendix, statistical code, and dataset are available from the corresponding author at [satoken@jikei.ac.jp](mailto:satoken@jikei.ac.jp).

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Kenichi Satoh, Department of Gastroenterology and Hepatology, Internal Medicine of Jikei University Katsushika Medical Center, 6-41-2 Aoto, Katsushikaku, Tokyo 125-8506, Japan. [satoken@jikei.ac.jp](mailto:satoken@jikei.ac.jp)  
Telephone: +81-33-6032111  
Fax: +81-33-8389944

Received: August 27, 2015  
Peer-review started: August 31, 2015  
First decision: September 28, 2015  
Revised: December 30, 2015  
Accepted: January 27, 2016  
Article in press: January 29, 2016  
Published online: February 18, 2016

### Abstract

**AIM:** To determine the significance of cholesteryl ester transfer protein (CETP) in lipoprotein abnormalities in chronic hepatitis C virus (HCV) infection.

**METHODS:** We evaluated the significance of the serum concentration of CETP in 110 Japanese patients with chronic HCV infection. Fifty-five patients had active HCV infection, and HCV eradication had been achieved in 55. The role of CETP in serum lipoprotein abnormalities, specifically, in triglyceride (TG) concentrations in the four major classes of lipoproteins, was investigated using Pearson correlations in conjunction with multiple regression analysis and compared them between those with active HCV infection and those in whom eradication had been achieved.

**RESULTS:** The serum CETP levels of patients with active HCV infection were significantly higher than those of patients in whom HCV eradication was achieved (mean  $\pm$  SD,  $2.84 \pm 0.69$   $\mu$ g/mL vs  $2.40 \pm 1.00$   $\mu$ g/mL,  $P = 0.008$ ). In multiple regression analysis, HCV infection status (active or eradicated) was an independent factor significantly associated with the serum CETP level. TG concentrations in low-density lipoprotein (mean  $\pm$  SD,  $36.25 \pm 15.28$   $\mu$ g/mL vs  $28.14 \pm 9.94$   $\mu$ g/mL,  $P = 0.001$ ) and high-density lipoprotein (HDL) (mean  $\pm$  SD,  $25.9 \pm 7.34$   $\mu$ g/mL vs  $17.17 \pm 4.82$   $\mu$ g/mL,  $P < 0.001$ ) were significantly higher in patients

with active HCV infection than in those in whom HCV eradication was achieved. The CETP level was strongly correlated with HDL-TG in patients with active HCV infection ( $R = 0.557$ ,  $P < 0.001$ ), whereas CETP was not correlated with HDL-TG in patients in whom HCV eradication was achieved ( $R = -0.079$ ,  $P = 0.56$ ).

**CONCLUSION:** Our results indicate that CETP plays a role in abnormalities of lipoprotein metabolism in patients with chronic HCV infection.

**Key words:** Hepatitis C virus; Cholesteryl ester transfer protein; High-density lipoprotein triglyceride; Case control study; Lipoprotein metabolism

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Cholesteryl ester transfer protein (CETP) mediates the transfer of neutral lipids between lipoproteins. Although lipoprotein metabolism abnormalities have been extensively studied, the role of CETP in abnormal lipoprotein profiles in patients with hepatitis C virus (HCV) infection is unknown. Accordingly, we investigated, for the first time, high serum CETP level in patients with active HCV infection. HCV infection was a determinant of the serum CETP level in multiple regression analysis. A high CETP concentration in HCV infection was strongly correlated with excessive triglyceride accumulation in high-density lipoprotein. Thus, CETP may contribute to abnormal lipoprotein metabolism in HCV infection.

Sato K, Nagano T, Seki N, Tomita Y, Aida Y, Sugita T, Itagaki M, Sutoh S, Abe H, Aizawa Y. High level of serum cholesteryl ester transfer protein in active hepatitis C virus infection. *World J Hepatol* 2016; 8(5): 291-300 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i5/291.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i5.291>

## INTRODUCTION

Chronic hepatitis C virus (HCV) infection is one of the most important etiologies of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide<sup>[1-3]</sup>. HCV is a unique virus; its use of host lipid metabolism results in a persistent infection. Therefore, it is very important to understand how HCV uses host lipid metabolism and how host lipid metabolism is affected by HCV infection, because HCV infection represents a unique model in which the virus causes chronic infection while coexisting with the host, simultaneously taking over the host's metabolism<sup>[4]</sup>.

Infectious HCV forms a lipoviral particle that can enter into hepatocytes in the blood<sup>[5]</sup>. The characteristics of HCV lipoviral particles are similar to those of very-low-density lipoproteins (VLDLs)<sup>[6]</sup>. This suggests a close association between HCV infection and VLDL. Both VLDL and HCV lipoviral particles are synthesized,

assembled, and secreted from hepatocytes *via* similar metabolic pathways<sup>[7]</sup>. Consequently, dysregulated lipid metabolism in chronic HCV infection may primarily be caused by VLDL abnormalities. According to some *in vitro* studies, HCV core protein suppressed VLDL production and secretion from the liver by inhibiting microsomal triglyceride (TG) transfer protein<sup>[8,9]</sup>.

In clinical situations, chronic HCV infection alters serum lipid profiles by decreasing the low-density lipoprotein cholesterol (LDL-C) level<sup>[10]</sup> and the VLDL-TG/non-VLDL-TG ratio<sup>[11]</sup>. However, the abnormalities of lipoproteins as a whole in patients with chronic HCV infection have not been clarified. In particular, the abnormal distribution of TGs among lipoprotein subclasses has not been extensively studied, because TG content in each lipoprotein subclass cannot be measured easily by routine laboratory tests.

Cholesterol ester transfer protein (CETP) is a plasma glycoprotein that facilitates the transfer of cholesteryl ester (CE) from high-density lipoprotein (HDL) to other subclasses of lipoprotein [chylomicrons (CM), VLDL, and LDL]<sup>[12]</sup>. The principal effect of CETP on lipoproteins is considered to be the reduction of HDL-C levels and facilitation of reverse cholesterol transport to the liver<sup>[13]</sup>. Accordingly, CETP adjusts the distribution of TG among the different lipoprotein subclasses. Therefore, we speculated that CETP may play an important role in the abnormalities of lipoprotein metabolism in patients with active HCV infection.

In this study, we determined the serum concentration of CETP in patients with HCV infection and in those in whom HCV was eradicated, to determine the significance of CETP in HCV infection. Furthermore, we investigated the influence of CETP on lipoprotein abnormalities in HCV infection, with particular attention to TG concentrations.

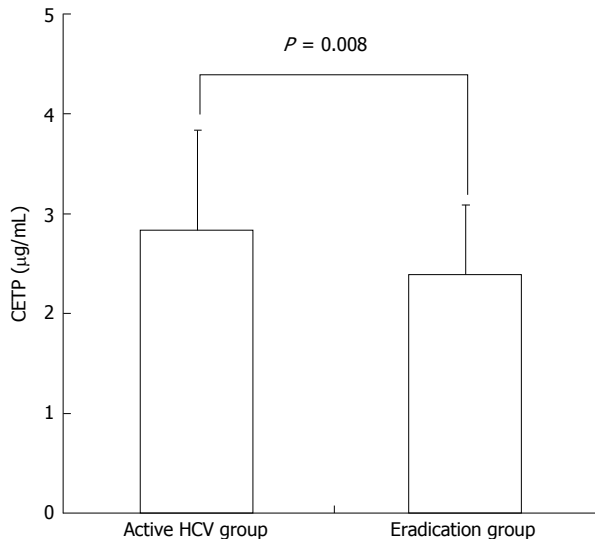
## MATERIALS AND METHODS

The protocol of this case control study was in accordance with the 2004 standards of the Declaration of Helsinki and current ethical guidelines, and was approved by the human ethics review committee of the Jikei University School of Medicine. Written informed consent was obtained from all patients who enrolled in this study.

### Patient population

Japanese patients with active chronic HCV infection (active HCV group) or successfully eradicated chronic HCV infection [negative serum HCV-RNA 6 mo after the end of interferon (IFN)-based therapy] (eradication group) who had been followed up at Jikei University Katsushika Medical Center between September 2013 and October 2014 were randomly considered for enrollment. Patients receiving treatment for diabetes (DM) or hyperlipidemia or hormone replacement therapy and those with hepatitis B virus or human immunodeficiency virus infection were excluded. Additionally, patients who had received IFN within 6 mo or who





**Figure 1** Comparison of the serum cholesteryl ester transfer protein level between the active hepatitis C virus infection group and the hepatitis C virus eradication group. The serum CETP level was significantly higher in patients with active HCV infection than those in whom HCV eradication was achieved ( $2.84 \pm 1.00$  µg/mL vs  $2.40 \pm 0.70$  µg/mL,  $P = 0.008$ ). CETP: Cholesterol ester transfer protein; HCV: Hepatitis C virus.

had been diagnosed with hepatocellular carcinoma or decompensated cirrhosis were excluded.

#### Laboratory tests and demographic data

Demographic data, including age, sex, and body mass index (BMI), and basic laboratory data were obtained from the medical records. The collected basic laboratory data included aspartate 2-oxoglutarate aminotransferase (AST), alanine 2-oxoglutarate aminotransferase (ALT), gamma-glutamyl transpeptidase ( $\gamma$ -GTP), albumin, total bilirubin, fasting blood glucose (FBG), and hemoglobin A1c (HbA1c) levels, hemoglobin (Hb) levels and the platelet count. In addition, basic serum lipid data, including total cholesterol, TG, HDL-cholesterol (HDL-C), and LDL-C were collected. HDL-C had been directly measured by a commercial kit (Kyowa Medex, Tokyo, Japan), and the LDL-C level had been calculated by the Friedewald equation.

#### CETP measurement

The CETP concentration was measured in sera collected after at least a 10-h overnight fast. The CETP mass concentration was measured using a sandwich enzyme-linked immunosorbent assay with two monoclonal antibodies specific to human CETP, JHC1, and JHC2, as previously described<sup>[14]</sup>. The assay was performed in duplicate, and the mean was adopted as the measured value<sup>[15]</sup>.

#### Measurement of cholesterol and TG concentration in the major classes of serum lipoproteins

To examine the distribution of cholesterol and TG in lipoprotein fractions, fresh sera from the collected patient samples were fractionated by high-performance liquid chromatography, and the cholesterol and TG

concentration in the major four lipoprotein classes was measured using the online detection system (Skylight Biotech, Inc., Akita, Japan)<sup>[16,17]</sup>. Serum lipoproteins were classified into four classes according to particle size: CM ( $> 80$  nm), VLDL (30–80 nm), LDL (16–30 nm), and HDL ( $< 16$  nm).

#### Biostatistics

A statistical review of the study was performed by a biomedical statistician.

#### Statistical analysis

Continuous data are expressed as mean  $\pm$  SD. Categorical data are expressed as numbers (%). We used Welch's *t* test or the  $\chi^2$  test for comparisons between the two groups. Correlations between two parameters were evaluated by the Pearson product-moment correlation coefficient. To determine the significance of HCV infection on the serum CETP level, multiple regression analysis was performed, with demographic and basic laboratory data including the HCV infection status (active infection or HCV eradication) as independent variables. The most suitable regression model for explanation of the serum CETP level was constructed by backward elimination of candidate variables.

We performed statistical analyses using STATISTICA software, version 6 (StatSoft Japan Inc. Tokyo, Japan), and two-tailed *P* values of  $\leq 0.05$  were considered significant; *P* values  $> 0.05$  but  $\leq 0.1$  were considered to indicate marginal significance. *P* values less than 0.001 are expressed as  $P < 0.001$ . We determined the multicollinearity of the multiple regression analysis to verify the reliability; the variance inflation factor was  $< 5$ , indicating that our models were reliable.

## RESULTS

#### Study population

In total, 110 patients were included in the study. Fifty-five had active HCV infection, and HCV eradication with IFN-based anti-viral therapy had been achieved in the remaining 55 patients. In the active HCV group, 48 (87%) had HCV genotype (G) 1b infection and 7 (13%) had HCV G2 infection. In the eradication group, 34 (62%) were previously infected with HCV G1b and 21 (38%) were previously infected with HCV G2.

#### Increase in the CETP level in active chronic HCV infection

The serum CETP level was significantly higher in the active HCV group than in the eradication group ( $2.84 \pm 1.00$  µg/mL vs  $2.40 \pm 0.70$  µg/mL,  $P = 0.008$ , Figure 1).

#### Characteristics of the active HCV group and the eradication group

The clinical features of patients in the active HCV group and those in the HCV eradication group are summarized in Table 1. There were significant differences in the proportion of patients with HCV G1b infection. There

**Table 1 Clinical features of patients with active hepatitis C virus infection (active hepatitis C virus group) and of those in whom hepatitis C virus infection was eradicated (eradication group) *n* (%)**

Discrete traits	Active HCV group ( <i>n</i> = 55)	Eradication group ( <i>n</i> = 55)	<i>P</i> value
Sex			0.254
Male	22 (40)	28 (51)	
Female	33 (60)	27 (49)	
HCV genotype			0.003
1b	48 (87)	36 (65)	
2	7 (13)	19 (35)	
Quantitative traits	Mean ± SD	Mean ± SD	
Age (yr)	66.9 ± 11.2	64.3 ± 12.1	0.200
BMI (kg/m <sup>2</sup> )	22.5 ± 3.2	22.8 ± 3.4	0.631
AST (IU/L)	53.1 ± 27.5	24.0 ± 7.2	< 0.001
ALT (IU/L)	49.9 ± 37.1	20.0 ± 11.7	< 0.001
Total bilirubin (mg/dL)	0.8 ± 0.3	0.7 ± 0.3	0.242
γ-GTP (IU/L)	50.4 ± 64	27.1 ± 20	0.011
Albumin (g/dL)	3.9 ± 0.4	4.4 ± 0.3	< 0.001
Hb (g/dL)	13.4 ± 1.7	14.2 ± 1.5	0.010
Platelet (10 <sup>4</sup> /μL)	15.0 ± 6.3	20.0 ± 17.8	0.050
FBG (mg/dL)	108 ± 32	107 ± 16	0.740
HbA1c (%)	5.4 ± 0.6	6.1 ± 0.5	0.030
Lipid profiles			
Total cholesterol (mg/dL)	173.6 ± 31	200.5 ± 37.8	< 0.001
Triglyceride (mg/dL)	107.5 ± 52.0	103 ± 53.1	0.712
LDL cholesterol (mg/dL)	92.1 ± 25.7	117 ± 29.9	< 0.001
HDL cholesterol (mg/dL)	59.9 ± 17.4	64.9 ± 19.0	0.159
CETP level (μg/mL)	2.84 ± 1.00	2.40 ± 0.69	0.008

There were significant differences in the proportion of patients with HCV G1b infection. There were no significant differences in BMI, FBG levels, and HbA1c levels, whereas AST, ALT, and albumin levels differed significantly. TC and LDL-C levels were significantly lower in the active infection group than in the eradication group, whereas TG and HDL-C levels were similar in the two groups. HCV: Hepatitis C virus; BMI: Body mass index; AST: Aspartate-2-oxoglutarate aminotransferase; ALT: Alanine-2-oxoglutarate aminotransferase; γ-GTP: Gamma-glutamyl transpeptidase; Hb: Hemoglobin; FBG: Fasting blood glucose; HbA1c: Glycosylated hemoglobin; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; CETP: Cholesterol ester transfer protein; LDL-C: Low-density lipoprotein cholesterol.

**Table 2 Influence of the hepatitis C virus infection status on the serum cholesterol ester transfer protein level as analyzed by multiple regression analysis**

	B	SE	<i>P</i> value
Constant	0.770	0.541	0.157
HCV infection	0.415	0.158	0.010
Age	0.014	0.006	0.042
Female sex	0.391	0.182	0.034
HDL	0.008	0.004	0.099

HCV infection status was an independent factor that significantly influenced the serum cholesterol ester transfer protein level. HCV: Hepatitis C virus; HDL: High-density lipoprotein.

were no significant differences in BMI, FBG levels, and HbA1c levels, whereas AST, ALT, and albumin levels differed significantly. TC and LDL-C levels were significantly lower in patients with active infection than in patients whose infection was eradicated, whereas TG and HDL-C levels were similar between the two groups.

#### **HCV infection status (active infection or eradication) as an independent determinant that significantly contributed to the serum CETP level**

To construct a multiple regression model that can suitably

explain the serum CETP concentration, six variables were selected as candidate independent variables. Among categorical data, HCV infection status and sex were selected, because the serum CETP level was significantly higher in female than in male patients ( $2.90 \pm 0.91 \mu\text{g/mL}$  vs  $2.28 \pm 0.73 \mu\text{g/mL}$ ,  $P < 0.001$ ). However, difference in HCV genotype was not selected as a candidate variable because there was no difference in serum CETP level between HCV G1b and G2 patients ( $2.51 \pm 0.68$  vs  $2.66 \pm 0.94$ ,  $P = 0.472$ ). Furthermore, the serum CETP level was similar between HCV G1b patients and G2 patients in the active group ( $2.82 \pm 1.02$  vs  $2.99 \pm 0.76$ ,  $P = 0.685$ ) and the eradication group ( $2.42 \pm 0.75$  vs  $2.36 \pm 0.57$ ,  $P = 0.734$ ).

Among the continuous variables, age ( $R = 0.246$ ,  $P = 0.010$ ), albumin level ( $R = -0.194$ ,  $P = 0.046$ ), Hb level ( $R = 0.249$ ,  $P = 0.009$ ), and HDL-C level ( $R = 0.236$ ,  $P = 0.014$ ) were significantly correlated with the serum CETP level and were thus selected as candidates. Other factors including HbA1c ( $R = 0.084$ ,  $P = 0.538$ ) and FBS ( $R = 0.074$ ,  $P = 0.589$ ) were not selected as candidates, because significant correlation was not verified.

Of these six candidates, HCV infection status, age, sex, and HDL-C level were selected as independent

**Table 3** Comparison of the cholesterol and triglyceride concentrations in the four major lipoprotein fractions according to hepatitis C virus infection status

Major class	Active HCV group	Eradication group	P value	Active HCV group	Eradication group	P value
	Cholesterol (mg/dL)			Triglyceride (mg/dL)		
CM	0.19 ± 0.19	0.19 ± 0.18	0.86	1.21 ± 1.53	1.28 ± 1.31	0.774
VLDL	29.04 ± 12.31	26.12 ± 10.66	0.187	57.66 ± 42.06	65.97 ± 44.18	0.314
LDL	87.94 ± 24.11	117.31 ± 26.91	< 0.001	36.25 ± 15.28	28.14 ± 9.94	0.001
HDL	49.58 ± 15.19	55.19 ± 16.29	0.064	25.9 ± 7.34	17.17 ± 4.82	< 0.001

The cholesterol concentration in LDL-C was significantly lower and HDL-C was marginally lower in the active HCV group, whereas cholesterol concentrations in CM and VLDL were similar between groups. The TG concentrations in LDL-TG and HDL-TG were significantly higher in the active HCV group, whereas TG concentrations in CM and VLDL were similar between groups. HCV: Hepatitis C virus; CM: Chylomicron; VLDL: Very-low-density lipoprotein; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglyceride.

variables for the most suitable multiple regression model to explain the serum CETP level. As shown in Table 2, HCV infection status was an independent factor that significantly influenced the serum CETP level. The R<sup>2</sup> value of this model was 0.221, and the adjusted value was 0.191. The R<sup>2</sup> value did not improve significantly by the addition of any other candidate factors. However, elimination of any of these four factors significantly decreased the R<sup>2</sup> values.

The most suitable regression equation was as follows: Serum CETP (μg/mL) = 0.770 + 0.0139 (age) + 0.391 (sex: male: 0, female: 1) + 0.00818 HDL-C (mg/dL) + 0.416 HCV infection status (eradication: 0, active infection: 1).

#### **Differences in the TG concentration in the four major classes of lipoproteins according to HCV infection status**

The cholesterol concentration in LDL-C was significantly lower and the HDL-C concentration was marginally lower in the active HCV group compared to those in the eradication group, whereas cholesterol concentrations in CM and VLDL were similar between the groups.

The TG concentrations in LDL-TG and HDL-TG were significantly higher in the active HCV group compared to the eradication group, whereas TG concentrations in CM and VLDL were similar between groups (Table 3).

#### **Correlation between the serum CETP concentration and TG concentration in the four major lipoprotein classes**

The CETP level had a weak, inverse correlation with CM-C (R = -0.290, P = 0.031) in the eradication group; it had a positive correlation with HDL-C in the active HCV group (R = 0.324, P = 0.015), but this correlation was marginal in the eradication group (R = 0.250, P = 0.064). Significant correlations were not found between groups for the other lipoprotein fractions (Figure 2A).

According to the TG concentration, the CETP level was inversely correlated with chylomicron-TG (R = -0.348, P = 0.009) and VLDL-TG (R = -0.415, P = 0.002) and marginally correlated with LDL-TG (R = -0.247, P = 0.069), but was not correlated with HDL-TG (R = -0.079, P = 0.566) in the eradication group. In contrast, the CETP level was strongly correlated with HDL-TG in

the active HCV group (R = 0.557, P < 0.001). However, significant correlations with TG for other lipoprotein classes were not detected in the active HCV group (Figure 2B).

## **DISCUSSION**

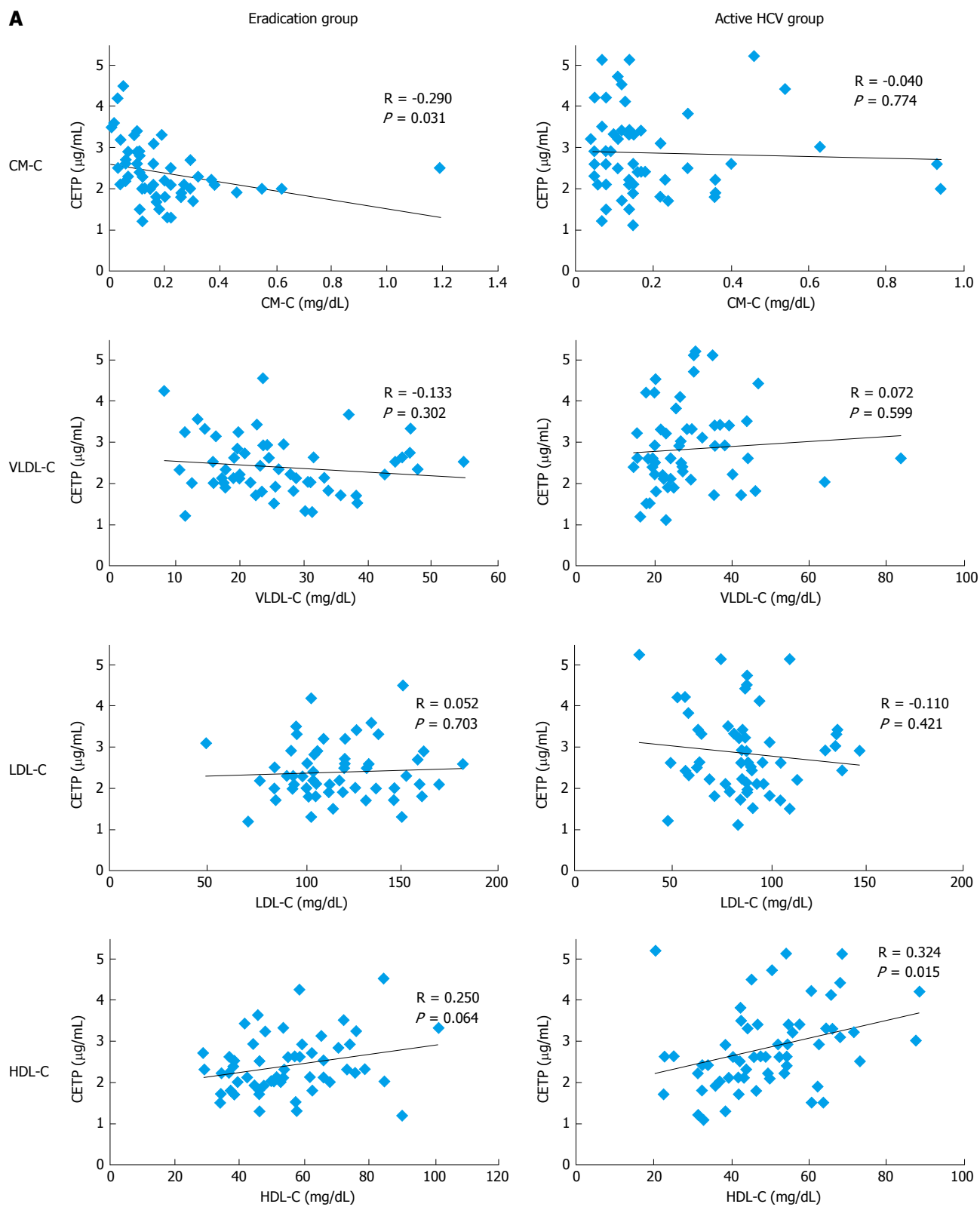
CETP is a glycoprotein that mediates the exchange of CE in HDL for TG in other lipoproteins. In the present study, we investigated the serum concentration of CETP and found that serum CETP levels were significantly higher in the active group than in the eradication group. Moreover, HCV infection was found to be an independent factor in determining the serum CETP level in multiple regression analysis. This suggests that HCV infection promotes the exchange of CE for TG in HDL by increasing the serum CETP concentration.

Although we did not evaluate the activity of CETP, it has generally been accepted that the serum CETP concentration reflects CETP activity<sup>[18]</sup>. Thus, an increase in the CETP level in patients with active HCV infection may indicate enhanced CETP activity.

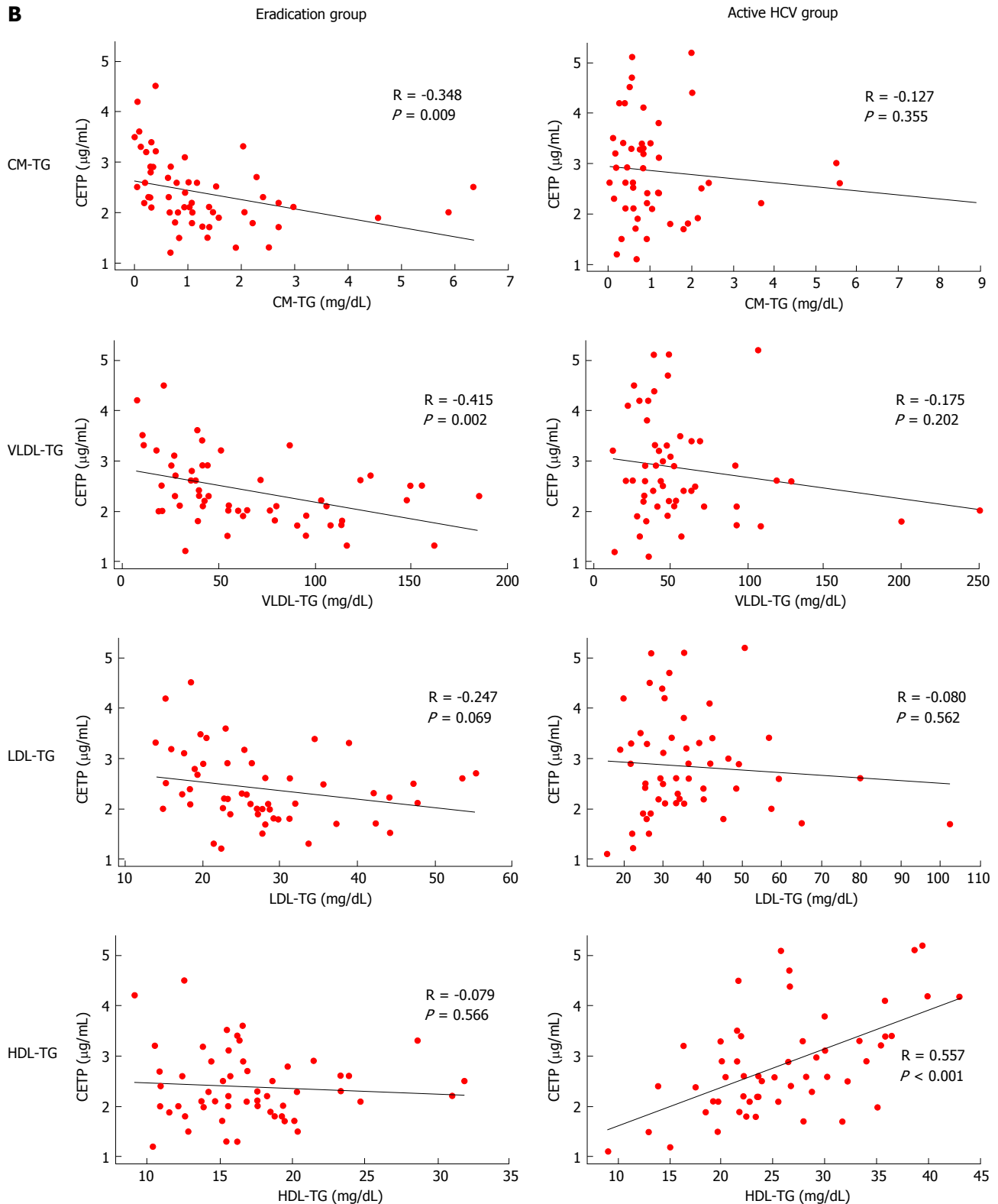
CETP activity is regulated by the amount of dietary cholesterol<sup>[19,20]</sup> and hypertriglyceridemia<sup>[21]</sup>. In addition, CETP activity is dependent on genetic variations in the CETP gene<sup>[22-24]</sup>. However, the effect of HCV infection on the activity of CETP has not been investigated previously. Our results indicate that active HCV infection may promote CETP activity. This enhanced CETP activity may play a role in lipoprotein metabolism abnormalities in patients with active HCV infection<sup>[22-24]</sup>.

An unexpected finding in our study was a positive correlation between the serum CETP level and HDL-C, although the correlation was relatively weak and did not reach the level of significance in the eradication group. One of the major functions of CETP is the removal of CE from HDL. In fact, CETP inhibitors substantially increase HDL-C levels and moderately decrease LDL-C levels in humans<sup>[25]</sup>. There may be a weak negative correlation between CETP and HDL-C in patients with type 2 DM<sup>[26]</sup>. However, the correlation between serum level of HDL-C and CETP in a healthy population has not been observed<sup>[27]</sup>. Therefore, the HDL-C level is not simply determined by the function of CETP because the serum

**A**





**B**

**Figure 2** Correlation between the cholesteryl ester transfer protein level and triglyceride concentration in the four major lipoprotein classes in the hepatitis C virus eradication group and the active hepatitis C virus infection group. A: The CETP level had a weak correlation with HDL cholesterol in the active HCV infection group ( $R = 0.324$ ,  $P = 0.015$ ) and a weak, inverse correlation with chylomicron-cholesterol ( $R = -0.290$ ,  $P = 0.031$ ); B: The CETP level had a strong correlation with HDL-TG in the active HCV infection group ( $R = 0.557$ ,  $P < 0.001$ ). However, significant correlations with TG for other lipoprotein classes were not detected in the active HCV group. HCV: Hepatitis C virus; CM: Chylomicron; VLDL: Very-low-density lipoprotein; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglyceride.

level of HDL-C may be dynamically controlled by the balance between HDL synthesis and catabolism, which is not mediated by CETP.

Although there is a consensus that a decrease in serum TC<sup>[28]</sup> and LDL-C<sup>[10]</sup> is a feature of HCV infection, little is known about TG abnormalities. A previous study reported that chronic hepatitis C patients had a lower serum VLDL-TG/non-VLDL-TG ratio<sup>[11]</sup>. Moreover, there was a reported increase in the TG concentration in HDL and LDL in patients with active HCV infection, although the total serum TG level was similar in the active HCV and eradication groups<sup>[29]</sup>.

In a correlation study between CETP and TG concentration in four lipoprotein classes, we found positive and strong correlations between CETP and HDL-TG in the active HCV group. However, this correlation was not found in the eradication group. Conversely, CETP was correlated with CM-TG and VLDL-TG in the eradication group, but was not significantly correlated with CM-TG or VLDL-TG in the active HCV group. These findings indicate that the significance of CETP in the regulation of TG concentration differs according to the HCV infection status. The most striking difference was found in HDL-TG.

The major source of serum TG is a TG-enriched VLDL that is secreted from the liver. TG in VLDL or LDL is transferred to HDL by the action of CETP. In active HCV infection, an increased serum CETP may enhance the transport of TG to HDL. Therefore, the increase in CETP and the strong positive correlation between CETP and HDL-TG in active HCV infection may indicate that HDL has abundant TG, promptly transferred from VLDL, but is not effectively catabolized and eliminated from the serum. Accordingly, as the major metabolic pathway that degrades and eliminates TG in HDL is mediated by hepatic lipase (HL)<sup>[30]</sup>, we speculate that HL activity in active HCV infection is impaired. Our hypothesis of reduced HL activity in active HCV infection is concordant with a previous result that the HL messenger RNA level is lower in the liver of patients with chronic hepatitis C than in the liver of patients with other etiologies and similar disease progression<sup>[31]</sup>. As a consequence of the abnormal retention of TG in HDL, the multifaceted functions of HDL on atherosclerosis<sup>[32]</sup> may be affected, and this could contribute to the progression of atherosclerosis in patients with active HCV infection<sup>[33]</sup>. Furthermore, dyslipidemia, which is caused by high serum CETP activity in active HCV infection, may contribute to intravascular lipoviral particle formation and thus for sustaining HCV infection<sup>[34,35]</sup>.

Our study had some limitations. It included a relatively small sample size, and the degree of atherosclerosis was not determined in the enrolled patients. In addition, we did not examine lipoviral particle and non-lipoviral particle viral load in the active HCV patients. To strengthen our hypothesis that an increase of TG in HDL contributes to atherosclerosis, further large-scale studies including the evaluation of the anti-inflammatory<sup>[36,37]</sup> and proinflammatory<sup>[38]</sup> functions of HDL and measurement

of the degree of atherosclerosis are warranted.

In summary, HCV infection was an independent factor contributing to the increase in serum CETP. The increase in CETP resulted in abnormal retention of TG in HDL. These findings suggest that CETP is one of the factors that contribute to abnormal lipoprotein metabolism in patients with active HCV infection.

## COMMENTS

### Background

Hepatitis C virus (HCV) is a unique virus; its use of host lipid metabolism results in a persistent infection. It is important to understand how HCV uses host lipid metabolism and how host lipid metabolism is affected by HCV infection, because HCV infection represents a unique model in which the virus causes chronic infection while coexisting with the host by taking over the host's metabolism.

### Research frontiers

The effect of HCV infection on the activity of cholesteryl ester transfer protein (CETP) has not been investigated previously. The authors have been the first to clarify that CETP may be increased with HCV.

### Innovations and breakthroughs

The authors confirmed that CETP plays a role in abnormal lipoprotein metabolism in patients with HCV infection.

### Applications

An increase of triglyceride (TG) in high-density lipoprotein (HDL) as a consequence of activated CETP may contribute to progression of atherosclerosis in HCV infection. Furthermore, disturbed lipoprotein metabolism induced by activated CETP may contribute to intravascular formation of HCV lipoviral particles.

### Terminology

CETP is a glycoprotein mediating the exchange of cholesteryl ester in HDL for TG in other lipoproteins.

### Peer-review

In this study, the authors found that HCV infection was an independent factor contributing to the increase in serum CETP, the increase in CETP resulted in abnormal retention of TG in HDL. These findings suggest that CETP is one of the factors that contribute to abnormal lipoprotein metabolism in patients with active HCV infection. This study has scientific basis and is interesting.

## REFERENCES

- 1 Seeff LB. Natural history of chronic hepatitis C. *Hepatology* 2002; **36**: S35-S46 [PMID: 12407575 DOI: 10.1002/hep.1840360706]
- 2 Hoofnagle JH. Course and outcome of hepatitis C. *Hepatology* 2002; **36**: S21-S29 [PMID: 12407573 DOI: 10.1002/hep.1840360704]
- 3 Casiraghi MA, De Paschale M, Romanò L, Biffi R, Assi A, Binelli G, Zanetti AR. Long-term outcome (35 years) of hepatitis C after acquisition of infection through mini transfusions of blood given at birth. *Hepatology* 2004; **39**: 90-96 [PMID: 14752827 DOI: 10.1002/hep.20030]
- 4 Bassendine MF, Sheridan DA, Bridge SH, Felmlee DJ, Neely RD. Lipids and HCV. *Semin Immunopathol* 2013; **35**: 87-100 [PMID: 23111699 DOI: 10.1007/s00281-012-0356-2]
- 5 Barth H, Liang TJ, Baumert TF. Hepatitis C virus entry: molecular biology and clinical implications. *Hepatology* 2006; **44**: 527-535 [PMID: 16941688 DOI: 10.1002/hep.21321]
- 6 Scholtes C, Ramière C, Rainteau D, Perrin-Cocon L, Wolf C, Humbert L, Carreras M, Guironnet-Paquet A, Zoulim F, Bartschlag R, Lotteau V, André P, Diaz O. High plasma level of nucleocapsid-free envelope glycoprotein-positive lipoproteins in

- hepatitis C patients. *Hepatology* 2012; **56**: 39-48 [PMID: 22290760 DOI: 10.1002/hep.25628]
- 7 **Huang H**, Sun F, Owen DM, Li W, Chen Y, Gale M, Ye J. Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low-density lipoproteins. *Proc Natl Acad Sci USA* 2007; **104**: 5848-5853 [PMID: 17376867 DOI: 10.1073/pnas.0700760104]
- 8 **Perlemuter G**, Sabile A, Letteron P, Vona G, Topilco A, Chrétien Y, Koike K, Pessayre D, Chapman J, Barba G, Bréchet C. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *FASEB J* 2002; **16**: 185-194 [PMID: 11818366 DOI: 10.1096/fj.01-0396com]
- 9 **Yamaguchi A**, Tazuma S, Nishioka T, Ohishi W, Hyogo H, Nomura S, Chayama K. Hepatitis C virus core protein modulates fatty acid metabolism and thereby causes lipid accumulation in the liver. *Dig Dis Sci* 2005; **50**: 1361-1371 [PMID: 16047488 DOI: 10.1007/s10620-005-2788-1]
- 10 **Petit JM**, Benichou M, Duvillard L, Jooste V, Bour JB, Minello A, Verges B, Brun JM, Gambert P, Hillon P. Hepatitis C virus-associated hypobetalipoproteinemia is correlated with plasma viral load, steatosis, and liver fibrosis. *Am J Gastroenterol* 2003; **98**: 1150-1154 [PMID: 12809841]
- 11 **Nishimura M**, Yamamoto H, Yoshida T, Seimiya M, Sawabe Y, Matsushita K, Umemura H, Sogawa K, Takizawa H, Yokosuka O, Nomura F. Decreases in the serum VLDL-TG/non-VLDL-TG ratio from early stages of chronic hepatitis C: alterations in TG-rich lipoprotein levels. *PLoS One* 2011; **6**: e17309 [PMID: 21364889 DOI: 10.1371/journal.pone.0017309]
- 12 **Kuivenhoven JA**, Jukema JW, Zwinderman AH, de Krijff P, McPherson R, Bruschke AV, Lie KI, Kastelein JJ. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. *N Engl J Med* 1998; **338**: 86-93 [PMID: 9420339 DOI: 10.1056/NEJM199801083380203]
- 13 **deGoma EM**, deGoma RL, Rader DJ. Beyond high-density lipoprotein cholesterol levels evaluating high-density lipoprotein function as influenced by novel therapeutic approaches. *J Am Coll Cardiol* 2008; **51**: 2199-2211 [PMID: 18534265 DOI: 10.1016/j.jacc.2008.03.016]
- 14 **Nagano M**, Yamashita S, Hirano K, Kujiraoka T, Ito M, Sagehashi Y, Hattori H, Nakajima N, Maruyama T, Sakai N, Egashira T, Matsuzawa Y. Point mutation (-69 G--> A) in the promoter region of cholesteryl ester transfer protein gene in Japanese hyperalphalipoproteinemic subjects. *Arterioscler Thromb Vasc Biol* 2001; **21**: 985-990 [PMID: 11397708 DOI: 10.1161/01.ATV.21.6.985]
- 15 **Nagano M**, Yamashita S, Hirano K, Ito M, Maruyama T, Ishihara M, Sagehashi Y, Oka T, Kujiraoka T, Hattori H, Nakajima N, Egashira T, Kondo M, Sakai N, Matsuzawa Y. Two novel missense mutations in the CETP gene in Japanese hyperalphalipoproteinemic subjects: high-throughput assay by Invader assay. *J Lipid Res* 2002; **43**: 1011-1018 [PMID: 12091484 DOI: 10.1194/jlr.M200024-JLR200]
- 16 **Usui S**, Hara Y, Hosaki S, Okazaki M. A new on-line dual enzymatic method for simultaneous quantification of cholesterol and triglycerides in lipoproteins by HPLC. *J Lipid Res* 2002; **43**: 805-814 [PMID: 11971952]
- 17 **Okazaki M**, Usui S, Ishigami M, Sakai N, Nakamura T, Matsuzawa Y, Yamashita S. Identification of unique lipoprotein subclasses for visceral obesity by component analysis of cholesterol profile in high-performance liquid chromatography. *Arterioscler Thromb Vasc Biol* 2005; **25**: 578-584 [PMID: 15637308 DOI: 10.1161/01.ATV.0000155017.60171.88]
- 18 **Yamashita S**, Hui DY, Wetterau JR, Sprecher DL, Harmony JA, Sakai N, Matsuzawa Y, Tarui S. Characterization of plasma lipoproteins in patients heterozygous for human plasma cholesteryl ester transfer protein (CETP) deficiency: plasma CETP regulates high-density lipoprotein concentration and composition. *Metabolism* 1991; **40**: 756-763 [PMID: 1870431 DOI: 10.1016/0026-0495(91)90097-G]
- 19 **Quinet EM**, Agellon LB, Kroon PA, Marcel YL, Lee YC, Whitlock ME, Tall AR. Atherogenic diet increases cholesteryl ester transfer protein messenger RNA levels in rabbit liver. *J Clin Invest* 1990; **85**: 357-363 [PMID: 2298910 DOI: 10.1172/JCI114446]
- 20 **Jiang XC**, Moulin P, Quinet E, Goldberg IJ, Yacoub LK, Agellon LB, Compton D, Schnitzer-Polokoff R, Tall AR. Mammalian adipose tissue and muscle are major sources of lipid transfer protein mRNA. *J Biol Chem* 1991; **266**: 4631-4639 [PMID: 1999438]
- 21 **Rashid S**, Sniderman A, Melone M, Brown PE, Otvos JD, Mente A, Schulze K, McQueen MJ, Anand SS, Yusuf S. Elevated cholesteryl ester transfer protein (CETP) activity, a major determinant of the atherogenic dyslipidemia, and atherosclerotic cardiovascular disease in South Asians. *Eur J Prev Cardiol* 2015; **22**: 468-477 [PMID: 24659026 DOI: 10.1177/2047487314528461]
- 22 **Nakamura A**, Niimura H, Kuwabara K, Takezaki T, Morita E, Wakai K, Hamajima N, Nishida Y, Turin TC, Suzuki S, Ohnaka K, Uemura H, Ozaki E, Hosono S, Mikami H, Kubo M, Tanaka H. Gene-gene combination effect and interactions among ABCA1, APOA1, SR-B1, and CETP polymorphisms for serum high-density lipoprotein-cholesterol in the Japanese population. *PLoS One* 2013; **8**: e82046 [PMID: 24376512 DOI: 10.1371/journal.pone.0082046]
- 23 **Suhly A**, Hartmann K, Papp AC, Wang D, Sadee W. Regulation of cholesteryl ester transfer protein expression by upstream polymorphisms: reduced expression associated with rs247616. *Pharmacogenet Genomics* 2015; **25**: 394-401 [PMID: 26061659 DOI: 10.1097/FPC.0000000000000151]
- 24 **Zhong S**, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD, Tall AR. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. *J Clin Invest* 1996; **97**: 2917-2923 [PMID: 8675707 DOI: 10.1172/JCI118751]
- 25 **Barter PJ**, Kastelein JJ. Targeting cholesteryl ester transfer protein for the prevention and management of cardiovascular disease. *J Am Coll Cardiol* 2006; **47**: 492-499 [PMID: 16458126 DOI: 10.1016/j.jacc.2005.09.042]
- 26 **Inukai Y**, Ito K, Hara K, Yamazaki A, Takebayashi K, Aso Y, Inukai T. Serum cholesteryl ester transfer protein concentrations are associated with serum levels of total cholesterol, beta-lipoprotein and apoproteins in patients with type 2 diabetes mellitus. *Med Princ Pract* 2007; **16**: 367-372 [PMID: 17709925 DOI: 10.1159/000104810]
- 27 **Jones RJ**, Owens D, Brennan C, Collins PB, Johnson AH, Tomkin GH. Increased esterification of cholesterol and transfer of cholesteryl ester to apo B-containing lipoproteins in Type 2 diabetes: relationship to serum lipoproteins A-I and A-II. *Atherosclerosis* 1996; **119**: 151-157 [PMID: 8808492 DOI: 10.1016/0021-9150(95)05639-4]
- 28 **Maggi G**, Bottelli R, Gola D, Perricone G, Posca M, Zavaglia C, Ideo G. Serum cholesterol and chronic hepatitis C. *Ital J Gastroenterol* 1996; **28**: 436-440 [PMID: 9032585]
- 29 **Nagano T**, Seki N, Tomita Y, Sugita T, Aida Y, Itagaki M, Sutoh S, Abe H, Tsubota A, Aizawa Y. Impact of Chronic Hepatitis C Virus Genotype 1b Infection on Triglyceride Concentration in Serum Lipoprotein Fractions. *Int J Mol Sci* 2015; **16**: 20576-20594 [PMID: 26334270 DOI: 10.3390/ijms160920576]
- 30 **Clay MA**, Newnham HH, Barter PJ. Hepatic lipase promotes a loss of apolipoprotein A-I from triglyceride-enriched human high density lipoproteins during incubation in vitro. *Arterioscler Thromb* 1991; **11**: 415-422 [PMID: 1900192 DOI: 10.1161/01.ATV.11.2.415]
- 31 **Shinohara Y**, Imajo K, Yoneda M, Tomeno W, Ogawa Y, Fujita K, Kirikoshi H, Takahashi J, Funakoshi K, Ikeda M, Kato N, Nakajima A, Saito S. Hepatic triglyceride lipase plays an essential role in changing the lipid metabolism in genotype 1b hepatitis C virus replicon cells and hepatitis C patients. *Hepatol Res* 2013; **43**: 1190-1198 [PMID: 23607715 DOI: 10.1111/hepr.12072]
- 32 **Ansell BJ**, Watson KE, Fogelman AM, Navab M, Fonarow GC. High-density lipoprotein function recent advances. *J Am Coll Cardiol* 2005; **46**: 1792-1798 [PMID: 16286161]
- 33 **Olubamwo OO**, Onyeka IN, Miettola J, Kauhanen J, Tuomainen

- TP. Hepatitis C as a risk factor for carotid atherosclerosis - a systematic review. *Clin Physiol Funct Imaging* 2015; Epub ahead of print [PMID: 25620553 DOI: 10.1111/cpf.12229]
- 34 **Felmlee DJ**, Sheridan DA, Bridge SH, Nielsen SU, Milne RW, Packard CJ, Caslake MJ, McLauchlan J, Toms GL, Neely RD, Bassendine MF. Intravascular transfer contributes to postprandial increase in numbers of very-low-density hepatitis C virus particles. *Gastroenterology* 2010; **139**: 1774-1783, 1783.e1-6 [PMID: 20682323 DOI: 10.1053/j.gastro.2010.07.047]
  - 35 **Bridge SH**, Sheridan DA, Felmlee DJ, Nielsen SU, Thomas HC, Taylor-Robinson SD, Neely RD, Toms GL, Bassendine MF. Insulin resistance and low-density apolipoprotein B-associated lipoviral particles in hepatitis C virus genotype 1 infection. *Gut* 2011; **60**: 680-687 [PMID: 20940286 DOI: 10.1136/gut.2010.222133]
  - 36 **Nofer JR**, Kehrel B, Fobker M, Levkau B, Assmann G, von Eckardstein A. HDL and arteriosclerosis: beyond reverse cholesterol transport. *Atherosclerosis* 2002; **161**: 1-16 [PMID: 11882312]
  - 37 **Zhang H**, Reilly MP. Anti-inflammatory effects of high-density lipoprotein through activating transcription factor 3: benefit beyond cholesterol transport-dependent processes. *Arterioscler Thromb Vasc Biol* 2014; **34**: e11-e12 [PMID: 24743432 DOI: 10.1161/ATVBAHA.114.303553]
  - 38 **Fogelman AM**. When good cholesterol goes bad. *Nat Med* 2004; **10**: 902-903 [PMID: 15340411]

**P- Reviewer:** Felmlee DJ, Wang Y, Zeng Z **S- Editor:** Song XX

**L- Editor:** A **E- Editor:** Liu SQ





Prospective Study

# Blood DNA methylation markers in prospectively identified hepatocellular carcinoma cases and controls from Taiwan

Hui-Chen Wu, Jing Shen, Hwai-I Yang, Wei-Yann Tsai, Chien-Jen Chen, Regina M Santella

Hui-Chen Wu, Jing Shen, Regina M Santella, Department of Environmental Health Sciences, Mailman School of Public Health of Columbia University, New York, NY 10032, United States

Hwai-I Yang, Chien-Jen Chen, Genomics Research Center, Academia Sinica, Taipei 11529, Taiwan

Hwai-I Yang, Graduate Institute of Clinical Medical Science, China Medical University, Taichung 40402, Taiwan

Hwai-I Yang, Molecular and Genomic Epidemiology Center, China Medical University Hospital, Taichung 40402, Taiwan

Wei-Yann Tsai, Departments of Biostatistics, Mailman School of Public Health of Columbia University, New York, NY 10032, United States

Wei-Yann Tsai, Department of Statistics, National Chen Kung University, Tainan 70101, Taiwan

Chien-Jen Chen, Graduate Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei 10617, Taiwan

Regina M Santella, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY 10032, United States

**Author contributions:** Wu HC analyzed the data and drafted the manuscript; Shen J generated the 450k array data and helped to select the genes to evaluate; Yang HI coordinated the followup of the cohort; Tsai WY reviewed the data analysis; Chen CJ designed the cohort and supervises all projects using samples; Santella RM designed the study; all authors reviewed the manuscript.

**Supported by** National Institutes of Health grants, RO1ES005116 (Santella RM) and P30ES009089 (Santella RM).

**Institutional review board statement:** The study was reviewed and approved by the Columbia University Medical Center Institutional Review Board.

**Informed consent statement:** All study participants provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** There are no conflicts for all authors.

**Data sharing statement:** Detailed data is available from the corresponding author.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Hui-Chen Wu, Department of Environmental Health Sciences, Mailman School of Public Health of Columbia University, 650 West 168<sup>th</sup> St, New York, NY 10032, United States. [hw2057@columbia.edu](mailto:hw2057@columbia.edu)  
 Telephone: +1-212-3058158  
 Fax: +1-212-3053857

**Received:** March 14, 2015  
**Peer-review started:** March 16, 2015  
**First decision:** April 10, 2015  
**Revised:** January 8, 2016  
**Accepted:** January 21, 2016  
**Article in press:** January 22, 2016  
**Published online:** February 18, 2016

## Abstract

**AIM:** To determine if gene-specific DNA methylation in prospectively collected blood samples is associated with later development of hepatocellular carcinoma (HCC).

**METHODS:** Comparing genome-wide DNA methylation profiles using Illumina Human methylation 450K arrays, we previously identified a list of loci that were differentially methylated between tumor and adjacent nontumor tissues. To examine if dysregulation of DNA

methylation patterns observed in tumor tissues can be detected in white blood cell (WBC) DNA, we conducted a prospective case-control study nested within a community-based cancer screening cohort in Taiwan with 16 years of follow up. We measured methylation levels in ninety-six loci that were aberrant in DNA methylation in HCC tumor tissues compared to adjacent tissues. Baseline WBC DNA from 159 HCC cases and 312 matched controls were bisulfite treated and assayed by Illumina BeadArray. We used the  $\chi^2$  test for categorical variables and student's *t*-test for continuous variables to assess the difference in selected characteristics between cases and controls. To estimate associations with HCC risk, we used conditional logistic regression models stratified on the matching factors to calculate odds ratios (OR) and 95%CI.

**RESULTS:** We found that high methylation level in cg10272601 in *WNK2* was associated with increased risk of HCC, with an OR of 1.91 (95%CI: 1.27-2.86). High methylation levels in both cg12680131 in *TPO* and cg22511877 in *MYT1L*, however, were associated with decreased risk. The ORs (95%CI) were 0.59 (0.39-0.87) and 0.50 (0.33-0.77), respectively, for those with methylation levels of cg12680131 and cg22511877 above the median compared with those with levels below the median. These associations were still statistically significant in multivariable conditional logistic regression models after adjusting for hepatitis B virus infection and alcohol consumption.

**CONCLUSION:** These findings support the measurement of methylation markers in WBC DNA as biomarkers of HCC susceptibility but should be replicated in additional prospective studies.

**Key words:** DNA methylation; Epigenetics; Hepatitis B virus; Hepatocellular carcinoma; White blood cell DNA

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatocellular carcinoma (HCC) is a highly fatal disease thus, the identification of biomarkers that could predict risk for development could enhance screening/early detection and prognosis. DNA methylation alterations are well established in HCC but whether changes in DNA methylation in white blood cells (WBC) are associated with increased risk of developing HCC is unknown. Taking advantage of a cancer screening program in Taiwan, we measured baseline WBC DNA methylation in prospectively collected blood samples at 96 CpG sites that were identified as differentially methylated in HCC tumors compared to adjacent tissues. Three were significantly associated with later development of HCC suggesting potential utility as a marker of risk.

Wu HC, Shen J, Yang HI, Tsai WY, Chen CJ, Santella RM. Blood DNA methylation markers in prospectively identified hepatocellular carcinoma cases and controls from Taiwan. *World*

*J Hepatol* 2016; 8(5): 301-306 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i5/301.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i5.301>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is among the most common cancers around the world<sup>[1]</sup>. Hepatitis B and C virus infection are the most important risk factors of HCC<sup>[2-4]</sup>. More recent studies have also identified the importance of exposure to alcohol, dietary aflatoxins and cigarette smoke<sup>[5-7]</sup>.

The mechanisms of liver cancer induction are now known to include mutations in specific genes and epigenetic alterations such as changes in DNA methylation and microRNA expression. These changes lead to changes in expression of oncogenes and tumor suppressor genes<sup>[8-10]</sup>. DNA hypermethylation can silence tumor suppressor genes while hypomethylation can activate oncogenes<sup>[11,12]</sup>. Using Illumina HumanMethylation 27K and 450K BeadChips, we previously reported a distinct DNA methylation pattern between HCC tumor and paired adjacent nontumor tissues (NCBI's GEO database accession numbers GSE54503 and GSE37988)<sup>[13,14]</sup>. In one of the studies, we found 28017 CpG sites hypermethylated and 102495 hypomethylated in tumor tissues compared with paired adjacent tissues<sup>[14]</sup>, suggesting their role in HCC tumorigenesis.

Using data on baseline white blood cell (WBC) DNA banked up to 16 years before diagnosis, we recently reported that global hypomethylation of Sat2, a repetitive element, was associated with increased HCC risk<sup>[15]</sup> and was also associated with high AFB<sub>1</sub> exposure<sup>[16]</sup>. These results suggest that decreased overall DNA methylation in WBC DNA can be used as a biomarker for HCC risk.

The main aim of this study was to examine whether the dysregulation of DNA methylation markers observed in tumor tissues can be detected in WBC DNA. We measured methylation levels in ninety-six loci in WBC DNA from 159 HCCs who developed cancer after enrollment in a community-based cancer screening program in Taiwan<sup>[5,6,15]</sup> and compared them with 312 controls who remained cancer free in the same cohort.

## MATERIALS AND METHODS

### Study population

This study included individuals who participated in a Cancer Screening Program cohort in Taiwan. This study was approved by both the Institutional Review Board of Columbia University and the Research Ethics Committee of the College of Public Health at National Taiwan University. We obtained written informed consent from all study subjects in this study.

Detail information regarding the cohort description and screening procedure and follow-up was provided in previous publications<sup>[5,6,15,16]</sup>. Between July 1990 and June 1992, 12020 males and 11924 females aged from

**Table 1 Sociodemographic characteristics of hepatocellular carcinoma cases and matched controls**

Variable	Cases <i>n</i> = 159	%	Controls <i>n</i> = 312	%	<i>P</i>
Age (yr, mean, SD)	52.8 (8.0)		53.1 (7.8)		0.72
BMI (mean, SD)	24.3 (3.6)		24.8 (3.7)		0.13
Gender					
Female	77	48	148	47	0.92
Male	82	52	164	53	
HBsAg					
Negative	65	41	238	76	< 0.0001
Positive	93	59	72	23	
Missing	1	< 1	2	< 1	
Anti-HCV					
Negative	109	69	243	78	< 0.0001
Positive	29	18	15	5	
Missing	21	13	54	17	
Smoking					
Never	97	61	184	59	0.67
Ever	62	39	128	41	
Alcohol					
Never	130	82	276	89	0.046
Ever	29	18	36	12	

HBsAg: Hepatitis B virus surface antigen; BMI: Body mass index; HCV: Hepatitis C virus.

30 to 65 years old and who lived in seven towns in Taiwan were enrolled in this study. Each participant filled out a structured questionnaire to collect information including demographic characteristics, history of alcohol intake and cigarette smoking, history of chronic disease and family history of cancers, including HCC. Each participant also donated a fasting blood sample during the time of recruitment.

In this study, we used blood collected from 159 participants who were diagnosed with HCC during the interval between their blood draw and June 2008. We also used blood from 312 controls who remained cancer free in the same cohort. Controls were selected by matching to each case by age (within 5 years), sex, residential area and time of recruitment (within 3 mo). Baseline WBCs were shipped to Columbia University on dry ice for DNA isolation and DNA methylation measurement.

#### DNA bisulfite conversion

We extracted genomic DNA from WBC using a salting out procedure. We bisulfite-treated an aliquot of DNA (500 ng) with EZ DNA methylation kits (Zymo Research, Orange, CA). The bisulfite DNA was resuspended in 20  $\mu$ L of distilled water and stored at -20 °C until use.

#### Loci selection and methylation measurement

We selected 96 CpG sites that previously had shown either hyper- or hypomethylation in HCC tumor compared to paired adjacent nontumor tissues in our 450k array data<sup>[14]</sup>. We selected our target CpG sites from among the top 250 most hyper or hypomethylated sites. Our selection of targets was based on the following criteria: (1) the largest methylation differences

between tumor and adjacent tissues; (2) half of the CpG sites showing hypomethylation and half hypermethylation; and (3) one site per gene. Due to the inability to design primers for some sites, we have 65 CpG sites with hypermethylation and 31 CpG sites with hypomethylation. DNA methylation analysis was measured using an Illumina GoldenGate assay with BeadArray technology. The arrays were customized to measure methylation covering the CpG sites identified in the 450k array. DNA methylation values were scored as  $\beta$ -values which ranges between 0 and 1.

#### Statistical analysis

We used the  $\chi^2$  test and/or student's *t*-test to assess the difference in selected variables between cases and controls. To estimate associations between methylation markers and HCC risk, we used a conditional logistic regression model using PROC PHREG procedure. Subjects were divided into different methylation groups: Those with methylation levels above the median value for all controls sample vs those below the median. In the multivariable model, we modeled the associations of methylation in cg10272601 in *WNK2*, cg12680131 in *TPO* and cg22511877 in *MYT1L* adjusting for, hepatitis B virus surface antigen (HBsAg) (Yes vs No), and history of alcohol intake (Ever vs Never) in the model. All analyses were performed with SAS software 9.2 (SAS Institute, Cary, NC).

## RESULTS

The distributions of subjects' characteristics at baseline for cases and matched controls is given in Table 1. The distributions of matching factors including age, sex were similar between cases and controls. There were 51.7% and 52.5% males in cases and controls, respectively. The distribution of smoking was also similar, while the percentage of ever alcohol consumption was slightly lower in controls (11.5%) than in cases (18.2%). The percents positive for HBsAg and anti-HCV were higher in cases than in matched controls [58.5% vs 23.1% for HBsAg (+) and 18.2% vs 4.8% for anti-HCV (+)].

Table 2 presents the distributions of the 96 methylation markers by HCC status. The mean values of methylation vary by methylation markers. Fifty DNA methylation markers had mean methylation values below 10% in cases and controls. Nineteen DNA methylation markers had mean methylation values above 90%. About 27 DNA methylation markers had mean methylation levels between 10% and 90%. The mean levels of three DNA methylation markers were statistically significantly different between cases and controls, including cg10272601, cg12680131, and cg22511877. The mean methylation beta values for cg1027261 were  $0.30 \pm 0.07$  for cases and  $0.28 \pm 0.08$  for controls ( $P = 0.04$ ). Values for cg12680131 were  $0.80 \pm 0.09$  and  $0.82 \pm 0.11$  for cases and controls, respectively ( $P = 0.02$ ) and for cg22511877,  $0.56 \pm 0.17$  for cases and  $0.60 \pm 0.16$  for controls ( $P = 0.01$ ).

**Table 2** Distribution of DNA methylation by hepatocellular carcinoma status

Locus	Gene	HCC cases		Controls		<i>P</i> <sup>1</sup>
		Mean	SD	Mean	SD	
cg00028598	GABRA5	0.92	0.04	0.92	0.07	0.81
cg00108164	ACP1	0.01	0.02	0.00	0.01	0.55
cg00249511	SCT	0.01	0.04	0.01	0.04	0.80
cg00753478	LDHB	0.09	0.08	0.08	0.06	0.12
cg00817367	GRASP	0.01	0.04	0.01	0.01	0.23
cg00939495	DRD5	0.22	0.10	0.22	0.12	0.95
cg01530024	STK32B	0.97	0.08	0.97	0.07	0.79
cg01566592	RIMS2	0.10	0.09	0.09	0.08	0.32
cg01860297	BASP1	0.96	0.03	0.95	0.08	0.49
cg02527669	OBSL1	0.02	0.02	0.03	0.05	0.53
cg02553663	SECTM1	0.03	0.04	0.03	0.03	0.65
cg02710296	C1orf14	0.33	0.11	0.33	0.11	0.92
cg02736548	FAM109B	0.08	0.09	0.08	0.09	0.46
cg03306486	APC2	0.02	0.02	0.01	0.02	0.44
cg03396005	APCDD1	0.92	0.04	0.92	0.06	0.99
cg03621881	BRUNOL6	0.04	0.05	0.03	0.04	0.70
cg04920951	GSTP1	0.01	0.07	0.00	0.02	0.22
cg05328339	PTPRN2	0.89	0.09	0.88	0.10	0.55
cg05661282	ZNF154	0.03	0.05	0.03	0.08	0.75
cg05699035	KCNK2	0.86	0.07	0.86	0.08	0.99
cg05833351	CUGBP2	0.95	0.07	0.95	0.08	0.70
cg05970721	HS3ST2	0.90	0.10	0.91	0.10	0.49
cg06382344	TBR1	0.02	0.03	0.03	0.05	0.13
cg06445348	ILDR2	0.02	0.06	0.01	0.01	0.24
cg06641285	TIMP2	0.02	0.02	0.02	0.05	0.74
cg07061738	SMOC2	0.94	0.08	0.94	0.11	0.75
cg07689503	MTHFD2	0.00	0.00	0.00	0.01	0.27
cg07759394	GLB1L2	0.01	0.03	0.01	0.02	0.44
cg07765706	KCNQ3	0.95	0.03	0.95	0.08	0.12
cg08328777	DUOX1	0.07	0.05	0.07	0.06	0.30
cg08714590	FZD1	0.86	0.12	0.86	0.12	0.43
cg08738570	C1orf70	0.09	0.10	0.09	0.08	0.69
cg09210956	SNTG2	0.67	0.09	0.67	0.12	0.93
cg09433131	KCNB2	0.94	0.06	0.93	0.10	0.43
cg09489445	ZNF788	0.01	0.03	0.01	0.04	0.92
cg09901035	PLEKHG4B	0.87	0.06	0.87	0.08	0.66
cg10272601	WNK2	0.30	0.07	0.28	0.08	0.04
cg10342963	IGFIR	0.81	0.13	0.79	0.15	0.07
cg11349423	OPCML	0.48	0.15	0.48	0.16	0.93
cg1137136	PKDREJ	0.03	0.03	0.03	0.03	0.69
cg11686528	ABR	0.01	0.07	0.01	0.06	0.60
cg12296772	MTMR7	0.07	0.06	0.07	0.07	0.78
cg12610564	SLC39A12	0.98	0.01	0.97	0.07	0.09
cg12680131	TPO	0.80	0.09	0.82	0.11	0.02
cg12852139	MYO10	0.96	0.02	0.95	0.06	0.70
cg13204512	RNF135	0.01	0.06	0.01	0.02	0.23
cg13517866	SMOC2	0.89	0.11	0.89	0.10	0.58
cg13564825	PPP1R14A	0.01	0.05	0.01	0.02	0.98
cg13604246	ANKMY1	0.11	0.08	0.11	0.09	0.68
cg13611121	COL5A1	0.80	0.08	0.80	0.10	0.73
cg13782274	KCNQ2	0.94	0.08	0.93	0.11	0.39
cg13791254	FOXE1	0.02	0.02	0.01	0.03	0.62
cg13879483	USP44	0.08	0.06	0.07	0.07	0.34
cg13895235	PRKAR1B	0.01	0.01	0.01	0.03	0.39
cg14183206	HLA-L	0.24	0.10	0.23	0.09	0.61
cg14486338	KCN52	0.12	0.07	0.12	0.07	0.53
cg14644001	PRRT1	0.04	0.03	0.04	0.05	0.63
cg14645545	SLC11A1	0.20	0.12	0.19	0.12	0.83
cg14715697	HRNBP3	0.70	0.08	0.71	0.08	0.20
cg14866200	SHISA3	0.02	0.07	0.02	0.06	0.74
cg14988503	CDKL2	0.02	0.03	0.02	0.03	0.85
cg15092343	MSX1	0.07	0.05	0.07	0.04	0.48
cg15167871	TCERG1L	0.92	0.10	0.92	0.11	0.98
cg15549700	AJAP1	0.96	0.05	0.96	0.08	0.53
cg15760257	SARM1	0.01	0.01	0.01	0.05	0.35

cg17264670	RGS17	0.08	0.06	0.08	0.08	0.94
cg17497608	FZD1	0.83	0.11	0.84	0.12	0.43
cg17725364	COL6A3	0.96	0.10	0.96	0.09	0.86
cg18537730	IZUMO1	0.16	0.07	0.16	0.08	0.63
cg19429281	ZNF702P	0.02	0.01	0.02	0.03	0.40
cg19464917	ISL2	0.06	0.04	0.05	0.03	0.17
cg20129213	RIMS2	0.01	0.05	0.01	0.05	0.47
cg20399616	BCAT1	0.05	0.08	0.04	0.08	0.40
cg21385746	LOC150568	0.96	0.10	0.95	0.11	0.80
cg21472506	OTX1	0.01	0.04	0.01	0.04	0.98
cg21790626	ZNF154	0.04	0.04	0.05	0.05	0.32
cg22403469	RIMBP2	0.83	0.05	0.83	0.08	0.63
cg22511877	MYT1L	0.56	0.17	0.60	0.16	0.01
cg22524061	OSR2	0.23	0.09	0.22	0.09	0.48
cg22655988	CRMP1	0.96	0.08	0.96	0.10	0.77
cg22789900	MIXL1	0.00	0.01	0.01	0.04	0.55
cg23004031	MGMT	0.55	0.31	0.58	0.32	0.41
cg23391785	DNM3	0.02	0.06	0.01	0.04	0.28
cg23498518	POM121L12	0.79	0.07	0.80	0.10	0.36
cg23864180	ADARB2	0.90	0.06	0.91	0.07	0.26
cg24274117	C20orf195	0.03	0.07	0.04	0.07	0.52
cg24425838	C2CD4D	0.05	0.08	0.05	0.07	0.98
cg24432073	CDKL2	0.02	0.03	0.02	0.04	0.84
cg24563094	FAM59B	0.10	0.04	0.10	0.05	0.55
cg24602704	ATP10A	0.97	0.02	0.97	0.07	0.46
cg24816460	CDYL	0.03	0.07	0.03	0.07	0.51
cg25480336	ZFP64	0.01	0.02	0.01	0.01	0.16
cg25577023	AMN	0.09	0.09	0.09	0.09	0.82
cg25622366	OTX1	0.02	0.07	0.02	0.05	0.66
cg26010734	EPHX3	0.05	0.05	0.05	0.04	0.43
cg26841013	WNT3A	0.03	0.02	0.03	0.03	0.45

<sup>1</sup>*P* value for student's *t*-test.**Table 3** White blood cell DNA methylation and hepatocellular carcinoma risk

Locus		Cases/ controls	OR (95%CI)
WNK2 cg10272601	Below median (< 0.279)	56/157	1.0
	Above median (≥ 0.279)	103/155	1.91 (1.27-2.86)
TPO cg12680131	Below median (< 0.836)	102/157	1.0
	Above median (≥ 0.836)	57/155	0.59 (0.39-0.87)
MYT1L cg22511877	Below median (< 0.636)	105/159	1.0
	Above median (≥ 0.636)	54/153	0.50 (0.33-0.77)

The association between DNA methylation of cg10272601, cg12680131, and cg22511877 and HCC are given in Table 3. The OR for those with cg10272601 methylation above the median was 1.91 (95%CI: 1.27-2.86). Individuals with a cg12680131 methylation level above the median had lower risk of HCC, with an OR of 0.59 (95%CI: 0.39-0.87). The OR was 0.50 (95%CI: 0.33-0.77) for those with cg22511877 methylation above median.

Table 4 shows the multiple variables conditional logistic regression model. Overall, HBsAg (+) was associated with increased HCC risk (OR = 5.50, 95%CI: 3.34-9.03) compared with HBsAg(-). Ever smokers had a 2.1-fold increased risk of developing HCC (OR = 2.10, 95%CI: 1.08-4.07). The ORs (95%CI) were 2.26 (1.42-3.61), 0.55 (0.34-0.87), and 0.53 (0.32-0.88) for cg10272601, cg12680131, and cg22511877 hypermethylation.



**Table 4 Multiple variables model for DNA methylation and hepatocellular carcinoma risk**

Variable	OR (95%CI)	P
<i>WNK2</i> cg10272601 <sup>1</sup>	2.26 (1.42-3.61)	0.0006
<i>TPO</i> cg12680131 <sup>2</sup>	0.55 (0.34-0.87)	0.01
<i>MYT1L</i> cg22511877 <sup>3</sup>	0.53 (0.32-0.88)	0.01
HBsAg (positive <i>vs</i> negative)	5.50 (3.34-9.03)	< 0.0001
Alcohol (yes <i>vs</i> no)	2.10 (1.08-4.07)	0.03

<sup>1</sup>Above or below the median of 0.279; <sup>2</sup>Above or below the median of 0.836;

<sup>3</sup>Above or below the median of 0.636. HBsAg: Hepatitis B virus surface antigen.

## DISCUSSION

Alterations in methylation of cg10272601, cg12680131, and cg22511877 were associated with risk for later HCC development. Consistent with our tissue data, we found that a high methylation level in cg10272601 was associated with increased risk of HCC, while high methylation levels in both cg12680131 and cg22511877 were associated with decreased risk. In the 450k data, the mean beta values were  $0.52 \pm 0.22$  for cg10272601,  $0.28 \pm 0.21$  for cg12680131, and  $0.34 \pm 0.26$  for cg22511877 in HCC tumors<sup>[14]</sup>. The corresponding beta values were  $0.10 \pm 0.06$ ,  $0.79 \pm 0.08$ ,  $0.87 \pm 0.05$ , respectively, in adjacent nontumor tissues.

cg10272601 is located at transcription start site (TSS) 200 of *WNK2*, a gene encoding a serine-threonine kinase on chromosome 9q22.31<sup>[17]</sup>. *WNK2* acts as a tumor suppressor gene by suppressing the ERK/MAPK-pathway and downstream cell cycle progression<sup>[18]</sup> and *WNK2* expression inhibited colony formation<sup>[19]</sup>, suggesting a role in cell growth suppression. Dense high methylation at the CpG island was associated with decreased *WNK2* expression<sup>[19]</sup>. Hypermethylation of *WNK2* was reported in many cancers, including pancreatic ductal adenocarcinoma<sup>[20]</sup>, HCC<sup>[14,21]</sup>, and gliomas<sup>[22]</sup>.

cg12680131 is located on chromosome 2p25 at TSS 200 of thyroid peroxidase (*TPO*), a key enzyme in thyroid hormone synthesis. Mutations in *TPO* are associated with several disorders of thyroid hormonogenesis<sup>[23]</sup>. The association of methylation and expression of *TPO* has not been studied and the role of *TPO* in carcinogenesis has not been reported. cg22511877 is located at a shore region of myelin transcription factor 1-like (*MYT1L*) also on chromosome 2p25. *MYT1L* is a main member of the MYT/NZF family of transcription factors<sup>[24,25]</sup>. Limited data suggests a polymorphism in *MYT1L* is associated with gastric cancer outcome in a Chinese population<sup>[26]</sup>. Future studies are needed to understand the mechanisms of hypomethylation of both *TPO* and *MYT1L* in hepatocarcinogenesis.

The main limitation of this study is that we did not adjust for multiple comparisons due to the limited sample size. However, in further data analysis, we also observed significant associations of methylation in these 3 CpG sites with HCC risk after adjusting for HBV infection and alcohol consumption, suggesting an independent effect

in HCC risk.

This study, using prospective study design, allowed us to produce causal evidence on DNA methylation in WBC and cancer susceptibility<sup>[27]</sup>. Using information from HCC tumor tissues, our study investigated the associations of HCC-specific differentially methylated loci observed in tumor tissues in WBC DNA with HCC risk.

In summary, we provide new evidence that specific loci methylation in WBC DNA is associated with increased HCC susceptibility. These finding could lead to development of a simple non-invasive blood measure of DNA methylation to identify people at high risk of HCC.

## COMMENTS

### Background

Hepatocellular carcinoma (HCC) is a highly devastating disease with a poor prognosis. Thus, methods that allow the identification of individuals at elevated risk of HCC should greatly enhance screening for early diagnosis and improve prognosis. While several risk factors are well known such as infection with hepatitis B or C virus, not all viral-infected individuals develop cancer. Additional biomarkers of risk are therefore needed.

### Research frontiers

It is known that tumors release DNA into the blood stream and that this DNA contains the same DNA alterations both mutations and changes in DNA methylation that are found in the tumor. Thus, researchers have been able to develop assays for tumor DNA in plasma/serum for early diagnosis. There is also limited data in some cancers, not HCC, that DNA methylation changes in blood cells differs between cases and controls.

### Innovations and breakthroughs

This study is the first to investigate whether DNA methylation in specific genes in white blood cells is predictive of later HCC development.

### Applications

While the study needs confirmation in another population, it suggests that it may be possible to develop risk prediction models that include white blood cell DNA methylation markers.

### Peer-review

This is a very interesting paper. The authors found the correlation between DNA methylation and HCC occurring. The results provide sufficient experimental evidence or data to draw firm scientific conclusions.

## REFERENCES

- 1 Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]
- 2 Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. *Hepatology* 1995; **21**: 650-655 [PMID: 7875662 DOI: 10.1002/hep.1840210308]
- 3 Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; **347**: 168-174 [PMID: 12124405 DOI: 10.1056/NEJMoa013215]
- 4 Thein HH, Walter SR, Gidding HF, Amin J, Law MG, George J, Dore GJ. Trends in incidence of hepatocellular carcinoma after diagnosis of hepatitis B or C infection: a population-based cohort study, 1992-2007. *J Viral Hepat* 2011; **18**: e232-e241 [PMID: 21692938 DOI: 10.1111/j.1365-2893.2011.01440.x]

- 5 **Wang LY**, Hatch M, Chen CJ, Levin B, You SL, Lu SN, Wu MH, Wu WP, Wang LW, Wang Q, Huang GT, Yang PM, Lee HS, Santella RM. Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan. *Int J Cancer* 1996; **67**: 620-625 [PMID: 8782648]
- 6 **Wu HC**, Wang Q, Yang HI, Ahsan H, Tsai WY, Wang LY, Chen SY, Chen CJ, Santella RM. Aflatoxin B1 exposure, hepatitis B virus infection, and hepatocellular carcinoma in Taiwan. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 846-853 [PMID: 19273485 DOI: 10.1158/1055-9965.EPI-08-0697]
- 7 **Chen CJ**, Liang KY, Chang AS, Chang YC, Lu SN, Liaw YF, Chang WY, Sheen MC, Lin TM. Effects of hepatitis B virus, alcohol drinking, cigarette smoking and familial tendency on hepatocellular carcinoma. *Hepatology* 1991; **13**: 398-406 [PMID: 1847891 DOI: 10.1002/hep.1840130303]
- 8 **Lee S**, Lee HJ, Kim JH, Lee HS, Jang JJ, Kang GH. Aberrant CpG island hypermethylation along multistep hepatocarcinogenesis. *Am J Pathol* 2003; **163**: 1371-1378 [PMID: 14507645 DOI: 10.1016/S0002-9440(10)63495-5]
- 9 **Herath NI**, Leggett BA, MacDonald GA. Review of genetic and epigenetic alterations in hepatocarcinogenesis. *J Gastroenterol Hepatol* 2006; **21**: 15-21 [PMID: 16706806 DOI: 10.1111/j.1440-1746.2005.04043.x]
- 10 **Shen L**, Ahuja N, Shen Y, Habib NA, Toyota M, Rashid A, Issa JP. DNA methylation and environmental exposures in human hepatocellular carcinoma. *J Natl Cancer Inst* 2002; **94**: 755-761 [PMID: 12011226 DOI: 10.1093/jnci/94.10.755]
- 11 **Jones PA**, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002; **3**: 415-428 [PMID: 12042769]
- 12 **Tycko B**. Genetic and epigenetic mosaicism in cancer precursor tissues. *Ann N Y Acad Sci* 2003; **983**: 43-54 [PMID: 12724211 DOI: 10.1111/j.1749-6632.2003.tb05961.x]
- 13 **Shen J**, Wang S, Zhang YJ, Kappil M, Wu HC, Kibriya MG, Wang Q, Jasmine F, Ahsan H, Lee PH, Yu MW, Chen CJ, Santella RM. Genome-wide DNA methylation profiles in hepatocellular carcinoma. *Hepatology* 2012; **55**: 1799-1808 [PMID: 22234943 DOI: 10.1002/hep.25569]
- 14 **Shen J**, Wang S, Zhang YJ, Wu HC, Kibriya MG, Jasmine F, Ahsan H, Wu DP, Siegel AB, Remotti H, Santella RM. Exploring genome-wide DNA methylation profiles altered in hepatocellular carcinoma using Infinium HumanMethylation 450 BeadChips. *Epigenetics* 2013; **8**: 34-43 [PMID: 23208076 DOI: 10.4161/epi.23062]
- 15 **Wu HC**, Wang Q, Yang HI, Tsai WY, Chen CJ, Santella RM. Global DNA methylation levels in white blood cells as a biomarker for hepatocellular carcinoma risk: a nested case-control study. *Carcinogenesis* 2012; **33**: 1340-1345 [PMID: 22581841 DOI: 10.1093/carcin/bgs160]
- 16 **Wu HC**, Wang Q, Yang HI, Tsai WY, Chen CJ, Santella RM. Global DNA methylation in a population with aflatoxin B1 exposure. *Epigenetics* 2013; **8**: 962-969 [PMID: 23867725 DOI: 10.4161/epi.25696]
- 17 **Hong C**, Moorefield KS, Jun P, Aldape KD, Kharbanda S, Phillips HS, Costello JF. Epigenome scans and cancer genome sequencing converge on WNK2, a kinase-independent suppressor of cell growth. *Proc Natl Acad Sci USA* 2007; **104**: 10974-10979 [PMID: 17578925 DOI: 10.1073/pnas.0700683104]
- 18 **Moniz S**, Verissimo F, Matos P, Brazão R, Silva E, Kotelevets L, Chastre E, Gespach C, Jordan P. Protein kinase WNK2 inhibits cell proliferation by negatively modulating the activation of MEK1/ERK1/2. *Oncogene* 2007; **26**: 6071-6081 [PMID: 17667937 DOI: 10.1038/sj.onc.1210706]
- 19 **Jun P**, Hong C, Lal A, Wong JM, McDermott MW, Bollen AW, Plass C, Held WA, Smiraglia DJ, Costello JF. Epigenetic silencing of the kinase tumor suppressor WNK2 is tumor-type and tumor-grade specific. *Neuro Oncol* 2009; **11**: 414-422 [PMID: 19001526 DOI: 10.1215/15228517-2008-096]
- 20 **Dutruel C**, Bergmann F, Rooman I, Zucknick M, Weichenhan D, Geiselhart L, Kaffenberger T, Rachakonda PS, Bauer A, Giese N, Hong C, Xie H, Costello JF, Hoheisel J, Kumar R, Rehli M, Schirmacher P, Werner J, Plass C, Popanda O, Schmezer P. Early epigenetic downregulation of WNK2 kinase during pancreatic ductal adenocarcinoma development. *Oncogene* 2014; **33**: 3401-3410 [PMID: 23912455 DOI: 10.1038/ncr.2013.312]
- 21 **Tao R**, Li J, Xin J, Wu J, Guo J, Zhang L, Jiang L, Zhang W, Yang Z, Li L. Methylation profile of single hepatocytes derived from hepatitis B virus-related hepatocellular carcinoma. *PLoS One* 2011; **6**: e19862 [PMID: 21625442 DOI: 10.1371/journal.pone.0019862]
- 22 **Moniz S**, Martinho O, Pinto F, Sousa B, Loureiro C, Oliveira MJ, Moita LF, Honavar M, Pinheiro C, Pires M, Lopes JM, Jones C, Costello JF, Paredes J, Reis RM, Jordan P. Loss of WNK2 expression by promoter gene methylation occurs in adult gliomas and triggers Rac1-mediated tumour cell invasiveness. *Hum Mol Genet* 2013; **22**: 84-95 [PMID: 23035050]
- 23 **Cangul H**, Aycan Z, Olivera-Nappa A, Saglam H, Schoenmakers NA, Boelaert K, Cetinkaya S, Tarim O, Bober E, Darendeliler F, Bas V, Demir K, Aydin BK, Kendall M, Cole T, Höglér W, Chatterjee VK, Barrett TG, Maher ER. Thyroid dysmorphogenesis is mainly caused by TPO mutations in consanguineous community. *Clin Endocrinol (Oxf)* 2013; **79**: 275-281 [PMID: 23236987 DOI: 10.1111/cen.12127]
- 24 **Stevens SJ**, van Ravenswaaij-Arts CM, Janssen JW, Klein Wassink-Ruiter JS, van Essen AJ, Dijkhuizen T, van Rheeën J, Heuts-Vijgen R, Stegmann AP, Smeets EE, Engelen JJ. MYT1L is a candidate gene for intellectual disability in patients with 2p25.3 (2pter) deletions. *Am J Med Genet A* 2011; **155A**: 2739-2745 [PMID: 21990140 DOI: 10.1002/ajmg.a.34274]
- 25 **Kim JG**, Armstrong RC, v Agoston D, Robinsky A, Wiese C, Nagle J, Hudson LD. Myelin transcription factor 1 (Myt1) of the oligodendrocyte lineage, along with a closely related CCHC zinc finger, is expressed in developing neurons in the mammalian central nervous system. *J Neurosci Res* 1997; **50**: 272-290 [PMID: 9373037]
- 26 **Zhang Y**, Zhu H, Zhang X, Gu D, Zhou X, Wang M, Cao C, Zhang X, Wu X, Gong W, Tang Y, Zhou J, Tang C, Zhang Z, Chen J. Clinical significance of MYT1L gene polymorphisms in Chinese patients with gastric cancer. *PLoS One* 2013; **8**: e71979 [PMID: 24015200 DOI: 10.1371/journal.pone.0071979]
- 27 **Terry MB**, Delgado-Cruzata L, Vin-Raviv N, Wu HC, Santella RM. DNA methylation in white blood cells: association with risk factors in epidemiologic studies. *Epigenetics* 2011; **6**: 828-837 [PMID: 21636973 DOI: 10.4161/epi.6.7.16500]

**P- Reviewer:** Celikbilek M, Dang SS, Luo GH, Morales-Gonzalez J, Romero MR

**S- Editor:** Song XX **L- Editor:** A **E- Editor:** Liu SQ





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 February 28; 8(6): 307-344







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 469 members, representing a team of worldwide experts in hepatology. They are from 53 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (1), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (98), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (19), Greece (11), Hungary (5), India (15), Indonesia (2), Iran (4), Israel (1), Italy (52), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (11), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (17), and United States (56).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*

Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*  
Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*

Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *São Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*



**Chile**

Luis A Videla, *Santiago*



## China

Guang-Wen Cao, Shanghai  
 En-Qiang Chen, Chengdu  
 Gong-Ying Chen, Hangzhou  
 Jin-lian Chen, Shanghai  
 Jun Chen, Changsha  
 Alfred Cheng, Hong Kong  
 Chun-Ping Cui, Beijing  
 Shuang-Suo Dang, Xi'an  
 Ming-Xing Ding, Jinhua  
 Zhi-Jun Duang, Dalian  
 He-Bin Fan, Wuhan  
 Xiao-Ming Fan, Shanghai  
 James Yan Yue Fung, Hong Kong  
 Yi Gao, Guangzhou  
 Zuo-Jiong Gong, Wuhan  
 Zhi-Yong Guo, Guangzhou  
 Shao-Liang Han, Wenzhou  
 Tao Han, Tianjin  
 Jin-Yang He, Guangzhou  
 Ming-Liang He, Hong Kong  
 Can-Hua Huang, Chengdu  
 Bo Jin, Beijing  
 Shan Jin, Hohhot  
 Hui-Qing Jiang, Shijiazhuang  
 Wan-Yee Joseph Lau, Hong Kong  
 Guo-Lin Li, Changsha  
 Jin-Jun Li, Shanghai  
 Qiang Li, Jinan  
 Sheng Li, Jinan  
 Zong-Fang Li, Xi'an  
 Xu Li, Guangzhou  
 Xue-Song Liang, Shanghai  
 En-Qi Liu, Xi'an  
 Pei Liu, Shenyang  
 Zhong-Hui Liu, Changchun  
 Guang-Hua Luo, Changzhou  
 Yi Lv, Xi'an  
 Guang-Dong Pan, Liuzhou  
 Wen-Sheng Pan, Hangzhou  
 Jian-Min Qin, Shanghai  
 Wai-Kay Seto, Hong Kong  
 Hong Shen, Changsha  
 Xiao Su, Shanghai  
 Li-Ping Sun, Beijing  
 Wei-Hao Sun, Nanjing  
 Xue-Ying Sun, Harbin  
 Hua Tang, Tianjin  
 Ling Tian, Shanghai  
 Eric Tse, Hong Kong  
 Guo-Ying Wang, Changzhou  
 Yue Wang, Beijing  
 Shu-Qiang Wang, Chengdu  
 Mary MY Wayne, Hong Kong  
 Hong-Shan Wei, Beijing  
 Danny Ka-Ho Wong, Hong Kong  
 Grace Lai-Hung Wong, Hong Kong  
 Bang-Fu Wu, Dongguan  
 Feng Wu, Chongqing  
 Xiong-Zhi Wu, Tianjin  
 Chun-Fang Xu, Suzhou  
 Rui-An Xu, Quanzhou  
 Rui-Yun Xu, Guangzhou  
 Wei-Li Xu, Shijiazhuang  
 Shi-Ying Xuan, Qingdao  
 Ming-Xian Yan, Jinan  
 Lv-Nan Yan, Chengdu  
 Jin Yang, Hangzhou  
 Ji-Hong Yao, Dalian  
 Winnie Yeo, Hong Kong

Zheng Zeng, Beijing  
 Qi Zhang, Hangzhou  
 Shi-Jun Zhang, Guangzhou  
 Xiao-Lan Zhang, Shijiazhuang  
 Xiao-Yong Zhang, Guangzhou  
 Xin-Chen Zhang, Harbin  
 Yong Zhang, Xi'an  
 Hong-Chuan Zhao, Hefei  
 Ming-Hua Zheng, Wenzhou  
 Yu-Bao Zheng, Guangzhou  
 Ren-Qian Zhong, Shanghai  
 Fan Zhu, Wuhan  
 Xiao Zhu, Dongguan



## Czech Republic

Kamil Vysloulzil, Olomouc



## Denmark

Henning Gronbaek, Aarhus  
 Christian Mortensen, Hvidovre



## Egypt

Ihab T Abdel-Raheem, Damanhour  
 NGB G Bader EL Din, Cairo  
 Hatem Elalfy, Mansoura  
 Mahmoud M El-Bendary, Mansoura  
 Mona El SH El-Raziky, Cairo  
 Mohammad El-Sayed, Cairo  
 Yasser M Fouad, Minia  
 Mohamed AA Metwally, Benha  
 Hany Shehab, Cairo  
 Mostafa M Sira, Shebin El-koom  
 Ashraf Taye, Minia  
 MA Ali Wahab, Mansoura



## France

Laurent Alric, Toulouse  
 Sophie Conchon, Nantes  
 Daniel J Felmlee, Strasbourg  
 Herve Lerat, Creteil  
 Dominique Salmon, Paris  
 Jean-Pierre Vartanian, Paris



## Germany

Laura E Buitrago-Molina, Hannover  
 Enrico N De Toni, Munich  
 Oliver Ebert, Muenchen  
 Rolf Gebhardt, Leipzig  
 Janine V Hartl, Regensburg  
 Sebastian Hinz, Kiel  
 Benjamin Juntermanns, Essen  
 Roland Kaufmann, Jena  
 Viola Knop, Frankfurt  
 Veronika Lukacs-Kornek, Homburg  
 Benjamin Maasoumy, Hannover  
 Jochen Mattner, Erlangen  
 Nadja M Meindl-Beinker, Mannheim  
 Ulf P Neumann, Aachen  
 Margarete Odenthal, Cologne  
 Yoshiaki Sunami, Munich

Christoph Roderburg, Aachen  
 Frank Tacke, Aachen  
 Yuchen Xia, Munich



## Greece

Alex P Betrosian, Athens  
 George N Dalekos, Larissa  
 Ioanna K Delladetsima, Athens  
 Nikolaos K Gatselis, Larissa  
 Stavros Gourgiotis, Athens  
 Christos G Savopoulos, Thessaloniki  
 Tania Siahaniidou, Athens  
 Emmanouil Sinakos, Thessaloniki  
 Nikolaos G Symeonidi, Thessaloniki  
 Konstantinos C Thomopoulos, Larissa  
 Konstantinos Tziomalos, Thessaloniki



## Hungary

Gabor Banhegyi, Budapest  
 Peter L Lakatos, Budapest  
 Maria Papp, Debrecen  
 Ferenc Sipos, Budapest  
 Zsolt J Tulassay, Budapest



## India

Deepak N Amarapurkar, Mumbai  
 Girish M Bhopale, Pune  
 Sibnarayan Datta, Tezpur  
 Nutan D Desai, Mumbai  
 Sorabh Kapoor, Mumbai  
 Jaswinder S Maras, New Delhi  
 Nabeen C Nayak, New Delhi  
 C Ganesh Pai, Manipal  
 Amit Pal, Chandigarh  
 K Rajeshwari, New Delhi  
 Anup Ramachandran, Vellore  
 D Nageshwar Reddy, Hyderabad  
 Shivaram P Singh, Cuttack  
 Ajith TA, Thrissur  
 Balasubramaniyan Vairappan, Pondicherry



## Indonesia

Cosmas RA Lesmana, Jakarta  
 Neneng Ratnasari, Yogyakarta



## Iran

Seyed M Jazayeri, Tehran  
 Sedigheh Kafi-Abad, Tehran  
 Iradj Maleki, Sari  
 Fakhraddin Naghibalhossaini, Shiraz



## Israel

Stephen DH Malnick, Rehovot



## Italy

Francesco Angelico, Rome

Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Andrea Galli, *Florence*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Salvatore Gruttadauria, *Palermo*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*



**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*  
 Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*

Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhito Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*



**Jordan**

Kamal E Bani-Hani, *Zarqa*



**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*



**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*



**Moldova**

Angela Peltec, *Chishinev*



**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*



**Nigeria**

CA Asabamaka Onyekwere, *Lagos*



**Pakistan**

Bikha Ram Devrajani, *Jamshoro*



**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*



**Poland**

Jacek Zielinski, *Gdansk*



**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*



**Qatar**

Reem Al Olaby, *Doha*



**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*



**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*



**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*



**Singapore**

Ser Yee Lee, *Singapore*



**South Korea**

Young-Hwa Chung, *Seoul*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonju*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*



**Spain**

Ivan G Marina, *Madrid*  
 Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*

José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodríguez-Frias, *Córdoba*  
 Manuel L Rodríguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Traperó-Marugán, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpaboon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*  
 Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*

Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



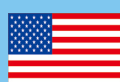
#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*



#### **United States**

Naim Alkhouri, *Cleveland*  
 Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*

Brian B Borg, *Jackson*  
 Ronald W Busuttill, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Grigoriy E Gurvits, *New York*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Kusum K Kharbanda, *Omaha*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



**REVIEW**

- 307 Bacterial infections in cirrhosis: A critical review and practical guidance  
*Bunchorntavakul C, Chamroonkul N, Chavalitdhamrong D*

**ORIGINAL ARTICLE****Basic Study**

- 322 Burn injury induces histopathological changes and cell proliferation in liver of rats  
*Bortolin JA, Quintana HT, Tomé TC, Ribeiro FAP, Ribeiro DA, de Oliveira F*

**Randomized Controlled Trial**

- 331 Boceprevir plus peginterferon/ribavirin for treatment of chronic hepatitis C in Russia  
*Isakov V, Nikitin I, Chulanov V, Ogurtsov P, Lukyanova E, Long J, Wahl J, Helmond FA; The P08160 Trial Investigators*

**CASE REPORT**

- 340 Primary hepatic amyloidosis: A case report and review of literature  
*Sonthalia N, Jain S, Pawar S, Zanwar V, Surude R, Rathi PM*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Yasuhito Tanaka, MD, PhD, Professor, Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjnet.com](mailto:editorialoffice@wjnet.com)  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjnet.com](mailto:bpgoffice@wjnet.com)  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

**PUBLICATION DATE**  
February 28, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

Full instructions are available online at [http://www.wjnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjnet.com/bpg/g_info_20160116143427.htm)

**ONLINE SUBMISSION**

<http://www.wjnet.com/esps/>

## Bacterial infections in cirrhosis: A critical review and practical guidance

Chalermrat Bunchorntavakul, Naichaya Chamroonkul, Disaya Chavalitdhamrong

Chalermrat Bunchorntavakul, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Rajavithi Hospital, College of Medicine, Rangsit University, Bangkok 10400, Thailand

Naichaya Chamroonkul, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand

Disaya Chavalitdhamrong, Division of Gastroenterology, Department of Internal Medicine, Harbor-UCLA Medical, Torrance, CA 90509, United States

**Author contributions:** Bunchorntavakul C conceptualized, searched and reviewed literature, created the figures and tables, drafted and reviewed the paper; Chamroonkul N searched and reviewed literature, drafted and reviewed the paper; Chavalitdhamrong D conceptualized and reviewed the paper.

**Conflict-of-interest statement:** The authors have nothing to disclose.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Chalermrat Bunchorntavakul, MD, Assistant Professor of Medicine, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Rajavithi Hospital, College of Medicine, Rangsit University, Rajavithi Road, Ratchathewi, Bangkok 10400, Thailand. [dr.chalermrat@gmail.com](mailto:dr.chalermrat@gmail.com)  
 Telephone: +66-2-3548081  
 Fax: +66-2-3548179

Received: August 18, 2015  
 Peer-review started: August 21, 2015  
 First decision: October 13, 2015  
 Revised: January 11, 2016

Accepted: January 27, 2016

Article in press: January 29, 2016

Published online: February 28, 2016

### Abstract

Bacterial infection is common and accounts for major morbidity and mortality in cirrhosis. Patients with cirrhosis are immunocompromised and increased susceptibility to develop spontaneous bacterial infections, hospital-acquired infections, and a variety of infections from uncommon pathogens. Once infection develops, the excessive response of pro-inflammatory cytokines on a pre-existing hemodynamic dysfunction in cirrhosis further predispose the development of serious complications such as shock, acute-on-chronic liver failure, renal failure, and death. Spontaneous bacterial peritonitis and bacteremia are common in patients with advanced cirrhosis, and are important prognostic landmarks in the natural history of cirrhosis. Notably, the incidence of infections from resistant bacteria has increased significantly in healthcare-associated settings. Serum biomarkers such as procalcitonin may help to improve the diagnosis of bacterial infection. Preventive measures (*e.g.*, avoidance, antibiotic prophylaxis, and vaccination), early recognition, and proper management are required in order to minimize morbidity and mortality of infections in cirrhosis.

**Key words:** Bacteria; Infection; Sepsis; Bacteremia; Liver cirrhosis; Vaccination; Spontaneous peritonitis; Immune dysfunction

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Bacterial infection is common and accounts for major morbidity and mortality in cirrhosis. Patients with cirrhosis are immunocompromised and increased susceptibility to develop spontaneous bacterial infec-

tions, hospital-acquired infections, and a variety of infections from uncommon pathogens. Once infection develops, the excessive response of pro-inflammatory cytokines on a pre-existing hemodynamic derangement in cirrhosis further predispose the development of serious complications such as shock, acute-on-chronic liver failure, renal failure, and death. The incidence of resistant bacteria has continually increased, especially in healthcare-associated settings. Preventive measures, early recognition and proper management are necessary to minimize morbidity and mortality of infections in cirrhosis.

Bunchorntavakul C, Chamroonkul N, Chavalitthamrong D. Bacterial infections in cirrhosis: A critical review and practical guidance. *World J Hepatol* 2016; 8(6): 307-321 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i6/307.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i6.307>

## INTRODUCTION

In the past decades, there have been several improvements in the management of cirrhotic patients, such as antiviral therapy and management of portal hypertension and liver transplantation (LT). However, the mortality of infection in cirrhosis is still high and has not changed substantially. Cirrhosis is an immunocompromised state that predisposes patients to spontaneous bacterial infections, hospital-acquired infections, and a variety of infections from uncommon pathogens. Once infection develops, the excessive response of pro-inflammatory cytokines on a pre-existing hemodynamic derangement in cirrhosis further facilitate the development of severe complications such as septic shock, acute-on-chronic liver failure (ACLF), multiple organ failure, and death. Accordingly, bacterial infection in patients with cirrhosis is very common in clinical practice and sepsis is the main reason of intensive care unit admission and death among such patients. The incidence of resistant bacteria has been increasing, especially in healthcare-associated settings. Preventive measures, early recognition, and proper management are necessary to minimize morbidity and mortality of infections in cirrhosis.

## MECHANISM OF INCREASED SUSCEPTIBILITY AND VULNERABILITY TO INFECTION IN PATIENTS WITH CIRRHOSIS

### *Immune dysfunction in cirrhosis*

Patients with cirrhosis are in a state of immune dysfunction, in parallel with a state of excessive activation of pro-inflammatory cytokines, referred to as cirrhosis-associated immune dysfunction syndrome, which predisposes the patient for infections<sup>[1,2]</sup>. Portosystemic

shunting allows less gut-derived bacteria and their products to be cleared from portal circulation by the liver, which contains about 90% of the reticuloendothelial cells in the body<sup>[1-5]</sup>. Nearly all components of systemic immune response are significantly impaired in cirrhosis, including a decrease in phagocytic activity, a reduction in serum albumin, complement and protein C activities, and an impaired opsonic activity both in serum and ascitic fluid<sup>[1-4,6-10]</sup>. Genetic polymorphisms of toll-like receptor (TLR) and nucleotide-binding oligomerisation domain 2 (NOD2) genes could be responsible for bacterial translocation (BT) and increase infection risk in cirrhosis by altering the TLR's ability to bind to lipopolysaccharide or endotoxins<sup>[11,12]</sup>. Further, cirrhosis-associated immune dysfunction may further complicate by additional factors such as malnourishment<sup>[13]</sup> and alcohol drinking<sup>[14]</sup> (Table 1).

### **BT**

BT is the migration of viable native bacteria from gut lumen through systemic circulation *via* mesenteric lymph nodes (MLN) and portal vein. Although this can be a healthy phenomenon, BT has increased pathologically compromising effects in cirrhosis<sup>[15-17]</sup>. The diagnosis of BT relies on the isolation of viable bacteria in MLN, while the detection of bacterial DNA in serum or ascitic fluid is proposed as a useful surrogated marker<sup>[15-18]</sup>. It has been shown that oral administration of radio-labeled *Escherichia coli* (*E. coli*) to cirrhotic rats revealed the detection of these bacteria not only in the gut lumen but also in the MLN and ascites<sup>[19]</sup>. Several experimental and clinical studies have suggested that small intestinal overgrowth, increased intestinal permeability, impaired intestinal motility, lack of bile acids, sympathetic overactivity, and local innate and adaptive immunological alterations (*e.g.*, impaired leukocyte recruitment, altered T-cell activation, TLR and NOD2 mutation) are important factors involved in the pathogenesis of BT<sup>[11,12,17,20,21]</sup>.

BT is pathogenetically linked to the development of infections, particularly spontaneous bacterial infections, and other serious complications in cirrhosis<sup>[15-17]</sup>. Apart from infections, bacterial DNA and bacterial products, such as endotoxin, can translocate to extra-intestinal sites and promote host immunological and hemodynamic responses, which is associated with the development of systemic pro-inflammatory and hyperdynamic circulatory state in cirrhosis<sup>[16,18]</sup>. The pathological translocation of viable bacteria occurs in the decompensated stage, while the rate and degree of translocating bacterial products also increases in the earlier stages of cirrhosis<sup>[15]</sup>. Notably, treatment with non-selective beta-blockers has been shown to ameliorate intestinal permeability and reduce BT<sup>[22]</sup>.

### **Systemic inflammatory response syndrome and circulatory dysfunction in cirrhosis**

Patients with cirrhosis are susceptible to the development of severe infection, septic shock, and organ



**Table 1** State of immune dysfunction in patients with cirrhosis

Natural barriers	Fragile, thin and/or edematous skin Alteration of GI motility and mucosal permeability Alteration of GI bacterial flora, bacterial overgrowth ↑ GI mucosal ulcerations
Hepatic RES activity	Portosystemic shunting Kupffer cells - ↓ number, impaired function
Cellular defense mechanisms	RES - ↓ activation, ↓ chemotaxis, ↓ phagocytosis, ↓ production of pro-inflammatory cytokines (IL-1, IL-6, IL-18, TNF-α) PMN - ↓ lifespan, ↓ intracellular killing activity, ↓ phagocytosis, ↓ chemotaxis
Serum factors	↓ Complement levels (C3, C4, CH50) ↓ Opsonic activity ↓ Protein C activity
Iatrogenic and treatment-related factors	↑ Invasive procedure and catheters Frequent hospitalization Immunosuppressive agents (autoimmune hepatitis, post-transplantation) Interferon therapy (viral hepatitis) Proton pump inhibitors
Other compelling factors	Malnutrition Alcohol drinking

Adapted from Bunchorntavakul C, Chavalitdharmong D. *World J Hepatol* 2012; 4: 158-168. RES: Reticuloendothelial system; GI: Gastrointestinal; IL: Interleukins; TNF: Tumor necrosis factors; PMN: Polymorphonuclear cells.

failure<sup>[1,2,23]</sup>. In cirrhosis, bacterial infection is associated with a dysregulated cytokine response, which transforms helpful responses against infections into excessive, damaging inflammation<sup>[1,2,23]</sup>. Nitric oxide is strikingly released in cirrhotic patients with sepsis and is a key driver of circulation dysfunction in this setting<sup>[23,24]</sup>. A pre-existing hyperdynamic circulatory state in patients with advanced cirrhosis predisposes detrimental complications from a sepsis-induced nitric oxide and cytokine storm which subsequently leads to intractable hypotension, insufficient tissue perfusion, multiple organ failure and death<sup>[1-3,23]</sup>.

### Epidemiology and types of infection

Bacterial infection accounts for about 30%-50% death in patients with cirrhosis<sup>[3,24,25]</sup>. Infections present in 32%-34% of hospitalized patients with cirrhosis, which is 4-5 folds higher than hospitalized patients in general, and is especially higher in those with gastrointestinal bleeding (45%-60%)<sup>[26-28]</sup>.

Common types of infections in patients with cirrhosis include spontaneous bacterial peritonitis (SBP) (25%-31%), urinary tract infection (UTI) (20%-25%), pneumonia (15%-21%), bacteremia (12%), and soft tissue infection (11%)<sup>[2,27,29]</sup>. The major causative organisms are gram-negative bacteria, *e.g.*, *E. coli*, *Klebsiella* spp. and *Enterobacter* spp., whereas gram positive bacteria, especially *Enterococci* and *Staphylococcus aureus*, comprise about 20% and anaerobes only 3%<sup>[2]</sup>. Risk factors of infection by gram positive bacteria are recent or current hospitalization, receiving quinolones prophylaxis, and invasive procedures<sup>[27,28,30]</sup>.

Healthcare-associated is defined as infections diagnosed within 48 h of hospital admission in patients with any prior 90-d healthcare contact and nosocomial is defined as infections diagnosed after 48 h of admission.

These infections are increasingly common in cirrhosis, frequently resistant to antibiotics (up to 64%) and are associated with bad outcomes<sup>[30]</sup>. In a large prospective study of cirrhotic patients with infections (> 650 infectious episodes)<sup>[31]</sup>, multi-resistant bacteria (18%) were isolated in 4%, 14%, and 35% of community-acquired, healthcare-associated, and nosocomial infections, respectively ( $P < 0.001$ ). The main resistant organism was extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Enterobacteriaceae*, followed by *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Enterococcus faecium*<sup>[31]</sup>. There was a significantly higher incidence of septic shock and death from infections caused by resistant bacteria. Notably, the efficacy of empirical antibiotic treatment was decreased in nosocomial infections (40%), compared to community-acquired and healthcare-associated episodes (83% and 73%, respectively;  $P < 0.0001$ ), especially in SBP, UTI, and pneumonia (26%, 29% and 44%, respectively)<sup>[31]</sup>. Due to an increasingly use of broad spectrum antibiotics (ATB), it is speculated that infections with multi-resistant gram-negative organisms and *Enterococci* will be largely more common and more problematic in the near future.

The common types of infections in cirrhosis and suggested empiric therapy are summarized in Table 2<sup>[32]</sup>. In addition, the common clinical features and risk factors of less common pathogens are summarized in Table 3<sup>[2]</sup>. It should be noted that the data regarding these less common pathogens derived from case reports and series from various regions of the world, in which the patterns of infection and ATB usage varies among reports. In real-life practice, empirical ATB should be selected based upon types of infection, individual risk factors, and the local epidemiological pattern of resistant bacteria, then narrow-downed according to the culture and ATB susceptibility testing.

**Table 2** Types of infection and suggested empirical antibiotic therapy in patients with cirrhosis

Types of infection	Common responsible bacteria	Suggested empirical antibiotic
SBP, spontaneous bacteremia, SBE	<i>Enterobacteriaceae</i> <i>S. pneumoniae</i> <i>S. viridans</i>	1 <sup>st</sup> line: Cefotaxime or ceftriaxone or BL-BI IV Options: Ciprofloxacin PO for uncomplicated SBP <sup>1</sup> ; carbapenems IV for nosocomial infections in areas with a high prevalence of ESBL BL-BI may prefer in those with suspicious for enterococcal infection <sup>2</sup>
Pneumonia	<i>Enterococci</i> <i>S. pneumoniae</i> <i>H. influenzae</i> <i>M. pneumoniae</i> <i>Legionella</i> spp. <i>Enterobacteriaceae</i> <i>P. aeruginosa</i> <i>S. aureus</i>	Community-acquired: ceftriaxone or BL-BI IV + macrolide or levofloxacin IV/PO Nosocomial and health care-associated infections: Meropenem or cefazidime IV + ciprofloxacin IV (IV vancomycin or linezolid should be added in patients with risk factors for MRSA <sup>3</sup> )
Urinary tract infection	<i>Enterobacteriaceae</i> <i>E. faecalis</i> <i>E. faecium</i>	1 <sup>st</sup> line: Ceftriaxone or BL-BI IV in patients with sepsis. Ciprofloxacin or cotrimoxazole PO in uncomplicated infections Options: In areas with a high prevalence of ESBL, IV carbapenems for nosocomial infections and sepsis (+ IV glycopeptides for severe sepsis); and nitrofurantoin PO for uncomplicated cases
Skin and soft tissue infections	<i>S. aureus</i> <i>S. pyogenes</i> <i>Enterobacteriaceae</i> <i>P. aeruginosa</i> <i>Vibrio vulnificus</i> <i>Aeromonas</i> spp.	Community-acquired: Ceftriaxone + cloxacillin IV or BL-BI IV Nosocomial: Meropenem or cefazidime IV + glycopeptides IV
Meningitis	<i>S. pneumoniae</i> <i>Enterobacteriaceae</i> <i>L. monocytogenes</i> <i>N. meningitidis</i>	Community-acquired: Cefotaxime or ceftriaxone IV + vancomycin IV Ampicillin IV should be added if <i>L. monocytogenes</i> is suspected <sup>4</sup> Nosocomial: Meropenem + vancomycin IV

Adapted from Fernandez J, Gustot T. *J Hepatol* 2012; 56 (Suppl 1): S1-12. <sup>1</sup>Quinolones should not be used in patients submitted to long-term norfloxacin prophylaxis or in geographical areas with a high prevalence of quinolone-resistant *Enterobacteriaceae*; <sup>2</sup>Risk factors for *Enterococci*: Quinolone prophylaxis, hospital-acquired infection; <sup>3</sup>Risk factors for MRSA: Ventilator-associated pneumonia, previous antibiotic therapy, nasal MRSA carriage; <sup>4</sup>Risk factors for *L. monocytogenes*: Hemochromatosis, detection of gram-positive bacilli/coccobacilli in cerebrospinal fluid. BL-BI: Beta-lactam/beta-lactamase inhibitors (e.g., amoxicillin/clavulanic acid, ampicillin/sulbactam, and piperacillin/tazobactam); MRSA: Methicillin-resistant *Staphylococcus aureus*; ESBL: Extended spectrum beta-lactamases; SBP: Spontaneous bacterial peritonitis; SBE: Spontaneous bacterial empyema; IV: Intravenous; *S. pneumoniae*: *Streptococcus pneumoniae*; *S. viridans*: *Streptococcus viridans*; *H. influenzae*: *Haemophilus influenzae*; *M. pneumoniae*: *Mycoplasma pneumoniae*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; *E. faecalis*: *Enterococcus faecalis*; *E. faecium*: *Enterococcus faecium*; *S. pyogenes*: *Streptococcus pyogenes*; *L. monocytogenes*: *Listeria monocytogenes*; *N. meningitidis*: *Neisseria meningitidis*.

### Biomarkers of bacterial infection in cirrhosis

It is crucial, but often difficult to make an early diagnosis of bacterial infections in cirrhosis due to non-specific manifestations, which are indistinguishable from other non-infectious causes of systemic inflammatory response syndrome (SIRS) and the symptoms of liver deterioration. Therefore, serum biomarkers that are sensitive, reliable and inexpensive are being pursued in order to improve the diagnosis of bacterial infection in the setting of cirrhosis. General inflammatory markers, such as C-reactive protein (CRP, synthesized by the liver), ferritin (synthesized by the liver) or white blood cells (WBC), lack specificity for bacterial infections. Procalcitonin (PCT) is potentially a more specific marker for bacterial infection. PCT is produced by nearly all tissues in response to endotoxin or mediators released in response to bacterial infections [interleukin (IL)-1b, tumor necrosis factor- $\alpha$ , and IL-6]. It highly correlates with the severity of bacterial infections and may be helpful to distinguish bacterial infections from viral infection or other non-infectious causes<sup>[33]</sup>.

In the meta-analysis included 10 diagnostic studies (1144 cirrhotic patients and 435 bacterial infection

episodes), PCT displayed an area under the curve of 0.92, a sensitivity of 0.79, and a specificity of 0.89 in diagnosing bacterial infection<sup>[34]</sup>. The pooled sensitivity estimates were 79% for PCT and 77% for CRP tests, whereas the pooled specificity were higher for both PCT (89%) and CRP tests (85%)<sup>[34]</sup>. The results were consistent when stratified to patients with SBP or patients with systemic infection. The authors suggested that the PCT test can be used as a rule-in diagnostic tool (positive likelihood ratio 7.38), CRP test can be used as a rule-out diagnostic tool (negative likelihood ratio 0.23) in patients without signs of infection<sup>[34]</sup>. However, the diagnostic accuracy of CRP in the detection of bacterial infections decreased in setting of advanced liver disease. The combination of CRP and PCT may slightly improve the diagnostic accuracy of bacterial infection<sup>[35]</sup>.

### SBP

#### Epidemiology and clinical features of SBP

SBP is common and quite unique in patients with cirrhosis. The prevalence of SBP in cirrhotic patients with ascites admitted to the hospital ranges from 10%-30%;

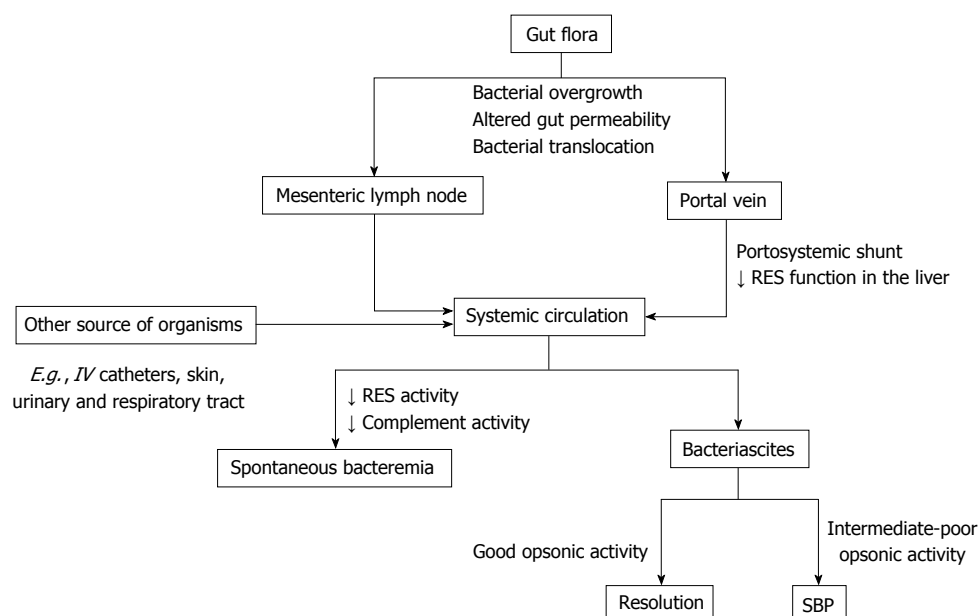
**Table 3** Common manifestations and risk factors of bacterial pathogens in patients with cirrhosis

Pathogens	Common clinical syndrome	Risk factors	Remarks
<i>Aeromonas</i> spp. ( <i>A. hydrophila</i> , <i>A. sobria</i> , <i>A. aquariorum</i> ) <sup>[120-126]</sup>	SBP, bacteremia, SSTI, enterocolitis	Contaminated food and water Diabetes	Increased incidence High mortality (20%-60%), especially when presence of hypotension on admission
<i>Campylobacter</i> spp. <sup>[127,128]</sup>	Bacteremia, SBP	Most reports were from East Asia Alcoholic	Increased incidence High mortality (10% in bacteremia)
<i>Clostridium</i> spp. ( <i>C. perfringens</i> , <i>C. bifermentans</i> , <i>C. septicum</i> ) <sup>[4,129,130]</sup>	SSTI	Diabetes	Increased incidence Very high mortality (54%-65%)
<i>Clostridium difficile</i> <sup>[108,131-133]</sup>	ATB-associated diarrhea and colitis	Broad-spectrum ATB Hospitalization PPIs	Increased incidence Higher mortality (14%) when compare to non-cirrhotics Increased cost and length of hospital stay
<i>Enterococcus</i> spp. ( <i>E. faecium</i> , <i>E. faecalis</i> , <i>E. gallinarum</i> ) <sup>[134-136]</sup>	SBP, bacteremia, UTI, endocarditis, biliary tract infection	Healthcare-associated infection Quinolone prophylaxis	Increased incidence High mortality (30% in bacteremia; 60% in SBP) Increased incidence of VRE colonization and infection in liver transplant setting
<i>Listeria monocytogenes</i> <sup>[137,138]</sup> <i>Mycobacterium</i> TB <sup>[2,139,140]</sup>	SBP, bacteremia, meningitis Pulmonary TB, TB peritonitis, TB lymphadenitis, disseminated TB	Hemochromatosis Alcoholic Developing countries Exposed to TB case	Increased incidence Increased incidence, especially extrapulmonary forms (> 50% of TB peritonitis cases in the United States had underlying cirrhosis) High mortality (22%-48%) Increased risk for multi-drug resistant TB Increased risk for anti-TB-induced hepatotoxicity
<i>Pasteurella multocida</i> <sup>[141-143]</sup>	SBP, bacteremia septic arthritis, meningitis	Presence of ascites (TB peritonitis) Domestic animal (cats or dogs) bites or scratches	Increased incidence High mortality (10%-40% in bacteremia)
<i>Staphylococcus aureus</i> <sup>[45,144,145]</sup>	SSTI, UTI, SBP, bacteremia, endocarditis	Alcoholic Invasive procedures Hospitalization	Increased incidence of MRSA carriage and infection High mortality (30% in bacteremia) Removal of the eradicable focus was associated with decreased mortality
<i>Streptococcus bovis</i> <sup>[146,147]</sup>	Bacteremia, SBP meningitis, endocarditis, septic arthritis	Quinolone prophylaxis Colonic lesion(s): Adenoma or adenocarcinoma (presence in 18%-40% of cases) Alcoholic	Increased incidence High mortality (up to 40% in bacteremia with advanced cirrhosis) Colonic lesion(s) was present in 18%-40% of cases
<i>Streptococcus group B</i> <sup>[148-150]</sup>	SSTI, bacteremia, SBP, meningitis, pneumonia	Post endoscopic sclerotherapy and banding ligation	Increased incidence High mortality (10%-25% in SBP and bacteremia; 45% in meningitis)
<i>Streptococcus pneumoniae</i> <sup>[89-92]</sup>	Pneumonia, SBP bacteremia, SSTI, meningitis	Alcoholic Post-splenectomy Not vaccinated	Increased incidence of invasive pneumococcal disease High mortality (10%-20%)
<i>Vibrio</i> spp. ( <i>V. vulnificus</i> , non-o1 <i>V. cholera</i> , <i>V. parahemolyticus</i> ) <sup>[151-153]</sup>	SSTI, bacteremia, gastroenteritis, diarrhea, SBP	Hemochromatosis Exposed to seawater and undercooked seafoods Most reports were from East Asia	Increased incidence Very high mortality (50%-60% in bacteremia; 24% in SSTI)
<i>Yersinia</i> spp. ( <i>Y. enterocolitica</i> , <i>Y. pseudotuberculosis</i> ) <sup>[154,155]</sup>	Bacteremia, SBP, hepatosplenic abscesses	Hemochromatosis Exposed to animals and contaminated foods	Increased incidence (in hemochromatosis) High mortality (50% in bacteremia)

SBP: Spontaneous bacterial peritonitis; SSTI: Skin and soft tissue infection; UTI: Urinary tract infection; ATB: Antibiotics; PPIs: Proton-pump inhibitors; TB: Tuberculosis; MRSA: Methicillin-resistant *Staphylococcus aureus*; *A. hydrophila*: *Aeromonas hydrophila*; *A. sobria*: *Aeromonas sobria*; *A. aquariorum*: *Aeromonas aquariorum*; *C. perfringens*: *Clostridium perfringens*; *C. bifermentans*: *Clostridium bifermentans*; *C. septicum*: *Clostridium septicum*; *E. faecium*: *Enterococcus faecium*; *E. faecalis*: *Enterococcus faecalis*; *E. gallinarum*: *Enterococcus gallinarum*; *Mycobacterium* TB: *Mycobacterium tuberculosis*; *V. vulnificus*: *Vibrio vulnificus*; *V. cholera*: *Vibrio cholera*; *V. parahemolyticus*: *Vibrio parahemolyticus*; *Y. enterocolitica*: *Yersinia enterocolitica*; *Y. pseudotuberculosis*: *Yersinia pseudotuberculosis*; VRE: Vancomycin-resistant *Enterococci*.

about 50% of cases are present at the time of hospitalization and 50% develop during the hospitalization<sup>[1,29,36]</sup>. BT, systemic, and local immune dysfunction, particularly a decreased opsonic activity in ascitic fluid, are the main elements in the pathogenesis of SBP<sup>[1,15,17,37]</sup> (Figure 1). Accordingly, gut microflora including *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Enterococci*, and *Streptococci* are common causative organisms<sup>[1,15,17,37]</sup>. The classical symptoms of SBP include fever, abdominal pain, and worsening of pre-existing ascites, although these

symptoms may be absent in up to one-third of cases<sup>[38]</sup>. Therefore, diagnostic paracentesis is recommended to perform in all cirrhotic patients with ascites at the time of admission and/or in case of gastrointestinal (GI) bleeding, shock, signs of inflammation, hepatic encephalopathy, worsening of liver or renal function<sup>[37,39-41]</sup>. The hospital mortality for SBP ranges from 10%-50% depending on various factors<sup>[37]</sup>. Predictors for poor prognosis in SBP include older age, higher Child-Pugh scores, nosocomial origin, encephalopathy, elevated serum creatinine



**Figure 1 Pathogenesis of spontaneous bacterial peritonitis and bacteremia (reproduced from Bonnel *et al*<sup>[41]</sup>. *Clin Gastroenterol Hepatol* 2011; 9: 729. With permission). SBP: Spontaneous bacterial peritonitis; RES: Reticuloendothelial system; IV: Intravenous.**

and bilirubin, ascites culture positivity, presence of bacteremia, and infections with resistant organisms<sup>[42-45]</sup>. Notably, the modifiable factors to reduce morbidity and mortality in SBP include prompt diagnosis, proper first-line ATB treatment and prevention of subsequent renal failure<sup>[37]</sup>. SBP is one of the important prognostic landmark in the natural course of cirrhosis as the overall one-year mortality rate after a first episode of SBP are 30%-93% regardless of its recurrence<sup>[37,46,47]</sup>.

### Diagnosis of SBP

The diagnosis of SBP is relied on the cell count of the ascitic fluid, determined either by microscope or appropriate automated cell counters, and bacterial culture<sup>[40,41,48]</sup>. Ascitic fluid culture is important and should be performed before initiating ATB therapy by bedside inoculation of ascites  $\geq 10$  mL into blood culture bottles<sup>[49]</sup>. Reagent strips to assess leucocyte esterase activity of activated polymorphonuclear cells (PMN) are not recommended for rapid diagnosis of SBP due to unacceptable false-negative rates<sup>[50]</sup>. To date, most of reagent strips (LERS) that had been evaluated were developed for UTI with a threshold of  $> 50$  PMN/mm<sup>3</sup><sup>[37]</sup>. More recently, ascites-calibrated reagent strips (cut-off of  $> 250$  PMN/mm<sup>3</sup>) have been introduced for SBP with promising preliminarily results<sup>[51]</sup>. Based on available evidences, LERS seem to have low sensitivity for SBP, but have reliably given a high negative predictive value ( $> 95\%$  in most studies), which supports the potential role of LERS as a screening tool for SBP<sup>[52]</sup>. In addition, neutrophil gelatinase-associated lipocalin (NGAL), a protein involved in iron metabolism and links to the inflammation, and bacterial DNA in ascitic fluid have the potential to improve the diagnosis of SBP. The pivot study of using NGAL to differentiate bacterial peritonitis (30% were SBP) from nonbacterial peritonitis reported

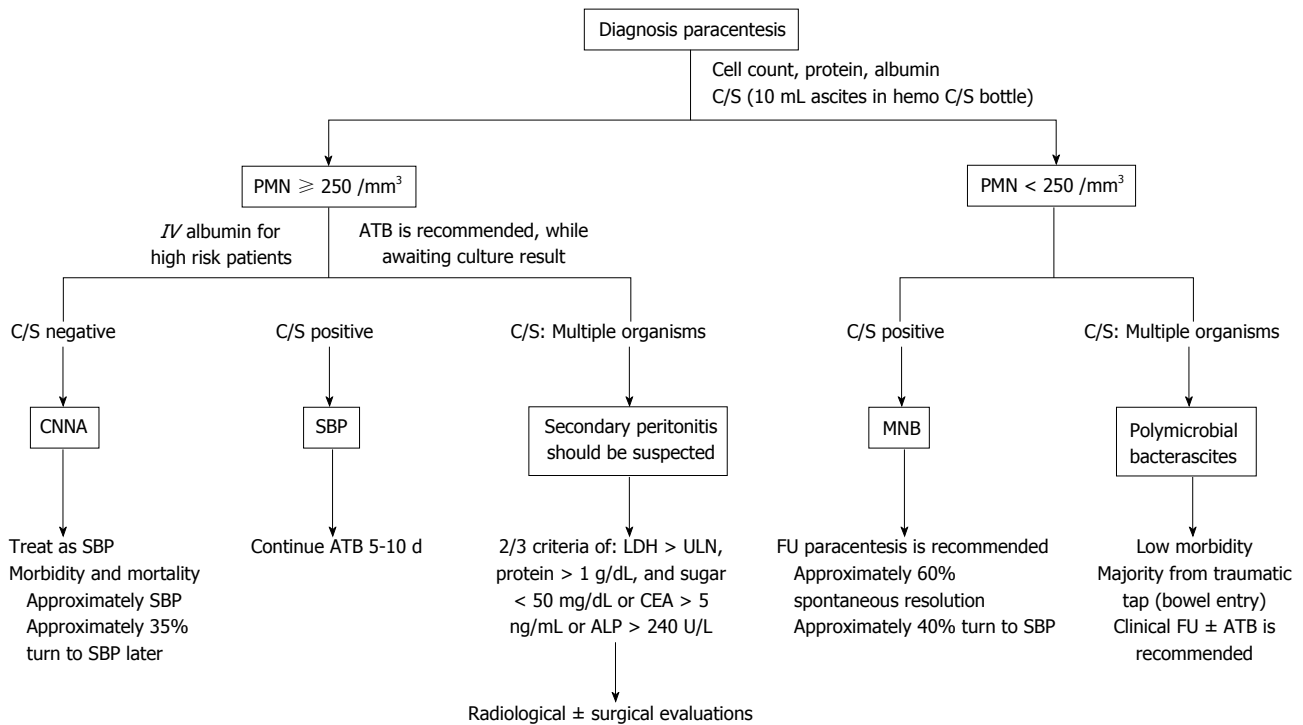
that AUC were 0.89 for NGAL and 0.94 for combination of NGAL and lactate dehydrogenase<sup>[53]</sup>. Detection of bacterial DNA by real-time polymerase chain reaction and sequencing of *16S rDNA* gene demonstrated poor results with negative results in almost half the culture-negative SBP episodes<sup>[54]</sup>. In contrast, another study using newly *in situ* hybridization method to detect global bacterial DNA demonstrated high sensitivity (91%) and specificity (100%) for detecting phagocytized bacterial DNA in the WBC of SBP ascites, with all test results obtained within one day<sup>[55]</sup>.

### Management of SBP

Empirical ATB should be given promptly to all cirrhotic patients with ascites PMN counts  $> 250$  cells/mm<sup>3</sup> in clinical settings that suggestive for ascitic fluid infection (culture results are often unavailable at this time)<sup>[40,41]</sup> (Figure 2). The choice of empirical ATB should be based on the origin of infection, individual risk factors for resistant organism and local microbial epidemiology. In general, the suggested initial treatments of community-acquired SBP are third-generation cephalosporins (mostly preferred), amoxicillin-clavulanate or quinolones (Table 2). These empirical ATB should be given intravenously for a duration of 5-10 d<sup>[40,41]</sup>. In countries with low rate of quinolone-resistant *Enterobacteriaceae*, oral quinolones may be used for uncomplicated SBP, as defined by cases without shock, ileus, GI bleeding, hepatic encephalopathy ( $\geq$  grade II) or renal impairment (creatinine  $> 3$  mg/dL)<sup>[56]</sup>. In nosocomial SBP, use of the antibiotics recommended above can be associated with unacceptable failure rates because resistance to third-generation cephalosporins (23%-44%) and quinolones (38%-50%) are increasingly reported<sup>[37,57,58]</sup>.

Notably, the incidence of SBP causing by with gram-positive and resistant bacteria (mainly ESBL-producing





**Figure 2** Algorithm for the management of cirrhotic patients with suspicious for ascitic fluid infection (adapted from Bonnel *et al*<sup>[1]</sup>. *Clin Gastroenterol Hepatol* 2011; 9: 732. With permission). PMN: Polymorphonuclear cells; SBP: Spontaneous bacterial peritonitis; ATB: Antibiotics; CNNA: Culture-negative neutrocytic ascites; MNB: Monobacterial non-neutrocytic bacterascites; LDH: Lactate dehydrogenase; CEA: Carcinoembryonic antigen; ALP: Alkaline phosphatase; ULN: Upper limit of normal; FU: Follow-up; C/S: Culture.

bacteria and multi-resistant gram-positive bacteria such as *Enterococci* or MRSA) has been increasingly reported in the healthcare associated and especially in nosocomial settings<sup>[37,57]</sup>. In patients with typical presentation and clinical improvement after ATB, a repeat of paracentesis is not necessary to assess for resolution of SBP<sup>[1,37,40,41]</sup>. However, in cases with questionable diagnosis or in those who did not satisfactorily improve with ATB, repeated paracentesis should be performed to document the response of treatment<sup>[37,40]</sup>. If the PMN count does not reduce by at least 25% after 2 d of ATB, changing treatment and/or reevaluation for other possible cause(s) of symptoms should be considered<sup>[37,59]</sup>.

Renal impairment develops in 30%-40% of SBP cases and is a strong predictor of death during hospitalization<sup>[39,40,60]</sup>. The use of intravenous albumin (1.5 g/kg within 6 h of SBP diagnosis followed by 1 g/kg on day 3) in conjunction with intravenous (IV) antibiotic was found to reduce the incidence of renal impairment from 33% to 10% and mortality from 29% to 10%<sup>[61]</sup>. Notably, albumin infusion was particularly effective in patients with baseline serum creatinine  $\geq 1$  mg/dL, blood urea nitrogen  $\geq 30$  mg/dL or bilirubin  $\geq 4$  mg/dL<sup>[39,61]</sup>. Unfortunately, albumin infusion in high-risk SBP has been underutilized, even in the United States, with > 50% of cases did not follow the guidelines<sup>[62]</sup>. It is unclear whether crystalloids or artificial colloids could replace albumin in this setting<sup>[39-41,63]</sup>.

### Prophylaxis of SBP

After recovering from SBP, the rate of recurrence is

around 43% at 6 mo and 69% at 1 year<sup>[46]</sup>. Therefore, secondary prophylaxis of SBP should be given indefinitely or until LT<sup>[37,40,61,64]</sup>. Intermittent dosing of prophylactic ATB may select resistant flora, thus daily dosing is preferred<sup>[37,40]</sup> (Table 4).

Primary prophylaxis of SBP is justified for patients with high risk for developing SBP. A meta-analysis of ATB prophylaxis in cirrhotic patients with GI hemorrhage (5 RCT;  $n = 534$ ) revealed 32% reduction of infections including SBP and/or bacteremia ( $P < 0.001$ ) and 9% increase in survival ( $P = 0.004$ )<sup>[28]</sup>. Further, a subsequent meta-analysis of 8 oral antibiotic trials ( $n = 647$ ) demonstrated 72% reduction in mortality at 3 mo; only 6 patients were additionally treated in order to prevent another death<sup>[65]</sup>. Oral norfloxacin is often utilized for primary prophylaxis in most settings, however IV ceftriaxone has been shown to be more effective than oral norfloxacin in patients with particularly advanced cirrhosis<sup>[66]</sup> (Table 4).

In cirrhotic patients with low ascitic fluid protein < 1.5 g/dL, the risk of developing a first episode of SBP is 13%-45% at 1 year<sup>[32,39]</sup>. However, several studies evaluating primary prophylaxis of SBP with norfloxacin in this setting yielded heterogeneous results<sup>[39]</sup>. Notably, a well-designed, randomized, controlled trial conducted in patients with severe liver disease and ascites protein < 1.5 g/dL without prior SBP demonstrated that norfloxacin (400 mg/d) reduced the development of SBP (from 61% to 7%) and improved survival at 1 year (from 48% to 60%)<sup>[67]</sup>. Notably, primary prophylactic ATB for SBP should be considered only for selected patients with

**Table 4** Vaccinations and other preventive measures for bacterial infections in patients with cirrhosis

Avoidance	
Raw/uncooked foods, especially seafood	
Close contact to at-risk animals or sick people	
Wound exposure to flood or seawater	
Vaccination <sup>[87]</sup>	
Influenza	Recommended yearly for all patients with chronic liver disease
Pneumococcal (polysaccharide)	Recommended for all cirrhotic patient Booster dose after 3-5 yr
Hepatitis A	Recommended for all non-immune, cirrhotic patient, 2 injections 6-12 mo apart Anti-HAV should be checked 1-2 mo after the second dose
Hepatitis B	Recommended for all cirrhotic patient without serological markers of HBV ( <i>e.g.</i> , negative HBsAg, anti-HBs, and anti-HBc antibodies) 3 injections (at month 0, 1 and 6) Anti-HBs should be checked 1-2 mo after the last dose Patients with advanced cirrhosis should receive 1 dose of 40 µg/mL (Recombivax HB) administered on a 3-dose schedule or 2 doses of 20 µg/mL (Engerix-B) administered simultaneously on a 4-dose schedule at 0, 1, 2 and 6 mo Recommendations are as same as general adult population
Other vaccines, <i>e.g.</i> , Td, Tdap, MMR, varicella	
Prophylactic antibiotics	
Secondary prophylaxis for SBP <sup>[32,41]</sup>	Recommended for all cirrhotic patients who recovered from SBP Norfloxacin 400 mg PO daily Alternatives: TMP/SMX 1 double-strength tablet or ciprofloxacin 500 mg PO daily
Primary prophylaxis in GI bleeding <sup>[32,41]</sup>	Recommended for all cirrhotic patients with GI hemorrhage Norfloxacin 400 mg PO twice daily or ceftriaxone 1 g IV daily for 7 d IV ceftriaxone is preferred, in patients with advanced cirrhosis as defined by the presence of at least two of the following: Ascites, severe malnutrition, encephalopathy or bilirubin > 3 mg/dL
Primary prophylaxis in patients with low ascitic fluid protein <sup>[32,41]</sup>	Recommended for cirrhotic patients with ascitic fluid protein < 1.5 g/dL and at least one of the following is present: Serum creatinine > 1.2 mg/dL, blood urea nitrogen > 25 mg/dL, serum sodium < 130 mEq/L or Child-Pugh > 9 points with bilirubin > 3 mg/dL
Prophylaxis before undergoing endoscopic and surgical procedures	Prophylactic antibiotics are recommended for the moderate-high risk invasive endoscopic or surgical procedures (choice of antibiotics should be individualized) Prophylactic antibiotics are not routinely recommended for diagnostic endoscopy, elective variceal band ligation or sclerotherapy, and abdominal paracentesis

HBV: Hepatitis B virus; SBP: Spontaneous bacterial peritonitis; Td: Tetanus-Diphtheria; Tdap: Tetanus-Diphtheria-Pertussis; MMR: Measles/Mumps/Rubella; GI: Gastrointestinal; TMP/SMX: Trimethoprim/sulfamethoxazole; PO: Per oral; IV: Intravenous.

advanced cirrhosis and ascitic fluid protein < 1.5 g/dL since more liberal use of these ATB in long-term would lead to subsequent infection by resistant bacteria as well as *Clostridium difficile*-associated diarrhea (Table 4)<sup>[39-41]</sup>.

### Consequences of bacterial infections in cirrhosis

Bacterial infections in cirrhosis are associated with poor outcomes (increased mortality about 4 folds)<sup>[47]</sup>. Both short- and long-term mortality rates of sepsis in cirrhotic patients are very high; 26%-44% of patients die within 1 mo after infection and another one-third die in 1 year<sup>[4,47]</sup>. The clinical predictors of death during or following infection are advanced liver disease, nosocomial origin, gastrointestinal hemorrhage, encephalopathy, liver cancer, presence of shock and organ failure (especially renal failure)<sup>[4,47]</sup>.

The suggested strategies for the management of cirrhotic patients with severe sepsis are discussed in depth in other articles<sup>[23,32,68,69]</sup>. Broad spectrum empirical ATB<sup>[70]</sup> and fluid resuscitation, with either crystalloids or colloids (albumin, gelatins or hydroxyethyl starches), should be promptly initiated and followed an early goal-directed therapy approach (stepwise emergent resuscitation with predefined goals to keep mean arterial pressure  $\geq$  65 mmHg, central venous

pressure between 8-12 mmHg, central venous oxygen saturation  $\geq$  70% and urine output  $\geq$  0.5 mL/kg per hour)<sup>[23,32,68]</sup>. Resuscitation with crystalloids requires more fluid to attain the same targets and results in more edema, particularly in cirrhotic patients with hypoalbuminemia<sup>[32]</sup>. The benefit of resuscitation with albumin in non-cirrhotic patients with sepsis has been reported<sup>[71]</sup>. However, the role of albumin infusion for sepsis other than from SBP in cirrhosis is still unclear. The RCT from Spain found beneficial effects on renal and circulatory functions with a potential benefit on survival<sup>[72]</sup>. Conversely, more recent RCT from France reported that albumin delayed the onset of renal failure, but did not significantly improve 3-mo renal failure and survival rates. Thus, pulmonary edema developed in 8% of patients in the albumin group<sup>[73]</sup>. Norepinephrine and dopamine have been considered as the first-choice vasopressor agents in patients with septic shock<sup>[23,32,68,69]</sup>. Cirrhotic patients with septic shock are often associated with vascular hyporeactivity to these vasopressor agents. Thus, inotropic drugs are not generally effective since they already present high cardiac outputs<sup>[23,32,68]</sup>. Relative adrenal insufficiency is common (51%-77%) in cirrhotic patients with septic shock, however the effects of corticosteroids on such patients' outcomes

are unclear<sup>[23,32,68]</sup>. Therefore, stress dose corticosteroid is currently recommended only for patients with vasopressor-unresponsive septic shock<sup>[23,32,68]</sup>. Blood sugar should be maintained in the range of 140-180 mg/dL<sup>[69]</sup>.

Acute kidney injury following infections develop in 27%-34% of patients with advanced cirrhosis<sup>[2,61,74,75]</sup>, and is a strong predictor of death (40%-50% mortality)<sup>[47,74,75]</sup>. Risk factors for infection-induced renal failure in cirrhosis include advanced liver disease<sup>[74-76]</sup>, pre-existing kidney disease<sup>[76]</sup>, hypovolemia or low cardiac output<sup>[2,75]</sup>, unresolved infection<sup>[74]</sup> and not receiving prompt albumin infusion<sup>[61]</sup>. It should be noted that most studies that reported poor survival in patients with infection-induced renal failure have defined renal failure as a serum creatinine level of > 1.5 mg/dL. Recently, the International Ascites Club and the Acute Dialysis Quality Initiative group proposed that acute kidney injury (AKI) in cirrhosis should be redefined as an increase in serum creatinine level of 0.3 mg/dL in less than 48 h or a 50% increase in serum creatinine level from a stable baseline reading within the previous 6 mo, irrespective of the final serum creatinine level<sup>[77,78]</sup>. This new definition was then evaluated and found to accurately predict 30-d mortality in patients with cirrhosis and infection (10-fold higher among those with irreversible AKI than those without AKI)<sup>[79]</sup>. Renal failure during infection (without septic shock) that does not respond to albumin infusion is considered hepatorenal syndrome<sup>[80]</sup>.

Bacterial infection can trigger a rapid deterioration of liver functions in patients with cirrhosis and it is one of the most common precipitating cause of ACLF, which represents > 30% of the cases<sup>[3,23,81,82]</sup>. The most common sites of bacterial infection are ascites and lungs<sup>[81]</sup>. Moreover, infections were the second most common cause of death at 28 d among patients with ACLF (28%), behind multiple organ failure without septic or hypovolemic shock (44%). However, there was no difference in 28 d mortality among ACLF patients with or without the bacterial infection at admission (37% and 33%, respectively)<sup>[81]</sup>. Independent predictors of poor survival in patients with bacterial infections and ACLF were presence of organ(s) failure, second infections, admission values of high MELD, low blood pressure, leukocytosis, and low albumin<sup>[83]</sup>.

Pulmonary complications are commonly observed in cirrhotic patients with infections. Aspiration is common in encephalopathic patients. Acute respiratory distress syndrome is increasingly seen in cirrhosis that may develop in association with exaggerated SIRS in severe sepsis<sup>[84]</sup>. Prognosis of cirrhotic patients with respiratory failure is poor, with a mortality rate up to 33%-60%<sup>[69,85]</sup>. Additionally, sepsis-induced cytokines can further worsen pre-existing coagulation and platelet abnormalities in patients with cirrhosis<sup>[2,24]</sup>.

### Prevention measures

Preventive measures must be emphasized to all patients with cirrhosis and prophylactic ATB is suggested for

those who are at high risk of developing infections (Table 4)<sup>[2]</sup>. Notably, antibiotic prophylaxis has been associated with the development of multi-drug resistant bacteria and *C. difficile* infection. Therefore it should be judiciously used in those patients with proper indications.

Active immunization against hepatitis A and B viruses, influenza and pneumococcus are recommended since these preventable infections carry accompanied by higher morbidity and mortality in patients with cirrhosis (Table 4)<sup>[86-88]</sup>. Both cellular and humoral immune responses are suboptimal in cirrhosis, particularly in the advanced stage, which can be associated with inadequate post-vaccination antibody response, as well as loss of immunogenicity in the long-term<sup>[86-88]</sup>. Therefore, it is important to address immunization needs in patients with chronic liver disease or compensated cirrhosis early on, when immunizations are most effective.

Although there is no clear recommendation whether we can safely utilize live and attenuated vaccines in patients with cirrhosis, inactivated or killed-type vaccinations are generally preferable<sup>[86-88]</sup>. The incidence and severity of *Streptococcus pneumoniae* infections are increased in patients with cirrhosis<sup>[89-92]</sup>. Pneumococcal vaccination is less effective in patients with cirrhosis, with a further decline in protective antibodies after LT<sup>[93]</sup>. It is therefore recommended with booster doses every 5 years<sup>[86-88]</sup>. Incidence of seasonal flu is not obviously increased in cirrhosis; however, influenza may precipitate liver decompensation<sup>[86,87,94]</sup>. Influenza vaccine is well-tolerated and effective in cirrhotic patients, despite a mildly decreased immunogenicity<sup>[95,96]</sup>. All other vaccinations recommended for general adult population are also indicated in patients with cirrhosis as the Centers for Disease Control and Prevention recommendation for adults<sup>[97]</sup>.

### Proton pump inhibitors and the risk of infections in cirrhosis

Proton pump inhibitors (PPIs) have been widely used in patients with cirrhosis (sometimes over-utilized)<sup>[98]</sup>. Patients with cirrhosis have high prevalence of gastroduodenal mucosal lesions<sup>[99,100]</sup> and are associated with increased mortality rate from peptic ulcer bleeding (adjusted OR = 3.3; 95%CI: 2.2-4.9)<sup>[101]</sup>. However, clear evidence for a protective role of PPIs in cirrhosis is limited.

A state of gastric acid suppression induced by PPIs, particularly in long-term users, is known to be associated with small bowel bacterial overgrowth, alteration of gut flora and reduction of gastrointestinal motility<sup>[102-104]</sup>. By these effects, PPIs may enhance BT and possibly increase the risk of various infections in patients with cirrhosis. In addition, impairment of neutrophil function caused by PPIs has also been reported<sup>[105-107]</sup>. There have been several studies, including case-control, retrospective and prospective cohorts, and meta-analyses, suggesting that PPIs are associated with increased risk of bacterial infections, such as SBP, bacteremia, *Clostridium difficile*-associated diarrhea, and enteric

**Table 5** Studies demonstrated risk of bacterial infections in cirrhotic patients receiving proton pump inhibitors

Ref.	Design	n	Results
Campbell <i>et al</i> <sup>[116]</sup>	Case-control	116	NS for SBP (OR = 1.05; 95%CI: 0.43-2.57)
Bajaj <i>et al</i> <sup>[108]</sup>	Case-control	83230	PPI use were significantly higher in those with CDAD (74% vs 31%, <i>P</i> = 0.0001)
Bajaj <i>et al</i> <sup>[112]</sup>	Retrospective, propensity-matched	1268	↑ Serious infections (HR = 1.66; 95%CI: 1.31-2.12)
de Vos <i>et al</i> <sup>[119]</sup>	Case-control	102	PPI were more frequently used in SBP patients than in controls, but did not influence prognosis of SBP
Min <i>et al</i> <sup>[113]</sup>	Retrospective cohort	1554	↑ SBP (HR = 1.39; 95%CI: 1.057-1.843)
Mandorfer <i>et al</i> <sup>[117]</sup>	Retrospective	607	PPI neither predisposes to SBP (HR = 1.38; 95%CI: 0.63-3.01) or other infections (HR = 1.71; 95%CI: 0.85-3.44)
Terg <i>et al</i> <sup>[118]</sup>	Prospective	770	PPI therapy was not associated with a higher risk of SBP and other infections
Merli <i>et al</i> <sup>[114]</sup>	Cross-sectional	400	↑ Bacterial infections (OR = 2; 95%CI: 1.2-3.2)
O'Leary <i>et al</i> <sup>[115]</sup>	Prospective	188	↑ Infections: CDAD and SBP (OR = 2.94; 95%CI: 1.39-6.20)

NS: Not significance; SBP: Spontaneous bacterial peritonitis; PPI: Proton pump inhibitor; CDAD: Clostridium difficile associate disease.

infections, in patients with cirrhosis<sup>[108-115]</sup>. However, the association between PPIs and infections in cirrhosis remains somewhat controversial since many studies have reported conflicting results<sup>[116-119]</sup> (Table 5). Though randomized controlled studies are required to draw firm conclusions whether or not PPIs increase infections in cirrhosis, PPI should be used only if clinically indicated.

## ACKNOWLEDGMENTS

The authors are grateful to Professor K Rajender Reddy at the University of Pennsylvania, PA, United States for supportive guidance.

## REFERENCES

- Bonnell AR, Bunchorntavakul C, Reddy KR. Immune dysfunction and infections in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2011; **9**: 727-738 [PMID: 21397731 DOI: 10.1016/j.cgh.2011.02.031]
- Bunchorntavakul C, Chavalitthamrong D. Bacterial infections other than spontaneous bacterial peritonitis in cirrhosis. *World J Hepatol* 2012; **4**: 158-168 [PMID: 22662285 DOI: 10.4254/wjh.v4.i5.158]
- Tandon P, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. *Semin Liver Dis* 2008; **28**: 26-42 [PMID: 18293275 DOI: 10.1055/s-2008-1040319]
- Christou L, Pappas G, Falagas ME. Bacterial infection-related morbidity and mortality in cirrhosis. *Am J Gastroenterol* 2007; **102**: 1510-1517 [PMID: 17509025 DOI: 10.1111/j.1572-0241.2007.01286.x]
- Ghassemi S, Garcia-Tsao G. Prevention and treatment of infections in patients with cirrhosis. *Best Pract Res Clin Gastroenterol* 2007; **21**: 77-93 [PMID: 17223498]
- Fiuza C, Salcedo M, Clemente G, Tellado JM. In vivo neutrophil dysfunction in cirrhotic patients with advanced liver disease. *J Infect Dis* 2000; **182**: 526-533 [PMID: 10915084 DOI: 10.1086/315742]
- Garfia C, García-Ruiz I, Solís-Herruzo JA. Deficient phospholipase C activity in blood polymorphonuclear neutrophils from patients with liver cirrhosis. *J Hepatol* 2004; **40**: 749-756 [PMID: 15094221 DOI: 10.1016/j.jhep.2004.01.004]
- Shawcross DL, Wright GA, Stadlbauer V, Hodges SJ, Davies NA, Wheeler-Jones C, Pitsillides AA, Jalan R. Ammonia impairs neutrophil phagocytic function in liver disease. *Hepatology* 2008; **48**: 1202-1212 [PMID: 18697192 DOI: 10.1002/hep.22474]
- Wasmuth HE, Kunz D, Yagmur E, Timmer-Stranghöner A, Vidacek D, Siewert E, Bach J, Geier A, Purucker EA, Gressner AM, Matern S, Lammert F. Patients with acute on chronic liver failure display "sepsis-like" immune paralysis. *J Hepatol* 2005; **42**: 195-201 [PMID: 15664244 DOI: 10.1016/j.jhep.2004.10.019]
- Ruot B, Béchereau F, Bayle G, Breuillé D, Obled C. The response of liver albumin synthesis to infection in rats varies with the phase of the inflammatory process. *Clin Sci (Lond)* 2002; **102**: 107-114 [PMID: 11749667]
- Guarner-Argente C, Sánchez E, Vidal S, Román E, Concepción M, Poca M, Sánchez D, Juárez C, Soriano G, Guarner C. Toll-like receptor 4 D299G polymorphism and the incidence of infections in cirrhotic patients. *Aliment Pharmacol Ther* 2010; **31**: 1192-1199 [PMID: 20222908 DOI: 10.1111/j.1365-2036.2010.04291.x]
- Nischalke HD, Berger C, Aldenhoff K, Thyssen L, Gentemann M, Grünhage F, Lammert F, Nattermann J, Sauerbruch T, Spengler U, Appenrodt B. Toll-like receptor (TLR) 2 promoter and intron 2 polymorphisms are associated with increased risk for spontaneous bacterial peritonitis in liver cirrhosis. *J Hepatol* 2011; **55**: 1010-1016 [PMID: 21356257 DOI: 10.1016/j.jhep.2011.02.022]
- Ledesma Castaño F, Echevarria Vierna S, Lozano Polo JL, Oloriz Rivas R, Alvarez Moreno C, Pons Romero F. Interleukin-1 in alcoholic cirrhosis of the liver: the influence of nutrition. *Eur J Clin Nutr* 1992; **46**: 527-533 [PMID: 1623857]
- Gomez F, Ruiz P, Schreiber AD. Impaired function of macrophage Fc gamma receptors and bacterial infection in alcoholic cirrhosis. *N Engl J Med* 1994; **331**: 1122-1128 [PMID: 7935636 DOI: 10.1056/nejm199410273311704]
- Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. *J Hepatol* 2014; **60**: 197-209 [PMID: 23993913 DOI: 10.1016/j.jhep.2013.07.044]
- Bellet P, Francés R, Such J. Pathological bacterial translocation in cirrhosis: pathophysiology, diagnosis and clinical implications. *Liver Int* 2013; **33**: 31-39 [PMID: 23121656 DOI: 10.1111/liv.12021]
- Wiest R, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. *Hepatology* 2005; **41**: 422-433 [PMID: 15723320 DOI: 10.1002/hep.20632]
- Bellet P, García-Pagán JC, Francés R, Abalde JG, Navasa M, Pérez-Mateo M, Such J, Bosch J. Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. *Hepatology* 2010; **52**: 2044-2052 [PMID: 20979050 DOI: 10.1002/hep.23918]
- Teltschik Z, Wiest R, Beisner J, Nuding S, Hofmann C, Schoelmerich J, Bevins CL, Stange EF, Wehkamp J. Intestinal bacterial translocation in rats with cirrhosis is related to compromised Paneth cell antimicrobial host defense. *Hepatology* 2012; **55**: 1154-1163 [PMID: 22095436 DOI: 10.1002/hep.24789]
- Campillo B, Pernet P, Bories PN, Richardet JP, Devanlay M, Aussel C. Intestinal permeability in liver cirrhosis: relationship with severe septic complications. *Eur J Gastroenterol Hepatol* 1999; **11**: 755-759 [PMID: 10445796]
- Chang CS, Chen GH, Lien HC, Yeh HZ. Small intestine dysmotility and bacterial overgrowth in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 1998; **28**: 1187-1190 [PMID: 9794900 DOI: 10.1002/hep.510280504]



- 22 **Reiberger T**, Ferlitsch A, Payer BA, Mandorfer M, Heinisch BB, Hayden H, Lammert F, Trauner M, Peck-Radosavljevic M, Vogelsang H. Non-selective betablocker therapy decreases intestinal permeability and serum levels of LBP and IL-6 in patients with cirrhosis. *J Hepatol* 2013; **58**: 911-921 [PMID: 23262249 DOI: 10.1016/j.jhep.2012.12.011]
- 23 **Gustot T**, Durand F, Lebre C, Vincent JL, Moreau R. Severe sepsis in cirrhosis. *Hepatology* 2009; **50**: 2022-2033 [PMID: 19885876 DOI: 10.1002/hep.23264]
- 24 **Wong F**, Bernardi M, Balk R, Christman B, Moreau R, Garcia-Tsao G, Patch D, Soriano G, Hoefs J, Navasa M. Sepsis in cirrhosis: report on the 7th meeting of the International Ascites Club. *Gut* 2005; **54**: 718-725 [PMID: 15831923 DOI: 10.1136/gut.2004.038679]
- 25 **Barnes PF**, Arevalo C, Chan LS, Wong SF, Reynolds TB. A prospective evaluation of bacteremic patients with chronic liver disease. *Hepatology* 1988; **8**: 1099-1103 [PMID: 3417230]
- 26 **Borzio M**, Salerno F, Piantoni L, Cazzaniga M, Angeli P, Bissoli F, Boccia S, Colloredo-Mels G, Corigliano P, Fornaciari G, Marengo G, Pistrà R, Salvagnini M, Sangiovanni A. Bacterial infection in patients with advanced cirrhosis: a multicentre prospective study. *Dig Liver Dis* 2001; **33**: 41-48 [PMID: 11303974]
- 27 **Fernández J**, Navasa M, Gómez J, Colmenero J, Vila J, Arroyo V, Rodés J. Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology* 2002; **35**: 140-148 [PMID: 11786970 DOI: 10.1053/jhep.2002.30082]
- 28 **Bernard B**, Grangé JD, Khac EN, Amiot X, Opolon P, Poynard T. Antibiotic prophylaxis for the prevention of bacterial infections in cirrhotic patients with gastrointestinal bleeding: a meta-analysis. *Hepatology* 1999; **29**: 1655-1661 [PMID: 10347104 DOI: 10.1002/hep.510290608]
- 29 **Caly WR**, Strauss E. A prospective study of bacterial infections in patients with cirrhosis. *J Hepatol* 1993; **18**: 353-358 [PMID: 8228129]
- 30 **Merli M**, Lucidi C, Giannelli V, Giusto M, Riggio O, Falcone M, Ridola L, Attili AF, Venditti M. Cirrhotic patients are at risk for health care-associated bacterial infections. *Clin Gastroenterol Hepatol* 2010; **8**: 979-985 [PMID: 20621200 DOI: 10.1016/j.cgh.2010.06.024]
- 31 **Fernández J**, Acevedo J, Castro M, Garcia O, de Lope CR, Roca D, Pavesi M, Sola E, Moreira L, Silva A, Seva-Pereira T, Corradi F, Mensa J, Ginès P, Arroyo V. Prevalence and risk factors of infections by multiresistant bacteria in cirrhosis: a prospective study. *Hepatology* 2012; **55**: 1551-1561 [PMID: 22183941 DOI: 10.1002/hep.25532]
- 32 **Fernández J**, Gustot T. Management of bacterial infections in cirrhosis. *J Hepatol* 2012; **56** Suppl 1: S1-S12 [PMID: 22300459 DOI: 10.1016/S0168-8278(12)60002-6]
- 33 **Schuetz P**, Albrich W, Mueller B. Procalcitonin for diagnosis of infection and guide to antibiotic decisions: past, present and future. *BMC Med* 2011; **9**: 107 [PMID: 21936959 DOI: 10.1186/1741-7015-9-107]
- 34 **Lin KH**, Wang FL, Wu MS, Jiang BY, Kao WL, Chao HY, Wu JY, Lee CC. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection in patients with liver cirrhosis: a systematic review and meta-analysis. *Diagn Microbiol Infect Dis* 2014; **80**: 72-78 [PMID: 24974271 DOI: 10.1016/j.diagmicrobio.2014.03.029]
- 35 **Papp M**, Vitalis Z, Altörjay I, Tornai I, Udvardy M, Harsfalvi J, Vida A, Kappelmayer J, Lakatos PL, Antal-Szalmas P. Acute phase proteins in the diagnosis and prediction of cirrhosis associated bacterial infections. *Liver Int* 2012; **32**: 603-611 [PMID: 22145664 DOI: 10.1111/j.1478-3231.2011.02689.x]
- 36 **Gioannini TL**, Zhang D, Teghanemt A, Weiss JP. An essential role for albumin in the interaction of endotoxin with lipopolysaccharide-binding protein and sCD14 and resultant cell activation. *J Biol Chem* 2002; **277**: 47818-47825 [PMID: 12372833 DOI: 10.1074/jbc.M206404200]
- 37 **Wiest R**, Krag A, Gerbes A. Spontaneous bacterial peritonitis: recent guidelines and beyond. *Gut* 2012; **61**: 297-310 [PMID: 22147550 DOI: 10.1136/gutjnl-2011-300779]
- 38 **Chinnock B**, Afarian H, Minnigan H, Butler J, Hendey GW. Physician clinical impression does not rule out spontaneous bacterial peritonitis in patients undergoing emergency department paracentesis. *Ann Emerg Med* 2008; **52**: 268-273 [PMID: 18433932 DOI: 10.1016/j.annemergmed.2008.02.016]
- 39 **European Association for the Study of the Liver**. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]
- 40 **Runyon BA**. Management of adult patients with ascites due to cirrhosis: an update. *Hepatology* 2009; **49**: 2087-2107 [PMID: 19475696 DOI: 10.1002/hep.22853]
- 41 **Runyon BA**. Introduction to the revised American Association for the Study of Liver Diseases Practice Guideline management of adult patients with ascites due to cirrhosis 2012. *Hepatology* 2013; **57**: 1651-1653 [PMID: 23463403 DOI: 10.1002/hep.26359]
- 42 **Toledo C**, Salmerón JM, Rimola A, Navasa M, Arroyo V, Llach J, Ginès A, Ginès P, Rodés J. Spontaneous bacterial peritonitis in cirrhosis: predictive factors of infection resolution and survival in patients treated with cefotaxime. *Hepatology* 1993; **17**: 251-257 [PMID: 8428722]
- 43 **Cheong HS**, Kang CI, Lee JA, Moon SY, Joung MK, Chung DR, Koh KC, Lee NY, Song JH, Peck KR. Clinical significance and outcome of nosocomial acquisition of spontaneous bacterial peritonitis in patients with liver cirrhosis. *Clin Infect Dis* 2009; **48**: 1230-1236 [PMID: 19302016 DOI: 10.1086/597585]
- 44 **Almdal TP**, Skinhøj P. Spontaneous bacterial peritonitis in cirrhosis. Incidence, diagnosis, and prognosis. *Scand J Gastroenterol* 1987; **22**: 295-300 [PMID: 3589498]
- 45 **Campillo B**, Richardet JP, Kheo T, Dupeyron C. Nosocomial spontaneous bacterial peritonitis and bacteremia in cirrhotic patients: impact of isolate type on prognosis and characteristics of infection. *Clin Infect Dis* 2002; **35**: 1-10 [PMID: 12060868 DOI: 10.1086/340617]
- 46 **Titó L**, Rimola A, Ginès P, Llach J, Arroyo V, Rodés J. Recurrence of spontaneous bacterial peritonitis in cirrhosis: frequency and predictive factors. *Hepatology* 1988; **8**: 27-31 [PMID: 3257456]
- 47 **Arvaniti V**, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, Burroughs AK. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* 2010; **139**: 1246-1256.e1-5 [PMID: 20558165 DOI: 10.1053/j.gastro.2010.06.019]
- 48 **Dever JB**, Sheikh MY. Review article: spontaneous bacterial peritonitis--bacteriology, diagnosis, treatment, risk factors and prevention. *Aliment Pharmacol Ther* 2015; **41**: 1116-1131 [PMID: 25819304 DOI: 10.1111/apt.13172]
- 49 **Bobadilla M**, Sifuentes J, Garcia-Tsao G. Improved method for bacteriological diagnosis of spontaneous bacterial peritonitis. *J Clin Microbiol* 1989; **27**: 2145-2147 [PMID: 2685014]
- 50 **Nousbaum JB**, Cadranet JF, Nahon P, Khac EN, Moreau R, Thévenot T, Silvain C, Bureau C, Nouel O, Pilette C, Paupard T, Vanbiervliet G, Oberti F, Davion T, Jouannaud V, Roche B, Bernard PH, Beaulieu S, Danne O, Thabut D, Chagneau-Derode C, de Lédinghen V, Mathurin P, Pauwels A, Bronowicki JP, Habersetzer F, Aberger A, Audigier JC, Sapay T, Grangé JD, Tran A. Diagnostic accuracy of the Multistix 8 SG reagent strip in diagnosis of spontaneous bacterial peritonitis. *Hepatology* 2007; **45**: 1275-1281 [PMID: 17464969 DOI: 10.1002/hep.21588]
- 51 **Mendler MH**, Agarwal A, Trimzi M, Madrigal E, Tsushima M, Joo E, Santiago M, Flores E, David G, Workman A, Runyon B. A new highly sensitive point of care screen for spontaneous bacterial peritonitis using the leukocyte esterase method. *J Hepatol* 2010; **53**: 477-483 [PMID: 20646775 DOI: 10.1016/j.jhep.2010.04.011]
- 52 **Koulouzidis A**. Diagnosis of spontaneous bacterial peritonitis: an update on leucocyte esterase reagent strips. *World J Gastroenterol* 2011; **17**: 1091-1094 [PMID: 21448413 DOI: 10.3748/wjg.v17.i9.1091]
- 53 **Lippi G**, Caleffi A, Pipitone S, Elia G, Ngah A, Aloe R, Avanzini P, Ferrari C. Assessment of neutrophil gelatinase-associated lipocalin and lactate dehydrogenase in peritoneal fluids for the screening

- of bacterial peritonitis. *Clin Chim Acta* 2013; **418**: 59-62 [PMID: 23318563 DOI: 10.1016/j.cca.2012.12.020]
- 54 **Soriano G**, Esparcia O, Montemayor M, Guarner-Argente C, Pericas R, Torras X, Calvo N, Román E, Navarro F, Guarner C, Coll P. Bacterial DNA in the diagnosis of spontaneous bacterial peritonitis. *Aliment Pharmacol Ther* 2011; **33**: 275-284 [PMID: 21083594 DOI: 10.1111/j.1365-2036.2010.04506.x]
  - 55 **Enomoto H**, Inoue S, Matsuhisa A, Aizawa N, Imanishi H, Saito M, Iwata Y, Tanaka H, Ikeda N, Sakai Y, Takashima T, Shimomura S, Iijima H, Nakamura H, Nishiguchi S. Development of a new in situ hybridization method for the detection of global bacterial DNA to provide early evidence of a bacterial infection in spontaneous bacterial peritonitis. *J Hepatol* 2012; **56**: 85-94 [PMID: 21835139 DOI: 10.1016/j.jhep.2011.06.025]
  - 56 **Navasa M**, Follo A, Llovet JM, Clemente G, Vargas V, Rimola A, Marco F, Guarner C, Forné M, Planas R, Bañares R, Castells L, Jimenez De Anta MT, Arroyo V, Rodés J. Randomized, comparative study of oral ofloxacin versus intravenous cefotaxime in spontaneous bacterial peritonitis. *Gastroenterology* 1996; **111**: 1011-1017 [PMID: 8831596]
  - 57 **Umgelter A**, Reindl W, Miedaner M, Schmid RM, Huber W. Failure of current antibiotic first-line regimens and mortality in hospitalized patients with spontaneous bacterial peritonitis. *Infection* 2009; **37**: 2-8 [PMID: 19169633 DOI: 10.1007/s15010-008-8060-9]
  - 58 **Castellote J**, Ariza X, Girbau A, Broquetas T, Lobatón T, Salord S, Rota R, Xiol X. Antibiotic-resistant bacteria in spontaneous bacterial peritonitis. Is it time to change? *J Hepatol* 2010; **52** (Suppl): S69 [DOI: 10.1016/S0168-8278(10)60158-4]
  - 59 **Runyon BA**, Hoefs JC. Spontaneous vs secondary bacterial peritonitis. Differentiation by response of ascitic fluid neutrophil count to antimicrobial therapy. *Arch Intern Med* 1986; **146**: 1563-1565 [PMID: 3729637]
  - 60 **Follo A**, Llovet JM, Navasa M, Planas R, Forns X, Francitorra A, Rimola A, Gassull MA, Arroyo V, Rodés J. Renal impairment after spontaneous bacterial peritonitis in cirrhosis: incidence, clinical course, predictive factors and prognosis. *Hepatology* 1994; **20**: 1495-1501 [PMID: 7982650]
  - 61 **Sort P**, Navasa M, Arroyo V, Aldegue X, Planas R, Ruiz-del-Arbol L, Castells L, Vargas V, Soriano G, Guevara M, Ginès P, Rodés J. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med* 1999; **341**: 403-409 [PMID: 10432325 DOI: 10.1056/nejm199908053410603]
  - 62 **Peeraphatdit T**, Gulleen EA, Anderson KB, Chaiteerakij R, Skarda PK. Letter: underutilisation of albumin infusion in high-risk spontaneous bacterial peritonitis. *Aliment Pharmacol Ther* 2015; **42**: 241-242 [PMID: 26081688 DOI: 10.1111/apt.13252]
  - 63 **Fernández J**, Monteagudo J, Bargallo X, Jiménez W, Bosch J, Arroyo V, Navasa M. A randomized unblinded pilot study comparing albumin versus hydroxyethyl starch in spontaneous bacterial peritonitis. *Hepatology* 2005; **42**: 627-634 [PMID: 16108036 DOI: 10.1002/hep.20829]
  - 64 **Ginès P**, Rimola A, Planas R, Vargas V, Marco F, Almela M, Forné M, Miranda ML, Llach J, Salmerón JM. Norfloxacin prevents spontaneous bacterial peritonitis recurrence in cirrhosis: results of a double-blind, placebo-controlled trial. *Hepatology* 1990; **12**: 716-724 [PMID: 2210673]
  - 65 **Saab S**, Hernandez JC, Chi AC, Tong MJ. Oral antibiotic prophylaxis reduces spontaneous bacterial peritonitis occurrence and improves short-term survival in cirrhosis: a meta-analysis. *Am J Gastroenterol* 2009; **104**: 993-1001; quiz 1002 [PMID: 19277033 DOI: 10.1038/ajg.2009.3]
  - 66 **Fernández J**, Ruiz del Arbol L, Gómez C, Durandez R, Serradilla R, Guarner C, Planas R, Arroyo V, Navasa M. Norfloxacin vs ceftriaxone in the prophylaxis of infections in patients with advanced cirrhosis and hemorrhage. *Gastroenterology* 2006; **131**: 1049-1056; quiz 1285 [PMID: 17030175 DOI: 10.1053/j.gastro.2006.07.010]
  - 67 **Fernández J**, Navasa M, Planas R, Montoliu S, Monfort D, Soriano G, Vila C, Pardo A, Quintero E, Vargas V, Such J, Ginès P, Arroyo V. Primary prophylaxis of spontaneous bacterial peritonitis delays hepatorenal syndrome and improves survival in cirrhosis. *Gastroenterology* 2007; **133**: 818-824 [PMID: 17854593 DOI: 10.1053/j.gastro.2007.06.065]
  - 68 **Ginès P**, Fernández J, Durand F, Saliba F. Management of critically-ill cirrhotic patients. *J Hepatol* 2012; **56** Suppl 1: S13-S24 [PMID: 22300462 DOI: 10.1016/S0168-8278(12)60003-8]
  - 69 **Olson JC**, Wendon JA, Kramer DJ, Arroyo V, Jalan R, Garcia-Tsao G, Kamath PS. Intensive care of the patient with cirrhosis. *Hepatology* 2011; **54**: 1864-1872 [PMID: 21898477 DOI: 10.1002/hep.24622]
  - 70 **Arabi YM**, Dara SI, Memish Z, Al Abdulkareem A, Tamim HM, Al-Shirawi N, Parrillo JE, Dodek P, Lapinsky S, Feinstein D, Wood G, Dial S, Zanotti S, Kumar A. Antimicrobial therapeutic determinants of outcomes from septic shock among patients with cirrhosis. *Hepatology* 2012; **56**: 2305-2315 [PMID: 22753144 DOI: 10.1002/hep.25931]
  - 71 **Delaney AP**, Dan A, McCaffrey J, Finfer S. The role of albumin as a resuscitation fluid for patients with sepsis: a systematic review and meta-analysis. *Crit Care Med* 2011; **39**: 386-391 [PMID: 21248514 DOI: 10.1097/CCM.0b013e3181ffe217]
  - 72 **Guevara M**, Terra C, Nazar A, Solà E, Fernández J, Pavesi M, Arroyo V, Ginès P. Albumin for bacterial infections other than spontaneous bacterial peritonitis in cirrhosis. A randomized, controlled study. *J Hepatol* 2012; **57**: 759-765 [PMID: 22732511 DOI: 10.1016/j.jhep.2012.06.013]
  - 73 **Thévenot T**, Bureau C, Oberti F, Anty R, Louvet A, Plessier A, Rudler M, Heurgué-Berlot A, Rosa I, Talbodec N, Dao T, Ozenne V, Carbonell N, Causse X, Gorla O, Minello A, De Ledinghen V, Amathieu R, Barraud H, Nguyen-Khac E, Becker C, Paupard T, Botta-Fridlung D, Abdelli N, Guillemot F, Monnet E, Di Martino V. Effect of albumin in cirrhotic patients with infection other than spontaneous bacterial peritonitis. A randomized trial. *J Hepatol* 2015; **62**: 822-830 [PMID: 25463545 DOI: 10.1016/j.jhep.2014.11.017]
  - 74 **Fasolato S**, Angeli P, Dallagnese L, Maresio G, Zola E, Mazza E, Salinas F, Donà S, Fagioli S, Sticca A, Zanusi G, Cillo U, Frasson I, Destro C, Gatta A. Renal failure and bacterial infections in patients with cirrhosis: epidemiology and clinical features. *Hepatology* 2007; **45**: 223-229 [PMID: 17187409 DOI: 10.1002/hep.21443]
  - 75 **Terra C**, Guevara M, Torre A, Gilabert R, Fernández J, Martín-Llahí M, Baccaro ME, Navasa M, Bru C, Arroyo V, Rodés J, Ginès P. Renal failure in patients with cirrhosis and sepsis unrelated to spontaneous bacterial peritonitis: value of MELD score. *Gastroenterology* 2005; **129**: 1944-1953 [PMID: 16344063 DOI: 10.1053/j.gastro.2005.09.024]
  - 76 **Terg R**, Gadano A, Cartier M, Casciato P, Lucero R, Muñoz A, Romero G, Levi D, Terg G, Miguez C, Abecasis R. Serum creatinine and bilirubin predict renal failure and mortality in patients with spontaneous bacterial peritonitis: a retrospective study. *Liver Int* 2009; **29**: 415-419 [PMID: 18803587 DOI: 10.1111/j.1478-3231.2008.01877.x]
  - 77 **Wong F**, Nadim MK, Kellum JA, Salerno F, Bellomo R, Gerbes A, Angeli P, Moreau R, Davenport A, Jalan R, Ronco C, Genyk Y, Arroyo V. Working Party proposal for a revised classification system of renal dysfunction in patients with cirrhosis. *Gut* 2011; **60**: 702-709 [PMID: 21325171 DOI: 10.1136/gut.2010.236133]
  - 78 **Angeli P**, Ginès P, Wong F, Bernardi M, Boyer TD, Gerbes A, Moreau R, Jalan R, Sarin SK, Piano S, Moore K, Lee SS, Durand F, Salerno F, Caraceni P, Kim WR, Arroyo V, Garcia-Tsao G. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. *J Hepatol* 2015; **62**: 968-974 [PMID: 25638527 DOI: 10.1016/j.jhep.2014.12.029]
  - 79 **Wong F**, O'Leary JG, Reddy KR, Patton H, Kamath PS, Fallon MB, Garcia-Tsao G, Subramanian RM, Malik R, Maliakkal B, Thacker LR, Bajaj JS. New consensus definition of acute kidney injury accurately predicts 30-day mortality in patients with cirrhosis and infection. *Gastroenterology* 2013; **145**: 1280-1288.e1 [PMID: 23999172 DOI: 10.1053/j.gastro.2013.08.051]

- 80 **Salerno F**, Gerbes A, Ginès P, Wong F, Arroyo V. Diagnosis, prevention and treatment of hepatorenal syndrome in cirrhosis. *Gut* 2007; **56**: 1310-1318 [PMID: 17389705 DOI: 10.1136/gut.2006.107789]
- 81 **Moreau R**, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, Durand F, Gustot T, Saliba F, Domenicali M, Gerbes A, Wendon J, Alessandria C, Laleman W, Zeuzem S, Trebicka J, Bernardi M, Arroyo V. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; **144**: 1426-1437, 1437.e1-9 [PMID: 23474284 DOI: 10.1053/j.gastro.2013.02.042]
- 82 **Jalan R**, Gines P, Olson JC, Mookerjee RP, Moreau R, Garcia-Tsao G, Arroyo V, Kamath PS. Acute-on chronic liver failure. *J Hepatol* 2012; **57**: 1336-1348 [PMID: 22750750 DOI: 10.1016/j.jhep.2012.06.026]
- 83 **Bajaj JS**, O'Leary JG, Reddy KR, Wong F, Biggins SW, Patton H, Fallon MB, Garcia-Tsao G, Maliakkal B, Malik R, Subramanian RM, Thacker LR, Kamath PS. Survival in infection-related acute-on-chronic liver failure is defined by extrahepatic organ failures. *Hepatology* 2014; **60**: 250-256 [PMID: 24677131 DOI: 10.1002/hep.27077]
- 84 **TenHoor T**, Mannino DM, Moss M. Risk factors for ARDS in the United States: analysis of the 1993 National Mortality Followback Study. *Chest* 2001; **119**: 1179-1184 [PMID: 11296187]
- 85 **Thomson SJ**, Moran C, Cowan ML, Musa S, Beale R, Treacher D, Hamilton M, Grounds RM, Rahman TM. Outcomes of critically ill patients with cirrhosis admitted to intensive care: an important perspective from the non-transplant setting. *Aliment Pharmacol Ther* 2010; **32**: 233-243 [PMID: 20456304 DOI: 10.1111/j.1365-2036.2010.04341.x]
- 86 **Leise MD**, Talwalkar JA. Immunizations in chronic liver disease: what should be done and what is the evidence. *Curr Gastroenterol Rep* 2013; **15**: 300 [PMID: 23250700 DOI: 10.1007/s11894-012-0300-6]
- 87 **Loulergue P**, Pol S, Mallet V, Sogni P, Launay O. Why actively promote vaccination in patients with cirrhosis? *J Clin Virol* 2009; **46**: 206-209 [PMID: 19501019 DOI: 10.1016/j.jcv.2009.05.006]
- 88 **Mehta G**, Rothstein KD. Health maintenance issues in cirrhosis. *Med Clin North Am* 2009; **93**: 901-915, viii-ix [PMID: 19577121 DOI: 10.1016/j.mcna.2009.03.005]
- 89 **Bouza E**, Pintado V, Rivera S, Blázquez R, Muñoz P, Cercenado E, Loza E, Rodríguez-Crèixems M, Moreno S. Nosocomial bloodstream infections caused by *Streptococcus pneumoniae*. *Clin Microbiol Infect* 2005; **11**: 919-924 [PMID: 16216109 DOI: 10.1111/j.1469-0691.2005.01260.x]
- 90 **Pirovino M**, Lydick E, Grob PJ, Arrenbrecht S, Altorf J, Schmid M. Pneumococcal vaccination: the response of patients with alcoholic liver cirrhosis. *Hepatology* 1984; **4**: 946-949 [PMID: 6479858]
- 91 **Viasus D**, Garcia-Vidal C, Castellote J, Adamuz J, Verdaguier R, Dorca J, Manresa F, Gudiol F, Carratalà J. Community-acquired pneumonia in patients with liver cirrhosis: clinical features, outcomes, and usefulness of severity scores. *Medicine (Baltimore)* 2011; **90**: 110-118 [PMID: 21358441 DOI: 10.1097/MD.0b013e318210504c]
- 92 **Choi SH**, Park HG, Jun JB, Lee SO, Choi SH, Woo JH, Kim YS. Clinical characteristics and outcomes of pneumococcal bacteremia in adult patients with liver cirrhosis. *Diagn Microbiol Infect Dis* 2009; **63**: 160-164 [PMID: 19150708 DOI: 10.1016/j.diagmicrobio.2008.10.018]
- 93 **McCashland TM**, Preheim LC, Gentry MJ. Pneumococcal vaccine response in cirrhosis and liver transplantation. *J Infect Dis* 2000; **181**: 757-760 [PMID: 10669371 DOI: 10.1086/315245]
- 94 **Duchini A**, Viernes ME, Nyberg LM, Hendry RM, Pockros PJ. Hepatic decompensation in patients with cirrhosis during infection with influenza A. *Arch Intern Med* 2000; **160**: 113-115 [PMID: 10632312]
- 95 **Cheong HJ**, Song JY, Park JW, Yeon JE, Byun KS, Lee CH, Cho HI, Kim TG, Kim WJ. Humoral and cellular immune responses to influenza vaccine in patients with advanced cirrhosis. *Vaccine* 2006; **24**: 2417-2422 [PMID: 16406176 DOI: 10.1016/j.vaccine.2005.11.064]
- 96 **Song JY**, Cheong HJ, Ha SH, Hwang IS, Kee SY, Jeong HW, Lee CG, Kim WJ. Clinical impact of influenza immunization in patients with liver cirrhosis. *J Clin Virol* 2007; **39**: 159-163 [PMID: 17560166 DOI: 10.1016/j.jcv.2007.04.018]
- 97 **Kim DK**, Bridges CB, Harriman KH. Advisory committee on immunization practices recommended immunization schedule for adults aged 19 years or older--United States, 2015. *MMWR Morb Mortal Wkly Rep* 2015; **64**: 91-92 [PMID: 25654609]
- 98 **Lodato F**, Azzaroli F, Di Girolamo M, Feletti V, Cecinato P, Lisotti A, Festi D, Roda E, Mazzella G. Proton pump inhibitors in cirrhosis: tradition or evidence based practice? *World J Gastroenterol* 2008; **14**: 2980-2985 [PMID: 18494046]
- 99 **Rabinovitz M**, Yoo YK, Schade RR, Dindzans VJ, Van Thiel DH, Gavalier JS. Prevalence of endoscopic findings in 510 consecutive individuals with cirrhosis evaluated prospectively. *Dig Dis Sci* 1990; **35**: 705-710 [PMID: 2344804]
- 100 **Luo JC**, Leu HB, Hou MC, Huang CC, Lin HC, Lee FY, Chang FY, Chan WL, Lin SJ, Chen JW. Cirrhotic patients at increased risk of peptic ulcer bleeding: a nationwide population-based cohort study. *Aliment Pharmacol Ther* 2012; **36**: 542-550 [PMID: 22817655 DOI: 10.1111/j.1365-2036.2012.05225.x]
- 101 **Venkatesh PG**, Parasa S, Njei B, Sanaka MR, Navaneethan U. Increased mortality with peptic ulcer bleeding in patients with both compensated and decompensated cirrhosis. *Gastrointest Endosc* 2014; **79**: 605-614.e3 [PMID: 24119507 DOI: 10.1016/j.gie.2013.08.026]
- 102 **Ge PS**, Runyon BA. Preventing future infections in cirrhosis: a battle cry for stewardship. *Clin Gastroenterol Hepatol* 2015; **13**: 760-762 [PMID: 25460013 DOI: 10.1016/j.cgh.2014.10.025]
- 103 **Lewis SJ**, Franco S, Young G, O'Keefe SJ. Altered bowel function and duodenal bacterial overgrowth in patients treated with omeprazole. *Aliment Pharmacol Ther* 1996; **10**: 557-561 [PMID: 8853759]
- 104 **Lo WK**, Chan WW. Proton pump inhibitor use and the risk of small intestinal bacterial overgrowth: a meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 483-490 [PMID: 23270866 DOI: 10.1016/j.cgh.2012.12.011]
- 105 **Agastya G**, West BC, Callahan JM. Omeprazole inhibits phagocytosis and acidification of phagolysosomes of normal human neutrophils in vitro. *Immunopharmacol Immunotoxicol* 2000; **22**: 357-372 [PMID: 10952036 DOI: 10.3109/08923970009016425]
- 106 **Yoshida N**, Yoshikawa T, Tanaka Y, Fujita N, Kassai K, Naito Y, Kondo M. A new mechanism for anti-inflammatory actions of proton pump inhibitors--inhibitory effects on neutrophil-endothelial cell interactions. *Aliment Pharmacol Ther* 2000; **14** Suppl 1: 74-81 [PMID: 10807407]
- 107 **Zedtwitz-Liebenstein K**, Wenisch C, Patruta S, Parschall B, Daxböck F, Graninger W. Omeprazole treatment diminishes intra- and extracellular neutrophil reactive oxygen production and bactericidal activity. *Crit Care Med* 2002; **30**: 1118-1122 [PMID: 12006811]
- 108 **Bajaj JS**, Ananthakrishnan AN, Hafeezullah M, Zadornova Y, Dye A, McGinley EL, Saeian K, Heuman D, Sanyal AJ, Hoffmann RG. *Clostridium difficile* is associated with poor outcomes in patients with cirrhosis: A national and tertiary center perspective. *Am J Gastroenterol* 2010; **105**: 106-113 [PMID: 19844204 DOI: 10.1038/ajg.2009.615]
- 109 **Bajaj JS**, Zadornova Y, Heuman DM, Hafeezullah M, Hoffmann RG, Sanyal AJ, Saeian K. Association of proton pump inhibitor therapy with spontaneous bacterial peritonitis in cirrhotic patients with ascites. *Am J Gastroenterol* 2009; **104**: 1130-1134 [PMID: 19337238 DOI: 10.1038/ajg.2009.80]
- 110 **Choi EJ**, Lee HJ, Kim KO, Lee SH, Eun JR, Jang BI, Kim TN. Association between acid suppressive therapy and spontaneous bacterial peritonitis in cirrhotic patients with ascites. *Scand J Gastroenterol* 2011; **46**: 616-620 [PMID: 21275825 DOI: 10.3109/00365521.2011.551891]
- 111 **Deshpande A**, Pasupuleti V, Thota P, Pant C, Mapara S, Hassan S, Rolston DD, Sfera TJ, Hernandez AV. Acid-suppressive therapy



- is associated with spontaneous bacterial peritonitis in cirrhotic patients: a meta-analysis. *J Gastroenterol Hepatol* 2013; **28**: 235-242 [PMID: 23190338 DOI: 10.1111/jgh.12065]
- 112 **Bajaj JS**, Ratliff SM, Heuman DM, Lapane KL. Proton pump inhibitors are associated with a high rate of serious infections in veterans with decompensated cirrhosis. *Aliment Pharmacol Ther* 2012; **36**: 866-874 [PMID: 22966967 DOI: 10.1111/apt.12045]
  - 113 **Min YW**, Lim KS, Min BH, Gwak GY, Paik YH, Choi MS, Lee JH, Kim JJ, Koh KC, Paik SW, Yoo BC, Rhee PL. Proton pump inhibitor use significantly increases the risk of spontaneous bacterial peritonitis in 1965 patients with cirrhosis and ascites: a propensity score matched cohort study. *Aliment Pharmacol Ther* 2014; **40**: 695-704 [PMID: 25078671 DOI: 10.1111/apt.12875]
  - 114 **Merli M**, Lucidi C, Di Gregorio V, Giannelli V, Giusto M, Ceccarelli G, Riggio O, Venditti M. The chronic use of beta-blockers and proton pump inhibitors may affect the rate of bacterial infections in cirrhosis. *Liver Int* 2015; **35**: 362-369 [PMID: 24836902 DOI: 10.1111/liv.12593]
  - 115 **O'Leary JG**, Reddy KR, Wong F, Kamath PS, Patton HM, Biggins SW, Fallon MB, Garcia-Tsao G, Subramanian RM, Malik R, Thacker LR, Bajaj JS. Long-term use of antibiotics and proton pump inhibitors predict development of infections in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2015; **13**: 753-9.e1-753-9.e2 [PMID: 25130937 DOI: 10.1016/j.cgh.2014.07.060]
  - 116 **Campbell MS**, Obstein K, Reddy KR, Yang YX. Association between proton pump inhibitor use and spontaneous bacterial peritonitis. *Dig Dis Sci* 2008; **53**: 394-398 [PMID: 17616817 DOI: 10.1007/s10620-007-9899-9]
  - 117 **Mandorfer M**, Bota S, Schwabl P, Bucsics T, Pfisterer N, Summereder C, Hagmann M, Blacky A, Ferlitsch A, Sieghart W, Trauner M, Peck-Radosavljevic M, Reiberger T. Proton pump inhibitor intake neither predisposes to spontaneous bacterial peritonitis or other infections nor increases mortality in patients with cirrhosis and ascites. *PLoS One* 2014; **9**: e110503 [PMID: 25369194 DOI: 10.1371/journal.pone.0110503]
  - 118 **Terg R**, Casciato P, Garbe C, Cartier M, Stieben T, Mendizabal M, Niveyro C, Benavides J, Marino M, Colombato L, Berbara D, Silva M, Salgado P, Barreiro F, Fassio E, Gadano A. Proton pump inhibitor therapy does not increase the incidence of spontaneous bacterial peritonitis in cirrhosis: a multicenter prospective study. *J Hepatol* 2015; **62**: 1056-1060 [PMID: 25481567 DOI: 10.1016/j.jhep.2014.11.036]
  - 119 **de Vos M**, De Vroey B, Garcia BG, Roy C, Kidd F, Henrion J, Deltenre P. Role of proton pump inhibitors in the occurrence and the prognosis of spontaneous bacterial peritonitis in cirrhotic patients with ascites. *Liver Int* 2013; **33**: 1316-1323 [PMID: 23730823 DOI: 10.1111/liv.12210]
  - 120 **Choi JP**, Lee SO, Kwon HH, Kwak YG, Choi SH, Lim SK, Kim MN, Jeong JY, Choi SH, Woo JH, Kim YS. Clinical significance of spontaneous Aeromonas bacterial peritonitis in cirrhotic patients: a matched case-control study. *Clin Infect Dis* 2008; **47**: 66-72 [PMID: 18484880 DOI: 10.1086/588665]
  - 121 **Lau SM**, Peng MY, Chang FY. Outcomes of Aeromonas bacteremia in patients with different types of underlying disease. *J Microbiol Immunol Infect* 2000; **33**: 241-247 [PMID: 11269369]
  - 122 **Lay CJ**, Zhuang HJ, Ho YH, Tsai YS, Wang LS, Tsai CC. Different clinical characteristics between polymicrobial and monomicrobial Aeromonas bacteremia--a study of 216 cases. *Intern Med* 2010; **49**: 2415-2421 [PMID: 21088342]
  - 123 **Ko WC**, Chuang YC. Aeromonas bacteremia: review of 59 episodes. *Clin Infect Dis* 1995; **20**: 1298-1304 [PMID: 7620014]
  - 124 **Lee CC**, Chi CH, Lee NY, Lee HC, Chen CL, Chen PL, Chang CM, Wu CJ, Ko NY, Tsai MC, Ko WC. Necrotizing fasciitis in patients with liver cirrhosis: predominance of monomicrobial Gram-negative bacillary infections. *Diagn Microbiol Infect Dis* 2008; **62**: 219-225 [PMID: 18653302 DOI: 10.1016/j.diagmicrobio.2008.05.016]
  - 125 **Chao CM**, Lai CC, Tang HJ, Ko WC, Hsueh PR. Skin and soft-tissue infections caused by Aeromonas species. *Eur J Clin Microbiol Infect Dis* 2013; **32**: 543-547 [PMID: 23135756 DOI: 10.1007/s10096-012-1771-y]
  - 126 **Wu CJ**, Tsai PJ, Chen PL, Wu IC, Lin YT, Chen YH, Wang LR, Ko WC. Aeromonas aquariorum septicemia and enterocolitis in a cirrhotic patient. *Diagn Microbiol Infect Dis* 2012; **74**: 406-408 [PMID: 22995364 DOI: 10.1016/j.diagmicrobio.2012.08.005]
  - 127 **Pigrau C**, Bartolome R, Almirante B, Planes AM, Gavalda J, Pahissa A. Bacteremia due to Campylobacter species: clinical findings and antimicrobial susceptibility patterns. *Clin Infect Dis* 1997; **25**: 1414-1420 [PMID: 9431389]
  - 128 **Brann OS**. Infectious complications of cirrhosis. *Curr Gastroenterol Rep* 2001; **3**: 285-292 [PMID: 11469997]
  - 129 **Chen YM**, Lee HC, Chang CM, Chuang YC, Ko WC. Clostridium bacteremia: emphasis on the poor prognosis in cirrhotic patients. *J Microbiol Immunol Infect* 2001; **34**: 113-118 [PMID: 11456356]
  - 130 **Cheng NC**, Tai HC, Tang YB, Chang SC, Wang JT. Necrotizing fasciitis: clinical features in patients with liver cirrhosis. *Br J Plast Surg* 2005; **58**: 702-707 [PMID: 15992530 DOI: 10.1016/j.bjps.2005.01.019]
  - 131 **Garcia-Tsao G**, Surawicz CM. Editorial: Clostridium difficile infection: Yet another predictor of poor outcome in cirrhosis. *Am J Gastroenterol* 2010; **105**: 114-116 [PMID: 20054307 DOI: 10.1038/ajg.2009.604]
  - 132 **Siple JF**, Morey JM, Gutman TE, Weinberg KL, Collins PD. Proton pump inhibitor use and association with spontaneous bacterial peritonitis in patients with cirrhosis and ascites. *Ann Pharmacother* 2012; **46**: 1413-1418 [PMID: 23032651 DOI: 10.1345/aph.1R174]
  - 133 **Bajaj JS**, O'Leary JG, Reddy KR, Wong F, Olson JC, Subramanian RM, Brown G, Noble NA, Thacker LR, Kamath PS. Second infections independently increase mortality in hospitalized patients with cirrhosis: the North American consortium for the study of end-stage liver disease (NACSELD) experience. *Hepatology* 2012; **56**: 2328-2335 [PMID: 22806618 DOI: 10.1002/hep.25947]
  - 134 **Fernández Guerrero ML**, González López J, Górgolas M. Infectious endocarditis in patients with cirrhosis of the liver: a model of infection in the frail patient. *Eur J Clin Microbiol Infect Dis* 2010; **29**: 1271-1275 [PMID: 20549527 DOI: 10.1007/s10096-010-0998-8]
  - 135 **Lee JH**, Yoon JH, Kim BH, Chung GE, Myung SJ, Kim W, Kim YJ, Kim EC, Lee HS. Enterococcus: not an innocent bystander in cirrhotic patients with spontaneous bacterial peritonitis. *Eur J Clin Microbiol Infect Dis* 2009; **28**: 21-26 [PMID: 18612666 DOI: 10.1007/s10096-008-0578-3]
  - 136 **McNeil SA**, Malani PN, Chenoweth CE, Fontana RJ, Magee JC, Punch JD, Mackin ML, Kauffman CA. Vancomycin-resistant enterococcal colonization and infection in liver transplant candidates and recipients: a prospective surveillance study. *Clin Infect Dis* 2006; **42**: 195-203 [PMID: 16355329 DOI: 10.1086/498903]
  - 137 **El Sayed Zaki M**, El Shabrawy WO, El-Eshrawy MM, Aly Elatreby S. The high prevalence of Listeria monocytogenes peritonitis in cirrhotic patients of an Egyptian Medical Center. *J Infect Public Health* 2011; **4**: 211-216 [PMID: 22000850 DOI: 10.1016/j.jiph.2011.06.002]
  - 138 **Cabellos C**, Viladrich PF, Ariza J, Maiques JM, Verdager R, Gudiol F. Community-acquired bacterial meningitis in cirrhotic patients. *Clin Microbiol Infect* 2008; **14**: 35-40 [PMID: 18005179 DOI: 10.1111/j.1469-0691.2007.01839.x]
  - 139 **Cho YJ**, Lee SM, Yoo CG, Kim YW, Han SK, Shim YS, Yim JJ. Clinical characteristics of tuberculosis in patients with liver cirrhosis. *Respirology* 2007; **12**: 401-405 [PMID: 17539845 DOI: 10.1111/j.1440-1843.2007.01069.x]
  - 140 **Thulstrup AM**, Mølle I, Svendsen N, Sørensen HT. Incidence and prognosis of tuberculosis in patients with cirrhosis of the liver. A Danish nationwide population based study. *Epidemiol Infect* 2000; **124**: 221-225 [PMID: 10813146]
  - 141 **Tamaskar I**, Ravakhah K. Spontaneous bacterial peritonitis with Pasteurella multocida in cirrhosis: case report and review of literature. *South Med J* 2004; **97**: 1113-1115 [PMID: 15586605]
  - 142 **Migliore E**, Serraino C, Brignone C, Ferrigno D, Cardellicchio A, Pomero F, Castagna E, Osenda M, Fenoglio L. Pasteurella



- multocida infection in a cirrhotic patient: case report, microbiological aspects and a review of literature. *Adv Med Sci* 2009; **54**: 109-112 [PMID: 19366651 DOI: 10.2478/v10039-009-0005-8]
- 143 **Tseng HK**, Su SC, Liu CP, Lee CM. Pasteurella multocida bacteremia due to non-bite animal exposure in cirrhotic patients: report of two cases. *J Microbiol Immunol Infect* 2001; **34**: 293-296 [PMID: 11825011]
- 144 **Chapoutot C**, Pageaux GP, Perrigault PF, Jomaye Z, Perney P, Jean-Pierre H, Jonquet O, Blanc P, Larrey D. Staphylococcus aureus nasal carriage in 104 cirrhotic and control patients. A prospective study. *J Hepatol* 1999; **30**: 249-253 [PMID: 10068104]
- 145 **Park HJ**, Lee YM, Bang KM, Park SY, Moon SM, Park KH, Chong YP, Kim SH, Lee SO, Choi SH, Jeong JY, Woo JH, Kim YS. Clinical significance of Staphylococcus aureus bacteremia in patients with liver cirrhosis. *Eur J Clin Microbiol Infect Dis* 2012; **31**: 3309-3316 [PMID: 22833245 DOI: 10.1007/s10096-012-1697-4]
- 146 **Gonzalez-Quintela A**, Martínez-Rey C, Castroagudín JF, Rajo-Iglesias MC, Domínguez-Santalla MJ. Prevalence of liver disease in patients with Streptococcus bovis bacteraemia. *J Infect* 2001; **42**: 116-119 [PMID: 11531317 DOI: 10.1053/jinf.2001.0799]
- 147 **Vilaichone RK**, Mahachai V, Kullavanijaya P, Nunthapisud P. Spontaneous bacterial peritonitis caused by Streptococcus bovis: case series and review of the literature. *Am J Gastroenterol* 2002; **97**: 1476-1479 [PMID: 12094869 DOI: 10.1111/j.1572-0241.2002.05790.x]
- 148 **Cho SY**, Kang CI, Kim J, Joo EJ, Ha YE, Chung DR, Lee NY, Peck KR, Song JH. Association of liver cirrhosis with group B streptococcal bacteremia in non-pregnant adults. *J Infect* 2013; **67**: 617-619 [PMID: 23999150 DOI: 10.1016/j.jinf.2013.08.015]
- 149 **Farley MM**. Group B streptococcal disease in nonpregnant adults. *Clin Infect Dis* 2001; **33**: 556-561 [PMID: 11462195 DOI: 10.1086/322696]
- 150 **Tung BY**, Kowdley KV. Spontaneous group B streptococcal meningitis in a patient with cirrhosis. *West J Med* 1996; **165**: 229-230 [PMID: 8987435]
- 151 **Vollberg CM**, Herrera JL. Vibrio vulnificus infection: an important cause of septicemia in patients with cirrhosis. *South Med J* 1997; **90**: 1040-1042 [PMID: 9347818]
- 152 **Chiang SR**, Chuang YC. Vibrio vulnificus infection: clinical manifestations, pathogenesis, and antimicrobial therapy. *J Microbiol Immunol Infect* 2003; **36**: 81-88 [PMID: 12886957]
- 153 **Herrera JL**, Rodríguez R. Medical care of the patient with compensated cirrhosis. *Gastroen Hepatol* 2006; **2**: 124-133
- 154 **Vadillo M**, Corbella X, Pac V, Fernandez-Viladrich P, Pujol R. Multiple liver abscesses due to Yersinia enterocolitica discloses primary hemochromatosis: three cases reports and review. *Clin Infect Dis* 1994; **18**: 938-941 [PMID: 8086556]
- 155 **Khan FA**, Fisher MA, Khakoo RA. Association of hemochromatosis with infectious diseases: expanding spectrum. *Int J Infect Dis* 2007; **11**: 482-487 [PMID: 17600748 DOI: 10.1016/j.ijid.2007.04.007]

**P- Reviewer:** Hsieh CB, Montalto G **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Liu SQ



Basic Study

## Burn injury induces histopathological changes and cell proliferation in liver of rats

Jeferson André Bortolin, Hananiah Tardivo Quintana, Tabata de Carvalho Tomé, Flavia Andressa Pidone Ribeiro, Daniel Araki Ribeiro, Flavia de Oliveira

Jeferson André Bortolin, Hananiah Tardivo Quintana, Tabata de Carvalho Tomé, Flavia Andressa Pidone Ribeiro, Daniel Araki Ribeiro, Flavia de Oliveira, Department of Biosciences, Federal University of São Paulo (UNIFESP), Santos, SP 11060-001, Brazil

**Author contributions:** Bortolin JA performed the majority of experiments and analyzed the data; Quintana HT and Tomé TC participated in treatment of animals; Ribeiro FAP performed the molecular investigation; Ribeiro DA wrote the paper; de Oliveira F wrote the paper, designed and coordinated the research; all authors critically reviewed the manuscript and approved it.

**Supported by** São Paulo Research Foundation, FAPESP, No. 11/22034-9.

**Institutional review board statement:** None.

**Institutional animal care and use committee statement:** Committee of Ethics and Research from Federal University of São Paulo (protocol number 329/12).

**Conflict-of-interest statement:** The authors do not disclose any conflicts of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Flavia de Oliveira, PhD, Department of Biosciences, Federal University of São Paulo (UNIFESP), Rua Silva Jardim, 136 - Lab 328, Vila Mathias CEP, Santos, SP 11060-001, Brazil. [flavia.oliveira@unifesp.br](mailto:flavia.oliveira@unifesp.br)  
Telephone: +55-13-38783844

Received: June 27, 2015  
Peer-review started: June 30, 2015  
First decision: October 6, 2015  
Revised: January 7, 2016  
Accepted: January 21, 2016  
Article in press: January 22, 2016  
Published online: February 28, 2016

### Abstract

**AIM:** To investigate effects of severe burn injury (BI) in rat liver through the histopathological and inflammatory markers analysis.

**METHODS:** Forty-two male Wistar rats were distributed into two groups, control (C) and subjected to scald BI (SBI). The animals were euthanized one, four and 14 d post sham or 45% of the total body surface BI. Liver fragments were submitted to histopathological, morphoquantitative (hepatocyte area and cell density), ciclooxigenase-2 (COX-2) immunoexpression, and gene expression [real-time polymerase chain reaction for tumor necrosis factor (TNF)- $\alpha$ , inducible nitric oxide synthase (iNOS) and caspase-3] methods.

**RESULTS:** Histopathological findings showed inflammatory process in all periods investigated and hepatocyte degeneration added to increased amount of connective tissue 14 d post injury. Hepatocyte area, the density of binucleated hepatocytes and density of sinusoidal cells of SBI groups were increased when compared with control. COX-2 immunoexpression was stronger in SBI groups. No differences were found in TNF- $\alpha$ , iNOS and caspase-3 gene expression.

**CONCLUSION:** BI induces histopathological changes, upregulation of COX-2 immunoexpression, and cell proliferation in liver of rats.

**Key words:** Burn injury; Morphology; Histopathology; Liver; Cyclooxygenase-2

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Severe burn injuries result in serious complications that involve host response related to inflammation and multiple organ dysfunction. The goal of this study was to investigate the temporal effects of extensive experimental burn injury (BI) in rat liver through the histopathological and morphoquantitative aspects, immunoexpression of cyclooxygenase-2 (COX-2) and liver gene expression of tumor necrosis factor- $\alpha$ , inducible nitric oxide synthase and caspase-3. Our results revealed that BI induces histopathological changes, upregulation of COX-2 immunoexpression, and cell proliferation in liver of rats.

Bortolin JA, Quintana HT, Tomé TC, Ribeiro FAP, Ribeiro DA, de Oliveira F. Burn injury induces histopathological changes and cell proliferation in liver of rats. *World J Hepatol* 2016; 8(6): 322-330 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i6/322.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i6.322>

## INTRODUCTION

Burn injuries (BIs) represent one of the greatest public health problems, which induce to significant patient morbidity and mortality<sup>[1]</sup>. Scalds are most common cause of BI and preferentially occurs in children under the five years<sup>[2]</sup>. In pediatric patient population persistent protein catabolism may lead to delay in growth for up to 2 years after burn<sup>[3]</sup>.

Severe BI greater than 40% is a process that involves several host responses, including organ damage by inflammation and immune response<sup>[4]</sup>. Hypermetabolism is characterized by the inflammatory response, negative nitrogen balance, increase in resting energy consumption, and alterations in glucose and lipid metabolism<sup>[5]</sup>. Excessive systemic inflammatory response syndrome (SIRS) following burns to damage distant organ and provoke multiple organ dysfunction syndrome<sup>[6]</sup>.

According to Jeschke *et al.*<sup>[7]</sup> liver has been shown to play a crucial role after a BI. In a study of 102 children, the authors found that liver size and weight increased during the first week post BI, peaked at 2 wk post burn, and remained increased at 6, 9 and 12 mo after trauma. In autopsy of severely burned pediatric patients hepatomegaly with fatty infiltration was related to elevated occurrence of sepsis and mortality<sup>[8]</sup>. Jeschke *et al.*<sup>[9]</sup> showed liver weight was increased by 140% to 150% compared with estimated liver weight at 6, 9 and 12 mo post BI, indicating prolonged alterations in hepato-structure.

Apoptosis from liver cells was evaluated by terminal

deoxyuridine nick end labeling (TUNEL) assay in rats that have severe BI greater than 40% total body surface area<sup>[10]</sup>. Moreover, compensatory hepatocyte proliferation is detected due to increased apoptosis<sup>[7]</sup>.

Severe BI is associated with host responses related to inflammation and apoptotic process and liver clearly plays an important role in metabolic processes post burn. The present study proposes to investigate effects of severe BI in liver of rats through the histopathological and morphoquantitative aspects, immunoexpression of cyclooxygenase-2 (COX-2) and liver gene expression of tumor necrosis factor (TNF)- $\alpha$ , inducible nitric oxide synthase (iNOS) and caspase-3.

## MATERIALS AND METHODS

### Experimental design

Male Wistar rats ( $n = 42$ ), *Rattus Norvegicus*, with 21-d-old was chosen in the present study to mimic a developing organism. The rats were individually housed cages for five days, distributed into two groups: Control (C) and subjected to scald BI (SBI). The temperature room was controlled (22 °C) with regular light-dark cycle with 12 h, water and food were offered ad libitum. On the sixth day, the animals were anesthetized with an intraperitoneal (IP) injection of Ketamine (50 mg/mL) and Xilazine (10 mg/mL) and dorsal and ventral hair were removed. The group SBI ( $n = 21$ ) was submitted to nonlethal scald BI by immersing 45% of each rat's body, in 87 °C water as described by Walker *et al.*<sup>[11]</sup>. The C group ( $n = 21$ ) were submitted to sham of the SBI. Each animal had 30% of its dorsal and 15% of ventral area exposed to SBI for 10 and 3 s, respectively<sup>[12]</sup>. The rats in both groups were subcutaneously injected with the analgesic Buprenorphine (0.2 mg/kg) immediately after sham or SBI and again 24 h later. One, 4 and 14 d following the SBI, all animals from each group were euthanized with a lethal IP injection of Ketamine (150 mg/kg) and Xilazine (30 mg/kg).

### Compliance with ethical requirements

All institutional and national guidelines for the care and use of laboratory animals were followed. The procedures were approved by the Committee of Ethics and Research from Federal University of São Paulo (protocol No. 329/12).

### Histopathological and morphoquantitative analysis

Liver of euthanized rats from SBI and C groups were examined. The specimens was immediately fixed in 10% formalin phosphate buffer for 24 h for histological analyzes and routinely embedded in paraffin blocks and cut in transversal sections (4  $\mu$ m). The slides were stained with hematoxylin and eosin (H and E) and Sirius Red<sup>[13]</sup>, whose photomicrographs were made under normal and polarized light to differentiate type I (red and yellow) and III (green) collagen.

The hepatocyte area ( $\mu$ m<sup>2</sup>) was determined from

**Table 1** Primers sequences

Gene	Forward	Reverse
TNF- $\alpha$	5'-CCCAGAAAAGCAAGCAACCA-3'	5'-GCCTCGGGCCAGTGTATG-3'
Caspase-3	5'-TCTACCGCACCCGGTTACTA-3'	5'-TGTCGTCAATGCCACCACTG-3'
GAPDH	5'-GCTCTCTGCTCCTCCCTGTTTC-3'	5'-GACGCTGGCACTGCACAA-3'

TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase.

the measurement of 50 cells stained with H and E per animal. These fibers were randomly chosen from each animal comprising each experimental group. The cell density (number of cells/mm<sup>2</sup>) was determined as described by Mandarim-de-Lacerda *et al.*<sup>[14]</sup>. For this purpose, it was used five sections chosen randomly and stained with H and E and two fields of each section was analyzed, totaling ten photomicrographs per animal. It was determinate the density of mononucleated hepatocytes, binucleated hepatocytes and sinusoidal cells. For to investigate the hepatocyte area and density, it was used a computerized imaging equipment (Axio Visio 4.5 - Zeiss®) attached to a binocular microscope (Axio Observer D1, Zeiss®) with a 63 × objective.

### COX-2 immunohistochemical analysis

The paraffin of liver sections (4  $\mu$ m) was removed with xylene and cuts were rehydrated in graded ethanol, after pre-treated with 0.01 mol/L citric acid buffer (pH 6) in a microwave for 15 min at 850 W for antigen retrieval. The sections were pre-incubated for 5 min in 0.3% hydrogen peroxide in phosphate buffered saline (PBS) solution to inactive the endogenous peroxidase. Then the material blocked was with 5% normal goat serum in PBS solution for 10 min and then incubated with anti-COX-2 polyclonal primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA), at concentration of 1:200. Sections were incubated overnight at 4 °C in a refrigerator. After this was washes in PBS and incubated with biotin conjugated secondary antibody anti-rabbit IgG (Vector Laboratories, Burlingame, CA) at a concentration of 1:200 in PBS for 30 min, washed with PBS. Followed by the application of avidin-biotin complex conjugated to peroxidase (Vector Laboratories) for 30 min. Then continued with the application of a 0.05% solution of 3-3-diaminobenzidine solution and counterstained with Harris hematoxylin (Merck).

### Real-time polymerase chain reaction for TNF- $\alpha$ and caspase-3

Liver of animals was homogenized with 1 mL Trizol® (Invitrogen®, CA, United States), was added chloroform, isopropanol and ethanol 75% and centrifuged. In 40  $\mu$ L of DEPC-treated water the pellet formed was re-suspended. The RNA purity and integrity were guaranteed by optical density (260/280 nm ratio > 1.9; Nanodrop® 2000 c, Thermo Scientific, Canada). Successively, the samples were kept in -80 °C. And were treated with DNAase (deoxyribonuclease I Amp Grade®, Invitrogen®, CA, United States) as fixed by the

producer. The total RNA extraction was according with the protocol adapted by Chomczynski *et al.*<sup>[15]</sup>.

The total RNA was treated with DNAase and was built the cDNA by reverse transcriptase [real-time polymerase chain reaction (RT-PCR)], with the High-Capacity cDNA kit Reverser Transcription® (Applied Biosystems®, United States). The primers previously designed for genes of interest and endogenous control (Glyceraldehyde-3-Phosphate Dehydrogenase) were used for gene expression analysis and the detection of amplification was through intercalating DNA (Sybr Green®, Applied Biosystems®, United States). The primers sequences are show in the Table 1.

The samples in duplicate are pipetted on the equipment RT-PCR 7500 Fast (Applied Biosystems®, United States) and subsequent the program of cycling was selected: Holding stage -95 °C for 10 min, 30 cycles of 15 s at 95 °C and 60 °C for 1 min, finish with the melting curve: 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. The results were acquired by relative quantification (method2<sup>- $\Delta\Delta$ Ct</sup>) at which the Cycle-threshold (Ct) values obtained for C group was compared to the SBI group.

### Statistical analysis

Statistical analysis for hepatocyte area, cell density and RT-PCR were evaluated by analysis of variance with two factors (group and time), and followed with a Tukey's test for multiple comparisons, when necessary.  $P < 0.05$  was considered to statistical significance.

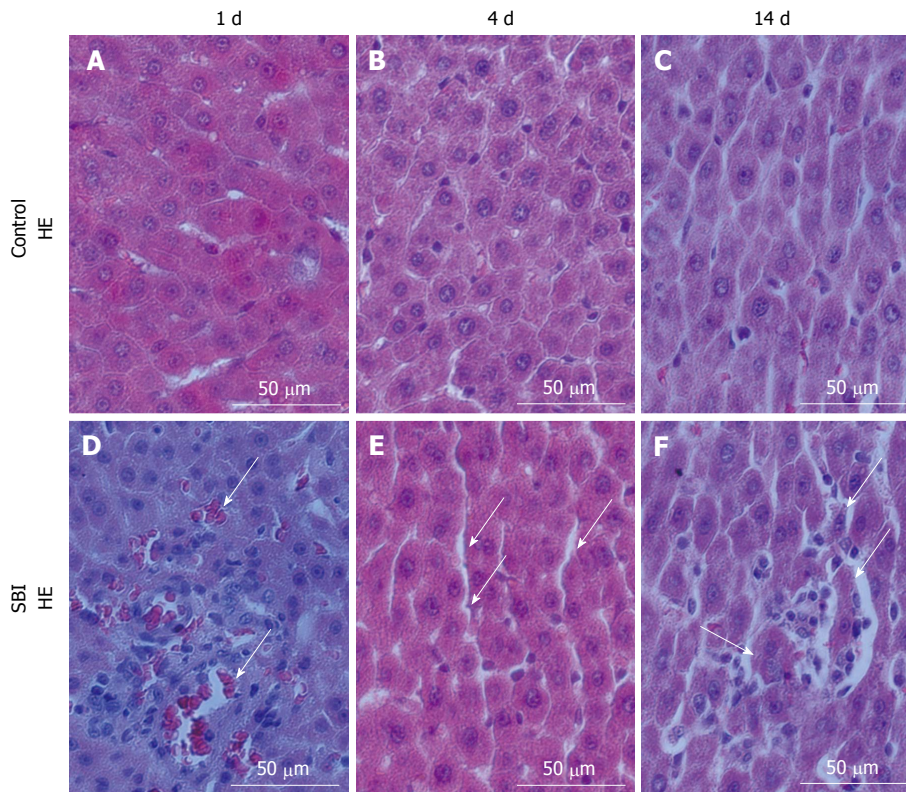
## RESULTS

### Histopathological and morphoquantitative analysis

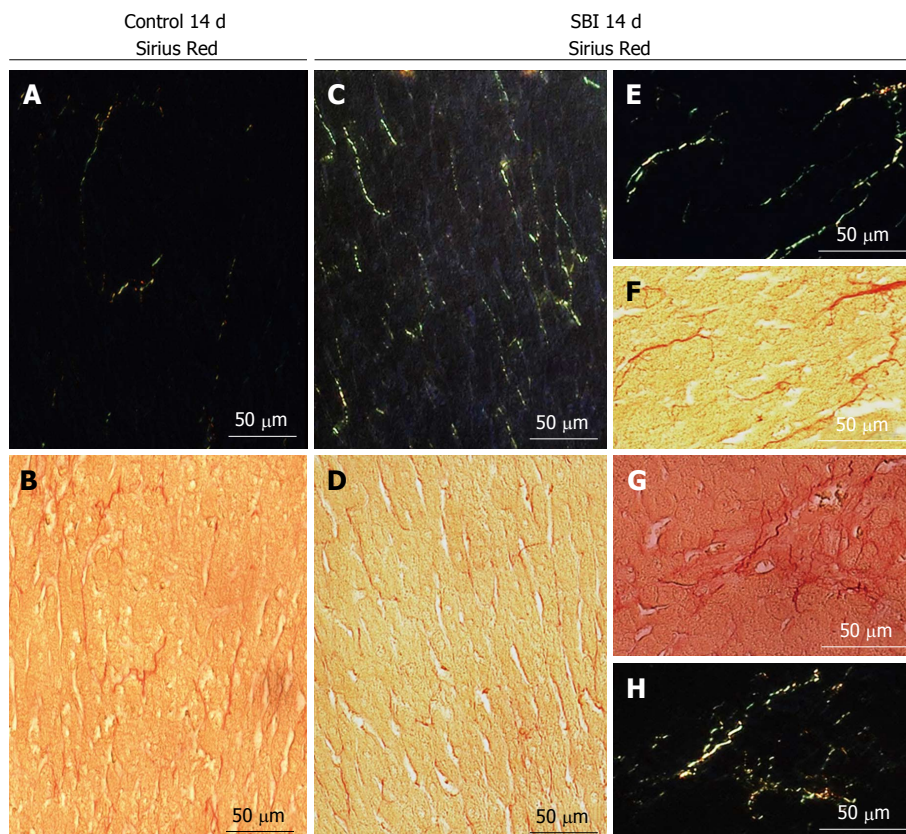
Liver cuts from Control group revealed hepatocytes arranged equidistantly with sinusoidal cells distributed in sinusoidal space (Figure 1A-C). Histopathological evaluation of SBI group investigated one day post injury revealed the presence of erythrocytes in sinusoidal space associated with inflammatory infiltrate (Figure 1D). Four days after injury, an increased sinusoidal space persisted (Figure 1E) and fourteen days post injury, liver sections showed inflammatory cells rounding hepatocytes in degeneration (Figure 1F).

SBI group following 14 d after lesion exhibited increase in connective tissue in the hepatic parenchyma (Figure 2C and D) when compared with controls (Figure 2A and B). The data concerning SBI group 1 and 4 d post BI not have been present for the reason that similar to control groups. Under polarized light connective tissue analysis showed type III collagen (green) preponderance.

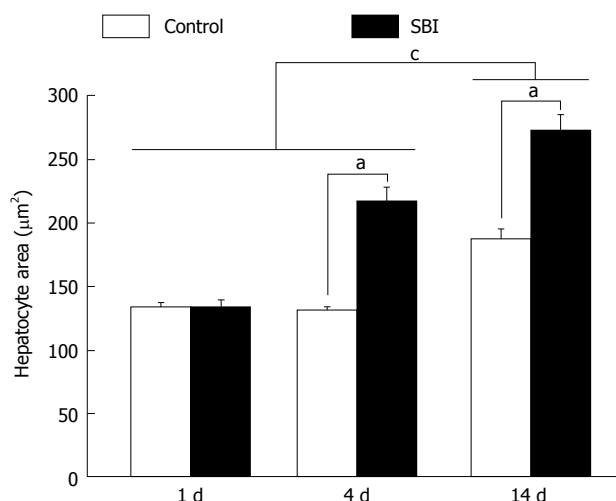




**Figure 1** Sections of rat liver stained with hematoxylin and eosin; panels show groups control (A-C) and submitted to scald burn injury (D-F) evaluated in different periods. A-C: Hepatocytes and sinusoidal cells with normal aspect; D: Sinusoidal space filled by erythrocytes (arrows) and inflammatory infiltrate; E: Sinusoidal space increased (arrows); F: Sinusoidal space increased and inflammatory cells rounding hepatocytes in degeneration process (arrows). SBI: Scald burn injury.



**Figure 2** Liver sections stained with Sirius Red with normal (B, D, F and G) and polarized light (A, C, E and H). Panels show control group 14 d after sham (A and B) or burn injury (C-H). Notes connective tissue was increased in SBI group when compared with Control. SBI: Scald burn injury.



**Figure 3** Mean + SD of hepatocyte area. <sup>a</sup> $P < 0.05$  - hepatocyte area of SBI group increased than control; <sup>c</sup> $P < 0.05$  - animals with 14 d sham or post burn injury showed hepatocyte larger than other periods investigated. SBI: Scald burn injury.

Further details in increased magnification of SBI group are demonstrated in Figure 2E-H.

Hepatocytes area was significantly higher in the SBI group investigated 4 and 14 d after BI (Figure 3). Moreover, the mean of cells area in animals with 14 d was statistically larger than 1 and 4 d post BI.

In relation to cell density (cells number/mm<sup>2</sup>) represented in Figure 4, mononucleated hepatocyte density decrease in SBI groups 1 and 14 d post BI ( $P < 0.05$ ). Binucleated hepatocyte density in SBI groups showed significantly decreased cell density one day post injury and increased cell density in 4 and 14 d after BI when compared with respectively controls. Sinusoidal cells presented significantly increased cell density for SBI group in all periods investigated.

### COX-2 immunohistochemistry

COX-2 immunoexpression was encountered in the cytoplasm of hepatocytes. Control groups presented weak immunoexpression for all periods investigated in this setting. However a strong and focal immunoexpression was detected in SBI groups after 1 and 4 d and persisted weakly 14 d post BI (Figure 5).

### RT-PCR for TNF- $\alpha$ , iNOS and caspase-3

TNF- $\alpha$  and iNOS, related to inflammation, and caspase-3 related to apoptosis, were evaluated in liver (Figure 6). The results showed no statistically differences between control and SBI groups for all periods investigated.

## DISCUSSION

Extensive burn injuries outcomes in critical complications that involve host response related to SIRS and multiple organ dysfunction being liver as a putative target-organ. The dynamic of organism adaptation as a result of liver response of hypermetabolism has been recognized in numerous studies but molecular

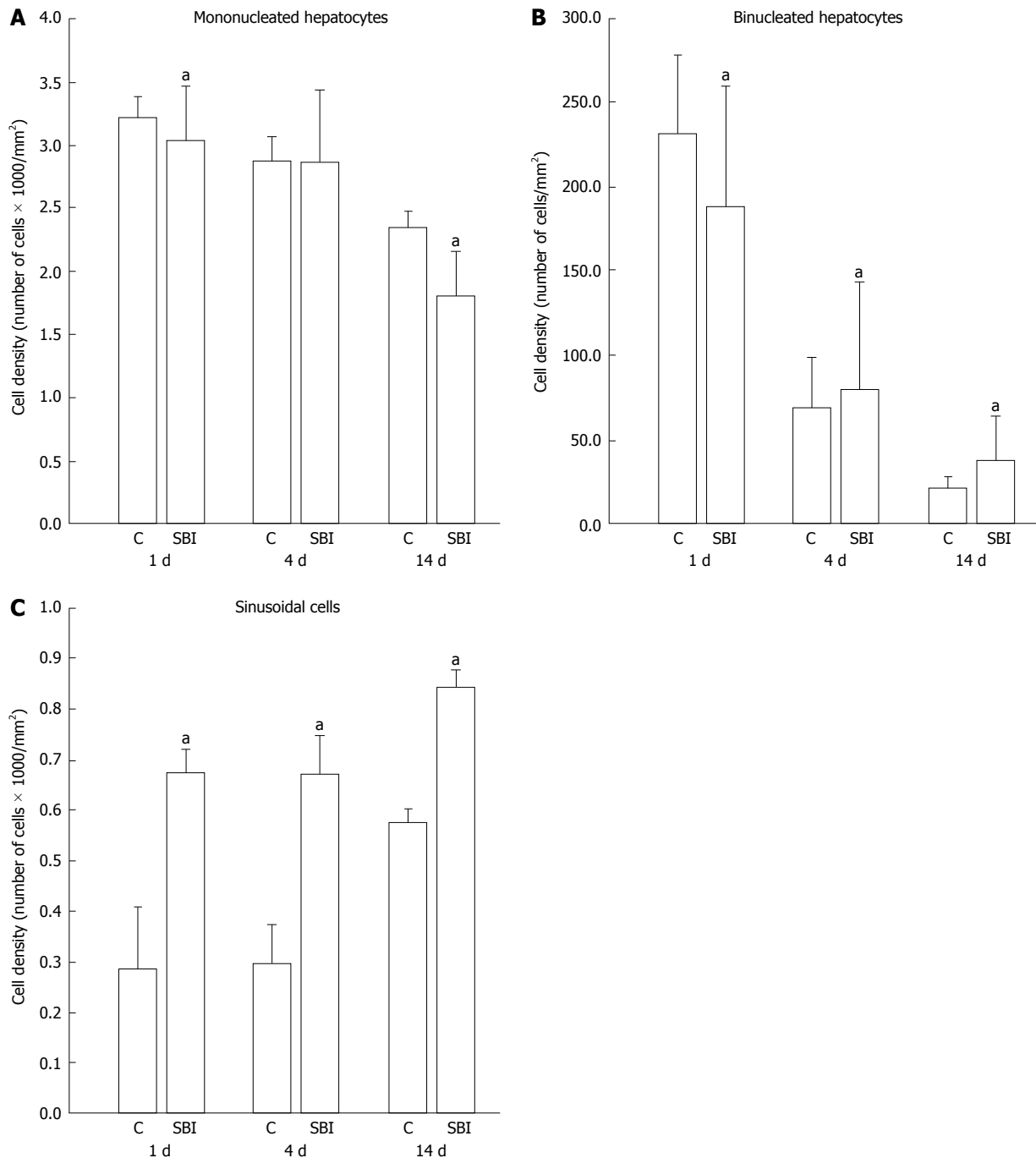
and morphological occurrences still need to be better clarified. The aim of this paper was to investigate effects of severe BI in rat liver through the histopathological and morphoquantitative aspects, immunoexpression of COX-2 and liver gene expression of TNF- $\alpha$ , iNOS and caspase-3.

The results showed morphological alterations in liver such as sinusoidal space filled by erythrocytes and inflammatory infiltrate associated with hepatocytes in degeneration following 14 d post injury. Damage resulting from severe BI initiates a SIRS as far as serious metabolic disturbances. Systemic signs manifested in the first hours post severe burns is related to enlarged systemic capillary permeability with protein escapement into the interstitial space<sup>[16]</sup>. Burns greater than 40% of body surface area commonly are followed by stress, inflammation, hypermetabolism, in addition the circulatory response associated to altered glycolysis, proteolysis, glycogenolysis, gluconeogenesis and lipolysis<sup>[9]</sup>. Our current results are consistent with this stress response of liver after BI.

BI causes liver injury which persists over a prolonged time. In children, at 6, 9 and 12 mo post burn, liver weight was incremented by 140% to 150% compared with estimated liver weight, showing longstanding alterations in liver morphology up to 12 mo after BI<sup>[7,9]</sup>. Morphoquantitative aspects on hepatocytes investigated in this setting detected increased hepatocyte area 4 and 14 d after BI in SBI group. Additionally, hepatocyte proliferation was present as result of increased binucleated hepatocyte density (number of cells/mm<sup>2</sup>) 4 and 14 d post BI. These data show that despite liver increase weight gain is caused by edema formation<sup>[7]</sup>, hepatocyte area gain and proliferation should be an important factor for hepatomegaly in burns. The compensatory hepatic cell proliferation are related to liver necrosis and liver apoptosis<sup>[10]</sup> but the underlying mechanisms, in which extensive burn provoke apoptosis in hepatocytes are not established so far<sup>[7]</sup>. This requires further study.

Regarding binucleated hepatocyte density, it is important to emphasize that because mitotic figures do not occur in adult liver, binucleated cells are usually assumed to be result of amitosis which implies splitting of the nucleus and these amitosis has been associated both with replacement of aged cells in abnormal tissue and with regenerative growth after injury<sup>[17]</sup>. Although binucleated hepatocytes are present in both groups (C and SBI), the presence of binucleated hepatocytes in SBI groups are related to regenerative growth as a response of skin BI. Interestingly mononucleated hepatocyte density decrease in SBI groups 1 and 14 d after trauma probably related to degeneration process showed in histopathological findings.

To further elucidate the molecular mechanisms induced by cutaneous BI, caspase-3 gene expression was evaluated in liver and no remarkable differences between groups were detected. Studies using TUNEL assay in liver of post BI experimental models showed apoptosis process<sup>[9,10]</sup>. Jayaraman *et al.*<sup>[18]</sup> related that

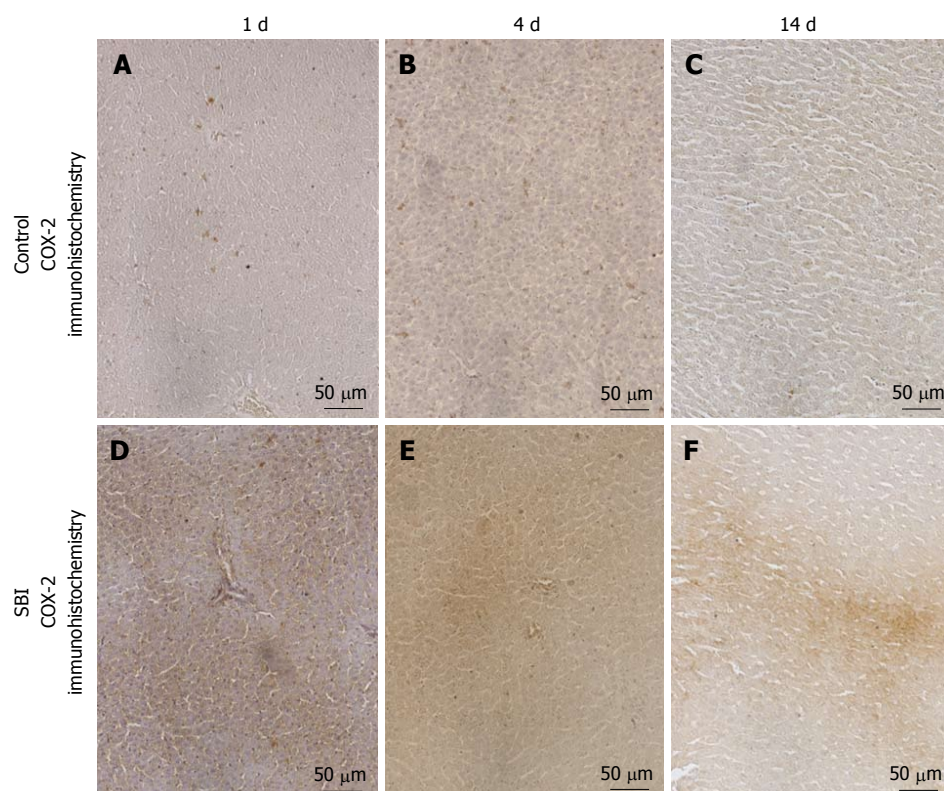


**Figure 4** Mean + SD of cell density (number of cells/mm<sup>2</sup>). Cell density of mononucleated hepatocytes (A), binucleated hepatocytes (B) and sinusoidal cells (C). <sup>a</sup>*P* < 0.05, SBI different from control. SBI: Scald burn injury; C: Control.

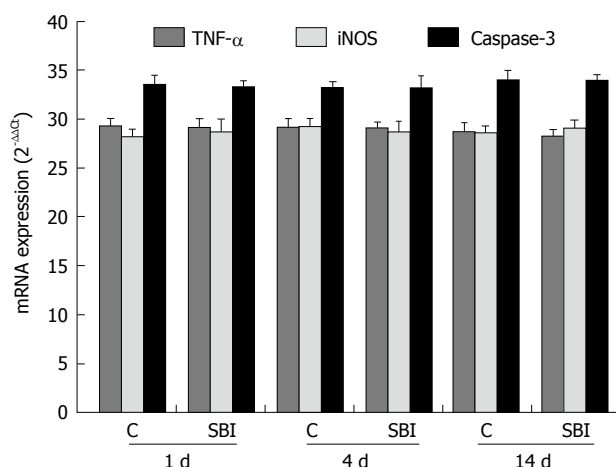
the up-regulation of some acute-phase genes as STAT3, leptin receptor and HNF4 $\alpha$ , are related to infection 7 d post injury in 20% of total body surface area in rats, suggesting bacterial infection of the wound post BI in animals. These authors showed up-regulation of Birc4, a protein that block caspase-3 and caspase-7 that are associated to apoptosis post burn. In this way, the present study suggests that the proliferation of hepatocytes cells was due to inflammatory process following necrosis. Following hepatocyte death, growth factors are secreted, and hepatocyte proliferation is triggered in liver.

Post BI, liver modulates the immune responses and the inflammatory processes. Protein catabolism owing to extensive BI is all guided by systemic inflammatory response, with enhanced activation of pro-inflammatory cytokines<sup>[19]</sup>. In a local investigation of liver, inflammatory infiltrate was viewed in histopathological findings and confirmed with immunohistochemical investigation a result of strong and focal immunoexpression COX-2 in SBI group. COX-2 is responsible for the conversion of arachidonic acid to prostaglandins<sup>[20]</sup>. Although severe burn in children is related to increased blood cytokine levels<sup>[21]</sup>, experimental studies with murine model





**Figure 5 Liver cyclooxygenase-2 immunohistochemistry.** Control (A-C) and SBI (D-F) evaluated 1, 4 or 14 d after burn injury. Notes stronger cytoplasmic immunoreactivity in SBI groups when compared with control. SBI: Scald burn injury; COX-2: Cyclooxygenase-2.



**Figure 6 Mean + SD of liver tumor necrosis factor- $\alpha$ , inducible nitric oxide synthase and caspase-3 mRNA expression.** TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; iNOS: Inducible nitric oxide synthase; C: Control; SBI: Scald burn injury.

submitted to BI for water vapor of 18% of the body surface showed enhanced expression of TNF- $\alpha$  and iNOS in initial stages of BI evaluated by means of peritoneal fluid of RT-PCR analysis<sup>[22]</sup>. Conversely, the present investigation of these inflammatory mediators (directly on the liver and not in a corporal fluid evaluation) showed no differences between groups.

Liver sinusoidal endothelial cells (LSECs) provide liver regeneration post injury of this organ<sup>[23]</sup>. In the sinusoidal space, there are four cell types: LSECs, Kupffer cells, stellate cells and pit cells. Kupffer cells

generate cytokines and pro-inflammatory factors that stimulate neutrophils activation and change sinusoids porosity and may lead to cirrhosis<sup>[24]</sup>. Herein, LSECs and Kupffer cells constitute the hepatic reticuloendothelial system<sup>[25]</sup>. In the present study the density of sinusoidal cells was significantly increased in SBI group for all periods when compared with controls. This should be associated to phagocytic activity of Kupffer cells since local inflammation, erythrocytes invasion activation of neutrophils and disturbance of porosity in the sinusoids walls leading to erythrocytes invasion of sinusoidal space. Severe haemolysis was observed immediately after BI<sup>[26]</sup>. LSECs are separated from liver parenchyma by space of Disse, which is represented for a perisinusoidal extravascular space. Space of Disse contains collagen type I, III, V and VI and the changes related with perisinusoidal basal lamina in livers, should increase collagen deposition in the space of Disse<sup>[25]</sup>. Furthermore, agglomeration of connective tissue inside the space of Disse may obstruct the normal traffic between blood and hepatocytes, reducing the release of macromolecules, diffculting the interaction between cells and leading to a liver dysfunction<sup>[27]</sup>.

Hepatocyte growth factor (HGF) develops a key function in cell regeneration, motility, growth and morphogenesis. Hepatic stellate cells provide as the main source of HGF in liver, however, after lesion, HGF expression is increased in LSECs<sup>[23,28]</sup>. In addition, hepatic stellate cells activated may initiate liver fibrosis process. Healthy LSECs inhibit the activation of hepatic stellate



cells<sup>[23]</sup>. In this study, increased density of sinusoidal cells should be related with enhanced of accumulation of the collagen, specially type III, in hepatic parenchyma in SBI animals when compared with controls 14 d after injury.

In conclusion, severe burn in greater than 40% of the body surface induces, in liver, histopathological changes, inflammation related to COX-2 immunoexpression, and cell proliferation not related to caspase-3 expression. Because modulating function of liver after burn injuries, the treatment of severe burns can be focused in liver disarrangements.

## COMMENTS

### Background

Scalds are most common cause of burn injury (BI) and preferentially occur in children under the five years. The persistent protein catabolism may lead to delay in growth for up to 2 years after injury. In addition, severe burn injuries result in serious complications that involve host response related to inflammation and multiple organ dysfunctions, including liver damage.

### Research frontiers

Studies involving autopsy of severely burned pediatric patients showed data about liver weight increased with fatty infiltration, but molecular and morphological investigation *in vivo* is necessary to elucidate better the liver damage process during great BI. For this, the present study investigated effects of severe BI in liver of young rats through the histopathological and morphoquantitative aspects, immunoexpression of COX-2 and liver gene expression of tumor necrosis factor- $\alpha$ , inducible nitric oxide synthase and caspase-3.

### Innovations and breakthroughs

The liver damage process during severe BI are related with histopathological and morphoquantitative changes such as presence of erythrocytes in sinusoidal space associated with inflammatory infiltrate and inflammatory cells rounding hepatocytes in degeneration. Moreover, increased connective tissue, hepatocyte area larger than control, altered binucleated hepatocyte and sinusoidal cells density, were described in the present study.

### Applications

Emphasize the importance of global treatment in burn great than 40% of total body surface area mainly in children. Highlight the damage caused in liver morphology clarifying morphological changes to possible treatments to prevent major consequences of BI.

### Terminology

Severe BI greater than 40% in children causes chronic morphological liver consequences ignored in numerous treatment centers. To emphasize the liver dysfunction as a result of extensive skin BI because hypermetabolic consequences was the purpose of the present paper.

### Peer-review

The aim of the authors was to investigate the temporal effects of extensive experimental burn injury in rat liver. There are some studies focused on this issue. This is well designed study. This article will provide new information about liver problems developing after BI.

## REFERENCES

- 1 Herndon DN, Hart DW, Wolf SE, Chinkes DL, Wolfe RR. Reversal of catabolism by beta-blockade after severe burns. *N Engl J Med* 2001; **345**: 1223-1229 [PMID: 11680441 DOI: 10.1056/NEJMoa010342]
- 2 Krishnamoorthy V, Ramaiah R, Bhananker SM. Pediatric burn injuries. *Int J Crit Illn Inj Sci* 2012; **2**: 128-134 [PMID: 23181206 DOI: 10.4103/2229-5151.100889]
- 3 Jeschke MG, Gauglitz GG, Kulp GA, Finnerty CC, Williams FN, Kraft R, Suman OE, Micak RP, Herndon DN. Long-term persistence of the pathophysiologic response to severe burn injury. *PLoS One* 2011; **6**: e21245 [PMID: 21789167 DOI: 10.1371/journal.pone.0021245]
- 4 Sun BW, Sun Y, Sun ZW, Chen X. CO liberated from CORM-2 modulates the inflammatory response in the liver of thermally injured mice. *World J Gastroenterol* 2008; **14**: 547-553 [PMID: 18203286 DOI: 10.3748/wjg.14.547]
- 5 Izamis ML, Sharma NS, Uygur B, Bieganski R, Saeidi N, Nahmias Y, Uygur K, Yarmush ML, Berthiaume F. In situ metabolic flux analysis to quantify the liver metabolic response to experimental burn injury. *Biotechnol Bioeng* 2011; **108**: 839-852 [PMID: 21404258 DOI: 10.1002/bit.22998]
- 6 Dahiya P. Burns as a model of SIRS. *Front Biosci (Landmark Ed)* 2009; **14**: 4962-4967 [PMID: 19482598]
- 7 Jeschke MG, Micak RP, Finnerty CC, Herndon DN. Changes in liver function and size after a severe thermal injury. *Shock* 2007; **28**: 172-177 [PMID: 17529902 DOI: 10.1097/shk.0b013e318047b9e2]
- 8 Barret JP, Jeschke MG, Herndon DN. Fatty infiltration of the liver in severely burned pediatric patients: autopsy findings and clinical implications. *J Trauma* 2001; **51**: 736-739 [PMID: 11586168 DOI: 10.1097/00005373-200110000-00019]
- 9 Jeschke MG. The hepatic response to thermal injury: is the liver important for postburn outcomes? *Mol Med* 2009; **15**: 337-351 [PMID: 19603107 DOI: 10.2119/molmed.2009.00005]
- 10 Jeschke MG, Low JF, Spies M, Vita R, Hawkins HK, Herndon DN, Barrow RE. Cell proliferation, apoptosis, NF-kappaB expression, enzyme, protein, and weight changes in livers of burned rats. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G1314-G1320 [PMID: 11352826]
- 11 Walker HL, Mason AD. A standard animal burn. *J Trauma* 1968; **8**: 1049-1051 [PMID: 5722120]
- 12 Newman JJ, Strome DR, Goodwin CW, Mason AD, Pruitt BA. Altered muscle metabolism in rats after thermal injury. *Metabolism* 1982; **31**: 1229-1233 [PMID: 6216391 DOI: 10.1016/0026-0495(82)90009-9]
- 13 Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J* 1979; **11**: 447-455 [PMID: 91593 DOI: 10.1007/BF01002772]
- 14 Mandarin-de-Lacerda CA. Stereological tools in biomedical research. *An Acad Bras Cienc* 2003; **75**: 469-486 [PMID: 14605681 DOI: 10.1590/S0001-37652003000400006]
- 15 Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. *Nat Protoc* 2006; **1**: 581-585 [PMID: 17406285 DOI: 10.1038/nprot.2006.83]
- 16 Farina JA, Rosique MJ, Rosique RG. Curbing inflammation in burn patients. *Int J Inflam* 2013; **2013**: 715645 [PMID: 23762773 DOI: 10.1155/2013/715645]
- 17 De Handt HA, Elwi AM, Soliman MA. Observations of the binucleate cells of the liver. *Nature* 1966; **212**: 827-829 [PMID: 5988215 DOI: 10.1038/212827a0]
- 18 Jayaraman A, Maguire T, Vemula M, Kwon DW, Vannucci M, Berthiaume F, Yarmush ML. Gene expression profiling of long-term changes in rat liver following burn injury. *J Surg Res* 2009; **152**: 3-17, e1-2 [PMID: 18755477 DOI: 10.1016/j.jss.2007.05.025]
- 19 Merritt EK, Thalacker-Mercer A, Cross JM, Windham ST, Thomas SJ, Bamman MM. Increased expression of atrogenes and TWEAK family members after severe burn injury in nonburned human skeletal muscle. *J Burn Care Res* 2013; **34**: e297-e304 [PMID: 23816995 DOI: 10.1097/BCR.0b013e31827a2a9c]
- 20 Paiotti AP, Marchi P, Miszputen SJ, Oshima CT, Franco M, Ribeiro DA. The role of nonsteroidal antiinflammatory drugs and cyclooxygenase-2 inhibitors on experimental colitis. *In Vivo* 2012; **26**: 381-393 [PMID: 22523290]

- 21 **Finnerty CC**, Herndon DN, Przkora R, Pereira CT, Oliveira HM, Queiroz DM, Rocha AM, Jeschke MG. Cytokine expression profile over time in severely burned pediatric patients. *Shock* 2006; **26**: 13-19 [PMID: 16783192 DOI: 10.1097/01.SHK.0000223120.26394.7d]
- 22 **Luo G**, Peng D, Zheng J, Chen X, Wu J, Elster E, Tadaki D. The role of NO in macrophage dysfunction at early stage after burn injury. *Burns* 2005; **31**: 138-144 [PMID: 15683683 DOI: 10.1016/j.burns.2004.09.009]
- 23 **DeLeve LD**. Liver sinusoidal endothelial cells and liver regeneration. *J Clin Invest* 2013; **123**: 1861-1866 [PMID: 23635783 DOI: 10.1172/JCI66025]
- 24 **Dobbs BR**, Rogers GW, Xing HY, Fraser R. Endotoxin-induced defenestration of the hepatic sinusoidal endothelium: a factor in the pathogenesis of cirrhosis? *Liver* 1994; **14**: 230-233 [PMID: 7997080]
- 25 **Svistounov D**, Zykova SN, Cogger VC, Warren A, McMahon AC, Fraser R, Le Counteur DG. Liver Sinusoidal Endothelial Cells and Regulation of Blood Lipoproteins. In: Kelishadi R. Dyslipidemia - From Prevention to Treatment. In Tech, 2012: 263-279 [DOI: 10.5772/29169]
- 26 **Okabayashi K**, Ohtani M, Morio M, Kajihara H. Structural changes of Kupffer cells in rat liver following experimental thermal injury. *Burns* 1990; **16**: 83-88 [PMID: 2350415 DOI: 10.1016/0305-4179(90)90162-P]
- 27 **Brandão DF**, Ramalho LN, Ramalho FS, Zucoloto S, Martinelli Ade L, Silva Ode C. Liver cirrhosis and hepatic stellate cells. *Acta Cir Bras* 2006; **21** Suppl 1: 54-57 [PMID: 17013515 DOI: 10.1590/S0102-86502006000700013]
- 28 **Maher JJ**. Cell-specific expression of hepatocyte growth factor in liver. Upregulation in sinusoidal endothelial cells after carbon tetrachloride. *J Clin Invest* 1993; **91**: 2244-2252 [PMID: 7683700 DOI: 10.1172/JCI116451]

**P- Reviewer:** Akarsu M, Morales-Gonzalez J **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Liu SQ



Randomized Controlled Trial

## Boceprevir plus peginterferon/ribavirin for treatment of chronic hepatitis C in Russia

Vasily Isakov, Igor Nikitin, Vladimir Chulanov, Pavel Ogurtsov, Ekaterina Lukyanova, Jianmin Long, Janice Wahl, Frans A Helmond; The P08160 Trial Investigators

Vasily Isakov, Department of Gastroenterology and Hepatology, Institute of Nutrition, Moscow 115446, Russia

Igor Nikitin, Department of Gastroenterology and Hematology, Russian Academy of Sciences, Moscow 117593, Russia

Vladimir Chulanov, Clinical Diagnostics and Research Center, Central Research Institute of Epidemiology, Moscow 111123, Russia

Pavel Ogurtsov, Hospital Medicine, Peoples' Friendship University of Russia, Moscow 117198, Russia

Ekaterina Lukyanova, Medical Affairs, MSD Pharmaceuticals LLC, Moscow 115093, Russia

Jianmin Long, Janice Wahl, Frans A Helmond, Clinical Research, Merck & Co., Inc., Kenilworth, NJ 07033, United States

**Author contributions:** Isakov V, Long J, Wahl J and Helmond FA designed the study; Isakov V, Nikitin I, Chulano V and Ogurtsov P enrolled patients; Isakov V, Lukyanova E, Long J and Helmond FA analyzed the data; Isakov V and Helmond FA wrote the first draft; all authors critically reviewed and approved the final draft of the paper.

Supported by Merck & Co., Inc., Kenilworth, NJ, United States.

**Institutional review board statement:** The protocol P08160 of the study registered at ClinicalTrials.gov with identifier NCT01425203 presented in the manuscript of Isakov V *et al* "Boceprevir plus peginterferon/ribavirin for treatment of chronic hepatitis C in Russia" was reviewed and approved by the Institute of Nutrition (Moscow, Russia) Institutional Review Board.

**Clinical trial registration statement:** Protocol P08160 (The Effect of Boceprevir in Russian Participants Diagnosed with Chronic Hepatitis C Genotype 1) is registered at ClinicalTrials.gov (Identifier: NCT01425203).

**Informed consent statement:** All procedures followed were in accordance with the ethical standards of the responsible

committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients included in the study.

**Conflict-of-interest statement:** Isakov V has received research/grant support from MSD, has served as a board member and or consultant for Abbvie, Vertex, Roche, Novartis, Janssen, and Bristol-Myers Squibb; has served on speakers' bureaus for Bristol-Myers Squibb, Janssen, Roche, and Novartis; and has received travel funding from Bristol-Myers Squibb and MSD Russia. Chulanov V has served on advisory boards for Roche, Bristol-Myers Squibb, Janssen, Novartis, and MSD Russia; has received research grants from Bristol-Myers Squibb; and has served on speakers' bureaus for Bristol-Myers Squibb, Roche, Janssen, Novartis, Gilead, AbbVie, and MSD. Ogurtsov P has served on advisory boards for Schering-Plough and delivered lectures on behalf of Abbott, Solvay, PRO.MED.CS Praha a.s., and Veropharm. Nikitin I has nothing to disclose. Lukyanova E, Long J, Wahl J and Helmond FA are current employees of Merck & Co., Inc., Kenilworth, NJ, United States.

**Data sharing statement:** These data were presented in part at the 64th Annual Meeting of the American Association for the Study of Liver Diseases; November 1-5, 2013; Washington, DC, United States.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Vasily Isakov, Professor, Department of Gastroenterology and Hepatology, Institute of Nutrition, Kashirskoe Shosse 21, Moscow 115446, Russia. [vasily.isakov@gmail.com](mailto:vasily.isakov@gmail.com)  
Telephone: +7-499-6130764  
Fax: +7-499-6130764

Received: July 3, 2015  
Peer-review started: July 3, 2015  
First decision: August 26, 2015  
Revised: October 7, 2015  
Accepted: December 19, 2015  
Article in press: December 23, 2015  
Published online: February 28, 2016

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Compared to the standard-of care treatment with peginterferon and ribavirin (PR), addition of boceprevir to PR results in a significant increase in rates of sustained virologic response achieved with substantially shorter treatment durations across a broad cross-section of patients with chronic hepatitis C virus infection in Russia.

## Abstract

**AIM:** To evaluate addition of boceprevir to peginterferon/ribavirin (PR) in Russian patients with chronic hepatitis C virus (HCV).

**METHODS:** Treatment-naïve (TN) and treatment-experienced (TE) patients (who had failed prior treatment with PR for  $\geq 12$  wk) with chronic HCV genotype 1 infection were enrolled in this placebo-controlled, double-blind study. All patients initially received PR for 4 wk. Patients randomized to control treatment then received PR for an additional 44 wk. TN patients randomized to triple therapy received boceprevir (800 mg three times daily) plus PR for 24 wk and then further therapy according to treatment week 8 (TW8) HCV RNA levels. TE patients received boceprevir plus PR for 32 wk and then further therapy according to TW8 HCV RNA levels. Treatment was discontinued for TN patients with detectable HCV RNA at TW24 and TE patients with detectable HCV RNA at TW12 because of futility. The primary efficacy end point was sustained virologic response (SVR) defined as undetectable HCV RNA 24 wk after completing all study therapy.

**RESULTS:** SVR was 74.8% in the boceprevir plus PR arm compared with 46.2% in the control arm, with a stratification-adjusted treatment difference of 29.2% (95%CI: 16.4-41.5;  $P < 0.0001$ ). Rates of SVR were higher in the boceprevir arm in both TN and TE patient groups (TN 78.4% *vs* 56.3%; TE 69.4% *vs* 30.0%). Within TE patients, the rates of SVR were higher with boceprevir plus PR compared with PR, regardless of treatment failure type (null responder, partial responder, and relapser). Most patients receiving boceprevir plus PR in both TN (86%) and TE (71%) populations were eligible for reduced treatment duration. Anemia was increased in patients receiving boceprevir plus PR *vs* PR alone (47.2% *vs* 24.4%); there was a corresponding increase in ribavirin dose reduction and erythropoietin use. Among patients receiving boceprevir plus PR, SVR rates were similar in patients with anemia ( $< 10$  g/dL) and those without anemia (71.2% *vs* 77.4%).

**CONCLUSION:** Regulatory approval has been obtained for boceprevir plus PR in Russian patients with HCV genotype 1 infection based on the results of this study.

**Key words:** Hepatitis C virus; Boceprevir; Peginterferon; Ribavirin; Randomized; Clinical trial; Sustained virologic response

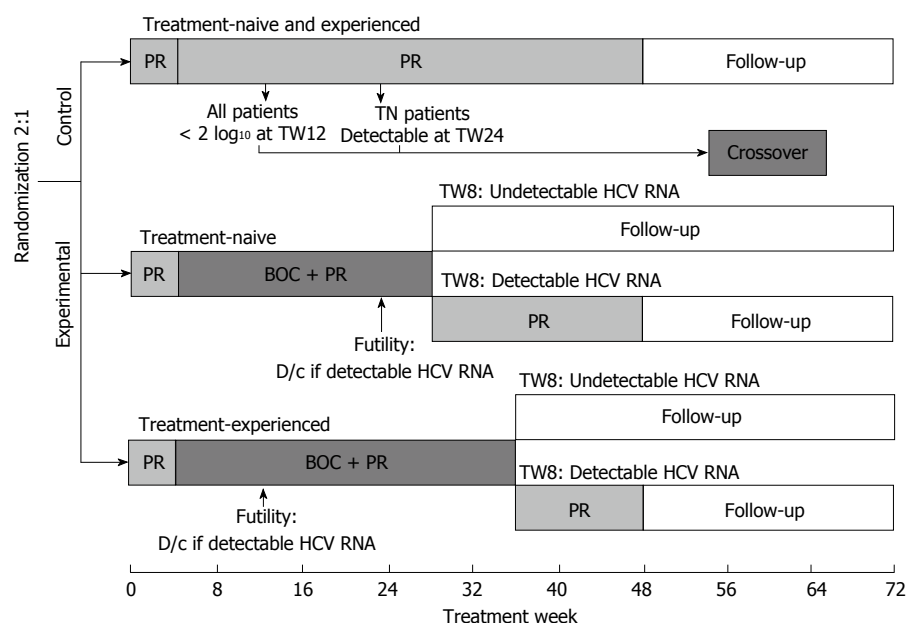
Isakov V, Nikitin I, Chulanov V, Ogurtsov P, Lukyanova E, Long J, Wahl J, Helmond FA; The P08160 Trial Investigators. Boceprevir plus peginterferon/ribavirin for treatment of chronic hepatitis C in Russia. *World J Hepatol* 2016; 8(6): 331-339 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i6/331.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i6.331>

## INTRODUCTION

Boceprevir is an orally administered, serine protease inhibitor of the hepatitis C virus (HCV) nonstructural protein 3 protease<sup>[1]</sup>. The addition of boceprevir to peginterferon and ribavirin (PR) improves rates of sustained virologic response (SVR) in adult patients with HCV genotype 1 (GT1) infection<sup>[2,3]</sup>. In the phase 3 SPRINT-2 study in previously untreated patients and the RESPOND-2 study in patients who had failed previous treatment, the addition of boceprevir to PR increased SVR rates compared with PR alone. In both studies, the implementation of response-guided therapy (RGT) permitted a shortened treatment duration for patients with an early response to therapy. In SPRINT-2, 44% of patients receiving boceprevir RGT required only 28 wk of treatment with triple therapy, and the SVR rate in this group was 96%<sup>[3]</sup>. Similarly, in RESPOND-2, 46% of patients had undetectable HCV RNA at treatment week 8 (TW8) and were eligible for a shortened 36-wk treatment regimen: SVR in this population was 86%<sup>[2]</sup>. In these studies, the safety profile of boceprevir plus PR largely resembled the safety profile of PR alone, with the notable exceptions of increased rates of dysgeusia and anemia in patients receiving boceprevir.

According to the World Health Organization (WHO), there were an estimated 5.8 million patients with HCV infection in Russia in 2010, accounting for 4.1% of the total Russian population<sup>[4]</sup>. In Western countries, treatment of HCV infection has advanced dramatically over the last 5 years with the introduction of new targeted therapies that substantially shorten treatment duration and improve SVR rates<sup>[5,6]</sup>. However, in resource-constrained countries, standard treatment protocols are lacking, and PR dual therapy frequently remains the cornerstone of treatment<sup>[7,8]</sup>. Recent guidelines from the WHO note the low rates of treatment uptake for patients in low- and middle-income countries. The aim of this study was to evaluate the safety and





**Figure 1 Study design.** BOC: Boceprevir; D/c: Discontinued; HCV: Hepatitis C virus; PR: Peginterferon/ribavirin; TW: Treatment week.

efficacy of boceprevir plus PR therapy in treatment-naïve (TN) and treatment-experienced (TE) Russian patients with chronic HCV GT1 infection.

## MATERIALS AND METHODS

This was a randomized, placebo-controlled, double-blind clinical trial (ClinicalTrials.gov identifier, NCT01425203; protocol P08160), carried out in accordance with the Declaration of Helsinki, current guidelines on Good Clinical Practice, and local ethical and legal requirements. All patients provided voluntary written informed consent before trial entry.

### Study design

Patients were randomized in a 2:1 ratio to receive experimental or control therapy, stratified by previous treatment (naïve vs experienced) and interleukin-28B (*IL28B*) status (CC allele vs non-CC allele) (Figure 1). All patients initially received PR [peginterferon alfa-2b (1.5 µg/kg per week) plus ribavirin (800-1400 mg/d)] for 4 wk. Patients in the control arm then received PR for an additional 44 wk. In the experimental arm, TN patients received boceprevir [800 mg three times daily (TID)] plus PR for 24 wk and then further therapy according to TW8 HCV RNA levels. Patients with undetectable HCV RNA at TW8 concluded treatment at week 28 while those with detectable HCV RNA at TW8 continued therapy with PR from weeks 28-48. TE patients received boceprevir (800 mg TID) plus PR for 32 wk and then further therapy according to TW8 HCV RNA levels. Patients with undetectable HCV RNA at TW8 concluded treatment at week 36, while those with detectable HCV RNA at TW8 continued PR therapy from weeks 36-48. Treatment was discontinued for TN patients with detectable HCV RNA at TW24 and TE patients with detectable HCV RNA at TW12

because of futility. Patients in the control arm (PR only) who failed treatment because of the futility rule could cross over to receive triple therapy. TN patients with  $< 2 \log_{10}$  decline in HCV RNA at TW12, or with detectable HCV RNA at TW24 could cross over to receive boceprevir plus PR for 32 wk. TE patients with detectable HCV RNA at TW12 could also cross over to receive boceprevir plus PR for 32 wk. Duration of further therapy depended on HCV RNA detectability at crossover week 4 (COW4). Crossover treatment duration was 32 (COW4 HCV RNA undetectable) or 44 wk (COW4 HCV RNA detectable).

### Patients

The study population included TN and TE adult patients with chronic HCV infection (enrollment ratio 60:40). TN patients had received no previous therapy for HCV infection, whereas TE patients were required to have received prior treatment with PR for  $\geq 12$  wk without interruption or dose reduction. Inclusion criteria for the study included a baseline viral load of  $\geq 10000$  IU/mL, and a liver biopsy consistent with chronic HCV infection. Cirrhotic patients were required to have an ultrasound within 6 mo of screening with no evidence of hepatocellular carcinoma. Exclusion criteria included a platelet count of  $< 100000/\text{mm}^3$ ; hemoglobin levels  $< 12$  g/dL for females or  $< 13$  g/dL for males; human immuno-deficiency virus or hepatitis B virus infection; previous discontinuation of PR due to a treatment-related adverse event (AE); or decompensated liver disease, including a history or presence of ascites, bleeding varices, or hepatic encephalopathy.

### Assessments

The primary efficacy end point was SVR, defined as undetectable HCV RNA 24 wk after completing treatment in randomized patients who received at least 1 dose

**Table 1 Patient demographics *n* (%)**

	Boceprevir plus PR ( <i>n</i> = 159)	PR ( <i>n</i> = 78)
Sex		
Male	94 (59.1)	45 (57.7)
Female	65 (40.9)	33 (42.3)
Age (yr), mean (SD)	38.6 (9.8)	38.1 (10.0)
Race		
White	158 (99.4)	77 (98.7)
Asian	1 (0.6)	1 (1.3)
Ethnicity		
Not Hispanic or Latino	159 (100)	78 (100)
Weight (kg), mean (SD)	78.1 (16.6)	78.5 (16.8)
BMI (kg/m <sup>2</sup> ), mean (SD)	25.9 (4.2)	26.0 (4.4)
Previous treatment		
Naive	97 (61.0)	48 (61.5)
Experienced	62 (39.0)	30 (38.5)
<i>IL28B</i> genotype		
CC allele	22 (13.8)	11 (14.1)
Non-CC allele	137 (86.2)	67 (85.9)
HCV genotype		
GT1a	4 (2.5)	0 (0)
GT1b	155 (97.5)	78 (100)
Baseline HCV RNA		
≤ 800000 IU/mL	89 (56.0)	53 (67.9)
> 800000 IU/mL	70 (44.0)	25 (32.1)
Hemoglobin (g/dL), mean (SD)	15.0 (1.5)	14.9 (1.5)
Liver histology		
Cirrhosis	7 (4.4)	2 (2.6)
No cirrhosis	152 (95.6)	76 (97.4)

GT: Genotype; HCV: Hepatitis C virus; PR: Peginterferon/ribavirin; SD: Standard deviation; BMI: Body mass index; *IL28B*: Interleukin-28B.

of any trial medication. HCV RNA was detected using COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, version 1.0 (Roche Diagnostics, Basel Switzerland); lower limit of quantification = 43 IU/mL; limit of detectability = 18.0 IU/mL. The key secondary end point was the achievement of SVR in randomized patients who received at least 1 dose of boceprevir or boceprevir placebo therapy. Other end points included the relationship between early virologic response and SVR (summarized using the proportion of patients who achieved SVR among those with undetectable HCV RNA at TW4, TW8 or TW12), the proportion of patients with virologic breakthrough (undetectable HCV RNA and subsequent HCV RNA above the limit of quantification while on study therapy), the proportion with incomplete virologic response (> 1 log<sub>10</sub> increase in HCV RNA from nadir value while on study therapy), and safety.

### Statistical analysis

The statistical methods of this study were reviewed by Jianmin Long from Merck and Co., Inc. Analyses were based on the full analysis set population, which included all randomized and treated patients. Target enrollment was 70 patients in the PR control group and 140 in the boceprevir plus PR arm, providing 98% power to demonstrate the superiority of boceprevir plus PR vs PR at an overall 1-sided, 2.5% alpha level, if the underlying difference in SVR was 30%. The power and sample size calculations were based on the assumption of an

underlying response rate of 30% for the PR control arm. The minimum criterion for success was that the *P* value for the comparison of SVR between the boceprevir plus PR arm and the control PR arm was < 0.05. An interim analysis was performed when all patients had completed at least 8 wk of treatment or had discontinued therapy. The results of this interim analysis were used as the basis for regulatory submission in Russia.

Achievement of SVR was summarized using descriptive statistics. The primary statistical comparison was conducted on the full analysis set using the stratified Miettinen and Nurminen method at alpha level of 0.05 adjusted for stratification factors (*IL28B* genotype CC vs non-CC and TN vs TE) as specified at the time of randomization. Multiplicity adjustment for controlling the type 1 error for the primary and key secondary comparisons was based on the step-down approach. The key secondary comparison was tested only if the statistical significance of the primary comparison reached an alpha level of 0.05. Any patient with missing data at, or after follow-up week 24, and undetectable HCV RNA at follow-up week 12, was considered a sustained virologic responder. For efficacy analyses, patients in the PR control arm who rolled over to the crossover arm were considered as failures at and after the time of the crossover.

## RESULTS

### Patients

A total of 238 patients were randomly assigned: 159 were assigned to receive boceprevir plus PR and 79 were assigned to PR (Figure 2). One patient assigned to PR did not receive any study medication and was therefore excluded from the full analysis set population. Four patients discontinued during lead-in (boceprevir plus PR, *n* = 3; PR, *n* = 1), yielding 233 patients in the modified intent-to-treat data set. Fifty-nine patients (boceprevir plus PR, *n* = 24; PR, *n* = 35) discontinued after adding boceprevir/placebo, with the most common reason for discontinuation being treatment failure (5% of patients receiving boceprevir plus PR and 34% of those receiving PR alone were discontinued based on futility criteria, Figure 2). Twenty-seven patients in the PR control arm entered crossover because of treatment failure at the futility time points. In total, 229 patients entered the follow-up phase (Figure 2). The majority of patients were white, with GT1b infection, and the *IL28B* non-CC genotype (Table 1). Few patients were cirrhotic. Compliance rates with boceprevir therapy were high (97.5% of patients had ≥ 80% compliance).

### SVR

SVR at follow-up week 24 was higher in the boceprevir plus PR arm compared with the control arm [74.8% (119/159) vs 46.2% (36/78)], with a stratification-adjusted treatment difference of 29.2% (95%CI: 16.4–41.5; *P* < 0.0001) (Figure 3). The end of treatment response rate was 87.4% (139/159) for the boceprevir

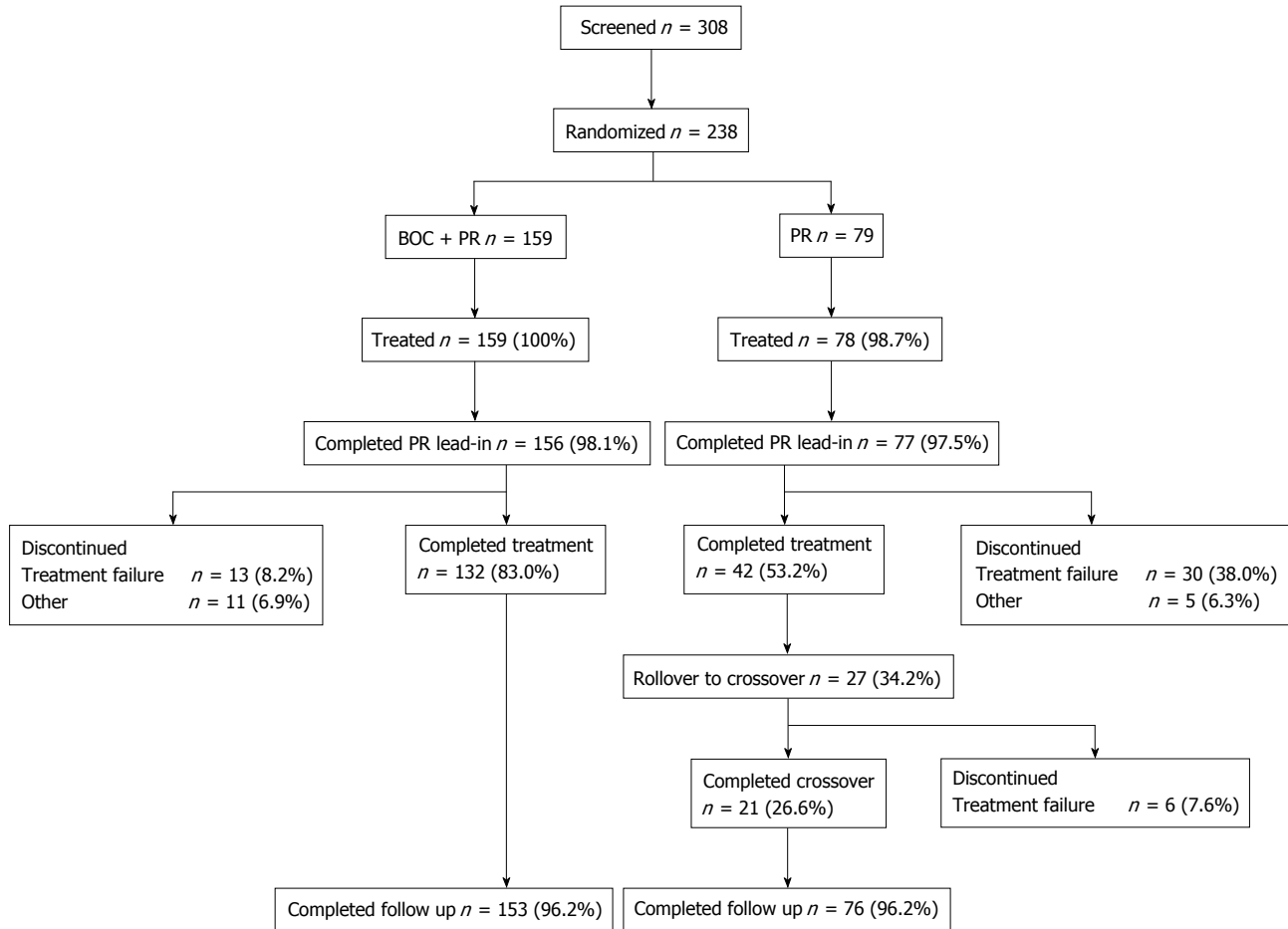


Figure 2 Patient disposition. BOC: Boceprevir; PR: Peginterferon/ribavirin.

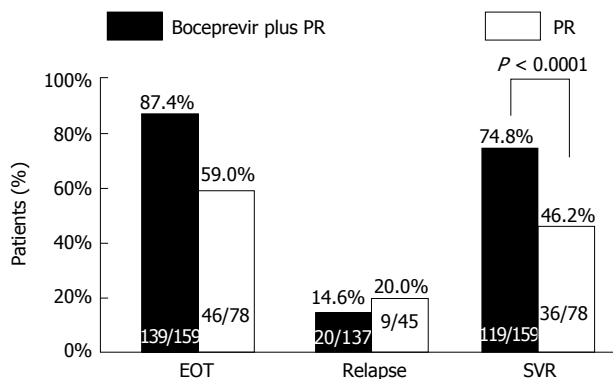


Figure 3 Analysis of sustained virologic response, end of treatment response, and relapse rate. If a patient had missing data at and after the FW24 window and had undetectable HCV RNA at FW12, the patient was considered a sustained virologic responder. The  $P$  value was adjusted for stratification factors of *IL28B* genotype (CC vs non-CC) and previous treatment (TN vs TE), based on the Miettinen and Nurminen method. EOT was defined as the last dose date in treatment phase  $\pm 14$  d inclusive. The closest value to the last dose date was considered to be the EOT value. If there was no value within this window, the closest available value after this window was used. Relapse was defined as any patient who had detectable HCV RNA following end of all study therapy, after becoming undetectable and remaining so until end of treatment. EOT: End of treatment; HCV: Hepatitis C virus; PR: Peginterferon/ribavirin; SVR: Sustained virologic response; TE: Treatment experienced; TN: Treatment naive; *IL28B*: Interleukin-28B.

plus PR arm, and 59.0% (46/78) for the PR control arm. The relapse rate was 14.6% (20/137) for the boceprevir plus PR arm, and 20.0% (9/45) for the PR control arm.

#### Virologic failure

Rates of virologic breakthrough were 3.8% (6/159) in the boceprevir plus PR arm, and 5.1% (4/78) in the PR control arm. No patients in the PR control arm exhibited virologic rebound. Incomplete virologic response/rebound rate in the boceprevir plus PR arm was 3.1% (5/159). Five patients with incomplete virologic response had samples sequenced, of which 3 samples had variants detected (V36M,  $n = 1$ ; T54A,  $n = 2$ ; T54S,  $n = 1$ ; T54T,  $n = 2$ ). Similarly, 5 patients with virologic breakthrough had samples sequenced, of which 3 had detectable HCV variants (T54A,  $n = 1$ ; T54S,  $n = 1$ ; T54T,  $n = 2$ ; V55A,  $n = 1$ ).

#### SVR according to on-treatment virologic response

All patients with undetectable HCV RNA at TW4 in both treatment arms attained SVR (Table 2). In both treatment arms, all patients received PR alone for the first 4 wk of therapy. The proportions of patients with  $< 1$  log drop [boceprevir 43/159 (27%) and PR 22/78

**Table 2** Sustained virologic response by previous treatment, interleukin-28B genotype, and on-treatment virologic response *n* (%)

	Boceprevir plus PR ( <i>n</i> = 159)	PR ( <i>n</i> = 78)
Treatment naive	76/97 (78.4)	27/48 (56.3)
Treatment experienced	43/62 (69.4)	9/30 (30.0)
Null responder	8/17 (47.1)	1/6 (16.7)
Partial responder	5/8 (62.5)	1/4 (25.0)
Relapser	30/37 (81.1)	7/20 (35.0)
Treatment naive		
<i>IL28B</i> CC genotype	19/20 (95.0)	11/11 (100.0)
<i>IL28B</i> non-CC genotype	57/77 (74.0)	16/37 (43.2)
Treatment experienced		
<i>IL28B</i> CC genotype	2/2 (100.0)	0/0
<i>IL28B</i> non-CC genotype	41/60 (68.3)	9/30 (30.0)
SVR according to baseline HCV RNA		
All patients		
≤ 800000 IU/mL	71/89 (79.8)	25/53 (47.2)
> 800000 IU/mL	48/70 (68.8)	11/25 (44.0)
Treatment naive		
≤ 800000 IU/mL	45/52 (86.5)	16/27 (59.3)
> 800000 IU/mL	31/45 (68.9)	11/21 (52.4)
Treatment experienced		
≤ 800000 IU/mL	26/37 (70.3)	9/26 (34.6)
> 800000 IU/mL	17/25 (68.0)	0/4 (0)
SVR according to TW4 response		
TW4 < 1 log drop	20/43 (46.5)	0/22 (0)
TW4 ≥ 1 log drop	75/90 (83.3)	26/45 (57.8)
TW4 undetectable	23/23 (100)	10/10 (100)
Missing	1/3	0/1
SVR according to TW8 response		
TW8 undetectable	115/139 (82.7)	29/33 (87.9)
TW8 detectable	4/16 (25)	7/44 (15.9)
Missing	0/4	0/1
SVR according to presence of anemia		
Yes	47/66 (71.2)	6/11 (54.5)
No	72/93 (77.4)	30/67 (44.8)
SVR according to EPO use		
Yes	10/15 (66.7)	3/3 (100)
No	109/144 (75.7)	33/75 (44)
SVR according to ribavirin dose reduction		
Yes	46/67 (68.7)	12/17 (70.6)
No	73/92 (79.4)	24/61 (39.3)

SVR is defined as the virologic response at follow-up week 24. If a patient had missing data at and after the follow-up week 24 window and had undetectable HCV RNA at follow-up week 12, the patient was considered a sustained virologic responder. EPO: Erythropoietin; HCV: Hepatitis C virus; SVR: Sustained virologic response; TW: Treatment week; PR: Peginterferon/ribavirin; *IL28B*: Interleukin-28B.

(28%)] and ≥ 1 log drop [boceprevir 90/159 (57%) and PR 45/78 (58%)] in HCV RNA at TW4 were similar in both treatment arms. However, SVR was higher in patients receiving boceprevir + PR compared with PR within the subgroups of patients with < 1 log drop in HCV RNA at TW4 (46.5% vs 0%) and those with ≥ 1 log drop in HCV RNA at TW4 (83.3% vs 57.8%).

A TW8 interim analysis was submitted for regulatory approval in Russia. In this analysis, rates of undetectable HCV RNA at TW8 in the boceprevir RGT and PR arms were 91% (88/97) vs 48% (23/48) in TN patients and 82% (51/62) vs 33% (22/67) in TE patients. Overall, the rates of undetectable HCV RNA at TW8 in all patients

were higher in patients receiving boceprevir plus PR compared with control therapy (87.4% vs 42.3%, *P* < 0.0001). SVR rates in patients with undetectable HCV RNA at TW8 were similar between treatment arms [boceprevir + PR 82.7% (115/139) vs PR 87.9% (29/33)].

### SVR according to baseline variables

SVR rates are presented by previous treatment and response, and *IL28B* genotype (Table 2). SVR rates were higher in patients receiving boceprevir plus PR compared with PR in both TN (78.4% vs 56.3%) and TE (69.4% vs 30.0%) subgroups. Within TE patients, the rates of SVR were higher with boceprevir plus PR compared with PR, regardless of treatment failure type (null responder, partial responder, and relapser). SVR rates were high among all patients with *IL28B* CC genotype, regardless of treatment arm or previous treatment history. Conversely, the rates of SVR in patients with *IL28B* CT or TT genotypes were higher with boceprevir plus PR compared with PR alone (Table 2). SVR rates were also higher with boceprevir compared with PR, regardless of baseline viral load. SVR was 87% in TN patients with baseline viral load ≤ 800000 IU/mL. Among patients receiving boceprevir, rates of SVR were generally higher in TN patients with low viral load compared with those with high baseline viral load (86.5% vs 68.9%); however, SVR was similar in TE patients with high vs low baseline viral load receiving boceprevir (70.3% vs 68.0%) (Table 2).

### SVR in patients requiring anemia management

Among patients receiving boceprevir plus PR, SVR rates were similar in patients with anemia (< 10 g/dL) and those without anemia (71.2% vs 77.4%). SVR rates were also relatively similar in boceprevir recipients requiring erythropoietin (EPO) for anemia management and those not using EPO (66.7% vs 75.7%, Table 2), and in those who received ribavirin dose reduction and those who did not (68.7% vs 79.4%).

### Crossover therapy

The SVR rates for the crossover group are presented in Table 3. Overall, 70.4% of patients who crossed over from PR alone to boceprevir plus PR had SVR at follow-up week 24.

### Safety

The reported AEs were consistent with the known safety profile of boceprevir (Table 4), with treatment-emergent AEs noted frequently in both treatment arms (97.5% in the boceprevir plus PR arm and 91.0% in the PR control arm). The number of patients discontinuing treatment because of AEs was 4.4% in the boceprevir plus PR arm (*n* = 7, of which 5 were considered treatment related) and 2.6% in the PR control arm (*n* = 2, of which 1 was considered treatment related). Serious AEs were reported in 10.7% (*n* = 17, of which



**Table 3 Sustained virologic response at follow-up week 24 in the crossover group *n* (%)**

	SVR
Total	19/27 (70.4)
TN TW12 failure (< 2 log decline HCV RNA)	8/11 (72.7)
TE TW12 failure (detectable HCV RNA)	11/16 (68.8)
TN TW24 failure (detectable HCV RNA)	0/0

HCV: Hepatitis C virus; SVR: Sustained virologic response; TE: Treatment-experienced; TN: Treatment-naïve; TW: Treatment week.

12 were considered drug related) and 11.5% (*n* = 9, of which 5 were considered drug related) of patients in the boceprevir plus PR and PR arms, respectively. Dose modifications due to an AE were reported in 56% (89/159) in the boceprevir plus PR arm, and 33.3% (26/78) for PR alone. There were no deaths reported during the study.

Anemia was reported at a higher rate in patients receiving boceprevir plus PR compared with those receiving PR alone (47.2% vs 24.4%). However, few patients in either treatment group had on-treatment hemoglobin levels < 8.5 g/dL (boceprevir + PR 6.3% vs PR 2.6%). EPO use was reported for 9.4% of patients receiving boceprevir plus PR and 3.8% of those receiving PR alone. Ribavirin dose reduction was required for 65 patients (40.9%) receiving boceprevir plus PR and 14 patients (17.9%) receiving PR alone.

## DISCUSSION

Data from the present study indicate that, similar to activity seen in Western populations, boceprevir added to PR results in a marked improvement in SVR rates compared with PR alone in TN and TE Russian patients with HCV GT1 infection. The high rate of undetectable HCV RNA at TW8 in TN and TE patients receiving boceprevir plus PR resulted in a high proportion of patients being deemed eligible for RGT with consequent reductions in their treatment durations. The treatment effect (*i.e.*, difference in response between boceprevir plus PR and PR alone) was comparable between this study in Russian patients, and the phase 3 trials (Table 5). However, whereas 42%-46% of patients receiving boceprevir RGT in the phase 3 studies had undetectable HCV RNA at TW8, in the present study 87.4% of boceprevir recipients had undetectable HCV RNA at TW8. This suggests that the proportion of Russian patients eligible for shortened treatment duration may be higher than reported in the phase 3 studies, and is suggestive of a favorable cost/efficacy ratio in Russian patients. Response rates were particularly high among patients with favorable disease characteristics such as the *IL28B* CC genotype. In patients with this genotype, SVR rates were high regardless of treatment regimen; however, patients with the *IL28B* non-CC genotype derived a substantial benefit from boceprevir therapy.

The tolerability profile seen with boceprevir in

**Table 4 Adverse events ( $\geq 20\%$  in any treatment arm) *n* (%)**

	Boceprevir plus PR ( <i>n</i> = 159)	PR ( <i>n</i> = 78)
Any AE	155 (97.5)	71 (91.0)
Neutropenia	84 (52.8)	31 (41.0)
Pyrexia	77 (48.4)	36 (46.2)
Anemia	75 (47.2)	19 (24.4)
Leukopenia	62 (39.0)	25 (32.1)
Dysgeusia	59 (37.1)	3 (3.8)
Asthenia	44 (27.7)	23 (29.5)
Headache	43 (27.0)	25 (32.1)
Influenza-like illness	39 (24.5)	14 (17.9)
Nausea	39 (24.5)	9 (11.5)
Anemia		
8.5-10 g/dL	56 (35.2)	9 (11.5)
< 8.5 g/dL	10 (6.3)	2 (2.6)
Ribavirin dose reduction	65 (40.9)	14 (17.9)
EPO use	15 (9.4)	3 (3.8)
Serious AE	17 (10.7)	9 (11.5)
Discontinued because of an AE	7 (4.4)	2 (2.6)
Dose modification due to an AE	89 (56.0)	26 (33.3)

AE: Adverse event; EPO: Erythropoietin; PR: Peginterferon/ribavirin.

Russian patients was consistent with the established tolerability profile documented in Western patients. The majority of AEs were associated with PR therapy. As seen in Western patients, anemia was increased with boceprevir, and there was also a corresponding increase in the use of anemia management strategies (ribavirin dose reduction and EPO use) among patients receiving boceprevir. In SPRINT-2 and RESPOND-2, approximately 3%-8% of patients receiving boceprevir plus PR had hemoglobin levels < 8.0 g/dL; EPO use was required in 41%-46% of patients, and 21% required dose reduction due to anemia<sup>[2,3]</sup>. In the present study, 6.3% of patients receiving boceprevir plus PR had nadir hemoglobin < 8.5 g/dL. There were also differences in the rates of anemia management strategies with lower rates of EPO use (9.4%) but higher rates of dose reduction (41%) in the present study compared with the phase 3 studies in Western patients<sup>[2,3]</sup>. These differences between studies are a reflection of the different anemia management strategies. In the phase 3 protocols, investigators were free to choose between ribavirin dose reduction and EPO use as a first-line strategy while in the present study ribavirin dose reduction was the first-line strategy and EPO use was the second-line strategy.

Response rates in this study are higher for both boceprevir plus PR and PR alone, compared with rates seen in previous phase 3 studies (Table 5). This increase in response may be explained by differences in the patient populations enrolled in the current study and the phase 3 studies<sup>[2,3]</sup>. Compared with patients enrolled in the boceprevir phase 3 studies, more Russian patients were aged  $\leq 40$  years (62% vs 13%), had baseline viral load  $\leq 800000$  IU/mL (60% vs 14%), and had HCV GT1b infection (98% vs 35%).

Data from the present study support the use of boceprevir in Russian patients with HCV GT1 infection. However, boceprevir-based triple therapy may not be

**Table 5 Comparison of virologic response rates between Russian patients and western patients receiving boceprevir-based triple therapy in the serine protease inhibitor therapy 2 and retreatment with hepatitis C virus serine protease inhibitor boceprevir and pegIntron/rebetol 2 studies *n* (%)**

	Russian patients		SPRINT-2		RESPOND-2	
	RGT of BOC	PR	RGT of BOC	PR	RGT of BOC	PR
TN						
EOT	89/97 (91.8)	33/48 (68.8)	277/366 (76)	191/363 (53)	-	-
SVR	76/97 (78.4)	27/48 (56.3)	242/366 (66)	137/363 (38)	-	-
Relapse	13/89 (14.6)	6/33 (18.2)	24/265 (9)	39/176 (22)	-	-
TE						
EOT	50/62 (80.6)	13/30 (43.3)	-	-	114/162 (70.4)	25/80 (31)
SVR	43/62 (69.4)	9/30 (30)	-	-	107/161 (66)	17/80 (21)
Relapse	7/48 (14.6)	3/12 (25.0)	-	-	14/121 (12)	8/25 (32)

BOC: Boceprevir; EOT: End of treatment response; PR: Peginterferon/ribavirin; RESPOND-2: Retreatment with hepatitis C virus serine protease inhibitor boceprevir and pegIntron/rebetol 2 study; RGT: Response-guided therapy; SPRINT-2: Serine protease inhibitor therapy 2 study; SVR: Sustained virologic response; TE: Treatment experienced; TN: Treatment naive.

appropriate for all patients with GT1 infection. Patients with low viral load at baseline who achieve undetectable HCV RNA at TW4 may achieve high SVR rates with 24-wk of therapy with PR alone and would not require the addition of boceprevir<sup>[9]</sup>. Despite the world-wide acceptance of interferon-free regimens as a standard of care due to the near 100% efficacy and low adverse events rate, some patients will continue to receive interferon-based treatment. This is due largely to the fact that the approval of interferon-free regimens is not immediately followed by total reimbursement in many countries, or that access to these regimens is dependent on the stage of the liver disease, prioritizing treatment of cirrhotic patients<sup>[10-12]</sup>. Easy-to-treat patients can be successfully treated with interferon-based regimens which may be easier to access through reimbursement.

In conclusion, data from the present study support the use of boceprevir plus PR for the treatment of Russian patients with HCV GT1 infection. The safety and efficacy profile of boceprevir in Russian patients was generally similar to that previously reported in phase 3 studies in Western patients; however, this treatment may be more cost-effective in Russia as approximately 88% of patients had undetectable HCV RNA at TW8, suggesting that a higher proportion of Russian patients receiving boceprevir plus PR would be eligible for reduced treatment duration with RGT compared with Western patients. Regulatory approval has been obtained for boceprevir in Russia based on the results of this study.

## ACKNOWLEDGMENTS

Medical writing and editorial assistance were provided by Tim Ibbotson, PhD, of ApotheCom, Yardley, PA, United States.

## COMMENTS

### Background

In the treatment of hepatitis C virus (HCV), genotype 1 infection, peginterferon

plus ribavirin is associated with low efficacy and poor tolerability. Phase 3 studies have shown that addition of a direct-acting antiviral agent such as boceprevir can improve efficacy and shorten treatment durations.

### Research frontiers

The safety and efficacy of boceprevir plus peginterferon and ribavirin in Russian patients with HCV infection is currently unknown.

### Innovations and breakthroughs

In the present study, patients receiving boceprevir plus peginterferon and ribavirin achieved significantly higher rates of sustained virologic response compared with patients treated with peginterferon and ribavirin alone. Patients receiving boceprevir-based therapy frequently required substantially shorter treatment durations compared to patients receiving PR alone. Rates of anemia were higher among patients receiving boceprevir.

### Applications

Regulatory approval has been obtained for boceprevir in Russia based on the results of this study.

### Peer-review

This manuscript evaluates the efficacy and safety of boceprevir plus peginterferon and ribavirin in treatment-naïve and treatment-experienced Russian patients with HCV genotype 1 infection.

## REFERENCES

- 1 **Malcolm BA**, Liu R, Lahser F, Agrawal S, Belanger B, Butkiewicz N, Chase R, Gheys F, Hart A, Hesk D, Ingrassia P, Jiang C, Kong R, Lu J, Pichardo J, Prongay A, Skelton A, Tong X, Venkatraman S, Xia E, Girijavallabhan V, Njoroge FG. SCH 503034, a mechanism-based inhibitor of hepatitis C virus NS3 protease, suppresses polyprotein maturation and enhances the antiviral activity of alpha interferon in replicon cells. *Antimicrob Agents Chemother* 2006; **50**: 1013-1020 [PMID: 16495264 DOI: 10.1128/AAC.50.3.1013-1020.2006]
- 2 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R; HCV RESPOND-2 Investigators. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]
- 3 **Poordad F**, McCone J Jr, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP;

- SPRINT-2 Investigators. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]
- 4 **Lavanchy D.** Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011; **17**: 107-115 [PMID: 21091831 DOI: 10.1111/j.1469-0691.2010.03432.x]
  - 5 **European Association for Study of Liver.** EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; **60**: 392-420 [PMID: 24331294 DOI: 10.1016/j.jhep.2013.11.003]
  - 6 **American Association for the Study of Liver Diseases,** Infectious Diseases Society of America, International Antiviral Society-USA. Recommendations for testing, managing, and treating hepatitis C. AASLD/IDSA/IAS-USA Web site. [Accessed 2014-05-26]. Available from: URL: <http://hcvguidelines.org/news/hcv-guidance>
  - 7 **Umar M,** Khan AG, Abbas Z, Arora S, Asifabbas N, Elewaut A, Esmat G, Foster G, Fried M, Goh KL, Hamama TB, Imawari M, Isakov V, Krabshuis J, LaBrecque D, Lemair A, Malfertheiner P, Ryder S, Schiedermaier P, Stimac D, Tandon R, Villamil F, Zapata R, Ferenci P; World Gastroenterology Organisation. World Gastroenterology Organisation global guidelines: diagnosis, management and prevention of hepatitis C April 2013. *J Clin Gastroenterol* 2014; **48**: 204-217 [PMID: 24504078 DOI: 10.1097/MCG.0000000000000050]
  - 8 **World Health Organization.** Guidelines for the screening, care and treatment of persons with hepatitis C infection. WHO Web site. [Accessed 2014-06-26]. Available from: URL: <http://www.who.int/hiv/pub/hepatitis/hepatitis-c-guidelines/en>
  - 9 **Zeuzem S,** Buti M, Ferenci P, Sperl J, Horsmans Y, Cianciara J, Ibranyi E, Weiland O, Noviello S, Brass C, Albrecht J. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. *J Hepatol* 2006; **44**: 97-103 [PMID: 16290907 DOI: 10.1016/j.jhep.2005.10.003]
  - 10 **Sadler MD,** Lee SS. Revolution in hepatitis C antiviral therapy. *Br Med Bull* 2015; **113**: 31-44 [PMID: 25680808 DOI: 10.1093/bmb/ldv004]
  - 11 **Pawlotsky JM.** New hepatitis C therapies: the toolbox, strategies, and challenges. *Gastroenterology* 2014; **146**: 1176-1192 [PMID: 24631495 DOI: 10.1053/j.gastro.2014.03.003]
  - 12 **Stepanova M,** Younossi ZM. Interferon-Free Regimens for Chronic Hepatitis C: Barriers Due to Treatment Candidacy and Insurance Coverage. *Dig Dis Sci* 2015; **60**: 3248-3251 [PMID: 25986525 DOI: 10.1007/s10620-015-3709-6]

**P- Reviewer:** Chuang WL, Messori A, Sirin G

**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Liu SQ



## Primary hepatic amyloidosis: A case report and review of literature

Nikhil Sonthalia, Samit Jain, Sunil Pawar, Vinay Zanwar, Ravindra Surude, Praveen M Rath

Nikhil Sonthalia, Samit Jain, Sunil Pawar, Vinay Zanwar, Ravindra Surude, Praveen M Rath, Department of Gastroenterology, Topiwala National Medical College and BYL Ch Hospital, Mumbai 400008, Maharashtra, India

**Author contributions:** All authors contributed equally to the manuscript.

**Institutional review board statement:** The case report was exempt from the institutional review board standards at the BYL Nair Ch. Hospital and Topiwala National Medical College.

**Informed consent statement:** The patient involved in the study gave her written informed consent authorizing use and disclosure of her protected health information.

**Conflict-of-interest statement:** Authors have no conflict of interest to be declared.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Nikhil Sonthalia, Senior Resident, Department of Gastroenterology, Topiwala National Medical College and BYL Ch Hospital, Dr. A.L Nair Road, Mumbai 400008, Maharashtra, India. [nikhil\\_zenith@yahoo.co.in](mailto:nikhil_zenith@yahoo.co.in)  
 Telephone: +91-900-4203165  
 Fax: +91-022-23016139

Received: October 1, 2015  
 Peer-review started: October 9, 2015  
 First decision: November 4, 2015  
 Revised: December 14, 2015  
 Accepted: January 27, 2016  
 Article in press: January 29, 2016  
 Published online: February 28, 2016

### Abstract

We describe a case of 42-year-old female presenting with abdominal pain associated with loss of weight and fever for 8 mo. On evaluation she had gross hepatomegaly with raised alkaline phosphatase and raised GGT levels with normal transaminases and bilirubin. On imaging she had diffuse enlargement of liver with heterogeneous contrast uptake in liver. Her viral marker and autoimmune markers were negative. Liver biopsy depicted massive deposition of amyloid in peri-sinusoidal spaces which revealed apple green birefringence on polarizing microscopy after Congo red staining. Cardiac and renal evaluation was unremarkable. Abdominal fat pad and rectum biopsy was negative for amyloid deposit. There was no evidence of primary amyloidosis as bone marrow examination was normal. Serum and urine immunofixation electrophoresis were normal. Immunoperoxidase staining for serum amyloid associated protein for secondary amyloidosis was negative from liver biopsy. We present this rare case of primary hepatic amyloidosis and review the literature regarding varied presentations of hepatic involvement in amyloidosis.

**Key words:** Amyloidosis; Congo red staining; Isolated hepatic amyloidosis; Amyloid associated protein; Immunofixation electrophoresis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Amyloidosis is a pathological process that encompasses a spectrum of disease resulting from the extracellular deposition of fibrillar amyloid protein. It can involve any organ isolated or in conjunction with other organs and can do so in the form of a focal, tumour-like lesion, or an infiltrative process. Amyloidosis localized to the liver has been rarely described. This case represents a rare instance of primary hepatic amyloidosis without



evidence of primary or secondary cause of amyloid deposit posing considerable diagnostic and therapeutic challenge for the clinicians.

Sonthalia N, Jain S, Pawar S, Zanwar V, Surude R, Rath PM. Primary hepatic amyloidosis: A case report and review of literature. *World J Hepatol* 2016; 8(6): 340-344 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i6/340.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i6.340>

## INTRODUCTION

Amyloidosis is a pathological process that encompasses a spectrum of disease resulting from the extracellular deposition of fibrillar amyloid protein, which can involve any organ in isolation or in conjunction with other organs and can do so in the form of a focal, tumour-like lesion, or an infiltrative process. Amyloidosis localized to the liver has been rarely described, although it is possible that these patients have yet to exhibit evidence of systemic disease. Hepatic involvement in both primary (AL) and secondary (AA) forms of systemic amyloidosis is common; however, clinically dominant hepatic amyloidosis is unusual<sup>[1]</sup>. Accumulation of amyloids in the liver produces hepatomegaly in 33%-92% of patients, as well as moderate jaundice and moderate to severe cholestasis<sup>[2,3]</sup>. Our patient presented with constitutional symptoms of fever, weight loss, and hepatomegaly without jaundice with evidence of amyloid deposit in perisinusoidal spaces without any systemic evidence of primary or secondary amyloidosis. Isolated hepatic amyloidosis has rarely been described in literature which poses great diagnostic and therapeutic challenge.

## CASE REPORT

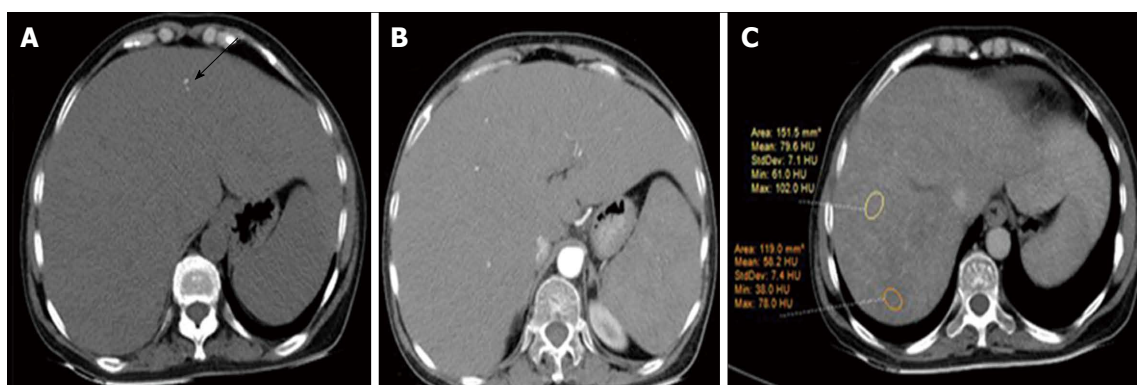
A 42-year-old female, presented with complaints of right hypochondriac and epigastric pain, which was dull aching, occurring intermittently, associated with weight loss of 15 kg over 8 mo. There was no history of jaundice, hematemesis, melena, and abdominal distension, alteration in bowel habit or bleeding from any site. There was no history of joint pain, rash, oral ulceration, cough, skin tightness, peripheral tingling, and weakness in limbs or breathlessness. She had an episode of acute febrile illness due to uncomplicated plasmodium vivax malaria in the recent past. There was history of acute viral hepatitis two times in remote past. She was hypothyroid on supplementation since 2 years. There was no history of tuberculosis in past.

On General examination patient was afebrile with pulse rate 80/min, blood pressure 16.2/10 kPa and had pallor. Systemic examination revealed liver enlarged for 2.4 inch below right costal margin which was firm, with sharp margin, smooth surface, non-tender, without hepatic rub with liver span of 8.8 inch. Spleen was also enlarged.

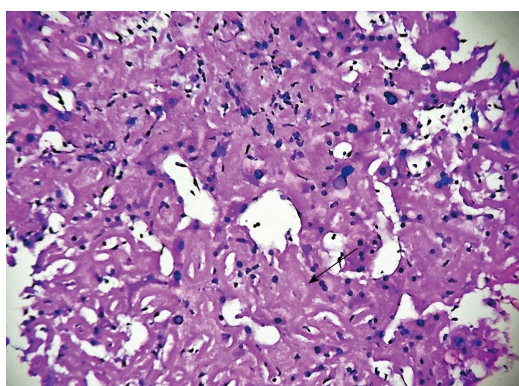
On evaluation investigations revealed normocytic normochromic anemia, aspartate aminotransferase level of 0.52  $\mu$ kat/L (upper normal limit 0.6  $\mu$ kat/L) and alanine aminotransferase level of 0.47  $\mu$ kat/L (upper normal limit 0.51  $\mu$ kat/L), alkaline phosphatase value of 7.46  $\mu$ kat/L (upper normal limit 2  $\mu$ kat/L) raised to 3.5 times the upper limit of normal, gamma glutamyl transferase value of 9.29  $\mu$ kat/L (upper normal limit 0.51  $\mu$ kat/L). Prothrombin time was 14 s with INR of 1, serum urea was 3.57 mmol/L (normal up to 8.2 mmol/L) and creatinine was 70.72  $\mu$ mol/L (normal upto 106  $\mu$ mol/L). Her thyroid stimulating hormone was 3.1  $\mu$ IU/mL (normal upto 5  $\mu$ IU/mL), FT4 was 14.16 pmol/L (normal 12 to 30 pmol/L), and FT3 was 4.62 pmol/L (normal 2 to 7 pmol/L). Erythrocyte sedimentation rate was 55 mm at end of 1<sup>st</sup> h by Westergreen method, C-reactive proteins was 47.62 nmol/L (normal up to 28.5 nmol/L). Her serum calcium was 2.23 mmol/L, fasting blood sugar was 5 mmol/L, triglycerides were 1.02 mmol/L and cholesterol was 3.37 mmol/L. Vitamin D3 was 239.2 nmol/L (normal > 150 nmol/L); intact-parathyroid hormone was 13 pg/mL (normal 10-65 ng/mL). Human immunodeficiency virus antibodies, HBsAg, anti-hepatitis C virus antibody were negative. Autoimmune markers including anti-nuclear antibodies, anti-Liver Kidney Microsome type 1 antibody (anti-LKM 1), anti-smooth muscle antibodies, anti-mitochondrial antibodies were negative.

Ultrasonography abdomen revealed hepatomegaly (7 inch) with coarse echo texture with compressed intrahepatic inferior vena cava and splenomegaly. Oesophagus-DuodenoScopy revealed mild antral gastritis. Pre-contrast computed tomography (CT) abdomen revealed gross hepatomegaly with tiny foci of calcification (Figure 1A). There was heterogeneous post contrast enhancement with diffuse low density areas in liver on venous phase (Figure 1). On further evaluation for infiltrative liver disorders, liver biopsy was done which revealed diffuse eosinophilic homogenous material throughout sinusoids with compressed hepatocytes (Figure 2). These areas were Congo red stain positive with apple green birefringence on polarizing microscopy suggestive of Amyloid deposits (Figure 3). Ultrasonography and fine needle aspiration cytology from the thyroid gland was done which was suggestive of colloid goiter without any evidence of amyloid deposit.

The patient had normal serum and urine protein immunofixation electrophoresis, with normal serum free light chain assay. There was no albuminuria or no bence-jones proteinuria. Her electrocardiogram and echocardiogram was normal. Bone marrow biopsy did not reveal any plasma cell dyscrasia or amyloid deposit. Contrast enhanced CT of thorax and nuclear medicine whole body bone scan was normal with no evidence of extra osseous uptake. Skeletal survey showed no gross abnormalities. Abdominal fat pad biopsy and rectum biopsy was negative for amyloid deposit. Her serum Rheumatoid factor, pAnti-neutrophilic cytoplasmic antibodies (ANCA), cANCA, anti-rho, anti-



**Figure 1** Pre-contrast computed tomography image of abdomen. A: Gross hepatomegaly with tiny foci of calcification in segment IV of liver (black arrow); arterial phase; B: Diffuse low contrast attenuation; C: Venous show heterogeneous contrast enhancement with diffuse low density areas scattered throughout liver parenchyma (yellow and orange spherical).



**Figure 2** Haematoxylin and eosin stain of the liver biopsy specimen shows diffuse extracellular amyloid deposit in peri-sinusoidal spaces with compression of hepatocytes (black arrow).

la antibody, tuberculin test, and tumour markers were negative. Immunohistochemistry using anti serum amyloid associated (SAA) protein immunoperoxidase staining for secondary amyloidosis was done from the liver biopsy specimen which was also negative. Immunohistochemistry of liver biopsy using anti-kappa and anti-lambda antibody was not done as the serum free light chain assay and serum protein immunofixation electrophoresis was normal. She was managed symptomatically with colchicine and other supportive therapies including intravenous fluids. The patient was followed up for 6 mo. Subsequently she was lost to follow up. Her constitutional symptoms improved marginally on colchicine. But hepatomegaly and altered biochemical parameters did not show any improvement.

## DISCUSSION

Though amyloidosis is considered as a systemic disease, 10%-20% cases can be localised<sup>[4]</sup>. To our knowledge primarily isolated hepatic involvement of liver in amyloidosis has rarely been described in the literature. Though it is possible that the patient has yet exhibited the evidence of systemic disease, hepatic involvement can occur in both primary and secondary

types of amyloidosis (AL/AA). In primary type the characteristic fibrillar protein is a fragment of the variable immunoglobulin light (and/or rarely heavy) chain and in secondary type the protein is the amino acid terminus of the acute phase protein SAA. Secondary amyloidosis with hepatic involvement can be seen in chronic inflammatory disorders and infections including multiple myeloma, tuberculosis, rheumatoid arthritis, familial Mediterranean fever, Crohn's disease, Reiter's syndrome, ankylosing spondylitis, Sjögren's syndrome, dermatomyositis, vasculitis, chronic osteomyelitis, bronchiectasis, cystic fibrosis, systemic lupus erythematosus (SLE)<sup>[4]</sup>, etc. Currently the AA/AL ratio has been 1:17 to 1:38 due to fewer chronic infections and an increasing recognition of AL amyloidosis. Other types of amyloidosis that are rarely seen include dialysis-related amyloidosis with the deposition of  $\beta$ 2-microglobulins, and autosomal dominant systemic amyloidosis, such as familial amyloidotic polyneuropathy with the deposition of genetically variant transthyretin<sup>[5]</sup>.

Hepatic amyloidosis is usually characterised by amyloid deposits in the liver parenchyma along the sinusoids within the spaces of disse or within the blood vessel walls. As a result of extensive compression of hepatocytes by the amyloid deposits there may be atrophy of hepatocyte. More massive infiltration results in enlarged liver with rubber elastic consistency. This results in "lardaceous liver" appearance on cut-surface<sup>[6]</sup>.

The clinical spectrum of hepatic amyloidosis can range from hepatomegaly and borderline abnormal liver function test to more severe form resulting in portal hypertension, hepatic failure and rarely spontaneous rupture<sup>[7]</sup>. Around 70%-80% of the cases have associated nephrotic syndrome, congestive cardiac failure, orthostatic hypotension or peripheral neuropathy. In another series, other frequent findings in cases of hepatic amyloidosis included proteinuria (88%), elevated serum alkaline phosphatase (86%), abnormal serum protein electrophoresis (monoclonal protein or hypogammaglobulinemia, 64%), hyposplenism on the peripheral blood smear (62%), defined by the presence

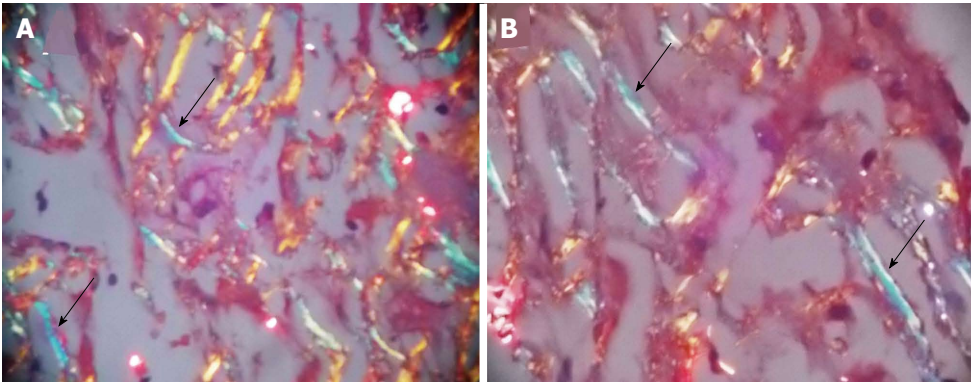


Figure 3 Apple green birefringence demonstrated by amyloid fibrils on polarizing light microscopy (black arrows in A and B) is in liver biopsy specimen.

of Howell-Jolly bodies; and hepatomegaly (81%) disproportional to the liver enzyme abnormalities<sup>[8]</sup>. The median survival rate in these patients is 9 mo<sup>[7,8]</sup>. Our patient had gross hepatomegaly with constitutional symptoms together with raised alkaline phosphatase but no other associated feature.

Radiological findings of hepatic involvement are non-specific. Pre-contrast and contrast enhanced CT reveals enlarged liver with heterogeneous decrease attenuation with delayed enhancement. There may be focal area of hypo attenuation owing to impaired blood flow due to extensive infiltration by amyloid deposit. Our patient had gross hepatomegaly with tiny foci of parenchymal calcification on pre-contrast CT which is rarely seen<sup>[9]</sup>. Her arterial phase CT shows lack of parenchymal enhancement in liver. Venous phase shows heterogeneous enhancement with diffuse low density areas in liver. Rarely typical hepatic contour characterised by asymmetric and triangular appearance of liver with apex towards falciform ligament may occur owing to selective atrophy of the lateral margins of both the lobes<sup>[10]</sup>.

Immunohistochemistry using anti-kappa and anti-lambda antibodies are useful in immunohistochemical classification and diagnosis of AL type amyloidosis. However it has its own limitations owing to cross reactivity between anti-kappa and anti-lambda antibodies. A study using antibodies against three different regions of immunoglobulin lambda light chain for the immunohistochemical analysis of liver biopsy samples from the cases of immunoglobulin lambda light chain amyloidosis showed that the amyloid deposits may not be homogeneous in the liver and that molecular heterogeneity of amyloid fibril protein or a difference in the mode of deposition results in the histopathological heterogeneity of AL amyloid deposits<sup>[11]</sup>.

Our case represents a diagnostic challenge where specific type of amyloid deposit in liver was difficult to determine. Normal bone marrow examination with normal serum and urine immunofixation electrophoresis ruled out primary amyloidosis. Anti-SAA immunoperoxide staining from liver biopsy was also negative. Hereditary forms of amyloidosis including lysosome form were considered. There was no facility for mass spectroscopic

analysis for varied type of amyloid protein at our centre. There was no evidence of hepatocellular failure or spontaneous rupture. There was no cardiac, renal or nervous system involvement. There was no evidence of tuberculosis, rheumatoid arthritis, SLE, crohns disease, or evidence of other common inflammatory diseases. This case represents need for high level of suspicion to diagnose a case of isolated hepatomegaly due to amyloidosis. There have been case reports of hepatic involvement in amyloidosis including one where the presentation was a liver SOL in the setting of plasma cell dyscrasias, but isolated hepatic involvement is a rare entity<sup>[12]</sup>. In absence of systemic evidence of amyloidosis liver transplant was considered for the patient but she did not have requisite Model for End Stage Liver Disease points to make her eligible candidate. She was managed symptomatically with colchicine and other supportive therapies.

This case represents diagnostic and therapeutic difficulty in managing a case of primary hepatic amyloidosis of undetermined aetiology.

## COMMENTS

### Case characteristics

A middle aged female presented with long-standing abdominal pain with loss of weight without jaundice.

### Clinical diagnosis

On examination her vitals were stable and she had gross hepatomegaly.

### Differential diagnosis

Infiltrative liver disorders (amyloidosis, lymphoma, sarcoidosis), metabolic liver disease (hemochromatosis), autoimmune liver disease.

### Laboratory diagnosis

She had normal alanine and aspartate aminotransferase levels, markedly raised alkaline phosphatase level of 447 U/L (upper normal limit 310 U/L) raised to 1.5 times the upper limit of normal, gamma Glutamyl transferase value of 566 U/L (upper normal limit 45 U/L) raised to 12 times the upper limit of normal and other parameters within normal limit.

### Imaging diagnosis

Computed tomography whole abdomen showed gross hepatomegaly with heterogeneous post contrast enhancement with diffuse low-density areas on



venous phase.

### Pathological diagnosis

Liver biopsy was suggestive of diffuse eosinophilic homogenous material throughout sinusoids with compressed hepatocytes which were Congo red stain positive showing apple green birefringence on polarizing microscopy suggestive of amyloid deposits.

### Treatment

She was managed symptomatically with colchicine and other supportive therapies as there was no definite cause of hepatic amyloidosis that could be found out.

### Related reports

There have been case reports of hepatic involvement in amyloidosis including being presented as a mass in setting of plasma cell dyscrasias, but isolated hepatic involvement is a rare entity published series have described varied presentation of liver involvement in amyloidosis which can range from asymptomatic hepatomegaly to fulminant hepatic failure.

### Term explanations

Amyloidosis is a pathological process that encompasses a spectrum of disease resulting from the extracellular deposition of fibrillar amyloid protein which can involve any organ isolated or in conjunction with other organs and can do so in the form of a focal, tumour-like lesion, or an infiltrative process.

### Experiences and lessons

This case represents diagnostic and therapeutic difficulty in managing a case of isolated hepatic amyloidosis of undetermined aetiology.

### Peer-review

The paper is well written.

## REFERENCES

- 1 **Lachmann HJ**, Goodman HJ, Gilbertson JA, Gallimore JR, Sabin CA, Gillmore JD, Hawkins PN. Natural history and outcome in systemic AA amyloidosis. *N Engl J Med* 2007; **356**: 2361-2371 [PMID: 17554117 DOI: 10.1056/NEJMoa070265]
- 2 **Bujanda L**, Beguiristain A, Alberdi F, Cosme A, Ruiz de la Hermosa J, Gutiérrez-Stampa JI. Spontaneous rupture of the liver

- in amyloidosis. *Am J Gastroenterol* 1997; **92**: 1385-1386 [PMID: 9260817]
- 3 **Tam M**, Seldin DC, Forbes BM, Connors LH, Skinner M, Oran B, Quillen K, Sanchowala V. Spontaneous rupture of the liver in a patient with systemic AL amyloidosis undergoing treatment with high-dose melphalan and autologous stem cell transplantation: a case report with literature review. *Amyloid* 2009; **16**: 103-107 [PMID: 20536404 DOI: 10.1080/13506120902879574]
- 4 **Urban BA**, Fishman EK, Goldman SM, Scott WW, Jones B, Humphrey RL, Hruban RH. CT evaluation of amyloidosis: spectrum of disease. *Radiographics* 1993; **13**: 1295-1308 [PMID: 8290725 DOI: 10.1148/radiographics.13.6.8290725]
- 5 **Sattianayagam PT**, Hawkins PN, Gillmore JD. Systemic amyloidosis and the gastrointestinal tract. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 608-617 [PMID: 19724253 DOI: 10.1038/nrgastro.2009.147]
- 6 **Monzawa S**, Tsukamoto T, Omata K, Hosoda K, Araki T, Sugimura K. A case with primary amyloidosis of the liver and spleen: radiologic findings. *Eur J Radiol* 2002; **41**: 237-241 [PMID: 11861098 DOI: 10.1016/S0720-048X(01)00407-7]
- 7 **Park MA**, Mueller PS, Kyle RA, Larson DR, Plevak MF, Gertz MA. Primary (AL) hepatic amyloidosis: clinical features and natural history in 98 patients. *Medicine (Baltimore)* 2003; **82**: 291-298 [PMID: 14530778 DOI: 10.1097/01.md.0000091183.93122.c7]
- 8 **Gertz MA**, Kyle RA. Hepatic amyloidosis (primary [AL], immunoglobulin light chain): the natural history in 80 patients. *Am J Med* 1988; **85**: 73-80 [PMID: 3389383 DOI: 10.1016/0002-9343(88)90505-0]
- 9 **Mainenti PP**, D'Agostino L, Soscia E, Romano M, Salvatore M. Hepatic and splenic amyloidosis: dual-phase spiral CT findings. *Abdom Imaging* 2003; **28**: 688-690 [PMID: 14628877]
- 10 **Kim SH**, Han JK, Lee KH, Won HJ, Kim KW, Kim JS, Park CH, Choi BI. Abdominal amyloidosis: spectrum of radiological findings. *Clin Radiol* 2003; **58**: 610-620 [PMID: 12887954 DOI: 10.1016/s0009-9260(03)00142-9]
- 11 **Kiyama M**, Hoshii Y, Cui D, Kawano H, Kanda T, Ishihara T. Immunohistochemical and immunochemical study of amyloid in liver affected by systemic AL amyloidosis with antibodies against three different regions of immunoglobulin lambda light chain. *Pathol Int* 2007; **57**: 343-350 [PMID: 17539965 DOI: 10.1111/j.1440-1827.2007.02106.x]
- 12 **Son RC**, Chang JC, Choi JH. Primary hepatic amyloidosis: report of an unusual case presenting as a mass. *Korean J Radiol* 2011; **12**: 382-385 [PMID: 21603298 DOI: 10.3348/kjr.2011.12.3.382]

**P- Reviewer:** Betrosian AP, Celikbilek M, Morales-Gonzalez J, Siahanidou T, Sonzogni A, Tanaka T

**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 March 8; 8(7): 345-384





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 469 members, representing a team of worldwide experts in hepatology. They are from 53 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (1), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (98), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (19), Greece (11), Hungary (5), India (15), Indonesia (2), Iran (4), Israel (1), Italy (52), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (11), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (17), and United States (56).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*

Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*  
Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*

Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *São Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*



**Chile**

Luis A Videla, *Santiago*



## China

Guang-Wen Cao, Shanghai  
 En-Qiang Chen, Chengdu  
 Gong-Ying Chen, Hangzhou  
 Jin-lian Chen, Shanghai  
 Jun Chen, Changsha  
 Alfred Cheng, Hong Kong  
 Chun-Ping Cui, Beijing  
 Shuang-Suo Dang, Xi'an  
 Ming-Xing Ding, Jinhua  
 Zhi-Jun Duang, Dalian  
 He-Bin Fan, Wuhan  
 Xiao-Ming Fan, Shanghai  
 James Yan Yue Fung, Hong Kong  
 Yi Gao, Guangzhou  
 Zuo-Jiong Gong, Wuhan  
 Zhi-Yong Guo, Guangzhou  
 Shao-Liang Han, Wenzhou  
 Tao Han, Tianjin  
 Jin-Yang He, Guangzhou  
 Ming-Liang He, Hong Kong  
 Can-Hua Huang, Chengdu  
 Bo Jin, Beijing  
 Shan Jin, Hohhot  
 Hui-Qing Jiang, Shijiazhuang  
 Wan-Yee Joseph Lau, Hong Kong  
 Guo-Lin Li, Changsha  
 Jin-Jun Li, Shanghai  
 Qiang Li, Jinan  
 Sheng Li, Jinan  
 Zong-Fang Li, Xi'an  
 Xu Li, Guangzhou  
 Xue-Song Liang, Shanghai  
 En-Qi Liu, Xi'an  
 Pei Liu, Shenyang  
 Zhong-Hui Liu, Changchun  
 Guang-Hua Luo, Changzhou  
 Yi Lv, Xi'an  
 Guang-Dong Pan, Liuzhou  
 Wen-Sheng Pan, Hangzhou  
 Jian-Min Qin, Shanghai  
 Wai-Kay Seto, Hong Kong  
 Hong Shen, Changsha  
 Xiao Su, Shanghai  
 Li-Ping Sun, Beijing  
 Wei-Hao Sun, Nanjing  
 Xue-Ying Sun, Harbin  
 Hua Tang, Tianjin  
 Ling Tian, Shanghai  
 Eric Tse, Hong Kong  
 Guo-Ying Wang, Changzhou  
 Yue Wang, Beijing  
 Shu-Qiang Wang, Chengdu  
 Mary MY Wayne, Hong Kong  
 Hong-Shan Wei, Beijing  
 Danny Ka-Ho Wong, Hong Kong  
 Grace Lai-Hung Wong, Hong Kong  
 Bang-Fu Wu, Dongguan  
 Feng Wu, Chongqing  
 Xiong-Zhi Wu, Tianjin  
 Chun-Fang Xu, Suzhou  
 Rui-An Xu, Quanzhou  
 Rui-Yun Xu, Guangzhou  
 Wei-Li Xu, Shijiazhuang  
 Shi-Ying Xuan, Qingdao  
 Ming-Xian Yan, Jinan  
 Lv-Nan Yan, Chengdu  
 Jin Yang, Hangzhou  
 Ji-Hong Yao, Dalian  
 Winnie Yeo, Hong Kong

Zheng Zeng, Beijing  
 Qi Zhang, Hangzhou  
 Shi-Jun Zhang, Guangzhou  
 Xiao-Lan Zhang, Shijiazhuang  
 Xiao-Yong Zhang, Guangzhou  
 Xin-Chen Zhang, Harbin  
 Yong Zhang, Xi'an  
 Hong-Chuan Zhao, Hefei  
 Ming-Hua Zheng, Wenzhou  
 Yu-Bao Zheng, Guangzhou  
 Ren-Qian Zhong, Shanghai  
 Fan Zhu, Wuhan  
 Xiao Zhu, Dongguan



## Czech Republic

Kamil Vyslouzil, Olomouc



## Denmark

Henning Gronbaek, Aarhus  
 Christian Mortensen, Hvidovre



## Egypt

Ihab T Abdel-Raheem, Damanhour  
 NGB G Bader EL Din, Cairo  
 Hatem Elalfy, Mansoura  
 Mahmoud M El-Bendary, Mansoura  
 Mona El SH El-Raziky, Cairo  
 Mohammad El-Sayed, Cairo  
 Yasser M Fouad, Minia  
 Mohamed AA Metwally, Benha  
 Hany Shehab, Cairo  
 Mostafa M Sira, Shebin El-koom  
 Ashraf Taye, Minia  
 MA Ali Wahab, Mansoura



## France

Laurent Alric, Toulouse  
 Sophie Conchon, Nantes  
 Daniel J Felmlee, Strasbourg  
 Herve Lerat, Creteil  
 Dominique Salmon, Paris  
 Jean-Pierre Vartanian, Paris



## Germany

Laura E Buitrago-Molina, Hannover  
 Enrico N De Toni, Munich  
 Oliver Ebert, Muenchen  
 Rolf Gebhardt, Leipzig  
 Janine V Hartl, Regensburg  
 Sebastian Hinz, Kiel  
 Benjamin Juntermanns, Essen  
 Roland Kaufmann, Jena  
 Viola Knop, Frankfurt  
 Veronika Lukacs-Kornek, Homburg  
 Benjamin Maasoumy, Hannover  
 Jochen Mattner, Erlangen  
 Nadja M Meindl-Beinker, Mannheim  
 Ulf P Neumann, Aachen  
 Margarete Odenthal, Cologne  
 Yoshiaki Sunami, Munich

Christoph Roderburg, Aachen  
 Frank Tacke, Aachen  
 Yuchen Xia, Munich



## Greece

Alex P Betrosian, Athens  
 George N Dalekos, Larissa  
 Ioanna K Delladetsima, Athens  
 Nikolaos K Gatselis, Larissa  
 Stavros Gourgiotis, Athens  
 Christos G Savopoulos, Thessaloniki  
 Tania Siahaniidou, Athens  
 Emmanouil Sinakos, Thessaloniki  
 Nikolaos G Symeonidi, Thessaloniki  
 Konstantinos C Thomopoulos, Larissa  
 Konstantinos Tziomalos, Thessaloniki



## Hungary

Gabor Banhegyi, Budapest  
 Peter L Lakatos, Budapest  
 Maria Papp, Debrecen  
 Ferenc Sipos, Budapest  
 Zsolt J Tulassay, Budapest



## India

Deepak N Amarapurkar, Mumbai  
 Girish M Bhopale, Pune  
 Sibnarayan Datta, Tezpur  
 Nutan D Desai, Mumbai  
 Sorabh Kapoor, Mumbai  
 Jaswinder S Maras, New Delhi  
 Nabeen C Nayak, New Delhi  
 C Ganesh Pai, Manipal  
 Amit Pal, Chandigarh  
 K Rajeshwari, New Delhi  
 Anup Ramachandran, Vellore  
 D Nageshwar Reddy, Hyderabad  
 Shivaram P Singh, Cuttack  
 Ajith TA, Thrissur  
 Balasubramaniyan Vairappan, Pondicherry



## Indonesia

Cosmas RA Lesmana, Jakarta  
 Neneng Ratnasari, Yogyakarta



## Iran

Seyed M Jazayeri, Tehran  
 Sedigheh Kafi-Abad, Tehran  
 Iradj Maleki, Sari  
 Fakhraddin Naghibalhossaini, Shiraz



## Israel

Stephen DH Malnick, Rehovot



## Italy

Francesco Angelico, Rome



Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Andrea Galli, *Florence*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Salvatore Gruttadauria, *Palermo*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*



**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*  
 Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*

Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhito Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*



**Jordan**

Kamal E Bani-Hani, *Zarqa*



**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*



**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*



**Moldova**

Angela Peltec, *Chishinev*



**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*



**Nigeria**

CA Asabamaka Onyekwere, *Lagos*



**Pakistan**

Bikha Ram Devrajani, *Jamshoro*



**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*



**Poland**

Jacek Zielinski, *Gdansk*



**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*



**Qatar**

Reem Al Olaby, *Doha*



**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*



**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*



**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*



**Singapore**

Ser Yee Lee, *Singapore*



**South Korea**

Young-Hwa Chung, *Seoul*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonju*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*



**Spain**

Ivan G Marina, *Madrid*  
 Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*

José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodríguez-Frias, *Córdoba*  
 Manuel L Rodríguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Traperó-Marugán, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpaboon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*  
 Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*

Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*



#### **United States**

Naim Alkhouri, *Cleveland*  
 Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*

Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Grigoriy E Gurvits, *New York*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Kusum K Kharbanda, *Omaha*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**MINIREVIEWS**

- 345 Human albumin solution for patients with cirrhosis and acute on chronic liver failure: Beyond simple volume expansion  
*Valerio C, Theocharidou E, Davenport A, Agarwal B*
- 355 Indocyanine green kinetics to assess liver function: Ready for a clinical dynamic assessment in major liver surgery?  
*De Gasperi A, Mazza E, Prosperi M*

**ORIGINAL ARTICLE****Retrospective Cohort Study**

- 368 Non-initiation of hepatitis C virus antiviral therapy in patients with human immunodeficiency virus/hepatitis C virus co-infection  
*Oramasionwu CU, Kashuba ADM, Napravnik S, Wohl DA, Mao L, Adimora AA*
- 376 Significant cohort of non-alcoholic fatty liver disease with portal vein thrombosis in transplant waiting list  
*Basaranoglu M, Najjar SM, Demirbag AE, Senturk H*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Zong-Fang Li, MD, PhD, Director, Head, Professor, Department of General Surgery, the Second Affiliated Hospital, College of Medicine, Xi'an Jiaotong University, Xi'an 710004, Shaanxi Province, China

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
March 8, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjgnet.com/bpg/g_info_20160116143427.htm)

**ONLINE SUBMISSION**

<http://www.wjgnet.com/esps/>



## Human albumin solution for patients with cirrhosis and acute on chronic liver failure: Beyond simple volume expansion

Christopher Valerio, Eleni Theocharidou, Andrew Davenport, Banwari Agarwal

Christopher Valerio, Banwari Agarwal, Intensive Care Unit, Royal Free Hospital, Royal Free Hampstead NHS Trust, University College London, London NW3 2QG, United Kingdom

Eleni Theocharidou, the Royal Free Sheila Sherlock Liver Centre, Royal Free Hospital, Royal Free Hampstead NHS Trust and Institute of Liver and Digestive Health, University College London, London NW3 2QG, United Kingdom

Andrew Davenport, UCL Centre for Nephrology, Royal Free Hospital, London NW3 2QG, United Kingdom

**Author contributions:** Davenport A and Agarwal B devised the idea; Valerio C wrote the first draft; Theocharidou E revised the draft; all authors contributed to reviewing articles, editing, revising and preparing the manuscript for publication.

**Conflict-of-interest statement:** The authors have no conflicts of interest to declare.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Banwari Agarwal, MD, Intensive Care Unit, Royal Free Hospital, Royal Free Hampstead NHS Trust, University College London, Pond Street, London NW3 2QG, United Kingdom. [banwari.agarwal@nhs.net](mailto:banwari.agarwal@nhs.net)  
 Telephone: +44-20-77940500

Received: October 29, 2015

Peer-review started: November 3, 2015

First decision: November 30, 2015

Revised: December 22, 2015

Accepted: February 14, 2016

Article in press: February 16, 2016

Published online: March 8, 2016

### Abstract

To provide an overview of the properties of human serum albumin (HSA), and to review the evidence for the use of human albumin solution (HAS) in critical illness, sepsis and cirrhosis. A MEDLINE search was performed using the terms "human albumin", "critical illness", "sepsis" and "cirrhosis". The references of retrieved articles were reviewed manually. Studies published between 1980 and 2014 were selected based on quality criteria. Data extraction was performed by all authors. HSA is the main plasma protein contributing greatly to its oncotic pressure. HSA demonstrates important binding properties for endogenous and exogenous toxins, drugs and drug metabolites that account for its anti-oxidant and anti-inflammatory properties. In disease states, hypoalbuminaemia is secondary to decreased HSA production, increased loss or transcapillary leakage into the interstitial space. HSA function can be also altered in disease with reduced albumin binding capacity and increased production of modified isoforms. HAS has been used as volume expander in critical illness, but received criticism due to cost and concerns regarding safety. More recent studies confirmed the safety of HAS, but failed to show any survival benefit compared to the cheaper crystalloid fluids, therefore limiting its use. On the contrary, in cirrhosis there is robust data to support the efficacy of HAS for the prevention of circulatory dysfunction post-large volume paracentesis and in the context of spontaneous bacterial peritonitis, and for the treatment of hepato-renal syndrome and hypervolaemic hyponatraemia. It is likely that not only the oncotic properties of HAS are beneficial in cirrhosis, but also its functional properties, as HAS replaces the dysfunctional HSA. The role of HAS as the resuscitation fluid of choice in critically ill patients with cirrhosis, beyond the established indications for HAS use, should be addressed in future studies.

**Key words:** Human serum albumin; Human albumin

solution; Critical illness; Cirrhosis; Resuscitation fluid; Large-volume paracentesis; Hepatorenal syndrome; Spontaneous bacterial peritonitis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Human serum albumin has several important functions beyond being the principal protein in plasma. In disease states, albumin levels may not only be low but there may also be functional hypoalbuminaemia. This may explain why human albumin solution is helpful in treating the complications of cirrhosis whereas its role (as a volume expander) in critical illness remains limited. However, in the presence of cirrhosis or acute liver failure the restoration of functional albumin may be beneficial, even in critically ill patients. This still needs to be addressed in clinical trials.

Valerio C, Theodoridou E, Davenport A, Agarwal B. Human albumin solution for patients with cirrhosis and acute on chronic liver failure: Beyond simple volume expansion. *World J Hepatol* 2016; 8(7): 345-354 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i7/345.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i7.345>

## INTRODUCTION

Human serum albumin (HSA) is produced in the liver, and is the main plasma protein fraction responsible for plasma oncotic pressure. Historically, the oncotic property of albumin has been the major determinant of its use in clinical practice. However, it is now clear that albumin is responsible for a number of other important biological functions, and hence should be treated as a drug and not just as a form of fluid used for resuscitation. A close look at the albumin molecule reveals that it consists of three specific domains which act as binding sites for various endogenous and exogenous toxins, and drugs and drug metabolites such that the overall binding capacity of albumin is reflected in its scavenging, antioxidant and anti-inflammatory properties<sup>[1]</sup>. Acute hypoalbuminaemia is common in hospitalised patients resulting from decreased synthesis due to acute organ dysfunction, malnutrition and increased trans-capillary escape due to increased endothelial permeability secondary to systemic inflammation<sup>[2]</sup>. This is particularly noticeable in patients who are chronically hypoalbuminaemic from chronic malnourishment, protein losing nephropathy and enteropathies, and cirrhosis of the liver. In cirrhosis, reduced albumin production (quantitative hypoalbuminaemia) is complicated by an increase in the proportion of irreversibly damaged isoforms (functional hypoalbuminaemia) thus further compromising overall binding capacity<sup>[3]</sup>. While human albumin solution (HAS) are often used for volume expansion and oncotic effect in critically ill patients, their superiority over crystalloid

fluids is not established. In cirrhosis, however, because of the functional dysfunction conferred to the albumin molecule, administration of HAS has been consistently shown to improve circulatory dysfunction, through oncotic but also extra-oncotic mechanisms, and survival. The common indications in this setting include large volume ascitic paracentesis (LVP), type 1 hepatorenal syndrome (HRS), and spontaneous bacterial peritonitis (SBP)<sup>[4]</sup>. The beneficial role of albumin function beyond volume expansion is an evolving field, and further research is required to explore this unique property of albumin in modulating biological functions and disease processes not just in liver disease and sepsis but also in other diseases where albumin dysfunction seems to play a central role in their pathophysiological processes.

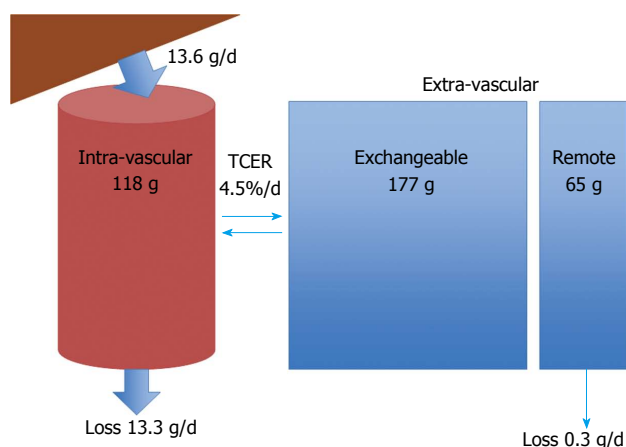
The aim of this review is to provide an overview of HSA structure, kinetics and function, and to explore the pathophysiological basis and clinical evidence for the use of HAS in various diseases, particularly in critical illness, sepsis and liver disease. We conducted a medline search for studies published between 1980 and 2014 using the terms "human albumin", "critical illness", "sepsis" and "cirrhosis". Studies were reviewed and selected for their quality and utility in producing this review.

## SYNTHESIS, METABOLISM, DISTRIBUTION AND FUNCTION OF HSA

HSA contributes around 50% of circulating plasma proteins with serum concentrations of 35-50 g/L in healthy subjects. This level reflects the synthesis, metabolism and distribution of HSA, but not its function. HSA synthesis (10-15 g/d) occurs within the hepatocyte from where it is released into the portal tract<sup>[5]</sup>. Synthesis is regulated by the colloid osmotic pressure of the interstitial fluid bathing the hepatocytes<sup>[6]</sup>. The rate of synthesis *in vivo* can increase up to 2.7 fold, provided there is adequate available messenger RNA<sup>[7]</sup>.

Only a minority of total body HSA remains within the bloodstream, with most albumin passing into the interstitial space (Figure 1). Injection of radio-labelled HSA demonstrates trans-capillary escape rate (TCER) of 4.5% per hour<sup>[8]</sup>. In fenestrated capillaries, TCER depends on capillary wall permeability, hydrostatic and oncotic pressure gradients (liver, small intestine, pancreas, bone marrow). In non-fenestrated capillaries, HSA binds to albondin and passes through to the interstitial space. This rate of transfer is increased with long-chain fatty acid binding, cationisation and glycosylation of HSA. Three quarters of extravascular albumin returns to the intravascular space *via* the lymphatic system.

HSA has a half-life of approximately 15 d. Degradation occurs in the liver and kidney, but the majority takes place in the skin and muscle (the main locations of extravascular HSA). Altered or denatured HSA binds to endothelial cell surface receptors; after uptake into intracellular vesicles, fusion with lysosomes results



**Figure 1** Albumin synthesis and distribution. TCER: Trans-capillary escape rate.

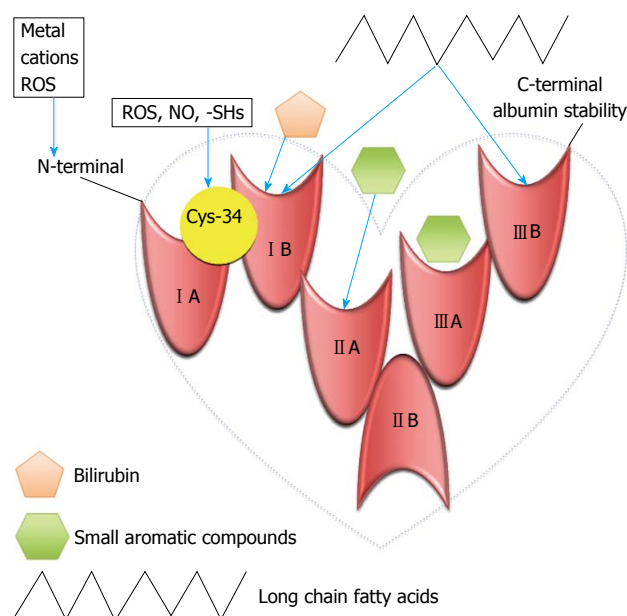
in breakdown into free amino acids. The fractional degradation rate of HSA is 3.7% which parallels the rate of synthesis in health.

The classical physiological role of HSA is to maintain colloid oncotic pressure. The high molecular weight of HSA combined with its concentration in blood result in an 80% contribution to the normal plasma oncotic pressure of around 25 mmHg. This direct osmotic effect provides 60% and the net negative charge 40% of the oncotic pressure. The presence of charged residues and the abundance of HSA account for its function as a physiological buffer. HSA is responsible for approximately half of the normal anion gap, and as such decreasing HSA concentration results in a metabolic alkalosis.

## STRUCTURE AND LIGAND BINDING PROPERTIES OF HSA

HSA consists of 585 amino acids with a molecular weight of 66500 Daltons. The globular structure of HSA determined by X-ray crystallography is "heart-shaped" with 17 disulphide bridges cross-linking cysteine residues and uniting the three domains<sup>[9,10]</sup>. These disulphide bridges give HSA strength, but also facilitate conformational changes in response to ligand binding. There is no carbohydrate moiety, but an abundance of charged lysine, arginine, glutamic acid and aspartic acid residues with a free cysteine and tryptophan residue<sup>[11]</sup>. The homologous domains (I, II and III) that make up HSA are in turn constructed from two sub-domains (A and B) that possess 6 and 4  $\alpha$ -helices respectively (Figure 2)<sup>[11]</sup>. Each domain has a binding site with different properties, but nine binding sites for fatty acids have been elucidated with electron magnetic resonance spectroscopy<sup>[11]</sup>. Flexible loops made of proline residues allow movement of subdomains to accommodate ligands. The HSA molecule serves as the transport vehicle for thyroid and steroid hormones, fatty acids, unconjugated bilirubin, and several drugs<sup>[12]</sup>.

Domain I contains the single cysteine residue that is not a part of the structural disulphide bridges<sup>[13]</sup>.



**Figure 2** Human serum albumin structure and binding sites. ROS: Reactive oxygen species; NO: Nitric oxide; SH: Sulfhydryl.

This creates a reactive thiol group which can form inter-molecular bridges and bind with metals, such as copper and iron. Covalent binding with molecules such as D-penicillamine may occur. There is a metal-binding site involving the N-terminus that can neutralize free copper and iron cations restricting catalysis of free radical production<sup>[14]</sup>. HSA contains two further functional cation binding sites, multi-metal binding site A and B<sup>[15]</sup>. The former lies in the interface of domain I and II binding zinc and cadmium. The latter is thought to be a secondary binding site and its location remains uncertain.

There is a single binding site for unconjugated bilirubin in domain I B within a narrow hydrophobic cavity. Usually, there are two fatty acids loaded on an HSA molecule. The long-chain fatty acid binding sites are found in subdomains I B and III B. These sites can also bind bacterial endotoxins so reducing their activity<sup>[16]</sup>.

The hydrophobic cavities in subdomains II A and III A are the principal ligand binding sites for small heterocyclic or aromatic compounds. Subdomain II A has a lone tryptophan residue that limits solvent accessibility. It is one of the principle binding sites of pharmacological agents (*i.e.*, Sudlow site 1) and shows affinity for bulky heterocyclic molecules, including drugs such as warfarin and furosemide<sup>[17]</sup>. Subdomain III A, corresponding to Sudlow site 2, demonstrates greater stereo-selectivity, but is less flexible and binds aromatic molecules, including diazepam and non-steroidal anti-inflammatory drugs<sup>[17]</sup>. The subdomains II A and III A actually face each other, and II A binding can utilise residues in subdomains II B and III A. An important pharmacological consequence of this configuration is that competitive displacement can then occur. Many compounds will also utilise secondary binding sites. Despite modern techniques there are aspects of the

HSA-drug interactions that remain unclear, such as the binding site of digoxin. The ligand binding activity of HSA may also generate a pseudo-enzymatic activity whereby HSA plays an active role in pro-drug modification by hydrolysis.

Most HSA exists with a free redox-active thiol group (due to the cysteine residue in domain I A), referred to as mercaptoalbumin. Due to the relative abundance of HSA this constitutes 80% of available plasma thiols and is a scavenger of many reactive oxygen and nitrogen species<sup>[18]</sup>. Oxidative stress initially converts HSA into the mixed disulfide non-mercaptoalbumin-1 (HNA-1) as reactive oxygen species are scavenged. The quantity of HNA-1 increases with aging<sup>[19]</sup>. HNA-1 can be further oxidised into HNA-2, which is thought to be an irreversibly damaged form. Nitroalbumin, the product of nitric oxide binding to the thiol group, may be a vasodilator and inhibitor of platelet aggregation.

HSA also has a role in clotting, transporting both anti-thrombin and heparin cofactor II, both of which increase the anticoagulant activity of natural heparinoids and exogenous heparins, by inhibiting thrombin generation. Hypoalbuminaemia has been linked to platelet hyper-aggregation in peritoneal dialysis patients<sup>[20]</sup>, and may play a role in the procoagulant tendency reported in acute on chronic liver failure, and with acute kidney injury<sup>[21,22]</sup>. HSA influences several immune pathways and may enhance intracellular protection from inflammation and oxidative stress. In experimental studies HSA inhibits tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) induced upregulation of vascular cell adhesion molecule 1 and nuclear factor- $\kappa$ B activation<sup>[23]</sup>. Intravascular HSA may promote endothelial stability by reducing oxidative stress, dampening inflammation and reducing neutrophil adhesion to endothelial cells. Vascular integrity may be aided by HSA binding in the sub-endothelium reducing endothelial permeability.

Isoforms of HSA as a result of genetic variation do occur but are not typically associated with disease. Exceptions are the variants with high affinity for tri-iodothyronine and levothyroxine, which are responsible for familial dysalbuminemic hypertri-iodothyroninaemia and hyperthyroxinaemia, respectively<sup>[24]</sup>. Patients with these clinical syndromes are euthyroid. Another isoform has been discovered with increased affinity for nitric oxide which has demonstrated anti-bacterial and anti-apoptotic properties.

## HYPOALBUMINAEMIA IN DISEASE

Disease can alter the synthesis, distribution and degradation of HSA. Decreased HSA synthesis occurs in malnutrition and malabsorption as a result of amino acid deficiency, and hypoalbuminaemia is often used as a surrogate of nutritional status<sup>[25]</sup>. In advanced liver disease, hepatocyte dysfunction or loss results in decreased HSA synthesis. HSA is a component of the Child-Pugh-Turcotte score<sup>[26]</sup>, a disease severity score widely used for patients with cirrhosis, although

the more recent model for end-stage liver disease (MELD) does not include HSA<sup>[27]</sup>. Hypoalbuminaemia is common in inflammatory disorders, as HSA synthesis is suppressed by pro-inflammatory cytokines, including interleukin 6 (IL-6) and TNF- $\alpha$ , in the context of the acute phase response<sup>[28]</sup>.

Increased HSA shift into the interstitial space occurs in cases of increased endothelial permeability. Vasodilatation and increased capillary leakage are the hallmarks of severe sepsis, and contribute greatly to multiple organ dysfunction<sup>[29,30]</sup>. Several vasoactive and pro-inflammatory mediators produce vasodilatation and loss of endothelial integrity in sepsis, such as endotoxins, TNF- $\alpha$ , IL-1, IL-6, prostacyclin and nitric oxide, leading to a three-fold increase in HSA TCER<sup>[2]</sup>. This leakage of HSA into the interstitial space is not associated with a concomitant increase in lymphatic return into the intravascular compartment; rather there is increased sequestration in the non-exchangeable sites in the body. Plasma HSA falls faster after a bolus of 20% HAS in patients with sepsis compared with healthy volunteers<sup>[31]</sup>. Furthermore, a reduction in HSA mRNA transcription occurs in the context of the acute phase response, mediated by IL-6 and TNF- $\alpha$ .

## HSA DYSFUNCTION IN CIRRHOSIS

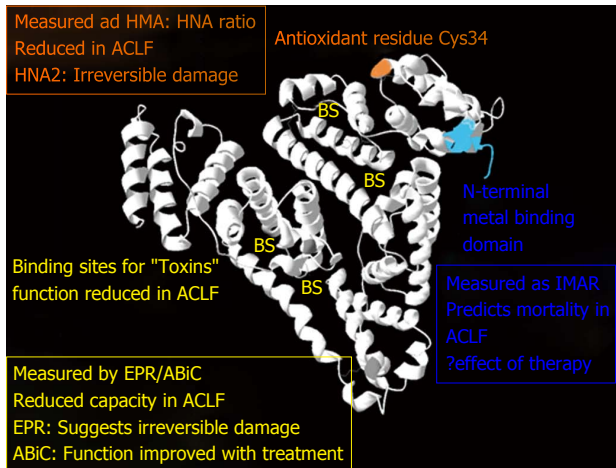
HSA concentration is used as a surrogate of liver function, and hypoalbuminaemia is a common feature in patients with cirrhosis. Recent research has shown that the function of HSA is impaired in patients with cirrhosis (Figure 3)<sup>[32]</sup>. HSA dysfunction may be due to either saturation with bilirubin or allosteric and structural modifications.

A recent study assessed post-transcriptional changes in HSA in patients with cirrhosis and healthy controls<sup>[33]</sup>. Seven isoforms of HSA resulting from post-transcriptional structural modification were identified in patients with cirrhosis, whereas the native unmodified HSA was reduced in the same group compared to controls. The presence of isoforms was associated with the severity of liver disease. The presence of oxidized and N-terminal truncated isoforms was associated with complications such as ascites, renal dysfunction and bacterial infections. The native HSA isoform was associated with greater one-year survival, and was a better predictor of survival than total HSA concentration, supporting the concept of the "effective HSA concentration".

Albumin binding capacity (ABIC) refers to assessment of binding site II by binding of a fluorescent marker (usually dansylsarcosine). ABIC was reduced (< 40%) in 22 patients with cirrhosis and high bilirubin<sup>[34]</sup>, and correlated inversely with the severity of liver disease and short-term mortality. This study showed improved ABIC in patients treated with the Molecular Adsorbents Recirculating System (MARS).

Cobalt binding assays can demonstrate defective metal cation scavenging N-terminal corresponding to ischaemia-modified albumin (IMA). Fatty acid binding





**Figure 3** Impaired albumin function in cirrhosis. ACLF: Acute-on-chronic liver failure; HMA: Mercaptoalbumin; HNA: Non-mercaptalbumin; IMAR: Ischaemia-modified albumin ratio; EPR: Electron paramagnetic resonance; ABiC: Albumin binding capacity.

capacity can be assessed using electron paramagnetic resonance spectroscopy. A study in 34 patients with acute-on-chronic liver failure (ACLF) assessed binding sites associated with main HSA functions using both these methods<sup>[3]</sup>. This study demonstrated impaired HSA ability to transport HSA-bound substances in ACLF. The ratio of IMA to normal HSA (IMAR) was significantly higher in non-survivors compared to survivors. The role of this ratio in novel prognostic scores is currently under investigation. MARS™ treatments did not improve HSA function or IMAR in this study.

Another study assessed the functional status of the HSA thiol moiety by measuring non-oxidized mercaptalbumin, reversibly oxidized HNA-1 and irreversibly oxidized HNA-2 with chromatography according to the redox state of cysteine-34<sup>[35]</sup>. ABiC assessed with dansylsarcosine as ligand was reduced in patients with cirrhosis and was associated with parameters of liver dysfunction. The proportion of oxidised forms was also increased in patients with cirrhosis. The irreversibly damaged HNA-2 form was a strong predictor of 30- and 90-d mortality with predictive accuracy comparable to MELD.

These studies demonstrated impaired HSA function in patients with cirrhosis, which increased with severity of underlying liver disease. Oxidative changes may account for the reduced binding capacity resulting in impaired detoxifying and antioxidant function. Extracorporeal liver support systems, MARS™ and Prometheus™, were developed to remove HSA-bound toxins, such as bilirubin and bile acids, but they are unable to restore HSA function, due to irreversible damage<sup>[36]</sup>. Although initial studies reported some improvement in ABiC with MARS™ treatments, subsequent studies did not show any benefit. Plasma exchange, on the other hand, removes and replaces damaged HSA, and has shown more encouraging clinical outcomes.

Impaired ABiC has been also demonstrated in

**Table 1** Composition of human plasma and different intra-venous fluids

	Human plasma	4% albumin solution	0.9% saline solution	Hartmann's solution
Osmolarity (mOsm/L)	291	250	308	280.6
Sodium (mmol/L)	135-145	148	154	131
Chloride (mmol/L)	94-111	128	154	111
Potassium (mmol/L)	4.5-5.0	0	0	5.4
Calcium (mmol/L)	2.2-2.6	0	0	2
Lactate (mmol/L)	1-2	0	0	29
Octanoate (mmol/L)	0	6.4	0	0

patients with chronic kidney disease, and correlates with the degree of renal dysfunction<sup>[37]</sup>. HSA dysfunction may contribute to the accumulation of HSA-bound uraemic toxins leading to uraemic complications. Renal dysfunction is not uncommon in patients with advanced liver disease, and may further aggravate HSA function. The impact of renal failure on HSA function in ACLF needs to be addressed in future studies.

## HAS COMPOSITION

HAS, produced by plasma fractionation since 1941, has been widely used in clinical practice - despite criticism - mainly for its intravascular volume expansion properties. There are differences that should be taken into consideration between HAS and endogenous HSA, as well as between different HAS formulations. HAS is hypo-osmolar compared to human plasma but with higher sodium and chloride concentrations (Table 1). There may also be differences in oxidation and metal ions among different HAS products, and storage conditions may lead to biochemical changes. These may not be relevant for volume expansion but could modify albumin function. Quantitative analysis of octanoate in HAS showed levels within 20% of the quoted product label value in 132 of 138 HAS tested<sup>[38]</sup>. Octanoate is used as a stabiliser but variations in levels are associated with embryotoxicity. It can also bind to HSA (binding site 1) inducing allostery and displacing compounds, such as non-steroidal anti-inflammatory drugs, at binding site 2<sup>[39,40]</sup>. The stability and binding capacity of different HAS preparations has been investigated for the use of albumin in liver support dialysis systems<sup>[41]</sup>. HAS is available in different concentrations, and experiments in a murine model of endotoxaemia suggest that only albumin at physiological concentrations of 4%, and not 20% HAS, had a protective effect<sup>[42]</sup>.

Recombinant human HAS has shown pharmacokinetic equivalence in studies, but has only been licensed as a pharmacological excipient due to concerns about immunogenic host cell products<sup>[43]</sup>. Industrial manufacture of recombinant HAS is currently not cost-effective. However, the potential production of genetic isoforms of HAS with desirable characteristics, such as antibacterial properties or bilirubin affinity, may expand the utility of recombinant HAS in the future.

## EVIDENCE FOR HAS USE IN CRITICAL ILLNESS AND CIRRHOSIS

### **Critically ill patients**

The utility of HAS in the management of critically ill patients has been a matter of great debate. A Cochrane meta-analysis of 30 clinical trials published in 1998 showed a 6% absolute increase in risk of death with HAS administration compared with crystalloid solutions in patients with hypovolaemia, burns or hypoalbuminaemia<sup>[44]</sup>. However further clinical trials and meta-analyses failed to confirm these findings.

The Saline vs Albumin Fluid Evaluation (SAFE) study was a large double-blind randomised trial comparing 4% HAS with normal saline (NS) fluid resuscitation in approximately 7000 critically ill patients<sup>[45]</sup>. This study did not show any difference in mortality, number of failing organs, length of intensive care unit (ICU) or hospital stay, or need for renal replacement therapy at day 28. In the subgroup of patients with severe sepsis 28-d mortality was lower in the HAS group (30.7%) compared to the NS group (35.3%), but this difference did not reach statistical significance. In multivariate analysis HAS administration was an independent predictor of survival in the same subgroup of patients. In the subgroup of patients with traumatic brain injury, however, mortality at 24 mo was higher in the HAS group (33.2%) compared with 20.4% in the NS group<sup>[46]</sup>.

Another study investigated the administration of 20% HAS in critically ill patients for the first seven days of ICU stay<sup>[47]</sup>. One hundred patients with hypoalbuminaemia were randomized to either 20% HAS or no HAS, with target HSA of 30 g/L. There was significant improvement in organ function, as assessed using the Sequential Organ Failure Assessment score, in the HAS group with a less positive fluid balance. There was, however, no significant difference in 28-d mortality (24% in the HAS vs 30% in the control group) and length of hospital stay.

A subsequent meta-analysis including 38 studies did not show any mortality benefit with HAS administration in critically ill patients with hypovolaemia, burns or hypoalbuminaemia<sup>[48]</sup>. The results of this meta-analysis were greatly influenced by the SAFE study population. A more recent meta-analysis compared colloid vs crystalloid fluid for resuscitation in critically ill patients<sup>[49]</sup>. Twenty four studies that compared HAS with crystalloid fluid were included in the analysis. There was no difference in mortality between the two groups. According to the results of the above meta-analyses, the administration of HAS in critically ill patients cannot be justified in view of the failure to demonstrate survival benefit and the higher cost of HAS.

### **Patients with cirrhosis**

Contrary to the controversial indications for HAS use in critical illness, there is robust evidence to support its use for the treatment or prevention of certain complications

of cirrhosis. Although initially the oncotic properties of HAS were thought to be of great benefit in cirrhosis, the emerging knowledge on the HSA binding properties and the idea of the "effective albumin concentration" shifted interest towards the non-oncotic properties of HAS.

Circulatory dysfunction is a hallmark of cirrhosis. Splanchnic vasodilatation in the arterial circulation, decreased vascular resistance and "effective intravascular blood volume", increased cardiac output and hyperdynamic circulation are the main features of this circulatory dysfunction, and are probably related to overproduction of vasoactive substances, mainly nitric oxide<sup>[50]</sup>. These changes lead to homeostatic activation of the renin-angiotensin system and the sympathetic nervous system, and increased release of antidiuretic hormone, resulting in sodium and water retention. Renal perfusion is reduced due to local vasoconstriction, and glomerular filtration rate decreases. Although HRS is often thought to be a vasomotor nephropathy, there is in addition an inflammatory component, with increased Toll like receptor expression in the renal tubules<sup>[51]</sup>. The use of HAS in cirrhosis has been largely based on its oncotic properties that increase the "effective intravascular blood volume" and improve the circulatory dysfunction. The European Association for the Study of the Liver guidelines suggest administration of HAS in patients with cirrhosis for the following indications<sup>[4]</sup>.

### **LVP to prevent paracentesis-induced circulatory**

**dysfunction:** Diuretic-refractory or diuretic-intolerant ascites occurs in 10% of patients with cirrhosis, and is associated with poor survival. LVP and transjugular intrahepatic portosystemic shunt (TIPS) are the main treatment options for these patients. TIPS not only is more effective in the treatment of refractory ascites compared to LVP, but has been also shown to improve transplant-free survival, as it addresses the underlying portal hypertension<sup>[52]</sup>. However, TIPS is associated with increased incidence of hepatic encephalopathy, thus it is contra-indicated in these patients, as well as in patients with severely impaired liver function or significant cardiac dysfunction<sup>[53]</sup>. TIPS may not be technically feasible in cases with non-compatible vascular anatomy or vascular occlusions.

It is evident that LVP remains the only available treatment option for a proportion of patients with refractory ascites. LVP, however, exacerbates the circulatory dysfunction already present in these patients by accentuating the arteriolar vasodilatation leading to overactivation of the compensatory endogenous neuro-humoral vasoactive systems<sup>[54]</sup>. This paracentesis induced circulatory dysfunction and effective reduction in blood volume may have detrimental effects in cirrhosis including: Rapid re-accumulation of ascites, development of dilutional hyponatraemia, HRS, increased portal pressures and shortened survival<sup>[55]</sup>. A randomised study comparing LVP with or without HAS administration as plasma expander showed that paracentesis without HAS was associated with higher frequency of renal

impairment, higher plasma renin activity and aldosterone concentration, and higher incidence of hyponatraemia<sup>[55]</sup>. Several strategies to prevent post-LVP circulatory dysfunction have been tested including administration of HAS, colloid fluids and vasoconstrictor agents. A meta-analysis including data from 17 randomised trials demonstrated significantly lower incidence of post-LVP circulatory dysfunction with HAS compared to each of the other treatment modalities<sup>[56]</sup>. The incidence of post-LVP hyponatraemia, and mortality were also lower in the HAS group. Current guidelines suggest HAS replacement at a dose of 8 g for every litre of ascitic fluid removed with LVP.

**Treatment of HRS:** HRS type 1 is characterised by progressive renal failure and is associated with increased mortality. Treatment of HRS includes vasoconstrictors (primarily terlipressin, or noradrenaline, or if these are not available then midodrine and octreotide) in combination with HAS. Terlipressin, a vasopressin analogue, is the vasoconstrictor most commonly used. A randomised, double-blind, placebo-controlled trial showed reversal of type 1 HRS in 34% of patients treated with terlipressin and HAS, vs 12% of those treated only with HAS<sup>[57]</sup>. HRS reversal in this study was associated with improved 6-mo survival. These results were confirmed in a randomised study published almost simultaneously by a different research group<sup>[58]</sup>. In this study renal function improved in 44% of patients treated with terlipressin and HAS, but only in 9% of those treated with HAS. Improvement in renal function was again an independent predictor of 3-mo survival.

The efficacy of terlipressin without HAS in treatment of HRS has been also assessed. HRS reversal was achieved in 77% of patients receiving terlipressin and HAS, and in 25% of those receiving terlipressin alone<sup>[59]</sup>. Improvement in arterial pressure and suppression of the renin-angiotensin system was observed only in the combination group, but not in the terlipressin monotherapy group. The recommended dose of HAS in HRS is 1 g/kg of body weight on day 1, followed by 20–40 g/d.

**SBP to prevent renal dysfunction:** One third of patients with SBP, another common complication in patients with cirrhosis and ascites, develop renal dysfunction secondary to rapidly progressive impairment in systemic haemodynamics<sup>[60]</sup>. SBP is also associated with increased mortality, in particular in the subgroup of patients who develop renal impairment. A randomised study assessed renal function and mortality in 126 patients with SBP treated with antibiotics with or without HAS<sup>[61]</sup>. HAS was administered at a dose of 1.5 g/kg of body weight at the time of diagnosis, followed by 1 g/kg of body weight on day 3. Renal impairment developed in 33% in the group treated only with antibiotics, and in 10% in the HAS group, and 3-mo mortality was 41% and 22%, respectively. Following this landmark study, the combination of antibiotics with HAS was established

for the treatment of SBP, and the recommended dose of HAS is that used in the initial study.

The beneficial effect of HAS has also been assessed in patients with cirrhosis and bacterial infections other than SBP<sup>[62]</sup>. A small study showed improvement in circulatory function in patients treated with antibiotics and HAS compared to those treated only with antibiotics, and a trend towards improved renal function, but no difference in 3-mo survival. Unless future studies provide more robust evidence, currently there is not enough evidence to support HAS administration in non-SBP infections.

### **Treatment of hypervolaemic hyponatraemia:**

Hyponatraemia in cirrhosis can be hypovolaemic or hypervolaemic according to extracellular fluid volume status<sup>[63]</sup>. Hypervolaemic or dilutional hyponatraemia is primarily the result of increased secretion of antidiuretic hormone resulting in greater renal water retention compared to sodium<sup>[64]</sup>. Hyponatraemia is a poor prognostic marker associated with high mortality. Treatment options are limited as fluid restriction is rarely effective, and crystalloid fluids are only indicated in hypovolaemic hyponatraemia. Previous studies have shown improvement in serum sodium concentration with HAS administration, most likely related to its volume expansion effect<sup>[65]</sup>, therefore HAS can be used for the treatment of hyponatraemia despite the scarcity of strong evidence. Preliminary reports have shown that increasing solute-free water excretion can improve hyponatraemia by blocking distal renal tubular vasopressin 2 receptors. The efficacy and safety of this class of drugs in patients with cirrhosis are currently under investigation, as too great a loss of water may lead to hypovolaemia and acute renal injury<sup>[66]</sup>.

Finally, the effect of HAS on hepatic encephalopathy has been investigated, with studies failing to show that HAS administration improved hepatic encephalopathy, although it was associated with improved 3-mo survival<sup>[67]</sup>.

### **Critically-ill patients with cirrhosis**

The prognosis for patients with cirrhosis admitted to the ICU is poor with mortality rates of approximately 30% reported in contemporary patient cohorts and up to 80% in older ones<sup>[68]</sup>. Terlipressin and TIPS have improved outcomes, but mortality still remains high. The role of HAS in this setting has not been investigated. The same indications for HAS administration apply to critically-ill patients with cirrhosis in the ICU setting. Beyond the established indications for HAS, however, the question regarding the optimal resuscitation fluid in these patients has not been addressed. HAS administration has been shown to improve circulatory dysfunction and survival in patients with cirrhosis. The use of HAS is limited in critical illness by the absence of survival benefit as demonstrated by the SAFE study and subsequent meta-analyses, and the higher economic cost. We strongly feel that the efficacy of HAS as the primary resuscitation



fluid in critically-ill patients with cirrhosis should be reassessed in prospective randomised studies.

## CONCLUSION

Beyond its well-known oncotic properties, HSA entails important binding capacity for endogenous and exogenous toxins which accounts for its antioxidant and anti-inflammatory properties. HSA concentrations are reduced in several disease states. There is increasing interest in HSA function in disease. In cirrhosis, hypoalbuminaemia is a common feature, but evolving research also suggests that HSA detoxifying function is impaired. The rationale for HAS administration in disease has been largely based on its volume expansion properties. In critical illness, however, fluid resuscitation with HAS has not been found to be superior to crystalloid fluids. In patients with cirrhosis, on the other hand, there are well-acknowledged indications for HAS, namely LVP, HRS and SBP. In critically ill patients with cirrhosis the optimal resuscitation fluid remains unknown. As such, future research should focus on the potential beneficial role of the functional properties of HAS, beyond simple volume expansion.

## REFERENCES

- Peters T. Serum albumin: recent progress in the understanding of its structure and biosynthesis. *Clin Chem* 1977; **23**: 5-12 [PMID: 318940]
- De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL. Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med* 2002; **166**: 98-104 [PMID: 12091178 DOI: 10.1164/rccm.200109-016OC]
- Jalan R, Schnurr K, Mookerjee RP, Sen S, Cheshire L, Hodges S, Muravsky V, Williams R, Matthes G, Davies NA. Alterations in the functional capacity of albumin in patients with decompensated cirrhosis is associated with increased mortality. *Hepatology* 2009; **50**: 555-564 [PMID: 19642174 DOI: 10.1002/hep.22913]
- European Association for the Study of the Liver. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]
- Miller LL, Bly CG, Watson ML, Bale WF. The dominant role of the liver in plasma protein synthesis; a direct study of the isolated perfused rat liver with the aid of lysine-epsilon-C14. *J Exp Med* 1951; **94**: 431-453 [PMID: 14888824 DOI: 10.1084/jem.94.5.431]
- Yamauchi A, Fukuhara Y, Yamamoto S, Yano F, Takenaka M, Imai E, Noguchi T, Tanaka T, Kamada T, Ueda N. Oncotic pressure regulates gene transcriptions of albumin and apolipoprotein B in cultured rat hepatoma cells. *Am J Physiol* 1992; **263**: C397-C404 [PMID: 1381147]
- Barle H, Januszkievicz A, Hållström L, Essén P, McNurlan MA, Garlick PJ, Wernerman J. Albumin synthesis in humans increases immediately following the administration of endotoxin. *Clin Sci (Lond)* 2002; **103**: 525-531 [PMID: 12401127 DOI: 10.1042/cs1030525]
- Nicholson JP, Wolmarans MR, Park GR. The role of albumin in critical illness. *Br J Anaesth* 2000; **85**: 599-610 [PMID: 11064620 DOI: 10.1093/bja/85.4.599]
- Sugio S, Kashima A, Mochizuki S, Noda M, Kobayashi K. Crystal structure of human serum albumin at 2.5 Å resolution. *Protein Eng* 1999; **12**: 439-446 [PMID: 10388840 DOI: 10.1093/protein/12.6.439]
- He XM, Carter DC. Atomic structure and chemistry of human serum albumin. *Nature* 1992; **358**: 209-215 [PMID: 1630489 DOI: 10.1038/358209a0]
- Hamilton JA. NMR reveals molecular interactions and dynamics of fatty acid binding to albumin. *Biochim Biophys Acta* 2013; **1830**: 5418-5426 [PMID: 23939311 DOI: 10.1016/j.bbagen.2013.08.002]
- Varshney A, Sen P, Ahmad E, Rehan M, Subbarao N, Khan RH. Ligand binding strategies of human serum albumin: how can the cargo be utilized? *Chirality* 2010; **22**: 77-87 [PMID: 19319989 DOI: 10.1002/chir.20709]
- Dockal M, Carter DC, Rüker F. The three recombinant domains of human serum albumin. Structural characterization and ligand binding properties. *J Biol Chem* 1999; **274**: 29303-29310 [PMID: 10506189 DOI: 10.1074/jbc.274.41.29303]
- Loban A, Kime R, Powers H. Iron-binding antioxidant potential of plasma albumin. *Clin Sci (Lond)* 1997; **93**: 445-451 [PMID: 9486090 DOI: 10.1042/cs0930445]
- Bal W, Sokołowska M, Kurowska E, Faller P. Binding of transition metal ions to albumin: sites, affinities and rates. *Biochim Biophys Acta* 2013; **1830**: 5444-5455 [PMID: 23811338 DOI: 10.1016/j.bbagen.2013.06.018]
- Kitano H, Fukui H, Okamoto Y, Kikuchi E, Matsumoto M, Kikukawa M, Morimura M, Tsujita S, Nagamoto I, Nakatani T, Takaya A, Tsujii T. Role of albumin and high-density lipoprotein as endotoxin-binding proteins in rats with acute and chronic alcohol loading. *Alcohol Clin Exp Res* 1996; **20**: 73A-76A [PMID: 8659697]
- Yamasaki K, Chuang VT, Maruyama T, Otagiri M. Albumin-drug interaction and its clinical implication. *Biochim Biophys Acta* 2013; **1830**: 5435-5443 [PMID: 23665585 DOI: 10.1016/j.bbagen.2013.05.005]
- Anraku M, Chuang VT, Maruyama T, Otagiri M. Redox properties of serum albumin. *Biochim Biophys Acta* 2013; **1830**: 5465-5472 [PMID: 23644037 DOI: 10.1016/j.bbagen.2013.04.036]
- Dröge W. Aging-related changes in the thiol/disulfide redox state: implications for the use of thiol antioxidants. *Exp Gerontol* 2002; **37**: 1333-1345 [PMID: 12559403]
- Kim SB, Chi HS, Park JS, Hong CD, Yang WS. Effect of increasing serum albumin on plasma D-dimer, von Willebrand factor, and platelet aggregation in CAPD patients. *Am J Kidney Dis* 1999; **33**: 312-317 [PMID: 10023644]
- Agarwal B, Wright G, Gatt A, Riddell A, Vemala V, Mallett S, Chowdary P, Davenport A, Jalan R, Burroughs A. Evaluation of coagulation abnormalities in acute liver failure. *J Hepatol* 2012; **57**: 780-786 [PMID: 22735303 DOI: 10.1016/j.jhep.2012.06.020]
- Agarwal B, Gatt A, Riddell A, Wright G, Chowdary P, Jalan R, Burroughs AK, Davenport A. Hemostasis in patients with acute kidney injury secondary to acute liver failure. *Kidney Int* 2013; **84**: 158-163 [PMID: 23515053 DOI: 10.1038/ki.2013.92]
- Zhang WJ, Frei B. Albumin selectively inhibits TNF alpha-induced expression of vascular cell adhesion molecule-1 in human aortic endothelial cells. *Cardiovasc Res* 2002; **55**: 820-829 [PMID: 12176131]
- Kragh-Hansen U, Minchiotti L, Galliano M, Peters T. Human serum albumin isoforms: genetic and molecular aspects and functional consequences. *Biochim Biophys Acta* 2013; **1830**: 5405-5417 [PMID: 23558059 DOI: 10.1016/j.bbagen.2013.03.026]
- Kirsch R, Frith L, Black E, Hoffenberg R. Regulation of albumin synthesis and catabolism by alteration of dietary protein. *Nature* 1968; **217**: 578-579 [PMID: 5641119]
- Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646-649 [PMID: 4541913]
- Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: 11172350 DOI: 10.1053/jhep.2001.22172]
- Moshage HJ, Janssen JA, Franssen JH, Hafkenscheid JC, Yap SH. Study of the molecular mechanism of decreased liver synthesis of albumin in inflammation. *J Clin Invest* 1987; **79**: 1635-1641



- [PMID: 3584463 DOI: 10.1172/jci113000]
- 29 **Aird WC.** The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. *Blood* 2003; **101**: 3765-3777 [PMID: 12543869 DOI: 10.1182/blood-2002-06-1887]
  - 30 **Fleck A, Raines G, Hawker F, Trotter J, Wallace PI, Ledingham IM, Calman KC.** Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury. *Lancet* 1985; **1**: 781-784 [PMID: 2858667]
  - 31 **Margarson MP, Soni NC.** Changes in serum albumin concentration and volume expanding effects following a bolus of albumin 20% in septic patients. *Br J Anaesth* 2004; **92**: 821-826 [PMID: 15064244 DOI: 10.1093/bja/ae111]
  - 32 **Leckie P, Davies N, Jalan R.** Albumin regeneration for extra-corporeal liver support using prometheus: a step in the right direction. *Gastroenterology* 2012; **142**: 690-692 [PMID: 22370211 DOI: 10.1053/j.gastro.2012.02.037]
  - 33 **Domenicali M, Baldassarre M, Giannone FA, Naldi M, Mastroberto M, Biselli M, Laggetta M, Patrono D, Bertucci C, Bernardi M, Caraceni P.** Posttranscriptional changes of serum albumin: clinical and prognostic significance in hospitalized patients with cirrhosis. *Hepatology* 2014; **60**: 1851-1860 [PMID: 25048618 DOI: 10.1002/hep.27322]
  - 34 **Klammt S, Mitzner SR, Stange J, Looock J, Heemann U, Emmrich J, Reisinger EC, Schmidt R.** Improvement of impaired albumin binding capacity in acute-on-chronic liver failure by albumin dialysis. *Liver Transpl* 2008; **14**: 1333-1339 [PMID: 18756471 DOI: 10.1002/lt.21504]
  - 35 **Oetfl K, Birner-Gruenberger R, Spindelboeck W, Stueger HP, Dorn L, Stadlbauer V, Putz-Bankuti C, Krisper P, Graziadei I, Vogel W, Lackner C, Stauber RE.** Oxidative albumin damage in chronic liver failure: relation to albumin binding capacity, liver dysfunction and survival. *J Hepatol* 2013; **59**: 978-983 [PMID: 23811308 DOI: 10.1016/j.jhep.2013.06.013]
  - 36 **Jalan R, Bernardi M.** Effective albumin concentration and cirrhosis mortality: from concept to reality. *J Hepatol* 2013; **59**: 918-920 [PMID: 23954671 DOI: 10.1016/j.jhep.2013.08.001]
  - 37 **Klammt S, Wojak HJ, Mitzner A, Koball S, Rychly J, Reisinger EC, Mitzner S.** Albumin-binding capacity (ABIC) is reduced in patients with chronic kidney disease along with an accumulation of protein-bound uraemic toxins. *Nephrol Dial Transplant* 2012; **27**: 2377-2383 [PMID: 22086973 DOI: 10.1093/ndt/gfr616]
  - 38 **Yu MW, Finlayson JS.** Quantitative determination of the stabilizers octanoic acid and N-acetyl-DL-tryptophan in human albumin products. *J Pharm Sci* 1984; **73**: 82-86 [PMID: 6694090]
  - 39 **Leonard PH, Charlesworth MC, Benson L, Walker DL, Fredrickson JR, Morbeck DE.** Variability in protein quality used for embryo culture: embryotoxicity of the stabilizer octanoic acid. *Fertil Steril* 2013; **100**: 544-549 [PMID: 23602317 DOI: 10.1016/j.fertnstert.2013.03.034]
  - 40 **Noctor TA, Wainer IW, Hage DS.** Allosteric and competitive displacement of drugs from human serum albumin by octanoic acid, as revealed by high-performance liquid affinity chromatography, on a human serum albumin-based stationary phase. *J Chromatogr* 1992; **577**: 305-315 [PMID: 1400761]
  - 41 **De Bruyn T, Meijers B, Evenepoel P, Laub R, Willems L, Augustijns P, Annaert P.** Stability of therapeutic albumin solutions used for molecular adsorbent recirculating system-based liver dialysis. *Artif Organs* 2012; **36**: 29-41 [PMID: 21955219 DOI: 10.1111/j.1525-1594.2011.01310.x]
  - 42 **Kremer H, Baron-Menguy C, Tesse A, Gallois Y, Mercat A, Henrion D, Andriantsitohaina R, Asfar P, Meziani F.** Human serum albumin improves endothelial dysfunction and survival during experimental endotoxemia: concentration-dependent properties. *Crit Care Med* 2011; **39**: 1414-1422 [PMID: 21336119 DOI: 10.1097/CCM.0b013e318211ff6e]
  - 43 **Otagiri M, Chuang VT.** Pharmaceutically important pre- and posttranslational modifications on human serum albumin. *Biol Pharm Bull* 2009; **32**: 527-534 [PMID: 19336879]
  - 44 **Cochrane Injuries Group Albumin Reviewers.** Human albumin administration in critically ill patients: systematic review of randomised controlled trials. *BMJ* 1998; **317**: 235-240 [PMID: 9677209]
  - 45 **Finfer S, Bellomo R, Boyce N, French J, Myburgh J, Norton R.** A comparison of albumin and saline for fluid resuscitation in the intensive care unit. *N Engl J Med* 2004; **350**: 2247-2256 [PMID: 15163774 DOI: 10.1056/NEJMoa040232]
  - 46 **Myburgh J, Cooper DJ, Finfer S, Bellomo R, Norton R, Bishop N, Kai Lo S, Vallance S.** Saline or albumin for fluid resuscitation in patients with traumatic brain injury. *N Engl J Med* 2007; **357**: 874-884 [PMID: 17761591 DOI: 10.1056/NEJMoa067514]
  - 47 **Dubois MJ, Orellana-Jimenez C, Melot C, De Backer D, Berre J, Leeman M, Brimiouille S, Appoloni O, Creteur J, Vincent JL.** Albumin administration improves organ function in critically ill hypoalbuminemic patients: A prospective, randomized, controlled, pilot study. *Crit Care Med* 2006; **34**: 2536-2540 [PMID: 16915107 DOI: 10.1097/01.ccm.0000239119.57544.0c]
  - 48 **Roberts I, Blackhall K, Alderson P, Bunn F, Schierhout G.** Human albumin solution for resuscitation and volume expansion in critically ill patients. *Cochrane Database Syst Rev* 2011; **(11)**: CD001208 [PMID: 22071799 DOI: 10.1002/14651858.CD001208.pub4]
  - 49 **Perel P, Roberts I, Ker K.** Colloids versus crystalloids for fluid resuscitation in critically ill patients. *Cochrane Database Syst Rev* 2013; **2**: CD000567 [PMID: 23450531 DOI: 10.1002/14651858.CD000567.pub6]
  - 50 **Arroyo V, Jiménez W.** Complications of cirrhosis. II. Renal and circulatory dysfunction. Lights and shadows in an important clinical problem. *J Hepatol* 2000; **32**: 157-170 [PMID: 10728802]
  - 51 **Adebayo D, Morabito V, Davenport A, Jalan R.** Renal dysfunction in cirrhosis is not just a vasomotor nephropathy. *Kidney Int* 2015; **87**: 509-515 [PMID: 25296092 DOI: 10.1038/ki.2014.338]
  - 52 **Salerno F, Cammà C, Enea M, Rössle M, Wong F.** Transjugular intrahepatic portosystemic shunt for refractory ascites: a meta-analysis of individual patient data. *Gastroenterology* 2007; **133**: 825-834 [PMID: 17678653 DOI: 10.1053/j.gastro.2007.06.020]
  - 53 **Saab S, Nieto JM, Lewis SK, Runyon BA.** TIPS versus paracentesis for cirrhotic patients with refractory ascites. *Cochrane Database Syst Rev* 2006; **(4)**: CD004889 [PMID: 17054221 DOI: 10.1002/14651858.CD004889.pub2]
  - 54 **Ruiz-del-Arbol L, Monescillo A, Jimenez W, Garcia-Plaza A, Arroyo V, Rodés J.** Paracentesis-induced circulatory dysfunction: mechanism and effect on hepatic hemodynamics in cirrhosis. *Gastroenterology* 1997; **113**: 579-586 [PMID: 9247479]
  - 55 **Ginès P, Titó L, Arroyo V, Planas R, Panés J, Viver J, Torres M, Humbert P, Rimola A, Llach J.** Randomized comparative study of therapeutic paracentesis with and without intravenous albumin in cirrhosis. *Gastroenterology* 1988; **94**: 1493-1502 [PMID: 3360270]
  - 56 **Bernardi M, Caraceni P, Navickis RJ, Wilkes MM.** Albumin infusion in patients undergoing large-volume paracentesis: a meta-analysis of randomized trials. *Hepatology* 2012; **55**: 1172-1181 [PMID: 22095893 DOI: 10.1002/hep.24786]
  - 57 **Sanyal AJ, Boyer T, Garcia-Tsao G, Regenstein F, Rossaro L, Appenrodt B, Blei A, Gülberg V, Sigal S, Teuber P.** A randomized, prospective, double-blind, placebo-controlled trial of terlipressin for type 1 hepatorenal syndrome. *Gastroenterology* 2008; **134**: 1360-1368 [PMID: 18471513 DOI: 10.1053/j.gastro.2008.02.014]
  - 58 **Martín-Llahí M, Pépin MN, Guevara M, Díaz F, Torre A, Monescillo A, Soriano G, Terra C, Fábrega E, Arroyo V, Rodés J, Ginès P.** Terlipressin and albumin vs albumin in patients with cirrhosis and hepatorenal syndrome: a randomized study. *Gastroenterology* 2008; **134**: 1352-1359 [PMID: 18471512 DOI: 10.1053/j.gastro.2008.02.024]
  - 59 **Ortega R, Ginès P, Uriz J, Cárdenas A, Calahorra B, De Las Heras D, Guevara M, Bataller R, Jiménez W, Arroyo V, Rodés J.** Terlipressin therapy with and without albumin for patients with hepatorenal syndrome: results of a prospective, nonrandomized study. *Hepatology* 2002; **36**: 941-948 [PMID: 12297842 DOI: 10.1053/jhep.2002.35819]
  - 60 **Ruiz-del-Arbol L, Urman J, Fernández J, González M, Navasa M, Monescillo A, Albillos A, Jiménez W, Arroyo V.** Systemic, renal,

- and hepatic hemodynamic derangement in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 2003; **38**: 1210-1218 [PMID: 14578859 DOI: 10.1053/jhep.2003.50447]
- 61 **Sort P**, Navasa M, Arroyo V, Aldeguer X, Planas R, Ruiz-del-Arbol L, Castells L, Vargas V, Soriano G, Guevara M, Ginès P, Rodés J. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med* 1999; **341**: 403-409 [PMID: 10432325 DOI: 10.1056/nejm199908053410603]
  - 62 **Guevara M**, Terra C, Nazar A, Solà E, Fernández J, Pavesi M, Arroyo V, Ginès P. Albumin for bacterial infections other than spontaneous bacterial peritonitis in cirrhosis. A randomized, controlled study. *J Hepatol* 2012; **57**: 759-765 [PMID: 22732511 DOI: 10.1016/j.jhep.2012.06.013]
  - 63 **Davenport A**, Argawal B, Wright G, Mantzoukis K, Dimitrova R, Davar J, Vasianopoulou P, Burroughs AK. Can non-invasive measurements aid clinical assessment of volume in patients with cirrhosis? *World J Hepatol* 2013; **5**: 433-438 [PMID: 24023982 DOI: 10.4254/wjh.v5.i8.433]
  - 64 **Ginès P**, Guevara M. Hyponatremia in cirrhosis: pathogenesis, clinical significance, and management. *Hepatology* 2008; **48**: 1002-1010 [PMID: 18671303 DOI: 10.1002/hep.22418]
  - 65 **Nguyen MK**, Ornekian V, Kao L, Butch AW, Kurtz I. Defining the role of albumin infusion in cirrhosis-associated hyponatremia. *Am J Physiol Gastrointest Liver Physiol* 2014; **307**: G229-G232 [PMID: 24833711 DOI: 10.1152/ajpgi.00424.2013]
  - 66 **Wong F**, Nadim MK, Kellum JA, Salerno F, Bellomo R, Gerbes A, Angeli P, Moreau R, Davenport A, Jalan R, Ronco C, Genyk Y, Arroyo V. Working Party proposal for a revised classification system of renal dysfunction in patients with cirrhosis. *Gut* 2011; **60**: 702-709 [PMID: 21325171 DOI: 10.1136/gut.2010.236133]
  - 67 **Simón-Talero M**, García-Martínez R, Torrens M, Augustin S, Gómez S, Pereira G, Guevara M, Ginès P, Soriano G, Román E, Sánchez-Delgado J, Ferrer R, Nieto JC, Sunyé P, Fuentes I, Esteban R, Córdoba J. Effects of intravenous albumin in patients with cirrhosis and episodic hepatic encephalopathy: a randomized double-blind study. *J Hepatol* 2013; **59**: 1184-1192 [PMID: 23872605 DOI: 10.1016/j.jhep.2013.07.020]
  - 68 **Theocharidou E**, Pieri G, Mohammad AO, Cheung M, Cholongitas E, Agarwal B, Burroughs AK. The Royal Free Hospital score: a calibrated prognostic model for patients with cirrhosis admitted to intensive care unit. Comparison with current models and CLIF-SOFA score. *Am J Gastroenterol* 2014; **109**: 554-562 [PMID: 24492755 DOI: 10.1038/ajg.2013.466]

**P- Reviewer:** Betrosian AP, Dang SS, Li YY, Liu EQ,  
Luo GH, Wong GLH

**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Liu SQ



## Indocyanine green kinetics to assess liver function: Ready for a clinical dynamic assessment in major liver surgery?

Andrea De Gasperi, Ernestina Mazza, Manlio Prosperi

Andrea De Gasperi, Ernestina Mazza, Manlio Prosperi, 2° Servizio Anestesia e Rianimazione, Ospedale Niguarda Ca' Granda, 20162 Milano, Italy

**Author contributions:** De Gasperi A, Mazza E and Prosperi M performed literature review and wrote the paper.

**Conflict-of-interest statement:** De Gasperi A had fees for serving as a speaker for lectures and travel reimbursements from Astellas, Pfizer, Edwards, SEDA Italia Gilead, MSD, Fresenius Kabi, Grifols; Mazza E and Prosperi M have no conflicts of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Andrea De Gasperi, MD, 2° Servizio Anestesia e Rianimazione, Ospedale Niguarda Ca' Granda, Piazza Ospedale Maggiore 3, 20162 Milano, Italy. [andrea.degasperi@ospedaleniguarda.it](mailto:andrea.degasperi@ospedaleniguarda.it)  
Telephone: +39-2-64444617  
Fax: +39-2-64444891

Received: March 23, 2015  
Peer-review started: March 25, 2015  
First decision: May 18, 2015  
Revised: February 1, 2016  
Accepted: February 23, 2016  
Article in press: February 24, 2016  
Published online: March 8, 2016

### Abstract

Indocyanine green (ICG) kinetics (PDR/R15) used to quantitatively assess hepatic function in the perioperative period of major resective surgery and liver

transplantation have been the object of an extensive, updated and critical review. New, non invasive bedside monitors (pulse dye densitometry technology) make this opportunity widely available in clinical practice. After having reviewed basic concepts of hepatic clearance, we analysed the most common indications ICG kinetic parameters have nowadays in clinical practice, focusing in particular on the diagnostic and prognostic role of PDR and R15 in the perioperative period of major liver surgery and liver transplantation. As recently pointed out, even if of extreme interest, ICG clearance parameters have still some limitations, to be considered when using these tests.

**Key words:** Liver function tests; Indocyanine green; Hepatic clearance; Liver surgery; Liver transplantation; Intraabdominal hypertension; Portal hypertension

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Non invasive monitors for indocyanine green (ICG) clearance (PDR and R15) are now available for a rapid assessment of liver function both in the intensive care unit and in major liver surgery. After having reviewed the basic concepts of hepatic clearance, we have analysed the most common indications of ICG kinetic parameters in clinical practice, focusing on the diagnostic and prognostic role of PDR and R15 in the perioperative period of major resective liver surgery and liver transplantation. Since ICG parameters have still some limitations, we will underline the conditions (mainly hyperbilirubinemia and severe peripheral hypoperfusion) able to alter the reliability of these tests.

De Gasperi A, Mazza E, Prosperi M. Indocyanine green kinetics to assess liver function: Ready for a clinical dynamic assessment in major liver surgery? *World J Hepatol* 2016; 8(7): 355-367 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i7/355.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i7.355>

## INTRODUCTION

In modern critical care medicine, extensive and accurate liver function assessment has a relevant place while caring for high risk medical patients or candidates to major liver surgery: At the moment, static and dynamic tests are available<sup>[1-7]</sup>. Static tests, since long included in scores able to quantify acute and chronic (CHILD PUGH, MELD) hepatic dysfunction, assess separately the different functions of the liver and describe the size of the hepatic injury<sup>[1-4]</sup>. On the contrary, information on the functional aspects of the remnant liver after resective surgery or of the quality of the liver graft recovery after transplantation remain elusive. In other words, available to the clinicians is a “frozen” representation of the hepatocytes integrity and of the (residual) metabolic and synthetic capacities (Figure 1).

## STATIC ASSESSMENT OF LIVER FUNCTION

A pivotal role in the amino acids metabolism is played by aspartate aminotransferase (AST) and alanine aminotransferase (ALT). AST, represented at various levels (mainly muscular and cardiac, but not only) are not liver specific and have shorter half life (12-22 h). On the contrary, ALT are liver - specific, have longer half life (30-40 h), are highly expressed in the hepatocytes and largely present in periportal areas. In case of centrilobular hypoxia, ALT show a moderate increase, while in case of acute hepatic injury (acute hepatitis) a significant increase in ALT serum concentration is almost always demonstrated: It is considered a consequence of necrosis or it should be secondary to the increased permeability after a cell membrane damage<sup>[2,3]</sup>. In case of ischemic injury, the AST and ALT peak may reflect the size of liver damage. As above mentioned, AST/ALT increase (longer for ALT, shorter for AST due to the different half life) does not provide information on the functional impairment of the liver nor, by force, of the (residual) hepatic functional reserve<sup>[2,3]</sup>. A rather non-specific marker of ischemic damage to the liver (but not only!) is lactate dehydrogenase (mainly fraction 5). Cholestatic alterations are usually described using gamma glutamyl transferase and alkaline phosphatase.

Plasma Bilirubin concentration reflects phase II metabolism and is the indirect expression of uptake, conjugation and excretion functions of the liver. Early (and perhaps self limiting) phases of ischemic injuries have a moderate impact on the phase II processes, defined as “relatively robust”<sup>[7]</sup>. Among the causes of hyperbilirubinemia (generally speaking due to an increased production or a reduced clearance) relevant are hemolysis, damage of cellular components and reduced intrahepatic bile excretion. One of the main functions of the hepatocytes is protein synthesis. Among synthesized proteins are large part of acute phase proteins, albumin, transport proteins, all the coagulation factors [apart from factor VIII (FVIII) and von Willebrand

factor], antithrombin, anticoagulant proteins (protein C, protein S and protein Z), Plasminogen, alpha 2 plasmin inhibitor, complement, lipoproteins<sup>[2]</sup>. Among coagulation factors, FV and FVII, due to a very short half-life (four to six hours), are included in the Clichy criteria to quantify the synthetic damage of the liver in case of acute liver failure. According to Clichy criteria, in case of acute hepatic failure (so called “fulminant hepatitis”), hepatic encephalopathy grade 3-4 and FV activity below 20% in patients < 30 years (< 30% in patients > 30 years) are the indications for urgent liver transplantation (OLT)<sup>[4]</sup>.

## DYNAMIC ASSESSMENT OF LIVER FUNCTION

Since long, dynamic liver function tests<sup>[2,3]</sup> are considered and used to assess “over time” the liver capacity to metabolize or to eliminate drugs or compounds. Dynamic quantitative liver function tests, unlike conventional (static) tests, rely upon a “quasi” exclusive clearance or metabolism of substances performed by the liver. Being repeatable in a short time span, dynamic tests are able to provide a fast and reliable liver functional evaluation, together with a general prognostic assessment (Figure 1). Indocyanine green (ICG) clearance parameters will be described and discussed in this paper, while Caffeine test, Bromsulphalein clearance, Aminoacid clearance, Galactose elimination capacity, Aminopyridine breath test and Monoethylglycinexylidide formation from lignocaine (MEGX test) are beyond the scope of this review (Figure 1).

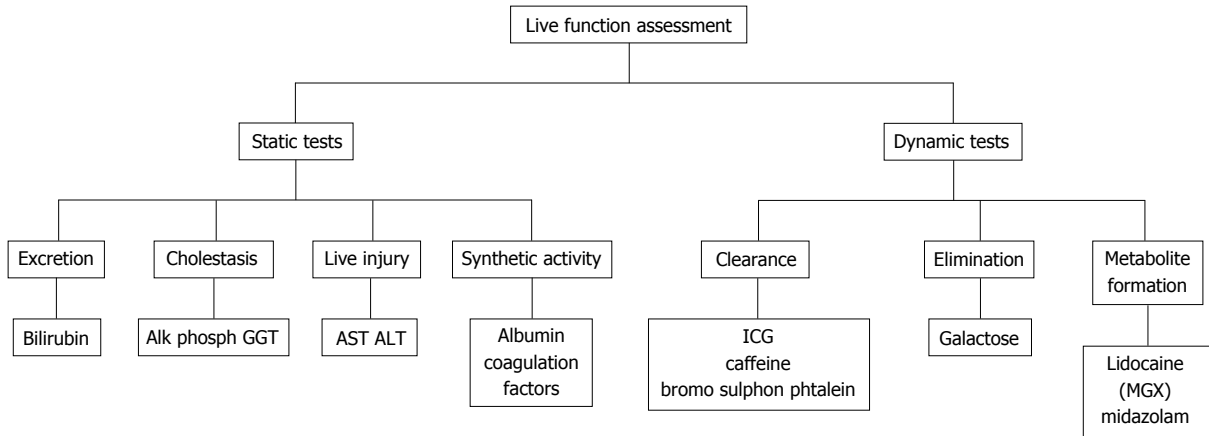
### *The hepatic clearance: Matching hepatic perfusion and liver function*

According to the clearance principle<sup>[5]</sup>, hepatic clearance (Cl) is the product of liver extraction capacity (Ex) and liver blood flow (Q):  $Cl = Q \times Ex$ . In general, the dynamic assessment of liver function relies upon this equation: According to the hepatic extraction capacity, the various drugs and compounds are considered at “low” or “high” extraction. Clearance of highly extracted substances approaches hepatic blood flow and is considered an indicator of liver blood flow, extraction rate being limited in case of reduced liver blood flow. Opposite is the case of the clearance of substances at low extraction rate: The clearance of these compounds, not dependent from the hepatic blood flow, becomes a measure of metabolism or elimination processes. A key point of this principle is that the intrinsic hepatic clearance ( $Cl_{int}$ ) becomes a measure of the capacity of the liver to remove substances when blood flow is not limited<sup>[5]</sup>.

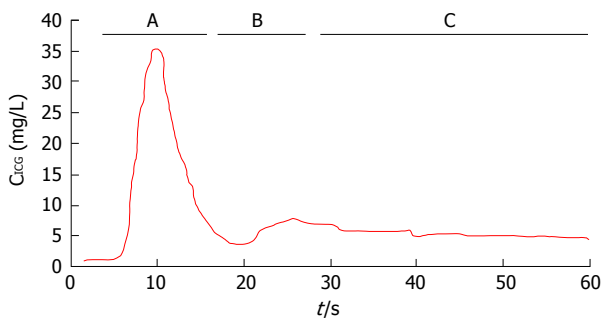
## ICG CLEARANCE FOR A DYNAMIC ASSESSMENT OF LIVER FUNCTION

Worldwide, ICG clearance is the most common and easy - to - use test for the perioperative dynamic assessment





**Figure 1** Liver function assessment: Static and dynamic tests (modified from Sakka<sup>[3]</sup>, 2007). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ICG: Indocyanine green; GGT: Gamma glutamyl transferase.



**Figure 2** Indocyanine green dilution curve. A: First peak; B: Second peak (re-circulation phase); C: Elimination phase (Modified from Vos *et al*<sup>[6]</sup>, 2014). ICG: Indocyanine green;  $C_{icg}$ : ICG blood concentration.

of liver function in case of major liver surgery (resective surgery and liver transplantation) and in the intensive care unit (ICU)<sup>[2,6-8]</sup>. ICG is an inert, water-soluble, fluorescent tricarbocyanine, with a protein binding close to 95% (mainly, alpha1- and beta-lipoproteins and albumin). In healthy individuals, ICG shows a high hepatic extraction rate, usually above 70%. Toxicity is very low, and very rare are the adverse effects, reported in 1/40000 cases. The presence of Iodine in the ICG molecule constitutes a contraindication to its use in case of thyrotoxicosis and iodine allergy (a reaction due to non-immunological histamine release)<sup>[6-8]</sup>. Since the early sixties, ICG elimination kinetics were used to measure blood volume and cardiac output, while in recent years an increased interest exists in using ICG clearance parameters for a dynamic assessment of liver function both in medical and surgical settings<sup>[6,9-11]</sup>. The "standard" determination of ICG clearance ( $ICG_{cl}$ ) relies upon a rather complex *ex vivo* photometric analysis of multiple arterial blood samples obtained in a short time frame (15 min) after the intravenous administration: In spite of being so far the gold standard, it is now used for research purposes only. New bedside, easy to use transcutaneous - non-invasive pulse dye densitometry (PDD) devices able to measure ICG concentrations are on the rise for the use in clinical practice<sup>[1,6,7]</sup>. Among

them are LiMon, (Pulsion Medical System, Germany) and DDG 2001 (Nihon Kohden, Japan): ICG elimination is expressed as ICG plasma disappearance rate ( $ICG_{PDR}$ ) or retention rate at fifteen minutes ( $ICG_{R15}$ ), assessing relative ICG concentration changes (Figure 2).

In hemodynamically stable or unstable ICU patients, in liver transplanted patients and in subjects involved in major liver surgery, good correlation exists between ICG elimination measurements performed with the standard "invasive" method and the PDD technology. In healthy subjects, the intravenous injection of ICG at the dosage of 0.5 mg/kg body weight (BW) generates a plasmatic concentration of 100 mg/mL: In recent experiences, reliable results are also reported with 0.25 mg/kg BW<sup>[3]</sup>. The  $K$  value (rate constant) of the ICG indicator-dilution curve is calculated by both devices applying monoexponential transformation of the ICG concentration and backward dynamic extrapolation of the curve of the elimination phase<sup>[6]</sup>. With appropriate calculations, functional parameters of extreme interest for the dynamic assessment of liver function are thus available.

After intravenous injection, ICG, almost completely bound to proteins, is distributed in the blood within 2 to 3 min: Volume of distribution is very close to plasma volume and half-life is very short (3 to 5 min<sup>[1,3,6]</sup>, longer in case of hepatic dysfunction). Extraction from the blood occurs almost exclusively by the liver, with selective uptake across the sinusoidal plasma membrane by 1 B3 and Na-taurocholate co-transporting polypeptides. ICG is excreted unchanged and almost completely (97%) into the bile in a non-conjugated form, following a two-compartmental model (excretion from the peripheral and not from the central compartment). The absence of metabolism and of enterohepatic recirculation supports the correlation between ICG elimination kinetics and liver function. Sinusoidal uptake (relevant in humans) and canalicular excretion are the two main processes involved in ICG hepatic clearance. The ATP-dependent-export pump multidrug resistance associated protein 2 (MDRP2) and the multi-drug resistance (MDR3)

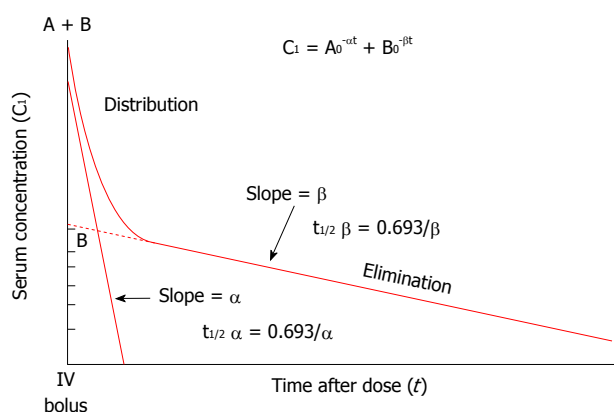


Figure 3 Schematic representation of indocyanine green kinetics (modified from Imamura *et al.*<sup>[5]</sup>, 2005).

P-glycoprotein are the specific carriers involved in this process, expression of the liver energy status and of the excretory function<sup>[1,3,6,7]</sup>.

Two peaks and one slope (the latter representing the elimination phase, usually lasting 10-20 min) are easily recognizable in the dye disappearance curve<sup>[5]</sup>. Of the two peaks, the first is used for the cardiac output determination, while the second is associated with the recirculation phase (elimination peak). Smaller peaks may follow the first two and are used for the estimation of circulating blood volume<sup>[6]</sup>. According to Imamura *et al.*<sup>[5]</sup>, in the ICG plasma disappearance curve (Figure 3) the initial sharp fall in concentration, (distribution phase, due to the rapid hepatic uptake of ICG from the plasma) is followed by a less steep fall (elimination phase, due to the passage from the liver into the bile). Twenty to 30 min are usually needed for the transition from the distribution to the elimination phase: *K* value (/min) is derived from the first fifteen minutes component of the disappearance curve.

In case of liver dysfunction/disease, a consistent prolongation of ICG half-life is usually recorded, as ICG hepatic clearance depends from both carriers capacity and liver blood flow. In individuals suffering for acute liver injury or steatohepatitis, release of cytokines (mainly tumor necrosis factor alpha and interleukine 6) by the reticuloendothelial cells (mainly Kupffer cells) is able to downregulate the expression of organic anion transporting polypeptide isoforms and sodium-taurocholate co-transporting polypeptide, reducing the hepatic uptake capacity. In contrast, ICG transport capacity is competitively inhibited in case of hyperbilirubinemia<sup>[6-8,10-13]</sup>, due to the same carrier system (ATP - export pump - MDRP2) shared by ICG and bilirubin: In case of hyperbilirubinemia (serum bilirubin > 3 mg/mL), "falsely" reduced ICG clearance values may be recorded due to the carrier competition (*vide infra*)<sup>[6,12,13]</sup>. This could be the case of OLT candidates with preoperative hyperbilirubinemia, in which functional recovery of the newly grafted liver is assessed early after transplant: In this specific context, "falsely" poor results may be found, making the ICG test useless

and possibly misleading (*vide infra*)<sup>[6,7]</sup>. Less common, but indeed not infrequent in the critically ill, is the case of high flow states: False reassuring findings (better than expected) due to "normal or near normal" results might be recorded, masking an altered liver excretory function<sup>[7]</sup>. In the cirrhotic population, measurements of liver blood flow using ICG<sub>CI</sub> are not to be considered completely reliable<sup>[14]</sup>: The hepatic extraction rate in this context is extremely reduced (close to 20%-30%) and ICG<sub>CI</sub> becomes a measure of the uptake clearance (*C<sub>int</sub>*, as demonstrated by Imamura *et al.*<sup>[5]</sup>)<sup>[14]</sup>. Interestingly enough, bile elimination constant was not altered, as reported by Kawasaki *et al.*<sup>[15]</sup>. Using the galactose clearance test to measure liver blood flow, the same AAs were able to demonstrate that in liver cirrhosis a reduced ICG<sub>CI</sub> (reported as ICG<sub>R15</sub>) was dependent from a reduction of both hepatic extraction and hepatic blood flow. Sinusoidal capillarization and intrahepatic shunts, largely represented in cirrhotic patients, are proposed as a possible explanation<sup>[6,15,16]</sup>. In normal conditions, the diffusion of drugs and substances (including proteins) is free between the sinusoids and the hepatocytes: In presence of sinusoid capillarization due to a barrier-limiting factor, it is impaired. ICG, which is highly protein-bound, is particularly prone to this phenomenon. Then, in cirrhotic patients ICG<sub>K</sub> and ICG<sub>R15</sub> (*vide infra*) might reflect not only the degree of sinusoidal capillarization and intrahepatic shunts but, at least in part, also the reduction of hepatic blood flow<sup>[15]</sup>. The logarithmic transformation of the distribution phase of the dye dilution curve is the key passage for the quantitative assessment of ICG removal by the liver cells.

ICG clearance parameters most commonly reported in the literature are<sup>[6,7]</sup>: (1) Plasma disappearance rate - ICG<sub>PDR</sub>; (2) Retention rate at 15 min - ICG<sub>R15</sub>; (3) Disappearance rate constant (or elimination rate constant) (*K* constant) - ICG<sub>K</sub>; and (4) ICG<sub>CI</sub> - ICG clearance.

ICG<sub>PDR</sub> and ICG<sub>R15</sub> are the two kinetic parameters most frequently used in clinical practice for the dynamic assessment of liver function<sup>[6-8,17]</sup> (Table 1, from Vos *et al.*<sup>[6]</sup>, 2014).

**ICG<sub>PDR</sub> - PDR:** Percentage change over time of the reduction of ICG blood concentration starting from a concentration of 100% (> 18% per minute). PDR is automatically calculated according to the time course of the ICG blood concentration using monoexponential transformation of the original ICG concentration curve and backward extrapolation to time point zero. In the critically ill, PDR is an accepted surrogate for clearance, due to the good correlation with ICG<sub>CI</sub> (*r*<sup>2</sup> = 0.77)<sup>[2]</sup>.

$$\text{PDR (\% per minute)} = \ln 2/t_{1/2} \times 100 \text{ or } C_{\text{ICG}}(t) = C_0 \times e^{-k \times t}$$

**ICG<sub>R15</sub> - R15:** The ratio between ICG concentration 15 min after injection and initial concentration (normal 0%-10%).

**Table 1** Quantitative indocyanine green kinetics variables (modified from Vos *et al.*<sup>[6]</sup>, 2014)

Variable	Denomination	Unit	Formula for calculation	Normal value
ICG <sub>PDR</sub>	ICG plasma disappearance rate	% per minute	Backward extrapolation of k, curve fitted as: $C_{ICG}(t) = C_0 \times e^{-k \times t}$	> 18%-24% per minute
ICG <sub>R15</sub>	ICG retention ratio after 15 min	%	$(C_{ICG(15)} / C_{ICG(0)}) \times 100$	< 10%
ICG <sub>t/2</sub>	ICG half life	min	$(\ln 2 \times V_D) / Cl_{ICG}$	3-5
Cl <sub>ICG</sub>	ICG clearance	mL/min per kilogram	$K \times V_D$	6-12

e: Euler's number (approximately 2.718); k: Fractional ICG concentration change per minute; V<sub>D</sub>: ICG volume of distribution; t: Time; C<sub>ICG</sub>(t): ICG concentration at time point t (min); Cl<sub>ICG</sub>: ICG clearance (mL/min per kilogram); ICG: Indocyanine green.

$$R15 (\%) = C_{ICG15} / C_{ICG0} \times 100$$

An initial ICG plasma concentration of 100 mg/mL is usually achieved after the intravenous administration of 0.5 mg/kg BW (considering an average plasma volume of 50 mL/kg). ICG<sub>R15</sub> is calculated transforming the ICG concentration curve to a "point zero" (100%) and then describing the decay (at minute fifteen) as a percentage change per time (% per minute) in a logarithmic graph. ICG<sub>R15</sub> has been widely used as an alternative to ICG<sub>K</sub>, being pharmacologically equivalent<sup>[5]</sup>. It could be considered a surrogate of liver blood flow.

**ICG plasma clearance (500-700 mL/min per square):** Volume of plasma entirely cleared off of ICG per unit time; plasma clearance is dependent on liver function, hepatic blood flow, bile flow (Table 1).

ICG<sub>PDR</sub> and ICG<sub>R15</sub> might be considered the two faces of the same phenomenon. ICG<sub>PDR</sub> quantifies ICG disappearance from the plasma over time (% per minute); ICG<sub>R15</sub> is the amount of the circulating ICG fifteen minutes after the administration (%). However, at variance of ICG<sub>R15</sub>, ICG<sub>PDR</sub> should be associated with ICG uptake by the hepatocytes mass, bile excretion, blood flow - dependent liver metabolism and the energy status<sup>[17]</sup>. Unfortunately, across the various studies the two parameters are used in a different and possibly confounding manner. ICG<sub>R15</sub> is almost always considered for the dynamic assessment of hepatic functional reserve in case of liver resection for hepatocellular carcinoma on cirrhosis (HCC)<sup>[5,8]</sup>; ICG<sub>PDR</sub> and ICG<sub>R15</sub> to assess liver graft function after liver transplantation<sup>[18]</sup>; ICG<sub>PDR</sub> in the critical care setting<sup>[2,17]</sup>.

ICG<sub>PDR</sub> and ICG<sub>R15</sub> are determined using either the high performance liquid chromatography with ultraviolet and fluorescence detection (cumbersome and time consuming methodology) or, as almost always reported nowadays, the modern, non-invasive PDD method (pulse dye densitometry method and spectrophotometry)<sup>[6-8]</sup>. A first "invasive" tool was available in the early nineties with the COLD System (Pulsion Medical System, Germany): ICG<sub>PDR</sub> was measured using an arterial fiberoptic catheter inserted in the femoral artery and connected to the COLD system. The system provided a complete and advanced volumetric hemodynamic profile and the ICG<sub>PDR</sub><sup>[19]</sup>. A non invasive, optical transcutaneous pulse spectrophotometric sensor (PDD

technology) is instead used by LiMON, (Pulsion Medical System, Germany) and DDG 2001, (Nihon Kohden, Japan) analysers<sup>[20-23]</sup>. The system measures ICG concentration determining the relative changes in light absorption by the arterial ICG at two different wave lengths, 805 nm (frequency of the ICG peak absorption) and 905 nm (frequency with no ICG absorption): No interference comes from oxidized or reduced hemoglobin and from bilirubin (peak absorption at 470 nm)<sup>[6,7]</sup>. PDD has been validated both in stable and unstable hemodynamic settings<sup>[18-21]</sup>. Purcell *et al.*<sup>[22]</sup> validated the PDD algorithm comparing ICG<sub>R15</sub> values obtained from direct measurement of blood samples and from LiMON. Stable hemodynamic conditions are imperative for reliable data on liver function<sup>[6,8]</sup>. Systemic or local conditions able to reduce hepatic blood flow (low cardiac output inducing hepatosplanchnic hypoperfusion or hepatic artery thrombosis and abdominal hypertension, respectively) have significant impact on ICG elimination, which is reduced in the above mentioned settings. On the contrary, splanchnic hyperperfusion, increasing ICG extraction, might produce (falsely) high ICG<sub>PDR</sub> readings. In case of liver dysfunction, true pathological ICG<sub>PDR</sub> or ICG<sub>R15</sub> values are present because of a decreased transport from the systemic circulation to the liver (reduced blood flow) and/or a decreased uptake by the hepatocytes from the sinusoids. In the liver transplant setting, for example, conditions able to negatively impact on liver blood flow and/or extraction capacities are hepatic artery thrombosis (HAT), primary graft non function (PGNF), severe early graft dysfunction, severe rejection<sup>[9,10]</sup>.

Altered ICG<sub>PDR</sub> and ICG<sub>R15</sub> might also be reported in case of elevated serum bilirubin levels: In the active transport process into the hepatocytes, competition between bilirubin and ICG for the same carrier "alters" ICG kinetic results. This specific condition could be quite common in the early postoperative period of liver transplantation in patients with pretransplant hyperbilirubinemia: Pathological results should be attributed to ICG/Bilirubin competition for the same carrier (Na Taurocolate-co-transporting peptide) and not necessarily to a graft dysfunction. Since pathological ICG<sub>R15</sub> or ICG<sub>PDR</sub> values might be recorded with serum bilirubin > 3 mg/dL<sup>[6,7]</sup>, extreme caution has to be used when interpreting ICG clearance results in hyperbilirubinemic patients. According to the available studies, a bilirubin

level > 3 mg/dL should be considered the cut-off value. In a series of 76 liver transplanted patients, a higher bilirubin level (6 mg/dL) was found by our group to be the cut-off value able to interfere with ICG kinetics (published in abstract)<sup>[24]</sup>.

ICG<sub>PDR</sub> and ICG<sub>R15</sub> are now used: (1) preoperatively, to assess the liver functional reserve before hepatic resection, particularly in cirrhotic patients<sup>[6,23]</sup>; (2) in the liver transplant setting, either in sequential assessments during the various phases of liver transplantation (rare) or (most often) to dynamically assess the recovery of the graft early after transplantation; and (3) following hepatic resection for a functional evaluation of the remnant liver both in cirrhotic and non cirrhotic patients and after partial hepatectomy (particularly the right hepatectomy) in case of living related liver donation. As above underlined, caution must be used while interpreting the results in case of hyperbilirubinemia<sup>[6,24]</sup>. Last but not least, ICG clearance parameters might be altered in case of repeated administrations if intervals between the sequential ICG injections are too short (less than 30 min): Residual ICG may change the baseline drift<sup>[6]</sup>.

In contemporary clinical liver medicine, a tentative list of indications of ICG kinetic parameters could be the following<sup>[2,6-8]</sup>: (1) Functional definition of the hepatic reserve in cirrhotic and non cirrhotic patients undergoing resective surgery; (2) Morbidity/mortality prediction in the same setting; (3) Functional assessment in cadaveric donors of liver function, particularly in case of extended criteria donors, and in case of living donation (beyond the scope of the review); (4) Non invasive assessment of portal hypertension (PH) and esophageal varices<sup>[25]</sup>; and (5) Early functional assessment of the newly grafted liver.

## THE ROLE OF ICG CLEARANCE KINETICS IN THE PREOPERATIVE ASSESSMENT OF LIVER RESECTION IN CIRRHOTIC PATIENTS

Nowadays, in the clinical management of HCC in cirrhotic and non cirrhotic patients relevant is the role played by the appropriate indication of surgery. Liver resection is considered for cirrhotic patients with compensated hepatic function, as assessed by scores, static or dynamic liver function tests, imaging<sup>[26]</sup>. In 2003, Imamura *et al.*<sup>[27]</sup> were able to report zero mortality in a series of 1056 hepatectomies: However, mortality rates ranging from 2% to 5% (and higher) are still reported by others<sup>[23,26,27]</sup>. Posthepatectomy liver dysfunction or failure remains an extremely feared complication, still reported in up to 30% of the cases: In spite of major innovations in surgical and anesthesiological techniques and in the postoperative care, mortality remains high<sup>[27-30]</sup>. Postoperative liver dysfunction is more frequent in cirrhotic patients who underwent hepatic resection: According to the literature,

major risk factors are inadequate preoperative assessment of liver functional reserve, too "aggressive" resection, perioperative hemorrhagic complications and transfusion needs, postoperative infective complications<sup>[30-35]</sup>.

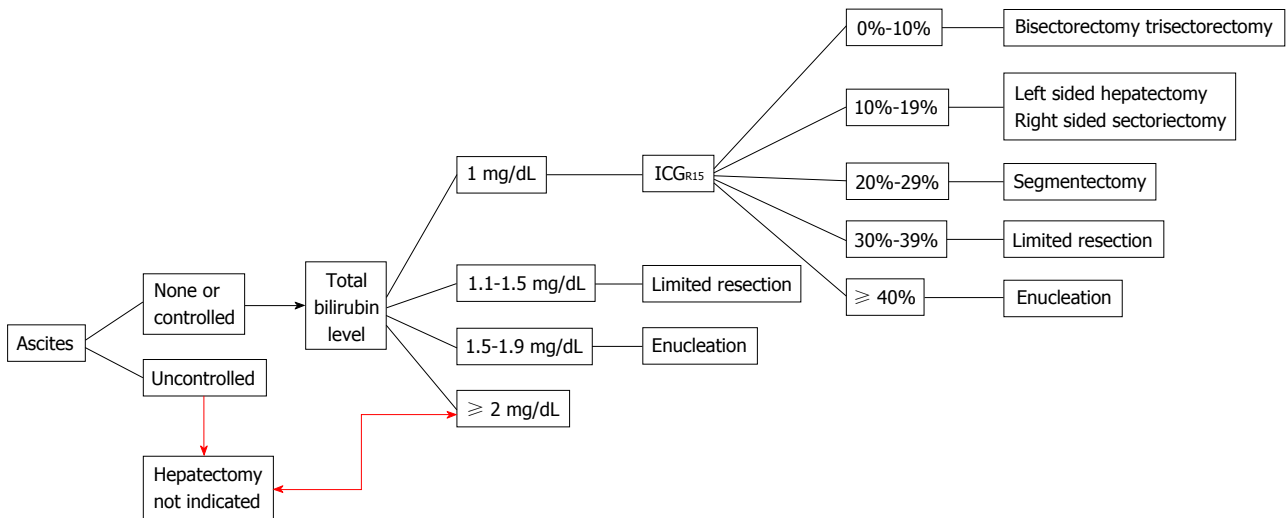
Usually (but not exclusively), indications and extension of resective surgery are tailored according to: (1) presence or absence of ascites and hepatic encephalopathy in the preoperative period; (2) results of conventional static liver function tests (AST/ALT, serum Bilirubin level); (3) imaging (magnetic resonance imaging/magnetic resonance imaging volumetric imaging to predict the remnant hepatic volume); and (4) CTP and MELD scoring systems<sup>[23,33-35]</sup>.

Scores systems widely used in liver medicine for a comprehensive assessment of liver function are CPT and MELD. The CTP score, proposed in 1964 by Child *et al.*<sup>[36]</sup>, and later modified by Pugh (CTP), was created to predict the morbidity/mortality risk of cirrhotic patients with severe PH admitted to shunt surgery<sup>[36,37]</sup>.

Using serum bilirubin, albumin and prothrombin time (PT), (common biochemical parameters, easy to determine in everyday clinical practice) and clinical findings (presence/absence of ascites and encephalopathy), the AAs defined three classes (A, B, C) able to identify the severity of the chronic liver disease. Within the three classes, Pugh *et al.*<sup>[37]</sup> later introduced a score for different values of the biochemical and clinical parameters to identify within the same class (A, B, C) subgroups of patients (A 5-6; B 7- 9; C 10-15) at increasing severity. The CTP score, still quite reliable in predicting mortality after general surgery (roughly, CTP A, 10%; CTP B, up to 30%; CTP C, as high as or above 50%)<sup>[6]</sup>, has some important limitations: Insufficient information on regional assessment of liver function (CTP is by definition a sort of broad classification of the severity of liver disease) and the absence of information on the volume of liver parenchima safely resectable are indeed relevant in the surgical setting<sup>[6,7]</sup>.

In spite of these reported limitations, in the Western surgical school CTP and the degree of PH (often qualitatively defined), together with imaging are often used to assess liver function in the preoperative period. Liver resective surgery should be considered for patients in class A and, limiting the extent of the resection to reduce the risk of postoperative hepatic dysfunction, in well selected Class B patients<sup>[5,27,30]</sup>. Controversial is the use of MELD score or its derivatives (NaMELD and iMELD) in the surgical context. MELD score, based on bilirubin, creatinine and PT as INR, was originally introduced to predict the outcome of patients candidates to transjugular intrahepatic portosystemic shunt procedure. Nowadays, MELD is mainly considered to define the severity of chronic liver disease and its prognosis, to prioritize the liver transplant procedure, to predict survival in liver transplant candidates<sup>[38-40]</sup>. However, reliability of MELD to predict mortality after liver resection is still a matter of debate: major concerns arise from the narrow range (9-14) in which the score





**Figure 4** Makuuchi decisional algorithm to select liver resective procedures in cirrhotic patients according to liver functional reserve (from Imamura *et al.*<sup>[5]</sup>, 2005). ICG<sub>R15</sub>: Indocyanine green retention ratio at 15 min.

**Table 2** Liver damage grading system (Mizuguchi *et al.*<sup>[8]</sup>, 2014, modified)

Parameters	Liver damage grade A	Liver damage grade B	Liver damage grade C
Albumin (g/L)	> 3.5	3.5-3	< 3
Bilirubin (mg/dL)	< 2	2-3	< 3
PT (%)	> 80	50-80	< 50
Ascites	None	Small or controlled	Tense
ICG <sub>R15</sub> (%)	< 15	15-40	> 40

PT: Prothrombin activity; ICG<sub>R15</sub>: Indocyanine green retention ratio at 15 min.

is used. In patients with MELD score > 10, Cucchetti *et al.*<sup>[41]</sup> found a high rate of postoperative liver dysfunction. Hepatic resection is contraindicated in CTP class C patients or in patients whose MELD score is above 14. Instead, in well selected class B patients or in subjects whose MELD score ranges from 9 to 14, the surgical option might be considered: Each single case mandates a thorough preoperative evaluation, including the type of liver resection and its feasibility<sup>[42,43]</sup>.

On the contrary, ICG clearance parameters (mainly ICG<sub>R15</sub>) are since the eighties championed by the Eastern surgical schools<sup>[5,32-34]</sup>: In particular dynamic tests were strongly supported to assess in advance the maximum extent of the resection of the hepatic parenchyma associated with a good functioning remnant liver. In the evidence-based guidelines for the treatment of hepatocellular carcinoma released in Japan in 2009, the use of ICG<sub>R15</sub> was recommended (level of evidence B) for the preoperative assessment of liver function<sup>[43]</sup>. Very recently, ICG<sub>R15</sub> was incorporated in a modified functional evaluation score [Liver Damage Grading System (LDGS)] derived from the CTP classification (Table 2). The Japanese Liver Cancer Study Group of Japan proposed the LDGS, instead of the CTP score, as a more accurate and appropriate tool for the functional

assessment of the hepatic reserve<sup>[8,23]</sup>.

In cirrhotic patients, liver resections should be performed with ICG<sub>R15</sub> < 15%: According to authoritative reports, appropriate candidates for right hepatectomies were patients with ICG<sub>R15</sub> > 10%, whereas left hepatectomies were considered also in surgical candidates with slightly longer ICG<sub>R15</sub> (range 10% to 19%)<sup>[43-45]</sup>. In other series, major liver resections were successfully performed with longer ICG<sub>R15</sub> (range 15% to 20%), if the volume of the residual liver was deemed "sufficient"<sup>[44]</sup>. The role of ICG<sub>R15</sub> in major liver resection became relevant and evident after the publication of the Makuuchi group's experience: Analyzing the results obtained between 1994 and 2002, the AAs were able to report zero mortality in 1056 hepatectomies<sup>[5]</sup>. Three variables were particularly highlighted in the preoperative assessment: (1) ascites (presence or absence); (2) bilirubinemia; and (3) ICG<sub>R15</sub><sup>[5,27]</sup>.

According to the original decisional tree proposed by Imamura *et al.*<sup>[5]</sup>, key points are: (1) contraindication to hepatic resection in presence of uncontrolled ascites or serum bilirubin > 1.9 mg/dL; (2) minor resections possible with serum bilirubin ranging between 1 and 1.9 mg/dL, the lower the bilirubin level, the larger the resection; and (3) according to ICG<sub>R15</sub> intervals different types of hepatic resection possible in case of serum bilirubin < 1.1 mg/dL and no ascites (Figure 4).

Nowadays, preoperative selective portal vein embolization is a challenging option in very well selected subjects candidates to liver resection: An example could be a patient with ICG<sub>R15</sub> 15%-20% whose remnant liver volume after the planned resection is considered "not sufficient". The aim of portal vein embolization is to induce hyperplasia of the hepatic lobules perfused by the contralateral portal vein to increase the volume of the "future remnant" liver<sup>[6,7,46]</sup>. ICG<sub>R15</sub> after embolization correlates with both the volumetric changes and the modification of the liver functional reserve:

It should allow a sort of functional prediction of the remnant liver before resective surgery<sup>[46]</sup>. In the original algorithm proposed by Poon and Fan, hepatic hyperplasia and preservation of "total" liver blood flow were the mainstays of this surgical strategy<sup>[33]</sup>. Definitive implementation of the procedure is still ongoing, even if available results seem promising.

In recent studies, postoperative morbidity [mainly represented by post-hepatectomy liver failure (PHLF)] is reliably predicted by R15 or PDR<sup>[44-47]</sup>. Still under debate is instead the ability of ICG kinetics to correctly predict mortality: The small number of negative events (death) might represent a possible cause<sup>[44,45]</sup>. Using intraoperative ICG<sub>PDR</sub> in a small series of patients, a value of < 9% per minute min predicted postresective liver failure with high sensitivity (88%) and specificity (82%)<sup>[44]</sup>. In another experience, liver failure occurring on postoperative day (POD) 2-5 was predicted by ICG<sub>PDR</sub> < 7% per minute on POD 1<sup>[45]</sup>. Prospectively studying postoperative complications in 100 cirrhotic patients admitted to different liver resections, our group was able to document a significant increase in postresective morbidity associated with ICG<sub>R15</sub> > 40%: Interestingly enough, mortality was not influenced by ICG<sub>R15</sub> (published in abstract)<sup>[47]</sup>.

The most recent intraoperative application of ICG kinetics (ICG<sub>PDR</sub>/ICG<sub>R15</sub>) in major liver surgery was proposed by Thomas *et al*<sup>[48]</sup>: Scope of the study was the definition of reliability of an intraoperative simulation of post-resection liver function. In 20 patients undergoing liver resection, ICG kinetics (LIMON, Pulsion Medical System, Germany) was assessed before and after selective arterial and portal venous inflow trial clamping (TC) of the resected liver segments: The aim was to prevent/avoid PHLF. Similar data were recorded under TC (a significant ICG<sub>PDR</sub> decrease from 16.5% to 10.5% per minute) and after resection (median ICG<sub>PDR</sub> after resection 10.5% per minute). Thomas *et al*<sup>[48]</sup> proposed ICG kinetics as able to reliably simulate post-resection liver function during TC: In their opinion, it might become a useful tool to prevent/avoid PHLF and to reduce hospital length of stay.

In a recent paper, combining the changes of total Bilirubin and INR on POD 1, 3, 5 and 7, Du *et al*<sup>[49]</sup> proposed a definition of postoperative liver failure (PLF). An hepatic damage score (HDs) was built up and used after liver resection to define the degree of the liver metabolic functional impairment (0 = mild; 1 = reversible hepatic "dysfunction"; 2 = fatal hepatic failure). Interestingly enough, in the most compromised patients (HDs = 2) a linear relationship was found between ICG<sub>R15</sub> and the number of the resected segments, possibly identifying preoperative criteria for the most appropriate and safest selection of hepatic resection to reduce PLF<sup>[49]</sup>.

Preoperative pathological ICG<sub>R15</sub> may be wrongly associated with liver dysfunction in case of biliary obstruction. If this is the case, caution should be exerted in interpreting the test results: While the programmed

surgical strategy should not be withheld, further and multimodal investigations are to be considered to adapt/optimize the surgical program<sup>[6]</sup>. In case of hyperbilirubinemia, the South Korean and Japanese surgical schools suggest, as very recently reported by Ge *et al*<sup>[17]</sup>, Tc - galactosyl serum albumin scintigraphy for a more precise functional assessment of the liver. According to the most updated literature, GSA seems to be the ideal agent to predict the volume of hepatocyte mass and its function, due, at least in part, to track the distribution of asialoglycoprotein receptors<sup>[17]</sup>.

## ICG<sub>R15</sub> IN PH: A ROLE AS A NON INVASIVE MARKER?

As above discussed, total liver blood flow and hepatic functional reserve are reflected by ICG<sub>R15</sub>, often used as a prognostic marker in decompensated cirrhotic patients and in candidates to resective liver surgery<sup>[50]</sup>. In cirrhotic patients admitted to resective surgery, preexisting PH and postoperative parenchymal dysfunction are among the most common causes of PHLF. Recently, Lisotti *et al*<sup>[25]</sup> in a cohort of CHILD. A cirrhotic patients with well-preserved liver function evaluated the accuracy of ICG<sub>R15</sub> in reflecting the alteration of hepatic blood flow and, indirectly, the presence and grade of PH and esophageal varices (EV). As comparators, the AAs used hepatic vein pressure gradient and upper gastrointestinal endoscopy, actually the gold standards in this setting. Interestingly enough, Lisotti *et al*<sup>[25]</sup> documented a good performance of ICG<sub>R15</sub> for the diagnosis of both PH and EV. In patients with compensated cirrhosis, ICG<sub>R15</sub> < 6.7% and < 6.9% ruled out clinically significant PH and severe PH respectively, while ICG<sub>R15</sub> < 10% was able to exclude the presence of EV. The AAs concluded for a role of ICG<sub>R15</sub> in identifying patients with advanced liver disease for whom the endoscopic study is warranted.

## ICG KINETICS IN LIVER TRANSPLANT SURGERY

An increased demand of grafts due to the expanded liver transplant (OLT) indications has to face organ shortage, perhaps the most relevant restraint when dealing with solid organ transplant surgery. To expand the donors pool, extended criteria donors and/or suboptimal ("marginal") grafts are ever and ever harvested to match the increasing transplant demand. Early after OLT, the results of conventional "static" liver function tests may raise doubts or uncertainties when used to assess the functional recovery of the liver grafts<sup>[6]</sup>. Recently, few, small single center studies reported on ICG<sub>PDR</sub> to assist and (more objectively support) the decision to harvest livers from suboptimal donors. ICG clearance kinetics, mainly expressed as ICG<sub>PDR</sub> or K constant of elimination, have been used in cadaveric donors before organ harvesting for a quantitative assessment of liver function<sup>[6]</sup>. Unfortunately, the value of ICG<sub>PDR</sub> to assist

graft suitability assessment before harvesting deserves further studies, as values  $< 15\%$  per minute during donor observation were consistently associated with a poor outcome of the graft<sup>[6]</sup>.

ICG kinetics have since long a place in the liver transplant setting. ICG kinetics were recently incorporated in the MELD score for a fine tuning of survival prediction in transplant candidates: As a matter of fact, in candidates whose MELD score ranged from 10 to 30, the ICG-MELD score further improved the prediction performance<sup>[50]</sup>. ICG kinetics into the MELD score add an estimation of liver blood flow, making the new score more accurate than the "simple" MELD and Na MELD in predicting survival in moderate to severe cirrhosis. The role played by hyperbilirubinemia, if present, has of course to be considered. Much more extensively studied is the use of ICG kinetics to predict early perioperative complications and graft and patient survival after OLT. Among the most feared complications in the early postoperative period are HAT and PGNF, conditions which warrant early diagnosis and a timely and appropriate treatment: Urgent retransplantation is mandatory in case of PGNF and very often is the only solution to avoid fatalities in cases of HAT. In the mid nineties, a number of relevant studies<sup>[51-53]</sup> strongly supported the use of ICG clearance parameters for an early assessment of graft function and to predict patient and graft survival. Jalan *et al.*<sup>[51]</sup>, using ICG clearance, correctly predicted both the immediate functional recovery of the new liver and the good graft function three months after OLT when ICG<sub>Cl</sub> on POD 1 was  $> 200$  mL/min. More recently, "low" ICG<sub>PDR</sub> values (5% to 12% per minute) early after OLT were associated with graft malfunction/failure. In the liver transplant setting, the definition of a reproducible and reliable "low" cut-off value is, even if eagerly awaited, still ill - defined: ideally, this value should not be affected by conditions able to create falsely pathological results. Unfortunately, no consensus exists in the literature on this critical point, so far. Faybik *et al.*<sup>[54]</sup>, studying ICG<sub>PDR</sub> using COLD System (Pulsion, Germany) and LiMon (Pulsion, Germany) in a series of patients who underwent OLT found ICG<sub>PDR</sub>  $< 10\%$  per minute as a predictor of postoperative complications. Hori *et al.*<sup>[55]</sup>, using ICG<sub>K</sub> (Nihon Kohden DDG 2001, Japan) in a cohort of thirty patients admitted to living donor liver transplant, assessed graft function daily for the first 14 postoperative days, and then on POD 21 and 28. The early outcome was defined "unfavourable" in case of increased morbidity or mortality. According to this definition, the AAs retrospectively allocated the transplanted patients to two groups, A (favourable outcome, 24 subjects) and B (unfavourable outcome, 6 subjects). ICG<sub>K</sub>  $< 0.180$  on POD 1 correctly predicted the poor outcome of the six patients of group B.

Levesque *et al.*<sup>[56,57]</sup> using LiMON (Pulsion Medical System, Germany) from POD 1 to POD 5 defined an ICG<sub>PDR</sub> value able to predict early postoperative complications. In a first study<sup>[56]</sup>, in a series of 70 consecutive procedures, the transplanted patients were divided

in two groups according to the early outcome: In the group of patients who did well, had immediate good graft function, favourable postoperative course and positive outcome, ICG<sub>PDR</sub> was  $24.4\% \pm 6.8\%$  per minute. Instead, the patients who had postoperative complications were retrospectively subdivided into two subgroups: The first group was composed by subjects who experienced PGNF, HAT, and hemorrhagic or septic shock (early complications); the second included patients who had rejection (late complications). While ICG<sub>PDR</sub> was low ( $8.8\% \pm 4.5\%$  per minute) during the first 5 d in the first subgroup, in the second the ICG<sub>PDR</sub>, initially normal, decreased significantly within 3 to 5 d (ICG<sub>PDR</sub>  $10.3\% \pm 2.5\%$  per minute). Levesque *et al.*<sup>[56]</sup> proposed ICG<sub>PDR</sub>  $< 12.85\%$  per minute as a marker of very early postoperative complications (mainly severe hepatocellular dysfunction, such as PGNF). In a second paper, the same AAs retrospectively reviewing ICG<sub>PDR</sub> in patients who had HAT in the early post OLT period found a significantly lower ICG<sub>PDR</sub> when HAT was documented (range 0.4 to 9.5, mean  $5.8\% \pm 4.3\%$  vs non HAT, range 15.3% to 32.9%, median  $23.8\% \pm 7.4\%$  per minute): ICG<sub>PDR</sub> increased significantly after the revascularization (mean  $15.6\% \pm 3.5\%$  per minute). The AAs concluded defining ICG<sub>PDR</sub> as an interesting diagnostic tool in the early posttransplant period to manage patients suspected for acute HAT<sup>[57]</sup>. The major concern that could be raised on this specific item is the absence of a clear cutoff value in the presence of HAT (see the wide range of ICG<sub>PDR</sub> in the HAT patients). As a matter of fact, this item is quite controversial in the literature. ICG kinetic parameters were used by Olmedilla *et al.*<sup>[58]</sup> at the end of OLT or on POD 1 to assess early graft function. In patients who suffered early severe hepatic dysfunction and had an increased mortality rate, ICG<sub>PDR</sub> was  $< 10.8\%$  per minute. Instead, a favorable outcome was recorded in transplanted patients who had ICG<sub>PDR</sub>  $> 10.8\%$  per minute: In the same study the AAs were also able to document a very high (99%) negative predictive value. In the most recent study coming from the same group, ICG<sub>PDR</sub> and INR were used to build a risk score to predict short term outcome after OLT. Cut-off values were  $\geq 2.2$  for INR (1 point) and  $< 10\%$  per minute for PDR (2 points). The AAs defined four categories (points 0 to 3) in which the risk of early death or retransplantation was described by the score, the higher the score, the higher the risk of adverse outcome (point 0, 4.4%; point 1, 6.5%; points 2, 12%; points 3, 50%). A similar trend was reported also for ICU length of stay and duration of mechanical ventilation. In a validation cohort of 70 patients the score had a good diagnostic performance with sensitivity 60%; specificity 95.5%; positive predictive value (PPV), 66.7%; negative predictive value (NPV) 94.1%. The AAs concluded for a simple and useful tool to be considered for the selection of diagnostic and therapeutic strategies in the early postoperative period<sup>[59]</sup>. Different result were proposed by Escorsell *et al.*<sup>[60]</sup>. In their experience, ICG<sub>PDR</sub> was not a predictor of liver dysfunction and short

term outcome. Using a cut off of 8.8% per minute the AAs subdivided the transplanted patients in two groups (A < 8.8% per minute; B > 8.8% per minute). Interestingly enough, outcome of patients in group A was similar to outcome of patients in group B: Since transplanted patients in group A showed significantly higher bilirubin levels, a false “low” reading of the ICG<sub>PDR</sub> might have occurred. The most probable explanation should be a non proper categorization of a graft as “malfunctioning” because of hyperbilirubinemia and not because of a real dysfunction. Confirmation of this interpretation comes from the reported outcome. Very similar were the results we proposed (in abstract) studying a cohort of 76 consecutive liver transplants<sup>[24]</sup>: ICG<sub>PDR</sub> < 10% per minute was not associated with a poor outcome of the patient and of the graft in the early postoperative period. Interestingly enough, serum bilirubin > 6 mg/dL was always present when ICG<sub>PDR</sub> was < 8% per minute<sup>[24]</sup>. We speculated that in this specific condition (hyperbilirubinemia), ICG<sub>PDR</sub> should be considered, as above underlined, unreliable<sup>[6,7,12,13]</sup>. This point is unfortunately not completely addressed, in our opinion, by Levesque *et al.*<sup>[61]</sup> in the most recent review on this item. The last two studies are, in our opinion, a further strong argument to support the relevant alteration introduced by hyperbilirubinemia, not infrequently observed early after OLT, on ICG kinetics. In both studies, ICG<sub>PDR</sub> falsely predicted an early hepatic dysfunction, not confirmed by the early and medium term outcome of both patients and grafts. Instead, Escorsell *et al.*<sup>[60]</sup> showed a strong correlation between lactate clearance and the functional recovery of the newly grafted livers, further stressing the high PPV of this test: A further confirmation of very similar results we obtained in an earlier study<sup>[62]</sup>. Last but not least, ICG kinetics might be altered by other factors or conditions quite common in the early post transplant period: Among them, the impact of different values of total proteins and hematocrit<sup>[63]</sup>.

Further confirmations for a cautious interpretation of low ICG<sub>PDR</sub> values while assessing liver function both after liver resection and OLT come from a series of recent studies performed with the Maximal Enzymatic Liver Function (LiMax test), a test which relies upon <sup>13</sup>C methacetin metabolism<sup>[64-67]</sup>. In patients who underwent liver resective surgery, Lock *et al.*<sup>[64]</sup> compared ICG<sub>PDR</sub> and Limax to identify patients at risk for postoperative liver failure: Limax showed a better predictive power, once again emphasizing how relevant could be the potential interference of various parameters on the ICG clearance variables.

In a cohort of liver transplant candidates suffering for chronic liver disease, patients who experienced six months liver-related death (primary end point of the study) had, when compared to survivors, significantly lower median Limax values. On the contrary, ICG<sub>PDR</sub> findings were similar in survivors and non survivors. In the same study LIMAX showed a slightly higher NPV (if compared to ICG<sub>PDR</sub> and MELD) when six months risk of

death was considered<sup>[65]</sup>.

Acute liver failure (ALF) is one of the most challenging conditions in liver medicine. Preliminary results on the use of ICG kinetic parameters were recently reported in small series of patients<sup>[7,61,65]</sup>: However hyperbilirubinemia, always present in patients with hyperacute, acute (“fulminant”) or subacute hepatic failure, should impact on ICG elimination kinetics, making problematic at best their interpretation. Lock *et al.*<sup>[67]</sup> recently tested the use of LiMax in ALF. Remarkably, LiMax values, contrary to MELD, were significantly lower in patients who had unfavourable outcome. If confirmed, the AAs concluded for an interesting relevant role of LiMax in ALF in predicting the individual prognosis, possibly supporting in the decision for urgent liver transplant<sup>[67]</sup>.

## CONCLUSION

In recent years reliable and easy-to-use non-invasive bedside analysers using the PDD technology, (LiMon and Nihon Kohden) have boosted the use of ICG kinetic parameters in hepatic surgery and, in general, while caring for the critically ill. Since long, the Eastern surgical schools have supported an extensive application of this technology, particularly when major surgical options are considered in patients affected by hepatocellular carcinoma on liver cirrhosis. The most relevant results, worth to be considered also by the Western surgical community, deal with liver cancer resectability and the potentials for preventing or avoiding postresective hepatic dysfunction/failure. In liver resective surgery, while firm results are available when dealing with morbidity, concern still exists in predicting mortality. In spite of the initial enthusiasms and some very recent results, the use of post OLT ICG kinetics to predict morbidity and mortality are to be considered, at least in our opinion, still under scrutiny. Notwithstanding the results proposed by the most recent publication<sup>[59]</sup>, mixed results or “false pathological findings” (false positives) are present in the literature: To be specifically addressed in the liver transplant setting is the presence of hyperbilirubinemia. In this context, according to ICG<sub>PDR</sub>, newly grafted liver might be falsely classified as severely dysfunctioning or at consistent risk of unfavourable outcome, when opposite is the real final outcome. In spite of the most recent evidence<sup>[59]</sup>, no consensus exists on the cut-off value of PDR/R15 below which a reliable assessment of early graft dysfunction is confidently available. In liver transplanted patients, the negative predictive value of ICG kinetics is indeed relevant: Good graft and patients outcome are almost always associated with “normal” ICG clearance parameters. Into our opinion, in this setting “low” or pathological values are still in a gray zone and caution in interpreting results is needed. As appropriately pointed out by Levesque *et al.*<sup>[61]</sup> when defining severity of complex and evolving diseases, a multistep dynamic approach (instead of single time point static result)



should become the rule. Ending up their review, Vos *et al.*<sup>[6]</sup> proposed a wise and prudent comment on the routine use of IGC kinetics in clinical practice, pushing for further large, prospective, randomized trials: A challenge worth to be considered, particularly in the field of liver transplantation, if gray has to turn to green.

## REFERENCES

- 1 **Wagener G.** Assessment of hepatic function, operative candidacy, and medical management after liver resection in the patient with underlying liver disease. *Semin Liver Dis* 2013; **33**: 204-212 [PMID: 23943101 DOI: 10.1055/s-0033-1351777]
- 2 **Hoekstra LT,** de Graaf W, Nibourg GA, Heger M, Bennink RJ, Stieger B, van Gulik TM. Physiological and biochemical basis of clinical liver function tests: a review. *Ann Surg* 2013; **257**: 27-36 [PMID: 22836216 DOI: 10.1097/SLA.0b013e31825d5d47]
- 3 **Sakka SG.** Assessing liver function. *Curr Opin Crit Care* 2007; **13**: 207-214 [PMID: 17327744 DOI: 10.1097/MCC.0b013e328012b268]
- 4 **Slack A,** Ladher N, Wendon J. Acute hepatic failure. In Wagener G, editor. *Liver Anesthesiology and Critical care Medicine*. New York, Heidelberg, Dordrecht, London: Springer, 2012: 21-42 [DOI: 10.1007/978-1-4614-5167-9\_2]
- 5 **Imamura H,** Sano K, Sugawara Y, Kokudo N, Makuuchi M. Assessment of hepatic reserve for indication of hepatic resection: decision tree incorporating indocyanine green test. *J Hepatobiliary Pancreat Surg* 2005; **12**: 16-22 [PMID: 15754094 DOI: 10.1007/s00534-004-0965-9]
- 6 **Vos JJ,** Wietasch JK, Absalom AR, Hendriks HG, Scheeren TW. Green light for liver function monitoring using indocyanine green? An overview of current clinical applications. *Anaesthesia* 2014; **69**: 1364-1376 [PMID: 24894115 DOI: 10.1111/anae.12755]
- 7 **Halle BM,** Poulsen TD, Pedersen HP. Indocyanine green plasma disappearance rate as dynamic liver function test in critically ill patients. *Acta Anaesthesiol Scand* 2014; **58**: 1214-1219 [PMID: 25307706 DOI: 10.1111/aas.12406]
- 8 **Mizuguchi T,** Kawamoto M, Meguro M, Hui TT, Hirata K. Preoperative liver function assessments to estimate the prognosis and safety of liver resections. *Surg Today* 2014; **44**: 1-10 [PMID: 23474700]
- 9 **Leevy CM,** Mendenhall CL, Lesko w, Howard MM. Estimation of hepatic blood flow with indocyanine green. *J Clin Invest* 1962; **41**: 1169-1179 [PMID: 14463639]
- 10 **Pessayre D,** Lebrec D, Descatoire V, Peignoux M, Benhamou JP. Mechanism for reduced drug clearance in patients with cirrhosis. *Gastroenterology* 1978; **74**: 566-571 [PMID: 631487]
- 11 **Lau H,** Man K, Fan ST, Yu WC, Lo CM, Wong J. Evaluation of preoperative hepatic function in patients with hepatocellular carcinoma undergoing hepatectomy. *Br J Surg* 1997; **84**: 1255-1259 [PMID: 9313707 DOI: 10.1046/j.1365-2168.1997.02770.x]
- 12 **Shinohara H,** Tanaka A, Kitai T, Yanabu N, Inomoto T, Satoh S, Hatano E, Yamaoka Y, Hirao K. Direct measurement of hepatic indocyanine green clearance with near-infrared spectroscopy: separate evaluation of uptake and removal. *Hepatology* 1996; **23**: 137-144 [PMID: 8550033]
- 13 **Cui Y,** König J, Leier I, Buchholz U, Keppler D. Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6. *J Biol Chem* 2001; **276**: 9626-9630 [PMID: 11134001 DOI: 10.1074/jbc.MOO49688200]
- 14 **Keiding S.** Hepatic clearance and liver blood flow. *J Hepatol* 1987; **4**: 393-398 [PMID: 3298417 DOI: 10.1016/S0168-8278(87)80552-4]
- 15 **Kawasaki S,** Sugiyama Y, Iga T, Hanano M, Sanjo K, Beppu T, Idezuki Y. Pharmacokinetic study on the hepatic uptake of indocyanine green in cirrhotic patients. *Am J Gastroenterol* 1985; **80**: 801-806 [PMID: 4036939]
- 16 **Huet PM,** Goresky CA, Villeneuve JP, Marleau D, Lough JO. Assessment of liver microcirculation in human cirrhosis. *J Clin Invest* 1982; **70**: 1234-1244 [PMID: 7174791]
- 17 **Ge PL,** Du SD, Mao YL. Advances in preoperative assessment of liver function. *Hepatobiliary Pancreat Dis Int* 2014; **13**: 361-370 [PMID: 25100120 DOI: 10.1016/S1499-3872(14)60267-8]
- 18 **Faybik P,** Krenn CG, Baker A, Lahner D, Berlakovich G, Steltzer H, Hetz H. Comparison of invasive and noninvasive measurement of plasma disappearance rate of indocyanine green in patients undergoing liver transplantation: a prospective investigator-blinded study. *Liver Transpl* 2004; **10**: 1060-1064 [PMID: 15390334]
- 19 **Kisch H,** Leucht S, Lichtwarck-Aschoff M, Pfeiffer UJ. Accuracy and reproducibility of the measurement of actively circulating blood volume with an integrated fiberoptic monitoring system. *Crit Care Med* 1995; **23**: 885-893 [PMID: 7736747 DOI: 10.1097/00003246-199505000-00017]
- 20 **Iijima T,** Aoyagi T, Iwao Y, Masuda J, Fuse M, Kobayashi N, Sankawa H. Cardiac output and circulating blood volume analysis by pulse dye-densitometry. *J Clin Monit* 1997; **13**: 81-89 [PMID: 9112203]
- 21 **Sakka SG,** Reinhart K, Meier-Hellmann A. Comparison of invasive and noninvasive measurements of indocyanine green plasma disappearance rate in critically ill patients with mechanical ventilation and stable hemodynamics. *Intensive Care Med* 2000; **26**: 1553-1556 [PMID: 11126271]
- 22 **Purcell R,** Kruger P, Jones M. Indocyanine green elimination: a comparison of the LiMON and serial blood sampling methods. *ANZ J Surg* 2006; **76**: 75-77 [PMID: 16483302 DOI: 10.1111/j.1445-2197.2006.03643.x]
- 23 **Seyama Y,** Kokudo N. Assessment of liver function for safe hepatic resection. *Hepatol Res* 2009; **39**: 107-116 [PMID: 19208031 DOI: 10.1111/j.1872-034X.2008.00441.x]
- 24 **Mazza E,** Prosperi M, DeGasperi A, Reggiori G, Corti A, Grugni C, Roselli E, Marchesi M, Amici O, Nichelatti M, Pavani M. Plasma disappearance rate of indocyanine green after liver transplantation: always a reliable tool to predict graft function and outcome? *Liver Transpl* 2008; **14**: S201: LB476
- 25 **Lisotti A,** Azzaroli F, Buonfiglioli F, Montagnani M, Cecinato P, Turco L, Calvanese C, Simoni P, Guardigli M, Arena R, Cucchetti A, Colecchia A, Festi D, Golfieri R, Mazzella G. Indocyanine green retention test as a noninvasive marker of portal hypertension and esophageal varices in compensated liver cirrhosis. *Hepatology* 2014; **59**: 643-650 [PMID: 24038116]
- 26 **Manizate F,** Hiotis SP, Labow D, Roayaie S, Schwartz M. Liver functional reserve estimation: state of the art and relevance for local treatments: the Western perspective. *J Hepatobiliary Pancreat Sci* 2010; **17**: 385-388 [PMID: 19936599]
- 27 **Imamura H,** Seyama Y, Kokudo N, Maema A, Sugawara Y, Sano K, Takayama T, Makuuchi M. One thousand fifty-six hepatectomies without mortality in 8 years. *Arch Surg* 2003; **138**: 1198-1206; discussion 1206 [PMID: 14609867 DOI: 10.1001/archsurg.138.11.1198]
- 28 **Bellavance EC,** Lumpkins KM, Mentha G, Marques HP, Capussotti L, Pulitano C, Majno P, Mira P, Rubbia-Brandt L, Ferrero A, Aldrighetti L, Cunningham S, Russolillo N, Philosophie B, Barroso E, Pawlik TM. Surgical management of early-stage hepatocellular carcinoma: resection or transplantation? *J Gastrointest Surg* 2008; **12**: 1699-1708 [PMID: 18709418]
- 29 **Jarnagin WR,** Gonen M, Fong Y, DeMatteo RP, Ben-Porat L, Little S, Corvera C, Weber S, Blumgart LH. Improvement in perioperative outcome after hepatic resection: analysis of 1,803 consecutive cases over the past decade. *Ann Surg* 2002; **236**: 397-406; discussion 406-407 [PMID: 12368667 DOI: 10.1097/01.SLA.0000029003.66466.B3]
- 30 **Fan ST.** Liver functional reserve estimation: state of the art and relevance for local treatments: the Eastern perspective. *J Hepatobiliary Pancreat Sci* 2010; **17**: 380-384 [PMID: 19865790]
- 31 **Bruix J,** Castells A, Bosch J, Feu F, Fuster J, Garcia-Pagan JC, Visa J, Bru C, Rodés J. Surgical resection of hepatocellular carcinoma in cirrhotic patients: prognostic value of preoperative portal pressure. *Gastroenterology* 1996; **111**: 1018-1022 [PMID: 8831597 DOI: 10.1016/S0016-5085(96)70070-7]
- 32 **Lee SG,** Hwang S. How I do it: assessment of hepatic functional

- reserve for indication of hepatic resection. *J Hepatobiliary Pancreat Surg* 2005; **12**: 38-43 [PMID: 15754098]
- 33 **Poon RT**, Fan ST. Assessment of hepatic reserve for indication of hepatic resection: how I do it. *J Hepatobiliary Pancreat Surg* 2005; **12**: 31-37 [PMID: 15754097 DOI: 10.1007/s00534-004-0945-0]
  - 34 **Nonami T**, Nakao A, Kurokawa T, Inagaki H, Matsushita Y, Sakamoto J, Takagi H. Blood loss and ICG clearance as best prognostic markers of post-hepatectomy liver failure. *Hepatogastroenterology* 1999; **46**: 1669-1672 [PMID: 10430318]
  - 35 **Capussotti L**, Viganò L, Giuliani F, Ferrero A, Giovannini I, Nuzzo G. Liver dysfunction and sepsis determine operative mortality after liver resection. *Br J Surg* 2009; **96**: 88-94 [PMID: 19109799 DOI: 10.1002/bjs.6429]
  - 36 **Child CG**, Turcotte JG. Surgery and portal hypertension. *Major Probl Clin Surg* 1964; **1**: 1-85 [PMID: 4950264]
  - 37 **Pugh RN**, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646-649 [PMID: 4541913 DOI: 10.1002/bjs.1800600817]
  - 38 **Malinchoc M**, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871 [PMID: 10733541 DOI: 10.1053/he.2000.5852]
  - 39 **Dutkowski P**, Oberkofler CE, Béchir M, Müllhaupt B, Geier A, Raptis DA, Clavien PA. The model for end-stage liver disease allocation system for liver transplantation saves lives, but increases morbidity and cost: a prospective outcome analysis. *Liver Transpl* 2011; **17**: 674-684 [PMID: 21618688]
  - 40 **Cholongitas E**, Marelli L, Shusang V, Senzolo M, Rolles K, Patch D, Burroughs AK. A systematic review of the performance of the model for end-stage liver disease (MELD) in the setting of liver transplantation. *Liver Transpl* 2006; **12**: 1049-1061 [PMID: 16799946]
  - 41 **Cucchetti A**, Ercolani G, Vivarelli M, Cescon M, Ravaioi M, La Barba G, Zanella M, Grazi GL, Pinna AD. Impact of model for end-stage liver disease (MELD) score on prognosis after hepatectomy for hepatocellular carcinoma in cirrhosis. *Liver Transpl* 2006; **12**: 966-971 [PMID: 16598792]
  - 42 **Teh SH**, Christein J, Donohue J, Que F, Kendrick M, Farnell M, Cha S, Kamath P, Kim R, Nagorney DM. Hepatic resection of hepatocellular carcinoma in patients with cirrhosis: Model of End-Stage Liver Disease (MELD) score predicts perioperative mortality. *J Gastrointest Surg* 2005; **9**: 1207-1215; discussion 1215 [PMID: 16332475]
  - 43 **Kokudo N**, Makuuchi M. Evidence-based clinical practice guidelines for hepatocellular carcinoma in Japan: the J-HCC guidelines. *J Gastroenterol* 2009; **44** Suppl 19: 119-121 [PMID: 19148805]
  - 44 **Ohwada S**, Kawate S, Hamada K, Yamada T, Sunose Y, Tsutsumi H, Tago K, Okabe T. Perioperative real-time monitoring of indocyanine green clearance by pulse spectrophotometry predicts remnant liver functional reserve in resection of hepatocellular carcinoma. *Br J Surg* 2006; **93**: 339-346 [PMID: 16498606 DOI: 10.1002/bjs.5258]
  - 45 **Greco E**, Nanji S, Bromberg IL, Shah S, Wei AC, Moulton CA, Greig PD, Gallinger S, Cleary SP. Predictors of peri-operative morbidity and liver dysfunction after hepatic resection in patients with chronic liver disease. *HPB (Oxford)* 2011; **13**: 559-565 [PMID: 21762299 DOI: 10.1111/j.1477-2574.2011.00329.x]
  - 46 **Shindoh J**, D Tzeng CW, Vauthey JN. Portal vein embolization for hepatocellular carcinoma. *Liver Cancer* 2012; **1**: 159-167 [PMID: 24159580 DOI: 10.1159/000343829]
  - 47 **Mazza E**, Kroeller D, Prosperi M, Grugni MC, Amici O, Roselli E, De Carlis L, Nichelatti M, De Gasperi A. Does ICG clearance (ICGR15) predict morbidity and mortality after hepatic resection for hepatocellular carcinoma in cirrhotic patients? *Intensive Care Med* 2012; **38**: S169, Abs 609
  - 48 **Thomas MN**, Weninger E, Angele M, Bösch F, Pratschke S, Andrassy J, Rentsch M, Stangl M, Hartwig W, Werner J, Guba M. Intraoperative simulation of remnant liver function during anatomic liver resection with indocyanine green clearance (LiMON) measurements. *HPB (Oxford)* 2015; **17**: 471-476 [PMID: 25581073 DOI: 10.1111/hpb.12380]
  - 49 **Du ZG**, Wei YG, Chen KF, Li B. An accurate predictor of liver failure and death after hepatectomy: a single institution's experience with 478 consecutive cases. *World J Gastroenterol* 2014; **20**: 274-281 [PMID: 24415882 DOI: 10.3748/wjg.v20.i1.274]
  - 50 **Zipprich A**, Kuss O, Rogowski S, Kleber G, Lotterer E, Seufferlein T, Fleig WE, Dollinger MM. Incorporating indocyanine green clearance into the Model for End Stage Liver Disease (MELD-ICG) improves prognostic accuracy in intermediate to advanced cirrhosis. *Gut* 2010; **59**: 963-968 [PMID: 20581243 DOI: 10.1136/gut.2010.208595]
  - 51 **Jalan R**, Plevris JN, Jalan AR, Finlayson ND, Hayes PC. A pilot study of indocyanine green clearance as an early predictor of graft function. *Transplantation* 1994; **58**: 196-200 [PMID: 8042238]
  - 52 **Plevris JN**, Jalan R, Bzeizi KI, Dollinger MM, Lee A, Garden OJ, Hayes PC. Indocyanine green clearance reflects reperfusion injury following liver transplantation and is an early predictor of graft function. *J Hepatol* 1999; **30**: 142-148 [PMID: 9927161]
  - 53 **Tsubono T**, Todo S, Jabbour N, Mizoe A, Warty V, Demetris AJ, Starzl TE. Indocyanine green elimination test in orthotopic liver recipients. *Hepatology* 1996; **24**: 1165-1171 [PMID: 8903393 DOI: 10.1002/hep.510240531]
  - 54 **Faybik P**, Hetz H. Plasma disappearance rate of indocyanine green in liver dysfunction. *Transplant Proc* 2006; **38**: 801-802 [PMID: 16647475]
  - 55 **Hori T**, Iida T, Yagi S, Taniguchi K, Yamamoto C, Mizuno S, Yamagiwa K, Isaji S, Uemoto S. K(ICG) value, a reliable real-time estimator of graft function, accurately predicts outcomes in adult living-donor liver transplantation. *Liver Transpl* 2006; **12**: 605-613 [PMID: 16555326]
  - 56 **Levesque E**, Saliba F, Benhamida S, Ichaï P, Azoulay D, Adam R, Castaing D, Samuel D. Plasma disappearance rate of indocyanine green: a tool to evaluate early graft outcome after liver transplantation. *Liver Transpl* 2009; **15**: 1358-1364 [PMID: 19790157]
  - 57 **Levesque E**, Hoti E, Azoulay D, Adam R, Samuel D, Castaing D, Saliba F. Non-invasive ICG-clearance: a useful tool for the management of hepatic artery thrombosis following liver transplantation. *Clin Transplant* 2011; **25**: 297-301 [PMID: 20412097]
  - 58 **Olmedilla L**, Pérez-Peña JM, Ripoll C, Garutti I, de Diego R, Salcedo M, Jiménez C, Bañares R. Early noninvasive measurement of the indocyanine green plasma disappearance rate accurately predicts early graft dysfunction and mortality after deceased donor liver transplantation. *Liver Transpl* 2009; **15**: 1247-1253 [PMID: 19790138]
  - 59 **Olmedilla L**, Lisbona CJ, Pérez-Peña JM, López-Baena JA, Garutti I, Salcedo M, Sanz J, Tisner M, Ascencio JM, Fernández-Quero L, Bañares R. Early Measurement of Indocyanine Green Clearance Accurately Predicts Short-Term Outcomes After Liver Transplantation. *Transplantation* 2016; **100**: 613-620 [PMID: 26569066]
  - 60 **Escorsell À**, Mas A, Fernández J, García-Valdecasas JC. Limitations of use of the noninvasive clearance of indocyanine green as a prognostic indicator of graft function in liver transplantation. *Transplant Proc* 2012; **44**: 1539-1541 [PMID: 22841207]
  - 61 **Levesque E**, Martin E, Dudau D, Lim C, Dhonneur G, Azoulay D. Current use and perspective of indocyanine green clearance in liver diseases. *Anaesth Crit Care Pain Med* 2016; **35**: 49-57 [PMID: 26477363 DOI: 10.1016/j.accpm.2015.06.006]
  - 62 **De Gasperi A**, Mazza E, Corti A, Zoppi F, Prosperi M, Fantini G, Scaiola A, Colella G, Amici O, Notaro P, Rocchini A, Ceresa F, Roselli E, Grugni MC. Lactate blood levels in the perioperative period of orthotopic liver transplantation. *Int J Clin Lab Res* 1997; **27**: 123-128 [PMID: 9266283]
  - 63 **Kim GY**, Bae KS, Noh GJ, Min WK. Estimation of indocyanine green elimination rate constant k and retention rate at 15 min using patient age, weight, bilirubin, and albumin. *J Hepatobiliary Pancreat Surg* 2009; **16**: 521-528 [PMID: 19365598]
  - 64 **Lock JF**, Schwabauer E, Martus P, Videv N, Pratschke J,

- Malinowski M, Neuhaus P, Stockmann M. Early diagnosis of primary nonfunction and indication for reoperation after liver transplantation. *Liver Transpl* 2010; **16**: 172-180 [PMID: 20104485]
- 65 **Jara M**, Malinowski M, Lüttgert K, Schott E, Neuhaus P, Stockmann M. Prognostic value of enzymatic liver function for the estimation of short-term survival of liver transplant candidates: a prospective study with the LiMAx test. *Transpl Int* 2015; **28**: 52-58 [PMID: 25263095]
- 66 **Merle U**, Sieg O, Stremmel W, Encke J, Eisenbach C. Sensitivity and specificity of plasma disappearance rate of indocyanine green as a prognostic indicator in acute liver failure. *BMC Gastroenterol* 2009; **9**: 91 [PMID: 19954554]
- 67 **Lock JF**, Kotobi AN, Malinowski M, Schulz A, Jara M, Neuhaus P, Stockmann M. Predicting the prognosis in acute liver failure: results from a retrospective pilot study using the LiMAx test. *Ann Hepatol* 2013; **12**: 556-562 [PMID: 23813133]

**P- Reviewer:** Lisotti A, Waisberg J **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Liu SQ



Retrospective Cohort Study

# Non-initiation of hepatitis C virus antiviral therapy in patients with human immunodeficiency virus/hepatitis C virus co-infection

Christine U Oramasionwu, Angela DM Kashuba, Sonia Napravnik, David A Wohl, Lu Mao, Adaora A Adimora

Christine U Oramasionwu, Angela DM Kashuba, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, United States

Angela DM Kashuba, Sonia Napravnik, David A Wohl, UNC Center for AIDS Research, University of North Carolina, Chapel Hill, NC 27599, United States

Angela DM Kashuba, Sonia Napravnik, David A Wohl, Adaora A Adimora, School of Medicine, University of North Carolina, Chapel Hill, NC 27599, United States

Lu Mao, Adaora A Adimora, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC 27599, United States

**Author contributions:** Oramasionwu CU designed and coordinated the research and wrote the manuscript; Kashuba ADM and Adimora AA help design the research and draft the manuscript; Napravnik S helped design the research and acquire clinical cohort data; Wohl DA helped design the research; Mao L conducted data analysis; all authors approved the final manuscript.

**Supported by** The University of North Carolina at Chapel Hill Center for AIDS Research (CFAR) an NIH funded program to Dr. Oramasionwu, No. P30 AI50410; Dr. Oramasionwu was also supported partially by the NIH Loan Repayment Program (LRP) through the National Institute on Minority Health and Health Disparities, No. L60 MD003770.

**Institutional review board statement:** This research was reviewed and approved by the University of North Carolina at Chapel Hill Institutional Review Board.

**Informed consent statement:** The clinical cohort, approved by the UNC Institutional Review Board, has ongoing enrollment and participants provide written informed consent.

**Conflict-of-interest statement:** The authors declare no other conflicts of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Christine U Oramasionwu, PharmD, PhD, Assistant Professor, UNC Eshelman School of Pharmacy, University of North Carolina, Kerr Hall 2215, Chapel Hill, NC 27599, United States. [oramsc@unc.edu](mailto:oramsc@unc.edu)  
 Telephone: +1-919-8434071  
 Fax: +1-919-9668486

**Received:** August 1, 2015  
**Peer-review started:** August 3, 2015  
**First decision:** September 14, 2015  
**Revised:** October 24, 2015  
**Accepted:** December 3, 2015  
**Article in press:** December 4, 2015  
**Published online:** March 8, 2016

## Abstract

**AIM:** To assess whether reasons for hepatitis C virus (HCV) therapy non-initiation differentially affect racial and ethnic minorities with human immunodeficiency virus (HIV)/HCV co-infection.

**METHODS:** Analysis included co-infected HCV treatment-naïve patients in the University of North Carolina CFAR HIV Clinical Cohort (January 1, 2004 and December 31, 2011). Medical records were abstracted to document non-modifiable medical (*e.g.*, hepatic decompensation, advanced immunosuppression), potentially modifiable medical (*e.g.*, substance abuse, severe depression, psychiatric illness), and non-medical (*e.g.*, personal,



social, and economic factors) reasons for non-initiation. Statistical differences in the prevalence of reasons for non-treatment between racial/ethnic groups were assessed using the two-tailed Fisher's exact test. Three separate regression models were fit for each reason category. Odds ratios and their 95% CIs (Wald's) were computed.

**RESULTS:** One hundred and seventy-one patients with HIV/HCV co-infection within the cohort met study inclusion. The study sample was racially and ethnically diverse; most patients were African-American (74%), followed by Caucasian (19%), and Hispanic/other (7%). The median age was 46 years (interquartile range = 39-50) and most patients were male (74%). Among the 171 patients, reasons for non-treatment were common among all patients, regardless of race/ethnicity (50% with  $\geq 1$  non-modifiable medical reason, 66% with  $\geq 1$  potentially modifiable medical reason, and 66% with  $\geq 1$  non-medical reason). There were no significant differences by race/ethnicity. Compared to Caucasians, African-Americans did not have increased odds of non-modifiable [adjusted odds ratio (aOR) = 1.47, 95%CI: 0.57-3.80], potentially modifiable (aOR = 0.72, 95%CI: 0.25-2.09) or non-medical (aOR = 0.90, 95%CI: 0.32-2.52) reasons for non-initiation.

**CONCLUSION:** Race/ethnicity alone is not predictive of reasons for HCV therapy non-initiation. Targeted interventions are needed to improve access to therapy for all co-infected patients, including minorities.

**Key words:** Human immunodeficiency virus; Hepatitis C virus; Co-infection; Antiviral therapy; Race

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Historically, hepatitis C virus (HCV) treatment rates have been low in patients with human immunodeficiency virus (HIV) co-infection, especially for African-American patients. Identifying the reasons for treatment non-initiation may help improve treatment rates among racially and ethnic minorities. In our study of patients with HIV/HCV coinfection, non-modifiable medical reasons, potentially modifiable medical reasons, and non-medical reasons for non-treatment were common among all patients, regardless of their race/ethnicity. There is a need to recognize and overcome potential treatment barriers in order to improve HCV treatment uptake in this patient population.

Oramasionwu CU, Kashuba ADM, Napravnik S, Wohl DA, Mao L, Adimora AA. Non-initiation of hepatitis C virus antiviral therapy in patients with human immunodeficiency virus/hepatitis C virus co-infection. *World J Hepatol* 2016; 8(7): 368-375 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i7/368.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i7.368>

## INTRODUCTION

Hepatitis C virus (HCV) treatment rates have been low in patients who are co-infected with human immunodeficiency virus (HIV). Up until 2011, when the first direct-acting antivirals (DAAs) became available, only one-third of co-infected patients were deemed eligible to receive HCV therapy, of whom less than one-third initiated HCV treatment<sup>[1-4]</sup>. Of great concern is the proportion of racial and ethnic minorities with co-infection that have not received HCV therapy. Nearly half of United States patients with HIV/HCV co-infection are African-American<sup>[5-7]</sup>. Previous studies involving older HCV regimens [pegylated interferon plus ribavirin (pegIFN-RBV)] reported that African-Americans were less likely than Caucasians to initiate HCV therapy<sup>[6,8,9]</sup>. Of co-infected patients in the HIV Outpatient Study during 1999-2007, African-Americans had a lower likelihood of HCV treatment than Caucasians (HR = 0.3, 95%CI: 0.2-0.6)<sup>[6]</sup>. African-American patients have been shown to not initiate therapy due to presence of IFN-related contraindications or to defer therapy due to lack of symptoms<sup>[10,11]</sup>.

Non-initiation of HCV therapy in co-infected patients is attributed to diverse factors such as patient- and provider-level barriers, perceived risks and benefits of therapy, and patient ineligibility to receive therapy due to medical contraindications<sup>[12]</sup>. Examples of medical conditions that sometimes precluded treatment with older regimens include hepatic decompensation, active injection drug use (IDU), alcohol abuse, severe depression, and advanced HIV-associated immunosuppression<sup>[13-16]</sup>.

Although these treatment-related barriers have been identified in the general co-infected population, scant research has documented their prevalence in co-infected minorities. Some reasons for non-treatment, such as substance abuse, are potentially modifiable. Addressing them could help improve access to HCV therapy in minorities. Despite the clinical promise of the DAAs, it is possible that some of the historical challenges to treating patients with HIV/HCV co-infection are still obstacles to treatment, particularly for minority patients<sup>[1,10,17]</sup>. The objectives of this study were to document reasons for non-treatment with HCV antiviral therapy and to assess how they differentially affect racial and ethnic minorities with HIV/HCV co-infection.

## MATERIALS AND METHODS

### Study design and population

This was a retrospective study of patients with HIV/HCV co-infection enrolled in the University of North Carolina (UNC) Center for AIDS Research HIV Clinical Cohort. This prospective cohort began enrolling patients in 1996 and includes over 4000 HIV-infected patients  $\geq 18$  years of age who receive HIV care at UNC. The cohort,

approved by the UNC Institutional Review Board, has ongoing enrollment and participants provide written informed consent. Data for the cohort are retrieved from two sources. Patient demographic characteristics and laboratory values are retrieved electronically, whereas patient medication histories and comorbid conditions are obtained by standardized and comprehensive electronic medical record reviews.

This study examined patients with HIV and HCV infection who had never received treatment for HCV and who had at least one outpatient clinic visit between January 1, 2004 and December 31, 2011. Patients were included in the study if they had the following: (1) a concomitant diagnosis of HCV based on positive HCV serostatus (as determined by HCV antibody test enzyme-linked immunosorbent assay/enzyme immunoassay); and (2) a positive HCV recombinant immunoblot assay (RIBA) test, detectable HCV RNA or HCV genotype test results. Patients with a history of HCV antiviral therapy were excluded. Anti-HCV therapy was defined as interferon, pegIFN, RBV, telaprevir, or boceprevir. The study period (2004-2011) was selected to best capture the timeframe when combination therapy with pegIFN-RBV was the standard of treatment for most patients with co-infection.

### Measurements

Baseline variables were retrieved from the cohort database and included patient demographics and clinical characteristics at time of HCV diagnosis. Baseline clinical characteristics were measurements taken proximal (allowing a 30-d window) to the date of the first positive HCV test. Demographic variables included age, gender, race/ethnicity (African-American, Caucasian, or Hispanic/other), and insurance coverage (private, public, none, or other). Clinical characteristics included CD4, HIV-1 RNA, HCV RNA, HCV genotype, HIV risk category (risk categories were not mutually exclusive), prior AIDS-defining clinical conditions, and use of highly active antiretroviral therapy (HAART), defined as a combination of three or more antiretroviral drugs. Prior to May 1, 2007, HCV RNA assays were measured in copies/mL, whereas subsequent HCV RNA assays were measured in IU/mL. Results for both assays are presented, where applicable, within the study period.

We reviewed individual medical records to identify reasons cited in the clinic notes by providers for not initiating HCV therapy. Reasons for treatment non-initiation were then categorized as non-modifiable medical reasons, potentially modifiable medical reasons, or non-medical reasons. Non-modifiable medical reasons included death (patients with a poor life expectancy or patients that died before treatment was ever initiated), hepatic decompensation, advanced immunosuppression (CD4 < 200) not controlled by antiretroviral therapy, renal insufficiency, uncontrolled autoimmune conditions, or hematological disease. Potentially modifiable medical reasons included active or recent (within the past six months) IDU/cocaine use, alcohol use, severe depressive

ssion (defined as depression with suicidal ideation), psychiatric illness, or pregnancy/unwillingness to use contraception. Lastly, non-medical reasons included personal factors (e.g., refusal of available therapies, poor adherence to care), social factors (e.g., social instability, homelessness/lack of housing, lack of transportation), and economic factors (e.g., lack of health insurance, prohibitive cost).

### Statistical analysis

Descriptive analyses were conducted on baseline variables, including demographic and clinical characteristics. For each type of reason for non-treatment, the prevalence of the sub-categories by racial/ethnic groups was computed. Statistical differences in the prevalence of reasons for non-treatment between racial/ethnic groups were assessed using the two-tailed Fisher's exact test. For each reason type (non-modifiable medical, potentially modifiable medical, and non-medical), risk factors such as age, gender, race/ethnicity, insurance status, and select HIV clinical characteristics were analyzed using multivariate logistic regression. Three separate regression models were fit for each reason type; the three reason types were the dependent variables in the respective models. Odds ratios and their 95% CIs (Wald's) were computed. All data analyses were conducted using SAS software (version 9.2; SAS Institute Inc., Cary, North Carolina, United States). All statistical analyses were performed by Lu Mao, a trained biostatistician with the UNC CFAR Biostatistics Core.

## RESULTS

### Baseline demographics and clinical characteristics

Within the cohort, 246 patients had a positive HCV serostatus and either a positive HCV RIBA test or detectable HCV RNA at baseline. Of these, 75 patients (30%) were excluded during the chart review process due to lack of HCV genotype results or due to reported history of antiviral therapy. We present results for the 171 patients (70%) that met criteria for this study. Baseline demographic and clinical characteristics are summarized in Table 1. The median age was 46 years [interquartile range (IQR) = 39-50] and most patients were male (74%). The study sample was racially and ethnically diverse; most patients were African-American (74%), followed by Caucasian (19%), and Hispanic/other (7%). This largely reflects the racial/ethnic makeup of the clinical cohort. More than one-third of patients lacked any insurance coverage (37%).

At baseline, patients had a median (IQR) HIV-1 RNA of 4.3 (2.7-5) log<sub>10</sub> copies/mL, a median (IQR) CD4 299 (91-517) cells/ $\mu$ L, and 73% of patients were treated with HAART. Twenty-five patients (15%) had a baseline median (IQR) HCV RNA of 5.8 (5.7, 5.8) log<sub>10</sub> copies/mL (values reported prior to May 1, 2007) and 10 patients (6%) had a baseline median (IQR) HCV RNA of 6.5 (6.2, 6.7) log<sub>10</sub> IU/mL (values reported after May 1, 2007). The most predominant HCV genotype was genotype 1

**Table 1** Baseline demographic and clinical characteristics of patients with human immunodeficiency virus/hepatitis C virus co-infection that did not initiate hepatitis C virus therapy

Variable	Patients (n = 171)
Patient demographics	
Age (median, IQR)	46 (39, 50)
Male gender, n (%)	126 (73.7)
Race/ethnicity, n (%)	-
Caucasian	32 (18.7)
African-American	126 (73.7)
Hispanic/other	13 (7.6)
Insurance, n (%)	-
Private	23 (13.5)
Public	67 (39.2)
None	64 (37.4)
Other	17 (9.9)
HIV clinical characteristics	
HIV-1 RNA (log <sub>10</sub> copies/mL) (median, IQR)	4.3 (2.7, 5)
CD4 (cells/ $\mu$ L) (median, IQR)	299 (91, 517)
HAART, n (%)	125 (73.1)
Prior AIDS-defining clinical condition, n (%)	37 (21.6)
HIV risk category, n (%) <sup>1</sup>	-
MSM	41 (24)
Injection drug use	97 (56.7)
HCV clinical characteristics	
HCV RNA log <sub>10</sub> copies/mL (median, IQR) <sup>2</sup>	5.8 (5.7, 5.8)
HCV RNA log <sub>10</sub> (IU/mL) (median, IQR) <sup>3</sup>	6.5 (6.2, 6.7)
HCV genotype, n (%) <sup>4</sup>	-
Genotype 1	157 (91.8)
Genotype 2	8 (4.7)
Genotype 3	6 (3.5)
Genotype 4	1 (0.6)

<sup>1</sup>HIV risk categories were not mutually exclusive; <sup>2</sup>n = 25 patients with RNA reported as copies/mL (prior to May 1, 2007); <sup>3</sup>n = 10 patients with RNA reported as IU/mL (following to May 1, 2007); <sup>4</sup>Genotypes 1 and 2 were both reported in one patient. IQR: Interquartile range; HIV: Human immuno-deficiency virus; HCV: Hepatitis C virus; HAART: Highly active antiretroviral therapy; MSM: Men who have sex with men.

(92%), followed by genotype 2 (5%), genotype 3 (3%), and genotype 4 (< 1%).

### Documented reasons for HCV non-treatment

Reasons for HCV non-treatment did not vary significantly by race/ethnicity (Table 2). Subcategories for each reason type are illustrated in Figure 1. At least one non-modifiable medical reason was documented in approximately half of all patients. Patient death was the most common non-modifiable medical reason in all three racial/ethnic groups, followed by advanced immunosuppression. Two-thirds of patients in each racial/ethnic group had at least one potentially modifiable reason for not initiating therapy (range 66%-69% across racial/ethnic groups); of these, IDU/cocaine use and psychiatric illness was the most common, followed by alcohol use and severe depression. Non-medical reasons were also common in each racial/ethnic group; these were most often due to personal and social reasons, and least commonly due to economic reasons.

### Factors associated with HCV non-treatment

We evaluated age, gender, race/ethnicity, insurance,

HIV-1 RNA, CD4, and prior AIDS-defining clinical conditions as factors independently associated with reasons for not initiating therapy (Table 3). Compared to Caucasian race/ethnicity, African-American race/ethnicity was not associated with having at least one non-modifiable medical reason [adjusted odds ratio (aOR) = 1.47, 95%CI: 0.57-3.80], potentially modifiable medical reason (aOR = 0.72, 95%CI: 0.25-2.09), or non-medical reason (aOR = 0.90, 95%CI: 0.32-2.52).

## DISCUSSION

While low uptake of older HCV antiviral regimens in HIV/HCV co-infected patients, particularly in racial and ethnic minorities, is well-documented in the literature, the reasons for low uptake are less clear<sup>[6,8,16]</sup>. This study evaluated reasons cited by the provider for non-initiation of HCV therapy in a cohort of untreated patients. Patients in our study were predominantly African-American and largely had genotype 1, which is comparable to other studies<sup>[1,18-21]</sup>. Our findings suggest that race/ethnicity alone is not predictive of having at least one reason for not initiating therapy. Rather, a key finding of this study was the high prevalence of multiple reasons for non-treatment, regardless of racial/ethnic group.

Nearly one-third of all patients in this study died without ever receiving HCV therapy. Of note, it cannot be assumed that patients who died would have ever initiated therapy while alive. Advanced immunosuppression (CD4 < 200) was also a common reason for non-treatment in our study. The majority (73%) of patients were on HAART at baseline, yet more than half of all patients had advanced immunosuppression documented as a reason for non-treatment, a finding that has been noted previously. In a study of patients with HIV/HCV co-infection at one of three Los Angeles HIV clinics, HAART use was common (> 90%), yet CD4  $\leq$  200 was independently associated with decreased HCV treatment acceptance (OR = 0.08, 95%CI: 0.01-0.40)<sup>[13]</sup>. Treatment guidelines suggest postponing HCV antiviral therapy in HIV/HCV co-infected patients with CD4 < 200 and recommend HAART initiation to preserve and restore immune function<sup>[22]</sup>. Treatment-related factors such as adherence and regimen appropriateness can influence immune response. CD4 and HIV-1 RNA are indirect, objective measures of these treatment-related factors. However, we only evaluated baseline CD4 and HIV-1 RNA values, which precluded us from drawing inferences about the effects of adherence and regimen appropriateness on immunosuppression and resultant non-initiation of HCV therapy.

As expected, IDU/cocaine use was reported as a potentially modifiable reason for not initiating therapy. Past studies have classified substance abuse as an absolute contraindication to HCV therapy<sup>[23]</sup>. In a recent systematic review evaluating barriers to HCV therapy in HIV/HCV co-infected patients, substance abuse was

**Table 2** Prevalence of reasons for hepatitis C virus non-treatment in patients with human immunodeficiency virus/hepatitis C virus co-infection

	Total ( <i>n</i> = 171)	Race/ethnicity			<i>P</i> value <sup>1</sup>
		African-American ( <i>n</i> = 126)	Caucasian ( <i>n</i> = 32)	Hispanic/other ( <i>n</i> = 13)	
≥ 1 non-modifiable medical reason, <i>n</i> (%)	85 (49.7)	64 (50.8)	14 (43.8)	7 (53.8)	0.806
≥ 1 potentially modifiable medical reason, <i>n</i> (%)	113 (66.1)	83 (65.9)	21 (65.6)	9 (69.2)	1.000
≥ 1 non-medical reason, <i>n</i> (%)	113 (66.1)	85 (67.5)	21 (65.6)	7 (53.8)	0.597

<sup>1</sup>Fisher's exact test.**Table 3** Multivariate analyses for associations between characteristics and reasons for hepatitis C virus non-treatment

Variable	Non-modifiable medical reason	Potentially modifiable medical reason	Non-medical reason
	aOR (95%CI)	aOR (95%CI)	aOR (95%CI)
Age (yr)	1.00 (0.96-1.05)	0.93 (0.87-0.98)	0.99 (0.94-1.04)
Gender			
Female	Ref	Ref	Ref
Male	1.07 (0.47-2.43)	0.77 (0.30-1.95)	1.57 (0.66-3.71)
Race/ethnicity			
Caucasian	Ref	Ref	Ref
African-American	1.47 (0.57-3.80)	0.72 (0.25-2.09)	0.90 (0.32-2.52)
Hispanic/other	1.94 (0.34-11.18)	1.65 (0.14-18.83)	0.46 (0.08-2.88)
Insurance			
Private	Ref	Ref	Ref
Public	1.30 (0.39-4.27)	2.27 (0.65-7.93)	0.69 (0.15-3.10)
None	0.64 (0.19-2.05)	1.13 (0.34-3.76)	0.44 (0.13-1.56)
Other	0.82 (0.32-3.13)	3.03 (0.59-15.6)	0.50 (0.09-2.68)
HIV-1 RNA log <sub>10</sub>	1.08 (0.79-1.49)	0.95 (0.67-1.34)	1.11 (0.80-1.56)
CD4	0.99 (0.99-1.00)	1.00 (0.99-1.00)	0.99 (0.99-1.00)
Prior AIDS-defining clinical condition	0.46 (0.09-2.37)	0.58 (0.10-3.28)	1.49 (0.15-14.65)

aOR: Adjusted odds ratio; HIV: Human immuno-deficiency virus; AIDS: Acquired immune deficiency syndrome.

the most frequently cited barrier<sup>[23]</sup>. However, substance users should not be routinely excluded from treatment, as substance use can fluctuate with time<sup>[24]</sup>. Recent guidelines recommend deferral of treatment if active or ongoing substance abuse is expected to interfere with regimen adherence, and frequent re-evaluation of each patient's adherence to routine medical care, other comorbidities, and potential for reinfection<sup>[15,24]</sup>.

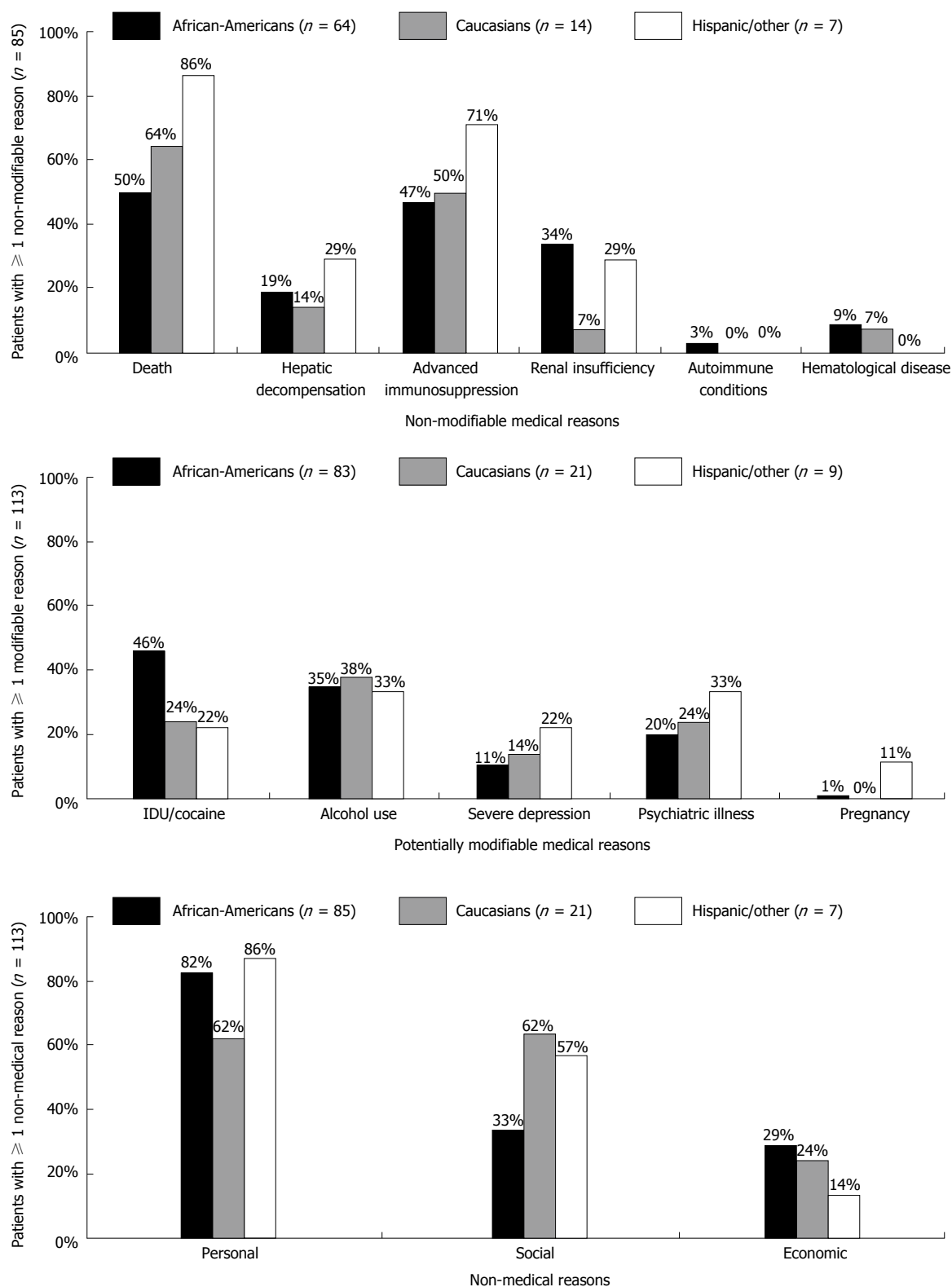
Personal and social factors were commonly reported in all racial/ethnic groups as reasons for not initiating therapy. Engagement in care and adherence are often perceived by the provider as an indicator of treatment readiness and are based on characteristics such as mental health, clinic attendance, substance use, and the patient's attitudes and beliefs about therapy<sup>[25]</sup>. These factors can decrease the likelihood of patient referral for HCV care.

Historically, patients with HIV/HCV, most often African-Americans, have had poor virologic response to HCV therapy<sup>[26-29]</sup>. African-Americans have been shown to be less likely to accept pegIFN-RBV antiviral therapy when it was recommended by their providers<sup>[13]</sup>. It has been suggested that patient awareness of low sustained virologic response (SVR) among African-Americans with genotype 1 may contribute to decisions to refuse therapy<sup>[11]</sup>. The high proportion of personal factors for non-treatment in our study could have also

been attributed to patient refusal of pegIFN-RBV in anticipation of more effective, and ultimately more convenient, therapies. DAA-based therapy demonstrates excellent efficacy in clinical trials; 87% SVR has been attained in African-Americans<sup>[30]</sup>. Nevertheless, some of the reasons for non-treatment identified in the present study are still likely barriers to the DAAs.

Current treatment guidelines acknowledge substance abuse, psychiatric disorders, and lack of access (e.g., cost, insurance, distance to provider) as barriers to current HCV treatment regimens that include DAAs<sup>[31]</sup>. Several strategies are proposed to increase HCV treatment, particularly DAA therapy, in patients with HIV/HCV. At the patient level, pre-treatment education, management of comorbidities and mental health conditions, and harm reduction counseling in individuals with continued substance abuse can be provided through patient referral for specialty services, such as substance abuse treatment and psychiatric therapy<sup>[24]</sup>. Strategies at the provider level include collaborative care models with primary care providers and HCV specialists<sup>[32]</sup>, and co-localization models that combine HCV treatment and care with other primary medical care or substance abuse treatment and social services<sup>[31]</sup>. Systems-level strategies are needed, such as medication patient assistance programs, removal of Medicaid state restrictions regarding substance abuse and HCV therapy,





**Figure 1** Documented reasons for hepatitis C virus non-treatment in patients with human immunodeficiency virus/hepatitis C virus coinfection patients, by race/ethnicity. Patients may have had more than one reason for non-treatment.

decrease prescription prior authorization requirements, and ultimately, to lower DAA drug prices in order to increase treatment access for patients<sup>[33]</sup>.

Our study is subject to limitations. The UNC clinic is a large academic center and may not be representative

of patients receiving care in other clinic settings. All baseline variables were retrieved from the clinical cohort database; however, individual patient records were reviewed to ascertain reasons for non-treatment in the medical record. Some limitations of medical record data

collection include variability in documentation across clinic providers, missing data due to errors that occur during clinic visit narrative dictation and transcription, and lack of specificity for patient information. Listed reasons were based on providers' cited reasons for not initiating HCV therapy. These may differ from patient-reported barriers to care that are specific to racial and ethnic groups, such as medical mistrust<sup>[34]</sup>. As our study was designed to focus on the untreated, we were unable to make any causal associations between documented reasons and why patients were not treated. We did not assess continuity of HAART. Given that advanced immunosuppression greatly contributed to having a non-modifiable medical reason, it is possible that patients who had advanced immunosuppression documented as a reason for non-treatment were maintained on HAART, but did not experience the full clinical benefits of HAART due to regimen adherence, regimen appropriateness, and/or due to inability for some patients to achieve immune reconstitution<sup>[35]</sup>. We did not measure these factors in our study. Lastly, we did not evaluate differences in HCV treatment by race/ethnicity, and were therefore, unable to determine if any treatment disparities exist among patients in the UNC clinic.

In summary, reasons for non-treatment did not differentially affect racial and ethnic minorities co-infected with HIV/HCV. Rather, there was a high prevalence of multiple reasons for non-treatment in patients, regardless of racial/ethnic group. The advent of DAAs has undoubtedly revolutionized HCV care, however, there is still a need to recognize and overcome potential treatment barriers in order to improve treatment uptake and eradicate HCV in this patient population.

## ACKNOWLEDGMENTS

The authors would like to thank Oksana Zakharova, Sam Stinnette, Christine Sun, Dan-Thanh Nguyen, Heather Moore, and Joshua Toliver for their assistance with data extraction and data collection for this study.

## COMMENTS

### Background

Historically, hepatitis C virus (HCV) treatment rates have been low in patients with human immunodeficiency virus (HIV) co-infection, especially for African-American patients. Identifying the reasons for treatment non-initiation may help improve treatment rates among racially and ethnic minorities.

### Research frontiers

The authors' findings suggest that race/ethnicity alone is not predictive of having at least one reason for not initiating therapy. Rather, a key finding of this study was the high prevalence of multiple reasons for non-treatment, regardless of racial/ethnic group.

### Innovations and breakthroughs

While low uptake of older HCV antiviral regimens in HIV/HCV co-infected patients, particularly in racial and ethnic minorities, is well documented in the literature, the reasons for low uptake are less clear. This study evaluated reasons cited by the provider for non-initiation of HCV therapy in a cohort of

untreated patients.

## Applications

This study demonstrates that there is a need to recognize and overcome potential treatment barriers in order to improve HCV treatment uptake in this patient population.

## Peer-review

This article is of interest to clinicians that manage patients with HIV/HCV coinfection.

## REFERENCES

- 1 Mehta SH, Lucas GM, Mirel LB, Torbenson M, Higgins Y, Moore RD, Thomas DL, Sulkowski MS. Limited effectiveness of antiviral treatment for hepatitis C in an urban HIV clinic. *AIDS* 2006; **20**: 2361-2369 [PMID: 17117023 DOI: 10.1097/QAD.0b013e32801086da]
- 2 Fleming CA, Craven DE, Thornton D, Tumilty S, Nunes D. Hepatitis C virus and human immunodeficiency virus coinfection in an urban population: low eligibility for interferon treatment. *Clin Infect Dis* 2003; **36**: 97-100 [PMID: 12491208 DOI: 10.1086/344907]
- 3 Restrepo A, Johnson TC, Widjaja D, Yarmus L, Meyer K, Clain DJ, Bodenheimer HC, Min AD. The rate of treatment of chronic hepatitis C in patients co-infected with HIV in an urban medical centre. *J Viral Hepat* 2005; **12**: 86-90 [PMID: 15655053 DOI: 10.1111/j.1365-2893.2005.00548.x]
- 4 Hooshyar D, Napravnik S, Miller WC, Eron JJ. Effect of hepatitis C coinfection on discontinuation and modification of initial HAART in primary HIV care. *AIDS* 2006; **20**: 575-583 [PMID: 16470122 DOI: 10.1097/01.aids.0000210612.37589.12]
- 5 Ananthakrishnan AN, McGinley EL, Fangman J, Saeian K. Hepatitis C/HIV co-infection is associated with higher mortality in hospitalized patients with hepatitis C or HIV. *J Viral Hepat* 2010; **17**: 720-729 [PMID: 20002558 DOI: 10.1111/j.1365-2893.2009.01232.x]
- 6 Vellozzi C, Buchacz K, Baker R, Spradling PR, Richardson J, Moorman A, Tedaldi E, Durham M, Ward J, Brooks JT. Treatment of hepatitis C virus (HCV) infection in patients coinfecting with HIV in the HIV Outpatient Study (HOPS), 1999-2007. *J Viral Hepat* 2011; **18**: 316-324 [PMID: 20367803 DOI: 10.1111/j.1365-2893.2010.01299.x]
- 7 Johnson TL, Toliver JC, Mao L, Oramasionwu CU. Differences in outpatient care and treatment utilization for patients with HIV/HCV coinfection, HIV, and HCV mono-infection, a cross-sectional study. *BMC Infect Dis* 2014; **14**: 217 [PMID: 24755037 DOI: 10.1186/1471-2334-14-217]
- 8 Backus LI, Boothroyd DB, Phillips BR, Mole LA. Pretreatment assessment and predictors of hepatitis C virus treatment in US veterans coinfecting with HIV and hepatitis C virus. *J Viral Hepat* 2006; **13**: 799-810 [PMID: 17109679 DOI: 10.1111/j.1365-2893.2006.00751.x]
- 9 Butt AA, Justice AC, Skanderson M, Good C, Kwok CK. Rates and predictors of hepatitis C virus treatment in HCV-HIV-coinfected subjects. *Aliment Pharmacol Ther* 2006; **24**: 585-591 [PMID: 16907891 DOI: 10.1111/j.1365-2036.2006.03020.x]
- 10 Schaeffer S, Khalili M. Reasons for HCV non-treatment in underserved African Americans: implications for treatment with new therapeutics. *Ann Hepatol* 2015; **14**: 234-242 [PMID: 25671833]
- 11 Khokhar OS, Lewis JH. Reasons why patients infected with chronic hepatitis C virus choose to defer treatment: do they alter their decision with time? *Dig Dis Sci* 2007; **52**: 1168-1176 [PMID: 17357838 DOI: 10.1007/s10620-006-9579-1]
- 12 Mehta SH, Thomas DL, Sulkowski MS, Safaei M, Vlahov D, Strathdee SA. A framework for understanding factors that affect access and utilization of treatment for hepatitis C virus infection among HCV-mono-infected and HIV/HCV-co-infected injection drug users. *AIDS* 2005; **19** Suppl 3: S179-S189 [PMID: 16251816]

- 13 **Osilla KC**, Wagner G, Garnett J, Ghosh-Dastidar B, Witt M, Bhatti L, Goetz MB. Patient and provider characteristics associated with the decision of HIV coinfecting patients to start hepatitis C treatment. *AIDS Patient Care STDS* 2011; **25**: 533-538 [PMID: 21823907 DOI: 10.1089/apc.2011.0048]
- 14 **Osilla KC**, Ryan G, Bhatti L, Goetz M, Witt M, Wagner G. Factors that influence an HIV coinfecting patient's decision to start hepatitis C treatment. *AIDS Patient Care STDS* 2009; **23**: 993-999 [PMID: 19929229 DOI: 10.1089/apc.2009.0153]
- 15 **Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents**. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medical Association of the Infectious Diseases Society of America. [Accessed 2015 Mar 12]. Available from: URL: [http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult\\_oi.pdf](http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf)
- 16 **Butt AA**, Tsevat J, Leonard AC, Shaikh OS, McMahon D, Khan UA, Dorey-Stein Z, Lo Re V 3rd. Effect of race and HIV co-infection upon treatment prescription for hepatitis C virus. *Int J Infect Dis* 2009; **13**: 449-455 [PMID: 18993100 DOI: 10.1016/j.ijid.2008.06.041]
- 17 **Walley AY**, White MC, Kushel MB, Song YS, Tulskey JP. Knowledge of and interest in hepatitis C treatment at a methadone clinic. *J Subst Abuse Treat* 2005; **28**: 181-187 [PMID: 15780548 DOI: 10.1016/j.jsat.2004.12.004]
- 18 **Adeyemi OM**, Jensen D, Attar B, Ghaoui R, Gallagher M, Wolen D, Cotler SJ. Hepatitis C treatment eligibility in an urban population with and without HIV coinfection. *AIDS Patient Care STDS* 2004; **18**: 239-245 [PMID: 15142354 DOI: 10.1089/108729104323038919]
- 19 **Backus LI**, Boothroyd D, Deyton LR. HIV, hepatitis C and HIV/hepatitis C virus co-infection in vulnerable populations. *AIDS* 2005; **19** Suppl 3: S13-S19 [PMID: 16251809]
- 20 **Butt AA**, Khan UA, Shaikh OS, McMahon D, Dorey-Stein Z, Tsevat J, Lo Re V 3rd. Rates of HCV treatment eligibility among HCV-monoinfected and HCV/HIV-coinfecting patients in tertiary care referral centers. *HIV Clin Trials* 2009; **10**: 25-32 [PMID: 19362993 DOI: 10.1310/hct1001-25]
- 21 **Pearlman BL**. Hepatitis C virus infection in African Americans. *Clin Infect Dis* 2006; **42**: 82-91 [PMID: 16323096 DOI: 10.1086/498512]
- 22 **Panel on Antiretroviral Guidelines for Adults and Adolescents**. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. [Accessed March 12, 2015]. Available from: URL: <http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>
- 23 **Oramasionwu CU**, Moore HN, Toliver JC. Barriers to hepatitis C antiviral therapy in HIV/HCV co-infected patients in the United States: a review. *AIDS Patient Care STDS* 2014; **28**: 228-239 [PMID: 24738846 DOI: 10.1089/apc.2014.0033]
- 24 **Robaey G**, Grebely J, Mauss S, Bruggmann P, Moussalli J, De Gottardi A, Swan T, Arain A, Kautz A, Stöver H, Wedemeyer H, Schaefer M, Taylor L, Backmund M, Dalgard O, Prins M, Dore GJ. Recommendations for the management of hepatitis C virus infection among people who inject drugs. *Clin Infect Dis* 2013; **57** Suppl 2: S129-S137 [PMID: 23884061 DOI: 10.1093/cid/cit302]
- 25 **Wagner GJ**, Ryan GW. Hepatitis C virus treatment decision-making in the context of HIV co-infection: the role of medical, behavioral and mental health factors in assessing treatment readiness. *AIDS* 2005; **19** Suppl 3: S190-S198 [PMID: 16251817 DOI: 10.1097/01.aids.0000192089.54130.b6]
- 26 **Sterling RK**, Stravitz RT, Luketic VA, Sanyal AJ, Contos MJ, Mills AS, Shiffman ML. A comparison of the spectrum of chronic hepatitis C virus between Caucasians and African Americans. *Clin Gastroenterol Hepatol* 2004; **2**: 469-473 [PMID: 15181614]
- 27 **Conjeevaram HS**, Fried MW, Jeffers LJ, Terrault NA, Wiley-Lucas TE, Afdhal N, Brown RS, Belle SH, Hoofnagle JH, Kleiner DE, Howell CD. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. *Gastroenterology* 2006; **131**: 470-477 [PMID: 16890601 DOI: 10.1053/j.gastro.2006.06.008]
- 28 **Ioannou GN**, Scott JD, Yang Y, Green PK, Beste LA. Rates and predictors of response to anti-viral treatment for hepatitis C virus in HIV/HCV co-infection in a nationwide study of 619 patients. *Aliment Pharmacol Ther* 2013; **38**: 1373-1384 [PMID: 24127691 DOI: 10.1111/apt.12524]
- 29 **Martel-Laferrrière V**, Brinkley S, Bichoupan K, Posner S, Stivala A, Perumalswami P, Schiano T, Sulkowski M, Dieterich D, Branch A. Virological response rates for telaprevir-based hepatitis C triple therapy in patients with and without HIV coinfection. *HIV Med* 2014; **15**: 108-115 [PMID: 24025147 DOI: 10.1111/hiv.12086]
- 30 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
- 31 **American Association for the Study of Liver Diseases and the Infectious Diseases Society of America**. Recommendations for testing, managing, and treating hepatitis C. [Accessed 2015 Jan 15]. Available from: URL: <http://www.hcvguidelines.org/full-report-view>
- 32 **Arora S**, Thornton K, Murata G, Deming P, Kalishman S, Dion D, Parish B, Burke T, Pak W, Dunkelberg J, Kistin M, Brown J, Jenkushy S, Komaromy M, Qualls C. Outcomes of treatment for hepatitis C virus infection by primary care providers. *N Engl J Med* 2011; **364**: 2199-2207 [PMID: 21631316 DOI: 10.1056/NEJMoa1009370]
- 33 **Grebely J**, Oser M, Taylor LE, Dore GJ. Breaking down the barriers to hepatitis C virus (HCV) treatment among individuals with HCV/HIV coinfection: action required at the system, provider, and patient levels. *J Infect Dis* 2013; **207** Suppl 1: S19-S25 [PMID: 23390301 DOI: 10.1093/infdis/jis928]
- 34 **Jordan AE**, Masson CL, Mateu-Gelabert P, McKnight C, Pepper N, Bouche K, Guzman L, Kletter E, Seewald RM, Des-Jarlais DC, Sorensen JL, Perlman DC. Perceptions of drug users regarding hepatitis C screening and care: a qualitative study. *Harm Reduct J* 2013; **10**: 10 [PMID: 23786800 DOI: 10.1186/1477-7517-10-10]
- 35 **Geng EH**, Deeks SG. CD4+ T cell recovery with antiretroviral therapy: more than the sum of the parts. *Clin Infect Dis* 2009; **48**: 362-364 [PMID: 19123869 DOI: 10.1086/595889]

**P- Reviewer:** Larrubia JR, Li ZF, Wang K **S- Editor:** Qi Y

**L- Editor:** A **E- Editor:** Liu SQ



Retrospective Cohort Study

## Significant cohort of non-alcoholic fatty liver disease with portal vein thrombosis in transplant waiting list

Metin Basaranoglu, Sonia M Najjar, Ali Ebag Demirbag, Hakan Senturk

Metin Basaranoglu, Hakan Senturk, Division of Gastroenterology, Department of Internal Medicine, Bezmialem Vakif University Faculty of Medicine, 34000 Fatih, Istanbul, Turkey

Metin Basaranoglu, Division of Gastroenterology, Türkiye-Yüksek İhtisas Hospital, 06010 Sıhhiye, Ankara

Ali Ebag Demirbag, Division of Gastrointestinal Surgery, Türkiye-Yüksek İhtisas Hospital, 06010 Sıhhiye, Ankara

Sonia M Najjar, Department of Physiology and Pharmacology, Center for Diabetes and Endocrine Research (CeDER), University of Toledo College of Medicine and Life Sciences, Toledo, OH 43614, United States

Vatan Caddesi, 34000 Fatih, Istanbul, Turkey. [metin\\_basaranoglu@yahoo.com](mailto:metin_basaranoglu@yahoo.com)  
Telephone: +90-212-5540000  
Fax: +90-212-5540000

Received: November 15, 2015  
Peer-review started: November 16, 2015  
First decision: December 18, 2015  
Revised: January 15, 2016  
Accepted: February 23, 2016  
Article in press: February 24, 2016  
Published online: March 8, 2016

**Author contributions:** Basaranoglu M was involved in the study concept and design; study supervision, data acquisition, analysis and interpretation; drafting of the manuscript; critical revision of the manuscript for important intellectual content; raising fund; and providing administrative, technical, and material support; Demirbag AE performed statistical analysis; Najjar SM critically analyzed and reviewed data analysis and interpretation, and provided critical revision of the manuscript for important intellectual content; Senturk H approved the final version of the manuscript.

**Conflict-of-interest statement:** The authors declare that they have no competing interests.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Metin Basaranoglu, MD, PhD, Division of Gastroenterology, Department of Internal Medicine, Bezmialem Vakif University Faculty of Medicine, Adnan Menderes Bulvarı

### Abstract

**AIM:** To characterize non-alcoholic fatty liver disease (NAFLD) presentation with esophageal varices.

**METHODS:** We carried out a retrospective cohort study on 258 patients with esophageal varices at a single tertiary referral center. These patients underwent diagnosis of several liver diseases, including: NAFLD-associated cirrhosis, hepatitis B, hepatitis C, Wilson disease, autoimmune liver diseases, and others.

**RESULTS:** Of the 258 patients, 39% of patients exhibited esophageal varices due to NAFLD-associated cirrhosis. Of the 38 (14.7%) patients developed hepatocellular carcinoma during follow-up, 52% were due to hepatitis B, 26% due to hepatitis C and 13.2% due to NAFLD. Of the 258 patients, 50.0% with NAFLD, 33.3% with hepatitis B, 26.3% with hepatitis C, and 58.3% with other diseases were alive at the end of the 5-year period with a significant difference according to the Kaplan-Meier log Rank test ( $P = 0.040$ ). Portal vein thrombosis was detected in 47.5% of patients with NAFLD, in 29% of patients with hepatitis B, in 17% of patients with hepatitis C, and in 62% of patients with other related diseases ( $P < 0.0001$ ).

**CONCLUSION:** Our study showed a proportionally



greater elevation in liver transplant candidacy in patients with NAFLD and portal vein thrombosis. Older patients were more prone to developing cirrhosis, hepatocellular carcinoma and a high mortality rate. However, younger patients exhibited more portal vein thrombosis and gastric varices.

**Key words:** Hepatocellular carcinoma; Non-alcoholic fatty liver disease; Portal vein thrombosis; Esophageal varices

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We aimed to characterize non-alcoholic fatty liver disease (NAFLD) presentation with esophageal varices. We carried out a retrospective cohort study on 258 patients with esophageal varices at a single tertiary referral center. Of the 258 patients, 39% exhibited esophageal varices due to NAFLD-associated cirrhosis. The incidence of portal vein thrombosis was 47.5% in patients with NAFLD, 29% in hepatitis B, 17% in hepatitis C, and 62% in patients with other related diseases ( $P < 0.0001$ ). Our study showed a proportionally greater elevation in liver transplant candidacy in patients with NAFLD and portal vein thrombosis.

Basaranoglu M, Najjar SM, Demirbag AE, Senturk H. Significant cohort of non-alcoholic fatty liver disease with portal vein thrombosis in transplant waiting list. *World J Hepatol* 2016; 8(7): 376-384 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i7/376.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i7.376>

## INTRODUCTION

Excessive accumulation of fat in hepatocytes in the absence of significant alcohol consumption occurs in up to 30% of adults<sup>[1,2]</sup>. This condition, termed non-alcoholic fatty liver disease (NAFLD), predisposes to non-alcoholic steatohepatitis (NASH), which progresses to cirrhosis and its complications, including hepatocellular carcinoma (HCC)<sup>[2-4]</sup>. Several studies have also reported that in some patients, NAFLD can lead to HCC without transitioning through cirrhosis<sup>[4,5-10]</sup>. Currently, it is estimated that NAFLD is the third leading cause of HCC after hepatitis C and B. Although earlier studies suggested that NAFLD may be less severe and progress slowly in Asian populations, the progression of fibrosis and cirrhosis in patients with NAFLD is no longer believed to differ significantly by ethnicity<sup>[11-13]</sup>.

The prevalence of NASH as a precursor of NAFLD-associated cirrhosis is 3% and 20% in non-obese and obese subjects, respectively<sup>[14]</sup>. The global obesity epidemic has been associated with the increasing burden of NAFLD. It has been estimated that the rising prevalence

of NAFLD will soon lead to large cohorts of patients with decompensated cirrhosis. In this respect, NAFLD is expected to become the leading indication for liver transplantation in the Western world, particularly in the United States. Longitudinal follow-up studies showed an increase in the mortality rate among patients with NAFLD due to hepatic decompensation<sup>[15-19]</sup>. These studies usually included a limited number of patients with short follow-up period and with selected patients such as compensated cirrhosis.

It is possible that risk factors for NAFLD-associated cirrhosis and HCC in Eastern countries differ from those in the West. Thus, we aimed to document the characteristics of patients with NAFLD-associated cirrhosis from Turkey, a European country sharing 97% of its borders with Asia. Relative to other Europeans, the Turkish population exhibits a higher rate of obesity that is comparable to that in the United States. In Turkey, 47.7% of all deaths have been attributed to cardiovascular diseases (most likely cerebrovascular and ischemic heart diseases), which are highly correlated with obesity<sup>[20]</sup>. Overall, 56% of the Turkish population is overweight, especially preobese (body-mass index: 25-29.9 kg/m<sup>2</sup>). This has been attributed in part, to the predominance of non-working women who manifest a higher incidence rate of obesity than their working counterparts (33% vs 14%).

In light of the epidemic spread of obesity in Turkey, and the association of this disease with NAFLD, the current follow-up study evaluated patients with esophageal varices from 2003 at a single tertiary referral liver center, with the aim to investigate the relationship between esophageal varices and NAFLD. The results were compared in terms of the development of portal vein thrombosis (PVT), HCC, survival and mortality. The association between esophageal varices and hepatitis B and hepatitis C was also examined, as these etiologies are also of importance to esophageal varices. According to the World Health Organization, Turkey is one of the countries with intermediate (2%-8%) endemic rate for hepatitis B and less than 2% (1.0%-1.9%) for hepatitis C<sup>[21,22]</sup>.

## MATERIALS AND METHODS

### Retrospective cohort study design

We have kept the records of patients with hepatitis B or C who have been followed prospectively at our hepatology unit and affiliated liver center. Confidentiality of records was maintained according to the guidelines issued by Türkiye Yüksek İhtisas Hospital Institutional Ethics Committee. Data were collected for esophageal varices only at the advanced endoscopy unit. A cohort of patients with esophageal varices from 2003 to 2014 was reviewed. All patients were of Turkish origin and were informed and consented about the investigation and treatment. Eligible patients were  $\geq 18$  years of age and have had esophageal varices diagnosed by upper

gastrointestinal endoscopy examination. They had regular clinical follow-up and endoscopic examinations at our clinic. Efficacy data were based on the last evaluation. Transplanted cases were excluded. The main inclusion criterion was the presence of esophageal varices with or without gastric varices.

Only 258 patients with endoscopically defined high risk varices had reliable data and were included in this study. Each patient was evaluated for fundal varices, PVT, cirrhosis, HCC, and mortality. After the first evaluation, patients were divided into 4 groups: Those with hepatitis B, hepatitis C, NAFLD and others related to autoimmune hepatitis, Wilson Disease, primary biliary cirrhosis, *etc.*

Alcohol history was determined through self-reporting and/or from information provided by family members. History of drug abuse, chronic hepatitis, hypertension, and diabetes was also recorded. Ultrasonographic evaluation of the hepatobiliary system was performed in each patient. Fatty liver was diagnosed by increased echogenicity or increased liver-kidney contrast. NAFLD was diagnosed according to standard criteria<sup>[19]</sup>. Serum serology of hepatitis B surface antigen, anti-hepatitis B surface, anti-hepatitis B core-total and anti-hepatitis C virus (anti-HCV) were measured by enzyme-linked immunosorbent assay. If necessary, liver biopsies were re-evaluated by an experienced pathologist according to established criteria.

The classification system of varices described by Sarin *et al.*<sup>[23]</sup> was used in our endoscopy unit. Accordingly, varices are endoscopically classified as gastroesophageal varices type I (lesser curvature), gastroesophageal varices type II (greater curvature), isolated gastric varices type I (gastric fundus), or isolated gastric varices type II (gastric-excluding the fundus).

### Statistical analysis

Data were coded and recorded electronically using an IBM Statistical Package for the Social Sciences (SPSS; Armonk, NY, United States) for Windows version 17.0 (2007). The  $\chi^2$  and Fisher's exact test was used to compare the groups for the distribution of cirrhosis, PVT, HCC, and mortality. Mean age compared by one-way ANOVA test in four groups and compared by Student's *t*-test between both genders. After the statistically significant ANOVA, we used post-hoc multiple comparison tests Bonferroni in order to identify statistically significant pairs. Kaplan-Meier Log Rank test was used to compare survival in four groups.  $P < 0.05$  was considered statistically significant in all of the tests.

## RESULTS

Primary end-point of the study was to use this cohort of patients with esophageal varices to evaluate the relationship between this disease and several etiologies, including NAFLD (in the presence or absence of cirrhosis), hepatitis B, hepatitis C or other liver-related

diseases. Second end-point was to draw this comparison in terms of PVT, HCC, survival and mortality.

### Etiology

As shown in Table 1, the etiology of the total 258 patients with esophageal varices was attributed to: NAFLD in 39.0% (101 patients), hepatitis B virus in 29.1% (75 patients) and HCV in 11.2% (29 patients). In the rest of the patients (20.5%, 53 patients), the etiology was: Hepatoportal sclerosis in 7.8%, isolated portal vein thrombosis without any other pathology in 4.3%, chronic alcohol consumption in 3.1%, primary sclerosing cholangitis in 1.9%, autoimmune hepatitis in 1.2%, primary biliary cirrhosis in 1.2%, Wilson Disease in 0.8% and chronic pancreatitis in 0.4% of this group of patients.

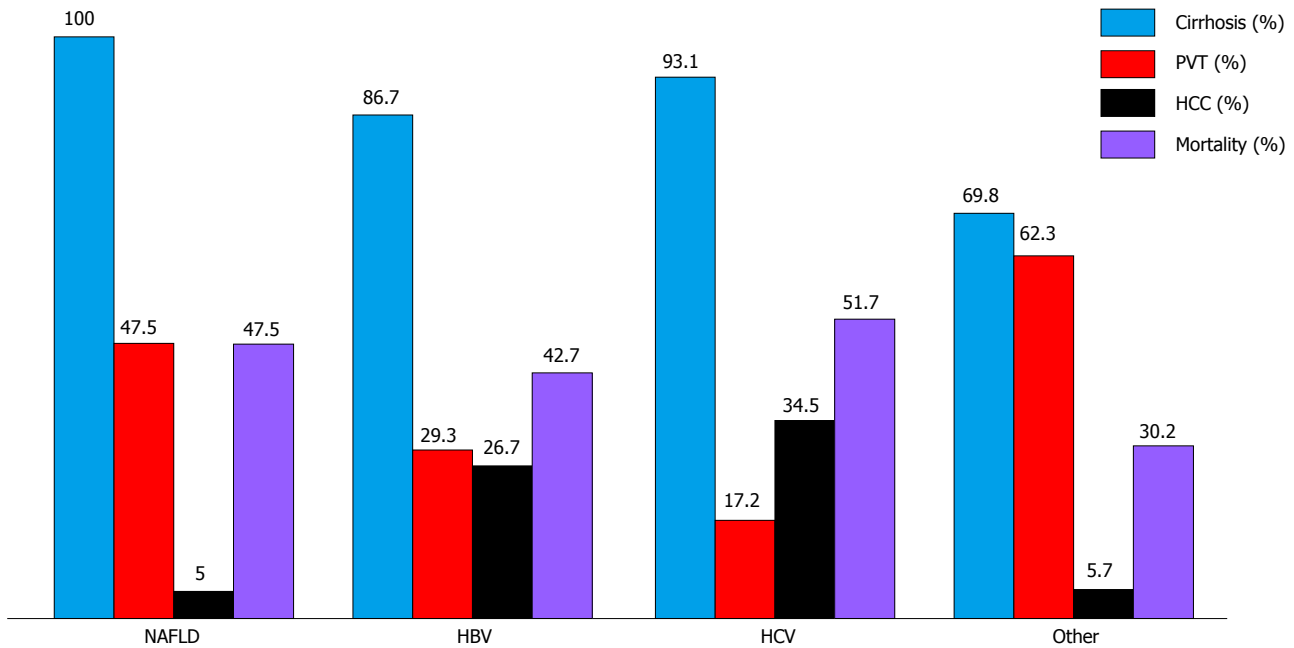
### Age

As Table 1 reveals, there is no statistical difference in the mean age between groups with NAFLD, hepatitis B and hepatitis C. However, the mean age of patients with these three etiologies (about 60 years) was higher than that in patients with other liver-related diseases (48 years) (Table 1;  $P < 0.0001$ ). The mean age of women with esophageal varices ( $60.4 \pm 14.8$  years, median: 64, and range: 27-90) was higher than that in men ( $53.5 \pm 14.6$ , median: 56; and range: 24-84),  $P < 0.001$ . In terms of etiology, men exhibited a higher percentage of hepatitis B than NAFLD, hepatitis C and others (80% vs 62.4%, 58.6% and 58.5%, respectively,  $P = 0.027$ ) (Table 1).

We also compared and found a difference in the mean age of patients with and without PVT ( $52 \pm 15$  years vs  $58.5 \pm 14.5$  years, respectively,  $P = 0.001$ ), with and without cirrhosis ( $56.3 \pm 15$  years vs  $51.9 \pm 13.8$  years, respectively,  $P < 0.05$ ), with and without HCC ( $62.7 \pm 9.7$  years vs  $54.6 \pm 15.4$  years, respectively,  $P = 0.001$ ) and the mean age of patients that have died and those that are still alive ( $61.1 \pm 13.3$  years vs  $51.8 \pm 15$  years, respectively;  $P < 0.0001$ ). However, there was no difference in the mean age of patients with and without fundic varices ( $54.9 \pm 15.3$  years vs  $56.5 \pm 14.8$  years, respectively,  $P > 0.05$ ).

### PVT

The incidence rate of PVT was 41.9% (being detected in 108 out of 258 patients with esophageal varices) (Table 2). As Figure 1 and Table 2 indicate, PVT was observed in 47.5% of patients with NAFLD, 29.3% of patients with hepatitis B, 17.2% of patients with hepatitis C, and 62.3% of patients with other liver-related diseases ( $P < 0.0001$ ). The incidence of PVT was 36.8% and 42.7% in patients with and without HCC, respectively ( $P > 0.05$ ), 40.4% and 53.6% in patients with and without cirrhosis, respectively ( $P > 0.05$ ), and 56.9% and 29.8% in patients with and without fundic varices, respectively ( $P < 0.0001$ ). Of the 111 patients (43%) that died during the study period, 72 patients (64.9%) had no PVT ( $P = 0.057$ ).



**Figure 1** Incidence of portal vein thrombosis, cirrhosis, and hepatocellular cancer, in addition to mortality rate in patients with non-alcoholic fatty liver disease, hepatitis B, hepatitis C and other liver-related diseases (others). PVT: Portal vein thrombosis; HCC: Hepatocellular cancer; NAFLD: Non-alcoholic fatty liver disease; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

**Table 1** Classification of study groups

	NAFLD	Hepatitis B	Hepatitis C	Others	Total	P value
n (%)	101 (39.0)	75 (29.1)	29 (11.2)	53 (20.5)	258 (100)	
Mean age (median; range of years)	56.4 ± 16.0 (59; 24-83)	57.8 ± 13.3 (58; 24-90)	62.9 ± 12.2 (65; 28-79)	48.1 ± 13.9 (48; 25-81) <sup>b</sup>	55.8 ± 15.0 (58; 24-90)	< 0.0001
% men	62.4%	80.0% <sup>a</sup>	58.6%	58.5%	66.3%	< 0.05

Study groups were classified per etiology: NAFLD, hepatitis B, hepatitis C and others. Mean age in patients with NAFLD, hepatitis B and C that was higher than the mean age of patients with other etiologies (<sup>b</sup> $P < 0.0001$  others *vs* each of NAFLD, hepatitis B and C *vs* other etiologies). Percentage of men with hepatitis B was higher than those with NAFLD, hepatitis C and other etiologies (<sup>a</sup> $P < 0.05$  patients with hepatitis B *vs* NAFLD, hepatitis C and other etiologies). NAFLD: Non-alcoholic fatty liver disease.

**Table 2** Incidence of pathologies and mortality

	Cirrhosis	PVT	HCC	Fundic varices	Mortality
NAFLD	100%	47.5%	5.0% <sup>d</sup>	45.5%	47.5%
Hepatitis B	86.7%	29.3%	26.7%	52.0%	42.7%
Hepatitis C	93.1%	17.2% <sup>b</sup>	34.5%	27.6%	51.7%
Others	69.8% <sup>f</sup>	62.3%	5.7% <sup>d</sup>	43.4%	30.2%
Total	89.1%	41.9%	14.7%	45.0%	43.0%
P value	< 0.0001	< 0.0001	< 0.0001	> 0.05	> 0.05

The distribution of portal vein thrombosis (PVT), cirrhosis, hepatocellular cancer (HCC), fundic varices, and mortality rate in patients with NAFLD, hepatitis B, hepatitis C and other liver-related diseases (others) is shown. <sup>b</sup> $P < 0.0001$ ; <sup>d</sup> $P < 0.0001$ ; and <sup>f</sup> $P < 0.0001$ . Different symbols were used in order to emphasize comparison within each etiology group. NAFLD: Non-alcoholic fatty liver disease.

### Fundic varices

The condition of fundic varices was found in 116 (45%) patients; evenly spread among women and men (46% and 44.4%, respectively,  $P > 0.05$ ). Etiology among patients with fundic varices was as follows: NAFLD in 39.7% (46 patients); hepatitis B in 33.6% (39 patients),

hepatitis C in 6.9% (8 patients) and other diseases in 19.8% (23 patients) ( $P > 0.05$ ). The incidence of fundic varices was 47.4% and 44.5% in patients with and without HCC, respectively ( $P > 0.05$ ), 43.9% and 53.6% in patients with and without cirrhosis, respectively ( $P > 0.05$ ), and 61% and 33% in patients with and without PVT, respectively ( $P < 0.0001$ ). Of the 111 patients (43%) that died during the follow-up study, 70 (63.1%) had no fundic varices ( $P = 0.024$ ). The mortality rate was 35.6% and 51.9% in those with and without fundic varices, respectively ( $P = 0.014$ ).

### HCC

HCC was detected in 14.7% of patients (38 out of 258 total study pool). As shown in Figure 1 and Table 2, the incidence rate of HCC was: 5.0% in patients with NAFLD, 26.7% in patients with hepatitis B, 34.5% in patients with hepatitis C, and 5.7% in other diseases ( $P < 0.0001$ ). Of the 38 patients with HCC, 13% had PVT (Table 3). Moreover, HCC increased the mortality rate in almost all the groups. The mortality rate in hepatitis B group increased from 31% (17/55) in patients with-

**Table 3** Incidence of portal vein thrombosis

	Cirrhosis ( $P > 0.05$ )	HCC ( $P > 0.05$ )	Fundic varices ( $P < 0.0001$ )	Mortality rate ( $P = 0.057$ )
PVT (+)	86% (93 patients)	13% (14 patients)	61% <sup>b</sup> (66 patients)	36.1% (39 patients)
PVT (-)	91% (137 patients)	16% (24 patients)	33% (50 patients)	48.0% (72 patients)

The incidence of portal vein thrombosis (PVT) in patients with cirrhosis, hepatocellular cancer (HCC) and fundic varices are shown. Also reported is the relationship between PVT and the mortality rate. Each of these pathologies and mortality rate was compared in patients with and without PVT. <sup>b</sup> $P < 0.0001$  in the presence *vs* absence of PVT.

**Table 4** Relationship among fundic varices, cirrhosis, hepatocellular cancer, and mortality rate

Fundic varices	Cirrhosis	HCC	Mortality rate
Yes	Yes	Yes	70.6%
		No	28.6%
	No	Yes	100%
No		No	28.6%
	Yes	Yes	85%
		No	23.1%
	No	Yes	No patients in this group
		No	23.1%

HCC: Hepatocellular cancer.

out HCC to 75% (15/20) in patients with HCC ( $P = 0.001$ ). In the group with hepatitis C, the mortality rate increased from 32% (6/19) in patients without HCC to 90% (9/10) in patients with HCC ( $P = 0.005$ ). The mortality rate in NAFLD patients increased from 47.5% during follow-up to 80% after HCC developed.

### Mortality

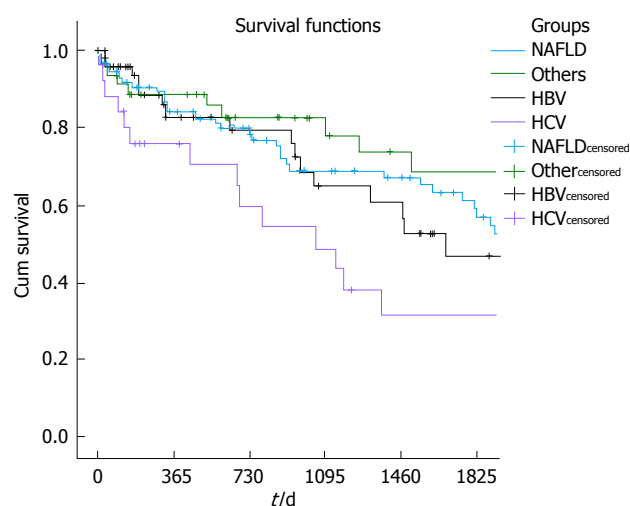
As shown in Table 4 and Figure 2, 111 (43%) patients died in this study during follow-up. Of the patients, 50.0% with NAFLD, 33.3% with hepatitis B, 26.3% with hepatitis C, and 58.3% with other diseases were alive at the end of the 5-year period with a significant difference according to the Kaplan-Meier log Rank test ( $P = 0.04$ ). Risk for mortality, measured by risk ratio (RR), did not change per gender (RR: male/female = 43.3%/42.5%,  $P > 0.05$ ) or with the occurrence of cirrhosis (RR: 44.8%/28.6%,  $P > 0.05$ ). However, it changed with the existence of fundic varices (RR: 49.3/35.3,  $P = 0.024$  in favor of fundic varices development) and HCC (RR: 78.9%/36.8%,  $P < 0.0001$  in favor of HCC development).

### NAFLD group

Of the 258 patients, 39.0% (101 patients) were diagnosed with NAFLD. The mean age of NAFLD was  $56.4 \pm 16.0$  years and 62% of these patients were men (Table 1). Moreover, 47.5% had PVT, 5.0% had HCC, and 45.5% had fundic varices (Table 2 and Figure 1). The mortality rate was 47.5% during follow-up (Table 2 and Figure 1), but increased to 80% in the presence of HCC.

## DISCUSSION

NAFLD-associated cirrhosis is predicted to rapidly



**Figure 2** Survival functions in patients with non-alcoholic fatty liver disease, hepatitis B, hepatitis C and other liver-related diseases (others). Of the patients, 50.0% with NAFLD, 33.3% with hepatitis B, 26.3% with hepatitis C, and 58.3% with other diseases were alive at the end of the 5-year period with a significant difference according to the Kaplan-Meier log Rank test ( $P = 0.040$ ). NAFLD: Non-alcoholic fatty liver disease; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

become the leading indicator for liver transplant in the Western world<sup>[24,25]</sup>. We herein show that NAFLD-associated cirrhosis is indeed the most common cause of end-stage liver disease at our liver center (Table 2 and Figure 1). By retrospectively evaluating national liver transplant database in the United States, Byrne *et al.*<sup>[26]</sup> and Charlton<sup>[27]</sup> and then, Wong *et al.*<sup>[24,27]</sup> showed a significant increase in the proportion of patients undergoing liver transplant due to NASH. These findings differ from studies carried out in Japan where the rate of seronegative cirrhotic patients was 5%-20%. This finding could be explained by the relatively lower incidence of NASH (1%-3%) by comparison to hepatitis B, hepatitis C and alcoholic liver disease in Japan. Among the cohort of our patients who needed liver transplant, a significantly larger proportion developed decompensated cirrhosis due to NAFLD than hepatitis B or C.

In a prospective longitudinal cohort study, Hui *et al.*<sup>[28]</sup> showed a comparable incidence and survival rate of cirrhosis related to hepatitis C to that related to NASH. Another large multi-center international study compared 247 patients with advanced fibrosis or cirrhosis secondary to NASH to 264 patients with chronic hepatitis C and similar stages of fibrosis<sup>[29]</sup>. In that study, 19.4% of NASH patients developed liver-related



complications and 13.4% either died or underwent liver transplantations during follow-up, as compared to patients with hepatitis C among whom 16.7% developed liver-related complications and 9.4% either died or required transplant surgery. Our observations of higher mortality rates in patients with NAFLD differ from previously reported survival data<sup>[28,30]</sup>. The first study<sup>[28]</sup> that investigated the survival rate in patients with NASH reported a 10-year survival rate of 84%. Then, Sanyal *et al.*<sup>[30]</sup> reported a 10-year mortality rate of 19.1% in patients with NASH cirrhosis as opposed to 4.1% in patients with compensated cirrhosis. Yatsuji *et al.*<sup>[31]</sup> observed a 5-year HCC rate of 11.3% for NASH-associated cirrhosis and 30.5% for HCV cirrhosis; and a 5-year survival rate was 75.2% in NASH-associated cirrhosis and 73.8% in HCV cirrhosis in a study carried out on Japanese patients. Our study found the mortality rate in NAFLD to be 46% during follow-up and 80% after HCC developed. These rates were higher than those in patients with hepatitis in whom mortality rate was 31%-32% in the absence of HCC, and increased to 75% and 90% in patients with hepatitis B and C after HCC developed, respectively. The cohort of this study showed significantly higher mortality in comparison to reports in other ethnic groups. This difference could be due to several factors, such as: (1) The severity of the disease in our cohort that included patients with cirrhosis and esophageal varices; and (2) a higher rate of consumption of diet rich in fat (red meat) and carbohydrates (sweets) in Turkey, as opposed to other countries where fish and white meat (chicken) are more commonly used. Although the role of ethnicity and/or genetics remains controversial, it is possible that the heterogeneity in terms of age, genetic and environmental factors in patients studied in other reports<sup>[1-4,14]</sup> contributes to the difference between their observations and those in the current studies. The observed higher mortality rate in our cohort could in part be attributed to its relative ethnical homogeneity since it basically consists of Caucasian patients from a Turkish origin.

Although NAFLD is a risk factor for HCC, the prevalence rate of HCC in cirrhotic NAFLD has not been well established, despite its reported range of 2.4% to 12.8%<sup>[32]</sup>. Scientists from Sweden described three and five cases of HCC in cohorts of 129 and 256 subjects with NAFLD followed for 13.7 and 21 years, respectively<sup>[33]</sup>. Previous reports indicated that the risk of HCC due to NAFLD is less than the risk resulting from chronic hepatitis C. In a 10-year prospective study, 10 out of 149 American patients with NAFLD-associated cirrhosis developed HCC compared to 25 out of 147 patients with hepatitis C virus-associated cirrhosis<sup>[4]</sup>. A large retrospective cohort study from South Korea evaluated 329 patients with HCC associated with fatty liver disease and demonstrated an increase in NAFLD-related HCC from 3.8% in 2001-2005 to 12.2% in 2006-2010<sup>[34]</sup>. A United States based study evaluated 195 NASH-cirrhosis patients from 2003-2007 with serial

abdominal computed tomography and serum alpha-fetoprotein every 6 mo with a median follow up of 3.2 years<sup>[35]</sup>. Among this cohort for NASH-related cirrhosis patients, 12.8% ( $n = 25$ ) developed HCC with an annual cumulative incidence rate of 2.6%. In a prospective cohort study, Yatsuji *et al.*<sup>[31]</sup> compared 68 patients with NASH-related cirrhosis to 69 age- and sex-matched patients with hepatitis C-related cirrhosis to determine HCC risk. Overall, the 5-year cumulative HCC rate was 11.3% for NASH patients and 30.5% for hepatitis C patients. This lower HCC risk among NAFLD-related cirrhosis patients compared with hepatitis C-related cirrhosis was also confirmed by our study. Our results with 5.0% NAFLD-related HCC with cirrhosis was lower than previously reported with 2.4%-12.8% in patients with NAFLD<sup>[32]</sup>.

The current studies revealed the prevalence of portal vein thrombosis in patients with NAFLD to be significantly higher than in patients with hepatitis B or hepatitis C ( $P < 0.0001$ ). This could be related to the predisposition of patients with NAFLD to developing pro-coagulation and impaired blood flow, as well as a pro-inflammatory state. It is well known that these patients are commonly obese. Obesity is associated with low-grade chronic inflammation and is strongly associated with chronic macrophage accumulation to the hypertrophied adipose tissue<sup>[36-39]</sup>. Adipose tissue macrophages produce proinflammatory cytokines such as tumor necrosis factor- $\alpha$ , interleukin-6, and C-reactive protein. These cytokines alter insulin signaling by protein kinase C  $\theta$ , inhibitor  $\kappa$ B kinase  $\beta$ , suppressors of cytokine signaling and inducible nitric oxide synthase to contribute to insulin resistance. Similarly, increased fat accumulation in liver alters its inflammatory milieu, thus modifying insulin action<sup>[40]</sup>. The metabolic syndrome and NAFLD are also independently associated with both atherosclerosis and endothelial vascular dysfunction, which are related to a prothrombotic state. Thus, increased systemic inflammation and increased procoagulant factor levels associated with insulin resistance could explain the higher prevalence of portal vein thrombosis in our cirrhotic patients with NAFLD.

Englesbe *et al.*<sup>[41]</sup> carried out a retrospective study evaluating the survival of 148 cirrhotic patients with occlusive portal vein thrombosis followed over a large period (1995-2007). The reported rate of death was 54.7%; significantly higher than the 37.2% in patients without portal vein thrombosis. These results are similar to our mortality data that show 65% incidence of death in cirrhotic patients with portal vein thrombosis vs 35% in patients without portal vein thrombosis. Additionally, the incidence of gastric varices was higher in NAFLD associated cirrhosis than other groups in our cohort.

It has also been reported that the incidence of portal vein thrombosis rises to 10%-40% in cirrhotic patients upon developing HCC<sup>[42]</sup>. Consistently, our study showed an elevated incidence of portal vein thrombosis in cirrhotic patients with hepatitis C or B after they developed HCC. In contrast, HCC failed to alter

the incidence of portal vein thrombosis in our cirrhotic patients with NAFLD.

Our studies suggest that increase in NASH-associated cirrhosis would be an indication for orthotopic liver transplantation in Turkey. Increased frequency of NASH-associated cirrhosis with portal vein thrombosis in clinical practice has been a subject of debate among transplant surgeons. Whereas the high incidence of PVT (up to 26%) in patients awaiting liver transplantation constitutes a risk factor for early post-liver transplantation mortality<sup>[43,44]</sup>, PVT is no longer considered an absolute contraindication for transplantation. Unfortunately, we could not reach the records of patients receiving transplant surgery in our studies to be able to assess more concretely the transplantation outcomes in our Turkish patients with NASH-associated cirrhosis and portal vein thrombosis. However, Quillin *et al*<sup>[16]</sup> have recently observed a strong indication for NASH in orthotopic liver transplantation in 2356 patients in the United States<sup>[16]</sup>, despite their older age by comparison to patients with hepatitis C and alcoholic cirrhosis. Whether this is related in part to the potential dominance of Caucasians in that study is unclear, but the study supports an equivalent, if not a more favorable, outcome for orthotopic liver transplantation in patients with fatty liver disease as compared to other common indications for surgery.

In conclusion, our data revealed a proportionally greater rise in liver transplant candidacy due to NAFLD-associated cirrhosis with portal vein thrombosis. The mortality rate of patients with NAFLD-associated cirrhosis did not differ from that in patients with virally caused cirrhosis. We confirmed that NAFLD was the third leading cause of HCC on the transplantation waiting list. Older patients were more prone to developing more cirrhosis, HCC and high mortality rates. However, the younger group had more portal vein thrombosis and fundic varices. These findings should constitute a reliable guideline for evaluating patients at the transplant center and for health policy makers to develop better strategic preventive measures against liver diseases.

## COMMENTS

### Background

Non-alcoholic fatty liver disease (NAFLD) predisposes to non-alcoholic steatohepatitis, which progresses to cirrhosis and hepatocellular carcinoma (HCC).

### Research frontiers

NAFLD-associated cirrhosis is predicted to rapidly become the leading indicator for liver transplant. The mortality rate of patients with NAFLD might differ from that in patients with virally caused cirrhosis.

### Innovations and breakthroughs

The authors' data revealed a proportionally greater rise in liver transplant candidacy due to NAFLD-associated cirrhosis with portal vein thrombosis. This could be related to the predisposition of patients with NAFLD to developing pro-coagulation and impaired blood flow, as well as a pro-inflammatory state. Their observations of higher mortality rates in patients with NAFLD differ from previously reported survival data. Older patients with esophageal varices were

more prone to developing cirrhosis, HCC and a high mortality rate.

### Applications

The authors' data revealed a proportionally greater rise in liver transplant candidacy due to NAFLD-associated cirrhosis with portal vein thrombosis. The underlying cause for this predisposition remains unclear, although both genetic and environmental factors could be implicated. These findings should constitute a reliable guideline to evaluate patients at the transplant center.

### Peer-review

This retrospective study describes NAFLD-related clinical diagnosis over 250 patients in Turkish origin. One unique strength of this study is to show higher risk of portal vein thrombosis and fundic varices, on the other hand, elderlies are more prone to cirrhosis, HCC and high mortality rates.

## REFERENCES

- 1 **Basaranoglu M**, Basaranoglu G, Sabuncu T, Sentürk H. Fructose as a key player in the development of fatty liver disease. *World J Gastroenterol* 2013; **19**: 1166-1172 [PMID: 23482247 DOI: 10.3748/wjg.v19.i8.1166]
- 2 **Caldwell S**, Argo C. The natural history of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 162-168 [PMID: 20460906 DOI: 10.1159/000282081]
- 3 **Mittal S**, Sada YH, El-Serag HB, Kanwal F, Duan Z, Temple S, May SB, Kramer JR, Richardson PA, Davila JA. Temporal trends of nonalcoholic fatty liver disease-related hepatocellular carcinoma in the veteran affairs population. *Clin Gastroenterol Hepatol* 2015; **13**: 594-601.e1 [PMID: 25148760 DOI: 10.1016/j.cgh.2014.08.013]
- 4 **Baffy G**, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. *J Hepatol* 2012; **56**: 1384-1391 [PMID: 22326465 DOI: 10.1016/j.jhep.2011.10.027]
- 5 **Schütte K**, Schulz C, Poranzke J, Antweiler K, Bornschein J, Bretschneider T, Arend J, Ricke J, Malfertheiner P. Characterization and prognosis of patients with hepatocellular carcinoma (HCC) in the non-cirrhotic liver. *BMC Gastroenterol* 2014; **14**: 117 [PMID: 24990270 DOI: 10.1186/1471-230X-14-117]
- 6 **Bencheqroun R**, Duvoux C, Luciani A, Zafrani ES, Dhumeaux D. [Hepatocellular carcinoma without cirrhosis in a patient with nonalcoholic steatohepatitis]. *Gastroenterol Clin Biol* 2004; **28**: 497-499 [PMID: 15243330 DOI: 10.1016/S0399-8320(04)94971-8]
- 7 **Bullock RE**, Zaitoun AM, Aithal GP, Ryder SD, Beekingham IJ, Lobo DN. Association of non-alcoholic steatohepatitis without significant fibrosis with hepatocellular carcinoma. *J Hepatol* 2004; **41**: 685-686 [PMID: 15464253 DOI: 10.1016/j.jhep.2004.05.008]
- 8 **Gonzalez L**, Blanc JF, Sa Cunha A, Rullier A, Saric J, Le Bail B, Balabaud C, Bioulac-Sage P. Obesity as a risk factor for hepatocellular carcinoma in a noncirrhotic patient. *Semin Liver Dis* 2004; **24**: 415-419 [PMID: 15605309 DOI: 10.1055/s-2004-860870]
- 9 **Hai S**, Kubo S, Shuto T, Tanaka H, Takemura S, Yamamoto T, Kanazawa A, Ogawa M, Hirohashi K, Wakasa K. Hepatocellular carcinoma arising from nonalcoholic steatohepatitis: report of two cases. *Surg Today* 2006; **36**: 390-394 [PMID: 16554999 DOI: 10.1007/s00595-005-3167-4]
- 10 **Hashizume H**, Sato K, Takagi H, Hirokawa T, Kojima A, Sohara N, Kakizaki S, Mochida Y, Shimura T, Sunose Y, Ohwada S, Mori M. Primary liver cancers with nonalcoholic steatohepatitis. *Eur J Gastroenterol Hepatol* 2007; **19**: 827-834 [PMID: 17873605 DOI: 10.1097/MEG.0b013e3282748ef2]
- 11 **Sung KC**, Ryan MC, Wilson AM. The severity of nonalcoholic fatty liver disease is associated with increased cardiovascular risk in a large cohort of non-obese Asian subjects. *Atherosclerosis* 2009; **203**: 581-586 [PMID: 18774133 DOI: 10.1016/j.atherosclerosis.2008.07.024]
- 12 **Sinn DH**, Gwak GY, Park HN, Kim JE, Min YW, Kim KM, Kim YJ, Choi MS, Lee JH, Koh KC, Paik SW, Yoo BC. Ultrasonographically detected non-alcoholic fatty liver disease is an independent predictor for identifying patients with insulin resistance in non-obese, non-

- diabetic middle-aged Asian adults. *Am J Gastroenterol* 2012; **107**: 561-567 [PMID: 22108448 DOI: 10.1038/ajg.2011.400]
- 13 **Farrell GC**, Wong VW, Chitturi S. NAFLD in Asia--as common and important as in the West. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 307-318 [PMID: 23458891 DOI: 10.1038/nrgastro.2013.34]
- 14 **Basaranoglu M**, Basaranoglu G, Sentürk H. From fatty liver to fibrosis: a tale of "second hit". *World J Gastroenterol* 2013; **19**: 1158-1165 [PMID: 23483818 DOI: 10.3748/wjg.v19.i8.1158]
- 15 **Siddiqui MS**, Fuchs M, Idowu MO, Luketic VA, Boyett S, Sargeant C, Stravitz RT, Puri P, Matherly S, Sterling RK, Contos M, Sanyal AJ. Severity of nonalcoholic fatty liver disease and progression to cirrhosis are associated with atherogenic lipoprotein profile. *Clin Gastroenterol Hepatol* 2015; **13**: 1000-1008.e3 [PMID: 25311381 DOI: 10.1016/j.cgh.2014.10.008]
- 16 **Quillin RC**, Wilson GC, Sutton JM, Hanseman DJ, Paterno F, Cuffy MC, Paquette IM, Diwan TS, Woodle ES, Abbott DE, Shah SA. Increasing prevalence of nonalcoholic steatohepatitis as an indication for liver transplantation. *Surgery* 2014; **156**: 1049-1056 [PMID: 25239365 DOI: 10.1016/j.surg.2014.06.075]
- 17 **Seko Y**, Sumida Y, Tanaka S, Taketani H, Kanemasa K, Ishiba H, Okajima A, Nishimura T, Yamaguchi K, Moriguchi M, Mitsuyoshi H, Yasui K, Minami M, Itoh Y. Predictors of malignancies and overall mortality in Japanese patients with biopsy-proven non-alcoholic fatty liver disease. *Hepatol Res* 2015; **45**: 728-738 [PMID: 25165040 DOI: 10.1111/hepr.12407]
- 18 **Önnerhag K**, Nilsson PM, Lindgren S. Increased risk of cirrhosis and hepatocellular cancer during long-term follow-up of patients with biopsy-proven NAFLD. *Scand J Gastroenterol* 2014; **49**: 1111-1118 [PMID: 24990583 DOI: 10.3109/00365521.2014.934911]
- 19 **Yeh MM**, Brunt EM. Pathological features of fatty liver disease. *Gastroenterology* 2014; **147**: 754-764 [PMID: 25109884 DOI: 10.1053/j.gastro.2014.07.056]
- 20 **Iseri A**, Arslan N. Obesity in adults in Turkey: age and regional effects. *Eur J Public Health* 2009; **19**: 91-94 [PMID: 19091784 DOI: 10.1093/eurpub/ckn107]
- 21 **Acar A**, Kemahli S, Altunay H, Kosan E, Oncul O, Gorenek L, Cavuslu S. HBV, HCV and HIV seroprevalence among blood donors in Istanbul, Turkey: how effective are the changes in the national blood transfusion policies? *Braz J Infect Dis* 2010; **14**: 41-46 [PMID: 20428653]
- 22 **Acar A**, Kemahli S, Altunay H, Kosan E, Oncul O, Gorenek L, Cavuslu S. The significance of repeat testing in Turkish blood donors screened with HBV, HCV and HIV immunoassays and the importance of S/CO ratios in the interpretation of HCV/HIV screening test results and as a determinant for further confirmatory testing. *Transfus Med* 2010; **20**: 152-159 [PMID: 20059750 DOI: 10.1111/j.1365-3148.2009.00987.x]
- 23 **Sarin SK**, Kumar A. Gastric varices: profile, classification, and management. *Am J Gastroenterol* 1989; **84**: 1244-1249 [PMID: 2679046]
- 24 **Wong RJ**, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, Ahmed A. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015; **148**: 547-555 [PMID: 25461851 DOI: 10.1053/j.gastro.2014.11.039]
- 25 **Wong RJ**, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014; **59**: 2188-2195 [PMID: 24375711 DOI: 10.1002/hep.26986]
- 26 **Byrne CD**, Targher G. NAFLD: a multisystem disease. *J Hepatol* 2015; **62**: S47-S64 [PMID: 25920090 DOI: 10.1016/j.jhep.2014.12.012]
- 27 **Charlton M**. Evolving aspects of liver transplantation for non-alcoholic steatohepatitis. *Curr Opin Organ Transplant* 2013; **18**: 251-258 [PMID: 23652610 DOI: 10.1097/MOT.0b013e3283615d30]
- 28 **Hui JM**, Kench JG, Chitturi S, Sud A, Farrell GC, Byth K, Hall P, Khan M, George J. Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. *Hepatology* 2003; **38**: 420-427 [PMID: 12883486 DOI: 10.1053/jhep.2003.50320]
- 29 **Bhala N**, Angulo P, van der Poorten D, Lee E, Hui JM, Saracco G, Adams LA, Charatcharoenwittaya P, Topping JH, Bugianesi E, Day CP, George J. The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. *Hepatology* 2011; **54**: 1208-1216 [PMID: 21688282 DOI: 10.1002/hep.24491]
- 30 **Sanyal AJ**, Banas C, Sargeant C, Luketic VA, Sterling RK, Stravitz RT, Shiffman ML, Heuman D, Coterrell A, Fisher RA, Contos MJ, Mills AS. Similarities and differences in outcomes of cirrhosis due to nonalcoholic steatohepatitis and hepatitis C. *Hepatology* 2006; **43**: 682-689 [PMID: 16502396 DOI: 10.1002/hep.21103]
- 31 **Yatsuji S**, Hashimoto E, Tobari M, Taniai M, Tokushige K, Shiratori K. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. *J Gastroenterol Hepatol* 2009; **24**: 248-254 [PMID: 19032450 DOI: 10.1111/j.1440-1746.2008.05640.x]
- 32 **White DL**, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. *Clin Gastroenterol Hepatol* 2012; **10**: 1342-1359.e2 [PMID: 23041539 DOI: 10.1016/j.cgh.2012.10.001]
- 33 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]
- 34 **Lee SS**, Jeong SH, Byoun YS, Chung SM, Seong MH, Sohn HR, Min BY, Jang ES, Kim JW, Park GJ, Lee YJ, Lee KH, Ahn S. Clinical features and outcome of cryptogenic hepatocellular carcinoma compared to those of viral and alcoholic hepatocellular carcinoma. *BMC Cancer* 2013; **13**: 335 [PMID: 23829392 DOI: 10.1186/1471-2407-13-335]
- 35 **Ascha MS**, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972-1978 [PMID: 20209604 DOI: 10.1002/hep.23527]
- 36 **Basaranoglu M**, Basaranoglu G. Pathophysiology of insulin resistance and steatosis in patients with chronic viral hepatitis. *World J Gastroenterol* 2011; **17**: 4055-4062 [PMID: 22039318 DOI: 10.3748/wjg.v17.i36.4055]
- 37 **Basaranoglu M**, Kayacetin S, Yilmaz N, Kayacetin E, Tarcin O, Sonsuz A. Understanding mechanisms of the pathogenesis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2010; **16**: 2223-2226 [PMID: 20458758 DOI: 10.3748/wjg.v16.i18.2223]
- 38 **Tetri LH**, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G987-G995 [PMID: 18772365 DOI: 10.1152/ajpgi.90272.2008]
- 39 **Neuschwander-Tetri BA**, Ford DA, Acharya S, Gilkey G, Basaranoglu M, Tetri LH, Brunt EM. Dietary trans-fatty acid induced NASH is normalized following loss of trans-fatty acids from hepatic lipid pools. *Lipids* 2012; **47**: 941-950 [PMID: 22923371]
- 40 **Najjar SM**, Russo L. CEACAM1 loss links inflammation to insulin resistance in obesity and non-alcoholic steatohepatitis (NASH). *Semin Immunopathol* 2014; **36**: 55-71 [PMID: 24258517 DOI: 10.1007/s00281-013-0407-3]
- 41 **Englesbe MJ**, Kubus J, Muhammad W, Sonnenday CJ, Welling T, Punch JD, Lynch RJ, Marrero JA, Pelletier SJ. Portal vein thrombosis and survival in patients with cirrhosis. *Liver Transpl* 2010; **16**: 83-90 [PMID: 20035521 DOI: 10.1002/lt.21941]
- 42 **Giorgio A**, Calisti G, Montesarchio L, Scognamiglio U, Matteucci P, Coppola C, Scarano F, Amendola F, Giorgio V. Hepatocellular carcinoma invading portal venous system in cirrhosis: long-term results of percutaneous radiofrequency ablation of both the nodule and portal vein tumor thrombus. A case control study. *Anticancer Res* 2014; **34**: 6785-6790 [PMID: 25368292]
- 43 **Tarantino G**, Citro V, Esposito P, Giaquinto S, de Leone A, Milan G, Tripodi FS, Cirillo M, Lobello R. Blood ammonia levels in liver cirrhosis: a clue for the presence of portosystemic collateral veins. *BMC Gastroenterol* 2009; **9**: 21 [PMID: 19292923 DOI:

- 10.1186/1471-230X-9-21]  
44 **Tarantino G**, Citro V, Conca P, Riccio A, Tarantino M, Capone D, Cirillo M, Lobello R, Iaccarino V. What are the implications

of the spontaneous spleno-renal shunts in liver cirrhosis? *BMC Gastroenterol* 2009; **9**: 89 [PMID: 19930687 DOI: 10.1186/1471-230X-9-89]

**P- Reviewer:** Kita K, Mikolasevic I, Tarantino G **S- Editor:** Qi Y  
**L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 March 18; 8(8): 385-420





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 469 members, representing a team of worldwide experts in hepatology. They are from 53 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (1), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (98), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (19), Greece (11), Hungary (5), India (15), Indonesia (2), Iran (4), Israel (1), Italy (52), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (11), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (17), and United States (56).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*

Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*  
Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*

Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *São Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*



**Chile**

Luis A Videla, *Santiago*



## China

Guang-Wen Cao, Shanghai  
 En-Qiang Chen, Chengdu  
 Gong-Ying Chen, Hangzhou  
 Jin-lian Chen, Shanghai  
 Jun Chen, Changsha  
 Alfred Cheng, Hong Kong  
 Chun-Ping Cui, Beijing  
 Shuang-Suo Dang, Xi'an  
 Ming-Xing Ding, Jinhua  
 Zhi-Jun Duang, Dalian  
 He-Bin Fan, Wuhan  
 Xiao-Ming Fan, Shanghai  
 James Yan Yue Fung, Hong Kong  
 Yi Gao, Guangzhou  
 Zuo-Jiong Gong, Wuhan  
 Zhi-Yong Guo, Guangzhou  
 Shao-Liang Han, Wenzhou  
 Tao Han, Tianjin  
 Jin-Yang He, Guangzhou  
 Ming-Liang He, Hong Kong  
 Can-Hua Huang, Chengdu  
 Bo Jin, Beijing  
 Shan Jin, Hohhot  
 Hui-Qing Jiang, Shijiazhuang  
 Wan-Yee Joseph Lau, Hong Kong  
 Guo-Lin Li, Changsha  
 Jin-Jun Li, Shanghai  
 Qiang Li, Jinan  
 Sheng Li, Jinan  
 Zong-Fang Li, Xi'an  
 Xu Li, Guangzhou  
 Xue-Song Liang, Shanghai  
 En-Qi Liu, Xi'an  
 Pei Liu, Shenyang  
 Zhong-Hui Liu, Changchun  
 Guang-Hua Luo, Changzhou  
 Yi Lv, Xi'an  
 Guang-Dong Pan, Liuzhou  
 Wen-Sheng Pan, Hangzhou  
 Jian-Min Qin, Shanghai  
 Wai-Kay Seto, Hong Kong  
 Hong Shen, Changsha  
 Xiao Su, Shanghai  
 Li-Ping Sun, Beijing  
 Wei-Hao Sun, Nanjing  
 Xue-Ying Sun, Harbin  
 Hua Tang, Tianjin  
 Ling Tian, Shanghai  
 Eric Tse, Hong Kong  
 Guo-Ying Wang, Changzhou  
 Yue Wang, Beijing  
 Shu-Qiang Wang, Chengdu  
 Mary MY Wayne, Hong Kong  
 Hong-Shan Wei, Beijing  
 Danny Ka-Ho Wong, Hong Kong  
 Grace Lai-Hung Wong, Hong Kong  
 Bang-Fu Wu, Dongguan  
 Feng Wu, Chongqing  
 Xiong-Zhi Wu, Tianjin  
 Chun-Fang Xu, Suzhou  
 Rui-An Xu, Quanzhou  
 Rui-Yun Xu, Guangzhou  
 Wei-Li Xu, Shijiazhuang  
 Shi-Ying Xuan, Qingdao  
 Ming-Xian Yan, Jinan  
 Lv-Nan Yan, Chengdu  
 Jin Yang, Hangzhou  
 Ji-Hong Yao, Dalian  
 Winnie Yeo, Hong Kong

Zheng Zeng, Beijing  
 Qi Zhang, Hangzhou  
 Shi-Jun Zhang, Guangzhou  
 Xiao-Lan Zhang, Shijiazhuang  
 Xiao-Yong Zhang, Guangzhou  
 Xin-Chen Zhang, Harbin  
 Yong Zhang, Xi'an  
 Hong-Chuan Zhao, Hefei  
 Ming-Hua Zheng, Wenzhou  
 Yu-Bao Zheng, Guangzhou  
 Ren-Qian Zhong, Shanghai  
 Fan Zhu, Wuhan  
 Xiao Zhu, Dongguan



## Czech Republic

Kamil Vysloulzil, Olomouc



## Denmark

Henning Gronbaek, Aarhus  
 Christian Mortensen, Hvidovre



## Egypt

Ihab T Abdel-Raheem, Damanhour  
 NGB G Bader EL Din, Cairo  
 Hatem Elalfy, Mansoura  
 Mahmoud M El-Bendary, Mansoura  
 Mona El SH El-Raziky, Cairo  
 Mohammad El-Sayed, Cairo  
 Yasser M Fouad, Minia  
 Mohamed AA Metwally, Benha  
 Hany Shehab, Cairo  
 Mostafa M Sira, Shebin El-koom  
 Ashraf Taye, Minia  
 MA Ali Wahab, Mansoura



## France

Laurent Alric, Toulouse  
 Sophie Conchon, Nantes  
 Daniel J Felmlee, Strasbourg  
 Herve Lerat, Creteil  
 Dominique Salmon, Paris  
 Jean-Pierre Vartanian, Paris



## Germany

Laura E Buitrago-Molina, Hannover  
 Enrico N De Toni, Munich  
 Oliver Ebert, Muenchen  
 Rolf Gebhardt, Leipzig  
 Janine V Hartl, Regensburg  
 Sebastian Hinz, Kiel  
 Benjamin Juntermanns, Essen  
 Roland Kaufmann, Jena  
 Viola Knop, Frankfurt  
 Veronika Lukacs-Kornek, Homburg  
 Benjamin Maasoumy, Hannover  
 Jochen Mattner, Erlangen  
 Nadja M Meindl-Beinker, Mannheim  
 Ulf P Neumann, Aachen  
 Margarete Odenthal, Cologne  
 Yoshiaki Sunami, Munich

Christoph Roderburg, Aachen  
 Frank Tacke, Aachen  
 Yuchen Xia, Munich



## Greece

Alex P Betrosian, Athens  
 George N Dalekos, Larissa  
 Ioanna K Delladetsima, Athens  
 Nikolaos K Gatselis, Larissa  
 Stavros Gourgiotis, Athens  
 Christos G Savopoulos, Thessaloniki  
 Tania Siahaniidou, Athens  
 Emmanouil Sinakos, Thessaloniki  
 Nikolaos G Symeonidi, Thessaloniki  
 Konstantinos C Thomopoulos, Larissa  
 Konstantinos Tziomalos, Thessaloniki



## Hungary

Gabor Banhegyi, Budapest  
 Peter L Lakatos, Budapest  
 Maria Papp, Debrecen  
 Ferenc Sipos, Budapest  
 Zsolt J Tulassay, Budapest



## India

Deepak N Amarapurkar, Mumbai  
 Girish M Bhopale, Pune  
 Sibnarayan Datta, Tezpur  
 Nutan D Desai, Mumbai  
 Sorabh Kapoor, Mumbai  
 Jaswinder S Maras, New Delhi  
 Nabeen C Nayak, New Delhi  
 C Ganesh Pai, Manipal  
 Amit Pal, Chandigarh  
 K Rajeshwari, New Delhi  
 Anup Ramachandran, Vellore  
 D Nageshwar Reddy, Hyderabad  
 Shivaram P Singh, Cuttack  
 Ajith TA, Thrissur  
 Balasubramaniyan Vairappan, Pondicherry



## Indonesia

Cosmas RA Lesmana, Jakarta  
 Neneng Ratnasari, Yogyakarta



## Iran

Seyed M Jazayeri, Tehran  
 Sedigheh Kafi-Abad, Tehran  
 Iradj Maleki, Sari  
 Fakhraddin Naghibalhossaini, Shiraz



## Israel

Stephen DH Malnick, Rehovot



## Italy

Francesco Angelico, Rome



Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Andrea Galli, *Florence*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Salvatore Gruttadauria, *Palermo*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verducci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*



**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*  
 Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*

Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhito Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*



**Jordan**

Kamal E Bani-Hani, *Zarqa*



**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*



**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*



**Moldova**

Angela Peltec, *Chishinev*



**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*



**Nigeria**

CA Asabamaka Onyekwere, *Lagos*



**Pakistan**

Bikha Ram Devrajani, *Jamshoro*



**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*



**Poland**

Jacek Zielinski, *Gdansk*



**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*



**Qatar**

Reem Al Olaby, *Doha*



**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*



**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*



**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*



**Singapore**

Ser Yee Lee, *Singapore*



**South Korea**

Young-Hwa Chung, *Seoul*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonju*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*



**Spain**

Ivan G Marina, *Madrid*  
 Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*

José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodríguez-Frias, *Córdoba*  
 Manuel L Rodríguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Traperó-Marugán, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpaboon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*  
 Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*

Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*



#### **United States**

Naim Alkhouri, *Cleveland*  
 Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*

Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Grigoriy E Gurvits, *New York*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Kusum K Kharbanda, *Omaha*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**MINIREVIEWS**

- 385 Management of hepatitis B reactivation in immunosuppressed patients: An update on current recommendations

*Bessone F, Dirchwolf M*

- 395 Management of human factors engineering-associated hemochromatosis: A 2015 update

*Sivakumar M, Powell LW*

**ORIGINAL ARTICLE****Basic Study**

- 401 Role of interleukin-1 and its antagonism of hepatic stellate cell proliferation and liver fibrosis in the Abcb4<sup>-/-</sup> mouse model

*Reiter FP, Wimmer R, Wotke L, Artmann R, Nagel JM, Carranza MO, Mayr D, Rust C, Fickert P, Trauner M, Gerbes AL, Hohenester S, Denk GU*

**Retrospective Study**

- 411 Retrocaval liver lifting maneuver and modifications of total hepatic vascular exclusion for liver tumor resection

*Ko S, Kirihataya Y, Matsumoto Y, Takagi T, Matsusaka M, Mukogawa T, Ishikawa H, Watanabe A*

## Contents

*World Journal of Hepatology*  
Volume 8 Number 8 March 18, 2016

### ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Xiao-Ming Fan, MD, PhD, Professor, Department of Gastroenterology, Jinshan Hospital of Fudan University, Shanghai 201508, China

### AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

### FLYLEAF

I-IV Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
March 18, 2016

#### COPYRIGHT

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

#### SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

#### INSTRUCTIONS TO AUTHORS

Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjgnet.com/bpg/g_info_20160116143427.htm)

#### ONLINE SUBMISSION

<http://www.wjgnet.com/esps/>



## Management of hepatitis B reactivation in immunosuppressed patients: An update on current recommendations

Fernando Bessone, Melisa Dirchwolf

Fernando Bessone, Department of Gastroenterology and Hepatology, School of Medicine, University of Rosario, Rosario 2000, Argentina

Melisa Dirchwolf, Department of Hepatology, Muñiz Hospital, Buenos Aires 1282, Argentina

**Author contributions:** Bessone F and Dirchwolf M contributed equally to this review article.

**Conflict-of-interest statement:** None of the authors have received fees for serving as a speaker or consultant, nor have they received research funding in relation to this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Fernando Bessone, MD, Full Professor of Gastroenterology, Department of Gastroenterology and Hepatology, School of Medicine, University of Rosario, Urquiza 3100, Rosario 2000, Argentina. [bessonefernando@gmail.com](mailto:bessonefernando@gmail.com)  
 Telephone: +54-341-4393511  
 Fax: +54-341-4393511

Received: May 23, 2015  
 Peer-review started: May 25, 2015  
 First decision: August 16, 2015  
 Revised: January 15, 2016  
 Accepted: March 7, 2016  
 Article in press: March 9, 2016  
 Published online: March 18, 2016

### Abstract

The proportion of hepatitis B virus (HBV) previously

exposed patients who receive immunosuppressive treatment is usually very small. However, if these individuals are exposed to potent immunosuppressive compounds, the risk of HBV reactivation (HBVr) increases with the presence of hepatitis B surface antigen (HBsAg) in the serum. Chronic HBsAg carriers have a higher risk than those who have a total IgG anticore as the only marker of resolved/occult HBV disease. The loss of immune control in these patients may result in the reactivation of HBV replication within hepatocytes. Upon reconstitution of the immune system, infected hepatocytes are once again targeted and damaged by immune surveillance in an effort to clear the virus. There are different virological scenarios, and a wide spectrum of associated drugs with specific and stratified risk for the development of HBVr. Some of these agents can trigger a severe degree of hepatocellular damage, including hepatitis, acute liver failure, and even death despite employment of effective antiviral therapies. Currently, HBVr incidence seems to be increasing around the world; a fact mainly related to the incessant appearance of more powerful immunosuppressive drugs launched to the market. Moreover, there is no consensus on the length of prophylactic treatment before the patients are treated with immunosuppressive therapy, and for how long this therapy should be extended once treatment is completed. Therefore, this review article will focus on when to treat, when to monitor, what patients should receive HBV therapy, and what drugs should be selected for each scenario. Lastly, we will update the definition, risk factors, screening, and treatment recommendations based on both current and different HBV management guidelines.

**Key words:** Anti-tumor necrosis factor- $\alpha$  drugs; Acute liver failure; Biologic therapy; Immunosuppressive therapy; Hepatitis B

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Chronic hepatitis B surface antigen carriers have a high risk to develop hepatitis B virus (HBV) reactivation (HBVr) when exposed to immunosuppressive therapy. The loss of immune control in these patients may result in an increase in HBV replication. There is a wide spectrum of associated drugs with specific and stratified risk for the development of HBVr. Currently, HBVr incidence seems to increase worldwide, mainly due to the appearance of more powerful immunosuppressive drugs. This review article focuses on when to treat, when to monitor, what patients should receive HBV therapy, and what drugs should be selected in each scenario. We updated here current HBV management guidelines.

Bessone F, Dirchwolf M. Management of hepatitis B reactivation in immunosuppressed patients: An update on current recommendations. *World J Hepatol* 2016; 8(8): 385-394 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i8/385.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i8.385>

## INTRODUCTION

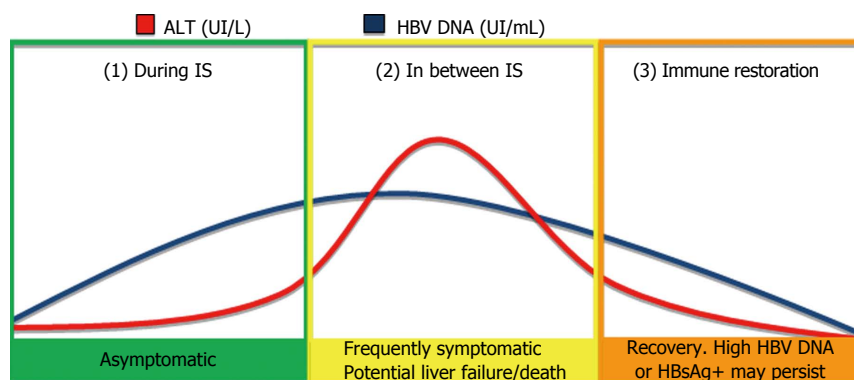
Hepatitis B virus (HBV) infection is a major public health problem worldwide; roughly, 30% of the world population shows serological evidence of current or past infection<sup>[1]</sup>, and it is largely considered that there are 350 million chronic carriers globally<sup>[2,3]</sup>. Although chronic infection can lead to progressive liver injury, most patients (60%-85%) are asymptomatic, and therefore the infection remains unrecognized until the appearance of signs or symptoms of chronic liver disease/cirrhosis<sup>[4,5]</sup>. Even though a small percentage of patients who were previously in contact with HBV will probably require immunosuppression as treatment of different illnesses (malignant, autoimmune, chronic rheumatic diseases) or to avoid post-transplantation rejection. However, treatment with such agents raises the risk of HBV reactivation (HBVr). This holds particularly true for patients with previously undetected chronic HBV infection, but also for those with resolved or occult infection [hepatitis B surface antigen (HBsAg)-negative, antibody to hepatitis B core antigen (antiHBc)-positive, with or without antibody to hepatitis B surface antigen (antiHBs)-positive serology]<sup>[6-10]</sup>. These events are referred to as HBVr; they were first described 40 years ago as a complication of renal transplantation and cancer chemotherapy. Since then, HBVr has become well recognized in numerous immunosuppression (IS) settings. Despite of that, HBVr due to IS continues to cause severe hepatitis, liver failure, and even death in spite of the availability of effective HBV vaccines, easily available and cheap tests to define patients at risk, and safe and effective antiviral therapies. A more worrisome issue is that the occurrence of this severe clinical event appears to increase around the globe<sup>[11]</sup>; perhaps, this is due to the permanent changing landscape of IS

agents involved, the heterogeneous screening, definition and treatment guidelines, and the multiple available therapeutic options<sup>[12]</sup>. This revision aims to update HBVr definition, risk factors, screening, and treatment recommendations based on the currently published evidence.

## HOST AND VIRAL INTERACTION: HBVR CLINICAL FEATURES

Hepatocellular inflammation and injury in HBV infection is suggested to be directly related to the intensity of host immune response<sup>[13]</sup>. In the initial phase of immune tolerance, infected children have high levels of viral replication, with no associated liver injury. As the immune system matures, the infected person enters a phase of immune clearance, in which the hepatocytes infected with HBV are targeted and damaged, resulting in hepatitis flares. In most individuals, the immune system is eventually able to control viremia, leading to hepatitis B e antigen clearance, suppression of HBV DNA levels, and normalization of liver biochemical test. The immune control phase usually endures; however, in cases of iatrogenic or natural IS, the loss of immune control results in reactivation of HBV replication inside hepatocytes. Upon reconstitution of the immune system, these hepatocytes are once again targeted and damaged by immune surveillance, in an effort to clear the virus<sup>[5,14,15]</sup>. HBVr has been described as a three-phase event (Figure 1). Initially, an increase in HBV DNA levels in an HBsAg positive person, or a reappearance of either HBsAg (seroreversion) or HBV DNA occurs; this period is usually asymptomatic. When the following phase takes place, HBV DNA levels show a sustained increase in viral load, accompanied by concomitant elevations in aminotransferase levels, which may also be associated to the development of severe hepatocellular damage; to note, even acute liver failure and ultimately death may occur. The aforementioned events result from a reconstitution syndrome of the host immune response. Finally, liver damage resolves due to recovery of the immune system strength (spontaneously or as a result of immunosuppressive therapy suspension) or due to the administration of antiviral drugs. This event may result in complete resolution of hepatic inflammation, or in fewer cases, a higher HBV DNA viral load in previously HBsAg positive patients can be observed<sup>[3,14,15]</sup>. Despite the fact that HBVr is usually found in chronically infected patients; it has also been reported in patients with resolved or occult HBV infection (*i.e.*, HBsAg negative, antiHBc positive), since these individuals still have traces of HBV DNA replication in their liver.

The reactivation risk depends on a combination between the degree and duration of IS<sup>[4,16]</sup>. Reactivation of HBV replication during IS can occur in an indirect fashion, through abolition of specific T-cell control, but also in a straightforward manner, when stimulation of a glucocorticoid-responsive element in the HBV



**Figure 1 Hepatitis B reactivation phases.** In the initial phase, there is an increase in HBV DNA levels, usually with an asymptomatic evolution. In the second phase, both ALT and HBV DNA are elevated; symptoms are frequently present, and they may be severe. The third phase is determined by resolution, although HBsAg (if reappeared), or elevated HBV DNA, may persist<sup>[3,17,55]</sup>. IS: Immunosuppression; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; HBsAg: Hepatitis B surface antigen.

genome occurs, leading to up regulation of HBV gene expression<sup>[17]</sup>.

## HBVR DEFINITIONS: A HETEROGENEOUS GROUP

One of the major difficulties assessing the impact of HBVr is the different diagnostic criteria found in the literature<sup>[16]</sup>. Although reports of HBVr and its consequences are not scarce, the data are often difficult to contrast, because of the different definitions used. Some studies consider HBV DNA level elevations<sup>[18]</sup>, some evaluate reappearance of HBsAg<sup>[19]</sup>, and others evaluate episodes of hepatitis syndrome, utilizing different grading (severity) systems<sup>[4]</sup>. Consensus has not been reached, even in the major clinical guidelines; both the European Association for the Study of the Liver (EASL) and Asian Pacific Association for the Study of the Liver (APASL) chronic hepatitis B guidelines, when addressing HBVr, considers HBsAg seroreversion and rise in HBV DNA levels as diagnostic criteria<sup>[20,21]</sup>, whereas the American Association for the Study of Liver Diseases (AASLD) defines HBVr as reappearance of active necro-inflammatory disease of the liver in an individual at an inactive HBsAg carrier state or who was known to have resolved hepatitis B<sup>[5]</sup>. Recently, at the Reactivation of Hepatitis B AASLD meeting held in 2013, the first attempt to establish a standardized nomenclature was made. Reactivation of HBV replication was defined as a marked increase in HBV replication ( $\geq 2$  log increase from baseline levels or a new appearance of HBV DNA to a level of  $\geq 100$  IU/mL) in a person with previously stable or undetectable levels. The types of reactivation were described as reverse HBsAg seroconversion (reappearance of HBsAg), or appearance of HBV DNA in serum in the absence of HBsAg. The severity of reactivation, defined by the presence or absence of jaundice and liver failure; and its outcome (return to baseline status or persistence in an activated state, need for liver transplantation or death) should also be reported<sup>[2,17]</sup>. A universal grading system that also

includes the consequences related to the IS therapy was recently proposed by Visram *et al.*<sup>[4]</sup>, in an additional effort to standardize HBVr grading and its consequences.

## MEDICAL INTERVENTIONS ASSOCIATED WITH HBVR

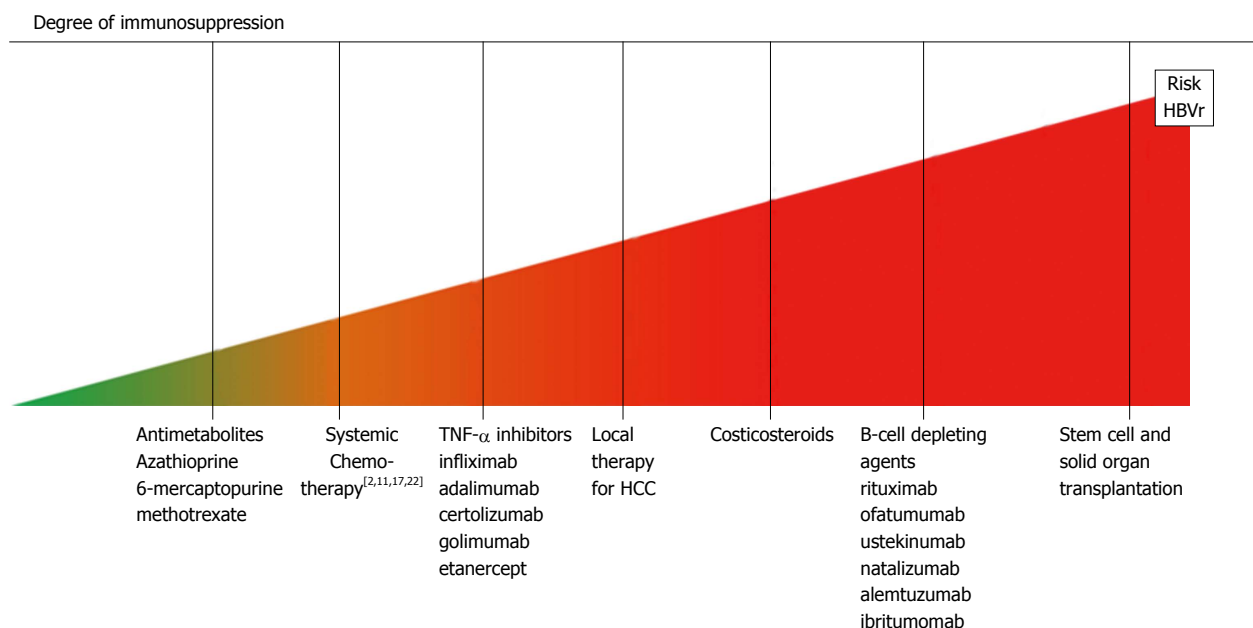
Several agents have been associated with the risk of HBVr, depending on the type and intensity of IS caused by these medical interventions<sup>[2]</sup>. The most relevant ones are described below, and displayed according to the risk of HBVr in Figure 2.

### Antimetabolites

HBVr during IS with low doses of azathioprine or methotrexate, when used as monotherapy, is uncommon<sup>[3,22]</sup>. In a thorough review recently published, no report was found in which azathioprine used alone was documented to cause HBVr<sup>[12]</sup>. Similarly, although several reports associated with methotrexate-induced HBVr are available, most of them involved the concomitant use of other immunomodulators<sup>[22]</sup>. Indeed, this antimetabolite has been in clinical use for more than 50 years, and only a small number of cases have been described in published reports in which HBVr was attributable to this agent when used alone. Based on these findings, they are considered to be drugs with low risk of HBVr<sup>[12,23]</sup>.

### Tumor necrosis factor- $\alpha$ inhibitors

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a pro-inflammatory and immunoregulatory cytokine involved in the pathogenesis of several inflammatory disorders. The inhibition of TNF- $\alpha$  signaling can lead to increased HBV replication and reactivation<sup>[2,24]</sup>. Anti-TNF- $\alpha$  agents are approved to treat rheumatoid arthritis, intestinal inflammatory diseases and psoriasis; in this context, several (*e.g.*, infliximab, adalimumab, certolizumab, golimumab and etanercept) have been associated with HBVr<sup>[22]</sup>. In a meta-analysis published by Lee *et al.*<sup>[25]</sup> evaluating the risk of HBVr in 468 isolated antiHBc patients with rheumatic conditions treated with different anti-TNF- $\alpha$



**Figure 2** Immunosuppressing agents and related risk of hepatitis B reactivation. HCC: Hepatocellular carcinoma; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; HBVr: Hepatitis B virus reactivation.

(mostly etanercept), HBVr was found in 1.7% of the cases. The same author reported much higher rates of HBVr in HBsAg-positive patients (12.3%) in a similar cohort of rheumatic patients<sup>[26]</sup>. Additionally, several severe HBVr have been communicated, particularly following infliximab administration<sup>[3,27]</sup>. However, it is unclear whether the risk of HBVr is the same with every TNF- $\alpha$  inhibitor. Most cases have been associated with the more potent IS drugs such as infliximab or adalimumab rather than etanercept. Comparative risk assessment between these agents is doubtful when incidence is derived from case report and retrospective rather than well-designed prospective studies. Therefore, a moderate level of confidence can be given to estimation that the risk of HBVr during anti-TNF- $\alpha$  monotherapy is between 1% and 10% in HBsAg carriers, and quite lower in isolated antiHBe<sup>[12]</sup>.

#### Locoregional therapy for hepatocellular carcinoma

Several therapeutic strategies for hepatocellular carcinoma (HCC) have been inferred to cause HBVr<sup>[28,29]</sup>. Transarterial chemoembolization (TACE) has been directly associated with an increased rate of HBVr<sup>[30]</sup>. Even though this procedure has little systemic effect due to the administration of chemotherapeutic agents directly into a branch of the hepatic artery<sup>[2]</sup>, it may cause systemic symptoms if arterio-venous shunts or peritumoral microcirculation are present; this is why host immune system is often compromised. Additionally, anthracyclines (*i.e.*, doxorubicin) are frequently used as part of intra-arterial chemotherapy. In experimental models, anthracyclines have stimulated HBV DNA secretion from HCC cell lines; this mechanism may help to explain the higher risk of HBVr in patients treated with doxorubicin-containing TACE<sup>[12]</sup>. HBVr during radio-

therapy, with or without TACE, has also been examined in several studies<sup>[27,31]</sup>. In a prospective study conducted by Huang evaluating 69 HBV patients with HCC treated with conformal radiotherapy, almost 25% of them suffered HBVr, and 21.7% HBVr induced hepatitis<sup>[32]</sup>. HBVr in patients who underwent HCC surgical resection and local ablation therapy have also been extensively reported<sup>[27,30]</sup>.

#### Corticosteroids

Prednisone is the cornerstone of several chemotherapeutic regimens, and an important agent to induce remission in inflammatory bowel disease<sup>[2]</sup>. This and other corticosteroids have been associated with an increased risk of HBVr (both in monotherapy and especially when combined with other IS drugs)<sup>[22]</sup>. The HBVr is thought to be mediated by abolition of specific T-cell control, and also by direct viral stimulation. The risk of infection has been stratified according to the dosage and time of exposure to the corticosteroid<sup>[33]</sup>. Based on these variables, in a meta-analysis that included every well-documented report on HBVr, a risk stratification score was proposed: HBsAg positive patients who received more than 10 mg/daily of prednisone for 4 wk or longer were included in the high-risk group (> 10% chance of HBVr); HBsAg positive patients that received less than 10 mg/daily of prednisone or HBsAg-negative, antiHBe positive patients that were treated with less than 20 mg/daily of prednisone for less than 4 wk are included in the moderate-risk group (1%-10% risk of HBVr). Finally, antiHBe positive patients treated with less than 10 mg/daily of prednisone for less than 4 wk, and patients with local steroid treatment (such as intra-articular infusion) were included in the low risk group<sup>[12]</sup>. Therefore, corticosteroid use is an independent risk



factor for HBVr<sup>[12,22]</sup>.

### Systemic chemotherapy

This is one of the therapeutic interventions more frequently related with HBVr; not only associated with the degree of immunosuppression but also with the type of malignancy treated<sup>[14,16,34]</sup>. HBsAg-positive chronic carriers with hematologic diseases are at the highest risk of developing HBVr during IS, reaching an incidence of 40%-50%, or even higher, according to different series<sup>[12,27]</sup>. One of the most frequently cited study is related to the risk of HBVr in lymphoma patients, with reactivation rates reaching almost 50%, and an associated mortality of 4%<sup>[14,17,18,22,35]</sup>. Other studies report on an incidence of HBVr in this setting between 24%-67%, and an elevated mortality rate of 4%-41%. One of the reasons for this elevated risk relies on the intensive chemotherapy necessary for lymphoma treatment, especially when most chemotherapies schemes include high doses of steroids and/or rituximab. It may also be due to the rather high prevalence of HBV observed in this cohort of patients<sup>[14,36]</sup>. HBVr has also been described in patients receiving chemotherapy for treatment of solid tumors (*i.e.*, breast, colon and lung cancer)<sup>[21,22,37]</sup>. These patients fall within the intermediate risk category (HBVr chance of 10%-30%). Finally, the low risk group includes patients with gastrointestinal malignancies receiving 5-fluorouracil based therapy<sup>[27]</sup>.

### Biologic antibodies

Rituximab is considered a high-risk factor for HBVr<sup>[38]</sup>. This cytolytic monoclonal antibody is directed against the CD20 antigen of immature and mature B cells; it is used for the treatment of numerous hematological malignancies, severe rheumatic conditions, and (off-label) solid organ transplantation (in the latter scenario, as an adjunctive agent to mitigate humoral allograft response)<sup>[13]</sup>. When combined with standard-of-care chemotherapy for non-Hodgkin's lymphoma, HBVr has been observed in up to 25% of patients with resolved infection; this reactivation may occur even 12 mo after the therapy has been completed; including those patients with isolated antiHBc<sup>[22,39]</sup>. A preliminary analysis of the post marketing data from the Food and Drug Administration (FDA) Adverse Event Reporting System found 109 cases of HBV-related acute liver failure associated with rituximab and ofatumumab (another anti-CD20 monoclonal antibody). They occurred during the 13 years of rituximab and 3 years of ofatumumab commercialization<sup>[17]</sup>. Due to these reports, in the year 2013 a boxed warning was included in the label issued by the FDA for both drugs, describing HBVr resulting in "fulminant hepatitis, hepatic failure and death". This advisement underlines the potential for HBVr especially in chronically infected patients, but also in those who have resolved a previous HBV infection<sup>[13,17]</sup>. Due to these events, all antibodies directed against CD20 have been compelled by the FDA to add HBVr to the boxed

warning; recommending HBV screening tests before initiation of therapy and therapy when positive results are found<sup>[17]</sup>. Other biologic agents, such as specific tyrosine kinase inhibitors imatinib and nilotinib, have been implied in well-documented cases of HBVr; however, newly developed drugs including cytokine and integrin inhibitors such as ustekinumab, natalizumab, alemtuzumab and vedolizumab, have few or no reports of HBVr as yet. Since they all share the same mechanism of action, it is expected for them to convey at least a low to moderate risk of this complication<sup>[12,22]</sup>.

### Stem cell and solid organ transplantation

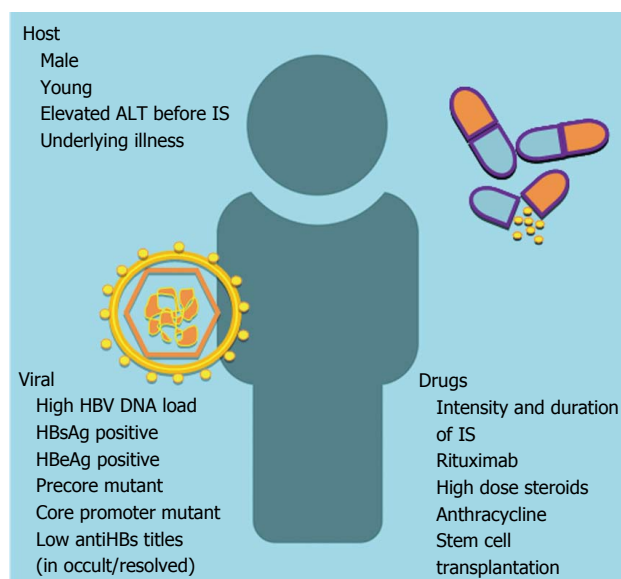
Patients undergoing stem cell/bone marrow transplantation are at the highest HBVr-risk position followed closely by those receiving solid organ transplantation.

Bone marrow/stem cell transplanted patients typically get intense chemotherapy to induce remission of the underlying malignancy, followed by additional chemotherapy and radiation therapy to ablate bone marrow<sup>[17,40]</sup>. The profound IS and loss of pre-existing HBV-specific immunity allows for HBVr in the liver to occur, and the return of active viral replication<sup>[19]</sup>. In this setting, the HBVr rate reaches 50% in both HBsAg-positive and isolated antiHBc-positive patients. In the latter group, antiHBs titles below 10 mIU/mL were a predictor of HBsAg seroreversion<sup>[17]</sup>. Due to the considerable delay in immune system reconstitution that typically occurs in this subset of patients, the risk of seroreversion can endure for several years; furthermore, these patients are prone to develop chronic infection once the virus is reactivated<sup>[19]</sup>. In a retrospective study that evaluated 137 HBsAg negative and antiHBc positive patients who underwent hematopoietic cell transplant patients, the prevalence of HBVr reached 10%, occurring within 9 to 77 mo after transplantation<sup>[40]</sup>.

When considering solid organ transplantation, HBVr risk has been known to reach 50%-90% in HBsAg-positive patients after kidney transplantation. In these cases, reactivation has the potential to cause liver failure, progression to cirrhosis, HCC, and increased liver-related mortality. Other solid transplant recipients (*e.g.*, heart, lung) have been the subject of similar reports<sup>[17]</sup>.

## WHOM TO CHECK: UNIVERSAL SCREENING VS HIGH-RISK PROFILING

The key to prevent HBVr is the timely identification of HBV-infected patients prior to immunosuppressive therapy<sup>[41,42]</sup>. The proportion of patients ignorant of their chronic HBV infection reaches 35% in United States; this proportion is far higher (90%) in the European Union<sup>[4,17]</sup>, whereas in Latin America the figures are unknown. To note, HBV screening is mandatory in high-risk groups in only 15% of the countries located of this region<sup>[43]</sup>. Lack of standardized risk factor assessment, the fact that many patients are not aware (or might not acknowledge)



**Figure 3 Risk factors for hepatitis B reactivation in patients with current/past hepatitis B infection.** ALT: Alanine aminotransferase; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; IS: Immunosuppression<sup>[2,4,27,56,57]</sup>.

that they have had risk behaviors, and the scarce time dedicated by most physicians to systematically screen their patients for HBV risk factors when they are about to start immunosuppressive therapy worsen the situation<sup>[14,17,44]</sup>. Risk factors for HBV infection are well-known (patients born in areas with intermediate-high HBV prevalence, patients who use intravenous drugs, patients that have had multiple sexual partners, patients with sexually transmitted diseases, etc.)<sup>[5,20]</sup>, but factors associated with HBVr have been less described; these are relevant for the decision and timing of HBV treatment. The most relevant ones are shown in Figure 3.

The combination of several of these risk factors has been suggested to stratify patients into high-, intermediate-, and low-risk for HBVr<sup>[6,12]</sup>. The relevance of risk assessment relies on the screening strategy adopted by the physician. Several HBV diagnostic consensus statements suggest universal screening, including the Centers for Disease Control and Prevention recommendations, as well as the EASL and the APASL guidelines<sup>[6,20]</sup>. The benefits of this approach are not only the identification of every chronically infected HBV patient, but also the recognition of patients previously exposed to the virus, thus eliminating the possibility of missing patients without clearly identified risk factors. The alternative screening strategy involves testing only patients at high risk for HBV infection; these recommendations are endorsed by the AASLD, the American Society of Clinical Oncology, and the National Comprehensive Cancer Network<sup>[6,17,45]</sup>. This targeted approach has been praised in relation to its lower cost, however, it may fail to identify chronic HBV carriers and previously exposed patients, and perhaps more importantly, it has been challenging for physicians to accomplish<sup>[6]</sup>.

**Table 1 Diagnostic tools suggested for hepatitis B screening prior to immunosuppression therapy by different major guidelines**

Recommendations	Hepatitis B screening tests before immunosuppression			
	HBsAg	AntiHBc	HBV DNA	AntiHBs
CDC	Yes	Yes	No	Yes
AASLD	High risk	High risk	No	No
EASL	Yes	Yes	No	No
APASL	Yes	No	No	No
ASCO	High risk	High risk	No	Yes <sup>1</sup>

<sup>1</sup>Only suggested in antiHBc-positive patients. CDC: Center for Disease Control; AASLD: American Association for the Study of Liver Disease; EASL: European Association for the Study of the Liver; APASL: Asian Pacific Association for the Study of the Liver; ASCO: American Society Clinical Oncology; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; AntiHBc: Antibody to hepatitis B core antigen; AntiHBs: Antibody to hepatitis B surface antigen.

## SCREENING TOOLS: HETEROGENEOUS RECOMMENDATIONS

Currently, there are no universally accepted screening tests adopted into clinical practice. Again, lack of consensus regarding testing for hepatitis B current/resolved infection complicates the picture<sup>[2]</sup>. Recommendations of different serologic testing for HBV screening prior to IS are shown in Table 1. HBsAg testing is endorsed by all major societies without consensus regarding risk evaluation, as already stated<sup>[6]</sup>. AntiHBc is not a required test by the APASL due to the high prevalence of HBV in this region (up to one third of the population)<sup>[46]</sup>. Whether to include antiHBs and HBV DNA testing among antiHBc-positive subjects is still controversial, since it is not based on prospective data<sup>[6,46]</sup>. Finally, regarding the moment for testing, it has been suggested that the major benefit is reached when it is done prior to initiation of therapy<sup>[16,27,47]</sup>.

## TREATMENTS FOR HBVR: SEVERAL MATTERS TO ADDRESS

The rationale for the identification of patients infected by HBV is to allow proper antiviral therapy, if needed, or otherwise to undertake careful monitoring<sup>[17]</sup>. Once again, several decisions have to be made by physicians on this point, some of which have different endorsements according to the consulted guideline. The decision-making stages are the following ones.

### When to treat

Several definitions have been used to classify treatment initiation timing. Treatment prophylaxis refers to antiviral therapy started before or concurrently as the initiation of immunosuppressive therapy, and before aminotransferase or HBV DNA levels rise occurs. On the other hand, in pre-emptive treatments, the occurrence of serum HBV DNA or aminotransferase elevation deter-

**Table 2** Recommendations for treatment and follow-up in different clinical scenarios, according to Asian Pacific Association for the Study of the Liver, American Association for the Study of Liver Disease and European Association for the Study of the Liver guidelines

Recommendations in different clinical scenarios					
	HBsAg (+) HBV DNA $\geq$ 2000 U/mL	HBsAg (+) HBV DNA < 2000 U/mL	HBsAg (-) antiHBc (+)	HBsAg (-) antiHBc (-) antiHBs (-)	HBV-HCC TACE
Action	Treat	Treat	Close mon/treat if HBV DNA (+) or rituximab/stem cell transplant <sup>1</sup>	Vaccination	Treat <sup>3</sup>
Onset	Before IS	Before IS	Before IS	-	Before IS
Duration	6-12 mo (except CI)	6-12 mo (except CI)	6-12 mo	-	-
Drug	Short IS: LAM (LdT) preferred ETV/TDF	Short IS: LAM (LdT) (ETV/TDF)	Short IS: LAM (LdT) (ETV/TDF)	-	LAM (ETV/TDF)
Follow-up	-	-	Every 1-3 mo/treat if HBV DNA (+) <sup>2</sup>	-	-

In HBsAg-positive patients, duration could be determined by CI as in immunocompetent patients. A 12-mo treatment was only endorsed by EASL. Drug selection depends on treatment duration and clinical setting. <sup>1</sup>In isolated antiHBc-positive patients when treated with biologic agents, close follow-up and treatment, if necessary, is suggested by AASLD/APASL; however, EASL proposes that isolated antiHBc-positive patients, if HBV DNA-positive, antiHBs-negative or undergoing rituximab/stem cell transplantation, should be treated with the same strategy as HBsAg positive patients; <sup>2</sup>When monitored, treatment should start when HBV DNA becomes positive, before ALT rise (EASL); <sup>3</sup>Treatment in all HBV-related HCC patients undergoing TACE is suggested by APASL guidelines. CI: Clinical indication; IS: Immunosuppression; HBsAg: Hepatitis B surface antigen; AntiHBc: Hepatitis B core antibody; AntiHBs: Hepatitis B surface antibody; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; TACE: Transarterial chemoembolization; LAM: Lamivudine; ETV: Entecavir; TDF: Tenofovir; LdT: Telbivudine (only listed as an option in AASLD guidelines); AASLD: American Association for the Study of Liver Disease; APASL: Asian Pacific Association for the Study of the Liver; EASL: European Association for the Study of the Liver.

mine the initiation of antivirals (before symptoms, if any, appear)<sup>[17]</sup>. The latter definition has been included in what referred to as “deferred treatment” in more recent publications<sup>[12]</sup>. Regarding HBsAg-positive patients, most treatment guidelines recommend prophylactic treatment; such as the AASLD (initiation of antivirals at the onset of IS), and the APASL guidelines (initiation of antivirals one week prior to chemotherapy)<sup>[5,30]</sup> (Table 2). The EASL mentions the “pre-emptive” treatment strategy, but defines it as antiviral administration during therapy regardless of HBV DNA levels, similar as the aforementioned guidelines<sup>[20]</sup>.

Several studies have compared these starting-point strategies. In the technical review by Perrillo *et al.*<sup>[12]</sup> where results of two randomized controlled trials of antiviral prophylaxis with lamivudine in HBsAg-positive patients undergoing chemotherapy were evaluated, an HBVr rate of 55% was found in the untreated group. There was biochemical evidence of hepatitis in 86% of these patients, which resulted in hepatic failure in 10% of the cohort<sup>[12]</sup>. In a recent meta-analysis published by Zheng *et al.*<sup>[48]</sup> where the efficacy of prophylactic use of lamivudine in HBsAg-positive patients undergoing chemotherapy for breast cancer was evaluated, the rate of HBVr was diminished by 91%, and there was a similar reduction in chemotherapy disruption in the prophylactic lamivudine group. HBsAg-negative and antiHBc-positive patients, when compared with HBsAg-positive patients, appear to have a lower risk of HBVr when exposed to moderate-risk immunosuppressive drugs. This would explain why certain scientific societies such as APASL suggest close monitoring and treatment in this patient’s population only when reactivation occurs<sup>[30]</sup>. In contrast, when high-risk agents such as rituximab are used in isolated antiHBc-positive patients, high rates of reactivation in excess of 10% occur, and antiviral prophylaxis can be expected to result in similar

absolute risk reduction, as described for HBsAg-positive patients<sup>[5,12,30]</sup>.

### Whom to treat and what antiviral to choose

Most guidelines agree on recommendations for HBsAg-positive patients<sup>[49]</sup>. In this group, the choice of antiviral and length of therapy will depend on the clinical status of the HBV infection. When considering HBsAg-positive patients, antiviral therapy should commence in the context of immunosuppression. If the patient has clinical indications for HBV treatment (*i.e.*, HBV DNA > 2000 IU/mL), either tenofovir or entecavir should be chosen, and therapy should be maintained until they reach therapeutic endpoints for chronic hepatitis B. Otherwise, prophylactic therapy could be initiated with lamivudine, although more powerful antiviral could be chosen as well<sup>[5,20,30,50]</sup>. This rule also applies to all HBV-related, HCC patients who are to undergo TACE<sup>[30]</sup>. Lamivudine will only be sufficient in a finite and short-term course of immunosuppressive therapy. Elseways, in those patients with elevated HBV DNA viral load and/or in those receiving prolonged cycles of IS, protection with entecavir or tenofovir is preferred, due to their higher potency and stronger barrier of resistance<sup>[20,51-53]</sup>. Lamivudine resistance increases according to treatment duration, reaching a 10%-20% rate in the first year, and increasing longitudinally with time (especially with high initial HBV DNA viremia). In addition, given that drug-resistant variants are archived and reemerge quickly on re-exposure to the antiviral drug, patients with a history of prior lamivudine or telbivudine treatment would be best treated with tenofovir, as this is the most effective drug for patients with prior resistance. The recommendation of both entecavir and tenofovir are based on the evidence of efficacy of these drugs in treating chronic HBV patients outside the prophylaxis setting, since their utility in the immunosuppressive

scenario has been less studied. There are no studies of tenofovir use, but several cohort studies and a randomized trial using entecavir<sup>[12,54]</sup>. Preliminary results of 121 patients with lymphoma treated with R-CHOP (chemotherapy treatment including rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) randomized to lamivudine and entecavir prophylaxis, HBVr was seen in 8% of the lamivudine-treated patients and none among the entecavir-treated patients. Recommendations for tenofovir use must be based on anticipated parallel benefits to entecavir, as both drugs are of high antiviral potency and have low risk of resistance with prolonged therapy<sup>[49]</sup>.

There is no consensus regarding the duration of treatment; AASLD and APASL societies suggest 6 mo of maintenance after IS cessation, whereas EASL recommends its extension to 12 mo<sup>[5,20,30]</sup>. Both AASLD and APASL guidelines consider that in HBsAg-negative but antiHBc- and antiHBs-positive patients, and in those with isolated antiHBc, reactivation is infrequent. Therefore these patients should be monitored and antiviral therapy initiated when HBVr occurs<sup>[5,30]</sup>. However, EASL suggests that this subgroup of patients should be tested for HBV DNA, and if present, they should be treated similarly as HBsAg-positive patients. Furthermore in antiHBs negative patients and/or when close monitoring of HBV DNA is not guaranteed, this guideline recommends prophylaxis therapy with antivirals in patients receiving rituximab, bone-marrow or stem-cell transplantation and/or combined regimens for hematological malignancies. The optimal duration of prophylaxis for these indications is unknown<sup>[20]</sup>.

## CONCLUSION

Ambiguity on the nomenclature of HBVr is a major problem that has led to the uncertain estimation of its incidence. A proper standardization of both terminology and definitions are required to reach better estimates of the frequency and associated risk factors of HBVr in different clinical settings. Furthermore, this standard definitions should be employed in safety and efficacy trials for new IS agents.

HBV screening before starting immunosuppressive therapy is a key factor to prevent HBVr. We need consensus on how and when to screen HBV in patients at high risk for HBVr. The call for large, collaborative, population-based studies is eagerly awaited to determine with confidence the efficiency of the HBV screening methods, and the consequent optimal antiviral prophylaxis, aimed to HBVr prevention.

Many HBV-infected patients are unconscious of their disease or risk factors. An appeal from scientific societies for physicians to spend enough time to assess patients for HBV risk factors prior to begin immunosuppression therapy is mandatory.

Finally, to make progress in this field, consensus from major societies composing the scientific hepatology community in the construction of clear guidelines to

define HBVr management (*i.e.*, antiviral selection, treatment onset and duration) and follow-up are essential.

## REFERENCES

- 1 Trépo C, Chan HL, Lok A. Hepatitis B virus infection. *Lancet* 2014; **384**: 2053-2063 [PMID: 24954675 DOI: 10.1016/s0140-6736(14)60220-8]
- 2 Seetharam A, Perrillo R, Gish R. Immunosuppression in Patients with Chronic Hepatitis B. *Curr Hepatol Rep* 2014; **13**: 235-244 [PMID: 25101233 DOI: 10.1007/s11901-014-0238-2]
- 3 Roche B, Samuel D. The difficulties of managing severe hepatitis B virus reactivation. *Liver Int* 2011; **31** Suppl 1: 104-110 [PMID: 21205146 DOI: 10.1111/j.1478-3231.2010.02396.x]
- 4 Visram A, Feld JJ. Defining and grading HBV reactivation: Defining and Grading HBV Reactivation. *Clinical Liver Disease* 2015; **5**: 35-38 [DOI: 10.1002/cld.426]
- 5 Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]
- 6 Etzion O, Ghany MG. Screening for hepatitis B virus to prevent viral reactivation - who and when? *Clinical Liver Disease* 2015; **5**: 47-50 [DOI: 10.1002/cld.458]
- 7 Lim R, Holt A. Hepatitis B and C prophylaxis in patients receiving chemotherapy. *Viral Hepat Pract* 2014; **6**: 10-13 [PMID: 25893086]
- 8 Larrubia JR. Occult hepatitis B virus infection: a complex entity with relevant clinical implications. *World J Gastroenterol* 2011; **17**: 1529-1530 [PMID: 21472115 DOI: 10.3748/wjg.v17.i12]
- 9 Gwak GY, Koh KC, Kim HY. Fatal hepatic failure associated with hepatitis B virus reactivation in a hepatitis B surface antigen-negative patient with rheumatoid arthritis receiving low dose methotrexate. *Clin Exp Rheumatol* 2007; **25**: 888-889 [PMID: 18173926]
- 10 Wu JM, Huang YH, Lee PC, Lin HC, Lee SD. Fatal reactivation of hepatitis B virus in a patient who was hepatitis B surface antigen negative and core antibody positive before receiving chemotherapy for non-Hodgkin lymphoma. *J Clin Gastroenterol* 2009; **43**: 496-498 [PMID: 19247200 DOI: 10.1097/MCG.0b013e3181945942]
- 11 Perrillo RP. Hepatitis B reactivation from immunosuppressive drug therapy: A global menace: Editor's comment for february issue of clinical liver disease. *Clinical Liver Disease* 2015; **5**: 39-42 [DOI: 10.1002/cld.448]
- 12 Perrillo RP, Gish R, Falck-Ytter YT. American Gastroenterological Association Institute technical review on prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. *Gastroenterology* 2015; **148**: 221-244.e3 [PMID: 25447852 DOI: 10.1053/j.gastro.2014.10.038]
- 13 Martin ST, Cardwell SM, Nailor MD, Gabardi S. Hepatitis B reactivation and rituximab: a new boxed warning and considerations for solid organ transplantation. *Am J Transplant* 2014; **14**: 788-796 [PMID: 24592928 DOI: 10.1111/ajt.12649]
- 14 Lubel JS, Angus PW. Hepatitis B reactivation in patients receiving cytotoxic chemotherapy: diagnosis and management. *J Gastroenterol Hepatol* 2010; **25**: 864-871 [PMID: 20546439 DOI: 10.1111/j.1440-1746.2010.06243.x]
- 15 Bessone F. Re-appraisal of old and new diagnostic tools in the current management of chronic hepatitis B. *Liver Int* 2014; **34**: 991-1000 [PMID: 25098191 DOI: 10.1111/liv.12499]
- 16 Hwang JP, Barbo AG, Perrillo RP. Hepatitis B reactivation during cancer chemotherapy: an international survey of the membership of the American Association for the Study of Liver Diseases. *J Viral Hepat* 2015; **22**: 346-352 [PMID: 25220947 DOI: 10.1111/jvh.12305]
- 17 Hwang JP, Lok AS. Management of patients with hepatitis B who require immunosuppressive therapy. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 209-219 [PMID: 24247262 DOI: 10.1038/nrgastro.2013.216]
- 18 Totani H, Kusumoto S, Ishida T, Masuda A, Yoshida T, Ito A,



- Ri M, Komatsu H, Murakami S, Mizokami M, Ueda R, Niimi A, Inagaki H, Tanaka Y, Iida S. Reactivation of hepatitis B virus (HBV) infection in adult T-cell leukemia-lymphoma patients with resolved HBV infection following systemic chemotherapy. *Int J Hematol* 2015; **101**: 398-404 [PMID: 25633779 DOI: 10.1007/s12185-015-1750-z]
- 19 **Palmore TN**, Shah NL, Loomba R, Borg BB, Lopatin U, Feld JJ, Khokhar F, Lutchman G, Kleiner DE, Young NS, Childs R, Barrett AJ, Liang TJ, Hoofnagle JH, Heller T. Reactivation of hepatitis B with reappearance of hepatitis B surface antigen after chemotherapy and immunosuppression. *Clin Gastroenterol Hepatol* 2009; **7**: 1130-1137 [PMID: 19577007 DOI: 10.1016/j.cgh.2009.06.027]
  - 20 **European Association For The Study Of The Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]
  - 21 **Liu CJ**, Chen PJ, Chen DS, Kao JH. Hepatitis B virus reactivation in patients receiving cancer chemotherapy: natural history, pathogenesis, and management. *Hepatol Int* 2013; **7**: 316-326 [PMID: 21670970 DOI: 10.1007/s12072-011-9279-6]
  - 22 **Reynolds JA**, Manch RA, Gish RG. Medical interventions associated with HBV reactivation: Common and less common. *Clinical Liver Disease* 2015; **5**: 32-34 [DOI: 10.1002/cld.413]
  - 23 **Laohapand C**, Arromdee E, Tanwandee T. Long-term use of methotrexate does not result in hepatitis B reactivation in rheumatologic patients. *Hepatol Int* 2015; **9**: 202-208 [PMID: 25788188 DOI: 10.1007/s12072-014-9597-6]
  - 24 **Manzano-Alonso ML**, Castellano-Tortajada G. Reactivation of hepatitis B virus infection after cytotoxic chemotherapy or immunosuppressive therapy. *World J Gastroenterol* 2011; **17**: 1531-1537 [PMID: 21472116 DOI: 10.3748/wjg.v17.i12.1531]
  - 25 **Lee YH**, Bae SC, Song GG. Hepatitis B virus (HBV) reactivation in rheumatic patients with hepatitis core antigen (HBV occult carriers) undergoing anti-tumor necrosis factor therapy. *Clin Exp Rheumatol* 2013; **31**: 118-121 [PMID: 23111095]
  - 26 **Lee YH**, Bae SC, Song GG. Hepatitis B virus reactivation in HBsAg-positive patients with rheumatic diseases undergoing anti-tumor necrosis factor therapy or DMARDs. *Int J Rheum Dis* 2013; **16**: 527-531 [PMID: 24164839 DOI: 10.1111/1756-185x.12154]
  - 27 **Jang JW**. Hepatitis B virus reactivation in patients with hepatocellular carcinoma undergoing anti-cancer therapy. *World J Gastroenterol* 2014; **20**: 7675-7685 [PMID: 24976705 DOI: 10.3748/wjg.v20.i24.7675]
  - 28 **Liu F**, Dan J, Zhang Y, Chen M, Huang J, Xie R. [Hepatitis B reactivation after treatment for HBV-related hepatocellular carcinoma: comparative analysis of radiofrequency ablation versus hepatic resection]. *Zhonghua Ganzangbing Zazhi* 2014; **22**: 38-42 [PMID: 24721242 DOI: 10.3760/cma.j.issn.1007-3418.2014.01.009]
  - 29 **von Weizsäcker F**, Blum HE. Out of control: hepatitis B reactivation by transarterial treatment of hepatocellular carcinoma. *J Hepatol* 2004; **41**: 482-484 [PMID: 15336452 DOI: 10.1016/j.jhep.2004.07.005]
  - 30 **Liaw YF**, Kao JH, Piratvisuth T, Chan HL, Chien RN, Liu CJ, Gane E, Locarnini S, Lim SG, Han KH, Amarapurkar D, Cooksley G, Jafri W, Mohamed R, Hou JL, Chuang WL, Lesmana LA, Sollano JD, Suh DJ, Omata M. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. *Hepatol Int* 2012; **6**: 531-561 [PMID: 26201469 DOI: 10.1007/s12072-012-9365-4]
  - 31 **Dan JQ**, Zhang YJ, Huang JT, Chen MS, Gao HJ, Peng ZW, Xu L, Lau WY. Hepatitis B virus reactivation after radiofrequency ablation or hepatic resection for HBV-related small hepatocellular carcinoma: a retrospective study. *Eur J Surg Oncol* 2013; **39**: 865-872 [PMID: 23597497 DOI: 10.1016/j.ejso.2013.03.020]
  - 32 **Huang W**, Zhang W, Fan M, Lu Y, Zhang J, Li H, Li B. Risk factors for hepatitis B virus reactivation after conformal radiotherapy in patients with hepatocellular carcinoma. *Cancer Sci* 2014; **105**: 697-703 [PMID: 24654677 DOI: 10.1111/cas.12400]
  - 33 **Gu HR**, Shin DY, Choi HS, Moon CH, Park SC, Kang HJ. HBV reactivation in a HBsAg-negative patient with multiple myeloma treated with prednisolone maintenance therapy after autologous HSCT. *Blood Res* 2015; **50**: 51-53 [PMID: 25830131 DOI: 10.5045/br.2015.50.1.51]
  - 34 **Dyson JK**, Hudson M, McPherson S. Lesson of the month 2: Severe reactivation of hepatitis B after immunosuppressive chemotherapy. *Clin Med (Lond)* 2014; **14**: 551-555 [PMID: 25301924 DOI: 10.7861/clinmedicine.14-5-551]
  - 35 **Tamori A**, Hino M, Kawamura E, Fujii H, Uchida-Kobayashi S, Morikawa H, Nakamae H, Enomoto M, Murakami Y, Kawada N. Prospective long-term study of hepatitis B virus reactivation in patients with hematologic malignancy. *J Gastroenterol Hepatol* 2014; **29**: 1715-1721 [PMID: 24730465 DOI: 10.1111/jgh.12604]
  - 36 **Hsu C**, Tsou HH, Lin SJ, Wang MC, Yao M, Hwang WL, Kao WY, Chiu CF, Lin SF, Lin J, Chang CS, Tien HF, Liu TW, Chen PJ, Cheng AL. Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: a prospective study. *Hepatology* 2014; **59**: 2092-2100 [PMID: 24002804 DOI: 10.1002/hep.26718]
  - 37 **Yang Y**, Du Y, Luo WX, Li C, Chen Y, Cheng K, Ding J, Zhou Y, Ge J, Yang X, Liu JY. Hepatitis B virus reactivation and hepatitis in gastrointestinal cancer patients after chemotherapy. *Cancer Chemother Pharmacol* 2015; **75**: 783-790 [PMID: 25687988 DOI: 10.1007/s00280-015-2700-4]
  - 38 **Mozessohn L**, Chan KK, Feld JJ, Hicks LK. Hepatitis B reactivation in HBsAg-negative/HBcAb-positive patients receiving rituximab for lymphoma: a meta-analysis. *J Viral Hepat* 2015; **22**: 842-849 [PMID: 25765930 DOI: 10.1111/jvh.12402]
  - 39 **Civan J**, Hann HW. Giving rituximab in patients with occult or resolved hepatitis B virus infection: are the current guidelines good enough? *Expert Opin Drug Saf* 2015; **14**: 865-875 [PMID: 25826452 DOI: 10.1517/14740338.2015.1032243]
  - 40 **Mikulska M**, Nicolini L, Signori A, Rivoli G, Del Bono V, Raiola AM, Di Grazia C, Dominietto A, Varaldo R, Ghiso A, Bacigalupo A, Viscoli C. Hepatitis B reactivation in HBsAg-negative/HBcAb-positive allogeneic haematopoietic stem cell transplant recipients: risk factors and outcome. *Clin Microbiol Infect* 2014; **20**: O694-O701 [PMID: 24575948 DOI: 10.1111/1469-0691.12611]
  - 41 **Sampedro B**, Hernández-López C, Ferrandiz JR, Illaro A, Fàbrega E, Cuadrado A, Iruzubietta P, Menéndez S, Cabezas J, Crespo J. Computerized physician order entry-based system to prevent HBV reactivation in patients treated with biologic agents: the PRESCRIB project. *Hepatology* 2014; **60**: 106-113 [PMID: 24585503 DOI: 10.1002/hep.27103]
  - 42 **Kusumoto S**, Tobinai K. Screening for and management of hepatitis B virus reactivation in patients treated with anti-B-cell therapy. *Hematology Am Soc Hematol Educ Program* 2014; **2014**: 576-583 [PMID: 25696914 DOI: 10.1182/asheducation-2014.1.576]
  - 43 **Diez-Padrisa N**, Castellanos LG. Viral hepatitis in Latin America and the Caribbean: a public health challenge. *Rev Panam Salud Publica* 2013; **34**: 275-281 [PMID: 24301739]
  - 44 **Sun WC**, Hsu PI, Yu HC, Lin KH, Tsay FW, Wang HM, Tsai TJ, Chen WC, Lai KH, Cheng JS. The compliance of doctors with viral hepatitis B screening and antiviral prophylaxis in cancer patients receiving cytotoxic chemotherapy using a hospital-based screening reminder system. *PLoS One* 2015; **10**: e0116978 [PMID: 25658926 DOI: 10.1371/journal.pone.0116978]
  - 45 **Artz AS**, Somerfield MR, Feld JJ, Giusti AF, Kramer BS, Sabichi AL, Zon RT, Wong SL. American Society of Clinical Oncology provisional clinical opinion: chronic hepatitis B virus infection screening in patients receiving cytotoxic chemotherapy for treatment of malignant diseases. *J Clin Oncol* 2010; **28**: 3199-3202 [PMID: 20516452 DOI: 10.1200/jco.2010.30.0673]
  - 46 **Lau GKK**. How do we handle the anti-HBc positive patient? (in highly endemic settings): The Anti-HBc positive patient. *Clinical Liver Disease* 2015; **5**: 29-31 [DOI: 10.1002/cld.399]
  - 47 **Ludwig E**. HBV reactivation in immunosuppressed patients: prevention or containment? *Hepatology* 2014; **59**: 2062-2064

- [PMID: 24753022 DOI: 10.1002/hep.27056]
- 48 **Zheng Y**, Zhang S, Tan Grahn HM, Ye C, Gong Z, Zhang Q. Prophylactic Lamivudine to Improve the Outcome of Breast Cancer Patients With HBsAg Positive During Chemotherapy: A Meta-Analysis. *Hepat Mon* 2013; **13**: e6496 [PMID: 23805156 DOI: 10.5812/hepatmon.6496]
  - 49 **Terrault N**. Management strategies for hepatitis B-infected patients undergoing immunomodulatory therapy: Is lamivudine enough? *Clinical Liver Disease* 2015; **5**: 43-46 [DOI: 10.1002/cld.447]
  - 50 **Mo YQ**, Liang AQ, Ma JD, Chen LF, Zheng DH, Schumacher HR, Dai L. Discontinuation of antiviral prophylaxis correlates with high prevalence of hepatitis B virus (HBV) reactivation in rheumatoid arthritis patients with HBV carrier state: a real-world clinical practice. *BMC Musculoskelet Disord* 2014; **15**: 449 [PMID: 25532827 DOI: 10.1186/1471-2474-15-449]
  - 51 **Tuna N**, Karabay O. Hepatitis B reactivation and timing for prophylaxis. *World J Gastroenterol* 2015; **21**: 2263-2264 [PMID: 25717269 DOI: 10.3748/wjg.v21.i7.2263]
  - 52 **Abramson JS**, Chung RT. Optimal antiviral prophylaxis against hepatitis B reactivation in patients receiving rituximab-based chemotherapy for lymphoma. *JAMA* 2014; **312**: 2505-2507 [PMID: 25514300 DOI: 10.1001/jama.2014.16095]
  - 53 **Brost S**, Schnitzler P, Stremmel W, Eisenbach C. Entecavir as treatment for reactivation of hepatitis B in immunosuppressed patients. *World J Gastroenterol* 2010; **16**: 5447-5451 [PMID: 21086562]
  - 54 **Baang J**. Treatment to prevent hepatitis B virus reactivation in patients with lymphoma receiving chemotherapy. *JAMA* 2015; **313**: 1269-1270 [PMID: 25803354 DOI: 10.1001/jama.2015.1438]
  - 55 **Philips CA**, Sarin SK. Potent antiviral therapy improves survival in acute on chronic liver failure due to hepatitis B virus reactivation. *World J Gastroenterol* 2014; **20**: 16037-16052 [PMID: 25473156 DOI: 10.3748/wjg.v20.i43.16037]
  - 56 **Kim HY**, Kim W. Chemotherapy-related reactivation of hepatitis B infection: updates in 2013. *World J Gastroenterol* 2014; **20**: 14581-14588 [PMID: 25356022 DOI: 10.3748/wjg.v20.i40.14581]
  - 57 **Salpini R**, Colagrossi L, Bellocchi MC, Surdo M, Becker C, Alteri C, Aragri M, Ricciardi A, Armenia D, Pollicita M, Di Santo F, Carioti L, Louzoun Y, Mastroianni CM, Lichtner M, Paoloni M, Esposito M, D'Amore C, Marrone A, Marignani M, Sarrecchia C, Sarmati L, Andreoni M, Angelico M, Verheyen J, Perno CF, Svicher V. Hepatitis B surface antigen genetic elements critical for immune escape correlate with hepatitis B virus reactivation upon immunosuppression. *Hepatology* 2015; **61**: 823-833 [PMID: 25418031 DOI: 10.1002/hep.27604]

**P- Reviewer:** Chiang TA, Lee WC, Li J **S- Editor:** Gong XM

**L- Editor:** A **E- Editor:** Liu SQ



## Management of human factors engineering-associated hemochromatosis: A 2015 update

Menaka Sivakumar, Lawrie W Powell

Menaka Sivakumar, School of Medicine, the University of Queensland, Brisbane QLD 4029, Australia

Lawrie W Powell, Centre for the Advancement of Clinical Research, Royal Brisbane and Women's Hospital Campus, Brisbane QLD 4029, Australia

**Author contributions:** Both authors contributed equally writing the review.

**Conflict-of-interest statement:** Neither author has any conflict of interest to declare (including but not limited to commercial, personal, political, intellectual, or religious interests).

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Lawrie W Powell, MD, PhD, Director, Professor, Centre for the Advancement of Clinical Research, Royal Brisbane and Women's Hospital Campus, Level 4, UQ Centre for Clinical Research, Building 71/918, Brisbane QLD 4029, Australia. [lawrie.powell@qimrberghofer.edu.au](mailto:lawrie.powell@qimrberghofer.edu.au)  
 Telephone: +61-7-36462352  
 Fax: +61-7-36462355

Received: August 7, 2015  
 Peer-review started: August 10, 2015  
 First decision: September 21, 2015  
 Revised: January 27, 2016  
 Accepted: March 7, 2016  
 Article in press: March 9, 2016  
 Published online: March 18, 2016

### Abstract

This review focuses on the management of iron meta-

bolism and iron overload experienced in the hereditary condition, human factors engineering (HFE)-associated hemochromatosis. Hemochromatosis refers to a group of genetic diseases that result in iron overload; the major one globally is HFE-associated hemochromatosis. The evolution in understanding of the most common form of hereditary hemochromatosis, being the substitution of cysteine to a tyrosine at position 282 in the *HFE* gene, has been extensively studied. Novel mutations in both *HFE* and non-*HFE* genes have been indicated in this disease which hold significance in its application for the Asia-Pacific region. In conditions with iron overload, the storage of excess iron in various body tissues leads to complications and toxic damage. The most common presenting complaint for this disease is malaise, lethargy and other non-specific symptoms. In order to diagnose hereditary hemochromatosis, there are biochemical, imaging and genetic testing options. Currently, cascade screening of affected families is preferred over population-level screening. The mainstay of treatment is venesection and the appropriate approach to treatment has been consolidated over the years. Recently, the indications for venesection therapy of hemochromatosis have been challenged and are the subject of ongoing research.

**Key words:** Human factors engineering; Iron storage diseases; Genetics; Venesections; Hemochromatosis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The concept of hemochromatosis as a single disease entity has changed to an iron storage disease resulting from several genetic disorders although the final common metabolic pathway is inappropriate iron absorption from the intestine and progressive tissue iron loading. The most common form of the disease is due to a mutation in the human factors engineering gene resulting in cysteine tyrosine substitution at position 282 in the molecule. This mutation is relatively common in populations of northern European extraction but is rare

in other populations. In contrast other rarer forms of hemochromatosis resulting from other mutations in the hepcidin pathway are quite ubiquitous. The main stay of treatment remains venesection although new oral iron-chelating agents show promise.

Sivakumar M, Powell LW. Management of human factors engineering-associated hemochromatosis: A 2015 update. *World J Hepatol* 2016; 8(8): 395-400 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i8/395.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i8.395>

## INTRODUCTION

The clinical and molecular research surrounding the clinical syndrome of hemochromatosis has been substantial in the last two decades even though it has been recognized in its advanced state for more than 100 years<sup>[1]</sup>. A mutation in the human factors engineering (*HFE*) gene was identified as the cause for more than 90% of cases of classic hemochromatosis<sup>[2]</sup> in most countries except for the Mediterranean region where it is responsible for around 65% of the cases. The genetic cause for hemochromatosis is more common in individuals with a northern European ancestry; however, the clinical manifestation, or incidence of biochemical abnormalities and clinical disease, is not as common in these populations. Although mutations in the *HFE* gene are most common, there are other forms of iron overload caused by mutations in other iron regulatory molecules that present as distinct clinical diseases. Over time, population studies have served the purpose of outlining the risk to an individual with a genetic mutation and the clinical investigations available for assessment and monitoring have improved. The treatment of hemochromatosis is the one aspect of this condition that has evolved the least over the years with phlebotomy still being the main therapy available. However, the treatment has potential for change with increased research on new therapeutic agents under trial. Although the European Association of the Study of Liver (EASL) and the American Association for the Study of Liver Disease have outlined appropriate treatment regimens, recent research have challenged these guidelines suggesting there is a benefit in beginning treatment early for patients with even mildly elevated iron levels but with or without clinical manifestation. According to current guidelines, the threshold of serum ferritin at which to start treatment is currently taken as above the normal range where the normal range for serum ferritin in men is 24-336 µg/L and in women is 11-307 µg/L. The current clinical standard is to maintain the serum ferritin at 50-100 µg/L<sup>[3]</sup>.

## PATHOPHYSIOLOGY

### Iron homeostasis

The role of iron in the body is a crucial one from oxygen

transport in hemoglobin and oxidative phosphorylation to the production of red blood cells and other functions<sup>[4,5]</sup>. In situations with overload, there are consequences in disease and mortality to be discussed later in this paper however the extent of this risk is still debated<sup>[6-9]</sup>. Beginning with iron, when it is consumed, it can enter the body in two forms: Either heme or non-heme<sup>[10,11]</sup>. Heme is mostly commonly ingested as animal protein and non-heme is *via* vegetables. However, there is no mechanism for the excretion of iron which is toxic in overload. Uncontrolled loss (1-2 mg) in menses, bleeding and the sloughing of skin are the only methods for iron removal.

In order to understand iron homeostasis, a discussion regarding the pathway of iron is necessary. Iron is absorbed on the apical surface of enterocytes in the duodenum and proximal small bowel. Non-heme iron can be either ferrous (Fe<sup>2+</sup>) or ferric (Fe<sup>3+</sup>)<sup>[4]</sup>. It is important to note that since ferrous iron is more soluble, it is necessary for ferric iron to be reduced to ferrous iron prior to absorption<sup>[12]</sup>. In order to reduce ferric iron found in non-heme iron to the ferrous state both gastric acidity and duodenal cytochrome B (DCyTB1) have been identified as well as other non-enzymatic pathways<sup>[13,14]</sup>. On the apical surface of enterocytes, the divalent metal transport 1 (DMT1) protein takes in ferrous iron<sup>[14]</sup>. The DMT1 protein also serves to transport manganese and copper (Figure 1).

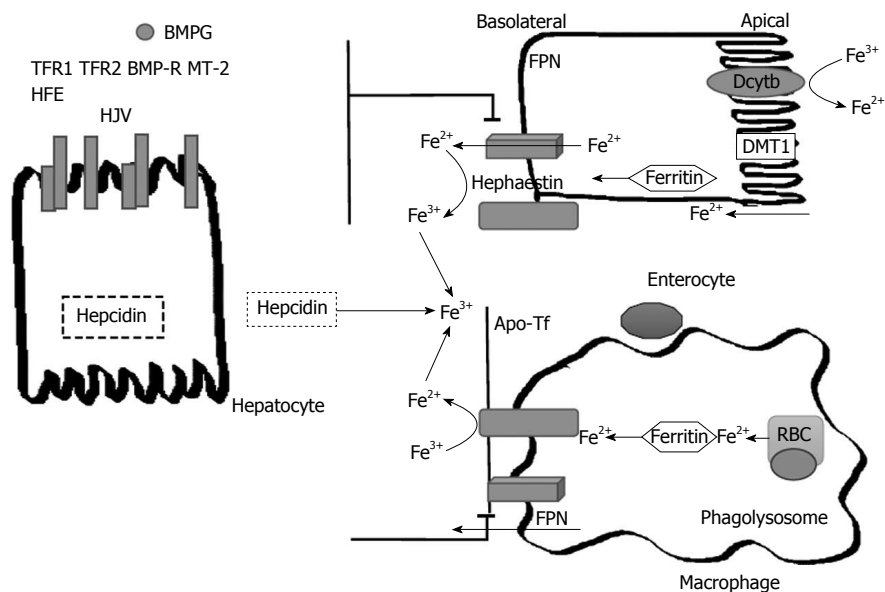
From the enterocyte, iron uptake into tissue is mediated by transferrin receptors (TfR1 and TfR2). In transportation, iron is consistently bound to a molecule due to its ability to form free radicals. Transferrin, the carrier protein for iron binds to the TfR1 and is taken up by endosomes, where transferrin is cleaved and the receptor recycled back to the cell surface<sup>[15]</sup>. In the case of iron overload, excess iron is stored in complexes of hemosiderin or ferritin. Another form of iron storage is hemosiderin which is a by-product of ferritin degradation<sup>[13]</sup>.

On the basal surface of enterocytes, ferroportin (FPN1) is the sole expressed exporter in cells. Iron is released into circulation when FPN1 interacts with ferroxidase and hepcidin. Hephestin next acts to oxidize the iron and the iron is then immediately bound to the transport molecule transferrin (Tf)<sup>[4]</sup>. Another important regulator of iron homeostasis is ferroportin, a protein which acts to export stored iron from enterocytes and other intracellular stores. A small hepatic peptide, hepcidin, negatively regulates ferroportin<sup>[12]</sup> by causing the internalization and degradation of this protein thereby affecting the export of iron. In summary, hepcidin reduces iron uptake and serum iron<sup>[12,16]</sup>. There have been certain factors such as iron, inflammation and oxidative stress that have been demonstrated to have an inhibitory effect on the expression hepcidin. However, hepcidin regulation is not a topic that is completely understood.

### Genetics

Hereditary Hemochromatosis is caused by different





**Figure 1 Pathways of Iron transport and metabolism.** The pathway of iron in enterocytes and macrophages as effected by hepcidin. Dietary non-heme iron is taken into the enterocyte via the DMT1. In order for iron to move across the brush border of the enterocyte via DMT1, it must first be reduced from  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by DcytB. Once inside of the cell, iron can be sequestered into storage as ferritin or continue along the pathway into circulation. In this process, the iron exporter FPN located on the basolateral surface of enterocytes is responsible for the transport of ferrous iron into circulation. Once iron is in circulation, hephaestin oxidizes the ferrous iron back into the ferric state and then it immediately binds to plasma transferrin. The iron is now able to travel to sites of iron storage or where iron is required. In macrophages, phagolysosomes containing senescent RBC release iron which is also then exported into circulation via ferroportin. Hepcidin, a protein derived from the liver, regulates iron transport in the body by causing the internalization and degradation of FPN transporters on macrophages and the basolateral surface of enterocytes. Hepcidin is regulated based on body iron requirements by signals produced from the interaction between different proteins on hepatocytes. The interaction of the HFE protein and transferrin receptors 1 and 2 (TFR1 and TFR2) and the interaction between bone morphogenic protein (BMP6), hemojuvelin (HJV) and the bone morphogenic protein receptor (BMP-R) and matriptase 2 (MT-2). RBC: Red blood cells; FPN: Ferroportin; DMT1: Divalent metal-ion transporter 1; DcytB: Duodenal cytochrome B; HFE: Human factors engineering.

**Table 1 Classification of iron overload and hemochromatosis**

Genetic iron overload (primary)
Type 1 HFE-associated hemochromatosis
C282Y homozygosity
C282Y/H63D compound heterozygosity
Type 2 juvenile hemochromatosis
2A hemojuvelin mutations
2B hepcidin mutations
Type 3 TFR2-related hemochromatosis
Transferrin receptor 2
Type 4 ferroportin disease
Loss of function mutations, also called type 4A or "M"
Hepcidin resistance mutations, also called type 4B or "H"
Aceruloplasminemia
Ceruloplasmin mutations
A(hypo)transferrinemia
Acquired iron overload (secondary)
Ineffective erythropoiesis
Thalassemia major
Sideroblastic anemia
Chronic hemolytic anemia
Dietary iron overload (African)
Parenteral iron overload (including transfusional overload)

HFE: Human factors engineering.

mutations that alter the regulatory proteins involved in iron homeostasis and hepcidin pathways. The genetic causes for hemochromatosis can be categorized into *HFE* gene mutations and non-*HFE* gene mutations (FPN, TFR HJV)<sup>[2]</sup>. While non-*HFE* gene mutations are not as

common as *HFE* gene mutations, there is an increased proportion of these mutations in non-Northern European populations<sup>[4]</sup>. Therefore, this information is of significance in Asia-Pacific populations<sup>[4]</sup>.

The knowledge and classification of hemochromatosis and other iron overload diseases has become more detailed in the last 2 decades (Table 1). Mutations in the genes encoding HFE, TFR2, hemojuvelin and hepcidin all lead to decreased hepcidin activity and increased iron absorption, resulting in the syndrome of hemochromatosis<sup>[5]</sup>. Mutations in *HFE*, *HJV*, *HAMP*, *TFR2* and *SLC40A1* have been linked to the various types of hemochromatosis<sup>[2,5]</sup>.

#### ***HFE-associated hereditary hemochromatosis***

In Northern European ancestry, an amino acid substitution specifically at position 282 of the HFE protein is the mutation most responsible for iron overload in this population<sup>[5]</sup>. The C282Y substitution is rare outside those of white ethnicity<sup>[17-19]</sup>. *HFE* is tightly linked to the HLA-A locus on chromosome 6p. Persons who are homozygous for the mutation are at increased risk of iron overload and account for 80% to 90% of clinical hereditary hemochromatosis in persons of northern European descent<sup>[6-9]</sup>. Pietrangelo suggest that between 10% and 33% of homozygous patients develop hereditary hemochromatosis<sup>[4,20]</sup>. This suggests that there are other genetic and non-genetic factors in the disease<sup>[21]</sup>.

There have been alternative mutations of HFE identified, primarily H63D and S65C; however, these mutations have not been proven to cause substantial iron overload<sup>[4]</sup>. In order to produce symptomatic disease, a heterozygous mutation is necessary. Since there is an increased prevalence of C282Y, and H63D is more relevant clinically, compound heterozygotes with symptomatic disease are usually C282Y/H63D<sup>[2,22-24]</sup>.

### **Non-HFE associated hereditary hemochromatosis**

Discussion regarding non-HFE associated hemochromatosis is beyond the scope of this paper.

## **CLINICAL MANIFESTATIONS**

Hereditary hemochromatosis is most commonly associated with liver disease including cirrhosis, but the clinical manifestations of iron overload are diverse and involve many other organs. Hemochromatosis is an overall underdiagnosed disease due to the idea that it is a rare condition and also associating diagnosis with clinical features seen in advanced disease such as cirrhosis, diabetes and skin pigmentation<sup>[3]</sup>. Genetic susceptibility for hemochromatosis is seen in approximately one in 250 Caucasians; however, fully expressed disease with end-organ manifestations is seen in fewer than 10% of these individuals<sup>[3]</sup>. Hemochromatosis patients mostly present with non-specific symptoms such as lethargy, arthralgia and weakness<sup>[25,26]</sup>. The other more commonly affected organ systems include liver, heart, pancreas, pituitary, skin and joints. Iron deposition in the conducting bundles and parenchyma of the heart result in cardiac arrhythmias and cardiomyopathy in 2%-19% of symptomatic patients<sup>[27,28]</sup>. Diabetes mellitus (DM) can be seen in up to 60% of symptomatic homozygotes but the rates of DM in asymptomatic patients are comparable to controls<sup>[7,29]</sup>. Endocrine dysfunction can occur as a result of iron deposition in pituitary and parathyroid glands<sup>[27,30]</sup>. Arthropathy is also observed in symptomatic and asymptomatic patients due to calcium pyrophosphate deposition in the articular cartilage, not iron sequestration and primarily involves the 2<sup>nd</sup> and 3<sup>rd</sup> metacarpophalangeal joints<sup>[25,31]</sup>.

## **MANAGEMENT AND TREATMENT**

Treatment for hemochromatosis with venesection (phlebotomy) has remained unchanged over the years<sup>[5]</sup>. Venesection as a treatment has two purposes: Directly reduce serum iron by depleting hemoglobin levels and to replace the depleted circulating serum iron by mobilizing iron stores from tissues. Early intervention, prior to the onset of symptoms, improves patient prognosis<sup>[32]</sup>. Furthermore, venesection in symptomatic individuals improves certain symptoms, such as skin pigmentation, while not having an effect on others such as cirrhosis and arthropathy<sup>[32]</sup>.

According to EASL clinical practice guidelines, the threshold of serum ferritin at which to start treatment is

currently taken as above the normal range. In regards to maintenance, the advocated standard practice is to maintain the serum ferritin at 50-100 µg/L and this is usually achieved with 3-6 mo of venesection<sup>[32]</sup>. It has been identified that the morbidity and mortality related to hereditary hemochromatosis can be greatly reduced by beginning treatment (phlebotomy) before the development of cirrhosis and/or diabetes. As a result of these findings, it is generally recommended that individuals at risk have prompt identification and pre-emptive treatment<sup>[32]</sup>. The pre-emptive treatment should be extended to involve those with homozygous HH that are asymptomatic and have markers of iron overload. Also, individuals with indications or evidence of increased level of hepatic iron should be treated. In summary, the American Association for the Study of Liver Disease recommends that in the absence of indicators suggestive of significant liver disease (alanine aminotransferase, aspartate transaminase elevation), C282Y homozygotes who have an elevated ferritin (but < 1000 µg/L) should proceed to prophylactic phlebotomy without a liver biopsy where target levels of phlebotomy should be a ferritin level of 50-100 µg/L<sup>[3,23,32]</sup>.

Traditionally, it was suggested that serum ferritin be maintained below 50 µg/L, but this has been updated to the range stated. Treatment guidelines also suggest yearly follow-up for the patients whose ferritin levels are at the normal range. This treatment strategy works for types 1-3 hereditary hemochromatosis but patients with type 4a may not tolerate venesection due to the irregular iron export from cells therefore treatment must be intermittent and is more complicated<sup>[32]</sup>.

Generally, 1 unit of blood is understood to contain approximately 200-250 mg of iron but the amount of iron that is removed each venesection can be variable<sup>[3]</sup>. It has been reported that on average, phlebotomy removes around 200-250 mg of iron per session<sup>[33]</sup>. Therefore, treatment must be provided on a personalized and case by case basis for each patient for appropriate venesection intervals and treatment regimens.

Although the treatment has remained the same for many years, there is still debate regarding the appropriate serum ferritin levels for maintenance of hemochromatosis. A recent study conducted by Bardou-Jacquet *et al*<sup>[6]</sup> found that early and sustained iron removal is beneficial as patients with serum ferritin levels between normal and 1000 µg/L, when treated, have reduced cardiovascular and extra-hepatic related mortality rates despite normal liver-related mortality rates. This study suggests that patients with even mild iron overload should be treated which builds on current management guidelines. However, this subject remains controversial.

## **CONCLUSION**

There is a continuing need to study the factors contributing to hemochromatosis due to the variable clinical penetrance of HFE mutations and the worldwide

prevalence of hemochromatosis in the absence of HFE mutations. Hemochromatosis has been divided into HFE-associated hemochromatosis related to mutations affecting iron transport and absorption and also HFE negative hemochromatosis or disease without HFE mutations. There is an incomplete understanding of the reasons for incomplete penetrance of disease phenotype in those with HFE mutations but recent research has revealed the presence of at least one other significant modifying genetic mutation<sup>[34]</sup>. Individuals at risk for hemochromatosis with genetic mutations and with or without symptomatic disease are recommended to pursue treatment at the earliest time possible and prior to any disease as this can help prevent further morbidity and mortality associated with hemochromatosis. Research advancement is opening doors for the management and treatment of iron overload as recent research has begun to develop the importance of treating mild iron overload due to its identified relation with reduced cardiovascular and extrahepatic related mortality rates.

## REFERENCES

- Von-Recklinghausen F.** Über haemochromatose. *Tageblatt Versammlung Deutsche Naturforscher Ärzte Heidelberg* 1889; **62**: 324-325
- Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R, Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS, Lauer P, Lee VK, Loeb DB, Mapa FA, McClelland E, Meyer NC, Mintier GA, Moeller N, Moore T, Morikang E, Prass CE, Quintana L, Starnes SM, Schatzman RC, Brunke KJ, Drayna DT, Risch NJ, Bacon BR, Wolff RK.** A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996; **13**: 399-408 [PMID: 8696333 DOI: 10.1038/ng0896-399]
- Bacon BR, Adams PC, Kowdley KV, Powell LW, Tavill AS.** Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011; **54**: 328-343 [PMID: 21452290 DOI: 10.1002/hep.24330]
- Ekanayake D, Roddick C, Powell LW.** Recent advances in hemochromatosis: a 2015 update : a summary of proceedings of the 2014 conference held under the auspices of Hemochromatosis Australia. *Hepatol Int* 2015; **9**: 174-182 [PMID: 25788196 DOI: 10.1007/s12072-015-9608-2]
- Wood MJ, Skoien R, Powell LW.** The global burden of iron overload. *Hepatol Int* 2009; **3**: 434-444 [PMID: 19669241 DOI: 10.1007/s12072-009-9144-z]
- Bardou-Jacquet E, Morcet J, Manet G, Lainé F, Perrin M, Jouanolle AM, Guyader D, Moirand R, Viel JF, Deugnier Y.** Decreased cardiovascular and extrahepatic cancer-related mortality in treated patients with mild HFE hemochromatosis. *J Hepatol* 2015; **62**: 682-689 [PMID: 25450707 DOI: 10.1016/j.jhep.2014.10.025]
- Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ, McLaren CE, Bahl M, Nisselle AE, Vulpe CD, Anderson GJ, Southey MC, Giles GG, English DR, Hopper JL, Olynyk JK, Powell LW, Gertig DM.** Iron-overload-related disease in HFE hereditary hemochromatosis. *N Engl J Med* 2008; **358**: 221-230 [PMID: 18199861 DOI: 10.1056/NEJMoa073286]
- Powell LW, Dixon JL, Ramm GA, Purdie DM, Lincoln DJ, Anderson GJ, Subramaniam VN, Hewett DG, Searle JW, Fletcher LM, Crawford DH, Rodgers H, Allen KJ, Cavanaugh JA, Bassett ML.** Screening for hemochromatosis in asymptomatic subjects with or without a family history. *Arch Intern Med* 2006; **166**: 294-301 [PMID: 16476869 DOI: 10.1001/archinte.166.3.294]
- Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T.** Penetrance of 845G->A (C282Y) HFE hereditary haemochromatosis mutation in the USA. *Lancet* 2002; **359**: 211-218 [PMID: 11812557 DOI: 10.1016/S0140-6736(02)07447-0]
- Han O.** Molecular mechanism of intestinal iron absorption. *Metallomics* 2011; **3**: 103-109 [PMID: 21210059 DOI: 10.1039/c0mt00043d]
- Bourdon E, Kang DK, Ghosh MC, Drake SK, Wey J, Levine RL, Rouault TA.** The role of endogenous heme synthesis and degradation domain cysteines in cellular iron-dependent degradation of IRP2. *Blood Cells Mol Dis* 2003; **31**: 247-255 [PMID: 12972033 DOI: 10.1016/S1079-9796(03)00161-X]
- Ganz T.** Systemic iron homeostasis. *Physiol Rev* 2013; **93**: 1721-1741 [PMID: 24137020 DOI: 10.1152/physrev.00008.2013]
- Lawen A, Lane DJ.** Mammalian iron homeostasis in health and disease: uptake, storage, transport, and molecular mechanisms of action. *Antioxid Redox Signal* 2013; **18**: 2473-2507 [PMID: 23199217 DOI: 10.1089/ars.2011.4271]
- Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA.** Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 1997; **388**: 482-488 [PMID: 9242408 DOI: 10.1038/41343]
- Bartnikas TB.** Known and potential roles of transferrin in iron biology. *Biometals* 2012; **25**: 677-686 [PMID: 22294463 DOI: 10.1007/s10534-012-9520-3]
- Ganz T, Nemeth E.** Hepcidin and iron homeostasis. *Biochim Biophys Acta* 2012; **1823**: 1434-1443 [PMID: 22306005 DOI: 10.1016/j.bbamer.2012.01.014]
- Khusainova RI, Khusnutdinova NN, Khusnutdinova EK.** [Analysis of the hemochromatosis gene (HFE) mutations, C282Y and H63D, in the populations of Central Asia]. *Genetika* 2006; **42**: 421-426 [PMID: 16649670]
- Hayashi H, Wakusawa S, Motonishi S, Miyamoto K, Okada H, Inagaki Y, Ikeda T.** Genetic background of primary iron overload syndromes in Japan. *Intern Med* 2006; **45**: 1107-1111 [PMID: 17106152 DOI: 10.2169/internalmedicine.45.1876]
- Lee JY, Yoo KH, Hahn SH.** HFE gene mutation, C282Y causing hereditary hemochromatosis in Caucasian is extremely rare in Korean population. *J Korean Med Sci* 2000; **15**: 179-182 [PMID: 10803694 DOI: 10.3346/jkms.2000.15.2.179]
- Pietrangelo A.** Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. *Gastroenterology* 2010; **139**: 393-408, 408.e1-2 [PMID: 20542038 DOI: 10.1053/j.gastro.2010.06.013]
- Fracanzani AL, Piperno A, Valenti L, Fraquelli M, Coletti S, Maraschi A, Consonni D, Coviello E, Conte D, Fargion S.** Hemochromatosis in Italy in the last 30 years: role of genetic and acquired factors. *Hepatology* 2010; **51**: 501-510 [PMID: 20101754 DOI: 10.1002/hep.23333]
- Bassett ML, Hickman PE, Dahlstrom JE.** The changing role of liver biopsy in diagnosis and management of haemochromatosis. *Pathology* 2011; **43**: 433-439 [PMID: 21716156 DOI: 10.1097/PAT.0b013e3283490e04]
- Kanwar P, Kowdley KV.** Diagnosis and treatment of hereditary hemochromatosis: an update. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 517-530 [PMID: 23985001 DOI: 10.1586/17474124.2013.816114]
- Pietrangelo A.** Hereditary hemochromatosis--a new look at an old disease. *N Engl J Med* 2004; **350**: 2383-2397 [PMID: 15175440 DOI: 10.1056/NEJMra031573]
- Cadet E, Capron D, Perez AS, Crépin SN, Arlot S, Ducroix JP, Dautréaux M, Fardellone P, Leflon P, Merryweather-Clarke AT, Livesey KJ, Pointon JJ, Harcourt J, Emery J, Sueur JM, Feyt R, Robson KJ, Rochette J.** A targeted approach significantly increases the identification rate of patients with undiagnosed haemochromatosis. *J Intern Med* 2003; **253**: 217-224 [PMID: 12542563 DOI: 10.1046/j.1365-2796.2003.01094.x]
- Swinkels DW, Aalbers N, Elving LD, Bleijenberg G, Swanink CM, van der Meer JW.** Primary haemochromatosis: a missed cause of chronic fatigue syndrome? *Neth J Med* 2002; **60**: 429-433 [PMID: 12685490]
- Adams PC, Deugnier Y, Moirand R, Brissot P.** The relationship

- between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. *Hepatology* 1997; **25**: 162-166 [PMID: 8985284 DOI: 10.1002/hep.510250130]
- 28 **Niederau C**, Fischer R, Pürschel A, Stremmel W, Häussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. *Gastroenterology* 1996; **110**: 1107-1119 [PMID: 8613000 DOI: 10.1053/gast.1996.v110.pm8613000]
  - 29 **Adams PC**, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, Dawkins FW, Acton RT, Harris EL, Gordeuk VR, Leiendecker-Foster C, Speechley M, Snively BM, Holup JL, Thomson E, Sholinsky P. Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med* 2005; **352**: 1769-1778 [PMID: 15858186 DOI: 10.1056/NEJMoa041534]
  - 30 **Bacon BR**, Olynyk JK, Brunt EM, Britton RS, Wolff RK. HFE genotype in patients with hemochromatosis and other liver diseases. *Ann Intern Med* 1999; **130**: 953-962 [PMID: 10383365 DOI: 10.7326/0003-4819-130-12-199906150-00002]
  - 31 **Timms AE**, Sathananthan R, Bradbury L, Athanasou NA, Wordsworth BP, Brown MA. Genetic testing for haemochromatosis in patients with chondrocalcinosis. *Ann Rheum Dis* 2002; **61**: 745-747 [PMID: 12117686 DOI: 10.1136/ard.61.8.745]
  - 32 **European Association For The Study Of The Liver**. EASL clinical practice guidelines for HFE hemochromatosis. *J Hepatol* 2010; **53**: 3-22 [PMID: 20471131 DOI: 10.1016/j.jhep.2010.03.001]
  - 33 **Harrison SA**, Bacon BR. Hereditary hemochromatosis: update for 2003. *J Hepatol* 2003; **38** Suppl 1: S14-S23 [PMID: 12591183 DOI: 10.1016/S0168-8278(02)00428-2]
  - 34 **McLaren CE**, Emond MJ, Subramaniam VN, Phatak PD, Barton JC, Adams PC, Goh JB, McDonald CJ, Powell LW, Gurrin LC, Allen KJ, Nickerson DA, Louie T, Ramm GA, Anderson GJ, McLaren GD. Exome sequencing in HFE C282Y homozygous men with extreme phenotypes identifies a GNPAT variant associated with severe iron overload. *Hepatology* 2015; **62**: 429-439 [PMID: 25605615 DOI: 10.1002/hep.27711]

**P- Reviewer:** Bardou-Jacquet E, Castro JA, Musci G  
**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Liu SQ





Basic Study

# Role of interleukin-1 and its antagonism of hepatic stellate cell proliferation and liver fibrosis in the *Abcb4*<sup>-/-</sup> mouse model

Florian P Reiter, Ralf Wimmer, Lena Wottke, Renate Artmann, Jutta M Nagel, Manuel O Carranza, Doris Mayr, Christian Rust, Peter Fickert, Michael Trauner, Alexander L Gerbes, Simon Hohenester, Gerald U Denk

Florian P Reiter, Ralf Wimmer, Lena Wottke, Renate Artmann, Jutta M Nagel, Alexander L Gerbes, Simon Hohenester, Gerald U Denk, Department of Medicine II, Liver Center Munich, University of Munich, D-81377 Munich, Germany

Manuel O Carranza, Doris Mayr, Institute of Pathology, University of Munich, D-80337 Munich, Germany

Christian Rust, Department of Medicine I, Krankenhaus Barmherzige Brüder, D-80639 Munich, Germany

Peter Fickert, Research Unit for Experimental and Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine, A-8036 Graz, Austria

Michael Trauner, Hans Popper Laboratory of Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, A-1090 Vienna, Austria

Gerald U Denk, Transplant Center Munich of the University of Munich, D-81377 Munich, Germany

**Author contributions:** Reiter FP, Wimmer R, Rust C, Fickert P, Trauner M, Gerbes AL, Hohenester S and Denk GU designed the research; Wimmer R, Nagel JM, Wottke L and Artmann R performed the experiments; Carranza MO and Mayr D performed the histological examinations and imaging; Reiter FP, Wimmer R, Hohenester S and Denk GU analysed the data; Reiter FP, Hohenester S and Denk GU prepared the manuscript; all of the authors discussed and approved the manuscript.

**Supported by** The Münchener Medizinische Wochenschrift (MMW); B. Braun-Stiftung (to Reiter FP); the Deutsche Forschungsgemeinschaft (HO 4460/2-1 to Hohenester S and RU 742/6-1 to Rust C).

**Institutional review board statement:** This study includes no data or material from patients. We confirm that all of the required permissions for this study were obtained from our local authorities as mentioned in the Institutional animal care and use

committee statement.

**Institutional animal care and use committee statement:** We confirm that the animals for our studies were kept according to the local regulations. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Regierung von Oberbayern; protocol number No.: 55.2.1.54-2532-104-11. All of the institutional and national guidelines for the care and use of laboratory animals were followed.

**Conflict-of-interest statement:** All of the authors declare that they have no conflict of interest.

**Data sharing statement:** The technical appendix, statistical code, and dataset are available from the corresponding author at [florian.reiter@med.uni-muenchen.de](mailto:florian.reiter@med.uni-muenchen.de).

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Florian P Reiter, MD, Department of Medicine II, Liver Center Munich, University of Munich, Marchioninstr. 15, D-81377 Munich, Germany. [florian.reiter@med.uni-muenchen.de](mailto:florian.reiter@med.uni-muenchen.de)  
 Telephone: +49-89-440073129  
 Fax: +49-89-440078864

**Received:** June 11, 2015

**Peer-review started:** June 11, 2015

**First decision:** September 18, 2015

**Revised:** January 7, 2016

**Accepted:** March 7, 2016

**Article in press:** March 9, 2016

**Published online:** March 18, 2016

## Abstract

**AIM:** To study the interleukin-1 (IL-1) pathway as a therapeutic target for liver fibrosis *in vitro* and *in vivo* using the ATP-binding cassette transporter b4<sup>-/-</sup> (Abcb4<sup>-/-</sup>) mouse model.

**METHODS:** Female and male Abcb4<sup>-/-</sup> mice from 6 to 13 mo of age were analysed for the degree of cholestasis (liver serum tests), extent of liver fibrosis (hydroxyproline content and Sirius red staining) and tissue-specific activation of signalling pathways such as the IL-1 pathway [quantitative polymerase chain reaction (qPCR)]. For *in vivo* experiments, murine hepatic stellate cells (HSCs) were isolated *via* pronase-collagenase perfusion followed by density gradient centrifugation using female mice. Murine HSCs were stimulated with up to 1 ng/mL IL-1 $\beta$  with or without 2.5  $\mu$ g/mL Anakinra, an IL-1 receptor antagonist, respectively. The proliferation of murine HSCs was assessed *via* the BrdU assay. The toxicity of Anakinra was evaluated *via* the fluorescein diacetate hydrolysis (FDH) assay. *In vivo* 8-wk-old Abcb4<sup>-/-</sup> mice with an already fully established hepatic phenotype were treated with Anakinra (1 mg/kg body-weight daily intraperitoneally) or vehicle and liver injury and liver fibrosis were evaluated *via* serum tests, qPCR, hydroxyproline content and Sirius red staining.

**RESULTS:** Liver fibrosis was less pronounced in males than in female Abcb4<sup>-/-</sup> animals as defined by a lower hydroxyproline content (274  $\pm$  64  $\mu$ g/g *vs* 436  $\pm$  80  $\mu$ g/g liver, respectively;  $n$  = 13-15;  $P$  < 0.001; Mann-Whitney  $U$ -test) and lower mRNA expression of the profibrogenic tissue inhibitor of metalloproteinase-1 (TIMP) (1  $\pm$  0.41 *vs* 0.66  $\pm$  0.33 fold, respectively;  $n$  = 13-15;  $P$  < 0.05; Mann-Whitney  $U$ -test). Reduced liver fibrosis was associated with significantly lower levels of F4/80 mRNA expression (1  $\pm$  0.28 *vs* 0.71  $\pm$  0.41 fold, respectively;  $n$  = 12-15;  $P$  < 0.05; Mann-Whitney  $U$ -test) and significantly lower IL-1 $\beta$  mRNA expression levels (1  $\pm$  0.38 *vs* 0.44  $\pm$  0.26 fold, respectively;  $n$  = 13-15;  $P$  < 0.001; Mann-Whitney  $U$ -test). No gender differences in the serum liver parameters [bilirubin; alanine aminotransferase (ALT); aspartate aminotransferase and alkaline phosphatase (AP)] were found. *In vitro*, the administration of IL-1 $\beta$  resulted in a significant increase in HSC proliferation [0.94  $\pm$  0.72 arbitrary units (A.U.) in untreated controls, 1.12  $\pm$  0.80 A.U. at an IL-1 $\beta$  concentration of 0.1 ng/mL and 1.18  $\pm$  0.73 A.U. at an IL-1 $\beta$  concentration of 1 ng/mL in samples from  $n$  = 6 donor animals;  $P$  < 0.001; analyses of variance (ANOVA)]. Proliferation was reduced significantly by the addition of 2.5  $\mu$ g/mL Anakinra (0.81  $\pm$  0.60 A.U. in untreated controls, 0.92  $\pm$  0.68 A.U. at an IL-1 $\beta$  concentration of 0.1 ng/mL, and 0.91  $\pm$  0.69 A.U. at an IL-1 $\beta$  concentration of 1 ng/mL; in samples from  $n$  = 6 donor animals;  $P$  < 0.001; ANOVA) suggesting an anti-proliferative effect of this clinically approved IL-1 receptor antagonist. The FDH assay showed this dose to be non-toxic in HSCs. *In vivo*, Anakinra had no effect on the hepatic hydroxyproline

content, liver serum tests (ALT and AP) and pro-fibrotic (collagen 1 $\alpha$ 1, collagen 1 $\alpha$ 2, transforming growth factor- $\beta$ , and TIMP-1) and anti-fibrotic [matrix metalloproteinase 2 (MMP2), MMP9 and MMP13] gene expression after 4 wk of treatment. Furthermore, the hepatic IL-1 $\beta$  and F4/80 mRNA expression levels were unaffected by Anakinra treatment.

**CONCLUSION:** IL-1 $\beta$  expression is associated with the degree of liver fibrosis in Abcb4<sup>-/-</sup> mice and promotes HSC proliferation. IL-1 antagonism shows antifibrotic effects *in vitro* but not in Abcb4<sup>-/-</sup> mice.

**Key words:** Cholestasis; Primary sclerosing cholangitis; The ATP-binding cassette transporter b4; Liver fibrosis; Interleukin-1

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Interleukin-1 (IL-1) critically participates in hepatic stellate cells (HSCs) pathophysiology and in the progression of liver injury to fibrosis. We found that fibrosis was more pronounced in female than in male ATP-binding cassette transporter b4<sup>-/-</sup> animals. This fibrosis was associated with higher IL-1 $\beta$  mRNA expression levels. We showed that IL-1 $\beta$  promoted the proliferation of murine HSCs and described an antifibrotic effect of the clinically approved IL-1 receptor antagonist Anakinra *in vitro*. Despite the promising antifibrotic effects *in vitro*, Anakinra failed to improve liver fibrosis in this preclinical primary sclerosing cholangitis model. Its potency in other models of liver injury and fibrosis remains to be determined.

Reiter FP, Wimmer R, Wottke L, Artmann R, Nagel JM, Carranza MO, Mayr D, Rust C, Fickert P, Trauner M, Gerbes AL, Hohenester S, Denk GU. Role of interleukin-1 and its antagonism of hepatic stellate cell proliferation and liver fibrosis in the Abcb4<sup>-/-</sup> mouse model. *World J Hepatol* 2016; 8(8): 401-410 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i8/401.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i8.401>

## INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease that is characterised by obliterative strictures of the intra- and extra-hepatic bile ducts. Because these strictures are associated with inflammation and fibrosis, PSC is supposed to be an immune-mediated liver disease. PSC primarily occurs in young adults and leads to liver cirrhosis with hepatic decompensation and portal hypertension and is frequently associated with malignancy of the biliary tract<sup>[1,2]</sup>. To date, there is no effective therapeutic option to halt disease progression. Thus, liver transplantation in end-stage liver disease is often required. The estimated median time for transplant-free survival may be as

short as 12 to 13 years at tertiary referral centres<sup>[1,3]</sup>. Therefore, new therapeutic targets need to be identified to establish an urgently required effective therapy for PSC.

PSC mainly affects the large bile ducts and causes periductal fibrosis<sup>[4]</sup>. This leads to obliteration of the bile ducts, resulting in impaired bile flow and cholestasis. During chronic cholestatic disease, toxic bile acids accumulate<sup>[5]</sup> and induce cholangio- and hepatocellular apoptosis by specific signalling pathways<sup>[6-8]</sup>. The clinical significance of cholangiocellular and hepatocellular apoptosis in patients with chronic cholestatic disease was recently supported by the detection of serum markers of liver cell apoptosis<sup>[9]</sup>.

Hepatocellular apoptosis is considered a trigger of liver fibrosis *via* the activation of hepatic stellate cells (HSCs)<sup>[10,11]</sup>. This results in the progression to liver fibrosis and cirrhosis in PSC.

HSCs are the major source of hepatic extracellular matrix deposition and crucially participate in the pathogenesis of liver fibrosis<sup>[12-14]</sup>. In addition to other factors, enhanced proliferation of this cell type is thought to be an important profibrotic mechanism<sup>[14]</sup>. In this regard, a recent study found that interleukin-1 $\beta$  (IL-1 $\beta$ ) induces the proliferation of rat HSCs<sup>[15]</sup>. Furthermore, IL-1 was identified as a factor in the progression of liver injury to fibrosis<sup>[16]</sup>. Therefore, we hypothesised that blocking the IL-1 pathway during liver fibrosis might be a therapeutic approach in chronic liver disease.

Anakinra is a clinically approved IL-1 receptor antagonist that is listed for the treatment of rheumatoid arthritis by the European Medicines Agency and the United States Food and Drug Administration. Its implication for the treatment of chronic liver disease and fibrosis has not been investigated thus far.

The ATP-binding cassette transporter b4<sup>-/-</sup> (Abcb4<sup>-/-</sup>) mouse is an established preclinical model for biliary fibrosis and PSC<sup>[17-19]</sup>. Therefore, this model is widely used to study therapeutic strategies for biliary fibrosis and PSC *in vivo*<sup>[20]</sup>.

Here, we tested the proliferative and pro-fibrotic properties of the IL-1 pathway *in vitro*, analysed its contribution to liver fibrosis in the Abcb4<sup>-/-</sup> model *in vivo* and tested the potential protective effects of the clinically approved IL-1 receptor antagonist Anakinra on the development of liver injury and fibrosis in this animal model.

## MATERIALS AND METHODS

### Animals

Animals were obtained from the Jackson Laboratory (United States) and Charles River (Germany). The animal protocol was designed to minimise pain or discomfort to the animals. The animals were housed at a 12/12 h light/dark cycle and were fed *ad libitum*. The animals were kept according to local regulations. The experiments were approved by local authorities. All

ethical, institutional and national guidelines for the care and use of laboratory animals were followed.

### Isolation and culture of primary murine HSCs

The isolation of primary murine HSC from female C57BL/6N wild-type animals was performed by pronase-collagenase perfusion followed by density gradient centrifugation in 13.2% Nycodenz<sup>®</sup> (Axis-Shield PoC, Norway)<sup>[21]</sup>. The cells were plated at a density of 25000 cells/cm<sup>2</sup>. The cells were kept in DMEM containing 10% fetal bovine serum and antibiotics (Sigma, Germany) in a humidified atmosphere with 5% CO<sub>2</sub> and 21% O<sub>2</sub> at 37 °C.

### Toxicity assay

To investigate the potential toxic effects of Anakinra on murine HSCs, we performed the fluorescein diacetate hydrolysis (FDH) assay according to the method of Jones *et al.*<sup>[22]</sup> after stimulation of murine HSCs for 24 h with 25 pg/mL to 2.5  $\mu$ g/mL Anakinra.

### Proliferation assay

The cells were stimulated with vehicle, 0.1 and 1 ng/mL IL-1 $\beta$  for 24 h at day three after isolation. Where indicated, the cells were co-incubated with 2.5  $\mu$ g/mL Anakinra. The proliferation of primary murine HSCs was measured using a BrdU-assay kit (Roche, Germany) according to the manufacturer's instructions.

### In vivo experiments

Available material from female and male Abcb4<sup>-/-</sup> mice was kindly provided by the Research Unit for Experimental and Molecular Hepatology, Graz, Austria (Fickert P and Trauner M). The material from 6- to 13-mo-old animals was studied.

Eight-week old female Abcb4<sup>-/-</sup> (FVB) mice received daily intraperitoneally injections of Anakinra (1 mg/kg body-weight, a dosage used previously for animal studies, corresponding to the recommended dose for the treatment of rheumatoid arthritis<sup>[23]</sup> or NaCl 0.9% as a control for 4 wk, a time period previously shown to be sufficient to identify the modulation of liver fibrosis in this model<sup>[20]</sup>. After narcotisation with isoflurane (Abbott GmbH, Germany), the animals were sacrificed by cervical dislocation.

### Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction was performed using a Sybr<sup>®</sup> green system. Glyceraldehyde-3-phosphate dehydrogenase or 18 s were used as housekeeping genes and were normalised against the means of the controls.

### Serum biochemistry

The levels of bilirubin, alkaline phosphatase (AP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were analysed using the Cobas Integra 800 analyser (Roche Diagnostics, Germany) or the

Hitachi 917 analyser (Boehringer Mannheim, Germany).

### Quantification of hydroxyproline

The hydroxyproline content was determined according to the method of Edwards *et al.*<sup>[24]</sup>.

### Sirius-red staining

Liver samples were fixed using 4% formaldehyde. After embedding in paraffin, 4- $\mu$ m sections were stained with Sirius red.

### Immunohistochemistry

Ki-67 immunohistochemistry was performed using the polyclonal rabbit anti-Ki-67 antibody (Novocastra, Germany). The ABC system with AEC as a substrate was used for the detection of antibody antigen binding. The number of positively stained cells was counted per mouse in 20 randomly chosen fields at 40-fold magnification.

### Statistical analysis

Statistical calculations were performed using the SPSS 23 software package (IBM, United States). Where appropriate, the differences between groups were verified by the Mann-Whitney *U*-test.

Where appropriate, the analyses of variance (ANOVA) were calculated with the procedure UNIANOVA. The normality and homogeneity of the variances of the residuals were assessed by inspection of residual plots from the UNIANOVA procedure and P-P plots. Furthermore, Levene's test was used to assess the equality of error variances (for details, see also supplemental material). The results are reported as the means  $\pm$  SD. *P*-values less than 0.05 were considered to be statistically significant. The statistical analysis of this study was supported and reviewed by Christoph Glasmacher (Christoph Glasmacher - Biometrics and SAS-Programming for Clinical Research).

## RESULTS

### Gender differences in the liver disease of *Abcb4*<sup>-/-</sup> mice

Hepatic injury and liver fibrosis were evaluated in male and female *Abcb4*<sup>-/-</sup> animals from 6 to 13 mo of age. We found that the hepatic hydroxyproline levels were 37% lower in male animals than in female animals (Figure 1A; 274  $\pm$  64  $\mu$ g/g vs 436  $\pm$  80  $\mu$ g/g liver, respectively; *n* = 13-15; *P* < 0.001; Mann-Whitney *U*-test). Sirius red staining illustrated reduced collagen deposition in male animals (Figure 1B). In accordance with these findings, the livers of male animals showed 34% lower tissue inhibitor of metalloproteinase (TIMP-1) mRNA expression (Figure 1C; 1  $\pm$  0.41 vs 0.66  $\pm$  0.33 fold; *n* = 13-15; *P* < 0.05; Mann-Whitney *U*-test).

The mRNA expression of IL-1 $\beta$  and F4/80 as markers of hepatic-inflammation was 56% (Figure 1C; 1  $\pm$  0.38 vs 0.44  $\pm$  0.26 fold; *n* = 13-15; *P* < 0.001; Mann-Whitney *U*-test) and 29% lower (Figure 1C; 1  $\pm$  0.28 vs 0.71  $\pm$  0.41 fold; *n* = 12-15; *P* < 0.05; Mann-Whitney *U*-test) in the liver tissue of male animals than in the

liver tissue of female animals.

No gender differences were observed regarding the hepatic mRNA expression of collagen 1 $\alpha$ 1, cytokeratin 19 and monocyte chemotactic protein-1; *n* = 13-15; Mann-Whitney *U*-test).

Additionally, no gender differences in serum biochemistry were observed regarding the bilirubin, ALT, AST, and AP levels (data not shown; *n* = 17-21; Mann-Whitney *U*-test). Furthermore, there were no sex differences regarding hepatic mitotic activity in Ki-67 immunohistochemistry (data not shown; *n* = 15; Mann-Whitney *U*-test).

In summary, we identified a lower grade of liver fibrosis in male than in female animals as illustrated by lower hydroxyproline levels and lower hepatic mRNA expression levels of the profibrogenic gene TIMP-1. Furthermore, lower fibrosis in male animals was associated with the reduced mRNA expression of the pro-inflammatory genes IL-1 $\beta$  and F4/80. These findings may support a potential role of the IL-1 pathway in the progression of liver injury to liver fibrosis.

### IL-1 $\beta$ exerts proliferative effects in murine HSCs, while proliferation is reduced by the IL-1 receptor antagonist Anakinra *in vitro*

The importance of IL-1 $\beta$  in stellate cell proliferation was reported previously<sup>[15]</sup>. This work implicates inhibition of the IL-1 pathway as a potential target in the treatment of liver fibrosis. However, blockade of this target by the clinically available IL-1 receptor antagonist Anakinra has not been tested yet regarding HSC pathophysiology and liver fibrosis.

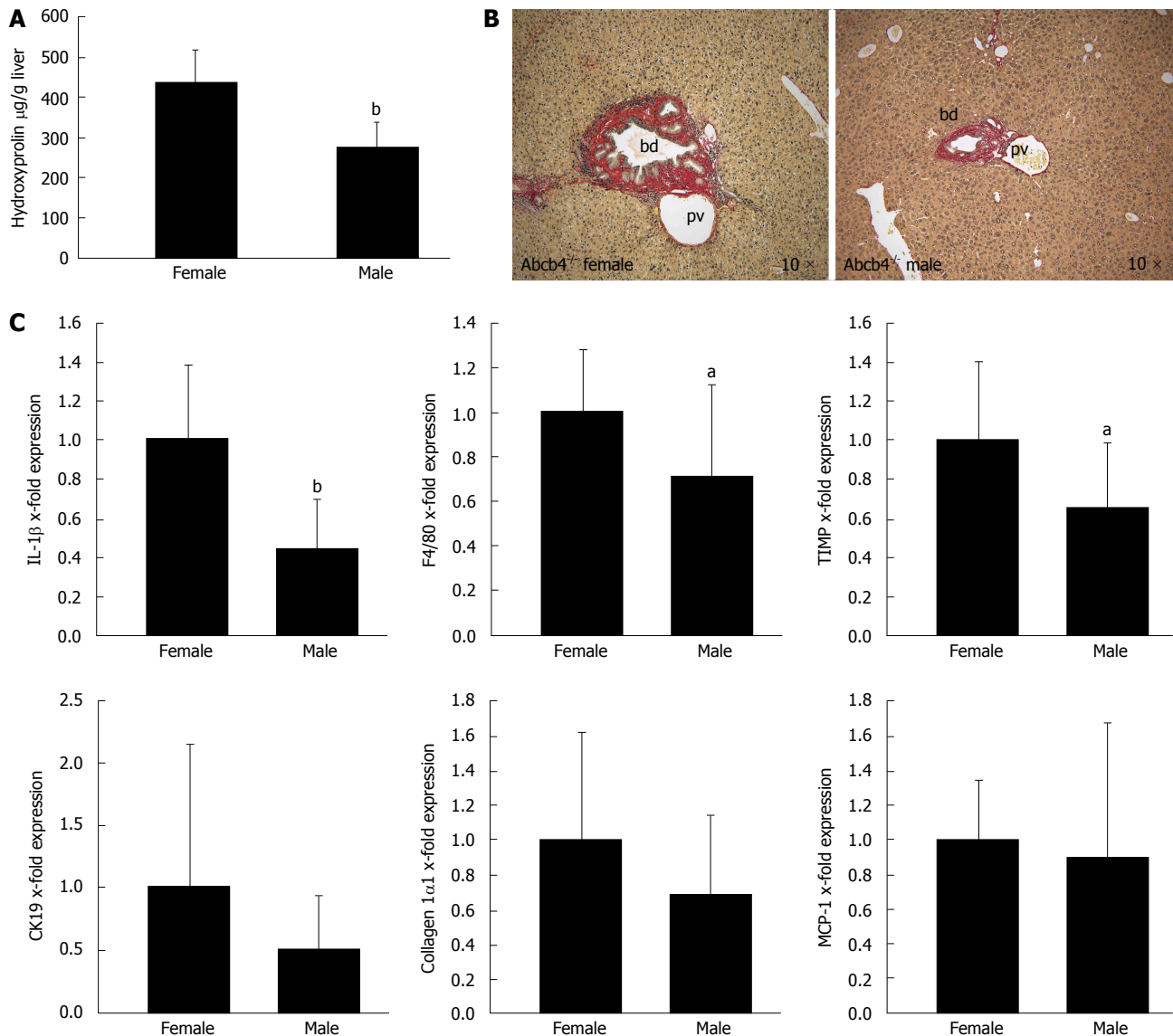
We addressed our *in vitro* experiments to investigate whether Anakinra could inhibit IL-1 $\beta$ -induced HSC proliferation.

In a first step, we evaluated the possible toxicity of Anakinra in primary murine HSCs using the FDH assay. This revealed no toxic effects of Anakinra at the tested doses, including the chosen dose of 2.5  $\mu$ g/mL (data not shown, *n* = 3).

Furthermore, we observed that IL-1 $\beta$  increased the proliferation of murine HSCs [Figure 2A; 0.94  $\pm$  0.72 (arbitrary units) A.U. in untreated controls, 1.12  $\pm$  0.80 A.U. at an IL-1 $\beta$  concentration of 0.1 ng/mL and 1.18  $\pm$  0.73 A.U. at an IL-1 $\beta$  concentration of 1 ng/mL in samples from *n* = 6 donor animals; general effect of IL-1 $\beta$  stimulation *P* < 0.001; control vs 0.1 ng/mL IL-1 $\beta$  *P* < 0.01; control vs 1 ng/mL IL-1 $\beta$  *P* < 0.001; ANOVA]. Due to the sufficient induction of HSC proliferation by IL-1 $\beta$ , the toxicity assay was not required for IL-1 $\beta$ .

Proliferation of murine HSCs was reduced significantly by treatment with 2.5  $\mu$ g/mL Anakinra (Figure 2B; 0.81  $\pm$  0.60 A.U. in untreated controls, 0.92  $\pm$  0.68 A.U. at an IL-1 $\beta$  concentration of 0.1 ng/mL, and 0.91  $\pm$  0.69 A.U. at an IL-1 $\beta$  concentration of 1 ng/mL; in sample from *n* = 6 donor animals; general effect of Anakinra: *P* < 0.001; effect of Anakinra without IL-1 $\beta$  stimulation: *P* < 0.01; effect of Anakinra with 0.1 ng/mL IL-1 $\beta$  stimulation: *P* < 0.01; effect of Anakinra with 1.0





**Figure 1** Gender differences in liver disease in ATP-binding cassette transporter *b4*<sup>-/-</sup> mice. Gender differences in liver fibrosis and liver inflammation were assessed with hydroxyproline measurement, Sirius red staining and quantitative real-time PCR in male and female *Abcb4*<sup>-/-</sup> mice between 6 and 13 mo of age. **A:** The hepatic hydroxyproline content of liver homogenates ( $\mu\text{g/g}$  liver) was lower in male animals than in female animals ( $n = 13-15$ ; <sup>b</sup> $P < 0.001$ ; Mann-Whitney *U*-test); **B:** Sirius-red staining illustrates the gender differences regarding fibrosis in *Abcb4*<sup>-/-</sup> mice. Images were taken from 9- to 10-mo-old animals (original magnification, 10 $\times$ ; bd: bile duct; pv: portal vein); **C:** Gene expression was assessed via quantitative real-time PCR and was normalised for 18 s as a housekeeping gene. Expressions were normalised against the means of female mice. The hepatic IL-1 $\beta$  and F4/80 mRNA expression levels as markers for hepatic inflammation were significantly lower in male *Abcb4*<sup>-/-</sup> mice than in female animals ( $n = 12-15$ ; <sup>b</sup> $P < 0.001$ ; <sup>a</sup> $P < 0.05$ ; Mann-Whitney *U*-test). The mRNA expression levels of the profibrotic gene TIMP-1 were also significantly lower in male *Abcb4*<sup>-/-</sup> mice than in female *Abcb4*<sup>-/-</sup> mice ( $n = 13-15$ ; <sup>a</sup> $P < 0.05$ ; Mann-Whitney *U*-test). PCR: Polymerase chain reaction; TIMP-1: Tissue inhibitor of metalloproteinase-1; *Abcb4*<sup>-/-</sup>: ATP-binding cassette transporter *b4*<sup>-/-</sup>; IL-1: Interleukin-1; CK19: Cytokeratin 19; MCP-1: Monocyte chemotactic protein-1.

ng/mL IL-1 $\beta$  stimulation:  $P < 0.001$ ; ANOVA), indicating a therapeutic effect of Anakinra on the proliferation of murine HSCs and, thereby, on liver fibrosis.

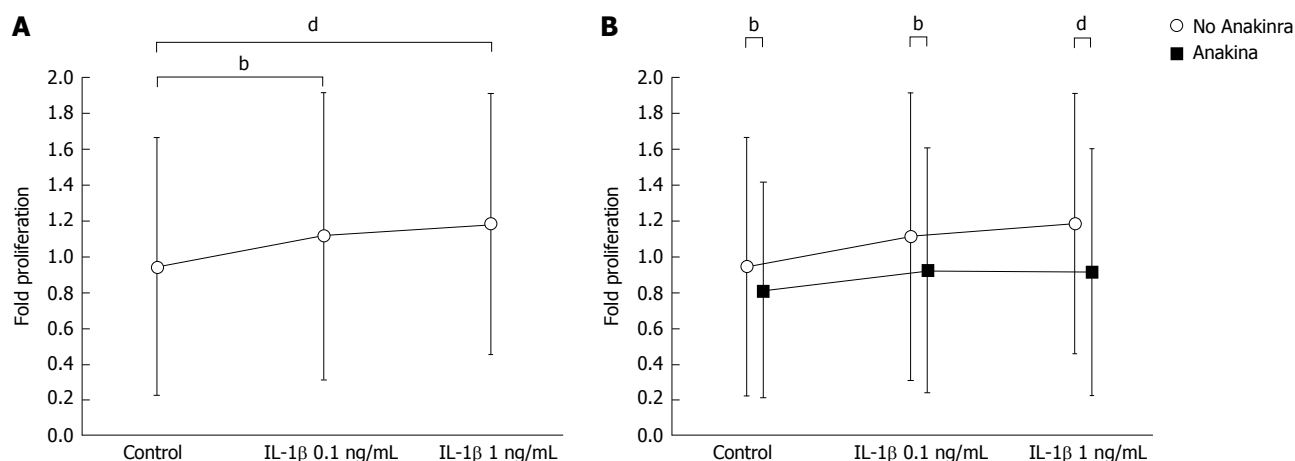
Taken together, Anakinra revealed promising anti-fibrotic effects *in vitro*.

#### **Anakinra has no therapeutic effects in *Abcb4*<sup>-/-</sup> mice**

The association of the degree of liver fibrosis and IL-1 $\beta$  expression in *Abcb4*<sup>-/-</sup> mice together with our *in vitro* data suggest a therapeutic effect of IL-1 antagonism on liver fibrosis *in vivo*. Therefore, we tested the therapeutic effects of Anakinra, a clinically approved

IL-1 receptor antagonist, on biliary fibrosis and hepatic injury in female *Abcb4*<sup>-/-</sup> mice.

The hepatic hydroxyproline levels as a marker of liver fibrosis were lower in untreated Wt animals than in untreated *Abcb4*<sup>-/-</sup> mice (Figure 3A;  $n = 11$ ;  $82 \pm 30 \mu\text{g/g}$  vs  $227 \pm 55 \mu\text{g/g}$  liver;  $P < 0.01$ ; Mann-Whitney *U*-test) but were unaffected by Anakinra treatment (Figure 3A;  $n = 10-11$ ; Mann-Whitney *U*-test). In accordance with this, we found differences regarding the serum parameters ALT (Figure 3A;  $89 \pm 87 \text{ U/L}$  vs  $454 \pm 133 \text{ U/L}$ ;  $n = 8-10$ ;  $P < 0.01$ ; Mann-Whitney *U*-test) and AP (Figure 3A;  $92 \pm 17 \text{ U/L}$  vs  $530 \pm 133 \text{ U/L}$ ;  $n$



**Figure 2** Interleukin-1 $\beta$  exerts proliferative effects in primary murine hepatic stellate cells, while Anakinra reduces hepatic stellate cell proliferation *in vitro*. The effects of IL-1 $\beta$   $\pm$  the IL-1 receptor antagonist Anakinra on the proliferation of murine HSCs were tested *in vitro*. A and B: The effects on HSC proliferation were examined using the BrdU assay after stimulation of cells with vehicle, and IL-1 $\beta$  at 0.1 and 1 ng/mL in the presence and absence of Anakinra (2.5  $\mu$ g/mL), respectively (samples from  $n = 6$  donor animals, <sup>b</sup> $P < 0.01$ ; <sup>d</sup> $P < 0.001$ ; ANOVA). HSC: Hepatic stellate cells; IL-1: Interleukin-1; ANOVA: Analyses of variance.

= 8-10;  $P < 0.01$ ; Mann-Whitney  $U$ -test) between the untreated Wt animals and Abcb4<sup>-/-</sup> mice but no effects of the treatment with Anakinra were observed (Figure 3A;  $n = 10-11$ ; Mann-Whitney  $U$ -test).

In a next step, we performed genetic profiling of fibrosis-related genes. Here, we found no influence of Anakinra on the profile of the profibrotic genes collagen 1 $\alpha$ 1, collagen 1 $\alpha$ 2, tissue growth factor- $\beta$ , and TIMP-1 (Figure 3B;  $n = 10-11$ ; Mann-Whitney  $U$ -test) between the groups.

In line with this finding, Anakinra treatment did not influence the expression of genes encoding the matrix-degrading enzymes matrix metalloproteinases 2, 9, and 13 (Figure 3C;  $n = 10-11$ ; Mann-Whitney  $U$ -test).

The mRNA expression levels of IL-1 $\beta$  and F4/80 as indicators of hepatic inflammation were also unaffected by Anakinra treatment (Figure 3D;  $n = 10-11$ ; Mann-Whitney  $U$ -test).

These missing antifibrotic effects were illustrated by Sirius red staining revealing no differences regarding periportal fibrosis (Figure 3E).

In summary, the *in vivo* administration of Anakinra showed no therapeutic effects on established fibrosis and hepatic injury in Abcb4<sup>-/-</sup> mice.

## DISCUSSION

Few treatment options are available to prevent disease progression to liver fibrosis and cirrhosis in chronic cholestatic liver disease such as PSC. In PSC, the frequently applied bile acid ursodeoxycholic acid (UDCA) failed to demonstrate a positive effect on the clinical outcome<sup>[25]</sup>, despite the beneficial effects of UDCA on serum biochemistry. Consequently, the American Association for the Study of Liver Disease no longer recommends the use of UDCA in PSC<sup>[2]</sup>. The loss of this therapeutic hope aggravates the need for an effective drug to treat PSC.

In the present study, we identified differences in

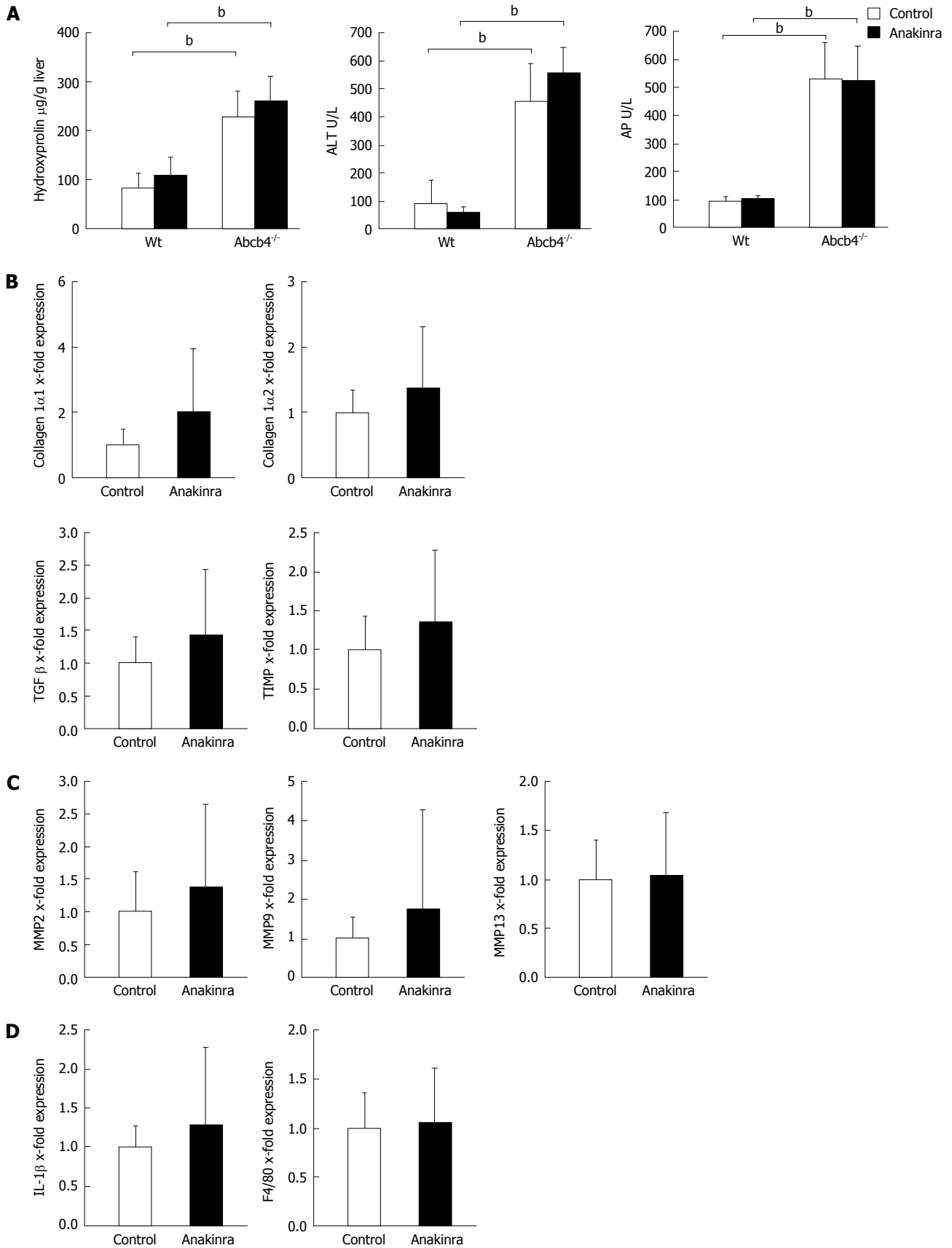
the levels of hepatic fibrosis between female and male Abcb4<sup>-/-</sup> mice. Hepatic fibrosis was more aggravated in female mice as assessed by the determination of the liver hydroxyproline content, a finding that is in line with that of previous studies, which identified a more severe histological phenotype in female animals<sup>[26]</sup>. Here, the pronounced phenotype in females was accompanied by higher mRNA expression levels of IL-1 $\beta$ , F4/80, and TIMP-1.

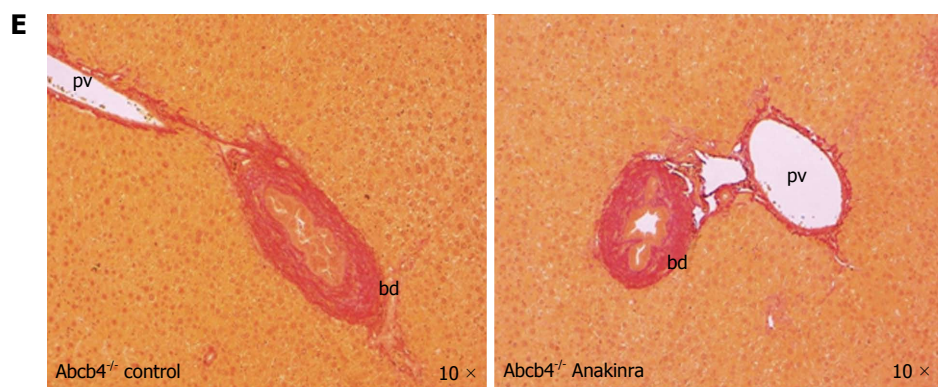
TIMP is a well-known profibrotic player in the regulation of matrix degradation during fibrogenesis and resolution *via* the inhibition of matrix metalloproteinases<sup>[27]</sup> but also *via* its anti-apoptotic effects on hepatic myofibroblasts<sup>[28,29]</sup>.

Therefore, the different TIMP levels might be a crucial factor for the observed gender differences in the degree of fibrosis. Hepatic inflammation, the role of chemokines, cytokines<sup>[30]</sup> and macrophages in the context of liver fibrosis are broadly discussed<sup>[31]</sup>. Macrophages may produce a wide spectrum of cytokines, including IL-1 $\beta$ , which are relevant for the pro-fibrogenic nature of HSCs<sup>[31-33]</sup>. Therefore, we hypothesised that the higher degree of fibrosis in female Abcb4<sup>-/-</sup> mice may be related to a more pronounced IL-1-driven hepatic inflammation and that the observed TIMP-1 alterations could reflect a consequence of inflammation. Thus, the IL-1 pathway might be a potential therapeutic target in PSC and fibrosis.

Supporting our hypothesis, we found that IL-1 $\beta$  stimulation exerts proliferative effects also on murine HSCs. These findings are in accordance with previously published data in rat HSCs<sup>[15]</sup>. Subsequently, we showed that the HSC proliferation was reduced by applying the clinically approved IL-1 receptor antagonist Anakinra. Because the proliferation of HSCs is a trigger of hepatic fibrosis<sup>[14]</sup>, these effects might be of consequence for disease progression *in vivo*.

In light of recently published data showing that IL-1 receptor antagonism ameliorates inflammasome-





**Figure 3** Anakinra does not reveal therapeutic effects on liver injury in ATP-binding cassette transporter b4<sup>-/-</sup> mice. Eight-week-old female Abcb4<sup>-/-</sup> animals were treated with daily intraperitoneal injections of saline (control) as vehicle or Anakinra (1 mg/kg body-weight) for 4 wk. A: The levels of hepatic hydroxyproline, alanine aminotransferase (ALT), and alkaline phosphatase (AP) were determined as described previously ( $n = 8-11$ ,  $^bP < 0.01$ ; Mann-Whitney *U*-test). Gene expression was assessed via quantitative real-time PCR using glyceraldehyde-3-phosphate dehydrogenase as the housekeeping gene. Data were normalised against the means of the controls (saline); B: No alterations in the profibrotic genes were found [ $n = 10-11$ ; not significant (n.s.); Mann-Whitney *U*-test]; C: No alterations in the antifibrotic genes were found ( $n = 10-11$ ; n.s.; Mann-Whitney *U*-test); D: Anakinra treatment did not result in a significant change in the mRNA expression of the pro-inflammatory gene IL-1 $\beta$  or F4/80 ( $n = 10-11$ , n.s., Mann-Whitney *U*-test); E: The Sirius-red staining illustrates the absence of antifibrotic effects (original magnification 10  $\times$ ). bd: Bile duct; pv: Portal-vein; PCR: Polymerase chain reaction; TIMP: Tissue inhibitor of metalloproteinase; Abcb4<sup>-/-</sup>: ATP-binding cassette transporter b4<sup>-/-</sup>; IL: Interleukin; MMP: Matrix metalloproteinase; TGF: Transforming growth factor.

dependent alcoholic steatohepatitis in mice<sup>[34]</sup>, our *in vitro* findings led us to test the efficacy of medical IL-1 receptor antagonism to prevent liver damage and fibrosis in the Abcb4<sup>-/-</sup> model.

We applied Anakinra at an established dose (1 mg/kg body-weight)<sup>[23]</sup> for 4 wk. This time span has been established to be sufficient to detect the therapeutic effects in the Abcb4<sup>-/-</sup> model<sup>[20,35]</sup>.

However, in the chosen setting, we found no effects on hepatic injury and fibrosis in this study. The serum markers of liver damage and cholestasis, as well as the analysis of liver tissue for fibrotic reaction, were unchanged.

This might be due to the distinctive pathophysiology of fibrosis in the Abcb4<sup>-/-</sup> model. The absence of phospholipids in Abcb4<sup>-/-</sup> mice results in a “toxic bile” constitution that leads to hepatic injury with scarring and obstruction of the biliary tree, finally causing cholestasis<sup>[36]</sup>. It is thought that portal myofibroblasts are primarily affected by the regurgitation of bile in this model<sup>[18]</sup>. These cells seem to rapidly acquire an activated phenotype in the early stages of biliary fibrosis<sup>[37]</sup> and might be the primary effectors of periportal fibrosis, which is the main type of fibrosis in the Abcb4<sup>-/-</sup> mouse<sup>[17,18]</sup>. This is also reflected by the previously observed increasing number in  $\alpha$ -SMA-positive cells per portal field of Abcb4<sup>-/-</sup> over time<sup>[18]</sup>. Our *in vitro* studies revealed the effects on HSCs, possibly might have different characteristics than portal myofibroblasts<sup>[37]</sup>. This could explain the missing transferability of the *in vitro* effects in HSCs on the *in vivo* situation in the Abcb4<sup>-/-</sup> model. Thus, it seems likely that the IL-1-dependent effects may be of pathophysiologic relevance in other types of hepatic fibrosis that are primarily caused by the activation and proliferation of perisinusoidal HSCs, a situation that might be different from cholestatic fibrosis. One might also suspect that

our results suggest that multiple pathways need to be considered for the therapeutic intervention of different types of liver fibrosis.

The observation that conventional immunosuppressive treatment has no therapeutic effect on PSC<sup>[2]</sup> is in accordance with the missing effects of our selective and putatively well-tolerable immunosuppressive approach by inhibition of the IL-1 pathway.

Taken together, this study demonstrated sex differences regarding hepatic inflammation and hepatic fibrosis in the Abcb4<sup>-/-</sup> model. Our *in vitro* experiments suggest the relevance of the IL-1 pathway in HSC proliferation and implicate the therapeutic potential of IL-1 antagonism. However, the treatment with the clinically approved IL-1 receptor antagonist Anakinra did not result in the amelioration of the hepatic phenotype in the Abcb4<sup>-/-</sup> model after 4 wk of treatment. Further studies might identify a therapeutic impact of this pathway in other types of liver fibrosis.

## ACKNOWLEDGMENTS

Parts of this work were presented at the annual meeting of the Bavarian Society for Gastroenterology 2015 in Garmisch-Partenkirchen. The statistical methods of this study were reviewed by Christoph Glasmacher, an external biostatistician (Christoph Glasmacher - Biometrics and SAS-Programming for Clinical Research).

## COMMENTS

### Background

Hepatic stellate cells (HSCs) are the major source of hepatic extracellular matrix deposition and crucially participate in the pathogenesis of liver fibrosis. In addition to other factors, enhanced proliferation of this cell type is thought to be an important profibrotic mechanism. In this regard, a recent study found that interleukin-1 $\beta$  (IL-1) $\beta$  induces the proliferation of rat HSCs. Furthermore, IL-1 was identified as a factor in the progression of liver injury to fibrosis.



Gender differences regarding hepatic injury in ATP-binding cassette transporter b4<sup>+</sup> (Abcb4<sup>+</sup>) mice were reported previously. However, differences in liver fibrosis were not evaluated in detail so far. In this study, the authors describe gender differences regard liver fibrosis in Abcb4<sup>+</sup> animals. Interestingly these findings are associated with coherent alterations of the IL-1 $\beta$  mRNA expression levels. Therefore, they hypothesised that the clinically approved IL-1 receptor antagonist Anakinra could reduce the proliferative effects of IL-1 $\beta$  in HSCs and improve fibrosis in Abcb4<sup>+</sup> mice. They also hypothesised that blocking the IL-1 pathway during liver fibrosis might be a therapeutic approach in this chronic cholestatic liver disease.

### Research frontiers

The proliferative effects of IL-1 $\beta$  on HSCs were described in former studies. However, a therapeutic approach via the clinically approved IL-1 receptor antagonist Anakinra was not investigated thus far regarding its efficacy in liver fibrosis.

### Innovations and breakthroughs

This is the first study to evaluate the efficacy of the clinically approved IL-1 receptor antagonist Anakinra on HSC proliferation *in vitro*. They report on the antiproliferative effects of this agent on HSC proliferation and identified a potential therapeutic approach. Furthermore, this study reported on gender differences regarding liver fibrosis in the Abcb4<sup>+</sup> mouse model.

### Applications

They *in vitro* findings support the importance of the IL-1 pathway in HSC proliferation. The effects of Anakinra on HSC proliferation suggest a therapeutic approach in liver fibrosis.

### Terminology

HSCs are the major source of hepatic extracellular matrix deposition and crucially participate in the pathogenesis of liver fibrosis. The Abcb4<sup>+</sup> mouse model is a preclinical model for primary sclerosing cholangitis and biliary liver fibrosis.

### Peer-review

This paper shows that the IL-1 signaling antagonist Anakinra can influence mouse HSC *in vitro* but not fibrosis in the Abcb4 mouse. Additional novel data is that female mice in this model are more affected than male mice.

## REFERENCES

- 1 Farrant JM, Hayllar KM, Wilkinson ML, Karani J, Portmann BC, Westaby D, Williams R. Natural history and prognostic variables in primary sclerosing cholangitis. *Gastroenterology* 1991; **100**: 1710-1717 [PMID: 1850376]
- 2 Chapman R, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ. Diagnosis and management of primary sclerosing cholangitis. *Hepatology* 2010; **51**: 660-678 [PMID: 20101749 DOI: 10.1002/hep.23294]
- 3 Boonstra K, Weersma RK, van Erpecum KJ, Rauws EA, Spanier BW, Poen AC, van Nieuwkerk KM, Drenth JP, Witteman BJ, Tuynman HA, Naber AH, Kingma PJ, van Buuren HR, van Hoek B, Vleggaar FP, van Geloven N, Beuers U, Ponsioen CY. Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. *Hepatology* 2013; **58**: 2045-2055 [PMID: 23775876 DOI: 10.1002/hep.26565]
- 4 Washington MK. Autoimmune liver disease: overlap and outliers. *Mod Pathol* 2007; **20** Suppl 1: S15-S30 [PMID: 17486048 DOI: 10.1038/modpathol.3800684]
- 5 Dilger K, Hohenester S, Winkler-Budenhofer U, Bastiaansen BA, Schaap FG, Rust C, Beuers U. Effect of ursodeoxycholic acid on bile acid profiles and intestinal detoxification machinery in primary biliary cirrhosis and health. *J Hepatol* 2012; **57**: 133-140 [PMID: 22414767 DOI: 10.1016/j.jhep.2012.02.014]
- 6 Hohenester S, Gates A, Wimmer R, Beuers U, Anwer MS, Rust C, Webster CR. Phosphatidylinositol-3-kinase p110 $\gamma$  contributes to bile salt-induced apoptosis in primary rat hepatocytes and human hepatoma cells. *J Hepatol* 2010; **53**: 918-926 [PMID: 20675006 DOI: 10.1016/j.jhep.2010.05.015]
- 7 Hohenester S, Wenniger LM, Paulusma CC, van Vliet SJ, Jefferson DM, Elferink RP, Beuers U. A biliary HCO<sub>3</sub><sup>-</sup> umbrella constitutes a protective mechanism against bile acid-induced injury in human cholangiocytes. *Hepatology* 2012; **55**: 173-183 [PMID: 21932391 DOI: 10.1002/hep.24691]
- 8 Rust C, Wild N, Bernt C, Vennegerts T, Wimmer R, Beuers U. Bile acid-induced apoptosis in hepatocytes is caspase-6-dependent. *J Biol Chem* 2009; **284**: 2908-2916 [PMID: 19017654 DOI: 10.1074/jbc.M804585200]
- 9 Denk G, Omary AJ, Reiter FP, Hohenester S, Wimmer R, Holdenrieder S, Rust C. Soluble intracellular adhesion molecule, M30 and M65 as serum markers of disease activity and prognosis in cholestatic liver diseases. *Hepatol Res* 2014; **44**: 1286-1298 [PMID: 24451045 DOI: 10.1111/hepr.12304]
- 10 Guicciardi ME, Gores GJ. Bile acid-mediated hepatocyte apoptosis and cholestatic liver disease. *Dig Liver Dis* 2002; **34**: 387-392 [PMID: 12132783]
- 11 Canbay A, Taimr P, Torok N, Higuchi H, Friedman S, Gores GJ. Apoptotic body engulfment by a human stellate cell line is profibrogenic. *Lab Invest* 2003; **83**: 655-663 [PMID: 12746475]
- 12 Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* 2000; **275**: 2247-2250 [PMID: 10644669]
- 13 Friedman SL. Mechanisms of disease: Mechanisms of hepatic fibrosis and therapeutic implications. *Nat Clin Pract Gastroenterol Hepatol* 2004; **1**: 98-105 [PMID: 16265071]
- 14 Lee UE, Friedman SL. Mechanisms of hepatic fibrogenesis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 195-206 [PMID: 21497738 DOI: 10.1016/j.bpg.2011.02.005]
- 15 Yaping Z, Ying W, Luqin D, Ning T, Xuemei A, Xixian Y. Mechanism of interleukin-1 $\beta$ -induced proliferation in rat hepatic stellate cells from different levels of signal transduction. *APMIS* 2014; **122**: 392-398 [PMID: 23992404 DOI: 10.1111/apm.12155]
- 16 Gieling RG, Wallace K, Han YP. Interleukin-1 participates in the progression from liver injury to fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G1324-G1331 [PMID: 19342509 DOI: 10.1152/ajpgi.90564]
- 17 Popov Y, Patsenker E, Fickert P, Trauner M, Schuppan D. Mdr2 (Abcb4)<sup>-/-</sup> mice spontaneously develop severe biliary fibrosis via massive dysregulation of pro- and antifibrogenic genes. *J Hepatol* 2005; **43**: 1045-1054 [PMID: 16223543]
- 18 Fickert P, Fuchsbichler A, Wagner M, Zollner G, Kaser A, Tilg H, Krause R, Lammert F, Langner C, Zatloukal K, Marschall HU, Denk H, Trauner M. Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. *Gastroenterology* 2004; **127**: 261-274 [PMID: 15236191]
- 19 Mauad TH, van Nieuwkerk CM, Dingemans KP, Smit JJ, Schinkel AH, Notenboom RG, van den Bergh Weerman MA, Verkruijsen RP, Groen AK, Oude Elferink RP. Mice with homozygous disruption of the mdr2 P-glycoprotein gene. A novel animal model for studies of nonsuppurative inflammatory cholangitis and hepatocarcinogenesis. *Am J Pathol* 1994; **145**: 1237-1245 [PMID: 7977654]
- 20 Fickert P, Wagner M, Marschall HU, Fuchsbichler A, Zollner G, Tsybrovskyy O, Zatloukal K, Liu J, Waalkes MP, Cover C, Denk H, Hofmann AF, Jaeschke H, Trauner M. 24-norUrsodeoxycholic acid is superior to ursodeoxycholic acid in the treatment of sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. *Gastroenterology* 2006; **130**: 465-481 [PMID: 16472600]
- 21 Friedman SL, Roll FJ. Isolation and culture of hepatic lipocytes, Kupffer cells, and sinusoidal endothelial cells by density gradient centrifugation with Stractan. *Anal Biochem* 1987; **161**: 207-218 [PMID: 3578783]
- 22 Jones KH, Senft JA. An improved method to determine cell viability by simultaneous staining with fluorescein diacetate-propidium iodide. *J Histochem Cytochem* 1985; **33**: 77-79 [PMID: 2578146]
- 23 Abbate A, Salloum FN, Vecile E, Das A, Hoke NN, Straino S, Biondi-Zoccai GG, Houser JE, Qureshi IZ, Ownby ED, Gustini E,

- Biasucci LM, Severino A, Capogrossi MC, Vetovec GW, Crea F, Baldi A, Kukreja RC, Dobrina A. Anakinra, a recombinant human interleukin-1 receptor antagonist, inhibits apoptosis in experimental acute myocardial infarction. *Circulation* 2008; **117**: 2670-2683 [PMID: 18474815]
- 24 **Edwards CA**, O'Brien WD. Modified assay for determination of hydroxyproline in a tissue hydrolyzate. *Clin Chim Acta* 1980; **104**: 161-167 [PMID: 7389130]
- 25 **Ali AH**, Carey EJ, Lindor KD. Current research on the treatment of primary sclerosing cholangitis. *Intractable Rare Dis Res* 2015; **4**: 1-6 [PMID: 25674381 DOI: 10.5582/ir.2014.01018]
- 26 **van Nieuwerck CM**, Groen AK, Ottenhoff R, van Wijland M, van den Bergh Weerman MA, Tytgat GN, Offerhaus JJ, Oude Elferink RP. The role of bile salt composition in liver pathology of mdr2 (-/-) mice: differences between males and females. *J Hepatol* 1997; **26**: 138-145 [PMID: 9148004]
- 27 **Ramachandran P**, Iredale JP. Liver fibrosis: a bidirectional model of fibrogenesis and resolution. *QJM* 2012; **105**: 813-817 [PMID: 22647759 DOI: 10.1093/qjmed/hcs069]
- 28 **Elsharkawy AM**, Oakley F, Mann DA. The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis. *Apoptosis* 2005; **10**: 927-939 [PMID: 16151628]
- 29 **Ismail MH**, Pinzani M. Reversal of hepatic fibrosis: pathophysiological basis of antifibrotic therapies. *Hepat Med* 2011; **3**: 69-80 [PMID: 24367223 DOI: 10.2147/HMER.S9051]
- 30 **Affö S**, Morales-Ibanez O, Rodrigo-Torres D, Altamirano J, Blaya D, Dapito DH, Millán C, Coll M, Caviglia JM, Arroyo V, Caballeria J, Schwabe RF, Ginès P, Bataller R, Sancho-Bru P. CCL20 mediates lipopolysaccharide induced liver injury and is a potential driver of inflammation and fibrosis in alcoholic hepatitis. *Gut* 2014; **63**: 1782-1792 [PMID: 24415562 DOI: 10.1136/gutjnl-2013-306098]
- 31 **Wynn TA**, Barron L. Macrophages: master regulators of inflammation and fibrosis. *Semin Liver Dis* 2010; **30**: 245-257 [PMID: 20665377 DOI: 10.1055/s-0030-1255354]
- 32 **Friedman SL**. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008; **88**: 125-172 [PMID: 18195085 DOI: 10.1152/physrev.00013.2007]
- 33 **Trautwein C**, Friedman SL, Schuppan D, Pinzani M. Hepatic fibrosis: Concept to treatment. *J Hepatol* 2015; **62**: S15-S24 [PMID: 25920084 DOI: 10.1016/j.jhep.2015.02.039]
- 34 **Petrasek J**, Bala S, Csak T, Lippai D, Kodys K, Menashy V, Barrieau M, Min SY, Kurt-Jones EA, Szabo G. IL-1 receptor antagonist ameliorates inflammasome-dependent alcoholic steatohepatitis in mice. *J Clin Invest* 2012; **122**: 3476-3489 [PMID: 22945633 DOI: 10.1172/JCI60777]
- 35 **Reiter FP**, Hohenester S, Nagel JM, Wimmer R, Artmann R, Wotke L, Makeschin MC, Mayr D, Rust C, Trauner M, Denk GU. 1,25-(OH)<sub>2</sub>-vitamin D<sub>3</sub> prevents activation of hepatic stellate cells in vitro and ameliorates inflammatory liver damage but not fibrosis in the Abcb4(-/-) model. *Biochem Biophys Res Commun* 2015; **459**: 227-233 [PMID: 25712522 DOI: 10.1016/j.bbrc.2015.02.074]
- 36 **Trauner M**, Fickert P, Halilbasic E, Moustafa T. Lessons from the toxic bile concept for the pathogenesis and treatment of cholestatic liver diseases. *Wien Med Wochenschr* 2008; **158**: 542-548 [PMID: 18998069 DOI: 10.1007/s10354-008-0592-1]
- 37 **Ramadori G**, Saile B. Portal tract fibrogenesis in the liver. *Lab Invest* 2004; **84**: 153-159 [PMID: 14688800]

**P- Reviewer:** Fan XM, Gorrell MD, Penkova-Radicheva MP, Xu J  
**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Liu SQ



Retrospective Study

# Retrocaval liver lifting maneuver and modifications of total hepatic vascular exclusion for liver tumor resection

Saiho Ko, Yuuki Kirihataya, Yayoi Matsumoto, Tadataka Takagi, Masanori Matsusaka, Tomohide Mukogawa, Hirofumi Ishikawa, Akihiko Watanabe

Saiho Ko, Yuuki Kirihataya, Yayoi Matsumoto, Tadataka Takagi, Masanori Matsusaka, Tomohide Mukogawa, Hirofumi Ishikawa, Akihiko Watanabe, Department of Surgery, Nara Prefecture General Medical Center, Nara 631-0846, Japan

**Author contributions:** Ko S conceived and designed the study, and wrote the manuscript; Kirihataya Y, Matsumoto Y and Takagi T acquired the data; Ko S, Matsusaka M and Mukogawa T analyzed and interpreted the data; Ishikawa H contributed to drafting of the manuscript; Watanabe A contributed to critical revision of the manuscript.

**Institutional review board statement:** The Institutional Ethical Committee of Nara Prefecture General Medical Center approved this retrospective clinical study.

**Informed consent statement:** Written informed consent was obtained from all patients for use of clinical data in research. The analysis is a retrospective study and the clinical data were anonymous.

**Conflict-of-interest statement:** None of the authors has any conflict of interest related to the publication of this manuscript.

**Data sharing statement:** There are no additional data available for this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Saiho Ko, Director, Department of Surgery, Nara Prefecture General Medical Center, 1-30-1 Nara, Nara 631-0846, Japan. [saihoko@naramed-u.ac.jp](mailto:saihoko@naramed-u.ac.jp)  
 Telephone: +81-742-466001  
 Fax: +81-742-466011

Received: November 28, 2015  
 Peer-review started: November 29, 2015  
 First decision: January 4, 2016  
 Revised: January 13, 2016  
 Accepted: March 7, 2016  
 Article in press: March 9, 2016  
 Published online: March 18, 2016

## Abstract

**AIM:** To evaluate the efficacy of technical modifications of total hepatic vascular exclusion (THVE) for hepatectomy involving inferior vena cava (IVC).

**METHODS:** Of 301 patients who underwent hepatectomy during the immediate previous 5-year period, 8 (2.7%) required THVE or modified methods of IVC cross-clamping for resection of liver tumors with massive involvement of the IVC. Seven of the patients had diagnosis of colorectal liver metastases and 1 had diagnosis of hepatocellular carcinoma. All tumors involved the IVC, and THVE was unavoidable for combined resection of the IVC in all 8 of the patients. Technical modifications of THVE were applied to minimize the extent and duration of vascular occlusion, thereby reducing the risk of damage.

**RESULTS:** Broad dissection of the space behind the IVC coupled with lifting up of the liver from the retrocaval space was effective for controlling bleeding around the IVC before and during THVE. The procedures facilitate modification of the positioning of the cranial IVC cross-clamp. Switching the cranial IVC cross-clamp from supra- to retrohepatic IVC or to the confluence of hepatic vein decreased duration of the THVE while restoring hepatic blood flow or systemic circulation *via* the IVC. Oblique cranial IVC cross-clamping avoided ischemia of the remnant hemi-liver. With these technical

modifications, the mean duration of THVE was  $13.4 \pm 8.4$  min, which was extremely shorter than that previously reported in the literature. Recovery of liver function was smooth and uneventful for all 8 patients. There was no case of mortality, re-operation, or severe complication (*i.e.*, Clavien-Dindo grade of III or more).

**CONCLUSION:** The retrocaval liver lifting maneuver and modifications of cranial cross-clamping were useful for minimizing duration of THVE.

**Key words:** Total hepatic vascular exclusion; Retrocaval liver lifting maneuver; Oblique clamping; Switching the clamp; Hepatectomy

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Total hepatic vascular exclusion (THVE) is needed for resection of liver tumors involving inferior vena cava (IVC). Because THVE has a high risk of morbidity, compared to inflow occlusion alone, its duration should be shortened. The technical modifications reported here minimized the risk of damage of THVE. Specifically, the procedures include the retrocaval liver lifting maneuver, switching of the cranial IVC cross-clamp, and oblique IVC cross-clamping. For the 8 patients retrospectively assessed, the duration of THVE was  $13.4 \pm 8.4$  min, which was remarkably shorter than that reported previously. Postoperative recovery was smooth for all patients, without severe complications.

Ko S, Kirihataya Y, Matsumoto Y, Takagi T, Matsusaka M, Mukogawa T, Ishikawa H, Watanabe A. Retrocaval liver lifting maneuver and modifications of total hepatic vascular exclusion for liver tumor resection. *World J Hepatol* 2016; 8(8): 411-420 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i8/411.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i8.411>

## INTRODUCTION

Despite recent advances in liver surgery, resection for tumors involving inferior vena cava (IVC) is still challenging<sup>[1-4]</sup>. This situation requires total hepatic vascular exclusion (THVE) for combined resection of the IVC. The THVE method was developed to control bleeding during hepatic parenchymal transection<sup>[5,6]</sup>; however, it is technically complicated and may cause profound liver damage and circulatory instability, and as a result inflow occlusion *via* Pringle's maneuver is more frequently used to control bleeding during usual hepatectomy, rather than THVE<sup>[7]</sup>. While THVE can effectively limit blood loss during hepatic parenchymal transection, especially that from hepatic veins<sup>[8,9]</sup>, its related rates of morbidity and mortality after hepatectomy for tumors involving IVC have been quite high<sup>[10,11]</sup>. Novel strategies to reduce the risk of damage and complications of THVE will benefit clinical practice and

patient outcome.

Long occlusion time is one of the most significant hazards of operative morbidity and mortality after hepatectomy with THVE<sup>[2]</sup>. There is no doubt about the importance of minimizing duration of THVE to reduce ischemic damage of the liver. Different from the procedure of portal triad clamping applied alone, addition of THVE necessitates taking into account both hepatic ischemia and instability of systemic circulation, including renal congestion. Selective hepatic vascular exclusion with hepatic venous occlusion preserving IVC flow is an alternative of THVE in usual hepatectomy<sup>[12,13]</sup>, but the method cannot be used for tumors involving IVC. Reports describing technical modifications aimed at minimizing hazards of THVE are available in the publicly available literature, but they are small in number<sup>[8,14]</sup>. Herein, we describe some technical modifications of THVE that were applied to patients in our hospital and which were found by retrospective analysis to have successfully minimized the duration and the extent of vascular occlusion. The purpose of reporting these results is to share these methods with clinicians and researchers in the field of hepatology so as to help reduce the rates of disastrous events during hepatectomy requiring THVE.

## MATERIALS AND METHODS

### Patients

From January 2010 to April 2015, 301 patients underwent hepatectomy at our hospital. Patients who underwent hepatectomy with THVE or modified THVE with IVC cross-clamping were selected for retrospective analysis, and those patients who had required only a small portion of the IVC to be resected without cross-clamping of the IVC were excluded from the study. A total of 8 patients (2.7% of the 301 hepatectomized patients) fit the criteria for inclusion, namely tumors involving IVC and treatment by combined resection of the IVC.

### Preoperative evaluation for indication of THVE

For all 8 patients, tumor status had been evaluated using three-phase contrast-enhanced computed tomography (CT). Contrast-enhanced magnetic resonance imaging (MRI) and/or (<sup>18</sup>F)-fluoro-D-glucose positron emission tomography had been performed in addition as necessary. The indication of THVE during hepatectomy was massive tumor involvement of IVC or tumor involvement of major hepatic vein extending to its confluence on IVC. Tumors that had been deemed as necessitating resection by side clamping of the IVC were excluded from the indication of THVE. THVE was not used merely for controlling bleeding from the parenchymal transection plane.

### Standard THVE procedures

By dissecting the coronary ligament around the suprahepatic IVC just below the diaphragm, the outer wall



**Table 1** Surgical procedures

Case No.	Diagnosis	No. of tumor	Size of the largest tumor, cm	THVE and modifications	THVE duration, min	Hepatectomy	Vascular reconstruction procedures	Outcome
1	HCC	1	20.0	Standard with switching <sup>1</sup>	5	Extended right hepatectomy	Direct suture of IVC	Alive without disease, 60 mo postoperative
2	Liver metastasis	1	20.0	Standard with switching <sup>1</sup>	9	Extended right hepatectomy	IMV patch for HV, Direct suture of IVC	Alive with disease, 17 mo postoperative
3	Liver metastasis	2	9.0	Standard with switching <sup>1</sup>	23	Extended right hepatectomy	IMV patch for HV and IVC	Alive with disease, 10 mo postoperative
4	Liver metastasis	1	6.0	Oblique <sup>2</sup> (right)	26	Right hepatectomy	IMV patch graft for IVC	Died of disease, 27 mo postoperative
5	Liver metastasis	4	2.5	Oblique <sup>2</sup> (right)	20	Partial resection	IMV patch graft for IVC and HV	Alive without disease, 15 mo postoperative
6	Liver metastasis	10	18.0	Oblique <sup>2</sup> (right)	7	Right hepatectomy, partial resection	Direct suture of IVC	Died of disease, 8 mo postoperative
7	Liver metastasis	15	5.5	Oblique <sup>2</sup> (right)	7	Extended anterior sectionectomy, partial resection	Direct suture of IVC	Died of disease, 9 mo postoperative
8	Liver metastasis	9	5.5	Oblique <sup>2</sup> (left)	10	Segmentectomy, partial resection	Direct suture of IVC and HVs	Alive without disease, 13 mo postoperative

<sup>1</sup>Switching the cross-clamp; <sup>2</sup>Oblique cross-clamping. IMV: Inferior mesenteric vein; IVC: Inferior vena cava; HVs: Hepatic veins; THVE: Total hepatic vascular exclusion.

of the IVC was made visible. Ligation and division of infradiaphragmatic veins had been made as necessary. For all 8 patients, the attachment between the liver and diaphragm was dissected, and the dorsal space of the suprahepatic IVC was dissected. Subsequently, the suprahepatic IVC was encircled gently and taped in preparation for cranial cross-clamping of the IVC. The infrahepatic IVC was also dissected circumferentially and encircled in preparation for caudal cross-clamping. Principally, THVE was not applied before completion of hepatic parenchymal transection. The final step of resection involved clamping of the caudal and cranial IVC prior to the resection and reconstruction of the involved IVC *via* THVE.

#### Other surgical procedures

The abdomen was opened *via* J-shape incision that included upper median and right oblique incisions. Tumor status and the relation between the tumors and major intrahepatic vasculature were confirmed by intraoperative ultrasonography. The liver was mobilized to the extent necessary for the planned surgery. The hepatic parenchyma was transected using the clamp-crushing method. Thin vessel branches were burned by electrocautery or soft coagulation devices. Thicker branches were ligated and divided. Intermittent Pringle's maneuver was applied routinely during the hepatic parenchymal transection using a 15-min/5-min period of clamping and release.

#### Evaluation of operative morbidity and mortality

The recorded postoperative complications were classified according to severity by using the Clavien-Dindo criteria (grades I - V). Hepatic failure was defined as serum level of total bilirubin > 5.0 mg/dL (equal to 85.5  $\mu$ mol/L) at postoperative day 5 or later. Operative mortality was defined as all in-hospital deaths and death

within 90 d after surgery.

#### Follow-up schedule and adjuvant chemotherapy

Follow-up after discharge involved CT or MRI evaluation every 4-6 mo and blood testing and physical examination every 1-6 mo for a total period of 5 years after the last intervention.

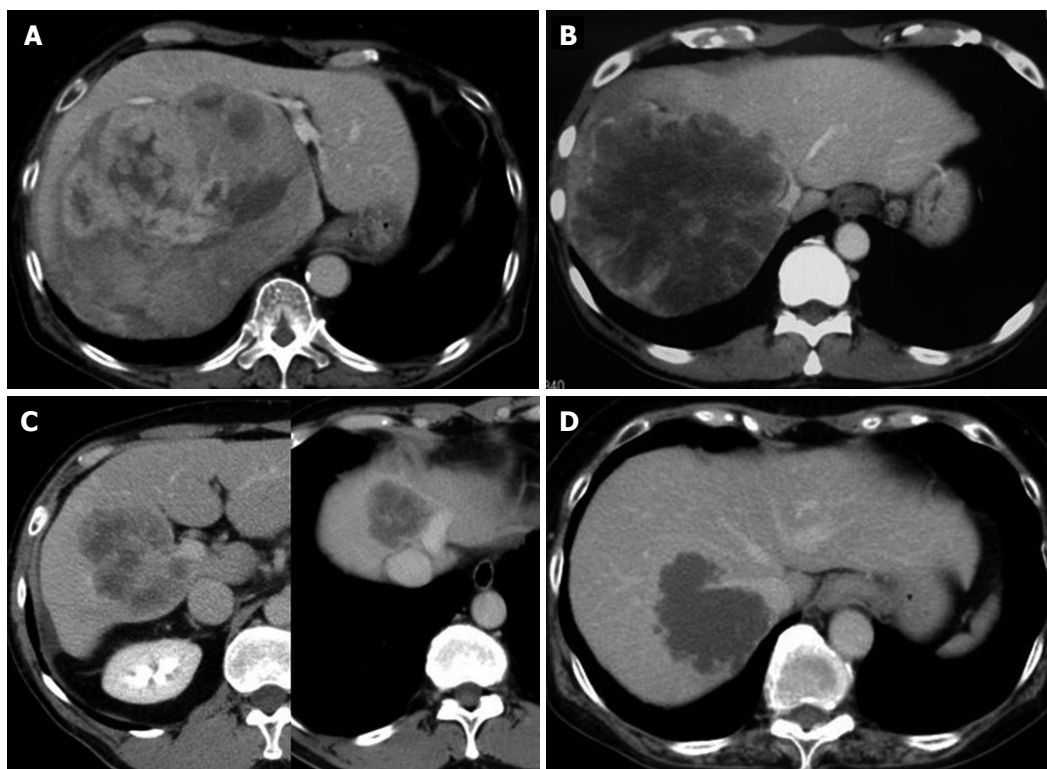
#### Statistical analysis

All numerical values were calculated as mean and SD or median and range.

## RESULTS

The 8 patients included in this analysis consisted of 6 men and 2 women, with median age of 72-years-old (range: 36-78 years). Seven of the patients had a diagnosis of colorectal liver metastases and 1 had a diagnosis of hepatocellular carcinoma (Table 1). The mean diameter of the total resected tumors was  $10.8 \pm 7.3$  cm. All patients had tumors involving the IVC, with or without confluence of the hepatic veins as shown in Figure 1.

The surgical procedures used for all 8 patients are listed in Table 1. For all, the initial surgical step was broad dissection of the space behind the IVC, after which the liver was lifted up by the surgeon's left hand to move it from the retrocaval space in order to control bleeding before and during THVE (retrocaval liver lifting maneuver). Standard THVE was applied to 3 patients (cases 1-3), for which the cranial cross-clamp for THVE was switched from the suprahepatic to retrohepatic levels or to the confluence of the major hepatic vein to shorten the duration of THVE (switching the cross-clamp). For the remaining 5 patients (cases 4-8), total ischemia of the whole liver was avoided by applying the



**Figure 1** Abdominal computed tomography scan images of cases 1-4. A: Case 1: Lumen of the IVC compressed by a huge right liver tumor with expansive growth; B: Case 2: A large right liver tumor involving the right aspect of the IVC and the confluence of middle and left hepatic veins; C: Case 3: Two tumors were present, a right liver tumor involving the right portal vein and the portal vein bifurcation, and another tumor occupying the cranial part of segments 4 and 8 and involving the left aspect of the trunk of the middle and left hepatic veins; D: Case 4: A tumor involving the right dorsal aspect of the IVC and its confluence of the right hepatic vein. IVC: Inferior vena cava.

cranial clamp obliquely to maintain the hepatic venous outflow of the liver remnant (oblique cross-clamping). Detailed descriptions of these techniques are provided below.

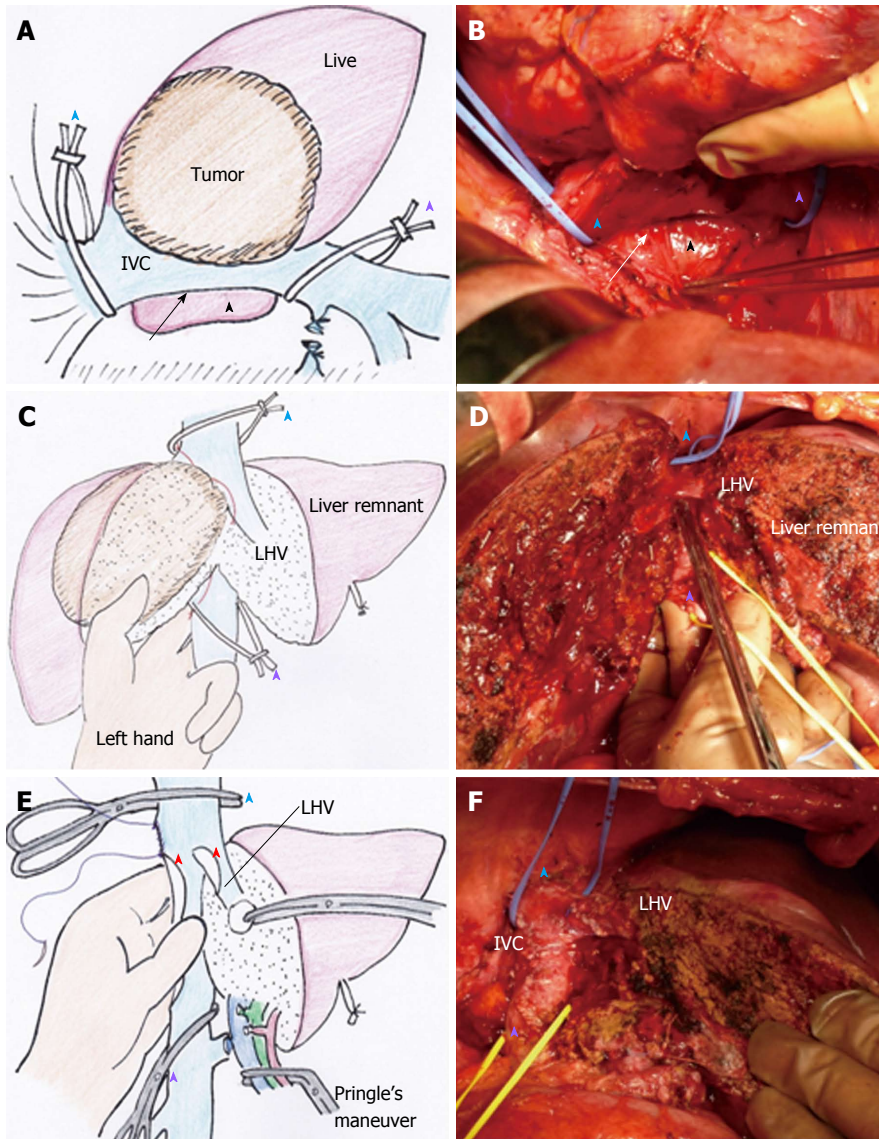
### Technical modifications for THVE

**Retrocaval liver lifting maneuver:** The liver cannot be detached from the IVC when the tumor involves the IVC. Instead, the retrocaval space behind the hepatic IVC should be dissected broadly to facilitate performance of standard or modified THVE (Figure 2A and B). In all 8 patients of this study, the IVC was compressed ventrally and the liver was lifted up from the retrocaval space by the surgeon's left hand (Figure 2C and D). This maneuver was quite useful for controlling bleeding during transection of the hepatic parenchyma near the IVC. With the help of this maneuver parenchymal transection can be completed safely before applying THVE, as shown in Figure 2D. The THVE procedure was applied at the last step of the combined resection and reconstruction of IVC, with the involved part of the IVC excised en-bloc with the liver specimen (Figure 2E). Theoretically, no bleeding should come from the IVC under THVE, but a significant amount of backflow bleeding often comes from the cut orifice of IVC during THVE. For all 8 patients in this study, compressing the IVC upward from the retrocaval space, by means of

the surgeon's left hand, was effective for controlling backflow bleeding from the cut orifice of the IVC under THVE (Figure 2E).

**Switching the cross-clamp:** Standard THVE cannot be avoided for combined resection of IVC involving the major hepatic vein of the liver remnant, as was performed in case 3 of this study (Figure 3A). After removal of the specimen and completion of the repair of the IVC under standard THVE, the position of the cranial IVC cross-clamp was switched from the suprahepatic IVC to the IVC confluence of the resected hepatic vein to be reconstructed (Figure 3C). Then, the IVC blood flow was restored to stabilize systemic hemodynamics.

Another surgical situation in this study involved switching of the cross-clamp of the suprahepatic IVC to the retrohepatic level, as performed in case 1. The tumor status is shown in Figure 1A. At the first step of this intervention, standard THVE was applied for resection of large hepatocellular carcinoma that involved retrohepatic IVC massively. Both the tumor and the involved wall of the IVC were resected under standard THVE. After repairing the IVC defect around the confluence of the hepatic vein, the suprahepatic IVC cross-clamp was switched to the level just below the hepatic vein confluence. Then, the portal triad occlusion was released to restore the blood flow of the liver remnant, after which



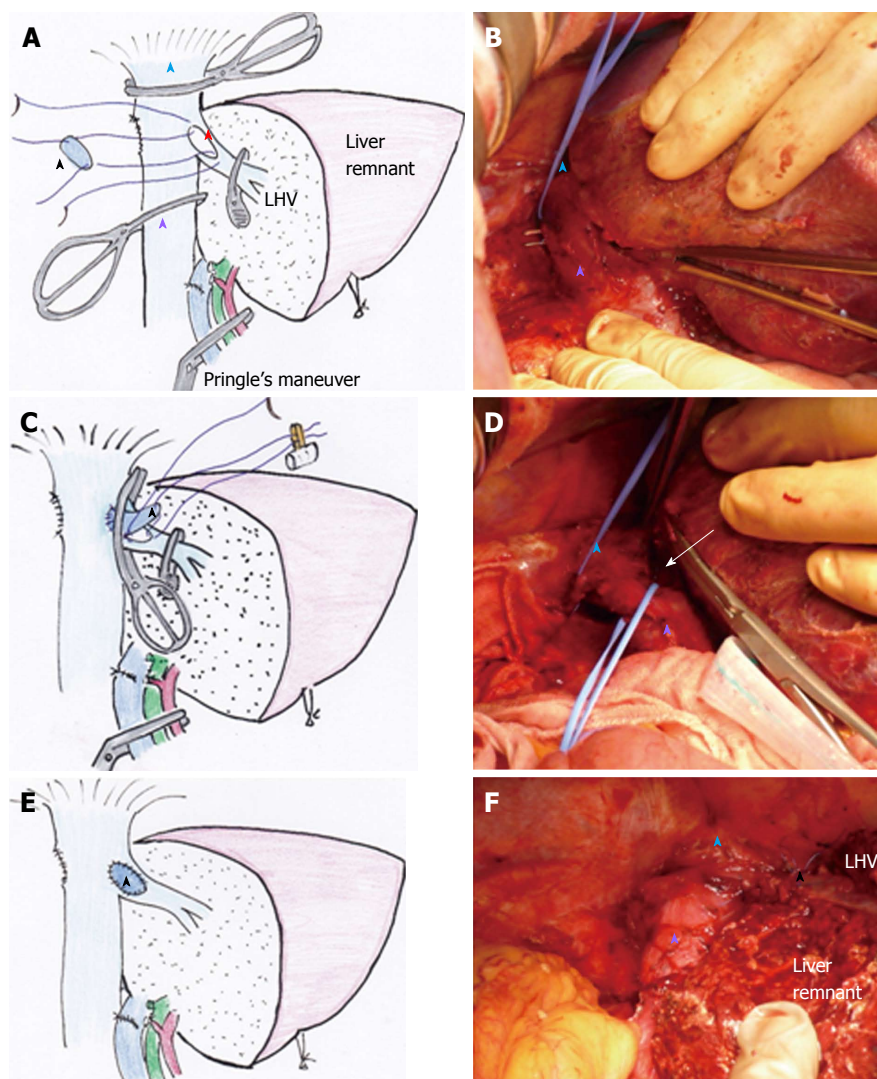
**Figure 2 Retrocaval liver lifting maneuver, performed in case 2.** A (illustration) and B (intraoperative image): The retrocaval space (arrow) was dissected broadly from the right lateral view toward the inner aspect of Spiegel's lobe (black arrowhead), after which the supra- and infrahepatic IVC were taped to prepare cross-clamping for THVE (blue arrowhead and purple arrowhead); C (illustration) and D (intraoperative image): The index and middle fingers of the surgeon's left hand were placed into the dissected retrocaval space, and the IVC was compressed ventrally to control bleeding around the IVC during deep parenchymal transection, after which the thumb finger of the surgeon's left hand was used to spread the transection plane of the liver (C, which also shows the tumor status); D: Hepatic parenchymal transection is completed, except for the IVC involved site, before applying THVE; E (illustration) and F (intraoperative image): The liver specimen was excised along with the involved IVC and LHV wall (red arrowheads), and the backflow bleeding from the cut orifice of IVC was controlled by pinching the IVC using the surgeon's left hand from its placement in the retrocaval space; F shows the view after reconstruction of IVC and LHV. See Figure 1B for tumor status. IVC: Inferior vena cava; LHV: Left hepatic vein; THVE: Total hepatic vascular exclusion.

the retrohepatic IVC was repaired without prolonging the ischemic time of the liver.

**Oblique cranial cross-clamping:** When the space between the right and middle hepatic veins is free from involvement, venous drainage of the residual liver can be preserved during vascular exclusion by applying the cranial cross-clamping obliquely (Figure 4A). For this purpose, the retrocaval space must be dissected sufficiently in advance, same as shown in Figure 2A. Prior to this step, hepatic parenchymal transection had been completed to facilitate visualization of the anterior aspect of the hepatic IVC (Figure 4A and B). In the next step,

the vascular clamp was inserted behind the IVC obliquely, preserving the outflow orifice of the hepatic vein of the liver remnant (Figure 4A and B). By applying this modification of the THVE procedure, ischemic damage was avoided, as was intestinal congestion. In patients of this study for whom the right liver tumor involved the IVC massively around the orifice of right hepatic vein (RHV) (as in cases 4-7), the cranial IVC cross-clamp was applied from the right cranial side to the left caudal side of the IVC to allow venous drainage of the trunk of middle and left hepatic veins (MHV + LHV) (Figure 4A). In patients of this study for whom the left liver tumor involved the trunk of MHV + LHV (as in case 8), the





**Figure 3 Switching the cross-clamp, performed in case 3.** A (illustration) and B (intraoperative image): The right liver and involved right wall of the LHV and a part of the IVC wall had been resected en-bloc under standard THVE (red star indicates the orifice of resected IVC and LHV), before which the suprahepatic IVC was taped (blue arrowhead, blue tape), and the caudal cross-clamp was placed just under the confluence of LHV to the retrocaval space that had been already dissected (B). The defect of the confluence of the IVC and LHV was to be reconstructed by using an IMV patch graft (black arrowhead); C (illustration) and D (intraoperative image): After suturing the IMV patch graft to the IVC part of the defect, the IVC cross-clamps were removed and the clamp was switched to the confluence of the LHV to restore the systemic circulation via the IVC. The space for switching the clamp was spread by the caudal blue tape (white arrow in D); E (illustration) and F (intraoperative image): The view after reconstruction is shown. See Figure 1C for tumor status. IMV: Inferior mesenteric vein; IVC: Inferior vena cava; LHV: Left hepatic vein; THVE: Total hepatic vascular exclusion.

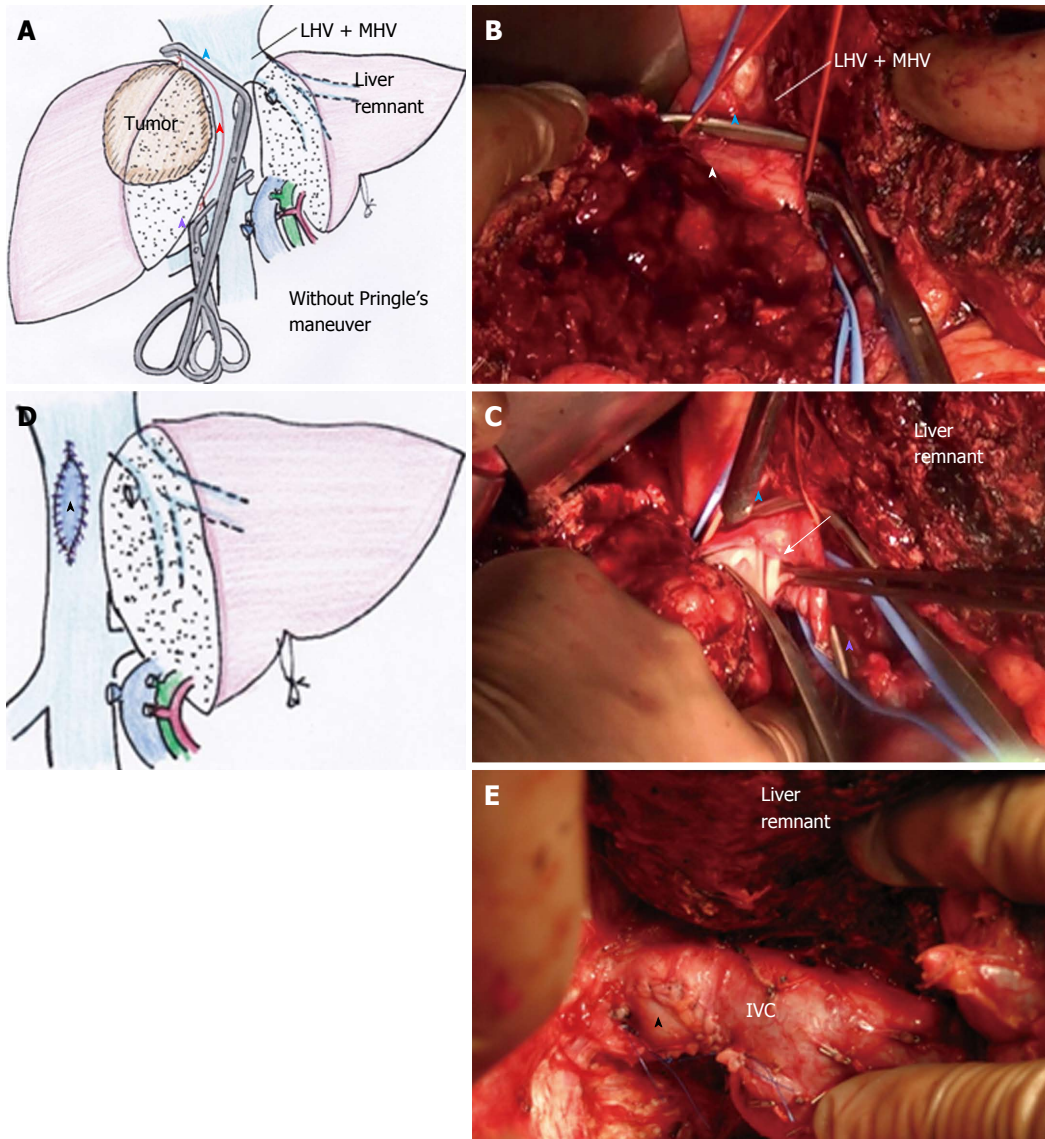
clamp was applied from the left cranial line to the right caudal line to preserve the outflow of the RHV. In the 5 patients who represented these two special situations, the THVE duration presented in Table 1 equates to the occlusion time of oblique cross-clamping of the IVC.

#### Operative parameters and postoperative course

The mean values of the operative parameters for the total 8 patients in this study are presented in Table 2. In no case did the THVE time exceed 30 min. Results from biochemical liver function tests and prothrombin time indicated the smooth recovery of liver function during the first postoperative week. In no case did the maximum serum total bilirubin level exceed 5 mg/mL, and no patient developed liver failure. For all of the

patients, the serum total bilirubin levels gradually decreased during the first postoperative week to below 2 mg/mL (equal to 34.2  $\mu$ mol/L) by day 7. No patient showed a prothrombin time less than 50% at any time during the postoperative period. In addition, no patient experienced a complication of Clavien-Dindo grade III or higher and there was no case of operative mortality. The postoperative complications that occurred included leg edema, pleural effusion, refractory ascites, and wound infection ( $n = 1$  each). No patient required reoperation, subsequent surgical or radiological interventions, or management in the intensive care unit, and all patients were discharged within a month after the surgery. All of the reconstructed vessels remained patent through the end of follow-up.





**Figure 4 Oblique cranial cross-clamping, performed in case 4.** A (illustration) and B (intraoperative image): The trunk of LHV + MHV was free from tumor involvement, while the right aspect of the IVC was involved extensively (red arrowhead). After completion of hepatic parenchymal transection, the cranial IVC cross-clamp (blue arrowhead) was applied obliquely between the tumor-involved IVC and the trunk of LHV + MHV preserving outflow of the LHV + MHV trunk, which was possible because the retrocaval space had been dissected sufficiently. Then, the involved IVC wall (white arrowhead in B) and the liver was resected *en-bloc* under modified THVE; C (intraoperative image): The involved IVC wall was cut away with scissors (arrow) between the oblique cranial (blue arrowhead) and caudal (purple arrowhead) cross-clamps; D (illustration) and E (intraoperative image): The large cut orifice of IVC was reconstructed with IMV patch graft (black arrowhead). See Figure 1D for tumor status. IMV: Inferior mesenteric vein; IVC: Inferior vena cava; LHV: Left hepatic vein; THVE: Total hepatic vascular exclusion.

### Survival

All patients returned to usual active life after discharge. The 1-year survival rate was 71%. Individual outcomes for each patient are presented in Table 1. At the date of this report, case 1 was alive without disease (5 years after surgery) and cases 6 and 7 presented with early multiple tumor recurrence in the liver remnant and died of rapid progression at 8 and 9 mo respectively.

### DISCUSSION

Liver tumors involving IVC or its junction of major hepatic veins represent situations in which THVE is needed for curative resection. Such surgery is fraught

with challenges, including unstable systemic circulation, ischemic damage of the liver and high risk of morbidity. Minimizing the duration and extent of exclusion is key to increasing the safety of THVE<sup>[2]</sup>. To this end, the present study highlights the feasibility of three technical modifications of THVE. The techniques include: (1) retrocaval liver lifting maneuver; (2) switching the cross-clamp; and (3) oblique cranial cross-clamping, which minimized duration of THVE and ischemic damage of the liver. It is noteworthy that application of these modified techniques minimized occlusion time; in addition, recovery of liver function was smooth, no severe complication developed, and operative mortality was not experienced. These operative results are quite favorable,

**Table 2** Operative parameters and postoperative course of 8 patients

Parameters	Values or number of patients
Operation time	482 ± 108 min
Blood loss	1778 ± 1233 mL
THVE duration	13.4 ± 8.4 min
Postoperative liver function	
TB (mg/dL)	
Maximum	2.01 (0.98-4.4)
POD7	1.08 (0.75-1.86)
AST (IU/L)	
Maximum	513 (238-1058)
POD7	30 (20-41)
ALT (IU/L)	
Maximum	319 (179-824)
POD7	64 (36-115)
PT (%)	
Minimum	64.4 (55.3-88.8)
POD7	88.1 (61.6-107.8)
Complications	
Clavien-Dindo classification	
I	2
II	1
≥ III	0
Operative mortality	0
Hospital stay (d)	15 (12-24)

AST: Aspartate amino transferase; PT: Prothrombin time; TB: Total bilirubin; ALT: Aspartate alanine aminotransferase; THVE: Total hepatic vascular exclusion.

particularly when compared to those reported by other studies<sup>[1-3,8,10,11]</sup>.

To date, only a limited number of studies of THVE for resection of liver tumors involving IVC are present in the publicly available literature, probably because the number of experiences in individual institutes has been small. The most distinctive features of the present study are the extremely short duration of THVE and favorable recovery after surgery. While the mean durations of THVE were 29 min to 78 min in the reports from very experienced institutes<sup>[1,2,7,8]</sup>, the mean THVE duration in the present study was only 13.4 ± 8.4 min. The mortality rates of hepatectomy with IVC resection were 4.5% to 25% in the previous reports<sup>[1-4,8,10,11,15]</sup>. In particular, the morbidity and mortality rates were quite high when standard THVE was applied frequently, even when hypothermic perfusion was used to attenuate the ischemic liver damage. In the previous reports, the major causes of mortality were liver failure and sepsis<sup>[1,3,4,8,11]</sup>, both of which are likely to be relevant to ischemic injury of the liver and intestinal congestion since they may facilitate bacterial translocation. Of course, simple comparison to the present case series is not possible due to the differences in severity of tumor status and underlying conditions. Nevertheless, minimized duration of THVE might have contributed to the favorable postoperative course in the present study.

Hand manipulation of the IVC from the broadly dissected retrocaval space is a unique method to decrease bleeding around the IVC. This procedure is also essential

as a preparation for modifying the THVE procedure to improve its safety. When the tumor involves the IVC, the liver cannot be freed from the IVC and Belghiti's liver hanging maneuver is not possible (and is rather a contraindication)<sup>[16]</sup>. In such a situation, wide dissection of the retrocaval space makes subsequent procedures safer. Lifting-up the liver by hand from the retrocaval space proved quite useful to control bleeding from the hepatic parenchyma near the IVC and backflow bleeding from the cut orifice of the IVC during THVE. Although no bleeding is supposed to come from the IVC under THVE theoretically, significant amount of backflow bleeding, which occurs frequently, serves to disrupt and complicate the vascular reconstruction procedure of IVC. Sources of such backflow bleeding are lumbar veins, short hepatic veins, or small venous branches that extend into the major hepatic veins. Even with portal triad occlusion by Pringle's maneuver, blood flow into the liver from the diaphragm or lesser omentum can exist. Compressing the IVC by hand, from the retrocaval space, was shown in the present study to be quite useful and the only way to control backflow bleeding during THVE.

The damage associated with THVE includes both systemic circulatory instability (due to absence of venous return *via* the IVC) and total cessation of hepatic blood flow. These conditions cause congestion of the kidney and intestine, which may explain why the damage and morbidity after THVE is much higher than that experienced after inflow occlusion alone<sup>[7]</sup>. In the current study, when the cranial IVC cross-clamp was switched from the supra- to the retrohepatic IVC (as performed in case 1), blood flow of the liver remnant was restored, thereby shortening the ischemic time of the liver remnant and intestine. When the clamp was switched to the confluence of hepatic vein of the liver remnant (as performed in case 3), IVC blood flow was restored, thereby resolving the systemic circulatory instability and renal congestion.

Oblique cranial IVC clamping is an option of standard THVE to avoid ischemic damage of the liver remnant as well as intestinal congestion; yet, this technique has not been precisely described in the literature. Even in the series of patients in this study with tumors with massive IVC involvement, the hepatic vein of the liver remnant side was free from involvement in many of the cases, and this is reported in other studies as well<sup>[1,8]</sup>. Such a situation is good indication for oblique cross-clamping. Sufficient dissection of the retrocaval space and completion of hepatic parenchymal transection in advance are essential for application of this method.

The timing of applying THVE may be one of the most important issues underlying its efficacy and safety. In most of the studies reported in the literature, THVE was applied during both parenchymal transection and combined resection/reconstruction of IVC<sup>[1,7,8]</sup>. This might be one of the reasons for the characteristic long occlusion time in the studies previous to ours. The primary policy of our hospital, however, is to make

every effort to minimize the duration of THVE. Because the retrocaval liver lifting maneuver makes it possible to complete the hepatic parenchymal transection without much bleeding, THVE was applied at the final step only for IVC resection and reconstruction. We believe that its success depends primarily on the procedures that had been applied prior to the application of THVE, including sufficient dissection of the retrocaval space and smooth completion of the hepatic parenchymal transection.

The small number of included patients may be a limitation of the present study. In general, the number of patients requiring THVE for hepatectomy with IVC reconstruction is rather small for a single institute, such as ours, as reflected in the previous studies<sup>[1-4,10,11,15]</sup>. The accumulated literature on this topic includes no reports that provide a definitive description of modified procedures of THVE, especially the retrocaval liver lifting maneuver and oblique cranial cross-clamping that are described here for the first time. These techniques can be strategies to decrease the risk of disastrous bleeding and ischemic liver damage. Sharing these data with other surgeons will serve to increase the safety and feasibility of surgery for liver tumors involving IVC.

In conclusion, the retrocaval liver lifting maneuver and modifications of IVC cross-clamping are useful to attenuate damage related to THVE. The knowledge and techniques that have arisen from the current analysis of our case series may help to improve the surgical techniques and outcomes for advanced liver tumors involving IVC.

## COMMENTS

### Background

Total hepatic vascular exclusion (THVE) is needed during hepatectomy for liver tumors involving inferior vena cava (IVC). However, THVE carries a much greater risk than inflow occlusion alone.

### Research frontiers

The authors performed technical modifications that shortened the duration of THVE, thereby reducing the risk of damage.

### Innovations and breakthroughs

The technical modifications described in this study for THVE included the retrocaval liver lifting maneuver, switching of the cranial IVC cross-clamp, and oblique cranial IVC cross-clamping. With these techniques, the mean duration of THVE was shortened remarkably, compared to that reported in the past literature. Moreover, postoperative recovery of liver function was smooth without any severe complications.

### Applications

The retrocaval liver lifting maneuver and modifications of cranial cross-clamping minimized the duration of THVE. Thus, these techniques might help to decrease risk of liver damage and increase likelihood of favorable postoperative courses in patients who undergo hepatectomy requiring THVE.

### Terminology

THVE is a method to control bleeding during hepatectomy by occluding both inflow and outflow of the liver. THVE is required especially for resection of liver tumors involving IVC.

## Peer-review

The authors suggest some technical modifications of the THVE. The study is clearly planned and correctly managed. The technical details of the three variations are clearly explained as well as the figures.

## REFERENCES

- 1 **Azoulay D**, Andreani P, Maggi U, Salloum C, Perdigao F, Sebah M, Lemoine A, Adam R, Castaing D. Combined liver resection and reconstruction of the supra-renal vena cava: the Paul Brousse experience. *Ann Surg* 2006; **244**: 80-88 [PMID: 16794392 DOI: 10.1097/01.sla.0000218092.83675.bc]
- 2 **Azoulay D**, Pascal G, Salloum C, Adam R, Castaing D, Tranecol N. Vascular reconstruction combined with liver resection for malignant tumours. *Br J Surg* 2013; **100**: 1764-1775 [PMID: 24227362 DOI: 10.1002/bjs.9295]
- 3 **Madariaga JR**, Fung J, Gutierrez J, Bueno J, Iwatsuki S. Liver resection combined with excision of vena cava. *J Am Coll Surg* 2000; **191**: 244-250 [PMID: 10989898 DOI: 10.1016/S1072-7515(00)00362-8]
- 4 **Hemming AW**, Mekeel KL, Zendejas I, Kim RD, Sicklick JK, Reed AI. Resection of the liver and inferior vena cava for hepatic malignancy. *J Am Coll Surg* 2013; **217**: 115-124; discussion 124-125 [PMID: 23376028 DOI: 10.1016/j.jamcollsurg.2012.12.003]
- 5 **Heaney JP**, Stanton WK, Halbert DS, Seidel J, Vice T. An improved technic for vascular isolation of the liver: experimental study and case reports. *Ann Surg* 1966; **163**: 237-241 [PMID: 4286023 DOI: 10.1097/0000658-196602000-00013]
- 6 **Huguet C**, Nordlinger B, Galopin JJ, Bloch P, Gallot D. Normo-thermic hepatic vascular exclusion for extensive hepatectomy. *Surg Gynecol Obstet* 1978; **147**: 689-693 [PMID: 715645]
- 7 **Belghiti J**, Noun R, Zante E, Ballet T, Sauvanet A. Portal triad clamping or hepatic vascular exclusion for major liver resection. A controlled study. *Ann Surg* 1996; **224**: 155-161 [PMID: 8757378 DOI: 10.1097/0000658-199608000-00007]
- 8 **Emre S**, Schwartz ME, Katz E, Miller CM. Liver resection under total vascular isolation. Variations on a theme. *Ann Surg* 1993; **217**: 15-19 [PMID: 8424696 DOI: 10.1097/0000658-199301000-00004]
- 9 **Smyrniotis VE**, Kostopanagiotou GG, Gamaletsos EL, Vassiliou JG, Voros DC, Fotopoulos AC, Contis JC. Total versus selective hepatic vascular exclusion in major liver resections. *Am J Surg* 2002; **183**: 173-178 [PMID: 11918884 DOI: 10.1016/S0002-9610(01)00864-9]
- 10 **Dubay D**, Gallinger S, Hawryluck L, Swallow C, McCluskey S, McGilvray I. In situ hypothermic liver preservation during radical liver resection with major vascular reconstruction. *Br J Surg* 2009; **96**: 1429-1436 [PMID: 19918862 DOI: 10.1002/bjs.6740]
- 11 **Malde DJ**, Khan A, Prasad KR, Toogood GJ, Lodge JP. Inferior vena cava resection with hepatectomy: challenging but justified. *HPB (Oxford)* 2011; **13**: 802-810 [PMID: 21999594 DOI: 10.1111/j.1477-2574.2011.00364.x]
- 12 **Fu SY**, Lai EC, Li AJ, Pan ZY, Yang Y, Sun YM, Lau WY, Wu MC, Zhou WP. Liver resection with selective hepatic vascular exclusion: a cohort study. *Ann Surg* 2009; **249**: 624-627 [PMID: 19300226 DOI: 10.1097/SLA.0b013e31819ed212]
- 13 **Cherqui D**, Malassagne B, Colau PI, Brunetti F, Rotman N, Fagniez PL. Hepatic vascular exclusion with preservation of the caval flow for liver resections. *Ann Surg* 1999; **230**: 24-30 [PMID: 10400032 DOI: 10.1097/0000658-199907000-00004]
- 14 **Azoulay D**, Maggi U, Lim C, Malek A, Compagnon P, Salloum C, Laurent A. Liver resection using total vascular exclusion of the liver preserving the caval flow, in situ hypothermic portal perfusion and temporary porta-caval shunt: a new technique for central tumors. *Hepatobiliary Surg Nutr* 2014; **3**: 149-153 [PMID: 25019076 DOI: 10.3978/j.issn.2304-3881.2014.05.02]
- 15 **Lodge JP**, Ammori BJ, Prasad KR, Bellamy MC. Ex vivo and in situ resection of inferior vena cava with hepatectomy for colorectal

metastases. *Ann Surg* 2000; **231**: 471-479 [PMID: 10749606 DOI: 10.1097/00000658-200004000-00004]

16 **Belghiti J**, Guevara OA, Noun R, Saldinger PF, Kianmanesh R.

Liver hanging maneuver: a safe approach to right hepatectomy without liver mobilization. *J Am Coll Surg* 2001; **193**: 109-111 [PMID: 11442247 DOI: 10.1016/S1072-7515(01)00909-7]

**P- Reviewer:** Aurello P **S- Editor:** Gong ZM

**L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 March 28; 8(9): 421-460





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 469 members, representing a team of worldwide experts in hepatology. They are from 53 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (1), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (98), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (19), Greece (11), Hungary (5), India (15), Indonesia (2), Iran (4), Israel (1), Italy (52), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (11), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (17), and United States (56).

### EDITORS-IN-CHIEF

Clara Balsano, Rome  
Wan-Long Chuang, Kaohsiung

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, Kaohsiung  
Tai-An Chiang, Tainan  
Chi-Tan Hu, Hualien  
Sen-Yung Hsieh, Taoyuan  
Wenya Huang, Tainan  
Liang-Yi Hung, Tainan  
Jih RU Hwu, Hsinchu  
Jing-Yi Lee, Taipei  
Mei-Hsuan Lee, Taipei  
Chih-Wen Lin, Kaohsiung  
Chun-Che Lin, Taichung  
Wan-Yu Lin, Taichung  
Tai-Long Pan, Tao-Yuan  
Suh-Ching Yang, Taipei  
Chun-Yan Yeung, Taipei

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, Batna



**Argentina**

Fernando O Bessone, Rosario  
Maria C Carrillo, Rosario  
Melisa M Dirchwolf, Buenos Aires  
Bernardo Frider, Buenos Aires

Jorge Quarleri, Buenos Aires  
Adriana M Torres, Rosario



**Armenia**

Narina Sargsyants, Yerevan



**Australia**

Mark D Gorrell, Sydney



**Austria**

Harald Hofer, Vienna  
Gustav Paumgartner, Vienna  
Matthias Pinter, Vienna  
Thomas Reiberger, Vienna



**Bangladesh**

Shahinul Alam, Dhaka  
Mamun Al Mahtab, Dhaka



**Belgium**

Nicolas Lanthier, Brussels  
Philip Meuleman, Ghent  
Luisa Vonghia, Antwerp



**Botswana**

Francesca Cainelli, Gaborone

Sandro Vento, Gaborone



**Brazil**

Edson Abdala, Sao Paulo  
Ilka FSF Boin, Campinas  
Niels OS Camara, Sao Paulo  
Ana Carolina FN Cardoso, Rio de Janeiro  
Roberto J Carvalho-Filho, Sao Paulo  
Julio CU Coelho, Curitiba  
Flavio Henrique Ferreira Galvao, São Paulo  
Janaina L Narciso-Schiavon, Florianopolis  
Sílvia HC Sales-Peres, Bauru  
Leonardo L Schiavon, Florianópolis  
Luciana D Silva, Belo Horizonte  
Vanessa Souza-Mello, Rio de Janeiro  
Jaques Waisberg, Santo André



**Bulgaria**

Mariana P Penkova-Radicheva, Stara Zagora  
Marieta Simonova, Sofia



**Canada**

Runjan Chetty, Toronto  
Michele Molinari, Halifax  
Giada Sebastiani, Montreal



**Chile**

Luis A Videla, Santiago



## China

Guang-Wen Cao, Shanghai  
 En-Qiang Chen, Chengdu  
 Gong-Ying Chen, Hangzhou  
 Jin-lian Chen, Shanghai  
 Jun Chen, Changsha  
 Alfred Cheng, Hong Kong  
 Chun-Ping Cui, Beijing  
 Shuang-Suo Dang, Xi'an  
 Ming-Xing Ding, Jinhua  
 Zhi-Jun Duang, Dalian  
 He-Bin Fan, Wuhan  
 Xiao-Ming Fan, Shanghai  
 James Yan Yue Fung, Hong Kong  
 Yi Gao, Guangzhou  
 Zuo-Jiong Gong, Wuhan  
 Zhi-Yong Guo, Guangzhou  
 Shao-Liang Han, Wenzhou  
 Tao Han, Tianjin  
 Jin-Yang He, Guangzhou  
 Ming-Liang He, Hong Kong  
 Can-Hua Huang, Chengdu  
 Bo Jin, Beijing  
 Shan Jin, Hohhot  
 Hui-Qing Jiang, Shijiazhuang  
 Wan-Yee Joseph Lau, Hong Kong  
 Guo-Lin Li, Changsha  
 Jin-Jun Li, Shanghai  
 Qiang Li, Jinan  
 Sheng Li, Jinan  
 Zong-Fang Li, Xi'an  
 Xu Li, Guangzhou  
 Xue-Song Liang, Shanghai  
 En-Qi Liu, Xi'an  
 Pei Liu, Shenyang  
 Zhong-Hui Liu, Changchun  
 Guang-Hua Luo, Changzhou  
 Yi Lv, Xi'an  
 Guang-Dong Pan, Liuzhou  
 Wen-Sheng Pan, Hangzhou  
 Jian-Min Qin, Shanghai  
 Wai-Kay Seto, Hong Kong  
 Hong Shen, Changsha  
 Xiao Su, Shanghai  
 Li-Ping Sun, Beijing  
 Wei-Hao Sun, Nanjing  
 Xue-Ying Sun, Harbin  
 Hua Tang, Tianjin  
 Ling Tian, Shanghai  
 Eric Tse, Hong Kong  
 Guo-Ying Wang, Changzhou  
 Yue Wang, Beijing  
 Shu-Qiang Wang, Chengdu  
 Mary MY Wayne, Hong Kong  
 Hong-Shan Wei, Beijing  
 Danny Ka-Ho Wong, Hong Kong  
 Grace Lai-Hung Wong, Hong Kong  
 Bang-Fu Wu, Dongguan  
 Feng Wu, Chongqing  
 Xiong-Zhi Wu, Tianjin  
 Chun-Fang Xu, Suzhou  
 Rui-An Xu, Quanzhou  
 Rui-Yun Xu, Guangzhou  
 Wei-Li Xu, Shijiazhuang  
 Shi-Ying Xuan, Qingdao  
 Ming-Xian Yan, Jinan  
 Lv-Nan Yan, Chengdu  
 Jin Yang, Hangzhou  
 Ji-Hong Yao, Dalian  
 Winnie Yeo, Hong Kong

Zheng Zeng, Beijing  
 Qi Zhang, Hangzhou  
 Shi-Jun Zhang, Guangzhou  
 Xiao-Lan Zhang, Shijiazhuang  
 Xiao-Yong Zhang, Guangzhou  
 Xin-Chen Zhang, Harbin  
 Yong Zhang, Xi'an  
 Hong-Chuan Zhao, Hefei  
 Ming-Hua Zheng, Wenzhou  
 Yu-Bao Zheng, Guangzhou  
 Ren-Qian Zhong, Shanghai  
 Fan Zhu, Wuhan  
 Xiao Zhu, Dongguan



## Czech Republic

Kamil Vyslouzil, Olomouc



## Denmark

Henning Gronbaek, Aarhus  
 Christian Mortensen, Hvidovre



## Egypt

Ihab T Abdel-Raheem, Damanhour  
 NGB G Bader EL Din, Cairo  
 Hatem Elalfy, Mansoura  
 Mahmoud M El-Bendary, Mansoura  
 Mona El SH El-Raziky, Cairo  
 Mohammad El-Sayed, Cairo  
 Yasser M Fouad, Minia  
 Mohamed AA Metwally, Benha  
 Hany Shehab, Cairo  
 Mostafa M Sira, Shebin El-koom  
 Ashraf Taye, Minia  
 MA Ali Wahab, Mansoura



## France

Laurent Alric, Toulouse  
 Sophie Conchon, Nantes  
 Daniel J Felmlee, Strasbourg  
 Herve Lerat, Creteil  
 Dominique Salmon, Paris  
 Jean-Pierre Vartanian, Paris



## Germany

Laura E Buitrago-Molina, Hannover  
 Enrico N De Toni, Munich  
 Oliver Ebert, Muenchen  
 Rolf Gebhardt, Leipzig  
 Janine V Hartl, Regensburg  
 Sebastian Hinz, Kiel  
 Benjamin Juntermanns, Essen  
 Roland Kaufmann, Jena  
 Viola Knop, Frankfurt  
 Veronika Lukacs-Kornek, Homburg  
 Benjamin Maasoumy, Hannover  
 Jochen Mattner, Erlangen  
 Nadja M Meindl-Beinker, Mannheim  
 Ulf P Neumann, Aachen  
 Margarete Odenthal, Cologne  
 Yoshiaki Sunami, Munich

Christoph Roderburg, Aachen  
 Frank Tacke, Aachen  
 Yuchen Xia, Munich



## Greece

Alex P Betrosian, Athens  
 George N Dalekos, Larissa  
 Ioanna K Delladetsima, Athens  
 Nikolaos K Gatselis, Larissa  
 Stavros Gourgiotis, Athens  
 Christos G Savopoulos, Thessaloniki  
 Tania Siahaniidou, Athens  
 Emmanouil Sinakos, Thessaloniki  
 Nikolaos G Symeonidi, Thessaloniki  
 Konstantinos C Thomopoulos, Larissa  
 Konstantinos Tziomalos, Thessaloniki



## Hungary

Gabor Banhegyi, Budapest  
 Peter L Lakatos, Budapest  
 Maria Papp, Debrecen  
 Ferenc Sipos, Budapest  
 Zsolt J Tulassay, Budapest



## India

Deepak N Amarapurkar, Mumbai  
 Girish M Bhopale, Pune  
 Sibnarayan Datta, Tezpur  
 Nutan D Desai, Mumbai  
 Sorabh Kapoor, Mumbai  
 Jaswinder S Maras, New Delhi  
 Nabeen C Nayak, New Delhi  
 C Ganesh Pai, Manipal  
 Amit Pal, Chandigarh  
 K Rajeshwari, New Delhi  
 Anup Ramachandran, Vellore  
 D Nageshwar Reddy, Hyderabad  
 Shivaram P Singh, Cuttack  
 Ajith TA, Thrissur  
 Balasubramaniyan Vairappan, Pondicherry



## Indonesia

Cosmas RA Lesmana, Jakarta  
 Neneng Ratnasari, Yogyakarta



## Iran

Seyed M Jazayeri, Tehran  
 Sedigheh Kafi-Abad, Tehran  
 Iradj Maleki, Sari  
 Fakhraddin Naghibalhossaini, Shiraz



## Israel

Stephen DH Malnick, Rehovot



## Italy

Francesco Angelico, Rome



Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Andrea Galli, *Florence*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Salvatore Gruttadauria, *Palermo*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*



**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*  
 Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*

Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhito Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*



**Jordan**

Kamal E Bani-Hani, *Zarqa*



**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*



**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*



**Moldova**

Angela Peltec, *Chishinev*



**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*



**Nigeria**

CA Asabamaka Onyekwere, *Lagos*



**Pakistan**

Bikha Ram Devrajani, *Jamshoro*



**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*



**Poland**

Jacek Zielinski, *Gdansk*



**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*



**Qatar**

Reem Al Olaby, *Doha*



**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*



**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*



**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*



**Singapore**

Ser Yee Lee, *Singapore*



**South Korea**

Young-Hwa Chung, *Seoul*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonju*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*



**Spain**

Ivan G Marina, *Madrid*  
 Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*

José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodríguez-Frias, *Córdoba*  
 Manuel L Rodríguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Traperó-Marugán, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpaboon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*  
 Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*

Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*



#### **United States**

Naim Alkhouri, *Cleveland*  
 Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*

Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Grigoriy E Gurvits, *New York*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Kusum K Kharbanda, *Omaha*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**TOPIC HIGHLIGHT**

- 421 New advances in hepatocellular carcinoma  
*Pascual S, Herrera I, Irurzun J*
- 439 Preoperative portal vein embolization for hepatocellular carcinoma: Consensus and controversy  
*Aoki T, Kubota K*

**MINIREVIEWS**

- 446 Focal liver lesions found incidentally  
*Algarni AA, Alshuhri AH, Alonazi MM, Mourad MM, Bramhall SR*

**SYSTEMATIC REVIEWS**

- 452 Comprehensive review of telbivudine in pregnant women with chronic hepatitis B  
*Piratvisuth T, Han GR, Pol S, Dong Y, Trylesinski A*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Fan Zhu, PhD, Professor, Department of Medical Microbiology, Medical School of Wuhan University, Wuhan 430072, Hubei Province, China

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
March 28, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjgnet.com/bpg/g_info_20160116143427.htm)

**ONLINE SUBMISSION**

<http://www.wjgnet.com/esps/>



## 2016 Hepatocellular Carcinoma: Global view

# New advances in hepatocellular carcinoma

Sonia Pascual, Iván Herrera, Javier Irurzun

Sonia Pascual, Iván Herrera, Javier Irurzun, Liver Unit, Gastroenterology Department, Interventional Radiological Unit, Hospital General Universitario de Alicante, 03010 Alicante, Spain

**Author contributions:** Pascual S, Herrera I and Irurzun J contributed equally to this work; Pascual S designed the research; Pascual S, Herrera I and Irurzun J wrote the paper.

**Conflict-of-interest statement:** None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content or the paper.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Sonia Pascual, MD, Liver Unit, Gastroenterology Department, Interventional Radiological Unit, Hospital General Universitario de Alicante, C/Pintor Baeza s/n, 03010 Alicante, Spain. [pascual\\_son@gva.es](mailto:pascual_son@gva.es)  
 Telephone: +34-965-934466  
 Fax: +34-965-934468

Received: April 29, 2015  
 Peer-review started: May 7, 2015  
 First decision: September 8, 2015  
 Revised: March 6, 2016  
 Accepted: March 14, 2016  
 Article in press: March 16, 2016  
 Published online: March 28, 2016

## Abstract

Hepatocellular carcinoma (HCC) is the leading cause of deaths in cirrhotic patients and the third cause of cancer related deaths. Most HCC are associated with

well known underlying risk factors, in fact, HCC arise in cirrhotic patients in up to 90% of cases, mainly due to chronic viral hepatitis and alcohol abuse. The worldwide prevention strategies are conducted to avoid the infection of new subjects and to minimize the risk of liver disease progression in infected patients. HCC is a condition which lends itself to surveillance as at-risk individuals can readily be identified. The American and European guidelines recommended implementation of surveillance programs with ultrasound every six months in patient at-risk for developing HCC. The diagnosis of HCC can be based on non-invasive criteria (only in cirrhotic patient) or pathology. Accurately staging patients is essential to oncology practice. The ideal tumour staging system in HCC needs to account for both tumour characteristics and liver function. Treatment allocation is based on several factors: Liver function, size and number of tumours, macrovascular invasion or extrahepatic spread. The recommendations in terms of selection for different treatment strategies must be based on evidence-based data. Resection, liver transplant and interventional radiology treatment are mainstays of HCC therapy and achieve the best outcomes in well-selected candidates. Chemoembolization is the most widely used treatment for unresectable HCC or progression after curative treatment. Finally, in patients with advanced HCC with preserved liver function, sorafenib is the only approved systemic drug that has demonstrated a survival benefit and is the standard of care in this group of patients.

**Key words:** Hepatocellular carcinoma; Surveillance; Staging system; Radiofrequency ablation; Liver surgery; Liver transplant; Transarterial chemoembolization

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Liver cancer is the fifth leading cause of cancer worldwide, and the third-leading cause of cancer death. Although some risk factors have been classically associated with development of hepatocellular carcinoma (HCC), in the last years, also, some protective factors

have been described, like coffee drink, and drugs like statins and beta-blockers. The current European Association for the Study of Liver and American Association for the Study of Liver Diseases guidelines recommended the barcelona clinic liver cancer classification as staging system for prognosis prediction and treatment allocation. The therapeutic approach in patients with HCC depends on factors such as liver function, tumour extension and comorbidities existence. Available treatments are: Surgical treatments, percutaneous ablation, chemoembolization, radioembolization and systemic treatment.

Pascual S, Herrera I, Irurzun J. New advances in hepatocellular carcinoma. *World J Hepatol* 2016; 8(9): 421-438. Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i9/421.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i9.421>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the leading cancer in the world. It is an important health problem especially in high incidence areas. Nowadays the global incidence is still growing, but with the development of hepatitis B vaccine and the new therapies in hepatitis C virus (HCV), a gradual decline in the incidence is expected in the next decades. Another important issue is the high mortality of the patients with this tumour. In spite of well established surveillance programs in patients with chronic liver disease, most tumours are diagnosed in intermediate-advanced stage, and only palliative measured can be applied.

In the next pages we will review the risk factors associated with the development of HCC, the new advances in diagnosis imaging, the main prognosis classification and finally the therapeutic approach.

## EPIDEMIOLOGY

Liver cancer is the fifth-leading cause of cancer diagnosed in men worldwide<sup>[1]</sup>, and the seventh cause of cancer in women, representing about 7% of the total number of cancer diagnoses. Globally, liver cancer is the third-leading cause of cancer death, after lung and stomach<sup>[2,3]</sup>. The annual incidence of HCC is similar to the deaths per year that it generates, which point out the aggressiveness of this disease<sup>[1]</sup>.

The HCC incidence increases progressively with advancing age in population with a peak at the age of 70-year-old<sup>[4]</sup>. In Chinese and black African population, mainly infected with hepatitis B virus (HBV), the patient are younger, and in Sub-Saharan Africa (an area with a high incidence of HBV infection) can appear in the third decade of life<sup>[5,6]</sup>.

The incidence of HCC is highest in men, with a male to female ratio of 2.4 and this difference is even higher in populations with a high incidence of HCC, with an

average of 3.7 to 1<sup>[3]</sup>. The differences in the geographical distribution of HCC reflects the differences in exposure to the hepatitis viruses and different environmental pathogens, so the incidence is highest in East Asia, Sub-Saharan Africa and Melanesia, with 85% of the total number of cases<sup>[2,3]</sup>, while in most industrialized countries the incidence is low, except in the South of Europe<sup>[7]</sup>. Globally there is a growing incidence of the number cases of HCC, even in United States and Europe, mainly due to the high number of people infected with the virus of HCV in these areas<sup>[3]</sup>. The universal vaccination against HBV in children born after 1980 in some endemic countries has decrease the rate of HCC in children and it is expected a reduction of the incidence of this tumour in the future in these areas<sup>[8,9]</sup>.

## ETIOLOGY AND RISK FACTORS

Multiple risk factors have been associated with the development of HCC, being the most frequent chronic viral hepatitis (B and C), alcohol abuse, and exposure to aflatoxins, however, this can occur in people without any known risk factor<sup>[10]</sup>.

Geographically in Africa and East Asia, the most frequently risk factor associated with HCC is chronic HBV infection, while in Western countries, HCV infection is the main risk factor<sup>[2]</sup>. Overall 54% of cases could be attributed to HBV infection, 31% to HCV infection and 15% to other causes. Cirrhosis is the main risk factor for the development of HCC and about 30%-35% of all cirrhotic patients will develop HCC in the course of their disease, which may be due to chronic viral hepatitis, alcohol, hereditary metabolic diseases, or autoimmune and non-alcoholic fatty liver disease<sup>[11]</sup>. It is estimated that the annual risk of developing HCC in the cirrhotic patients is between 1%-8% according to the aetiology<sup>[12]</sup>. The risk of developing HCC increases progressively in male patients, with advanced age, low platelet count, and oesophageal varices<sup>[13]</sup>, as well as it has also been associated with increasing pressure portal<sup>[14]</sup>, or with the degree of liver stiffness measured with transient elastography<sup>[15-17]</sup>.

### Viral hepatitis

HBV and HCV Chronic infection are the main risk factor for the development of HCC<sup>[18-21]</sup>. The higher prevalence of HBV infection occurs in China, Southeast Asia and Sub-Saharan Africa<sup>[8,21]</sup>. Globally, it is estimated that 54% of all liver cancers are attributable to HBV infection<sup>[22]</sup>. The prevalence of HCV infection is higher in Egypt, Japan and the South of Italy<sup>[21]</sup>.

The development of HCC associated with HBV infection usually occurs in patients with cirrhosis, but it can appear in patients without cirrhosis<sup>[5,23-28]</sup>. So screening for HCC will be recommended in this group of patients. Some risk factors for the development of HCC have been identified in patients with chronic HBV infection: The presence of hepatitis virus e antigen (as an indicator of viral replication)<sup>[28]</sup>, high viral load<sup>[29]</sup>,

genotype C (which is the most prevalent in Asia)<sup>[30]</sup> and infection in early childhood or perinatal period<sup>[31-33]</sup>. Several studies have demonstrated that the treatment of chronic HBV hepatitis with interferon or nucleotide analogues (suppressing viral load) reduces the relative risk of developing HCC<sup>[31,34-43]</sup>, but these benefits have not been observed in patients who develop resistance to the treatment. Some studies suggest that patients co-infected by HBV and HCV have greater risk of developing HCC<sup>[44-46]</sup>.

There is a very well known association between HCV chronic infection and the development of HCC, in fact, the risk of developing HCC in these patients increase between 20 and 30 times<sup>[21,47-49]</sup>. In very few cases it may occur in patients with HCV infection and lower grades of hepatic fibrosis<sup>[13,50]</sup>. High viral loads and HCV genotype 1b infection have been associated with higher risk of HCC occurrence<sup>[51]</sup>. The levels of inflammatory markers of oxidative stress are higher in patients infected with HCV and HCC<sup>[52]</sup> and the immune response can be another cofactor in the progression from cirrhosis to HCC in HCV infected patient<sup>[53]</sup>. In patients with HCV infection who achieve sustained viral response after treatment, there is a decrease in the risk of HCC<sup>[54,55]</sup>. The universal analysis of blood donations for anti-HCV has resulted in a substantial decrease in the number of cases of hepatitis C in blood donors and the use of needles and disposable syringes and other changes in medical procedures have substantially reduced new infections by HCV. As well as HCV and HBV co-infection may increase the risk of developing cirrhosis and HCC<sup>[56]</sup>, the HIV infection appears to be a cofactor that increases the risk of developing HCC in cirrhotic patients with viral hepatitis<sup>[57]</sup>.

### **Schistosomiasis**

The infection by trematode in blood is endemic in tropical areas of Africa, the Caribbean, Asia, and South America. The species of *Schistosoma japonicum*, already identified as possible human carcinogen, has been associated with risk of developing HCC in infected by HBV and HCV patients<sup>[58,59]</sup>.

### **Toxins**

The ingestion of food contaminated with aflatoxin B1 (fungi *Aspergillus flavus* and *Aspergillus parasiticus*), which can be found at staple foods of tropical and subtropical areas, is a co-factor of risk in the development of HCC, especially in some regions of Africa and Asia, associated with infection by HBV<sup>[60,61]</sup>. Several studies have shown increased HCC mortality in some rural Chinese areas associated with drinking water potentially contaminated with toxins of some algae (microcystins), with hepatotoxic effect<sup>[62,63]</sup>. Other studies have established a relationship between the consumption of betel nut, very common in Asia, with an increasing risk of developing cirrhosis and HCC<sup>[64,65]</sup>.

Many studies have associated chronic alcohol consumption with the development of liver cirrhosis and

HCC<sup>[66-72]</sup>, although quantity of alcohol ingestion and duration of consumption that supposes a significant risk for developing HCC is unknown. It has been described a relationship between genetic polymorphisms of the enzymes involved in the metabolic pathway of ethanol and increased risk of HCC in excessive drinkers. An increased risk of HCC in heavy alcohol drinkers has been associated to the polymorphism of the aldehyde dehydrogenase and the dysfunction of the enzyme Glutathion S-transferase<sup>[73,74]</sup>. Some studies have established that smoking is a significant co-factor in the development of HCC<sup>[66,75,76]</sup>.

### **Diabetes mellitus and obesity**

The obesity, diabetes and dyslipidemia have also been identified as cofactors of risk in the development of HCC, although the pathophysiological mechanisms have not been clarified. It is believed that the deposit of fat in the liver could alter some metabolic functions in patients with diabetes mellitus<sup>[77,78]</sup>. In these patients, liver steatosis can lead to a nonalcoholic fatty hepatitis, whose pathogenesis is unclear but it have been related to chronic inflammation, oxidative stress, insulin resistance and lipotoxicity, constituting a cofactor for the development of liver cirrhosis and HCC<sup>[79-82]</sup>.

The metabolic syndrome, which is defined by the presence of central obesity, dyslipemia, hypertension, and impaired glucose metabolism, has also been associated with an increased risk of developing HCC<sup>[83]</sup>.

### **Other causes of cirrhosis**

Patients with hemochromatosis may develop HCC by up 45% cases, according to some studies, iron overload can lead to the development of cirrhosis and HCC in these patients<sup>[84]</sup>. The protein alpha-1-antitrypsin deficiency is a documented risk factor in the development of cirrhosis and HCC that also could be without cirrhosis<sup>[85]</sup>. Occasionally, patients with cirrhosis secondary to Wilson's disease, autoimmune hepatitis or primary biliary cirrhosis can develop HCC<sup>[86-88]</sup>. Several studies suggest that porphyria may increase the risk of developing HCC, even in patients without cirrhosis<sup>[89-97]</sup>.

### **Other factors**

A meta-analysis showed an increase of significant risk of any primary liver cancer, and also of HCC in patients with cholelithiasis<sup>[98]</sup>. The oral contraceptive (OC) consumption has been rarely associated with the emergence of benign tumours of the liver in young women, like hepatic haemangioma, focal nodular hyperplasia and specially hepatocellular adenoma<sup>[99]</sup>. Some cases of malignant transformation of liver adenomas in women taking OC have been described<sup>[100,101]</sup>, but subsequent studies did not corroborate these results<sup>[102]</sup>. Some studies have suggested that the excessive consumption of saturated fats and meat may increase the risk of HCC<sup>[103,104]</sup>. Although others authors have not found this association<sup>[105]</sup>. Nitrogenous compounds (used in smoked fish, cheeses, bacon, sausages and other foods)

may increase the risk of liver disease and cancer<sup>[106]</sup>.

In an American study, individuals with a family history of first degree with liver cancer, had up to four times more likely to develop liver cancer than the general population, suggesting that certain shared genetic and environmental factors would influence the risk of developing liver cancer<sup>[107]</sup>. There is some evidence that there might be an association between a polymorphism of the gene of epidermal growth factor and the risk of developing HCC, although these data require further investigation<sup>[108-115]</sup>.

## PROTECTIVE FACTORS

### Statins

The use of statins has been associated with a decrease in the risk of developing HCC<sup>[116,117]</sup>. In a meta-analysis, including 10 studies, the risk of developing HCC was lower in people taking statins<sup>[118]</sup>.

### Beta-blockers

A recent retrospective, observational study establishes the hypothesis that treatment with propranolol may reduce the risk of HCC in cirrhotic patients<sup>[119]</sup>.

### Diet

The consumption of fish, vegetables and omega-3 fatty acids has been associated with a lower risk of developing HCC in different studies<sup>[107,120,121]</sup>. Similarly, the increased consumption of vitamin E has also been associated with lower risk of HCC rate<sup>[122]</sup>. The Mediterranean diet, characterized by high consumption of vegetables, olive oil and cereals, with moderate wine consumption and fish, and low consumption of meat, is associated with a lower risk of HCC<sup>[123]</sup>.

### Coffee

There are several studies that have associated coffee consumption with a reduced risk of liver cancer including HCC. In a recent meta-analysis, taking more than two cups of coffee a day reduces risk of liver cancer of up to 43%, which could be related to its antioxidant effect<sup>[124-126]</sup>.

## SURVEILLANCE

Surveillance is cost effective in high risk cirrhotic patient, with an expected annual incidence of HCC exceeding 1%-5% per year, and in some cases of non-cirrhotic patients with HBV chronic infection. The problem is that most of the studies of surveillance of HCC in chronic liver disease have been developed in endemic Asian countries with high incidence of HBV infection. In fact, the only prospective study has been developed in China, exclusively in patients with HBV infection. In this study, the mortality related to HCC was lower in patients under HCC surveillance<sup>[127]</sup>. Other retrospective studies conducted in Europe and America also have showed a better prognosis in patients diagnosed in

surveillance programs<sup>[128-130]</sup>. Both, European American and Asian guidelines recommended that patient with high risk of developing HCC should be entered into surveillance programs. This should be performed using ultrasonography every six months<sup>[131-133]</sup>.

## DIAGNOSIS OF HCC

According to the latest consensus conferences and practice guidelines, nowadays, to get to a definitive diagnosis of HCC, will not be necessary to perform a liver biopsy if the tumour is higher than 1 cm in diameter and the typical imaging features are present in a contrast enhanced study [dynamic computed tomography (CT) scan or magnetic resonance (MR)]. Thus, to properly documented the existence of HCC is required that the tumour enhances more intensely in the arterial phase than the surrounding liver and less than the surrounding liver in the venous phase. But these rules are only applicable if the patient has well diagnosed cirrhosis or a HBV chronic hepatitis. In any other cases (patient with typical lesion but without liver disease or patient with atypical lesion and cirrhosis), a liver biopsy must be performed to establish the diagnosis. The serum alphafetoprotein level has no longer be used for diagnosis of HCC, because is insufficiently sensitive or specific for use as a surveillance assay<sup>[130,131]</sup>.

In order to reduce the variability in liver lesion interpretation and standardize the report from CT and MR information, the American College of Radiology has developed a new classification: Liver Imaging-Reporting and Data System (LI-RADS). The LI-RADS assigns imaging findings to one of five categories, allowing radiologist to stratify individual observations according to the level of concern HCC. So LR-1 is an observation definitively benign and LR-5 is definitively HCC. The intermediate stages correlates with probably benign (LR-2), intermediate possibility of being HCC (LR-3) and probably HCC (LR-4) according to radiological features, lesion diameter and contrast enhanced behaviour<sup>[134]</sup>. As has been described recently, the nodules both LI-RADS category 4 and category 5 have high specificity for HCC diagnosis, and in addition, a relevant proportion of lesions categorized as LI-RADS category 2 and 3 could be HCC and a liver biopsy should be recommended in such patients<sup>[135]</sup>. A consensus is necessary between different organizations in order to optimize reporting of CT and MR imaging features in the patients at risk for HCC<sup>[136]</sup>.

## STAGING

The main prognosis predictors of survival in patients with HCC are: Liver function, tumour burden (size and number of HCC nodules, vascular invasion), serum alpha-fetoprotein level and performance status. Nowadays, there is no universally adopted staging system for HCC. The most widely and accepted staging system in oncology, the classification of malignant tumours



**Table 1** Factors included in each staging system

Staging system	Size	Nodules	Met	PVT	AFP	CH	Alb	Bil	ALP	Ascites	PS
TNM	Yes	Yes	Yes	No	No	No	No	No	No	No	No
Okuda	Yes	No	No	No	No	No	Yes	Yes	No	Yes	No
CLIP	Yes	No	No	Yes	Yes	Yes	No	No	No	No	No
FRENCH	No	No	No	Yes	Yes	No	No	Yes	Yes	No	Yes
BCLC	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	No	Yes
JIS	Yes	Yes	Yes	No	No	Yes	No	No	No	No	No
CUPI	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes	No

Met: Metastasis; PVT: Portal vein thrombosis; AFP: Alfafetoproteina; Alb: Albumin; Bil: Bilirubin; ALP: Alkaline phosphatase; PS: Performance status; CLIP: Cancer of the Liver Italian Program; BCLC: Barcelona clinic liver cancer classification; CUPI: Chinese University Prognosis Index; JIS: The Japan Integrated Staging; TNM: Classification of malignant tumours; FRENCH: French classification of hepatocellular carcinomas; CH: Child-Pugh.

(TNM), has been adapted for HCC by the American Joint Committee on Cancer. Currently, the United Network for Organ Sharing, the organ allocation administration in United States of America, allocates donors organs for liver transplantation for the treatment of HCC based on the revised TNM classification. The problem of this system is that it does not incorporate any measure of liver function reserve, which is critical in HCC. Prognosis for HCC is impacted by local spread and hepatic dysfunction, and any staging system in HCC should include parameters that represent both aspects because an advanced liver disease can contraindicate any therapeutic approach as much as an advanced and extended HCC. The first staging system specifically designed for HCC was the Okuda classification<sup>[137]</sup>, but other staging systems have been described in the last decades: Cancer of the Liver Italian Program<sup>[138]</sup>, French classification<sup>[139]</sup>, Barcelona clinic liver cancer classification (BCLC)<sup>[140]</sup>, Chinese University Prognosis Index<sup>[141]</sup>, the Japan Integrated Staging<sup>[142]</sup>, which has been redefined including biomarkers and the Taipei Integrated Scoring System, based on total tumour volume<sup>[143]</sup>. In Table 1 are represents the parameters included in these staging system. Some of these classifications have been externally validated in separated groups.

The current European Association for the Study of Liver (EASL)-EORTC GP guidelines and the American Association for the Study of Liver Diseases (AASLD) guidelines endorse the BCLC classification and recommend the use of this staging system for prognosis prediction and treatment allocation<sup>[132,133]</sup>. The BCLC classification divides HCC patients in five stages, from (0, A, B, C, D) according to pre-established prognosis variables: Size and number of nodules, vascular invasion, performance status and Child-Pugh stage. The five stages are: 0 very early stage, A early, B intermediate, C advanced and D terminal and each stage represents the first approach to the evaluation of the patients with expected prognosis and initial treatment option to be considered. Early stage patients may be treated with potential curative treatment: Percutaneous ablation, surgery or liver transplant (LT). Intermediate stage patients may be treated with chemoembolization, advanced stages may be treated with systemic therapy (sorafenib) and in terminal patients only best supportive approach

can be applied. But, as in all recommendations, the final treatment indication should take into account a detail evaluation of additional characteristics of the patients that imply a personalized decision making. So, a young patient with Child C and a small tumour should be considered for LT, not for best supportive care.

## TREATMENT

The therapeutic approach in patients with HCC depends on several factors such as liver function, size and number of nodules, tumour extension, age and comorbidities existence. Currently, available treatments can be divided into surgical treatments (resection or transplantation), percutaneous ablation (Chemistry: Acid ethanol acetic or thermal: Microwave, laser, radiofrequency and cryoablation), chemoembolization, radioembolization and systemic treatment. The goal of curative treatments should be to obtain a complete response, according to modified RECIST radiological criteria<sup>[144,145]</sup>. The recommendation of selection for different treatment strategies are based on evidence-based data and local experience and capacities. Is advisable that any decision of treatment should be adopted by multidisciplinary HCC teams including hepatologist, oncologist, surgeons, radiologist and interventional radiologist. Properly allocate each treatment in each case is a crucial decision and is mandatory to warrant a good results in terms of survival, treatment morbidity and mortality and recurrence.

### Surgery

As in any tumour, the surgical resection should be the first option to be considered in patients with HCC. The problem is the limitation that supposes the presence of liver cirrhosis, hypertension portal, coagulopathy, or hepatic dysfunction associated, that may contraindicate any surgery and resection of the tumour. The results of surgery to make appropriate estimated that survival at 5 years should reach 60% and 5 years tumour recurrence 70%, peri-operative mortality must be 2%-3% and less than 10% of transfusion requirements. Anatomic resection aiming 2 cm margins provides better results and survival but only could be applied in patients with preserved liver function. Adequate selection of patients for surgery involves a correct assessment of liver

**Table 2** Reported 5-year overall survival and recurrence in patients undergoing liver transplant for hepatocellular carcinoma within Milan criteria

Ref.	n	5-yr overall survival	5-yr recurrence
Mazzaferro <i>et al</i> <sup>[155]</sup>	48	74%	8%
Bismuth <i>et al</i> <sup>[149]</sup>	45	74%	11%
Llovet <i>et al</i> <sup>[147]</sup>	79	75%	4%
Jonas <i>et al</i> <sup>[151]</sup>	120	71%	15%
Yao <i>et al</i> <sup>[158]</sup>	64	72%	6.5%
Marsh <i>et al</i> <sup>[153]</sup>	248	67%	3.6%
Herrero <i>et al</i> <sup>[154]</sup>	47	70%	8.5%
Mazzaferro <i>et al</i> <sup>[155]</sup>	444	73%	4.3%

function, using Model End Stage Liver Disease punctuation, Child-Pugh class or more sophisticated estimation with the measurement of indocyanine green retention rate or hepatic venous pressure gradient (HVPG). Portal hypertension is an independent prognosis factor in patients undergoing resection and the extensive assessment is recommended before surgery using the component of portal hypertension: Platelet counts, splenomegaly, esophageal varices, and/or HVPG. In practice, BCLC recommendation is to avoid surgery in patient with advanced liver insufficiency, hypertension portal or high bilirubin<sup>[146]</sup>.

If the patient is properly selected, with preserved liver function and no clinically significant portal hypertension, the next step is to evaluate tumour extension: Size and number of nodules, vascular invasion and presence of microsatellites. Tumour size, multinodularity and vascular invasion, are well known predictors of recurrence and survival. Characteristically, microscopic vascular invasion is related to tumour size and involves 20% of tumours of 2 cm, 30%-60% of tumours 2-5 cm and up to 60%-90% of tumours up to 5 cm<sup>[147]</sup>. With all of this in mind, hepatic resection should be considered for small solitary tumours (and multifocal only if technically possible) with adequate hepatic function. In BCLC staging system, surgery is reserved for patient in the very/early stage, with well preserved liver function and a single tumour less than 2 cm, without portal hypertension and normal bilirubin.

## LT

Since Mazzaferro described the Milan criteria in 1996 (solitary tumour less than 50 mm in diameter or less than 3 tumours, and 30 mm in diameter each one, in the absence of extrahepatic vascular spread), numerous studies have validated the results of the initial study, both in terms of 5-year survival and recurrence of the tumour (Table 2)<sup>[148-155]</sup>. This study also allowed that transplantation became a feasible option for treatment in these patients, and also showed that to achieve acceptable rates of survival (*i.e.*, similar to that of the patients transplanted without HCC), the size and number of tumour should be limited. The situation of treatment of HCC has changed dramatically in the last decades. A better knowledge about the tumour behaviour, impro-

vement in surgical techniques and radiological therapies together with a better selection of potential candidates to each treatment have allowed to improve the survival of patients with HCC. The optimisation of the criteria as well as the management of patient already listed for LT remains a source of debate. Important questions, like the expansion of eligibility criteria for LT beyond Milan criteria, the role of down-staging as a bridge to LT or the possible need of adjuvant therapies in patient in waiting list in order to avoid tumour progression and eventual drop-out, are still unresolved.

## Expanded criteria for LT

Alternative eligibility criteria beyond Milan criteria have been proposed, and some of them have been incorporated into clinical practice. The main aim of all these new approaches is to permit the fair allocation of liver graft between more potential recipient with similar survival and tumour recurrences. Having in mind the recognised predictors of recurrence (size and number of nodules, presence of bi-lobar disease, tumour differentiation and presence of micro or macro vascular invasion or tumour satellites), some groups have proposed different expensive criteria. In fact, the limitation of some of the studies have been the used of pathological examination of the explants to determine the tumour burden (data that obviously is only disposable after the LT) instead of radiological staging, as it is showed in Table 3<sup>[155-162]</sup>. This fact, hinders the correct interpretation of the results a consequently the clinical application of the results. The University of California, San Francisco criteria constitutes a well recognised extension to Milan criteria and have been applied in clinical practice<sup>[151]</sup>. First published in 2001, demonstrated that patients with a single tumour less 65 mm in diameter, or 2-3 tumours each with less 45 mm diameter, with a total tumour diameter less than 80 mm, had similar survival than patients inside Milan criteria<sup>[155]</sup>. Subsequent studies (both prospective and retrospective) have reported favourable results with expanded criteria. A recent retrospective and multicentre study by Mazzaferro *et al*<sup>[155]</sup>, have been performed introducing "up to seven" criteria: the sum of the number of tumour nodules and the diameter of the largest nodule (in centimetres) being less than 7<sup>[154]</sup>. These results have been externally validated in an independent cohort<sup>[162,163]</sup>. The international consensus conference for liver transplantation for HCC recommended to consider the LT in patients with HCC inside Milan criteria and only a modest expansion of the number of potential candidates may be considered outside Milan criteria<sup>[164]</sup>.

## Downstaging

Another important question is the role of downstaging in patients with HCC exceeding Milan criteria, using locoregional therapies: Radiofrequency ablation (RFA), transarterial chemoembolization (TACE), transarterial radioembolization or surgery. The objective of these therapies should be to decrease tumour size or number

**Table 3** Summary of the characteristics of the published studies including patients within Milan criteria or with expanded criteria

Ref.	Patients MC/EC	HCC criteria	Staging method	Design	5-yr survival (%) MC/EC
Yao <i>et al</i> <sup>[158]</sup> UCSF criteria	46/14	1 < 6 cm 2-3 > 4, 5 cm Sum diameter < 8 cm	Explant	Retro	72
Herrero <i>et al</i> <sup>[154]</sup> Navarra Criteria	35/12	1 < 6 cm 2-3 < 5 cm	Rx	Pros	
Kneteman <i>et al</i> <sup>[157]</sup>	19/21	1 < 7.5 cm	Explant	Pros	87/83 (4-yr)
	18/9	Multinodular < 5 cm	Rx		92/77
Yao <i>et al</i> <sup>[152]</sup>	130/38	1 < 6 cm 2-3 > 4, 5 cm Sum diameter < 8 cm	Rx	Pros	90/93
Silva <i>et al</i> <sup>[159]</sup> Valencia Criteria	231/26 254/27	1 < 5 cm 2-3 < 5 cm Sum diameter 10 cm	Explant Rx	Retro	62/69
Herrero <i>et al</i> <sup>[156]</sup>	59/26	1 < 6 cm 2-3 < 5 cm	Explant Rx	Pros	70/56 66/68
Mazzaferro <i>et al</i> <sup>[155]</sup> Metroticket	444/283	Sum nodules/size 7 cm	Explant	Retro	73/71
Fan <i>et al</i> <sup>[160]</sup> Shanghai Criteria	394/176	1 < 9 cm 2-3 < 5 cm Sum diameter 9 cm	Explant	Retro	51/65
Guiteau <i>et al</i> <sup>[161]</sup>	363/82	1 < 6 cm 2-3 < 5 cm Sum diameter 9 cm	Rx	Pros	73/71 (3-yr)

Staging method: Pre LT with radiological features (Rx) or post LT according to histopathological features (Explant). Study design: retrospective (Retros), prospective (Pros). MC: Milan criteria; EC: Expanded criteria; LT: Liver transplant; UCSF: University of California, San Francisco; HCC: Hepatocellular carcinoma.

of tumours in order to achieve a pre-established locally criteria acceptable for LT. Some of the studies have reported successfully results with this strategy achieving 5 years survival similar to that of patients with HCC who meet Milan criteria without requiring downstaging<sup>[165,166]</sup>. Nevertheless, there are some unresolved issues. The defined upper limit for size and number of nodules eligibility for downstaging and the possible role of alpha-fetoprotein has not been well defined. The assessment of adequate response is variable in the different reports, although the recommendation should be to consider the amount of available tumour according to modified RECIST criteria. Otherwise, the acceptable criteria previously defined as successful downstaging in each study, has been different, as well as the observation period recommended after the tumour has been downstaged, before considering for LT. The recommendation of Consensus Conference was that LT may be considered after successful downstaging, without evidence for preferring a specific locoregional therapy and using criteria including size and number of viable tumour<sup>[164]</sup>.

### Interventional radiology treatment

HCC is the tumour that takes the greatest advantage from interventional radiology therapies for several reasons: Not only surgical difficulties in cirrhotic patients, but also ablative and endovascular treatments have demonstrated high response rates and survival benefits.

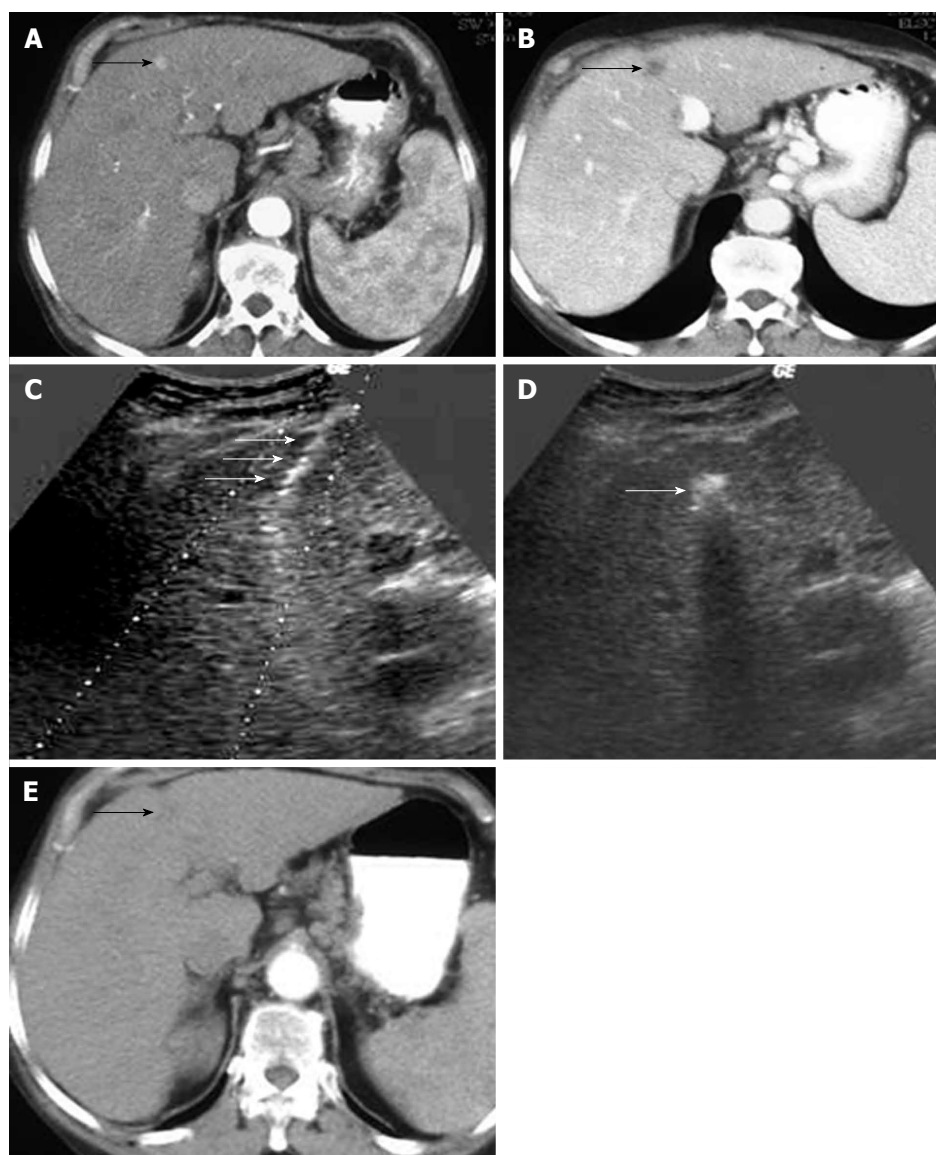
Among all chemical ablative treatments, percutaneous ethanol injection (PEI) has a widespread use, although it has more difficulties to treat encapsulated tumours against other substances as acetic acid. PEI

has been the most used ablative therapy until 1999<sup>[167]</sup>, but it has been disregarded after the emergence of more sophisticated techniques. Despite it has also evolved with multi-pronged needles that minimize some PEI disadvantages as the need of multiple sessions<sup>[168]</sup>, they have a limited use and nowadays PEI use is reserved for the treatment of HCC < 2 cm with unfavorable RFA locations (Figure 1).

Among 2000-2010 numerous cohort studies and some randomized control trials (RCTs) and meta-analysis<sup>[169]</sup> demonstrated that RFA gets better control of the disease compared to PEI. It has the ability to create bigger necrosis, including a peripheral ring to the tumour, and therefore higher complete necrotic rates - even sustained necrosis - particularly in tumours < 3 cm, where ablation is more effective.

Initial complete response has demonstrated a positive impact on survival, although there still will be high recurrence rates, comparable to surgical resection. HCC usually appears in the setting of underlying chronic hepatic disease and this conditioned the appearance of new nodules, but there are also same segment recurrence nodules as a result of the growth of small peritumoral satellites or vascular microinvasion out of the ablated zone.

There are some researches<sup>[170,171]</sup> with specimen from surgery, about the distance of microsatellites depending on tumour size that come to the conclusion that a reasonable limit of RFA is 2, 5-3 cm in order to create a security margin of 5 mm. This makes us use RFA needles 1 or 2 numbers of ablation greater than the tumour diameter. Other strategies to increase the



**Figure 1 Ethanol injection treatment for hepatocellular carcinoma.** A: Very early hepatocellular carcinoma pre-percutaneous ethanol injection treatment (Arterial phase); B: Very early hepatocellular carcinoma pre-percutaneous ethanol injection treatment (Portal phase); C: Percutaneous ethanol injection procedure (Ultrasound guidance fine needle puncture); D: Percutaneous ethanol injection procedure (Ethanol aggregation after ultrasound guidance percutaneous ethanol injection); E: Computed tomography control arterial phase after 1 year (Sustained complete response).

ablation zone are overlapping techniques or multi-pronged needles, but their clinical use is difficult and not widespread.

RFA creates a complete necrosis area with a predictable diameter, whenever is not affected by nearby medium-large-sized vessels that could condition the perfusion-mediated tissue cooling, known as the heat sink effect. This limitation and the presence of non-treated microsatellites make up their main theoretical limitations, but there are also others that limit their clinical use: Ultrasound visualization of the nodule within liver parenchyma (difficult at fatty liver, macronodular cirrhosis, VIII segment nodules...) and the risk of damage of nearby organs (yuxtahilar, gallbladder, stomach, duodenum, large intestine). This potential damage contraindicates RFA if we are not able to isolate them with sterile water instillation (spacing technique). Last,

sub capsular tumours are not good indication of RFA due to the risk of tumoral seeding.

BCLC protocol last review<sup>[140]</sup> considered RFA as the first therapy at HCC < 2 cm, when a patient is not candidate to LT. This stage is also known as very early stage 0 or carcinoma *in situ*. RFA is also considered an alternative curative treatment at early stage (A) (single or 3 nodules  $\leq$  3 cm), with survival benefit up to 70%.

Microwave ablation is emerging as an alternative to RFA with several advantages. It is able to induce greater intratumoral temperature and bigger ablation area during less time than RFA. Thus, it is less dependent from tissue impedance and less influenced by heat-sink effect. Nowadays, it has less scientific evidence than RFA and there is lack of comparative papers between both techniques, but it seems logical to use it at HCC nearby to large hepatic vessels.



Irreversible electroporation is the technique more expensive, less used in clinical practice and with less evidence, although it is not affected by heat-sink effect and it doesn't damage adjacent structures. Therefore, its use seems useful to treat complex location lesions<sup>[144,172]</sup>.

TACE has been established by a meta-analysis of RCTs<sup>[173]</sup> as the standard of care for nonsurgical patients with large or multinodular noninvasive HCC isolated to the liver and with preserved liver function, known as intermediate stage HCC.

It is frequently used to control tumour progression (palliative treatment) as primary therapy or while waiting for liver transplantation, but some considerations has to be remarked. Intermediate stage is actually a heterogeneous group of patients and TACE benefit should be assessed in subgroups of patients as it has already been remarked<sup>[174]</sup>. Moreover, large series treated by TACE reported patients with single nodule stage A HCC<sup>[175,176]</sup>.

This would be justified by the recent concept of treatment stage migration: If a subject in a given stage is not candidate to the recommended treatment, we should consider the treatment of the more advance stage<sup>[140]</sup>. In our experience more than 1/3 of patient candidates to RFA, due to ablation difficulties, were treated by TACE (Figure 2), as has also been remarked in the literature<sup>[177]</sup>.

Thus, early stage HCCs have been treated with TACE with reported maintained complete responses and it has been suggested to include TACE as an alternative curative intention therapy (stage A), in selected patients and performed with a concrete technique<sup>[178]</sup>.

TACE technique is an interesting underestimate debate. There are different accepted techniques to perform endovascular HCC treatments with no enough evidence to determine the best option and this implies huge difficulties to standardize the results. Bland embolization or simple chemoinfusion have evolved to combined techniques of intra-arterial chemotherapy followed by ischemic changes after intra-arterial embolic materials (TACE).

Conventional TACE involves the selective injection of a chemotherapeutic agent (usually Doxorubicine) emulsified in a viscous carrier (lipiodol), followed by embolic material into the feeding arteries of the tumour.

It has been the most common way to perform TACE since the beginning of the century-validated with level 1 of evidence<sup>[173]</sup> - and is still acceptable with widespread use, above all in eastern countries. There are different ways to perform it regarding on how to mix lipiodol and contrast, being more or less selective and types of lipiodol aggregation. The optimal way should include filling of the "rear door of the tumour", *i.e.*, small portal drainage veins<sup>[179]</sup>.

An alternative way to perform TACE is widespread in the clinical practice, known as drug-eluting beads-TACE (DEB-TACE). It concerns performed microspheres loaded with chemotherapeutic agents which allows the delivery of large amounts of drugs to the tumour for a prolonged

period of time (improve antitumoral efficacy), thereby decreasing plasma levels of the chemotherapeutic agent and potentially systemic effects (better tolerance).

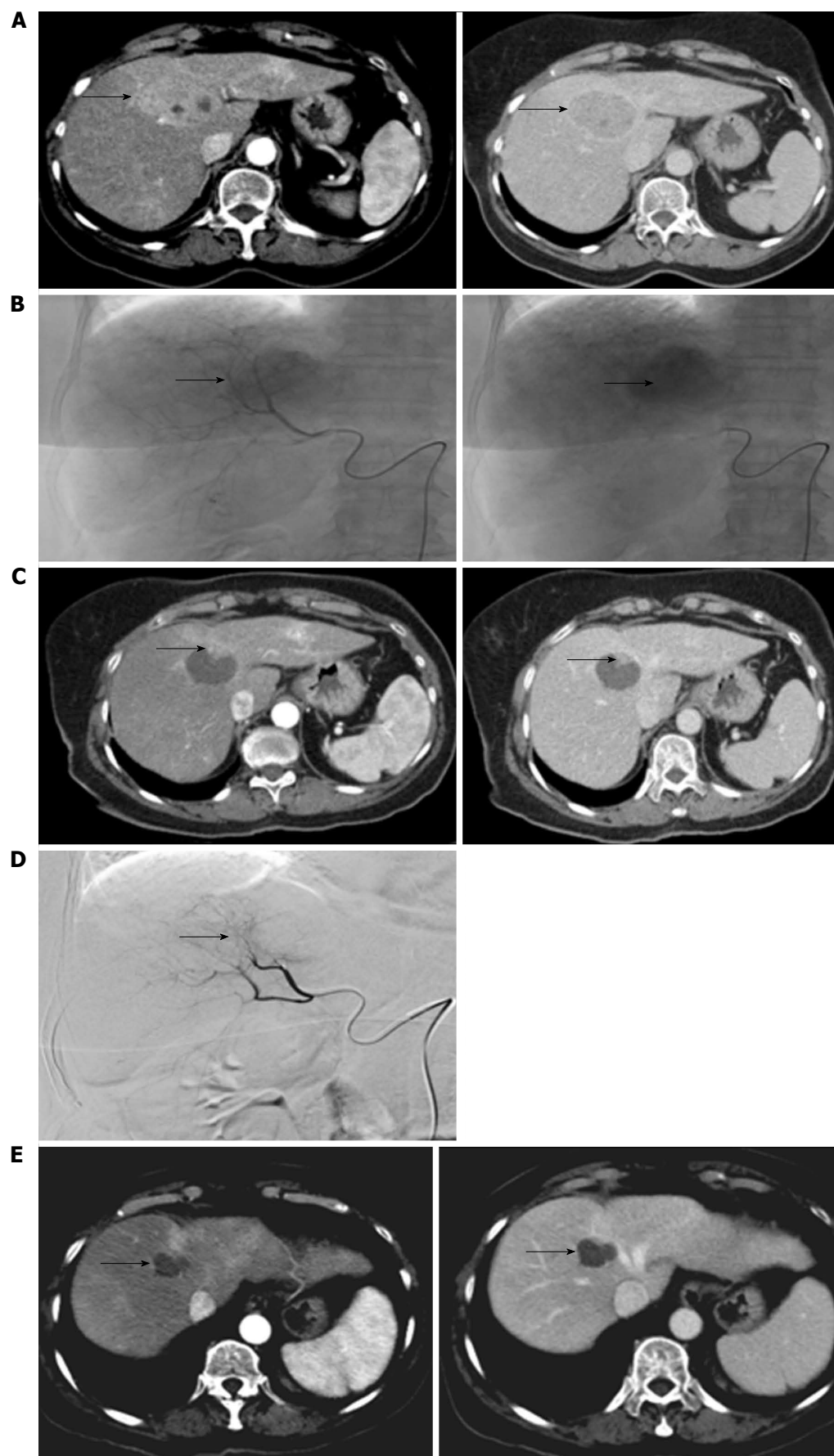
A prospective multi-institutional RCT (Precision V)<sup>[178]</sup> demonstrated significant better tolerance compared to cTACE, but only improved response in advanced disease (Child-Pugh B). Later several cohort studies and some RCTs favors DEB-TACE vs cTACE in response rates and survival, but nowadays it is a usual debate in HCC symposiums because more evidence is needed to evaluate the two modalities of TACE. Actually, DEB-TACE has implemented in the clinical practice of western countries based on some clear rationale: Maximize drug delivery, long lasting effect/slow and sustained release, tumour effect vs systemic side effects and better reproducibility.

Technical recommendations to perform it have been published to improve its efficacy, helping reproducibility and constitute clear working tendencies<sup>[180-182]</sup>: (1) Must use microcatheter with super-selective injection at feeding arteries; (2) Use angio-CT system technology for tumour targeting; (3) Mix beads with contrast 3-4:1 to increase visibility; (4) Avoid complete stasis (endpoint near stasis); (5) Inject slowly (1 mL/min) trying to introduce as much Doxorubicin as possible inside the tumour (maximum 150 mg); (6) Use of small size microspheres to increase penetrability. At present 100-300  $\mu$ m are recommended, but the use of smaller beads (M1 70-150  $\mu$ m) - commonly used at treating liver metastasis- is being evaluated in clinical trials. Many working groups have introduced them in their protocols, particularly with small size HCCs and they are extremely promising thanks to their bigger penetrability<sup>[183]</sup>; and (7) - Repeat TACE in 2-4 wk, if needed, to get initial complete response, which is being related to survival benefit<sup>[184]</sup>.

Ablative therapies and chemoembolization form the interventional treatments recommended by BCLC staging and treatment strategy, with simplicity as one of its known advantages. Other classifications as Japanese guidelines<sup>[185]</sup> stands for suggest other treatment options together with first line therapies in different stages or subgroups of them.

The huge variability of patients with HCC makes necessary to create a tailored approach that nowadays it is an undeniable clinical tendency<sup>[186]</sup>. We should adjust to each patient the most suitable treatment for its particular case, after a multidisciplinary assessment. The combination of locoregional therapies sometimes offers this maximal flexibility. This approach seems to be particularly valuable in patients with multifocal disease and nodules > 3 cm.

Among combine therapies, there are more experience with the combination of TACE and RFA (TACE first). Therefore, perfusion tissue is reduced and heat loss by perfusion mediated tissue cooling is minimized making possible larger ablation zone with wider safety margin<sup>[187]</sup>. Thus, sometimes downstaging is possible, above all with HCC 3-5 cm.



**Figure 2** Transarterial chemoembolization for hepatocellular carcinoma. A: Four centimeter hepatocellular carcinoma S-IV. Arterial and venous phase computed tomography; B: First transarterial chemoembolization procedure; C: Small residual foci after 1 transarterial chemoembolization; D: Second transarterial chemoembolization; E: Complete response after 2 transarterial chemoembolization.

In the recent years, several groups perform RFA followed by TACE (RFA first). This way, TACE acts over a transitional zone with sub lethal hyperthermia and increase vascular permeability. This forms an increase delivery, uptake and susceptibility to chemotherapeutics ideal to treat microsatellites outside RFA zone<sup>[188]</sup>.

Radioembolization is an alternative to TACE with less evidence and minor applicability. It needs to join interventional radiology and nuclear medicine units, which is restricted to only a few hospitals. Besides, technically is more complex than TACE and require an anatomical previous vascular map, because many times is necessary to embolize the arteries that communicate the target liver places with other adjacent organs as gallbladder or stomach that could be damaged.

Although is not included in the BCLC recommended treatments, it would be indicated in stage B HCC as an alternative to TACE and some stage C HCC with portal thrombosis that is not a contraindication of this technique. Some working groups consider it a first option in tumour > 5 cm or when > 4 nodules are present<sup>[174]</sup>. Ongoing RCTs are needed to unequivocally confirm the survival benefit provided by transarterial radioembolization in many cohort studies.

### Sorafenib

Sorafenib is a small molecule that inhibits tumour-cell proliferation, tumour angiogenesis and it is a multi-tyrosine kinase inhibitor and nowadays is the only drug that have demonstrated survival benefits in patients with advanced HCC. The initial phase II and phase III studies showed positive results with better survival in patients treated with sorafenib. The benefit of sorafenib was to increase the median survival from 7.9 mo in the placebo group to 10.7 mo in the sorafenib group. In addition, sorafenib showed a significant benefit in terms of time to progression, but objective responses rates were low<sup>[189]</sup>. These results were corroborated in other phase III study conducted in Asia<sup>[190]</sup>. This drug is only indicated in patients with preserved liver function and advanced disease not susceptible of other therapies and in this group of patients have an acceptable safety profile with manageable adverse events. The initial results were very promising because it was the first time that a systemic therapy demonstrated benefits effects in patients with HCC. Two subsequent trials, the Space (Sorafenib or placebo in combination with TACE for intermediate-stage HCC)<sup>[191]</sup> and the Storm (Sorafenib or placebo after resection or ablation to prevent recurrence of HCC)<sup>[192]</sup> have failed to demonstrated efficacy of sorafenib as adjuvant in combination with locally therapies. In the next years, new novel drugs, with a slightly different profile in terms of targets and intensity, have been tried both in first-line and second-line therapy. Until now, none of these drugs (sunitinib, brivanib, linifanib and combination of erlotinib and sorafenib) have proven to be better than sorafenib in first-line trials, in terms of survival. Second-line trails with brivanib, everolimus

and ramucirumab have also failed to show benefits compared with placebo.

The EASL and AASLD recommend the use of sorafenib in patients with HCC advanced stage and preserved liver function.

## CONCLUSION

HCC is a tumour with high incidence in patients with liver cirrhosis and is currently the leading cause of death in this group of patients. It is expected a decreases in incidence in the coming decades due to better management of patients infected with HBV and HCV. The vaccination against hepatitis B, the extended use of antiviral drugs with a high genetic barrier, which remain at undetectable viral load levels and the higher rate of sustained viral response in patients with chronic HCV with the new generation of antiviral drugs will reduce the incidence of this tumour in the future. On the other hand, increasingly numbers of studies have identified protective factors such as treatment with beta-blockers or statins, and perhaps in the future the use of some of these drugs will be recommended in selected cirrhotic patients. On the other hand, the improvement in the quality of imaging techniques allows establishing a diagnosis without histological confirmation in a high percentage of patients. New radiologic classifications, although promising, need more studies to be accepted universally. Once confirmed the diagnosis, the staging of the tumour allows us to decide the best therapeutic approach. Although several prognostic classifications have been described, the BCLC classification has been supported by American and European clinical practice guidelines. In addition, it allows deciding the best therapy according to the stage. The mainstays of treatment of HCC are surgery, radiological approach and systemic drugs. Since it is the treatment of choice to better outcomes in terms of survival, the indications of liver transplantation are in constant review. The expanded criteria and the downstaging have helped to expand the number of patients who are eligible for this option, with acceptable survival and recurrence after the transplant. On the other hand, the percutaneous ablative techniques have obtained good results in terms of response and survival, similar to surgical resection, in selected cases. In patients at intermediate stages, chemoembolization with particles has improved the results against the conventional chemoembolization with a similar rate of adverse effects. Sorafenib is the only systemic drug that has demonstrated survival benefits in advanced-stage patients and therefore remains the standard of care in this group. So far, any drug has shown survival benefits in second-line therapy after progression with sorafenib.

## REFERENCES

- 1 Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID:



- 21296855 DOI: 10.3322/caac.20107]
- 2 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]
- 3 **International Agency for Research on Cancer**. Available from: URL: <http://www-dep.iarc.fr/>
- 4 **El-Serag HB**, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999; **340**: 745-750 [PMID: 10072408 DOI: 10.1056/NEJM199903113401001]
- 5 **Tsukuma H**, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328**: 1797-1801 [PMID: 7684822 DOI: 10.1056/NEJM199306243282501]
- 6 **Prates MD**, Torres FO. A cancer survey in Lourenço Marques, Portuguese East Africa. *J Natl Cancer Inst* 1965; **35**: 729-757 [PMID: 5892211 DOI: 10.1093/jnci/35.5.729]
- 7 **Bosetti C**, Levi F, Boffetta P, Lucchini F, Negri E, La Vecchia C. Trends in mortality from hepatocellular carcinoma in Europe, 1980-2004. *Hepatology* 2008; **48**: 137-145 [PMID: 18537177 DOI: 10.1002/hep.22312]
- 8 **Franceschi S**, Raza SA. Epidemiology and prevention of hepatocellular carcinoma. *Cancer Lett* 2009; **286**: 5-8 [PMID: 19070421 DOI: 10.1016/j.canlet.2008.10.046]
- 9 **Chang MH**, You SL, Chen CJ, Liu CJ, Lee CM, Lin SM, Chu HC, Wu TC, Yang SS, Kuo HS, Chen DS; Taiwan Hepatoma Study Group. Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: a 20-year follow-up study. *J Natl Cancer Inst* 2009; **101**: 1348-1355 [PMID: 19759364 DOI: 10.1093/jnci/djp288]
- 10 **Bralet MP**, Régimbeau JM, Pineau P, Dubois S, Loas G, Degos F, Valla D, Belghiti J, Degott C, Terris B. Hepatocellular carcinoma occurring in nonfibrotic liver: epidemiologic and histopathologic analysis of 80 French cases. *Hepatology* 2000; **32**: 200-204 [PMID: 10915724 DOI: 10.1053/jhep.2000.9033]
- 11 **Sangiovanni A**, Prati GM, Fasani P, Ronchi G, Romeo R, Manini M, Del Ninno E, Morabito A, Colombo M. The natural history of compensated cirrhosis due to hepatitis C virus: A 17-year cohort study of 214 patients. *Hepatology* 2006; **43**: 1303-1310 [PMID: 16729298 DOI: 10.1002/hep.21176]
- 12 **Ioannou GN**, Splan MF, Weiss NS, McDonald GB, Beretta L, Lee SP. Incidence and predictors of hepatocellular carcinoma in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2007; **5**: 938-945, 945.e1-4 [PMID: 17509946 DOI: 10.1016/j.cgh.2007.02.039]
- 13 **Lok AS**, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Goodman ZD; HALT-C Trial Group. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009; **136**: 138-148 [PMID: 18848939 DOI: 10.1053/j.gastro.2008.09.014]
- 14 **Ripoll C**, Groszmann RJ, Garcia-Tsao G, Bosch J, Grace N, Burroughs A, Planas R, Escorsell A, Garcia-Pagan JC, Makuch R, Patch D, Matloff DS; Portal Hypertension Collaborative Group. Hepatic venous pressure gradient predicts development of hepatocellular carcinoma independently of severity of cirrhosis. *J Hepatol* 2009; **50**: 923-928 [PMID: 19303163 DOI: 10.1016/j.jhep.2009.01.014]
- 15 **Masuzaki R**, Tateishi R, Yoshida H, Goto E, Sato T, Ohki T, Imamura J, Goto T, Kanai F, Kato N, Ikeda H, Shiina S, Kawabe T, Omata M. Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. *Hepatology* 2009; **49**: 1954-1961 [PMID: 19434742 DOI: 10.1002/hep.22870]
- 16 **Jung KS**, Kim SU, Ahn SH, Park YN, Kim do Y, Park JY, Chon CY, Choi EH, Han KH. Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). *Hepatology* 2011; **53**: 885-894 [PMID: 21319193 DOI: 10.1002/hep.24121]
- 17 **Lok AS**. Prevention of hepatitis B virus-related hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S303-S309 [PMID: 15508098 DOI: 10.1053/j.gastro.2004.09.045]
- 18 **London WT**, McGlynn KA. Liver cancer. In: Schottenfeld D, Fraumeni Jr JF, editors. *Cancer epidemiology and prevention*. 3rd ed. New York: Oxford University Press, 2006: 763e86 [DOI: 10.1093/acprof:oso/9780195149616.001.0001]
- 19 **Stuver S**, Trichopoulos D. Cancer of the liver and biliary tract. In: Adami HO, Hunter D, Trichopoulos D, editors. *Textbook of cancer epidemiology*. 2nd ed. New York: Oxford University Press, 2008: 308e32 [DOI: 10.1093/acprof:oso/9780195311174.001.0001]
- 20 **Boffetta P**, Boccia S, La Vecchia C. Cancer of the liver and biliary tract. In: Boffetta P, Boccia S, La Vecchia C, editors. *A quick guide to cancer epidemiology*. Springer, 2014 [DOI 10.1007/978-3-319-05068-3]
- 21 **IARC**. IARC monographs on the evaluation of carcinogenic risks to humans. Hepatitis viruses, 1994: 59
- 22 **Parkin DM**. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; **118**: 3030-3044 [PMID: 16404738 DOI: 10.1002/ijc.21731]
- 23 **Beasley RP**, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet* 1981; **2**: 1129-1133 [PMID: 6118576 DOI: 10.1016/S0140-6736(81)90585-7]
- 24 **Yu MW**, Chen CJ. Hepatitis B and C viruses in the development of hepatocellular carcinoma. *Crit Rev Oncol Hematol* 1994; **17**: 71-91 [PMID: 7818788 DOI: 10.1016/1040-8428(94)90020-5]
- 25 **Sherman M**, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology* 1995; **22**: 432-438 [PMID: 7543434]
- 26 **Villeneuve JP**, Desrochers M, Infante-Rivard C, Willems B, Raymond G, Bourcier M, Côté J, Richer G. A long-term follow-up study of asymptomatic hepatitis B surface antigen-positive carriers in Montreal. *Gastroenterology* 1994; **106**: 1000-1005 [PMID: 8143967]
- 27 **Chen JD**, Yang HI, Iloeje UH, You SL, Lu SN, Wang LY, Su J, Sun CA, Liaw YF, Chen CJ; Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer in HBV (REVEAL-HBV) Study Group. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. *Gastroenterology* 2010; **138**: 1747-1754 [PMID: 20114048 DOI: 10.1053/j.gastro.2010.01.042]
- 28 **Beasley RP**. Hepatitis B virus. The major etiology of hepatocellular carcinoma. *Cancer* 1988; **61**: 1942-1956 [PMID: 2834034]
- 29 **Yang HI**, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ; Taiwan Community-Based Cancer Screening Project Group. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; **347**: 168-174 [PMID: 12124405 DOI: 10.1056/NEJMoa013215]
- 30 **Chen CJ**, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH; REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65-73 [PMID: 16391218 DOI: 10.1001/jama.295.1.65]
- 31 **Yu MW**, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, Shih WL, Kao JH, Chen DS, Chen CJ. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst* 2005; **97**: 265-272 [PMID: 15713961 DOI: 10.1093/jnci/dji043]
- 32 **Muñoz N**, Lingao A, Lao J, Estève J, Viterbo G, Domingo EO, Lansang MA. Patterns of familial transmission of HBV and the risk of developing liver cancer: a case-control study in the Philippines. *Int J Cancer* 1989; **44**: 981-984 [PMID: 2606583]
- 33 **Kuper H**, Hsieh C, Stuver SO, Mucci LA, Tzonou A, Zavitsanos X, Lagiou P, Trichopoulos D. Birth order, as a proxy for age at infection, in the etiology of hepatocellular carcinoma. *Epidemiology* 2000; **11**: 680-683 [PMID: 11055629]
- 34 **Bosch FX**, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; **127**: S5-S16 [PMID: 15508102 DOI: 10.1053/j.gastro.2004.09.011]



- 35 **Dragosics B**, Ferenci P, Hitchman E, Denk H. Long-term follow-up study of asymptomatic HBsAg-positive voluntary blood donors in Austria: a clinical and histologic evaluation of 242 cases. *Hepatology* 1987; **7**: 302-306 [PMID: 3557309]
- 36 **Chen CJ**, Yang HI, Iloeje UH, Su J, Jen CL, You SL, Liaw YF. Time-dependent relative risk of hepatocellular carcinoma for markers of chronic hepatitis B. The REVEAL HBV study (abstract). *Hepatology* 2005; **42** Suppl 1: 722A
- 37 **Tong MJ**, Blatt LM, Kao JH, Cheng JT, Corey WG. Basal core promoter T1762/A1764 and precore A1896 gene mutations in hepatitis B surface antigen-positive hepatocellular carcinoma: a comparison with chronic carriers. *Liver Int* 2007; **27**: 1356-1363 [PMID: 17900245 DOI: 10.1111/j.1478-3231.2007.01585.x]
- 38 **Simonetti J**, Bulkow L, McMahon BJ, Homan C, Snowball M, Negus S, Williams J, Livingston SE. Clearance of hepatitis B surface antigen and risk of hepatocellular carcinoma in a cohort chronically infected with hepatitis B virus. *Hepatology* 2010; **51**: 1531-1537 [PMID: 20087968 DOI: 10.1002/hep.23464]
- 39 **Yuen MF**, Wong DK, Fung J, Ip P, But D, Hung I, Lau K, Yuen JC, Lai CL. HBsAg Seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. *Gastroenterology* 2008; **135**: 1192-1199 [PMID: 18722377 DOI: 10.1053/j.gastro.2008.07.008]
- 40 **Sung JJ**, Tsoi KK, Wong VW, Li KC, Chan HL. Meta-analysis: Treatment of hepatitis B infection reduces risk of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2008; **28**: 1067-1077 [PMID: 18657133 DOI: 10.1111/j.1365-2036.2008.03816.x]
- 41 **Papatheodoridis GV**, Lampertico P, Manolakopoulos S, Lok A. Incidence of hepatocellular carcinoma in chronic hepatitis B patients receiving nucleos(t)ide therapy: a systematic review. *J Hepatol* 2010; **53**: 348-356 [PMID: 20483498 DOI: 10.1016/j.jhep.2010.02.035]
- 42 **Shen YC**, Hsu C, Cheng CC, Hu FC, Cheng AL. A critical evaluation of the preventive effect of antiviral therapy on the development of hepatocellular carcinoma in patients with chronic hepatitis C or B: a novel approach by using meta-regression. *Oncology* 2012; **82**: 275-289 [PMID: 22555181 DOI: 10.1159/000337293]
- 43 **Singal AK**, Salameh H, Kuo YF, Fontana RJ. Meta-analysis: the impact of oral anti-viral agents on the incidence of hepatocellular carcinoma in chronic hepatitis B. *Aliment Pharmacol Ther* 2013; **38**: 98-106 [PMID: 23713520 DOI: 10.1111/apt.12344]
- 44 **Yu MW**, You SL, Chang AS, Lu SN, Liaw YF, Chen CJ. Association between hepatitis C virus antibodies and hepatocellular carcinoma in Taiwan. *Cancer Res* 1991; **51**: 5621-5625 [PMID: 1655259]
- 45 **Huang YT**, Yang HI, Jen CL, Iloeje UH, Su J, You SL, Wang LY, Sun CA, Chen CJ. Suppression of hepatitis B virus replication by hepatitis C virus: combined effects on risk of hepatocellular carcinoma (abstract). *Hepatology* 2005; **42** (Suppl 1): 230A
- 46 **Benvenuto L**, Fattovich G, Noventa F, Tremolada F, Chemello L, Cecchetto A, Alberti A. Concurrent hepatitis B and C virus infection and risk of hepatocellular carcinoma in cirrhosis. A prospective study. *Cancer* 1994; **74**: 2442-2448 [PMID: 7922998]
- 47 **Bruix J**, Barrera JM, Calvet X, Ercilla G, Costa J, Sanchez-Tapias JM, Ventura M, Vall M, Bruguera M, Bru C. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet* 1989; **2**: 1004-1006 [PMID: 2572739 DOI: 10.1016/S0140-6736(89)91015-5]
- 48 **Colombo M**, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA, Dioguardi N, Houghton M. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989; **2**: 1006-1008 [PMID: 2572740 DOI: 10.1016/S0140-6736(89)91016-7]
- 49 **Omland LH**, Jepsen P, Krarup H, Christensen PB, Weis N, Nielsen L, Obel N, Sørensen HT, Stuver SO, DANVIR cohort study. Liver cancer and non-Hodgkin lymphoma in hepatitis C virus-infected patients: results from the DANVIR cohort study. *Int J Cancer* 2012; **130**: 2310-2317 [PMID: 21780099 DOI: 10.1002/ijc.26283]
- 50 **Lewis S**, Roayaie S, Ward SC, Shykevsky I, Jibara G, Taouli B. Hepatocellular carcinoma in chronic hepatitis C in the absence of advanced fibrosis or cirrhosis. *AJR Am J Roentgenol* 2013; **200**: W610-W616 [PMID: 23701091 DOI: 10.2214/AJR.12.9151]
- 51 **Raimondi S**, Bruno S, Mondelli MU, Maisonneuve P. Hepatitis C virus genotype 1b as a risk factor for hepatocellular carcinoma development: a meta-analysis. *J Hepatol* 2009; **50**: 1142-1154 [PMID: 19395111 DOI: 10.1016/j.jhep.2009.01.019]
- 52 **Maki A**, Kono H, Gupta M, Asakawa M, Suzuki T, Matsuda M, Fujii H, Rusyn I. Predictive power of biomarkers of oxidative stress and inflammation in patients with hepatitis C virus-associated hepatocellular carcinoma. *Ann Surg Oncol* 2007; **14**: 1182-1190 [PMID: 17195915]
- 53 **Suruki RY**, Mueller N, Hayashi K, Harn D, DeGruttola V, Raker CA, Tsubouchi H, Stuver SO. Host immune status and incidence of hepatocellular carcinoma among subjects infected with hepatitis C virus: a nested case-control study in Japan. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 2521-2525 [PMID: 17164379 DOI: 10.1158/1055-9965.EPI-06-0485]
- 54 **George SL**, Bacon BR, Brunt EM, Mihindukulasuriya KL, Hoffmann J, Di Bisceglie AM. Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009; **49**: 729-738 [PMID: 19072828 DOI: 10.1002/hep.22694]
- 55 **Liang TJ**, Ghany MG. Therapy of hepatitis C--back to the future. *N Engl J Med* 2014; **370**: 2043-2047 [PMID: 24795199 DOI: 10.1056/NEJMe1403619]
- 56 **Ikeda K**, Marusawa H, Osaki Y, Nakamura T, Kitajima N, Yamashita Y, Kudo M, Sato T, Chiba T. Antibody to hepatitis B core antigen and risk for hepatitis C-related hepatocellular carcinoma: a prospective study. *Ann Intern Med* 2007; **146**: 649-656 [PMID: 17470833 DOI: 10.7326/0003-4819-146-9-200705010-00008]
- 57 **Marcellin P**, Peignot F, Delarocque-Astagneau E, Zarski JP, Ganne N, Hillon P, Antona D, Bovet M, Mechain M, Asselah T, Desenclos JC, Jougla E. Mortality related to chronic hepatitis B and chronic hepatitis C in France: evidence for the role of HIV coinfection and alcohol consumption. *J Hepatol* 2008; **48**: 200-207 [PMID: 18086507 DOI: 10.1016/j.jhep.2007.09.010]
- 58 **Chou YH**, Chiou HJ, Tiu CM, Chiou SY, Lee SD, Hung GS, Wu SC, Kuo BI, Lee RC, Chiang JH, Chang T, Yu C. Duplex Doppler ultrasound of hepatic Schistosomiasis japonica: a study of 47 patients. *Am J Trop Med Hyg* 2003; **68**: 18-23 [PMID: 12556142]
- 59 **Ezzat S**, Abdel-Hamid M, Eissa SA, Mokhtar N, Labib NA, El-Ghorory L, Mikhail NN, Abdel-Hamid A, Hifnawy T, Strickland GT, Loffredo CA. Associations of pesticides, HCV, HBV, and hepatocellular carcinoma in Egypt. *Int J Hyg Environ Health* 2005; **208**: 329-339 [PMID: 16217918]
- 60 **Hsu IC**, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. *Nature* 1991; **350**: 427-428 [PMID: 1849234 DOI: 10.1038/350427a0]
- 61 **Bressac B**, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature* 1991; **350**: 429-431 [PMID: 1672732 DOI: 10.1038/350429a0]
- 62 **Yu SZ**. Primary prevention of hepatocellular carcinoma. *J Gastroenterol Hepatol* 1995; **10**: 674-682 [PMID: 8580413]
- 63 **Ueno Y**, Nagata S, Tsutsumi T, Hasegawa A, Watanabe MF, Park HD, Chen GC, Chen G, Yu SZ. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. *Carcinogenesis* 1996; **17**: 1317-1321 [PMID: 8681449 DOI: 10.1093/carcin/17.6.1317]
- 64 **Tsai JF**, Chuang LY, Jeng JE, Ho MS, Hsieh MY, Lin ZY, Wang LY. Betel quid chewing as a risk factor for hepatocellular carcinoma: a case-control study. *Br J Cancer* 2001; **84**: 709-713 [PMID: 11237396 DOI: 10.1054/bjoc.1999.1597]
- 65 **Tsai JF**, Jeng JE, Chuang LY, Ho MS, Ko YC, Lin ZY, Hsieh MY, Chen SC, Chuang WL, Wang LY, Yu ML, Dai CY, Ho C. Habitual betel quid chewing as a risk factor for cirrhosis: a case-control study. *Medicine (Baltimore)* 2003; **82**: 365-372 [PMID: 14530785]

- 66 **Trichopoulos D**, Bamia C, Lagiou P, Fedirko V, Trepo E, Jenab M, Pischon T, Nöthlings U, Overved K, Tjønneland A, Outzen M, Clavel-Chapelon F, Kaaks R, Lukanova A, Boeing H, Aleksandrova K, Benetou V, Zylis D, Palli D, Pala V, Panico S, Tumino R, Sacerdote C, Bueno-De-Mesquita HB, Van Kranen HJ, Peeters PH, Lund E, Quirós JR, González CA, Sanchez Perez MJ, Navarro C, Dorronsoro M, Barricarte A, Lindkvist B, Regnér S, Werner M, Hallmans G, Khaw KT, Wareham N, Key T, Romieu I, Chuang SC, Murphy N, Boffetta P, Trichopoulou A, Riboli E. Hepatocellular carcinoma risk factors and disease burden in a European cohort: a nested case-control study. *J Natl Cancer Inst* 2011; **103**: 1686-1695 [PMID: 22021666 DOI: 10.1093/jnci/djr395]
- 67 **Mayans MV**, Calvet X, Bruix J, Bruguera M, Costa J, Estève J, Bosch FX, Bru C, Rodés J. Risk factors for hepatocellular carcinoma in Catalonia, Spain. *Int J Cancer* 1990; **46**: 378-381 [PMID: 2168342]
- 68 **Tanaka K**, Hirohata T, Takeshita S, Hirohata I, Koga S, Sugimachi K, Kanematsu T, Ohryohji F, Ishibashi H. Hepatitis B virus, cigarette smoking and alcohol consumption in the development of hepatocellular carcinoma: a case-control study in Fukuoka, Japan. *Int J Cancer* 1992; **51**: 509-514 [PMID: 1318264]
- 69 **Mohamed AE**, Kew MC, Groeneveld HT. Alcohol consumption as a risk factor for hepatocellular carcinoma in urban southern African blacks. *Int J Cancer* 1992; **51**: 537-541 [PMID: 1318267]
- 70 **Donato F**, Taggar A, Gelatti U, Parrinello G, Boffetta P, Albertini A, Decarli A, Trevisi P, Ribero ML, Martelli C, Porru S, Nardi G. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. *Am J Epidemiol* 2002; **155**: 323-331 [PMID: 11836196 DOI: 10.1093/aje/k155.4.323]
- 71 **Lieber CS**. Alcohol and the liver: 1994 update. *Gastroenterology* 1994; **106**: 1085-1105 [PMID: 8143977]
- 72 **Chiesa R**, Donato F, Taggar A, Favret M, Ribero ML, Nardi G, Gelatti U, Bucella E, Tomasi E, Portolani N, Bonetti M, Bettini L, Pelizzari G, Salmi A, Savio A, Garatti M, Callea F. Etiology of hepatocellular carcinoma in Italian patients with and without cirrhosis. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 213-216 [PMID: 10698484]
- 73 **Munaka M**, Kohshi K, Kawamoto T, Takasawa S, Nagata N, Itoh H, Oda S, Katoh T. Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2003; **129**: 355-360 [PMID: 12759747]
- 74 **Covolo L**, Gelatti U, Talamini R, Garte S, Trevisi P, Franceschi S, Franceschini M, Barbone F, Taggar A, Ribero ML, Parrinello G, Donadon V, Nardi G, Donato F. Alcohol dehydrogenase 3, glutathione S-transferase M1 and T1 polymorphisms, alcohol consumption and hepatocellular carcinoma (Italy). *Cancer Causes Control* 2005; **16**: 831-838 [PMID: 16132793]
- 75 **Yu MC**, Tong MJ, Govindarajan S, Henderson BE. Nonviral risk factors for hepatocellular carcinoma in a low-risk population, the non-Asians of Los Angeles County, California. *J Natl Cancer Inst* 1991; **83**: 1820-1826 [PMID: 1660542 DOI: 10.1093/jnci/83.24.1820]
- 76 **Kuper H**, Tzonou A, Kaklamani E, Hsieh CC, Lagiou P, Adami HO, Trichopoulos D, Stuver SO. Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. *Int J Cancer* 2000; **85**: 498-502 [PMID: 10699921]
- 77 **El-Serag HB**, Richardson PA, Everhart JE. The role of diabetes in hepatocellular carcinoma: a case-control study among United States Veterans. *Am J Gastroenterol* 2001; **96**: 2462-2467 [PMID: 11513191 DOI: 10.1111/j.1572-0241.2001.04054.x]
- 78 **Marrero JA**, Fontana RJ, Fu S, Conjeevaram HS, Su GL, Lok AS. Alcohol, tobacco and obesity are synergistic risk factors for hepatocellular carcinoma. *J Hepatol* 2005; **42**: 218-224 [PMID: 15664247 DOI: 10.1016/j.jhep.2004.10.005]
- 79 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140 [PMID: 12105842 DOI: 10.1053/gast.2002.34168]
- 80 **Hashimoto E**, Yatsuji S, Tobari M, Taniai M, Torii N, Tokushige K, Shiratori K. Hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *J Gastroenterol* 2009; **44** Suppl 19: 89-95 [PMID: 19148800 DOI: 10.1007/s00535-008-2262-x]
- 81 **Ascha MS**, Hanounieh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972-1978 [PMID: 20209604 DOI: 10.1002/hep.23527]
- 82 **Yasui K**, Hashimoto E, Komorizono Y, Koike K, Arai S, Imai Y, Shima T, Kanbara Y, Saibara T, Mori T, Kawata S, Uto H, Takami S, Sumida Y, Takamura T, Kawanaka M, Okanoue T; Japan NASH Study Group, Ministry of Health, Labour, and Welfare of Japan. Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2011; **9**: 428-433; quiz e50 [PMID: 21320639 DOI: 10.1016/j.cgh.2011.01.023]
- 83 **Welzel TM**, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary liver cancer in the United States: a study in the SEER-Medicare database. *Hepatology* 2011; **54**: 463-471 [PMID: 21538440 DOI: 10.1002/hep.24397]
- 84 **Deugnier YM**, Guyader D, Crantock L, Lopez JM, Turlin B, Yaouanq J, Jouanolle H, Campion JP, Launois B, Halliday JW. Primary liver cancer in genetic hemochromatosis: a clinical, pathological, and pathogenetic study of 54 cases. *Gastroenterology* 1993; **104**: 228-234 [PMID: 8419246]
- 85 **Perlmutter DH**. Pathogenesis of chronic liver injury and hepatocellular carcinoma in alpha-1-antitrypsin deficiency. *Pediatr Res* 2006; **60**: 233-238 [PMID: 16864711 DOI: 10.1203/01.pdr.0000228350.61496.90]
- 86 **Polio J**, Enriquez RE, Chow A, Wood WM, Atterbury CE. Hepatocellular carcinoma in Wilson's disease. Case report and review of the literature. *J Clin Gastroenterol* 1989; **11**: 220-224 [PMID: 2472436]
- 87 **Dawn BM**, Todd S, Kim SI, Glucksman M. Biochemistry and molecular biology. Philadelphia: Wolters Kluwer Health/Lippincott Williams and Wilkins, 2007
- 88 **Liang Y**, Yang Z, Zhong R. Primary biliary cirrhosis and cancer risk: a systematic review and meta-analysis. *Hepatology* 2012; **56**: 1409-1417 [PMID: 22504852 DOI: 10.1002/hep.25788]
- 89 **Stewart MF**. Review of hepatocellular cancer, hypertension and renal impairment as late complications of acute porphyria and recommendations for patient follow-up. *J Clin Pathol* 2012; **65**: 976-980 [PMID: 22851509 DOI: 10.1136/jclinpath-2012-200791]
- 90 **Andant C**, Puy H, Bogard C, Faivre J, Soule JC, Nordmann Y, Deybach JC. Hepatocellular carcinoma in patients with acute hepatic porphyria: frequency of occurrence and related factors. *J Hepatol* 2000; **32**: 933-939 [PMID: 10898313 DOI: 10.1016/S0168-8278(00)80097-5]
- 91 **Andant C**, Puy H, Faivre J, Deybach JC. Acute hepatic porphyrias and primary liver cancer. *N Engl J Med* 1998; **338**: 1853-1854 [PMID: 9634374 DOI: 10.1056/NEJM199806183382518]
- 92 **Andersson C**, Bjersing L, Lithner F. The epidemiology of hepatocellular carcinoma in patients with acute intermittent porphyria. *J Intern Med* 1996; **240**: 195-201 [PMID: 8918510]
- 93 **Bengtsson NO**, Hardell L. Porphyrias, porphyrins and hepatocellular cancer. *Br J Cancer* 1986; **54**: 115-117 [PMID: 3015181]
- 94 **Gubler JG**, Bargetzi MJ, Meyer UA. Primary liver carcinoma in two sisters with acute intermittent porphyria. *Am J Med* 1990; **89**: 540-541 [PMID: 2171334]
- 95 **Hardell L**, Bengtsson NO, Jonsson U, Eriksson S, Larsson LG. Aetiological aspects on primary liver cancer with special regard to alcohol, organic solvents and acute intermittent porphyria--an epidemiological investigation. *Br J Cancer* 1984; **50**: 389-397 [PMID: 6087869]
- 96 **Kaappinen R**, Mustajoki P. Acute hepatic porphyria and hepatocellular carcinoma. *Br J Cancer* 1988; **57**: 117-120 [PMID: 2831925]
- 97 **Lithner F**, Wetterberg L. Hepatocellular carcinoma in patients with

- acute intermittent porphyria. *Acta Med Scand* 1984; **215**: 271-274 [PMID: 6328897]
- 98 **Liu Y**, He Y, Li T, Xie L, Wang J, Qin X, Li S. Risk of primary liver cancer associated with gallstones and cholecystectomy: a meta-analysis. *PLoS One* 2014; **9**: e109733 [PMID: 25290940 DOI: 10.1371/journal.pone.0109733]
- 99 **Tajada M**, Nerin J, Ruiz MM, Sánchez-Dehesa M, Fabre E. Liver adenoma and focal nodular hyperplasia associated with oral contraceptives. *Eur J Contracept Reprod Health Care* 2001; **6**: 227-230 [PMID: 11848652]
- 100 **Korula J**, Yellin A, Kanel G, Campofiori G, Nichols P. Hepatocellular carcinoma coexisting with hepatic adenoma. Incidental discovery after long-term oral contraceptive use. *West J Med* 1991; **155**: 416-418 [PMID: 1663298]
- 101 **Gordon SC**, Reddy KR, Livingstone AS, Jeffers LJ, Schiff ER. Resolution of a contraceptive-steroid-induced hepatic adenoma with subsequent evolution into hepatocellular carcinoma. *Ann Intern Med* 1986; **105**: 547-549 [PMID: 3019201 DOI: 10.7326/0003-4819-105-4-547]
- 102 **Maheshwari S**, Sarraj A, Kramer J, El-Serag HB. Oral contraception and the risk of hepatocellular carcinoma. *J Hepatol* 2007; **47**: 506-513 [PMID: 17462781 DOI: 10.1016/j.jhep.2007.03.015]
- 103 **Freedman ND**, Cross AJ, McGlynn KA, Abnet CC, Park Y, Hollenbeck AR, Schatzkin A, Everhart JE, Sinha R. Association of meat and fat intake with liver disease and hepatocellular carcinoma in the NIH-AARP cohort. *J Natl Cancer Inst* 2010; **102**: 1354-1365 [PMID: 20729477 DOI: 10.1093/jnci/djq301]
- 104 **Cross AJ**, Leitzmann MF, Gail MH, Hollenbeck AR, Schatzkin A, Sinha R. A prospective study of red and processed meat intake in relation to cancer risk. *PLoS Med* 2007; **4**: e325 [PMID: 18076279 DOI: 10.1371/journal.pmed.0040325]
- 105 **Luo J**, Yang Y, Liu J, Lu K, Tang Z, Liu P, Liu L, Zhu Y. Systematic review with meta-analysis: meat consumption and the risk of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2014; **39**: 913-922 [PMID: 24588342 DOI: 10.1111/apt.12678]
- 106 **Agency for Toxic Substances and Disease Registry**. Toxicological profile for N nitrosodimethylamine. Atlanta, GA: US Department of Health and Human Services, 1989
- 107 **Turati F**, Edefonti V, Talamini R, Ferraroni M, Malvezzi M, Bravi F, Franceschi S, Montella M, Polesel J, Zucchetto A, La Vecchia C, Negri E, Decarli A. Family history of liver cancer and hepatocellular carcinoma. *Hepatology* 2012; **55**: 1416-1425 [PMID: 22095619 DOI: 10.1002/hep.24794]
- 108 **Tanabe KK**, Lemoine A, Finkelstein DM, Kawasaki H, Fujii T, Chung RT, Lauwers GY, Kulu Y, Muzikansky A, Kuruppu D, Lanuti M, Goodwin JM, Azoulay D, Fuchs BC. Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. *JAMA* 2008; **299**: 53-60 [PMID: 18167406 DOI: 10.1001/jama.2007.65]
- 109 **De Luca A**, Carotenuto A, Rachiglio A, Gallo M, Maiello MR, Aldinucci D, Pinto A, Normanno N. The role of the EGFR signaling in tumor microenvironment. *J Cell Physiol* 2008; **214**: 559-567 [PMID: 17894407 DOI: 10.1002/jcp.21260]
- 110 **Iavarone M**, Lampertico P, Iannuzzi F, Manenti E, Donato MF, Arosio E, Bertolini F, Primignani M, Sangiovanni A, Colombo M. Increased expression of vascular endothelial growth factor in small hepatocellular carcinoma. *J Viral Hepat* 2007; **14**: 133-139 [PMID: 17244253 DOI: 10.1111/j.1365-2893.2006.00782.x]
- 111 **Park YN**, Kim YB, Yang KM, Park C. Increased expression of vascular endothelial growth factor and angiogenesis in the early stage of multistep hepatocarcinogenesis. *Arch Pathol Lab Med* 2000; **124**: 1061-1065 [PMID: 10888784]
- 112 **Suzuki K**, Hayashi N, Miyamoto Y, Yamamoto M, Ohkawa K, Ito Y, Sasaki Y, Yamaguchi Y, Nakase H, Noda K, Enomoto N, Arai K, Yamada Y, Yoshihara H, Tujimura T, Kawano K, Yoshikawa K, Kamada T. Expression of vascular permeability factor/vascular endothelial growth factor in human hepatocellular carcinoma. *Cancer Res* 1996; **56**: 3004-3009 [PMID: 8674055]
- 113 **Ito Y**, Takeda T, Sasaki Y, Sakon M, Yamada T, Ishiguro S, Imaoka S, Tsujimoto M, Higashiyama S, Monden M, Matsuura N. Expression and clinical significance of the erbB family in intrahepatic cholangiocellular carcinoma. *Pathol Res Pract* 2001; **197**: 95-100 [PMID: 11261824]
- 114 **Zhong JH**, You XM, Gong WF, Ma L, Zhang Y, Mo QG, Wu LC, Xiao J, Li LQ. Epidermal growth factor gene polymorphism and risk of hepatocellular carcinoma: a meta-analysis. *PLoS One* 2012; **7**: e32159 [PMID: 22403631 DOI: 10.1371/journal.pone.0032159]
- 115 **Clifford RJ**, Zhang J, Meerzaman DM, Lyu MS, Hu Y, Cultraro CM, Finney RP, Kelley JM, Efroni S, Greenblum SI, Nguyen CV, Rowe WL, Sharma S, Wu G, Yan C, Zhang H, Chung YH, Kim JA, Park NH, Song IH, Buetow KH. Genetic variations at loci involved in the immune response are risk factors for hepatocellular carcinoma. *Hepatology* 2010; **52**: 2034-2043 [PMID: 21105107 DOI: 10.1002/hep.23943]
- 116 **Tsan YT**, Lee CH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis B virus infection. *J Clin Oncol* 2012; **30**: 623-630 [PMID: 22271485 DOI: 10.1200/JCO.2011]
- 117 **Tsan YT**, Lee CH, Ho WC, Lin MH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis C virus infection. *J Clin Oncol* 2013; **31**: 1514-1521 [PMID: 23509319 DOI: 10.1200/JCO.2012.44.6831]
- 118 **Singh S**, Singh PP, Singh AG, Murad MH, Sanchez W. Statins are associated with a reduced risk of hepatocellular cancer: a systematic review and meta-analysis. *Gastroenterology* 2013; **144**: 323-332 [PMID: 23063971 DOI: 10.1053/j.gastro.2012.10.005]
- 119 **Nkontchou G**, Aout M, Mahmoudi A, Roulot D, Bourcier V, Grando-Lemaire V, Ganne-Carrie N, Trinchet JC, Vicaut E, Beaugrand M. Effect of long-term propranolol treatment on hepatocellular carcinoma incidence in patients with HCV-associated cirrhosis. *Cancer Prev Res (Phila)* 2012; **5**: 1007-1014 [PMID: 22525582 DOI: 10.1158/1940-6207.CAPR-11-0450]
- 120 **Sawada N**, Inoue M, Iwasaki M, Sasazuki S, Shimazu T, Yamaji T, Takachi R, Tanaka Y, Mizokami M, Tsugane S; Japan Public Health Center-Based Prospective Study Group. Consumption of n-3 fatty acids and fish reduces risk of hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1468-1475 [PMID: 22342990 DOI: 10.1053/j.gastro.2012.02.018]
- 121 **Huang RX**, Duan YY, Hu JA. Fish intake and risk of liver cancer: a meta-analysis. *PLoS One* 2015; **10**: e0096102 [PMID: 25615823 DOI: 10.1371/journal.pone.0096102]
- 122 **Zhang W**, Shu XO, Li H, Yang G, Cai H, Ji BT, Gao J, Gao YT, Zheng W, Xiang YB. Vitamin intake and liver cancer risk: a report from two cohort studies in China. *J Natl Cancer Inst* 2012; **104**: 1173-1181 [PMID: 22811438 DOI: 10.1093/jnci/djs277]
- 123 **Turati F**, Trichopoulos D, Polesel J, Bravi F, Rossi M, Talamini R, Franceschi S, Montella M, Trichopolou A, La Vecchia C, Lagiou P. Mediterranean diet and hepatocellular carcinoma. *J Hepatol* 2014; **60**: 606-611 [PMID: 24240052 DOI: 10.1016/j.jhep.2013]
- 124 **Larsson SC**, Wolk A. Coffee consumption and risk of liver cancer: a meta-analysis. *Gastroenterology* 2007; **132**: 1740-1745 [PMID: 17484871 DOI: 10.1053/j.gastro.2007.03.044]
- 125 **Bravi F**, Bosetti C, Tavani A, Gallus S, La Vecchia C. Coffee reduces risk for hepatocellular carcinoma: an updated meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 1413-1421.e1 [PMID: 23660416 DOI: 10.1016/j.cgh.2013.04.039]
- 126 **Setiawan VW**, Wilkens LR, Lu SC, Hernandez BY, Le Marchand L, Henderson BE. Association of coffee intake with reduced incidence of liver cancer and death from chronic liver disease in the US multiethnic cohort. *Gastroenterology* 2015; **148**: 118-125; quiz e15 [PMID: 25305507 DOI: 10.1053/j.gastro.2014.10.005]
- 127 **Zhang BH**, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004; **130**: 417-422 [PMID: 15042359]
- 128 **Sangiovanni A**, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R, Morabito A, De Franchis R, Colombo M. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. *Gastroenterology* 2004; **126**: 1005-1014 [PMID: 15057740 DOI: 10.1053/j.gastro.2003.12.049]
- 129 **Trevisani F**, De Notariis S, Rapaccini G, Farinati F, Benvegnù



- L, Zoli M, Grazi GL, Del PP, Di N, Bernardi M; Italian Liver Cancer Group. Semiannual and annual surveillance of cirrhotic patients for hepatocellular carcinoma: effects on cancer stage and patient survival (Italian experience). *Am J Gastroenterol* 2002; **97**: 734-744 [PMID: 11922571 DOI: 10.1111/j.1572-0241.2002.05557.x]
- 130 **Pascual S**, Irurzun J, Zapater P, Such J, Sempere L, Carnicer F, Palazón JM, de la Iglesia P, Gil S, de España F, Perez-Mateo M. Usefulness of surveillance programmes for early diagnosis of hepatocellular carcinoma in clinical practice. *Liver Int* 2008; **28**: 682-689 [PMID: 18433394 DOI: 10.1111/j.1478-3231.2008.01710.x]
- 131 **Poon D**, Anderson BO, Chen LT, Tanaka K, Lau WY, Van Cutsem E, Singh H, Chow WC, Ooi LL, Chow P, Khin MW, Koo WH. Management of hepatocellular carcinoma in Asia: consensus statement from the Asian Oncology Summit 2009. *Lancet Oncol* 2009; **10**: 1111-1118 [PMID: 19880065 DOI: 10.1016/S1470-2045(09)70241-4]
- 132 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
- 133 **European Association For The Study Of The Liver**; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]
- 134 **Jha RC**, Mitchell DG, Weinreb JC, Santillan CS, Yeh BM, Francois R, Sirlin CB. LI-RADS categorization of benign and likely benign findings in patients at risk of hepatocellular carcinoma: a pictorial atlas. *AJR Am J Roentgenol* 2014; **203**: W48-W69 [PMID: 24951229 DOI: 10.2214/AJR.13.12169]
- 135 **Darnell A**, Forner A, Rimola J, Reig M, Garcia-Criado Á, Ayuso C, Bruix J. Liver Imaging Reporting and Data System with MR Imaging: Evaluation in Nodules 20 mm or Smaller Detected in Cirrhosis at Screening US. *Radiology* 2015; **275**: 698-707 [PMID: 25658038 DOI: 10.1148/radiol.15141132]
- 136 **Mitchell DG**, Bruix J, Sherman M, Sirlin CB. LI-RADS (Liver Imaging Reporting and Data System): summary, discussion, and consensus of the LI-RADS Management Working Group and future directions. *Hepatology* 2015; **61**: 1056-1065 [PMID: 25041904 DOI: 10.1002/hep.27304]
- 137 **Okuda K**, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, Nakajima Y, Ohnishi K. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. *Cancer* 1985; **56**: 918-928 [PMID: 2990661]
- 138 A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. *Hepatology* 1998; **28**: 751-755 [PMID: 9731568 DOI: 10.1002/hep.510280322]
- 139 **Chevret S**, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C. A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire. *J Hepatol* 1999; **31**: 133-141 [PMID: 10424293 DOI: 10.1016/S0168-8278(99)80173-1]
- 140 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]
- 141 **Leung TW**, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, Lau JT, Yu SC, Johnson PJ. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system: a study based on 926 patients. *Cancer* 2002; **94**: 1760-1769 [PMID: 11920539 DOI: 10.1002/cncr.10384]
- 142 **Ikai I**, Takayasu K, Omata M, Okita K, Nakanuma Y, Matsuyama Y, Makuuchi M, Kojiro M, Ichida T, Arii S, Yamaoka Y. A modified Japan Integrated Stage score for prognostic assessment in patients with hepatocellular carcinoma. *J Gastroenterol* 2006; **41**: 884-892 [PMID: 17048053]
- 143 **Hsu CY**, Huang YH, Hsia CY, Su CW, Lin HC, Loong CC, Chiou YY, Chiang JH, Lee PC, Huo TI, Lee SD. A new prognostic model for hepatocellular carcinoma based on total tumor volume: the Taipei Integrated Scoring System. *J Hepatol* 2010; **53**: 108-117 [PMID: 20451283 DOI: 10.1016/j.jhep.2010.01.038]
- 144 **Lencioni R**, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: 20175033 DOI: 10.1055/s-0030-1247132]
- 145 **Reig M**, Darnell A, Forner A, Rimola J, Ayuso C, Bruix J. Systemic therapy for hepatocellular carcinoma: the issue of treatment stage migration and registration of progression using the BCLC-refined RECIST. *Semin Liver Dis* 2014; **34**: 444-455 [PMID: 25369306 DOI: 10.1055/s-0034-1394143]
- 146 **Bruix J**, Castells A, Bosch J, Feu F, Fuster J, Garcia-Pagan JC, Visa J, Bru C, Rodés J. Surgical resection of hepatocellular carcinoma in cirrhotic patients: prognostic value of preoperative portal pressure. *Gastroenterology* 1996; **111**: 1018-1022 [PMID: 8831597]
- 147 **Llovet JM**, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. *Semin Liver Dis* 2005; **25**: 181-200 [PMID: 15918147 DOI: 10.1055/s-2005-871198]
- 148 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]
- 149 **Bismuth H**, Majno PE, Adam R. Liver transplantation for hepatocellular carcinoma. *Semin Liver Dis* 1999; **19**: 311-322 [PMID: 10518310 DOI: 10.1055/s-2007-1007120]
- 150 **Llovet JM**, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999; **30**: 1434-1440 [PMID: 10573522 DOI: 10.1002/hep.510300629]
- 151 **Jonas S**, Bechstein WO, Steinmüller T, Herrmann M, Radke C, Berg T, Settmacher U, Neuhaus P. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. *Hepatology* 2001; **33**: 1080-1086 [PMID: 11343235 DOI: 10.1053/jhep.2001.23561]
- 152 **Yao FY**, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403 [PMID: 11391528 DOI: 10.1053/jhep.2001.24563]
- 153 **Marsh JW**, Dvorchik I. Liver organ allocation for hepatocellular carcinoma: are we sure? *Liver Transpl* 2003; **9**: 693-696 [PMID: 12827554 DOI: 10.1053/jlts.2003.50086]
- 154 **Herrero JI**, Sangro B, Pardo F, Quiroga J, Iñarrairaegui M, Rotellar F, Montiel C, Alegre F, Prieto J. Liver transplantation in patients with hepatocellular carcinoma across Milan criteria. *Liver Transpl* 2008; **14**: 272-278 [PMID: 18306328 DOI: 10.1002/lt.21368]
- 155 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]
- 156 **Herrero JI**, Sangro B, Quiroga J, Pardo F, Herraiz M, Cienfuegos JA, Prieto J. Influence of tumor characteristics on the outcome of liver transplantation among patients with liver cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2001; **7**: 631-636 [PMID: 11460231 DOI: 10.1053/jlts.2001.25458]
- 157 **Kneteman NM**, Oberholzer J, Al Saghier M, Meeberg GA, Blitz M, Ma MM, Wong WW, Gutfreund C, Mason AL, Jewell LD, Shapiro AM, Bain VG, Bigam DL. Sirolimus-based immunosuppression for liver transplantation in the presence of extended criteria for hepatocellular carcinoma. *Liver Transpl* 2004; **10**: 1301-1311 [PMID: 15376305 DOI: 10.1002/lt.20237]



- 158 **Yao FY**, Xiao L, Bass NM, Kerlan R, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. *Am J Transplant* 2007; **7**: 2587-2596 [PMID: 17868066 DOI: 10.1111/j.1600-6143.2007.01965.x]
- 159 **Silva M**, Moya A, Berenguer M, Sanjuan F, López-Andujar R, Pareja E, Torres-Quevedo R, Aguilera V, Montalva E, De Juan M, Mattos A, Prieto M, Mir J. Expanded criteria for liver transplantation in patients with cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2008; **14**: 1449-1460 [PMID: 18825681 DOI: 10.1002/lt.21576]
- 160 **Fan J**, Yang GS, Fu ZR, Peng ZH, Xia Q, Peng CH, Qian JM, Zhou J, Xu Y, Qiu SJ, Zhong L, Zhou GW, Zhang JJ. Liver transplantation outcomes in 1,078 hepatocellular carcinoma patients: a multi-center experience in Shanghai, China. *J Cancer Res Clin Oncol* 2009; **135**: 1403-1412 [PMID: 19381688 DOI: 10.1007/s00432-009-0584-6]
- 161 **Guiteau JJ**, Cotton RT, Washburn WK, Harper A, O'Mahony CA, Sebastian A, Cheng S, Klintmalm G, Ghobrial M, Halford G, Miele L, Goss J. An early regional experience with expansion of Milan Criteria for liver transplant recipients. *Am J Transplant* 2010; **10**: 2092-2098 [PMID: 20883543 DOI: 10.1111/j.1600-6143.2010.03222.x]
- 162 **Raj A**, McCall J, Gane E. Validation of the "Metroticket" predictor in a cohort of patients transplanted for predominantly HBV-related hepatocellular carcinoma. *J Hepatol* 2011; **55**: 1063-1068 [PMID: 21354447 DOI: 10.1016/j.jhep.2011.01.052]
- 163 **Lei JY**, Wang WT, Yan LN. "Metroticket" predictor for assessing liver transplantation to treat hepatocellular carcinoma: a single-center analysis in mainland China. *World J Gastroenterol* 2013; **19**: 8093-8098 [PMID: 24307805 DOI: 10.3748/wjg.v19.i44.8093]
- 164 **Clavien PA**, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A; OLT for HCC Consensus Group. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13**: e11-e22 [PMID: 22047762 DOI: 10.1016/S1470-2045(11)70175-9]
- 165 **Gordon-Weeks AN**, Snaith A, Petrinic T, Friend PJ, Burls A, Silva MA. Systematic review of outcome of downstaging hepatocellular cancer before liver transplantation in patients outside the Milan criteria. *Br J Surg* 2011; **98**: 1201-1208 [PMID: 21618496 DOI: 10.1002/bjs.7561]
- 166 **Toso C**, Mentha G, Kneteman NM, Majno P. The place of downstaging for hepatocellular carcinoma. *J Hepatol* 2010; **52**: 930-936 [PMID: 20385428 DOI: 10.1016/j.jhep.2009.12.032]
- 167 **Shiina S**. Image-guided percutaneous ablation therapies for hepatocellular carcinoma. *J Gastroenterol* 2009; **44** Suppl 19: 122-131 [PMID: 19148806 DOI: 10.1007/s00535-008-2263-9]
- 168 **Lencioni R**, Crocetti L, Cioni D, Pina CD, Oliveri F, De Simone P, Brunetto M, Filippini F. Single-session percutaneous ethanol ablation of early-stage hepatocellular carcinoma with a multi-pronged injection needle: results of a pilot clinical study. *J Vasc Interv Radiol* 2010; **21**: 1533-1538 [PMID: 20817558 DOI: 10.1016/j.jvir.2010.06.019]
- 169 **Bouza C**, López-Cuadrado T, Alcázar R, Saz-Parkinson Z, Amate JM. Meta-analysis of percutaneous radiofrequency ablation versus ethanol injection in hepatocellular carcinoma. *BMC Gastroenterol* 2009; **9**: 31 [PMID: 19432967 DOI: 10.1186/1471-230X-9-31]
- 170 **Sasaki A**, Kai S, Iwashita Y, Hirano S, Ohta M, Kitano S. Microsatellite distribution and indication for locoregional therapy in small hepatocellular carcinoma. *Cancer* 2005; **103**: 299-306 [PMID: 15578688 DOI: 10.1002/cncr.20798]
- 171 **Ikeda K**, Seki T, Umehara H, Inokuchi R, Tamai T, Sakaida N, Uemura Y, Kamiyama Y, Okazaki K. Clinicopathologic study of small hepatocellular carcinoma with microscopic satellite nodules to determine the extent of tumor ablation by local therapy. *Int J Oncol* 2007; **31**: 485-491 [PMID: 17671673 DOI: 10.3892/ijo.31.3.485]
- 172 **Thomson KR**, Cheung W, Ellis SJ, Federman D, Kavnoudias H, Loader-Oliver D, Roberts S, Evans P, Ball C, Haydon A. Investigation of the safety of irreversible electroporation in humans. *J Vasc Interv Radiol* 2011; **22**: 611-621 [PMID: 21439847 DOI: 10.1016/j.jvir.2010.12.014]
- 173 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794 DOI: 10.1053/jhep.2003.50047]
- 174 **Bolondi L**, Burroughs A, Dufour JF, Galle PR, Mazzaferro V, Piscaglia F, Raoul JL, Sangro B. Heterogeneity of patients with intermediate (BCLC B) Hepatocellular Carcinoma: proposal for a subclassification to facilitate treatment decisions. *Semin Liver Dis* 2012; **32**: 348-359 [PMID: 23397536 DOI: 10.1055/s-0032-1329906]
- 175 **Takayasu K**, Arii S, Kudo M, Ichida T, Matsui O, Izumi N, Matsuyama Y, Sakamoto M, Nakashima O, Ku Y, Kokudo N, Makuuchi M. Superselective transarterial chemoembolization for hepatocellular carcinoma. Validation of treatment algorithm proposed by Japanese guidelines. *J Hepatol* 2012; **56**: 886-892 [PMID: 22173160 DOI: 10.1016/j.jhep.2011.10.021]
- 176 **Terzi E**, Golfieri R, Piscaglia F, Galassi M, Dazzi A, Leoni S, Giampalma E, Renzulli M, Bolondi L. Response rate and clinical outcome of HCC after first and repeated cTACE performed "on demand". *J Hepatol* 2012; **57**: 1258-1267 [PMID: 22871502 DOI: 10.1016/j.jhep.2012.07.025]
- 177 **Leoni S**, Piscaglia F, Serio I, Terzi E, Pettinari I, Croci L, Marinelli S, Benevento F, Golfieri R, Bolondi L. Adherence to AASLD guidelines for the treatment of hepatocellular carcinoma in clinical practice: experience of the Bologna Liver Oncology Group. *Dig Liver Dis* 2014; **46**: 549-555 [PMID: 24630947 DOI: 10.1016/j.dld.2014.02.012]
- 178 **Matsui O**, Miyayama S, Sanada J, Kobayashi S, Khoda W, Minami T, Kozaka K, Gabata T. Interventional oncology: new options for interstitial treatments and intravascular approaches: superselective TACE using iodized oil for HCC: rationale, technique and outcome. *J Hepatobiliary Pancreat Sci* 2010; **17**: 407-409 [PMID: 19885639 DOI: 10.1007/s00534-009-0234-z]
- 179 **Miyayama S**, Matsui O, Yamashiro M, Ryu Y, Takata H, Takeda T, Aburano H, Shigenari N. Visualization of hepatic lymphatic vessels during transcatheter arterial chemoembolization for hepatocellular carcinoma. *J Vasc Interv Radiol* 2007; **18**: 1111-1117 [PMID: 17804773]
- 180 **Lammer J**, Malagari K, Vogl T, Pilleul F, Denys A, Watkinson A, Pitton M, Sergent G, Pfammatter T, Terraz S, Benhamou Y, Avajon Y, Gruenberger T, Pomoni M, Langenberger H, Schuchmann M, Dumortier J, Mueller C, Chevallier P, Lencioni R. Prospective randomized study of doxorubicin-eluting-bead embolization in the treatment of hepatocellular carcinoma: results of the PRECISION V study. *Cardiovasc Intervent Radiol* 2010; **33**: 41-52 [PMID: 19908093 DOI: 10.1007/s00270-009-9711-7]
- 181 **Lencioni R**, de Baere T, Burrel M, Caridi JG, Lammer J, Malagari K, Martin RC, O'Grady E, Real MI, Vogl TJ, Watkinson A, Geschwind JF. Transcatheter treatment of hepatocellular carcinoma with Doxorubicin-loaded DC Bead (DEBDOX): technical recommendations. *Cardiovasc Intervent Radiol* 2012; **35**: 980-985 [PMID: 22009576 DOI: 10.1007/s00270-011-0287-7]
- 182 **Basile A**, Carrafiello G, Ierardi AM, Tsetis D, Brountzos E. Quality-improvement guidelines for hepatic transarterial chemoembolization. *Cardiovasc Intervent Radiol* 2012; **35**: 765-774 [PMID: 22648700 DOI: 10.1007/s00270-012-0423-z]
- 183 **Spriafico C**, Cascella T, Facciorusso A, Sposito C, Rodolfo L, Morosi C, Civelli EM, Vaiani M, Bhooi S, Pellegrianni A, Marchianò A, Mazzaferro V. Transarterial chemoembolization for hepatocellular carcinoma with a new generation of beads: clinical-radiological outcomes and safety profile. *Cardiovasc Intervent Radiol* 2015; **38**: 129-134 [PMID: 24870698 DOI: 10.1007/s00270-014-0907-0]
- 184 **Malagari K**, Pomoni M, Moschouris H, Kelekis A, Charokopakis A, Bouma E, Spyridopoulos T, Chatziioannou A, Sotirchos V, Karampelas T, Tamvakopoulos C, Filippiadis D, Karagiannis E, Marinis A, Koskinas J, Kelekis DA. Chemoembolization of hepatocellular carcinoma with HepaSphere 30-60 µm. Safety and efficacy study. *Cardiovasc Intervent Radiol* 2014; **37**: 165-175 [PMID: 24263774 DOI: 10.1007/s00270-013-0777-x]

- 185 **Kudo M**, Izumi N, Kokudo N, Matsui O, Sakamoto M, Nakashima O, Kojiro M, Makuuchi M; HCC Expert Panel of Japan Society of Hepatology. Management of hepatocellular carcinoma in Japan: Consensus-Based Clinical Practice Guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. *Dig Dis* 2011; **29**: 339-364 [PMID: 21829027 DOI: 10.1159/000327577]
- 186 **Bolondi L**, Cillo U, Colombo M, Craxi A, Farinati F, Giannini EG, Golfieri R, Leviero M, Pinna AD, Piscaglia F, Raimondo G, Trevisani F, Bruno R, Caraceni P, Ciano A, Coco B, Fraquelli M, Rendina M, Squadrito G, Toniutto P. Position paper of the Italian Association for the Study of the Liver (AISF): the multidisciplinary clinical approach to hepatocellular carcinoma. *Dig Liver Dis* 2013; **45**: 712-723 [PMID: 23769756 DOI: 10.1016/j.dld.2013.01.012]
- 187 **Sugimori K**, Morimoto M, Shirato K, Kokawa A, Tomita N, Saito T, Nozawa A, Hara M, Sekihara H, Tanaka K. Radiofrequency ablation in a pig liver model: effect of transcatheter arterial embolization on coagulation diameter and histologic characteristics. *Hepatol Res* 2002; **24**: 164 [PMID: 12270746 DOI: 10.1016/S1386-6346(02)00030-X]
- 188 **Higuchi T**, Kikuchi M, Okazaki M. Hepatocellular carcinoma after transcatheter hepatic arterial embolization. A histopathologic study of 84 resected cases. *Cancer* 1994; **73**: 2259-2267 [PMID: 7513245]
- 189 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Goret TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]
- 190 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]
- 191 **Lencioni R**, Llovet JM, Han G, Tak WY, Yang J, Leberre MA, Niu W, Nicholson K, Meinhardt G, Bruix J. SPACE: Sorafenib or placebo in combination with transarterial chemoembolization with doxorubicin-eluting beads for intermediate-stage hepatocellular carcinoma: Phase II, randomized, double-blind SPACE trial. *J Clin Oncol* 2012; **30**: A154
- 192 **Bruix J**, Takayama T, Mazzaferro V, Chau GY, Yang J, Kudo M, Cai J, Poon RT, Han KH, Tak WY, Lee HC, Song T, Roayaie S, Bolondi L, Lee KS, Makuuchi M, Souza F, Berre MA, Meinhardt G, Llovet JM; STORM investigators. Adjuvant sorafenib for hepatocellular carcinoma after resection or ablation (STORM): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2015; **16**: 1344-1354 [PMID: 26361969 DOI: 10.1016/S1470-2045(15)00198-9]
- 193 **Sangro B**, Iñarrairaegui M, Bilbao JL. Radioembolization for hepatocellular carcinoma. *J Hepatol* 2012; **56**: 464-473 [PMID: 21816126 DOI: 10.1016/j.jhep.2011.07.012]
- 194 **Salem R**, Lewandowski RJ, Kulik L, Wang E, Riaz A, Ryu RK, Sato KT, Gupta R, Nikolaidis P, Miller FH, Yaghami V, Ibrahim SM, Senthilnathan S, Baker T, Gates VL, Atassi B, Newman S, Memon K, Chen R, Vogelzang RL, Nemcek AA, Resnick SA, Chrisman HB, Carr J, Omary RA, Abecassis M, Benson AB, Mulcahy MF. Radioembolization results in longer time-to-progression and reduced toxicity compared with chemoembolization in patients with hepatocellular carcinoma. *Gastroenterology* 2011; **140**: 497-507.e2 [PMID: 21044630 DOI: 10.1053/j.gastro.2010.10.049]
- 195 **Carr BI**, Kondragunta V, Buch SC, Branch RA. Therapeutic equivalence in survival for hepatic arterial chemoembolization and yttrium 90 microsphere treatment in unresectable hepatocellular carcinoma: a two cohort study. *Cancer* 2010; **116**: 1305-1314 [DOI: 10.1002/CNCR.24884]
- 196 **Kooby DA**, Egnatashvili V, Srinivasan S, Chamsuddin A, Delman KA, Kauh J, Staley CA, Kim HS. Comparison of yttrium-90 radioembolization and transcatheter arterial chemoembolization for the treatment of unresectable hepatocellular carcinoma. *J Vasc Interv Radiol* 2010; **21**: 224-230 [PMID: 20022765 DOI: 10.1016/j.jvir.2009.10.013]

**P- Reviewer:** Lau WY, Morris DLL, Zhu ZH

**S- Editor:** Song XX **L- Editor:** A **E- Editor:** Liu SQ



2016 Hepatocellular Carcinoma: Global view

## Preoperative portal vein embolization for hepatocellular carcinoma: Consensus and controversy

Taku Aoki, Keiichi Kubota

Taku Aoki, Keiichi Kubota, Second Department of Surgery, Dokkyo Medical University, Tochigi 321-0293, Japan

Author contributions: Aoki T and Kubota K analyzed the literature and wrote the manuscript.

Conflict-of-interest statement: The authors have no conflict of interest to report.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Taku Aoki, MD, PhD, Second Department of Surgery, Dokkyo Medical University, 880 Kitakobayashi Mibu, Tochigi 321-0293, Japan. [aoki-2su@dokkyomed.ac.jp](mailto:aoki-2su@dokkyomed.ac.jp)  
 Telephone: +81-282-872158  
 Fax: +81-282-866317

Received: April 29, 2015

Peer-review started: May 8, 2015

First decision: September 8, 2015

Revised: January 18, 2016

Accepted: March 7, 2016

Article in press: March 9, 2016

Published online: March 28, 2016

### Abstract

Thirty years have passed since the first report of portal vein embolization (PVE), and this procedure is widely adopted as a preoperative treatment procedure for patients with a small future liver remnant (FLR). PVE has been shown to be useful in patients with hepatocellular carcinoma (HCC) and chronic liver disease.

However, special caution is needed when PVE is applied prior to subsequent major hepatic resection in cases with cirrhotic livers, and volumetric analysis of the liver segments in addition to evaluation of the liver functional reserve before PVE is mandatory in such cases. Advances in the embolic material and selection of the treatment approach, and combined use of PVE and transcatheter arterial embolization/chemoembolization have yielded improved outcomes after PVE and major hepatic resections. A novel procedure termed the associating liver partition and portal vein ligation for staged hepatectomy has been gaining attention because of the rapid hypertrophy of the FLR observed in patients undergoing this procedure, however, application of this technique in HCC patients requires special caution, as it has been shown to be associated with a high morbidity and mortality even in cases with essentially healthy livers.

**Key words:** Hepatocellular carcinoma; Future liver remnant; Portal vein embolization; Liver functional reserve; The associating liver partition and portal vein ligation for staged hepatectomy

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Preoperative portal vein embolization (PVE) has been developed to secure the safety of a major hepatic resection by inducing the hypertrophy of the future liver remnant. PVE has been shown to be useful for patients with hepatocellular carcinoma and chronic liver disease. However, the indications should be carefully judged based on the volumetric analysis and evaluation of the liver functional reserve. Recently, a novel technique called the associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) has been introduced to gain a rapid hypertrophy of the future liver remnant; however, at present, data supporting ALPPS in hepatocellular carcinoma with

cirrhosis are still very weak.

Aoki T, Kubota K. Preoperative portal vein embolization for hepatocellular carcinoma: Consensus and controversy. *World J Hepatol* 2016; 8(9): 439-445 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i9/439.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i9.439>

## INTRODUCTION

Currently, hepatic resection is the treatment of choice for large hepatocellular carcinomas (HCC), colorectal liver metastases (CLM) and hilar cholangiocarcinomas, and extensive liver resection is often required in patients with these malignancies. Preoperative portal vein embolization (PVE), which induces atrophy of the liver segments to be resected and hypertrophy of the future liver remnant (FLR), has been introduced in an attempt to expand the indications for major (the resection of 3 or more Couinaud segments<sup>[1]</sup>) hepatic resection and prevent postoperative liver insufficiency. Thirty years have passed since the first report of PVE by Makuuchi *et al.*<sup>[2]</sup>, and the usefulness of PVE is currently widely accepted. However, the beneficial effect of preoperative PVE may be impaired in patients with chronic liver disease, especially liver cirrhosis<sup>[3]</sup>, and caution is required when PVE is applied in patients with large HCCs and underlying liver cirrhosis. In such patients, volumetric analysis of the liver segments in addition to evaluation of the liver functional reserve is mandatory<sup>[4]</sup>. On the other hand, some European groups have recently advocated the usefulness of a new procedure termed the associating liver partition and portal vein ligation for staged hepatectomy (ALPPS)<sup>[5]</sup>, as this procedure has been shown to induce rapid hypertrophy of the FLR within a short interval<sup>[6]</sup>. However, application of ALPPS to HCC patients with underlying liver cirrhosis is debatable from the point of view of the safety. In this manuscript, we have reviewed the recent advances in preoperative PVE and other procedures aimed at increasing the FLR.

## HISTORY OF PVE

In the first report, Makuuchi *et al.*<sup>[7]</sup> applied preoperative PVE for patients with hilar cholangiocarcinoma. They stated that the purposes of PVE were: (1) to initiate compensatory hypertrophy of the FLR; and (2) to avoid a sudden increase of the portal venous pressure during and after the surgery<sup>[7]</sup>. The second goal is especially important in HCC patients with portal hypertension, where PVE may serve as a preoperative "tolerance test"; if the FLR cannot tolerate the higher portal pressure induced by PVE, sufficient hypertrophy of the FLR cannot be expected. Two approaches were used for PVE: Transileocolic portal embolization (TIPE) *via* laparotomy under general anesthesia, and percutaneous transhepatic portal embolization (PTPE) using a puncture technique

with ultrasonic guidance under local anesthesia. The embolic material consisted of a mixture of absorbable gelatin powder, contrast material, and antibiotics.

Kinoshita *et al.*<sup>[8]</sup> performed selective PVE (THPE), wherein they used a contralateral approach to occlude the portal vein branch bearing the HCC tumor. The aim of selective PVE was to enhance the effect of transcatheter arterial embolization (TAE) and the accompanying hypertrophy of the nonembolized segments. They used gelatin sponge, thrombin mixed with glucose, or an adhesive mixture of fibrin with contrast material as the embolic material.

Subsequently, the indication of preoperative PVE was expanded to other liver tumors, including CLM and HCC without cirrhosis. Among patients with CLM, PVE is indicated in patients with: (1) small multiple lesions of the right lobe; or (2) a small solitary tumor located adjacent to the hilum of the liver<sup>[9,10]</sup>. Reports dealing with PVE for HCC with underlying cirrhosis or chronic hepatitis were at first mainly small patient series from Asian countries, while documentations of large patient series have appeared after the year 2000<sup>[11-18]</sup>. The indications for PVE in cases of HCC is determined by the relationship between the liver functional reserve and the volumetric ratio of the FLR to the total liver volume. In general, major hepatic resection is contraindicated in Child-Pugh class B or C patients; these patients are therefore also not suitable candidates for PVE. In addition, Child-Pugh class A patients should undergo assessment by the indocyanine green retention rate at 15 min (ICG-R15). An ICG-R15 value of > 20% is generally considered as a contraindication for major hepatic resection and therefore also for PVE (Figure 1)<sup>[4]</sup>.

## MODIFICATION OF PVE

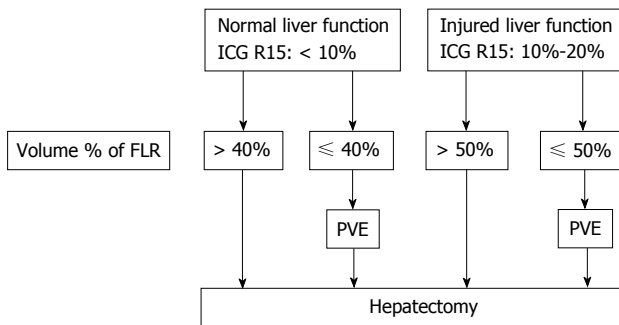
### Approach

Several approaches have been advocated for PVE, which can be mainly categorized as TIPE or PTPE; the PTPE approach is further subdivided into an ipsilateral approach and a contralateral approach. TIPE is a safe approach; complete portography can be achieved using this approach, and insertion of the catheter into the segmental portal branches is relatively easy; however, it requires general anesthesia and laparotomy, and carries the risk of post-PVE bowel obstruction.

PTPE can be performed under local anesthesia, and is therefore considered to be a less invasive procedure; however, the possible risk of hemorrhage/subcapsular hematoma or peritonitis cannot be ignored, and if the contralateral approach is selected, injury to the vessels in the FLR may make the subsequent liver resection impossible. A meta-analysis showed that despite the absence of any significant difference in the rate of major complications between TIPE and PTPE, the rate for minor complications was significantly higher for PTPE<sup>[19]</sup>.

Nagino *et al.*<sup>[20]</sup> recommended the ipsilateral approach occluded the right anterior and posterior portal branches using different types of catheters. This technique is





**Figure 1** Indications of portal vein embolization for patients with hepatocellular carcinoma. PVE: Portal vein embolization; FLR: Future liver remnant; ICG R15: Indocyanine green retention ratio at 15 min.

advantageous from the standpoint of safety, as the portal branch of the resected segments is punctured. Currently, PTPE using the ipsilateral approach, although the most technically demanding, is the most popularly used approach; however, the optimal approach must be selected according to the tumor location and past history of laparotomy.

#### Segment 4 embolization

When a more extended hepatic resection, such as right trisegmentectomy, is needed, embolization of the segment 4 portal branch in addition to the right portal vein branch may yield additional beneficial effects<sup>[21]</sup>. Embolization of the segment 4 branch is easy when the ipsilateral PTPE approach or TIPE is used. Two previous reports have confirmed the additional beneficial effect of embolization of the segment 4 portal branch on segment 2 + 3 hypertrophy, however, both reports dealt with non-injured livers, and no data are available for patients with underlying liver cirrhosis<sup>[22,23]</sup>.

#### Embolic material

A number of embolic materials have been used for PVE, including gelatin sponge, gelatin powder, thrombin, fibrin glue, polyvinyl alcohol particles, absolute ethanol, cyanoacrylate, absolute ethanol, small spherical particles, and metallic coils<sup>[19]</sup>. The ideal agent would be the one that would lead to rapid, reproducible, and substantial functional hypertrophy of the FLR in the majority of patients without producing significant toxicity or adverse events. Currently, a combination of absolute ethanol and microcoils is widely used for HCC patients, as these agents have been shown to induce a greater degree of hypertrophy of the FLR as compared with other embolic materials<sup>[24]</sup>. However, there have been no randomized controlled trials to compare the efficiency of the embolic materials.

## BASIC ASPECTS OF PVE

#### Liver regeneration after PVE

The mechanism of liver hypertrophy/regeneration after PVE has been widely studied using animal models or in clinical settings. Several experimental results imply

that the mechanism of liver regeneration after PVE/portal vein ligation (PVL) is different from that after hepatectomy, as indicated by the different response to follistatin<sup>[25]</sup>. The difference is fundamentally attributed to maintained or enhanced arterial blood flow to the embolized liver segments after PVE, or the presence *per se* of the embolized segments, and the atrophying embolized liver segments are supposed to retain their specific functions. In addition, negative regulators of hepatocytes proliferation (such as transforming growth factor- $\beta$  and interleukin-1 $\beta$ ) are strongly expressed in the embolized segments. These factors in the embolized segments may modify the whole process of regeneration after PVE, although no definitive conclusions have been made yet<sup>[26]</sup>.

#### Enhancement of the effect of PVE

Various factors have been shown to influence the effect of PVE: Age, gender, body mass index, nutrition status, previous chemotherapy, diabetes mellitus, *etc*<sup>[26]</sup>. It has been shown that liver regeneration is impaired in chronically diseased livers<sup>[3]</sup>. Sugawara *et al*<sup>[14]</sup> have examined the clinical factors associated with liver hypertrophy after PVE in HCC patients, and have found that the hypertrophic effect was significantly enhanced when PVE was combined with TAE. Recently, Beppu *et al*<sup>[27]</sup> have shown a favorable effect of branched-chain amino acid supplementation on functional liver regeneration after PVE.

## CLINICAL IMPLICATIONS OF PVE

#### Clinical outcomes after PVE and major hepatic resection

Clinically, the percent increase in the volume of the FLR in cirrhotic livers within the first 2-3 wk after PVE is reported to be in the range of 5% to 10%<sup>[10-12]</sup>, and the hypertrophy ratio of the FLR has also been reported to be approximately 1.3 to 1.5<sup>[10,11,13]</sup>. Others have reported a rate of hypertrophy in cirrhotic livers of 9 cm<sup>2</sup>/d at 2 wk<sup>[14]</sup>. These figures are significantly smaller than those reported in non-cirrhotic livers<sup>[14-17]</sup>. Nevertheless, most previous reports have documented the safety of the PVE procedure and of subsequent major hepatic resection even in cases with a cirrhotic liver<sup>[28-32]</sup>.

Previous reports have documented satisfactory long-term results after PVE and subsequent major hepatic resection for HCC (Table 1)<sup>[11-18]</sup>. The reported 5-year survival rates range from 44% to 72%, and the reported 5-year disease-free survival rates range from 21% to 56%. These figures are comparable to those after major hepatic resections for HCC without PVE. It may be deduced that PVE does not have any adverse effect on the risk of oncogenesis (*i.e.*, intrahepatic HCC recurrence or development of new primary lesions) in the FLR after hepatic resection.

PVE also has significance as a preoperative "tolerance test". Indeed, if the liver cannot tolerate PVE, sufficient hypertrophy of the FLR cannot be expected, and a subsequent major hepatic resection is precluded. In

**Table 1 Clinical outcomes of portal vein embolization for hepatocellular carcinoma**

Ref.	Year	Technique	No. of patients	Morbidity (%)	Mortality (%)	5-yr disease-free survival (%)	5-yr overall survival (%)
Azoulay <i>et al</i> <sup>[11]</sup>	2000	PVE	10	55	0	21	44
Tanaka <i>et al</i> <sup>[12]</sup>	2000	PVE	33	-	3	33	50
Wakabayashi <i>et al</i> <sup>[13]</sup>	2001	PVE	26	-	11.5	40	46
Sugawara <i>et al</i> <sup>[14]</sup>	2002	PVE	66	-	0	37.9	58.9
Aoki <i>et al</i> <sup>[15]</sup>	2004	TACE + PVE	24	24	0	47	56
Ogata <i>et al</i> <sup>[16]</sup>	2006	TACE + PVE	18	39	-	37	-
		PVE	18	56	-	19	-
Seo <i>et al</i> <sup>[17]</sup>	2007	PVE	32	19	0	37	72
Palavecino <i>et al</i> <sup>[18]</sup>	2009	PVE	21	24	0	56	72

PVE: Portal vein embolization; TACE: Transcatheter arterial chemoembolization.

addition to the volumetric increase of the FLR, the kinetic growth rate (speed of increase in the volume of the FLR) has also been shown to be a predictor of the morbidity and mortality after subsequent major hepatic resections<sup>[33]</sup>.

### Tumor growth after PVE

On the other hand, tumors in the nonembolized liver segments have been reported to grow more rapidly than tumors in the embolized segments. Alternatively, tumors in the nonembolized segments show an enhanced rate of progression as compared to their natural history. This possible underlying mechanisms for this observation are that: (1) the increased arterial blood supply to the nonembolized liver segments after PVE can promote tumor growth; and (2) the cytokines associated with the atrophy-hypertrophy complex can also promote the progression of tumors. Several previous reports have addressed this issue. Despite some conflicting results, accumulating evidence suggests an adverse effect of PVE on tumor growth<sup>[34-38]</sup>, although most previous studies investigating the risk of tumor growth after PVE have dealt with patients having colorectal liver metastases.

Tumor growth after PVE, especially tumor growth in the nonembolized FLR and/or extrahepatic tumor progression, may preclude curative resection. Indeed, a recent meta-analysis reported that about 15% of patients could not undergo curative resection after PVE, and about a half of these patients showed severe tumor progression or extrahepatic tumor spread<sup>[19]</sup>.

### Sequential transcatheter arterial chemoembolization and PVE and two-staged hepatectomy

As mentioned above, the risk of tumor growth after PVE may counteract the beneficial effect of PVE. Therefore, measures to prevent tumor growth during the waiting period before hepatectomy should be considered.

Our group has employed combined transcatheter arterial chemoembolization (TACE) with PVE as a preoperative treatment in HCC patients. The antitumor effect of TACE in cases of HCC has been reported previously<sup>[39]</sup>. TACE is also useful for embolizing the arterio-portal shunts in the tumor. Thus, the combination

of TACE plus PVE before planned major hepatic resection may strengthen the effect of PVE while simultaneously preventing tumor progression. Our study showed satisfactory short- and long-term outcomes after sequential preoperative TACE and PVE in 17 patients with HCC<sup>[15]</sup>. During the waiting period after PVE, tumor progression, as evaluated by measurements of the tumor volume, serum alpha-fetoprotein level, and plasma des- $\gamma$ -carboxy prothrombin level, was significantly suppressed.

Another European group compared 18 patients who underwent sequential preoperative TACE and PVE with 18 patients who underwent PVE alone prior to hepatic resection<sup>[16]</sup>. All the patients underwent a right hepatectomy 4-8 wk after the PVE. They found that the degree of hypertrophy of the FLR was greater in the TACE + PVE group, and that the recurrence-free survival period was also significantly longer in the TACE + PVE group than that in the PVE alone group.

A potential concern of sequential TACE and PVE is infarction or necrosis of the non-cancerous liver parenchyma. Our previous results showed, however, that necrosis of the non-cancerous liver parenchyma in the resected specimens was minimal. Possibly, recanalization of the hepatic artery abrogates the possible adverse effect of dual embolization.

Two-stage hepatectomy with preoperative PVE can also be applied in patients with metastatic liver tumors<sup>[40]</sup>. The tumors in the FLR are removed by limited resections as the first step, PVE is performed as the second step, and finally, the major hepatic resection is carried out as the third and final step. This strategy is fascinating, but is rarely performed in HCC patients as the surgical indications for bilobar multiple HCC are extremely limited.

## ALTERNATIVES TO PVE

### PVE vs PVL vs ALPPS

In general, PVL at the right branch is believed to induce canalization of the intrahepatic communications of the peripheral portal branches within a few days, therefore, PVE is considered to be more efficient as compared to PVL. However, a meta-analysis has shown only a borderline difference in the increase of the FLR volume

after PVE and PVL. The morbidity and mortality of the two procedures are similar<sup>[41]</sup>.

Recently, European groups have reported a novel approach to rapid liver regeneration in patients scheduled for extended right hepatectomy. This procedure, termed ALPPS, consists of right portal ligation and in situ splitting of the liver parenchyma on the right side of the umbilical portion of the portal vein. Schnitzbauer *et al.*<sup>[5]</sup>, who published the first report of this procedure, reported a marked and rapid hypertrophy of about 75% of the left lateral lobe within a median of 9 d. This growth rate has been reported to be 11 times higher as compared to that after PVE/PVL, and comparable to that in donors after living donor liver transplantation<sup>[42]</sup>. The mechanisms of the apparent profound hepatic growth of the FLR after ALPPS are unknown, although probably this noteworthy phenomenon may be attributable to an abrupt of the arterial blood flow between the two parts of the liver. The same group and others also documented that ALPPS significantly improved the chance of curative resection for initially unresectable liver tumors as compared to conventional PVE/PVL<sup>[6,43]</sup>.

The concern about this procedure, however, is the high morbidity and mortality rates associated with it<sup>[44,45]</sup>. The reported 90-d mortality after ALPPS is 15%, while that after PVE/PVL is 6%, and the odds ratio for perioperative death was 2.7-fold higher in the patients who underwent ALPPS<sup>[6]</sup>. In addition, a high recurrence rate within a short follow-up period has also been reported<sup>[46]</sup>. Based on these observations, Shindoh *et al.*<sup>[47]</sup> concluded that PVE (right portal branch plus segment 4) and interval surgery remain the standard for patients with small FLRs.

Is ALPPS applicable to HCC patients with cirrhosis? The ALPPS series included some patients with HCC (about 10% of the patients), and some recent papers have documented that ALPPS can be safely performed in HCC patients with cirrhosis; however, no detailed data are available because of the small number of patients<sup>[5,6,48]</sup>. Currently, the indications of ALPPS for HCC patients are extremely limited and each patient should be carefully examined as to his/her suitability to undergo ALPPS.

### Radioembolization

Our group has applied a combination of preoperative TACE and PVE to prevent tumor progression during the waiting period before surgery. An alternative to this strategy is radioembolization, which treats the tumor in the embolized lobe along with induction of contralateral hypertrophy. An increase in the size of the non-embolized lobe by 42% after radioembolization has been reported in cirrhotic livers<sup>[49]</sup>. A comparison of PVE and radioembolization in non-cirrhotic livers has shown that PVE induces a greater degree of hypertrophy of the FLR than that radioembolization<sup>[50]</sup>. Nevertheless, this novel procedure is promising, as it enables both embolization and treatment of the tumor(s) in a single step.

## CONCLUSION

Much basic and clinical evidence associated with PVE has been accumulated, however, especially for cases of HCC with underlying liver cirrhosis or chronic hepatitis, the available clinical data are limited. Development of safe and reliable novel approaches that can be used in combination with PVE to induce rapid hypertrophy of FLR, which can be applied even to chronically diseased livers, is needed.

## REFERENCES

- 1 **Couinaud C.** Le Foie, Etude Anatomiques et Chirurgicales. Paris, France: Masson, 1957
- 2 **Makuuchi M,** Takayasu K, Takuma T, Yamazaki S, Hasegawa H, Nishimura S, Shimamura Y. Preoperative transcatheter embolization of the portal venous branch for patients receiving extended lobectomy due to the bile duct carcinoma. *J Jpn Soc Clin Surg* 1984; **45**: 14-20 [DOI: 10.3919/ringe1963.45.1558]
- 3 **Chen MF,** Hwang TL, Hung CF. Human liver regeneration after major hepatectomy. A study of liver volume by computed tomography. *Ann Surg* 1991; **213**: 227-229 [PMID: 1998403 DOI: 10.1097/0000658-199103000-00008]
- 4 **Kubota K,** Makuuchi M, Kusaka K, Kobayashi T, Miki K, Hasegawa K, Harihara Y, Takayama T. Measurement of liver volume and hepatic functional reserve as a guide to decision-making in resectional surgery for hepatic tumors. *Hepatology* 1997; **26**: 1176-1181 [PMID: 9362359]
- 5 **Schnitzbauer AA,** Lang SA, Goessmann H, Nadalin S, Baumgart J, Farkas SA, Fichtner-Feigl S, Lorf T, Goralczyk A, Hörbelt R, Kroemer A, Loss M, Rümmele P, Scherer MN, Padberg W, Königsrainer A, Lang H, Obed A, Schlitt HJ. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann Surg* 2012; **255**: 405-414 [PMID: 22330038 DOI: 10.1097/SLA.0b013e31824856f5]
- 6 **Schadde E,** Ardiles V, Slankamenac K, Tschuor C, Sergeant G, Amacker N, Baumgart J, Croome K, Hernandez-Alejandro R, Lang H, de Santibañes E, Clavien PA. ALPPS offers a better chance of complete resection in patients with primarily unresectable liver tumors compared with conventional-staged hepatectomies: results of a multicenter analysis. *World J Surg* 2014; **38**: 1510-1519 [PMID: 24748319 DOI: 10.1007/s00268-014-2513-3]
- 7 **Makuuchi M,** Thai BL, Takayasu K, Takayama T, Kosuge T, Gunvén P, Yamazaki S, Hasegawa H, Ozaki H. Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. *Surgery* 1990; **107**: 521-527 [PMID: 2333592]
- 8 **Kinoshita H,** Sakai K, Hirohashi K, Igawa S, Yamasaki O, Kubo S. Preoperative portal vein embolization for hepatocellular carcinoma. *World J Surg* 1986; **10**: 803-808 [PMID: 3022488 DOI: 10.1007/BF01655244]
- 9 **Kawasaki S,** Makuuchi M, Kakazu T, Miyagawa S, Takayama T, Kosuge T, Sugihara K, Moriya Y. Resection for multiple metastatic liver tumors after portal embolization. *Surgery* 1994; **115**: 674-677 [PMID: 8197557]
- 10 **Imamura H,** Shimada R, Kubota M, Matsuyama Y, Nakayama A, Miyagawa S, Makuuchi M, Kawasaki S. Preoperative portal vein embolization: an audit of 84 patients. *Hepatology* 1999; **29**: 1099-1105 [PMID: 10094953 DOI: 10.1002/hep.510290415]
- 11 **Azoulay D,** Castaing D, Krissat J, Smail A, Hargreaves GM, Lemoine A, Emile JF, Bismuth H. Percutaneous portal vein embolization increases the feasibility and safety of major liver resection for hepatocellular carcinoma in injured liver. *Ann Surg* 2000; **232**: 665-672 [PMID: 11066138 DOI: 10.1097/0000658-20001000-00008]
- 12 **Tanaka H,** Hirohashi K, Kubo S, Shuto T, Higaki I, Kinoshita H.

- Preoperative portal vein embolization improves prognosis after right hepatectomy for hepatocellular carcinoma in patients with impaired hepatic function. *Br J Surg* 2000; **87**: 879-882 [PMID: 10931022 DOI: 10.1046/j.1365-2168.2000.01438.x]
- 13 **Wakabayashi H**, Ishimura K, Okano K, Izuishi K, Karasawa Y, Goda F, Maeba T, Maeta H. Is preoperative portal vein embolization effective in improving prognosis after major hepatic resection in patients with advanced-stage hepatocellular carcinoma? *Cancer* 2001; **92**: 2384-2390 [PMID: 11745294 DOI: 10.1002/1097-0142(20011101)92:9<2384::AID-CNCR1586>3.0.CO;2-H]
  - 14 **Sugawara Y**, Yamamoto J, Higashi H, Yamasaki S, Shimada K, Kosuge T, Takayama T, Makuuchi M. Preoperative portal embolization in patients with hepatocellular carcinoma. *World J Surg* 2002; **26**: 105-110 [PMID: 11898042 DOI: 10.1007/s00268-001-0189-y]
  - 15 **Aoki T**, Imamura H, Hasegawa K, Matsukura A, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Sequential preoperative arterial and portal venous embolizations in patients with hepatocellular carcinoma. *Arch Surg* 2004; **139**: 766-774 [PMID: 15249411 DOI: 10.1001/archsurg.139.7.766]
  - 16 **Ogata S**, Belghiti J, Farges O, Varma D, Sibert A, Vilgrain V. Sequential arterial and portal vein embolizations before right hepatectomy in patients with cirrhosis and hepatocellular carcinoma. *Br J Surg* 2006; **93**: 1091-1098 [PMID: 16779884 DOI: 10.1002/bjs.5341]
  - 17 **Seo DD**, Lee HC, Jang MK, Min HJ, Kim KM, Lim YS, Chung YH, Lee YS, Suh DJ, Ko GY, Lee YJ, Lee SG. Preoperative portal vein embolization and surgical resection in patients with hepatocellular carcinoma and small future liver remnant volume: comparison with transarterial chemoembolization. *Ann Surg Oncol* 2007; **14**: 3501-3509 [PMID: 17899289 DOI: 10.1245/s10434-007-9553-y]
  - 18 **Palavecino M**, Chun YS, Madoff DC, Zorzi D, Kishi Y, Kaseb AO, Curley SA, Abdalla EK, Vauthey JN. Major hepatic resection for hepatocellular carcinoma with or without portal vein embolization: Perioperative outcome and survival. *Surgery* 2009; **145**: 399-405 [PMID: 19303988 DOI: 10.1016/j.surg.2008.10.009]
  - 19 **Abulkhir A**, Limongelli P, Healey AJ, Damrah O, Tait P, Jackson J, Habib N, Jiao LR. Preoperative portal vein embolization for major liver resection: a meta-analysis. *Ann Surg* 2008; **247**: 49-57 [PMID: 18156923 DOI: 10.1097/SLA.0b013e31815f6e5b]
  - 20 **Nagino M**, Nimura Y, Kamiya J, Kondo S, Kanai M. Selective percutaneous transhepatic embolization of the portal vein in preparation for extensive liver resection: the ipsilateral approach. *Radiology* 1996; **200**: 559-563 [PMID: 8685357 DOI: 10.1148/radiology.200.2.8685357]
  - 21 **Nagino M**, Nimura Y, Kamiya J, Kondo S, Uesaka K, Kin Y, Kutsuna Y, Hayakawa N, Yamamoto H. Right or left trisegment portal vein embolization before hepatic trisegmentectomy for hilar bile duct carcinoma. *Surgery* 1995; **117**: 677-681 [PMID: 7778031 DOI: 10.1016/S0039-6060(95)80012-3]
  - 22 **Nagino M**, Kamiya J, Kanai M, Uesaka K, Sano T, Yamamoto H, Hayakawa N, Nimura Y. Right trisegment portal vein embolization for biliary tract carcinoma: technique and clinical utility. *Surgery* 2000; **127**: 155-160 [PMID: 10686980 DOI: 10.1067/msy.2000.101273]
  - 23 **Kishi Y**, Madoff DC, Abdalla EK, Palavecino M, Ribero D, Chun YS, Vauthey JN. Is embolization of segment 4 portal veins before extended right hepatectomy justified? *Surgery* 2008; **144**: 744-751 [PMID: 19081016 DOI: 10.1016/j.surg.2008.05.015]
  - 24 **Madoff DC**, Hicks ME, Abdalla EK, Morris JS, Vauthey JN. Portal vein embolization with polyvinyl alcohol particles and coils in preparation for major liver resection for hepatobiliary malignancy: safety and effectiveness--study in 26 patients. *Radiology* 2003; **227**: 251-260 [PMID: 12616006 DOI: 10.1148/radiol.2271012010]
  - 25 **Kogure K**, Omata W, Kanzaki M, Zhang YQ, Yasuda H, Mine T, Kojima I. A single intraportal administration of follistatin accelerates liver regeneration in partially hepatectomized rats. *Gastroenterology* 1995; **108**: 1136-1142 [PMID: 7698581 DOI: 10.1016/0016-5085(95)90212-0]
  - 26 **Yokoyama Y**, Nagino M, Nimura Y. Mechanisms of hepatic regeneration following portal vein embolization and partial hepatectomy: a review. *World J Surg* 2007; **31**: 367-374 [PMID: 17219273 DOI: 10.1007/s00268-006-0526-2]
  - 27 **Beppu T**, Nitta H, Hayashi H, Imai K, Okabe H, Nakagawa S, Hashimoto D, Chikamoto A, Ishiko T, Yoshida M, Yamashita Y, Baba H. Effect of branched-chain amino acid supplementation on functional liver regeneration in patients undergoing portal vein embolization and sequential hepatectomy: a randomized controlled trial. *J Gastroenterol* 2015; **50**: 1197-1205 [PMID: 25847401 DOI: 10.1007/s00535-015-1067-y]
  - 28 **de Baere T**, Roche A, Elias D, Lasser P, Lagrange C, Bousson V. Preoperative portal vein embolization for extension of hepatectomy indications. *Hepatology* 1996; **24**: 1386-1391 [PMID: 8938166 DOI: 10.1002/hep.510240612]
  - 29 **Lee KC**, Kinoshita H, Hirohashi K, Kubo S, Iwasa R. Extension of surgical indications for hepatocellular carcinoma by portal vein embolization. *World J Surg* 1993; **17**: 109-115 [PMID: 8383379 DOI: 10.1007/BF01655721]
  - 30 **Nagino M**, Nimura Y, Kamiya J, Kondo S, Uesaka K, Kin Y, Hayakawa N, Yamamoto H. Changes in hepatic lobe volume in biliary tract cancer patients after right portal vein embolization. *Hepatology* 1995; **21**: 434-439 [PMID: 7843717 DOI: 10.1016/0270-9139(95)90104-3]
  - 31 **Yamanaka N**, Okamoto E, Kawamura E, Kato T, Oriyama T, Fujimoto J, Furukawa K, Tanaka T, Tomoda F, Tanaka W. Dynamics of normal and injured human liver regeneration after hepatectomy as assessed on the basis of computed tomography and liver function. *Hepatology* 1993; **18**: 79-85 [PMID: 8392029 DOI: 10.1002/hep.1840180114]
  - 32 **Shimamura T**, Nakajima Y, Une Y, Namieno T, Ogasawara K, Yamashita K, Haneda T, Nakanishi K, Kimura J, Matsushita M, Sato N, Uchino J. Efficacy and safety of preoperative percutaneous transhepatic portal embolization with absolute ethanol: a clinical study. *Surgery* 1997; **121**: 135-141 [PMID: 9037224 DOI: 10.1016/S0039-6060(97)90282-8]
  - 33 **Shindoh J**, Truty MJ, Aloia TA, Curley SA, Zimmiti G, Huang SY, Mahvash A, Gupta S, Wallace MJ, Vauthey JN. Kinetic growth rate after portal vein embolization predicts posthepatectomy outcomes: toward zero liver-related mortality in patients with colorectal liver metastases and small future liver remnant. *J Am Coll Surg* 2013; **216**: 201-209 [PMID: 23219349 DOI: 10.1016/j.jamcollsurg.2012.10.018]
  - 34 **Elias D**, De Baere T, Roche A, Mducreux J, Lasser P. During liver regeneration following right portal embolization the growth rate of liver metastases is more rapid than that of the liver parenchyma. *Br J Surg* 1999; **86**: 784-788 [PMID: 10383579 DOI: 10.1046/j.1365-2168.1999.01154.x]
  - 35 **Kokudo N**, Tada K, Seki M, Ohta H, Azekura K, Ueno M, Ohta K, Yamaguchi T, Matsubara T, Takahashi T, Nakajima T, Muto T, Ikari T, Yanagisawa A, Kato Y. Proliferative activity of intrahepatic colorectal metastases after preoperative hemihepatic portal vein embolization. *Hepatology* 2001; **34**: 267-272 [PMID: 11481611 DOI: 10.1053/jhep.2001.26513]
  - 36 **Barbaro B**, Di Stasi C, Nuzzo G, Vellone M, Giuliani F, Marano P. Preoperative right portal vein embolization in patients with metastatic liver disease. Metastatic liver volumes after RPVE. *Acta Radiol* 2003; **44**: 98-102 [PMID: 12631007]
  - 37 **Hayashi S**, Baba Y, Ueno K, Nakajo M, Kubo F, Ueno S, Aikou T, Komokata T, Nakamura N, Sakata R. Acceleration of primary liver tumor growth rate in embolized hepatic lobe after portal vein embolization. *Acta Radiol* 2007; **48**: 721-727 [PMID: 17729001 DOI: 10.1080/02841850701424514]
  - 38 **Hoekstra LT**, van Lienden KP, Doets A, Busch OR, Gouma DJ, van Gulik TM. Tumor progression after preoperative portal vein embolization. *Ann Surg* 2012; **256**: 812-817; discussion 817-818 [PMID: 23095626 DOI: 10.1097/SLA.0b013e3182733f09]
  - 39 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794 DOI: 10.1053/jhep.2003.50047]
  - 40 **Jaeck D**, Oussoultzoglou E, Rosso E, Greget M, Weber JC,



- Bachellier P. A two-stage hepatectomy procedure combined with portal vein embolization to achieve curative resection for initially unresectable multiple and bilobar colorectal liver metastases. *Ann Surg* 2004; **240**: 1037-1049; discussion 1049-1051 [PMID: 15570209 DOI: 10.1097/01.sla.0000145965.86383.89]
- 41 **Pandanaboyana S**, Bell R, Hidalgo E, Toogood G, Prasad KR, Bartlett A, Lodge JP. A systematic review and meta-analysis of portal vein ligation versus portal vein embolization for elective liver resection. *Surgery* 2015; **157**: 690-698 [PMID: 25704417 DOI: 10.1016/j.surg.2014.12.009]
- 42 **Croome KP**, Hernandez-Alejandro R, Parker M, Heimbach J, Rosen C, Nagorney DM. Is the liver kinetic growth rate in ALPPS unprecedented when compared with PVE and living donor liver transplant? A multicentre analysis. *HPB (Oxford)* 2015; **17**: 477-484 [PMID: 25728543 DOI: 10.1111/hpb.12386]
- 43 **Alvarez FA**, Ardiles V, de Santibañes M, Pekolj J, de Santibañes E. Associating liver partition and portal vein ligation for staged hepatectomy offers high oncological feasibility with adequate patient safety: a prospective study at a single center. *Ann Surg* 2015; **261**: 723-732 [PMID: 25493362 DOI: 10.1097/SLA.0000000000001046]
- 44 **Schadde E**, Ardiles V, Robles-Campos R, Malago M, Machado M, Hernandez-Alejandro R, Soubrane O, Schnitzbauer AA, Raptis D, Tschuor C, Petrowsky H, De Santibanes E, Clavien PA. Early survival and safety of ALPPS: first report of the International ALPPS Registry. *Ann Surg* 2014; **260**: 829-836; discussion 836-838 [PMID: 25379854 DOI: 10.1097/SLA.0000000000000947]
- 45 **Schadde E**, Schnitzbauer AA, Tschuor C, Raptis DA, Bechstein WO, Clavien PA. Systematic review and meta-analysis of feasibility, safety, and efficacy of a novel procedure: associating liver partition and portal vein ligation for staged hepatectomy. *Ann Surg Oncol* 2015; **22**: 3109-3120 [PMID: 25448799 DOI: 10.1245/s10434-014-4231-5]
- 46 **Sala S**, Ardiles V, Ulla M, Alvarez F, Pekolj J, de Santibañes E. Our initial experience with ALPPS technique: encouraging results. *Updates Surg* 2012; **64**: 167-172 [PMID: 22903531 DOI: 10.1007/s13304-012-175-y]
- 47 **Shindoh J**, Vauthey JN, Zimmitti G, Curley SA, Huang SY, Mahvash A, Gupta S, Wallace MJ, Aloia TA. Analysis of the efficacy of portal vein embolization for patients with extensive liver malignancy and very low future liver remnant volume, including a comparison with the associating liver partition with portal vein ligation for staged hepatectomy approach. *J Am Coll Surg* 2013; **217**: 126-133; discussion 133-134 [PMID: 23632095 DOI: 10.1016/j.amcollsurg.2013.03.004]
- 48 **Vennarecci G**, Laurenzi A, Levi Sandri GB, Busi Rizzi E, Cristofaro M, Montalbano M, Piselli P, Andreoli A, D'Offizi G, Ettorre GM. The ALPPS procedure for hepatocellular carcinoma. *Eur J Surg Oncol* 2014; **40**: 982-988 [PMID: 24767805 DOI: 10.1016/j.ejso.2014.04.002]
- 49 **Edeline J**, Lenoir L, Boudjema K, Rolland Y, Boulic A, Le Du F, Pracht M, Raoul JL, Clément B, Garin E, Boucher E. Volumetric changes after (90)y radioembolization for hepatocellular carcinoma in cirrhosis: an option to portal vein embolization in a preoperative setting? *Ann Surg Oncol* 2013; **20**: 2518-2525 [PMID: 23494107 DOI: 10.1245/s10434-013-2906-9]
- 50 **Garlipp B**, de Baere T, Damm R, Irmscher R, van Buskirk M, Stübs P, Deschamps F, Meyer F, Seidensticker R, Mohnike K, Pech M, Amthauer H, Lippert H, Ricke J, Seidensticker M. Left-liver hypertrophy after therapeutic right-liver radioembolization is substantial but less than after portal vein embolization. *Hepatology* 2014; **59**: 1864-1873 [PMID: 24259442 DOI: 10.1002/hep.26947]

**P-Reviewer:** de Santibañes E, Edeline J **S-Editor:** Gong ZM  
**L-Editor:** A **E-Editor:** Liu SQ



## Focal liver lesions found incidentally

Abdullah A Algarni, Abdullah H Alshuhri, Majed M Alonazi, Moustafa Mabrouk Mourad, Simon R Bramhall

Abdullah A Algarni, Abdullah H Alshuhri, Majed M Alonazi, Liver Unit, Prince Sultan Military Medical City, Riyadh 11159, Saudi Arabia

Moustafa Mabrouk Mourad, Simon R Bramhall, Department of General Surgery, Wye Valley NHS Trust, Hereford HR1 2ER, United Kingdom

**Author contributions:** Algarni AA designed the study; Algarni AA, Alshuhri AH, Alonazi MM and Mourad MM collected, analysed, interpreted the data, and drafted the article; Algarni AA and Bramhall SR designed the conception, critically revised the manuscript for important intellectual content, and made the final approval of the version to be published.

**Conflict-of-interest statement:** The investigators have not received any financial support for this study. None of the authors has any potential conflicting financial interests relevant to this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Abdullah A Algarni, MD, Liver Unit, Prince Sultan Military Medical City, PO Box 7897, Riyadh 11159, Saudi Arabia. [aaalgarni@psmmc.med.sa](mailto:aaalgarni@psmmc.med.sa)  
 Telephone: +966-504-264119  
 Fax: +966-114-757863

Received: September 17, 2015  
 Peer-review started: September 21, 2015  
 First decision: October 30, 2015  
 Revised: January 31, 2016  
 Accepted: March 9, 2016  
 Article in press: March 14, 2016  
 Published online: March 28, 2016

### Abstract

Incidentally found focal liver lesions are a common

finding and a reason for referral to hepatobiliary service. They are often discovered in patients with history of liver cirrhosis, colorectal cancer, incidentally during work up for abdominal pain or in a trauma setting. Specific points should be considered during history taking such as risk factors of liver cirrhosis; hepatitis, alcohol consumption, substance exposure or use of oral contraceptive pills and metabolic syndromes. Full blood count, liver function test and tumor markers can act as a guide to minimize the differential diagnosis and to categorize the degree of liver disease. Imaging should start with B-mode ultrasound. If available, contrast enhanced ultrasound is a feasible, safe, cost effective option and increases the ability to reach a diagnosis. Contrast enhanced computed tomography should be considered next. It is more accurate in diagnosis and better to study anatomy for possible operation. Contrast enhanced magnetic resonance is the gold standard with the highest sensitivity. If doubt still remains, the options are biopsy or surgical excision.

**Key words:** Focal liver lesions; B-mode ultrasound; Ultrasound; Magnetic resonance; Fine needle biopsy; Computed tomography

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Focal liver lesions are being found more commonly, which may need further investigations. History and physical examination is essential part of work up. Blood work is an important adjunct in the patient's journey. There are different modalities of imaging (B-mode ultrasound, contrast enhanced ultrasound, contrast enhanced computed tomography and contrast enhanced magnetic resonance); each has advantages and disadvantages. The decision of biopsy or surgery is kept for the treating team.

Algarni AA, Alshuhri AH, Alonazi MM, Mourad MM, Bramhall SR. Focal liver lesions found incidentally. *World J Hepatol* 2016; 8(9): 446-451 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i9/446.htm> DOI: <http://dx.doi.org/10.4254/>

## INTRODUCTION

Focal liver lesions (FLLs) are a common reason for consultation to a hepatobiliary service, they often need further work up, and investigations. They are often discovered in patients with a cirrhotic liver or colorectal cancer but can be found incidentally during work up for abdominal pain and sometimes in the trauma setting.

Incidental liver lesions are being found more commonly due to advancement in imaging modalities. In some reports, incidental FLLs were found in up to 33% of radiological studies. In autopsy cases, it reached more than 50%<sup>[1,2]</sup>.

Unfortunately, there is no clear pathway for work up and with a wide differential diagnosis; these lesions may need multiple imaging modalities to characterize whether they are benign or malignant.

A cornerstone in evaluating these patients is history and physical examination. A deferential diagnosis of metastasis vs hepatocellular carcinoma (HCC) should be considered for patients with family history of previous malignancies or chronic liver diseases. However, in a healthy population without significant medical background, the differential diagnosis should include wider possibilities, both benign and malignant.

Different modalities are being used to reach a definitive diagnosis. These include: B-mode ultrasound (B-US), contrast enhanced ultrasound (C-US), elastography, contrast enhanced computed tomography (C-CT) scan and contrast enhanced magnetic resonance (C-MR) imaging. Due to the lack of guidelines, most institutions are using all available modalities to establish a diagnosis, which is time consuming, uncomfortable, and not cost effective.

## HISTORY AND PHYSICAL EXAMINATION

Specific points should be taken in consideration as a part of history taking; risk factors for liver cirrhosis like hepatitis and alcohol consumption, exposure to substances known to cause liver lesions, use of the oral contraceptive pill should be elucidated especially in childbearing aged women. Obesity and metabolic syndromes and diabetes are know pathognomic factors for non alcoholic fatty liver disease which is know to increase hepatocellular cancer<sup>[3]</sup>. Patients with a previous cancer should raise the suspicion of a liver metastatic lesion. A family history of malignancy should also be clarified<sup>[4]</sup>. A history of fever and travel should raise the suspicion of infective process.

During physical examination of the patient-jaundice, cachexia, palpable masses, palpable lymph nodes and stigmata of liver disease - should be looked for (Table 1)<sup>[4]</sup>.

The differential diagnosis of a liver lesion is wide, and

can be benign requiring no treatment or an advanced malignant condition beyond cure. The list can be minimized with a careful clinical, chemical and radiological assessment (Table 2).

## BLOOD WORKS

When requesting blood investigation for patients with FLL the results should answer three essential points.

The general condition of the patient; using a full blood count, renal profile, liver function test and albumin level.

The assessment of liver status using the above with the addition of a coagulation profile. These will help obtain a Childs-Pugh score and can be determinant in planning proper management plan.

Tumor markers such as carcinoembryonic antigen (CEA), alpha-feto protein (AFP) and cancer antigen 19-9 (CA19-9) should be requested. A high-level of CEA should raise the possibilities of metastatic colorectal cancer. HCC and cholangiocarcinoma could have raised level of AFP and CA19-9 respectively. An elevated AFP (over 400 ng/mL) may confirm the diagnosis if combined with the addition of two confirmatory imaging techniques<sup>[5]</sup>.

## B-US

The limitation of any type of ultrasonography (USS) (B-mode or contrast enhanced) is the visualization of the whole liver. When the whole liver can be seen USS is a very useful screening test but in certain patients views of parts of the liver can be very limited which limits the usefulness of the investigation.

B-US is one of the most commonly used modalities to investigate the liver and can help to diagnose different pathology. In patients presenting with liver disease, abdominal pain and jaundice a B-US is usually requested. In the Focused Assessment with Sonography for Trauma examination, liver lesions are found in approximately 12 of every 1000 patients examined<sup>[6]</sup>. B-US is also recommended in the surveillance for patients at a high risk of developing HCC<sup>[7,8]</sup>.

The role of B-US in the diagnosing FLLs in a healthy patient is limited to a few diagnoses, of which hemangioma is the most common. Haematomas, hydatid cysts, and abscesses can be conveniently identified using B-US alone. The diagnosis of other FLLs with B-US alone is more challenging and rarely possible.

The use of pulsed and color Doppler USS is limited to focal nodular hyperplasia (FNH) in which the central artery with radial distribution is a characteristic element present in approximately 80% of cases<sup>[9]</sup>.

## C-US

There are two main types of contrast used with ultrasound, micro-bubbles (MBs) and Sonazoid. MBs can be

**Table 1 Clinical signs in-patients with liver disease**

General	Compensated	Decompensated
Jaundice	Xanthelasma	Disorientation
Fever	Parotid enlargement	Drowsiness
Loss of body hair	Spider naevi	Coma
	Gynecomastia	Hepatic flap
	Large or small liver	Fetor hepaticus
	Splenomegaly	Ascites
	Clubbing	Dilated veins on abdominal wall
	Liver palms	Oedema
	Dupuytren's contracture	
	Xanthoma	
	Scratch marks	
	Testicular atrophy	
	Purpura	
	Pigmented ulcers	

defined within different vascular phases: Arterial, portal and the delayed venous phase and are very useful in the detection of malignancies. Sonazoid is approved only in Japan and has an extra post-vascular phase (also called the Kupffer phase), MBs become phagocytosed by Kupffer cells and hence there is no post vascular phase when MBs are used.

Malignancies are characterized by hypo enhancement in the portal and venous phases as well as in the post-vascular phase, making their detection with C-US possible. C-US has been shown to be a reliable imaging technique for follow-up of metastatic liver disease with an accuracy of 91% compared to CT scan and MR imaging<sup>[10]</sup>.

In imaging of HCCs C-US is more complicated. Well-differentiated HCC lesions are iso enhancing in late phases in 51% of cases only, meaning that other imaging modalities are required<sup>[11]</sup>.

The use of USS contrast agents has radically changed the approach to the characterization of FLLs. C-US allows the classification of the majority of FLLs with a high diagnostic accuracy. The typical pattern of FLLs has been well described in the European Federation of Societies for Ultrasound in Medicine and Biology guidelines for C-US, originally published in 2004, updated in 2008, and soon to be updated again<sup>[12,13]</sup>.

Excluding simple cysts (without enhancement in all phases), benign FLLs are generally characterized by an iso echoic pattern in the portal and late phases; because of the persistence of USS contrast agents in the sinusoidal space. In contrast, the washout of these agents in late phases is characteristic of malignant lesions.

Hervé Trillaud confirmed the superior results of real-time C-US for FLLs characterization, compared to that of unenhanced ultrasound. Furthermore, it was demonstrated that the diagnostic accuracy of SonoVue®-enhanced ultrasound was better in comparison to C-CT and C-MR<sup>[14]</sup>.

Hohmann *et al.*<sup>[15]</sup> using MBs agents in C-US with a long-lasting late phase, showed no significant difference in lesion detection compared with C-MR imaging.

**Table 2 Common differential diagnosis of focal liver lesions**

Benign lesions	Malignant lesions
Cystic lesion (5%-14%)	Metastasis (14.4)
Simple, infectious, pre malignant	Cystic lesions (8%)
Hemangioma (2%-20%)	Hepatocellular carcinoma (2%-6%)
Hepatic adenoma (3%)	Cholangiocarcinoma (2%)
Biliary hamartoma (1.5%)	Lymphoma
Regenerative nodule (11%)	Sarcoma

## ELASTOGRAPHY

Real-time (RT) elastography is a technique that can estimate the strain modules from radiofrequency signals in response to external compression and provide an estimation of tissue elasticity. This technique has been studied for the characterization of nodules in superficial structures such as the breast, thyroid, and prostate. Few studies are available concerning its application to the liver, particularly for the evaluation of liver fibrosis. Apart from its use for characterization, RT elastography has been studied for the detection of liver nodules in animal models and during surgery<sup>[16,17]</sup>. In the latter setting, it has been demonstrated to have a higher diagnostic accuracy than B-mode intraoperative USS in detecting lesions surrounded by a heterogeneous background or with an iso echoic pattern (96% vs 89%). Nevertheless, its role in the detection of FLLs is yet to be definitively assessed.

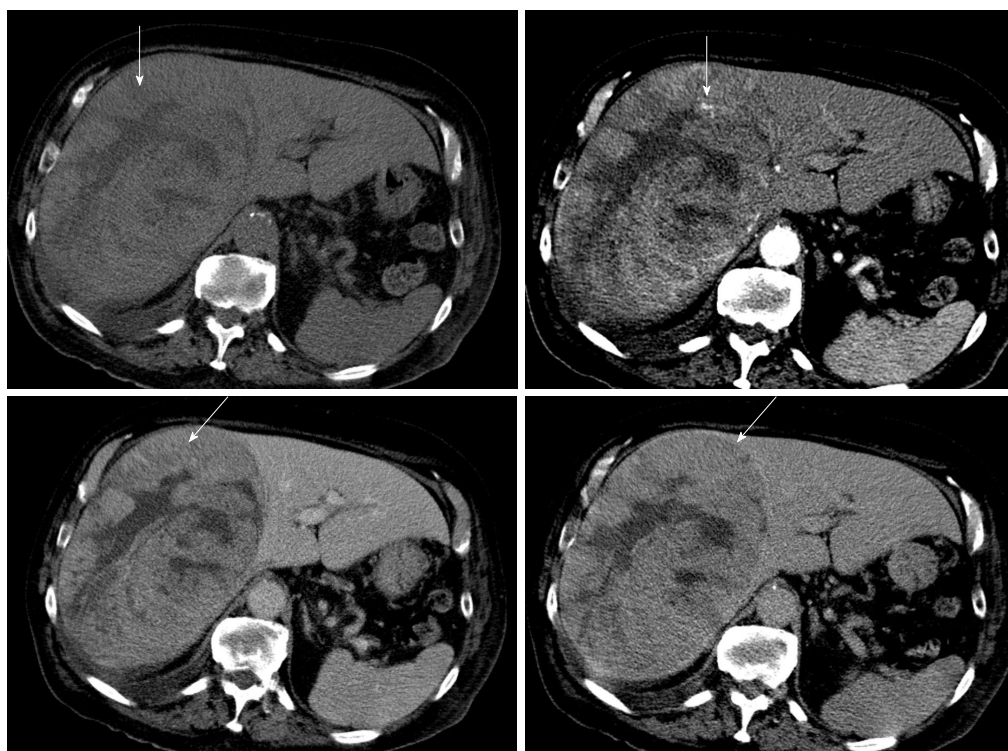
## C-CT SCAN

C-CT scan is one of the essential imaging studies of FLL. The protocol and ability to acquire a multiphasic study is paramount in characterizing liver lesions. Triphasic images are the method of choice, which give a significant improvement in the result compared to single-phase studies<sup>[18]</sup>. The ability for three-dimensional reconstruction helps in assessing the vascular anatomy, the liver and tumor volumes. It also provides a good screening tool to the rest of the abdomen as well as to stage a malignant pathology. Differentiation between benign and malignant conditions is based on the degree of uptake of the contrast agent at different phases of the study. For example, hepatocellular cancer has an early uptake of contrast in the arterial phase with an early washout in the portal and delayed phases (Figure 1)<sup>[8]</sup>. One of the limitations of C-CT is the large dose of radiation given to the patient and the nephrotoxic effect of the iodine contrast that limit its use in patients with renal impairment.

## C-MR SCAN

C-MR is the best modality for FLLs assessment, in both primary and metastatic malignancy. C-MR represents the current technique of choice in this setting since it is free of ionizing radiation as well as demonstrating a high contrast resolution using several sequences and different





**Figure 1** Contrast enhanced computed tomography images of hepatocellular carcinoma. A 55 years old male, diabetic, presented with upper abdominal pain (arrows shows the lesion in different phases with clear washout at the venous phase).

types of contrast media. The commonly used contrast media are gadolinium-chelates, which have an extra-cellular hepatic distribution which help in differentiating liver lesions and obtaining angiography. Other types of contrast agent have an intra-cellular distribution such as ferrumoxides and hence help to detect liver parenchymal lesions<sup>[19-21]</sup>. There is general agreement about the superiority of C-MR with extra-cellular contrast medium compared to the baseline study without contrast or with other types of contrast<sup>[22-26]</sup>.

Primovist (Gd-EOB-DTPA) is a biphasic hepatobiliary magnetic resonance contrast agent. Dynamic C-MR imaging can be performed with the Gd-based extracellular contrast agents where the hemodynamic characteristics of the lesion can be studied. Following that, the hepatobiliary phase can be obtained when the contrast agents are excreted in both renal and biliary systems. Obtaining hepatobiliary phase can provide histological as well as functional information about lesions which might improve the diagnostic accuracy of FLLs<sup>[27]</sup>. Gd-EOB-DTPA-enhanced MR can provide useful information to help characterizing benign and malignant focal lesions and not only to detect them (Figure 2)<sup>[28]</sup>.

Soussan *et al*<sup>[29]</sup> reported that using gadolinium-based C-MR gives a diagnostic accuracy of 52%-66% for incidentally found solid liver lesions compared to 52%-53% with C-US.

Chung *et al*<sup>[30]</sup> demonstrated that Gd-EOB-DTPA-enhanced MR is more accurate to differentiate between benign and malignant lesions and more specific to diagnosis FNH and focal eosinophilic infiltration. Both

dynamic C-CT and Gd-EOB-DTPA-enhanced MR had similar high diagnostic accuracy for hemangiomas and HCCs, whereas other relatively uncommon lesions such as inflammatory myofibroblastic tumor, embryonal sarcoma or schwannoma are rarely diagnosed accurately on both modalities<sup>[30]</sup>.

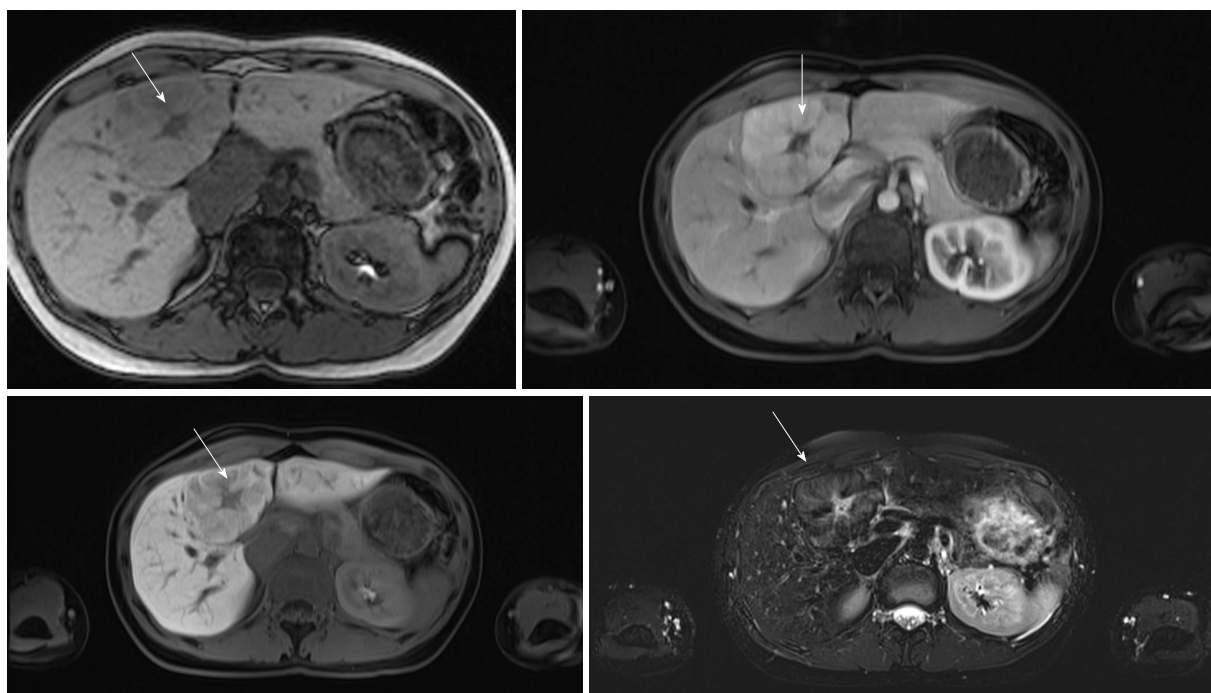
An advantage of C-MR is lack of ionizing radiation and the ability to use in renal impairment patients. It also provides a better characterization of liver lesions compared to other modalities. A drawback is the high cost and the longer procedure duration<sup>[30]</sup>.

## BIOPSY VS SURGERY

Radiological imaging, tumor markers and other information gathered through the assessment process are often diagnostic, and therefore biopsy is rarely needed. Biopsy increases risks of bleeding and needle-track seeding. Biopsy of hepatic adenomas, FNH, and hemangioma has an increased risk of bleeding<sup>[31]</sup>. It has been reported that biopsy of HCCs are associated with a significant risk of needle-track seeding (1.6%-5%)<sup>[4,32,33]</sup>.

A group of investigators studied 160 patients with FLLs. Preoperative fine needle biopsy was not performed. After surgery, 98% of preoperative diagnosis was confirmed histologically<sup>[34]</sup>.

In rare cases imaging might not be conclusive, and hence, a surgical resection for definitive diagnosis might be needed. Resection will confirm the diagnosis, prevent progression of premalignant conditions and will reduce the risk of bleeding or seeding if biopsy were done.



**Figure 2** Contrast enhanced magnetic resonance images of focal nodular hyperplasia. A 30 years old female, medically free, had abdominal pain; ultrasonography showed gallstones and liver lesion (arrows shows the lesion with the characteristic central scar of FNH).

Other indication for surgery is resectable lesion, which has been characterized on imaging, and a diagnosis has been made.

Fine-needle liver biopsy of FLLs is generally reserved for patients who are not surgical candidates and can be done at the same time of non-surgical treatments such as radiofrequency ablation or trans arterial chemoembolization.

## CONCLUSION

Incidentally found FLLs should be thoroughly assessed using history and physical examination in association with blood tests as the starting point to formulate a differential diagnosis. Imaging modalities should be used wisely to save cost but to get the highest sensitivity possible. Ultrasound is fast, feasible, safe, cost effective and if combined with contrast, has an increased sensitivity in reaching the diagnosis but C-CT has a greater accuracy in diagnosis, is more widely applicable (less influenced by body morphology) and is helpful to study liver anatomy. C-MR is the modality of choice with the highest sensitivity. Biopsy should be reserved for questionable lesions where surgery is not an option.

## REFERENCES

- 1 **Boutros C**, Katz SC, Espat NJ. Management of an incidental liver mass. *Surg Clin North Am* 2010; **90**: 699-718 [PMID: 20637942 DOI: 10.1016/j.suc.2010.04.005]
- 2 **Karhunen PJ**. Benign hepatic tumours and tumour like conditions in men. *J Clin Pathol* 1986; **39**: 183-188 [PMID: 3950039]
- 3 **Scalera A**, Tarantino G. Could metabolic syndrome lead to hepatocarcinoma via non-alcoholic fatty liver disease? *World J Gastroenterol* 2014; **20**: 9217-9228 [PMID: 25071314 DOI: 10.3748/wjg.v20.i28.9217]
- 4 **Huang GT**, Sheu JC, Yang PM, Lee HS, Wang TH, Chen DS. Ultrasound-guided cutting biopsy for the diagnosis of hepatocellular carcinoma--a study based on 420 patients. *J Hepatol* 1996; **25**: 334-338 [PMID: 8895013]
- 5 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607]
- 6 **Sgourakis G**, Lanitis S, Korontzi M, Kontovounisios C, Zacharioudakis C, Armoutidis V, Karaliotas C, Dedemadi G, Lepida N, Karaliotas C. Incidental findings in focused assessment with sonography for trauma in hemodynamically stable blunt trauma patients: speaking about cost to benefit. *J Trauma* 2011; **71**: E123-E127 [PMID: 22182913 DOI: 10.1097/TA.0b013e3182249eaa]
- 7 **European Association For The Study Of The Liver**; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]
- 8 **Tan CH**, Low SC, Thng CH. APASL and AASLD Consensus Guidelines on Imaging Diagnosis of Hepatocellular Carcinoma: A Review. *Int J Hepatol* 2011; **2011**: 519783 [PMID: 22007313 DOI: 10.4061/2011/519783]
- 9 **Lim KJ**, Kim KW, Jeong WK, Kim SY, Jang YJ, Yang S, Lee JJ. Colour Doppler sonography of hepatic haemangiomas with arteriportal shunts. *Br J Radiol* 2012; **85**: 142-146 [PMID: 21385916 DOI: 10.1259/bjr/96605786]
- 10 **Dietrich CF**, Kratzer W, Strobe D, Danse E, Fessl R, Bunk A, Vossas U, Hauenstein K, Koch W, Blank W, Oudkerk M, Hahn D, Greis C. Assessment of metastatic liver disease in patients with primary extrahepatic tumors by contrast-enhanced sonography versus CT and MRI. *World J Gastroenterol* 2006; **12**: 1699-1705 [PMID: 16586537]
- 11 **Nicolau C**, Catalá V, Vilana R, Gilibert R, Bianchi L, Solé M, Pagés M, Brú C. Evaluation of hepatocellular carcinoma using

- SonoVue, a second generation ultrasound contrast agent: correlation with cellular differentiation. *Eur Radiol* 2004; **14**: 1092-1099 [PMID: 15007620 DOI: 10.1007/s00330-004-2298-0]
- 12 **Albrecht T**, Blomley M, Bolondi L, Claudon M, Correas JM, Cosgrove D, Greiner L, Jäger K, Jong ND, Leen E, Lencioni R, Lindsell D, Martegani A, Solbiati L, Thorelius L, Tranquart F, Weskott HP, Whittingham T. Guidelines for the use of contrast agents in ultrasound. January 2004. *Ultraschall Med* 2004; **25**: 249-256 [PMID: 15300497 DOI: 10.1055/s-2004-813245]
- 13 **Claudon M**, Cosgrove D, Albrecht T, Bolondi L, Bosio M, Calliada F, Correas JM, Darge K, Dietrich C, D'Onofrio M, Evans DH, Filice C, Greiner L, Jäger K, Jong Nd, Leen E, Lencioni R, Lindsell D, Martegani A, Meairs S, Nolsøe C, Piscaglia F, Ricci P, Seidel G, Skjoldbye B, Solbiati L, Thorelius L, Tranquart F, Weskott HP, Whittingham T. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) - update 2008. *Ultraschall Med* 2008; **29**: 28-44 [PMID: 18270887 DOI: 10.1055/s-2007-963785]
- 14 **Xu HX**, Liu GJ, Lu MD, Xie XY, Xu ZF, Zheng YL, Liang JY. Characterization of focal liver lesions using contrast-enhanced sonography with a low mechanical index mode and a sulfur hexafluoride-filled microbubble contrast agent. *J Clin Ultrasound* 2006; **34**: 261-272 [PMID: 16788957 DOI: 10.1002/jcu.20234]
- 15 **Hohmann J**, Müller A, Skrok J, Wolf KJ, Martegani A, Dietrich CF, Albrecht T. Detection of hepatocellular carcinoma and liver metastases with BR14: a multicenter phase IIA study. *Ultrasound Med Biol* 2012; **38**: 377-382 [PMID: 22261514 DOI: 10.1016/j.ultrasmedbio.2011.11.018]
- 16 **Melodelima D**, Chenot J, Souchon R, Rivoire M, Chapelon JY. Visualisation of liver tumours using hand-held real-time strain imaging: results of animal experiments. *Br J Radiol* 2012; **85**: e556-e565 [PMID: 22253340 DOI: 10.1259/bjr/25132680]
- 17 **Inoue Y**, Takahashi M, Arita J, Aoki T, Hasegawa K, Beck Y, Makuuchi M, Kokudo N. Intra-operative freehand real-time elastography for small focal liver lesions: "visual palpation" for non-palpable tumors. *Surgery* 2010; **148**: 1000-1011 [PMID: 20363009 DOI: 10.1016/j.surg.2010.02.009]
- 18 **Chi Y**, Zhou J, Venkatesh SK, Tian Q, Liu J. Content-based image retrieval of multiphase CT images for focal liver lesion characterization. *Med Phys* 2013; **40**: 103502 [PMID: 24089935 DOI: 10.1118/1.4820539]
- 19 **Semelka RC**, Helmberger TK. Contrast agents for MR imaging of the liver. *Radiology* 2001; **218**: 27-38 [PMID: 11152776 DOI: 10.1148/radiology.218.1.r01ja2427]
- 20 **Harisinghani MG**, Jhaveri KS, Weissleder R, Schima W, Saini S, Hahn PF, Mueller PR. MRI contrast agents for evaluating focal hepatic lesions. *Clin Radiol* 2001; **56**: 714-725 [PMID: 11585393 DOI: 10.1053/crad.2001.0764]
- 21 **Hammerstingl R**, Huppertz A, Breuer J, Balzer T, Blakeborough A, Carter R, Fusté LC, Heinz-Peer G, Judmaier W, Laniado M, Manfredi RM, Mathieu DG, Müller D, Mortelè K, Reimer P, Reiser MF, Robinson PJ, Shamsi K, Strotzer M, Taupitz M, Tombach B, Valeri G, van Beers BE, Vogl TJ. Diagnostic efficacy of gadoxetic acid (Primovist)-enhanced MRI and spiral CT for a therapeutic strategy: comparison with intraoperative and histopathologic findings in focal liver lesions. *Eur Radiol* 2008; **18**: 457-467 [PMID: 18058107 DOI: 10.1007/s00330-007-0716-9]
- 22 **Mueller GC**, Hussain HK, Carlos RC, Nghiem HV, Francis IR. Effectiveness of MR imaging in characterizing small hepatic lesions: routine versus expert interpretation. *AJR Am J Roentgenol* 2003; **180**: 673-680 [PMID: 12591673 DOI: 10.2214/ajr.180.3.180 0673]
- 23 **Mainenti PP**, Mancini M, Mainolfi C, Camera L, Maurea S, Manchia A, Tanga M, Persico F, Addeo P, D'Antonio D, Speranza A, Bucci L, Persico G, Pace L, Salvatore M. Detection of colo-rectal liver metastases: prospective comparison of contrast enhanced US, multidetector CT, PET/CT, and 1.5 Tesla MR with extracellular and reticulo-endothelial cell specific contrast agents. *Abdom Imaging* 2010; **35**: 511-521 [PMID: 19562412 DOI: 10.1007/s00261-009-95 55-2]
- 24 **Ward J**, Guthrie JA, Scott DJ, Atchley J, Wilson D, Davies MH, Wyatt JJ, Robinson PJ. Hepatocellular carcinoma in the cirrhotic liver: double-contrast MR imaging for diagnosis. *Radiology* 2000; **216**: 154-162 [PMID: 10887242 DOI: 10.1148/radiology.216.1.r00 j124154]
- 25 **Kim YK**, Kim CS, Han YM. Detection of small hepatocellular carcinoma: comparison of conventional gadolinium-enhanced MRI with gadolinium-enhanced MRI after the administration of ferucarbotran. *Br J Radiol* 2009; **82**: 468-484 [PMID: 19124563 DOI: 10.1259/bjr/76535286]
- 26 **Matsuo M**, Kanematsu M, Itoh K, Ito K, Maetani Y, Kondo H, Kako N, Matsunaga N, Hoshi H, Shiraishi J. Detection of malignant hepatic tumors: comparison of gadolinium-and ferumoxide-enhanced MR imaging. *AJR Am J Roentgenol* 2001; **177**: 637-643 [PMID: 11517061 DOI: 10.2214/ajr.177.3.1770637]
- 27 **Kim MJ**. Current limitations and potential breakthroughs for the early diagnosis of hepatocellular carcinoma. *Gut Liver* 2011; **5**: 15-21 [PMID: 21461067 DOI: 10.5009/gnl.2011.5.1.15]
- 28 **Purysko AS**, Remer EM, Veniero JC. Focal liver lesion detection and characterization with GD-EOB-DTPA. *Clin Radiol* 2011; **66**: 673-684 [PMID: 21524416 DOI: 10.1016/j.crad.2011.01.014]
- 29 **Soussan M**, Aubé C, Bahrami S, Boursier J, Valla DC, Vilgrain V. Incidental focal solid liver lesions: diagnostic performance of contrast-enhanced ultrasound and MR imaging. *Eur Radiol* 2010; **20**: 1715-1725 [PMID: 20069427 DOI: 10.1007/s00330-009-1700 -3]
- 30 **Chung YE**, Kim MJ, Kim YE, Park MS, Choi JY, Kim KW. Characterization of incidental liver lesions: comparison of multi-detector CT versus Gd-EOB-DTPA-enhanced MR imaging. *PLoS One* 2013; **8**: e66141 [PMID: 23776623 DOI: 10.1371/journal. pone.0066141]
- 31 **Reddy KR**, Schiff ER. Approach to a liver mass. *Semin Liver Dis* 1993; **13**: 423-435 [PMID: 8303323 DOI: 10.1055/s-2007-1007370]
- 32 **Durand F**, Regimbeau JM, Belghiti J, Sauvanet A, Vilgrain V, Terris B, Moutardier V, Farges O, Valla D. Assessment of the benefits and risks of percutaneous biopsy before surgical resection of hepatocellular carcinoma. *J Hepatol* 2001; **35**: 254-258 [PMID: 11580148]
- 33 **Takamori R**, Wong LL, Dang C, Wong L. Needle-tract implantation from hepatocellular cancer: is needle biopsy of the liver always necessary? *Liver Transpl* 2000; **6**: 67-72 [PMID: 10648580 DOI: 10.1002/lt.500060103]
- 34 **Torzilli G**, Minagawa M, Takayama T, Inoue K, Hui AM, Kubota K, Ohtomo K, Makuuchi M. Accurate preoperative evaluation of liver mass lesions without fine-needle biopsy. *Hepatology* 1999; **30**: 889-893 [PMID: 10498639 DOI: 10.1002/hep.510300411]

**P- Reviewer:** Abu-Zidan FM, Tarantino G, Wang JS  
**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Liu SQ





## Comprehensive review of telbivudine in pregnant women with chronic hepatitis B

Teerha Piratvisuth, Guo Rong Han, Stanislas Pol, Yuhong Dong, Aldo Trylesinski

Teerha Piratvisuth, Department of Medicine, NKC Institute of Gastroenterology and Hepatology, Prince of Songkla University, Songkhla 90110, Thailand

Guo Rong Han, Department of Gynecology and Obstetrics, the Second Affiliated Hospital of the Southeast University, Nanjing 210003, Jiangsu Province, China

Stanislas Pol, Département d'Hépatologie, Inserm USM20, Institut Pasteur, AP-HP, hôpital Cochin, Université Paris Descartes, 75006 Paris, France

Yuhong Dong, Aldo Trylesinski, Novartis Pharma AG, 4056 Basel, Switzerland

**Author contributions:** Piratvisuth T, Han GR, Pol S, Dong Y and Trylesinski A contributed to the concept, inception, design, interpretation of data, and critical revision of the manuscript; Dong Y collected the data and performed the analysis; all authors approved the final version of the manuscript, including the authorship list.

Supported by Novartis Pharma AG.

**Conflict-of-interest statement:** Dr. Teerha Piratvisuth is an advisory board member of BMS, Roche, MSD and Novartis and has received fees for serving as a speaker for BMS, Roche, MSD, Novartis and GSK; he has received research grants from BMS, Roche, MSD, Novartis, Fibrogen and Bayer. Dr. Guo Rong Han has received fees for serving as a speaker for Novartis; Dr. Stanislas Pol has received fees for serving as a speaker for the following companies: GSK, BMS, Boehringer Ingelheim, Janssen, Gilead, Roche, MSD, Sanofi, Novartis, Vertex, and Abbvie; he has received grants from BMS, Gilead, Roche, and MSD and is also a board member of the following companies: GSK, BMS, Boehringer Ingelheim, Janssen, Gilead, Roche, MSD, Sanofi, Novartis, Vertex, and Abbvie. Dr. Aldo Trylesinski and Dr. Yuhong Dong are employees of Novartis.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and

the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Teerha Piratvisuth, MD, Department of Medicine, NKC Institute of Gastroenterology and Hepatology, Prince of Songkla University, 15 Equipment Acantholysis SOI Road, Hat Yai District, Songkhla 90110, Thailand. [teerha.p@psu.ac.th](mailto:teerha.p@psu.ac.th)  
Telephone: +66-74-451966  
Fax: +66-74-429436

Received: December 21, 2015

Peer-review started: December 22, 2015

First decision: January 20, 2016

Revised: February 22, 2016

Accepted: March 14, 2016

Article in press: March 16, 2016

Published online: March 28, 2016

### Abstract

**AIM:** To achieve an evidence-based conclusion regarding the safety and efficacy of telbivudine during pregnancy.

**METHODS:** A pooled analysis of data from a literature search reported 1739 pregnancy outcomes (1673 live births) from 1725 non-overlapping pregnant women treated with telbivudine. The prevalence of live birth defects (3.6/1000) was similar to that of the non-antiviral controls (3.0/1000) and not increased as compared with overall prevalence (14.5 to 60/1000). No target organ toxicity was identified. The prevalence of spontaneous abortion in pregnant women treated with telbivudine (4.2/1000) was not increased compared with the overall prevalence (16/1000). The mother-to-child transmission rate was significantly reduced in pregnant women treated with telbivudine (0.70%) compared to those treated with the non-antiviral controls (11.9%;  $P < 0.0001$ ) or compared to the historical rates of hepatitis B virus (HBV)-infected population without antiviral treatment (10%-15%).



**RESULTS:** Cumulatively 489 pregnancy cases have been reported in the telbivudine pharmacovigilance database (with a cut-off date 31 August 2014), of those, 308 had known pregnancy outcomes with 249 cases of live births (239 cases of live birth without congenital anomaly and 10 cases of live birth with congenital anomaly). In the latest antiretroviral pregnancy registry report (1 January 1989 through 31 January 2015) of 27 patients exposed to telbivudine during pregnancy (18, 6 and 3 during first, second and third trimester, respectively) 19 live births were reported and there were no cases of birth defects reported.

**CONCLUSION:** Telbivudine treatment during pregnancy presents a favorable safety profile without increased rates of live birth defects, spontaneous abortion or elective termination, or fetal/neonatal toxicity. Exposure to telbivudine in the first, second and third trimester of pregnancy has been shown to significantly reduce the risk of HBV transmission from mother to child on the basis of standard immune prophylaxis procedure.

**Key words:** Telbivudine; Hepatitis B virus; Pregnancy; Mother-to-child transmission; Vertical transmission

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The data from literatures, pharmacovigilance reports on telbivudine exposure and antiretroviral pregnancy registry during pregnancy in women with hepatitis B virus (HBV) infection showed no increased rates of live birth defects, spontaneous abortion or elective termination. No fetal/neonatal toxicity was reported during telbivudine treatment. Telbivudine exposure in the second and/or third trimesters of pregnancy has been shown to reduce the risk of HBV transmission from mother to child if administered in addition to hepatitis B immunoglobulin and HBV vaccination with a favorable safety profile.

Piratvisuth T, Han GR, Pol S, Dong Y, Trylesinski A. Comprehensive review of telbivudine in pregnant women with chronic hepatitis B. *World J Hepatol* 2016; 8(9): 452-460 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i9/452.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i9.452>

## INTRODUCTION

Chronic hepatitis B (CHB) infection is a major public health problem. Perinatal or childhood transmission of hepatitis B virus (HBV) commonly leads to chronic hepatitis which causes necroinflammation and progression of fibrosis resulting in higher risk of developing cirrhosis and hepatocellular carcinoma<sup>[1]</sup>. Over 50% of CHB carriers in endemic areas acquired their infection perinatally<sup>[2,3]</sup>. In the absence of prevention, infants born to hepatitis B e antigen (HBeAg) positive mothers have a 40%-90% risk of acquiring CHB *via* vertical

transmission<sup>[4]</sup>. In addition, 15%-90% of infected infants develop chronic infection (according to the HBeAg status of the mother), compared with < 5% of patients who acquire infection during adulthood<sup>[5-7]</sup>.

It was reported that 42.1% of infants born to HBsAg-positive mothers globally acquired HBV infection perinatally, because those infants did not receive any active or passive immunoprophylaxis for HBV. In contrast only 2.9% of infants who received immunoprophylaxis acquired HBV infection perinatally<sup>[8]</sup>. HBV perinatal transmission or mother-to-child transmission (MTCT) is considered to occur mainly at delivery. Therefore, standard immunoprophylaxis procedures to prevent perinatal transmission are recommended<sup>[9]</sup>. This standard procedure is based on the combination of passive and active immunization with hepatitis B immunoglobulin (HBIG) and HBV vaccination. However, immunoprophylaxis may not be effective in a proportion of newborns from highly viremic mothers (serum HBV DNA > 10<sup>6-7</sup> IU/mL) who are mostly HBeAg positive, who carry a > 10% risk of vertical HBV transmission despite efficient HBIG and vaccination<sup>[10]</sup>. The vaccine failure cases were reported in previous studies<sup>[11-13]</sup>. There was an earlier report from Mayotte, a French territory in Africa, that newborns who had received complete and timely sero-vaccination had a low immunoprophylaxis failure rate (3%)<sup>[14]</sup>.

Antiviral therapy administered to HBV carrier mothers during pregnancy plus appropriate immunoprophylaxis to newborns have been suggested to effectively prevent MTCT by reducing maternal HBV DNA levels and developing passive immunization in newborns. The European Association for the Study of the Liver (EASL) guidelines recommend the use of a nucleos(t)ide analogue to reduce viral loads in pregnant women who are hepatitis B surface antigen (HBsAg) positive and have high HBV DNA levels (> 10<sup>6-7</sup> IU/mL) to enhance the effectiveness of HBIG and vaccination<sup>[15,16]</sup>. Pregnant women with cirrhosis have an increased risk of developing maternal complications, significant perinatal complications, and poor pregnancy outcomes<sup>[9]</sup>. Therefore, it is often recommended that woman of child-bearing age with advanced fibrosis or cirrhosis should be treated with nucleos(t)ide analogues and that their treatment regimen must be maintained during a future pregnancy<sup>[13]</sup>.

No anti-HBV therapies are currently approved for the prevention of MTCT in pregnancy. Each antiviral has been assigned by the Food and Drug Administration (FDA) to one pregnancy drug class based on preclinical evaluation of the potential teratogenicity. Of the seven antiviral drugs for CHB currently available, alpha interferons and pegylated alpha interferons have anti-proliferative actions and are contraindicated during pregnancy<sup>[15]</sup>. Of the currently approved five oral nucleos(t)ide analogues, tenofovir and telbivudine belong to pregnancy category B (animal reproduction studies have failed to demonstrate a risk to the fetus and studies in pregnant women failed to demonstrate a risk to the fetus), while the other three

**Table 1 Food and drug administration pregnancy categories for hepatitis B virus antiviral therapy<sup>[15]</sup>**

Pregnancy category	definition	HBV therapy categorization
A	Adequate and well controlled studies have failed to demonstrate a risk to the fetus in the first trimester of pregnancy (and there is no evidence of risk in later trimesters)	None
B	Animal reproduction studies have failed to demonstrate a risk to the fetus, and there are no adequate and well-controlled studies in pregnant women or animal studies that have shown an adverse effect, but adequate and well-controlled studies in pregnant women have failed to demonstrate a risk to the fetus in any trimester	Telbivudine; Tenofovir
C	Animal reproduction studies have shown an adverse effect on the fetus, and there are no adequate and well controlled studies in humans, but potential benefits might warrant use of the drug in pregnant women despite potential risks	Lamivudine; Entecavir; Adefovir
D	There is positive evidence of human fetus risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits might warrant use of the drug in pregnant women despite potential risks	None
X	Studies in animals or humans have demonstrated fetus abnormalities, and/or there is positive evidence of human fetus risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits	Interferon

drugs, lamivudine, adefovir and entecavir, belong to pregnancy category C (animal reproduction studies have shown an adverse effect on the fetus and no adequate or well controlled studies in humans)<sup>[15]</sup> (Table 1). Of the aforementioned drugs, there are limited data on treating HBV infection during pregnancy. A prospective randomized controlled trial of tenofovir in HBV infected mothers have been reported<sup>[17]</sup>. Treatment with lamivudine in late pregnancy has shown reduced mother-to-infant transmission but drug resistance is a potential concern<sup>[15]</sup>.

Telbivudine has shown no carcinogenicity, teratogenicity, mutagenicity or mitochondrial toxicity in pre-clinical studies. Telbivudine has demonstrated greater antiviral and clinical efficacy than lamivudine in CHB patients<sup>[18-20]</sup>. In a prospective cohort study, telbivudine showed better preventive effect in reducing perinatal transmission when used in early trimesters of pregnancy than latter in pregnancy. There were no complications or severe adverse events observed in telbivudine-treated mothers or infants<sup>[21]</sup>. Another study showed that telbivudine treatment in chronic HBV-infected mothers was effective in blocking the MTCT of HBV and growth and development of the children were normal<sup>[22]</sup>. As recommended by EASL and the Asian-Pacific Association for the Study of the Liver guidelines, telbivudine is listed as one of the preferred drugs to be used for the prevention of MTCT in the last trimester of pregnancy in HBsAg-positive women with high levels of viremia (serum HBV DNA > 10<sup>6-7</sup> IU/mL)<sup>[15,23]</sup>.

Here we present a summary of the information available on the safety and efficacy of telbivudine when used during pregnancy. This analysis was based on scientific literature, and analysis of a Novartis pharmacovigilance database and a public Antiretroviral Pregnancy Registry. The objective of this analysis was to achieve an evidence-based conclusion regarding the safety and efficacy of telbivudine use in HBV infected pregnant mothers and to confirm the observations from telbivudine preclinical studies.

## MATERIALS AND METHODS

### Preclinical studies

Several preclinical studies of reproductive and developmental toxicity have been conducted with telbivudine to assess its potential adverse effects on fertility, general reproductive performance, development of the conceptus, gestation, birth and post-natal performance (Novartis; data on file). An overview of these studies conducted is summarized in Table 2.

### Clinical studies

Programmed searches were conducted in literature databases for an extensive literature review. The cut-off periods were set as no starting limit till May 2015. Databases included BIOSIS Previews, EBM Reviews (Cochrane Database of Systematic Reviews, ACP Journal Club, Database of Abstracts of Reviews of Effects, Cochrane Central Register of Controlled Trials, Cochrane Methodology Register, Health Technology Assessment, and NHS Economic Evaluation Database), Embase, International Pharmaceutical Abstracts, MEDLINE (including in-process and other non-indexed citations, MEDLINE Daily Update, and OLDMEDLINE). The search strategy included the following keywords in all fields using different combinations with the Boolean operators OR and AND: "telbivudine" or equivalent names ("2' deoxy beta thymidine", "beta thymidine", "epavudine", "LdT 600", "NV 02B", "NV02B", "Sebivo" or "Tyzeka"); pregnant or pregnancy; hepatitis. Another search was conducted in Chinese databases to review Chinese literatures in the following Chinese databases: Wanfang Med Online (med.wanfangdata.com) and China Knowledge Resource Integrated Database (www.cnki.net). Keywords for search in Chinese databases included "telbivudine", "gestation", "pregnancy", "intrauterine infection", "mother-to-child transmission" and "vertical transmission".

A consistent methodology was used when reviewing each paper. The main criterion for selecting a publication

**Table 2** Reproductive and developmental toxicity with telbivudine

Study type	Route of administration	Species	No. of animals	Doses (mg/kg per day)	Treatment	Reference
Rat studies						
Fertility, reproduction, developmental	Oral gavage	Sprague Dawley rats	25 males 25 females	0, 100, 500, 1000	Males: -28 AC to DG 17 Females: -15 AC to DG 17	Study 1314-001
Fertility	Oral gavage	Sprague Dawley rats	25 males 25 females	0, 1000, 2000	Males: -28 AC to DG 13	Study 1314-005
Fertility	Oral gavage	Sprague Dawley rats	25 males 25 females	0, 2000	Females: -15 AC to DG 7	Study 1314-006
Peri/postnatal	Oral gavage	Sprague Dawley rats	25 females	0, 100, 250, 1000	Females: DG 7 to DL 20	Study 1314-003
Rabbit study						
Developmental	Oral gavage	New Zealand White rabbits	20 females	0, 50, 250, 1000	Females: DG 6-18	Study 1314-002

AC: Ante coitum; DG: Gestation day; DL: Lactation day.

was completeness of safety data ("adequate safety information" was defined as including both pregnancy and pregnancy/infant outcome to address the safety profile of telbivudine use in pregnancy) and non-overlapping cases. For articles reported more than once by the same author, the corresponding author was contacted for clarification of the case details. Systemic reviews or meta-analysis were not included in this analysis. Studies with non-overlapping data and safety information were selected and analyzed. All pregnant women who were treated with telbivudine during the period of pregnancy and were reported with a pregnancy outcome were included in the analysis of this review. All those pregnancies without a pregnancy outcome reported or lost to follow up were excluded from this review.

### Pharmacovigilance database

Pregnancy cases from Novartis pharmacovigilance database were collected with a cut-off date of 31 August 2014. Data collected prospectively (acquired prior to the knowledge of the pregnancy outcome or prior to the detection of a congenital malformation at prenatal examination (e.g., fetal ultrasound or serum markers) were separated from data collected retrospectively (acquired after the outcome of the pregnancy was known or after the detection of a congenital malformation on prenatal test). Only safety data were collected from the cases; data on perinatal and intrauterine information was not adequately collected.

### Antiretroviral pregnancy registry

The Antiretroviral Pregnancy Registry (APR; [www.APRRegistry.com](http://www.APRRegistry.com)) is designed to collect and evaluate data on the outcomes of pregnancies exposed to antiretroviral products. It has been actively collecting relevant data since January 2003 and telbivudine has been included in the list of evaluated drugs. An interim analysis report is issued online semi-annually including data from 1 January 1989 through the latest period. The interim report contains analyses of voluntary prospective reports (i.e., reports made to the Registry prior to the outcome of pregnancy being known) of prenatal exposures. Additionally, data from retrospective reports are collected,

but the outcomes are reviewed and evaluated separately. The present analysis was based on the latest available APR Interim Report<sup>[24]</sup> (1 January 1989 through 31 January 2015)

### Endpoints assessment and variables of analysis

The following endpoints were selected in pregnant women with HBV infection: Pregnancy outcome and efficacy of preventing MTCT.

According to the Committee for Medicinal Products for Human Use (CHMP) 2005 guidelines on the exposure to medicinal products during pregnancy, "pregnancy outcome" is defined as the end products of pregnancy, which include three main categories: (1) fetal death; (2) termination of pregnancy; and (3) live birth<sup>[25]</sup>.

Fetal death (intrauterine death or *in utero* death) is defined as death prior to complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of pregnancy; the death is indicated by the fact that after such separation the fetus does not show any evidence of life [World Health Organization (WHO) International Classification of Diseases (ICD) 10]. There are 2 types of fetal death: (1) early fetal death (before 22 completed weeks of gestation) comprises ectopic pregnancy (extra-uterine pregnancy or early fetal death most often in the Fallopian tube) and miscarriage (spontaneous abortion or molar pregnancy); and (2) late fetal death (after 22 completed weeks of gestation) is known as stillbirth.

Termination of pregnancy (induced abortion or elective abortion) is artificial interruption of pregnancy.

Live birth is the complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of pregnancy, which breathes or shows any evidence of life after separation (WHO ICD 10).

The same guidelines also defined the variables used to measure prevalence of birth defects<sup>[25]</sup>: (1) live birth prevalence rate = (number of cases among live born infants/total number of live born infants) × 1000; (2) birth prevalence rate = [number of cases among live and stillborn infants/total number of (live + still) born infants] × 1000; and (3) Total prevalence rate = (number of cases among live births, stillborn and terminated pregnancies)/(number of live births, stillbirths and

**Table 3** Non-overlapping literature references of telbivudine exposure during pregnancy

Ref.	Original language	Study design	LdT starting trimester during pregnancy	No. of pregnancy with exposure to LdT	Maternal HBV DNA (at inclusion)
Chen <i>et al</i> <sup>[46]</sup>	Chinese	Prospective	1 <sup>st</sup> trimester	43	$\geq 1 \times 10^7$ copies/mL
Han <i>et al</i> <sup>[47]</sup>	English	Prospective	2 <sup>nd</sup> and 3 <sup>rd</sup> trimesters	362	$> 1.0 \times 10^6$ copies/mL
Jiang <i>et al</i> <sup>[53]</sup>	Chinese	Prospective	3 <sup>rd</sup> trimesters	28	$> 10^3$ copies/mL (at inclusion)
Liu <i>et al</i> <sup>[38]</sup>	Chinese	Prospective	3 <sup>rd</sup> trimester	5	$\geq 1 \times 10^7$ copies/mL (before treatment)
Liu <i>et al</i> <sup>[28]</sup>	English	Prospective	1 <sup>st</sup> trimester	89	$> 1 \times 10^5$ copies/mL
Liu <i>et al</i> <sup>[21]</sup>	English	Prospective	1 <sup>st</sup> , 2 <sup>nd</sup> or 3 <sup>rd</sup> trimesters	82	$\geq 10^6$ IU/mL
Mohan <i>et al</i> <sup>[54]</sup>	English	Prospective	1 <sup>st</sup> trimester	1	$4.0433 \times 10^4$ copies/mL
Peng <i>et al</i> <sup>[39]</sup>	Chinese	Prospective	3 <sup>rd</sup> trimester	40	$\geq 1 \times 10^6$ copies/mL
Wu <i>et al</i> <sup>[27]</sup>	English	Prospective	2 <sup>nd</sup> or 3 <sup>rd</sup> trimester	279	$> 10^6$ IU/mL
Yu <i>et al</i> <sup>[44]</sup>	English	Prospective	1 <sup>st</sup> , 2 <sup>nd</sup> or 3 <sup>rd</sup> trimester	233	$> 1.0 \times 10^6$ copies/mL
Zeng <i>et al</i> <sup>[40]</sup>	Chinese	Prospective	3 <sup>rd</sup> trimester	22	$\geq 10^5$ copies/mL
Zeng <i>et al</i> <sup>[22]</sup>	English	Prospective	1 <sup>st</sup> or 3 <sup>rd</sup> trimester	54	Not reported
Zhao <i>et al</i> <sup>[55]</sup>	Chinese	Prospective	3 <sup>rd</sup> trimester	30	Not reported
Zhang <i>et al</i> <sup>[41]</sup>	Chinese	Prospective	3 <sup>rd</sup> trimester	31	$> 1 \times 10^7$ copies/mL
Zhang <i>et al</i> <sup>[42]</sup>	Chinese	Prospective	3 <sup>rd</sup> trimester	60	$\geq 1 \times 10^6$ copies/mL
Zhang <i>et al</i> <sup>[26]</sup>	English	Prospective	3 <sup>rd</sup> trimester	257	$> 6 \log_{10}$ copies/mL
Zhou <i>et al</i> <sup>[43]</sup>	Chinese	Prospective	3 <sup>rd</sup> trimester	36	$\geq 1 \times 10^7$ copies/mL
Zhou <i>et al</i> <sup>[45]</sup>	Chinese	Prospective	1 <sup>st</sup> trimester	73	$\geq 1 \times 10^7$ copies/mL

terminated pregnancies)  $\times 1000$ .

The efficacy variable is the rate of MTCT, which is conservatively defined as evidence of HBV infection (detectable HBV DNA or detectable HBsAg) at the age of 6-12 mo or older in the source literature references.

## RESULTS

### Preclinical studies

Studies in pregnant rats (Study 7245-112) and rabbits (Study GVA00010) showed that telbivudine crosses the placenta. Developmental toxicity studies in rats (Study 1314-001) and rabbits (Study 1314-002) at doses up to 1000 mg/kg per day and with exposure levels 6- to 37-times higher indicated that telbivudine was not a developmental toxin in either species (Table 2). Similarly, the high doses (1000 mg/kg per day) given to rats during the peri- and post-natal developmental periods showed no evidence of post-natal developmental toxicity or change in behavior (Study 1314-003). Based on these findings, it is concluded that telbivudine is not teratogenic and has shown no adverse effects in developing embryos and fetuses, as well as in pre- and postnatal development. Telbivudine use is considered to pose a negligible risk to fetus during pregnancy.

### Clinical studies from literature search

**Characteristics of the selected cases:** A total of 18 publications with non-overlapping data and safety information were identified through the literature search, in which 1725 mothers were treated with telbivudine during pregnancy period. These 1725 non-overlapping pregnancy cases were all prospective cases where mothers were exposed to telbivudine during different trimester of pregnancy. The 18 selected publications are listed in Table 3.

**MTCT rate:** Based on the literature review, MTCT rate of telbivudine treatment during pregnancy with the

standard immunoprophylaxis procedure was reported to be 0.70% (11/1572; Table 4). Of the 11 infants with MTCT, 8 mothers started telbivudine treatment from 3<sup>rd</sup> trimester and 3 mothers started from 1<sup>st</sup> trimester. Of the 11 infants with MTCT, 6 mothers had  $> 6 \log$  copies/mL HBV DNA prior to telbivudine treatment, 2 mothers had  $> 5 \log$  copies/mL and 2 mothers had  $> 3 \log$  copies/mL HBV DNA. There was no report on HBV DNA level for 1 mother.

Of the 18 selected literature references, 14 had a non-antiviral control group. The MTCT rate in the non-antiviral treatment group was 11.9% (124/1041; Table 4). The MTCT rate calculated in telbivudine treated patients (0.70%) was significantly lower vs MTCT rate calculated in patients from non-antiviral control group (11.9%) ( $P < 0.0001$ , Fisher's exact test).

**Rates of birth defects:** A total of 1739 pregnancy outcomes were reported from 1725 pregnancies (Figure 1). The safety outcomes of infants in terms of rates of birth defects were calculated according to the three definitions of the CHMP guidelines<sup>[25]</sup>.

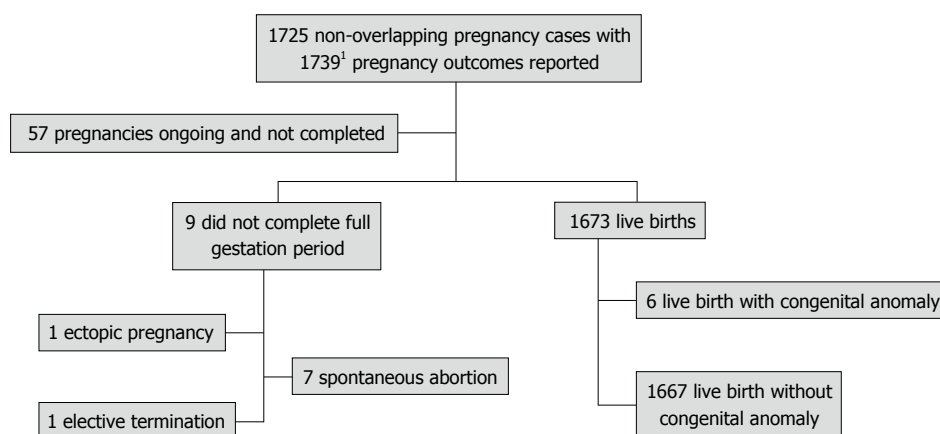
Of the 1673 live births, a total of 6 infants had birth defects (3 infants with ankyloglossia, cutaneous hemangioma, and vaginal canal leak<sup>[22]</sup>; 1 infant with unilateral cleft palate<sup>[26]</sup>; 2 infants with a congenital cleft lip, palate and ear accessories<sup>[27,28]</sup>). Of the 6 infants with birth defects, 4 were born to mothers starting telbivudine treatment in 1<sup>st</sup> trimester and 2 were born to a mother starting telbivudine treatment in 2<sup>nd</sup> or 3<sup>rd</sup> trimester. The "live birth prevalence rate" was  $6/1673 = 3.6/1000$  which was not significantly different from the non-antiviral control ( $3.0/1000$ ) ( $P = 1.0000$ ) (Table 4). Since no stillbirth was reported, the "birth prevalence rate" was same as the "live birth prevalence rate"  $6/1673 = 3.6/1000$ , which was not significantly different from the non-antiviral control ( $3.0/1000$ ) ( $P = 1.0000$ ). The "total prevalence rate" of birth defects with telbivudine exposure was  $7/1674 = 4.2/1000$  (Table



**Table 4** Summary of prevalence rates of birth defects, abortion and perinatal transmission rates with telbivudine exposure during pregnancy in literature studies

	Events ( <i>n</i> )	Population ( <i>N</i> )	Rate in telbivudine treated patients ( <i>n/N</i> )	Non-antiviral treatment control in literature studies	Background rates in overall population (prevalence based on surveillance reports)
Birth defects: Live birth prevalence	6	1673	3.6/1000 <sup>a</sup>	3.0/1000	14.5-60/1000 <sup>1</sup>
Birth defects: Birth prevalence	6	1673	3.6/1000 <sup>a</sup>	3.0/1000	NA
Birth defects: Total prevalence	7	1674	4.2/1000 <sup>b</sup>	3.0/1000	NA
Spontaneous abortion	7	1682	4.2/1000	NA	16/1000 <sup>2</sup>
Elective termination	1	1682	0.6/1000	NA	230/1000 <sup>3</sup>
MTCT	11	1572	0.70% (11/1572) <sup>d</sup>	11.9% (124/1041)	10%-15% <sup>4</sup>

<sup>1</sup>EUROCAT data<sup>[48]</sup>; MACDP data<sup>[49]</sup>; Christianson *et al*<sup>[50]</sup> (2006); Dai *et al*<sup>[54]</sup> (2011); <sup>2</sup>US CDC data<sup>[51]</sup>; <sup>3</sup>WHO data<sup>[52]</sup>; <sup>4</sup>Historical data from HBV-infected population without antiviral treatment<sup>[10-12]</sup>. <sup>a</sup>*P* = 1.0000 *vs* non-antiviral treatment control in the same literature studies (Fisher's exact test); <sup>b</sup>*P* = 0.7502 *vs* non-antiviral treatment control in the same literature studies (Fisher's exact test); <sup>d</sup>*P* < 0.0001 *vs* non-antiviral treatment control in the same literature studies (Fisher's exact test). NA: Not available; MTCT: Mother-to-child transmission.



**Figure 1** Analysis of the pregnancy outcomes from non-overlapping literature references. <sup>1</sup>1734 pregnancy outcomes from 1721 pregnancy mothers due to multiple births.

4), which was not significantly different from the non-antiviral control (3.0/1000) (*P* = 0.7502).

**Pharmacovigilance database:** A total of 489 cumulative pregnancy cases have been reported in the telbivudine pharmacovigilance database (with a cut-off date 31 August 2014). Of the 489 cases, 308 had known pregnancy outcomes with 249 cases of live births (239 cases of live birth without congenital anomaly and 10 cases of live birth with "congenital anomaly" including medical conditions that were not birth defects). Of these 10 cases, 6 cases were considered with congenital birth defects (one case each of hypertrophic pyloric stenosis, cryptorchism, atrial septal defect, syndactyly, hemangioma, and congenital heart disease). Of these 6 cases, 3 had telbivudine exposure during the first trimester.

Fifty-nine cases were reported with the following situations: Ectopic pregnancy (*n* = 2), spontaneous abortion (*n* = 11), intrauterine death (*n* = 3), neonatal death (*n* = 1), elective termination with fetal defects (*n* = 5) and elective termination without fetal defects or unknown (*n* = 37).

**Antiretroviral pregnancy registry:** Based on the cumulative current APR report (1 January 1989 through 31 January 2015), a total of 17332 evaluable prospective cases treated with anti-retroviral drugs during pregnancy period [most with human immunodeficiency virus (HIV) infection] were included in the primary analysis. Of the 8602 birth outcomes with a 1<sup>st</sup> trimester exposure to an antiretroviral drug, there were 219 reports of birth defects. Of the 9026 birth outcomes in the combined second and/or third trimester exposure to antiretroviral drugs, 249 were reported birth defects. Of 27 patients who were exposed to telbivudine during pregnancy (18, 6 and 3 during first, second and third trimester, respectively), 19 live births were reported and there were no cases of birth defects reported.

## DISCUSSION

The prevention of vertical transmission of HBV from mothers to their infants, while limiting toxicity is the key for treating pregnant women with HBV infection and is a significant unmet medical need. Telbivudine, classified as a FDA pregnancy category B drug, is listed

as one of the preferred drugs and may be used for the prevention of MTCT in the last trimester of pregnancy in HBsAg-positive women with high levels of viremia (serum HBV DNA  $> 10^{6-7}$  IU/mL)<sup>[15,23]</sup>. Preclinical studies have demonstrated that telbivudine is not teratogenic and has not shown any adverse effects in developing embryos and fetuses, as well as in pre- and postnatal development. However, certain other antiviral drugs are associated with some potential teratogenic risks during fetal development. A French long-term perinatal cohort study in HIV-infected mothers reported the risks of lamivudine exposure during pregnancies as it causes birth defects in children<sup>[29]</sup>. In the present analysis, based on a systematic literature review of clinical studies, the total prevalence rate of live birth defects in telbivudine-treated pregnancies was not significantly different as compared to the non-antiviral controls in the same literature studies or did not increase as compared to overall prevalence. In the six cases that were reported with congenital anomalies, no particular organ toxicity emerged. Three infants were reported with ankyloglossia, cutaneous hemangioma, and vaginal canal leak; 1 infant with unilateral cleft palate; 2 infants with a congenital cleft lip, palate and ear accessories. The reported prevalence of accessory auricle (0.06%) in this study was not higher than in studies from China (0.3%)<sup>[30]</sup>, Taiwan (0.2%)<sup>[31]</sup>, or Turkey (0.47%-0.7%)<sup>[32,33]</sup>. The reported prevalence of cleft lip and palate (0.12%) in this study was similar to those rates reported in studies performed in China (0.13%)<sup>[34]</sup>, in United States (cleftlip with or without palate 0.114% or cleft palate without cleft lip 0.109%)<sup>[35]</sup>.

The present analysis provides evidence that telbivudine usage in pregnant women in all pregnancy trimesters is generally safe and efficacious, which is in accordance with the EASL guidelines<sup>[15]</sup>. Moreover, at least 297 mothers with telbivudine exposure during 1<sup>st</sup> trimester were included in our study. Of note, 4/6 infants with birth defects were born to mothers who were exposed to telbivudine in the 1<sup>st</sup> trimester; and 8/11 infants with MTCT were born to mothers who were exposed to telbivudine in the 3<sup>rd</sup> trimester of pregnancy. Accordingly, the starting trimester of telbivudine treatment should be a balanced decision considering the maternal HBV DNA load and the need of minimizing risk of birth defects to achieve a best efficacy and safety outcome.

The pharmacovigilance database setting is different from clinical trials in terms of nature, objective or data completeness. In a clinical trial setting, all pregnancy cases treated with telbivudine are required to be collected either prospectively or retrospectively according to a predefined protocol. In contrast, pharmacovigilance database is an observational setting which is targeted to collect adverse event cases reported from all sources and physicians (or consumers) are trained to report cases when any "adverse" event occurs. However, pregnancy is usually not regarded by physicians and consumer as an "adverse" event. As a result, a majority of pregnancy cases with normal outcomes are not reported to the pharmacovigilance database, but those with unfavorable

pregnancy or infants' outcome are more likely to be regarded as "adverse" events and reported. In other words, pregnancy cases with normal outcomes are either under-reported by physicians or cannot be sufficiently collected in the current safety database settings. Therefore, in this review, data from the pharmacovigilance database was cited as another source of data, and it was not pooled with data from literature studies to calculate the prevalence rates of birth defects.

Several recent reviews on telbivudine use in pregnancy have reported results of pregnancy outcomes and prevention of HBV transmission, which were consistent with our results. A meta-analysis of telbivudine use in pregnancy (two randomized controlled trials and four non-randomized controlled trials) analyzed 306 mothers who received telbivudine treatment (vs no treatment,  $n = 270$ ). After a follow-up of 6-12 mo after delivery, HBV DNA positive rates were 0.9% in the telbivudine group vs 14.6% in the control group<sup>[36]</sup>.

In another review of 8 studies, a total of 663 infants born to telbivudine-treated mothers had significantly lower rates of HBsAg positivity and HBV DNA positivity measured post-partum at 6 mo (OR = 0.06,  $P < 0.00001$ ; OR = 0.05,  $P = 0.0003$ ) and 12 mo (OR = 0.13,  $P = 0.007$ ; OR = 0.08,  $P = 0.001$ ) vs the non-treatment control<sup>[37]</sup>.

Although the mechanism of MTCT of HBV is not yet fully elucidated, there are three proposed mechanisms (intrauterine transmission, transmission during delivery and post-partum transmission)<sup>[9]</sup>. Maternal serum HBV DNA level has been identified as the most important independent risk factor for MTCT<sup>[15]</sup>.

A majority of patients in our analysis had HBeAg-positive CHB and high HBV DNA levels prior to treatment with HBV DNA levels and HBeAg status being evenly matched between the telbivudine-treated patient and control groups. Telbivudine use during pregnancy resulted in a low rate of MTCT at 0.70% despite high HBV DNA levels at baseline. The MTCT rate in the non-antiviral control groups of the 14 literature references was 11.9%, which was similar to the rates reported in previous literature references (10%-15%)<sup>[10,11]</sup>. These results from 18 different studies with 1725 pregnancies indicate that the overall blocking of vertical transmission is 99.3% (MTCT 0.70%). Of the 18 literature studies, 15 studies did not report antiviral resistance associated with telbivudine treatment; 3 studies had reported a resistance rate of 1.2%, 2.3% or 6.5%.

A limitation of the analysis is the follow-up period in literature references which was a maximum of 12 mo for most of infants; therefore, long-term effects on such infants remain to be assessed.

In conclusion, the data from literatures, post-marketing pharmacovigilance reports on telbivudine exposure and APR during pregnancy in women with HBV infection showed no increased rates of live birth defects, spontaneous abortion or elective termination. No fetal/neonatal toxicity was reported during telbivudine treatment. The favorable safety profile observed from telbivudine reproductive and developmental preclinical

studies have been confirmed in various clinical settings. Importantly, based on the evidences from more than 1700 of HBV infected mothers reported from literature, telbivudine exposure in pregnancy has been shown to reduce the risk of HBV transmission from mother to child if administered in addition to HBIG and HBV vaccination with a favorable safety profile.

## ACKNOWLEDGMENTS

The authors wish to thank Rajeeb Ghosh from Novartis Healthcare for editorial assistance in the development of this manuscript.

## COMMENTS

### Background

Currently, no anti-hepatitis B virus (HBV) therapies are approved for the prevention of mother-to-child transmission (MTCT) of HBV during pregnancy. In this comprehensive review, data were collected from the published literature, a pharmacovigilance database and an ongoing public registry antiretroviral pregnancy registry (APR).

### Research frontiers

Here the authors present a summary of the information available on the safety and efficacy of telbivudine when used during pregnancy. This analysis was based on scientific literature, and analysis of a Novartis pharmacovigilance database and a public APR.

### Innovations and breakthroughs

The favorable safety profile observed from telbivudine reproductive and developmental preclinical studies have been confirmed in various clinical settings. Importantly, based on the evidences from more than 1700 of HBV infected mothers reported from literature, telbivudine exposure in pregnancy has been shown to reduce the risk of HBV transmission from mother to child if administered in addition to hepatitis B immunoglobulin and HBV vaccination with a favorable safety profile.

### Peer-review

This is a very interesting study on the safety of telbivudine administration in pregnancy and its efficacy in preventing MTCT of HBV infection. The data are well analysed and written and the conclusions are useful particularly for the hepatitis B e antigen positive mothers with high viral loads.

## REFERENCES

- Gish RG, Given BD, Lai CL, Locarnini SA, Lau JY, Lewis DL, Schlup T. Chronic hepatitis B: Virology, natural history, current management and a glimpse at future opportunities. *Antiviral Res* 2015; **121**: 47-58 [PMID: 26092643 DOI: 10.1016/j.antiviral.2015.06.008]
- Lavanchy D. Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. *J Clin Virol* 2005; **34** Suppl 1: S1-S3 [PMID: 16461208 DOI: 10.1016/S1386-6532(05)00384-7]
- Jonas MM. Hepatitis B and pregnancy: an underestimated issue. *Liver Int* 2009; **29** Suppl 1: 133-139 [PMID: 19207977 DOI: 10.1111/j.1478-3231.2008.01933.x]
- Stevens CE, Beasley RP, Tsui J, Lee WC. Vertical transmission of hepatitis B antigen in Taiwan. *N Engl J Med* 1975; **292**: 771-774 [PMID: 1113797 DOI: 10.1056/NEJM197504102921503]
- Tassopoulos NC, Papaevangelou GJ, Sjogren MH, Roumeliotou-Karayannis A, Gerin JL, Purcell RH. Natural history of acute hepatitis B surface antigen-positive hepatitis in Greek adults. *Gastroenterology* 1987; **92**: 1844-1850 [PMID: 3569758]
- Chang MH. Natural history of hepatitis B virus infection in children. *J Gastroenterol Hepatol* 2000; **15** Suppl: E16-E19 [PMID: 10921376 DOI: 10.1046/j.1440-1746.2000.02096.x]
- McMahon BJ, Alward WL, Hall DB, Heyward WL, Bender TR, Francis DP, Maynard JE. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. *J Infect Dis* 1985; **151**: 599-603 [PMID: 3973412 DOI: 10.1093/infdis/151.4.599]
- Li Z, Hou X, Cao G. Is mother-to-infant transmission the most important factor for persistent HBV infection? *Emerg Microbes Infect* 2015; **4**: e30 [PMID: 26060603 DOI: 10.1038/emi.2015.30]
- Piratvisuth T. Optimal management of HBV infection during pregnancy. *Liver Int* 2013; **33** Suppl 1: 188-194 [PMID: 23286864 DOI: 10.1111/liv.12060]
- del Canho R, Grosheide PM, Mazel JA, Heijntink RA, Hop WC, Gerards LJ, de Gast GC, Fetter WP, Zwijneberg J, Schalm SW. Ten-year neonatal hepatitis B vaccination program, The Netherlands, 1982-1992: protective efficacy and long-term immunogenicity. *Vaccine* 1997; **15**: 1624-1630 [PMID: 9364693 DOI: 10.1016/S0264-410X(97)00080-7]
- Grosheide PM, del Canho R, Heijntink RA, Nuijten AS, Zwijneberg J, Bänffer JR, Wladimiroff YW, Botman MJ, Mazel JA, de Gast GC. Passive-active immunization in infants of hepatitis Be antigen-positive mothers. Comparison of the efficacy of early and delayed active immunization. *Am J Dis Child* 1993; **147**: 1316-1320 [PMID: 8249953 DOI: 10.1001/archpedi.1993.02160360058019]
- Farmer K, Gunn T, Woodfield DG. A combination of hepatitis B vaccine and immunoglobulin does not protect all infants born to hepatitis B e antigen positive mothers. *N Z Med J* 1987; **100**: 412-414 [PMID: 2967932]
- Shi Z, Yang Y, Ma L, Li X, Schreiber A. Lamivudine in late pregnancy to interrupt in utero transmission of hepatitis B virus: a systematic review and meta-analysis. *Obstet Gynecol* 2010; **116**: 147-159 [PMID: 20567182 DOI: 10.1097/AOG.0b013e3181e45951]
- Chakvetadze C, Roussin C, Roux J, Mallet V, Petinelli ME, Pol S. Efficacy of hepatitis B sero-vaccination in newborns of African HBsAg positive mothers. *Vaccine* 2011; **29**: 2846-2849 [PMID: 21338675 DOI: 10.1016/j.vaccine.2011.01.101]
- European Association For The Study Of The Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]
- Wiseman E, Fraser MA, Holden S, Glass A, Kidson BL, Heron LG, Maley MW, Ayres A, Locarnini SA, Levy MT. Perinatal transmission of hepatitis B virus: an Australian experience. *Med J Aust* 2009; **190**: 489-492 [PMID: 19413519]
- Pan CQ, Duan ZP, Dai E, Zhang S, Han GR, Wang Y, Zhang H, Zou H, Zhu BS, Zhao WJ, Jiang HX. Tenofovir disoproxil fumarate (TDF) reduces perinatal transmission of hepatitis B virus in highly viremic mothers: a multi-center, prospective, randomized and controlled study. *Hepatology* 2015; **62** (Suppl): 316A
- Liaw YF, Gane E, Leung N, Zeuzem S, Wang Y, Lai CL, Heathcote EJ, Manns M, Bzowej N, Niu J, Han SH, Hwang SG, Cakaloglu Y, Tong MJ, Papatheodoridis G, Chen Y, Brown NA, Albanis E, Galil K, Naoumov NV. 2-Year GLOBE trial results: telbivudine is superior to lamivudine in patients with chronic hepatitis B. *Gastroenterology* 2009; **136**: 486-495 [PMID: 19027013 DOI: 10.1053/j.gastro.2008.10.026]
- Wang Y, Thongsawat S, Gane EJ, Liaw YF, Jia J, Hou J, Chan HL, Papatheodoridis G, Wan M, Niu J, Bao W, Trylesinski A, Naoumov NV. Efficacy and safety of continuous 4-year telbivudine treatment in patients with chronic hepatitis B. *J Viral Hepat* 2013; **20**: e37-e46 [PMID: 23490388 DOI: 10.1111/jvh.12025]
- Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, Chen Y, Heathcote EJ, Rasenack J, Bzowej N, Naoumov NV, Di Bisceglie AM, Zeuzem S, Moon YM, Goodman Z, Chao G, Constance BF, Brown NA. Telbivudine versus lamivudine in patients with chronic hepatitis B. *N Engl J Med* 2007; **357**: 2576-2588 [PMID: 18094378 DOI: 10.1056/NEJMoa066422]
- Liu Y, Wang M, Yao S, Yuan J, Lu J, Li H, Zeng W, Deng Y, Zou R, Li J, Xiao J. Efficacy and safety of telbivudine in perinatal trimesters of pregnancy with high viremia for interrupting perinatal

- transmission of hepatitis B virus. *Hepatol Res* 2015; Epub ahead of print [PMID: 25869545 DOI: 10.1111/hepr.12525]
- 22 **Zeng H**, Cai H, Wang Y, Shen Y. Growth and development of children prenatally exposed to telbivudine administered for the treatment of chronic hepatitis B in their mothers. *Int J Infect Dis* 2015; **33**: 97-103 [PMID: 25449229 DOI: 10.1016/j.ijid.2014.09.002]
- 23 **Liaw YF**, Kao JH, Piratvisuth T, Chan HL, Chien RN, Liu CJ, Gane E, Locarnini S, Lim SG, Han KH, Amarapurkar D, Cooksley G, Jafri W, Mohamed R, Hou JL, Chuang WL, Lesmana LA, Sollano JD, Suh DJ, Omata M. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. *Hepatol Int* 2012; **6**: 531-561 [PMID: 26201469 DOI: 10.1007/s12072-012-9365-4]
- 24 Antiretroviral Pregnancy Registry Interim Report. Issued, 2015. Available from: URL: [http://www.apregistry.com/forms/interim\\_report.pdf](http://www.apregistry.com/forms/interim_report.pdf)
- 25 Guideline on the exposure to medicinal products during pregnancy: need for post-authorisation data. EMEA/CHMP/313666/2005. [Accessed 2013 Sept 16]. Available from: URL: <http://www.ema.europa.eu/docs/en...guideline/.../WC500011303.pdf>
- 26 **Zhang H**, Pan CQ, Pang Q, Tian R, Yan M, Liu X. Telbivudine or lamivudine use in late pregnancy safely reduces perinatal transmission of hepatitis B virus in real-life practice. *Hepatology* 2014; Epub ahead of print [PMID: 25227594 DOI: 10.1002/hep.27034]
- 27 **Wu Q**, Huang H, Sun X, Pan M, He Y, Tan S, Zeng Y, Li L, Deng G, Yan Z, He D, Li J, Wang Y. Telbivudine prevents vertical transmission of hepatitis B virus from women with high viral loads: a prospective long-term study. *Clin Gastroenterol Hepatol* 2015; **13**: 1170-1176 [PMID: 25251571 DOI: 10.1016/j.cgh.2014.08.043]
- 28 **Liu M**, Cai H, Yi W. Safety of telbivudine treatment for chronic hepatitis B for the entire pregnancy. *J Viral Hepat* 2013; **20** Suppl 1: 65-70 [PMID: 23458527 DOI: 10.1111/jvh.12066]
- 29 **Sibiude J**, Mandelbrot L, Blanche S, Le Chenadec J, Boullag-Bonnet N, Faye A, Dollfus C, Tubiana R, Bonnet D, Lelong N, Khoshnood B, Warszawski J. Association between prenatal exposure to antiretroviral therapy and birth defects: an analysis of the French perinatal cohort study (ANRS CO1/CO11). *PLoS Med* 2014; **11**: e1001635 [PMID: 24781315 DOI: 10.1371/journal.pmed.1001635]
- 30 **Sun G**, Xu ZM, Liang JF, Li L, Tang DX. Twelve-year prevalence of common neonatal congenital malformations in Zhejiang Province, China. *World J Pediatr* 2011; **7**: 331-336 [PMID: 22015725 DOI: 10.1007/s12519-011-0328-y]
- 31 **Shih IH**, Lin JY, Chen CH, Hong HS. A birthmark survey in 500 newborns: clinical observation in two northern Taiwan medical center nurseries. *Chang Gung Med J* 2007; **30**: 220-225 [PMID: 17760272]
- 32 **Altıntaş EE**, Nur N, Cerrah YS, Müderris S. A study of the prevalence of developmental anomalies of the external ear among preschool children in Sivas, Turkey. *Turk J Pediatr* 2011; **53**: 528-531 [PMID: 22272453]
- 33 **Beder LB**, Kemaloğlu YK, Maral I, Serdaroğlu A, Bumin MA. A study on the prevalence of accessory auricle anomaly in Turkey. *Int J Pediatr Otorhinolaryngol* 2002; **63**: 25-27 [PMID: 11879926 DOI: 10.1016/S0165-5876(01)00639-5]
- 34 **Dai L**, Zhu J, Liang J, Wang YP, Wang H, Mao M. Birth defects surveillance in China. *World J Pediatr* 2011; **7**: 302-310 [PMID: 22015723 DOI: 10.1007/s12519-011-0326-0]
- 35 US National Birth Defects Prevention Network. [Accessed 2013 Sept 17]. Available from: URL: <http://www.nbdpn.org/>
- 36 **Deng M**, Zhou X, Gao S, Yang SG, Wang B, Chen HZ, Ruan B. The effects of telbivudine in late pregnancy to prevent intrauterine transmission of the hepatitis B virus: a systematic review and meta-analysis. *Virol J* 2012; **9**: 185 [PMID: 22947333 DOI: 10.1186/1743-422X-9-185]
- 37 **Xu HX**, Wang LJ, Yu YX, Wu YP, Xu YF, Liu XX, Chen Y. [Efficacy and safety of telbivudine treatment to block mother-to-child transmission of hepatitis B virus: a meta-analysis]. *Zhonghua Gan Zang Bing Za Zhi* 2012; **20**: 755-760 [PMID: 23207336]
- 38 **Liu M**, Li L, Wang L, Cai H. Preliminary observation on efficacy and safety of telbivudine for preventing mother-to-infant HBV vertical transmission in five HBV-infected pregnant women. *Adverse Drug React* 2008; **10**: 19-21
- 39 **Peng B**, Zhao Y, Yang X. Evaluation of the efficacy and safety of telbivudine in preventing mother-to-infant HBV transmission. *Zhongguo Yaolixue Tongbao* 2012; **47**: 855-857
- 40 **Zeng Y**, Zhang S, Lou G. Clinical study on preventing baby infections in utero from hepatitis B virus with telbivudine. *Zhongguo Linchuang Yaolixue Zazhi* 2010; **15**: 443-445
- 41 **Zhang LJ**, Wang L. [Blocking intrauterine infection by telbivudine in pregnant chronic hepatitis B patients]. *Zhonghua Gan Zang Bing Za Zhi* 2009; **17**: 561-563 [PMID: 19719910]
- 42 **Zhang Y**, Hu Y. Efficacy and safety of telbivudine in blocking mother to child transmission of hepatitis B. *Adverse Drug React* 2010; **12**: 157-159
- 43 **Zhou YJ**, Zheng JL, Pan HJ, Jiang S. [Efficacy and safety of telbivudine in pregnant chronic hepatitis B patients]. *Zhonghua Gan Zang Bing Za Zhi* 2011; **19**: 861-862 [PMID: 22553840]
- 44 **Yu MM**, Jiang Q, Ji Y, Wu KH, Ju LL, Tang X, Yang YF. Comparison of telbivudine versus lamivudine in interrupting perinatal transmission of hepatitis B virus. *J Clin Virol* 2014; **61**: 55-60 [PMID: 24994007 DOI: 10.1016/j.jcv.2014.06.005]
- 45 **Zhou Y**, Zheng J, Pan H, Lu C. [Long-term efficacy and safety of telbivudine in the treatment of childbearing patients with chronic hepatitis B]. *Zhonghua Gan Zang Bing Za Zhi* 2014; **22**: 573-576 [PMID: 25243955 DOI: 10.3760/cma.j.issn.1007-3418.2014.08.004]
- 46 **Chen C**, Tu X, Cheng Q, Chen F, Dai Y, Gong F, Lin X. [Clinical observation of telbivudine's antiviral efficacy and protection against mother-to-infant transmission of chronic hepatitis B during the first trimester of pregnancy]. *Zhonghua Gan Zang Bing Za Zhi* 2015; **23**: 9-12 [PMID: 25751379 DOI: 10.3760/cma.j.issn.1007-3418.2015.01.004]
- 47 **Han GR**, Jiang HX, Yue X, Ding Y, Wang CM, Wang GJ, Yang YF. Efficacy and safety of telbivudine treatment: an open-label, prospective study in pregnant women for the prevention of perinatal transmission of hepatitis B virus infection. *J Viral Hepat* 2015; **22**: 754-762 [PMID: 25641421 DOI: 10.1111/jvh.12379]
- 48 European Surveillance of Congenital Anomalies. [Accessed 2013 Sept 17]. Available from: URL: <http://www.eurocat-network.eu/>
- 49 Metropolitan Atlanta Congenital Defects Program. [Accessed 2013 Sept 18]. Available from: URL: <http://www.cdc.gov/ncbddd/birthdefects/macdp.html>
- 50 **Christianson A**, Howson C, Modell B. Global report on birth defects. The hidden toll of dying and disabled children. March of Dimes. New York: March of Dimes Birth Defects Foundation White Plains, 2006
- 51 Center for Disease Control in US. [Accessed 2013 Sept 18]. Available from: URL: <http://www.cdc.gov/>
- 52 Abortion in Europe. *Entre Nous* 59. 2005. Available from: URL: <http://www.euro.who.int/en/what-we-do/health-topics/Life-stages/sexual-and-reproductive-health/publications/entre-nous/entre-nous/abortion-in-europe.-entre-nous-59>
- 53 **Jiang Q**, Liang W, Zhang S. New research for efficacy of telbivudine blocking HBV transmission from mother to child. *Zhonghua Shiyan He Linchang Bingduxue Zazhi* 2010; **24**: 286-288
- 54 **Mohan A**, Hariharan M. Efficacy and safety of telbivudine during pregnancy in a patient with HBeAg-negative chronic hepatitis B. *Hepatitis B Ann* 2009; **6**: 157-162 [DOI: 10.4103/0972-9747.76912]
- 55 **Zhao D**, Liao X, Peng G. Efficacy of telbivudine combined with hepatitis B vaccine and hepatitis B immune globulin for preventing mother-to-infant transmission in sixty HBV-infected pregnant women. *Zhongguo Xiandai Yaowu Yingyong* 2010; **4**: 37-38

**P- Reviewer:** Alexopoulou A, Bock CT, Iwasaki Y, Larrubia JR, Wang K

**S- Editor:** Qi Y **L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 April 8; 8(10): 461-508





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vysloulzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**TOPIC HIGHLIGHT**

- 461 Management issues in post living donor liver transplant biliary strictures  
*Wadhawan M, Kumar A*

**REVIEW**

- 471 Hepatocellular carcinoma: Review of disease and tumor biomarkers  
*Kim JU, Shariff MIF, Crossey MME, Gomez-Romero M, Holmes E, Cox IJ, Fye HKS, Njie R, Taylor-Robinson SD*
- 485 Host nucleotide polymorphism in hepatitis B virus-associated hepatocellular carcinoma  
*Mathew S, Abdel-Hafiz H, Raza A, Fatima K, Qadri I*

**ORIGINAL ARTICLE****Basic Study**

- 499 Metabolomics studies identify novel diagnostic and prognostic indicators in patients with alcoholic hepatitis  
*Ascha M, Wang Z, Ascha MS, Dweik R, Zein NN, Grove D, Brown JM, Marshall S, Lopez R, Hanounieh IA*

## Contents

*World Journal of Hepatology*  
Volume 8 Number 10 April 8, 2016

### ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Young-Hwa Chung, MD, PhD, Professor, Department of Gastroenterology, University of Ulsan College of Medicine, Asan Medical Center, Seoul 138-736, South Korea

### AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

### FLYLEAF

I-IV Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
April 8, 2016

#### COPYRIGHT

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

#### SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

#### INSTRUCTIONS TO AUTHORS

Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjgnet.com/bpg/g_info_20160116143427.htm)

#### ONLINE SUBMISSION

<http://www.wjgnet.com/esps/>



2016 Liver Transplantation: Global view

## Management issues in post living donor liver transplant biliary strictures

Manav Wadhawan, Ajay Kumar

Manav Wadhawan, Ajay Kumar, Fortis Escorts Liver and Digestive Diseases Institute, Okhla, New Delhi 110025, India

**Author contributions:** Wadhawan M designed research, collected data, performed ERCP's, analyzed data, and wrote paper; Kumar A designed research, performed ERCP's and wrote paper.

**Conflict-of-interest statement:** There is no conflict of interest with anyone on the data published.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Ajay Kumar, MD, DM, MAMS, FRCP (Glasgow), Chief and Executive Director, Fortis Escorts Liver and Digestive Diseases Institute, Okhla, New Delhi 110025, India. [ajaykge@hotmail.com](mailto:ajaykge@hotmail.com)

Received: May 14, 2015

Peer-review started: May 15, 2015

First decision: September 8, 2015

Revised: March 12, 2016

Accepted: March 22, 2016

Article in press: March 23, 2016

Published online: April 8, 2016

### Abstract

Biliary complications are common after living donor liver transplant (LDLT) although with advancements in surgical understanding and techniques, the incidence is decreasing. Biliary strictures are more common than leaks. Endoscopic retrograde cholangiopancreatography (ERCP) is the first line modality of treatment of post

LDLT biliary strictures with a technical success rate of 75%-80%. Most of ERCP failures are successfully treated by percutaneous transhepatic biliary drainage (PTBD) and rendezvous technique. A minority of patients may require surgical correction. ERCP for these strictures is technically more challenging than routine as well post deceased donor strictures. Biliary strictures may increase the morbidity of a liver transplant recipient, but the mortality is similar to those with or without strictures. Post transplant strictures are short segment and soft, requiring only a few session of ERCP before complete dilatation. Long-term outcome of patients with biliary stricture is similar to those without stricture. With the introduction of new generation cholangioscopes, ERCP success rate may increase, obviating the need for PTBD and surgery in these patients.

**Key words:** Living donor liver transplant; Biliary complications; Biliary strictures; Endoscopic retrograde cholangiopancreatography; Percutaneous transhepatic biliary drainage

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Biliary complications are the Achilles heel of liver transplantation and are more common in live related liver transplant than cadaver liver transplant. Endoscopic retrograde cholangiopancreatography along with percutaneous transhepatic biliary drainage is successful in managing more than 90% of biliary complications after liver transplant. Although strictures increase morbidity after liver transplant, the mortality rates are not influenced by biliary strictures. This review provides diagnostic approach and management algorithm of these biliary structures in the setting of right lobe liver transplant.

Wadhawan M, Kumar A. Management issues in post living donor liver transplant biliary strictures. *World J Hepatol* 2016;

8(10): 461-470 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i10/461.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i10.461>

## INTRODUCTION

Liver transplantation has become a well-established treatment for end stage liver disease<sup>[1]</sup>. Living donor liver transplant (LDLT) is still the predominant form for transplantation in eastern part of the world including India. Biliary leaks and strictures are still recognized as the most common complications after LDLT. Biliary complications after orthotopic liver transplantation (OLT) are evaluated and treated by endoscopy; only a few require percutaneous interventions. Surgical intervention is necessary only in treatment failures as a backup option<sup>[2]</sup>. Non-anastomotic stricture (NAS) are uncommon and difficult to treat with endoscopic retrograde cholangiopancreatography (ERCP)/percutaneous transhepatic biliary drainage (PTBD). NAS's often require re-transplant as the only effective treatment option. This review will focus on the diagnosis and management of anastomotic biliary strictures (ABS) after LDLT.

## MAGNITUDE OF THE PROBLEM AND CONTRIBUTING FACTORS

Incidence of biliary complications after liver transplantation has been variably reported between 5%-40% (Table 1). The incidence is higher after LDLT compared to deceased donor liver transplant (DDLTL)<sup>[3]</sup>. Over last 3 decades the reported incidence of bile leaks as well strictures is decreasing (Table 1)<sup>[4-15]</sup>. This can be ascribed to better understanding of the technical causes leading to biliary complications.

Although the cause of biliary complications is mainly technical, various factors have been implicated in the development of these complications. Overview of the possible contributory factors and role of each has been listed in Table 2<sup>[12,14-22]</sup>.

In LDLT the anastomosis is made between right anterior and posterior ducts of the donor with the common hepatic duct of the recipient. The various types of anastomoses are shown in Figure 1. There could be one anastomosis if common trunk of right hepatic duct is available (Figure 2) or there could be two or more anastomoses (Figure 3). Usually, in case of double duct anastomosis, native right anterior and right posterior are used to anastomose to donor ducts. If the two ducts are close together, sometimes ductoplasty with single recipient duct is done (Figure 4). In rare circumstances, surgeons have used cystic duct for anastomosis to one of the ducts of the donor. In our own experience, the use of cystic duct for anastomosis leads to stricture formation in almost all cases (unpublished data). Once the stricture develops in cystic duct anastomosis, it is technically almost impossible to handle with endoscopy

**Table 1 Evolution of post living donor liver transplant biliary complications with the changing time**

Ref.	Year	Country	n	Follow-up (mo)	Leaks	Strictures
Sugawara <i>et al</i> <sup>[4]</sup>	2003	Japan	92	45	20.6%	9.7%
Gondolesi <i>et al</i> <sup>[5]</sup>	2004	United States	96	24.2	21.9%	22.9%
Lee <i>et al</i> <sup>[6]</sup>	2004	South Korea	31	10.5	6.5%	12.9%
Liu <i>et al</i> <sup>[7]</sup>	2004	China	41	13.3	7.3%	24.3%
Soejima <i>et al</i> <sup>[8]</sup>	2006	Japan	182	21	11.5%	25.3%
Shah <i>et al</i> <sup>[9]</sup>	2007	Canada	128	23	14.8%	17.1%
Mita <i>et al</i> <sup>[10]</sup>	2008	Japan	231			9.5%
Marubashi <i>et al</i> <sup>[11]</sup>	2009	Japan	83	32.4	1.2%	7.2%
Kim <i>et al</i> <sup>[12]</sup>	2010	South Korea	22	51.3	0%	9.1%
Wadhawan <i>et al</i> <sup>[14]</sup>	2013	India	65	28	8.8%	10.3%
Mizuno <i>et al</i> <sup>[13]</sup>	2014	Japan	108	58.4	5.6%	13.9%
Vij <i>et al</i> <sup>[15]</sup>	2015	India	127	9.32	0.7%	0%

(Figures 5 and 6). At our center, we have abandoned using cystic duct of the recipient for ductal anastomosis.

## DIAGNOSIS

Biliary complications related to anastomosis could be leaks or strictures. The diagnosis of biliary complications is made on the basis of clinical symptoms (jaundice, itching, bilious drainage, and cholangitis), deranged liver function tests (LFT), and/or radiologic imaging. Imaging plays a very important role in diagnosis as well management of biliary problems. Ultrasonography (USG), magnetic resonance cholangiopancreatography (MRCP), hepatobiliary scintigraphy (HBS) as well as computerized tomogram (CT) have an important role in diagnosis and management of biliary problems.

The timeline for biliary complications after transplant is shown in Figure 7<sup>[23]</sup>.

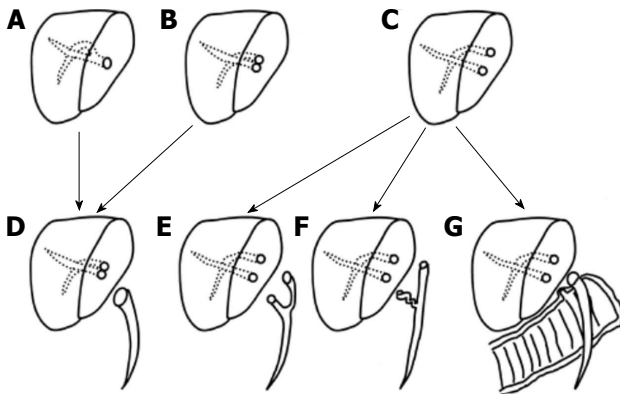
Most bile leaks would present early after transplant (within first few weeks), almost all would manifest in 3 mo<sup>[23]</sup>. Leaks presenting early after transplants are usually diagnosed clinically by the presence of bile in the drains. Sometimes, even early bile leaks may be tricky to diagnose as many patients produce large amount of peritoneal fluid for a few days after transplant, thus diluting the bile. On the other hand, late leaks may present after drain removal with pain abdomen and fever with or without jaundice and septicemia. Role of static imaging (USG, CT, MRCP) in diagnosis of leaks is mainly to diagnose collections. However, HBS may be useful to diagnose subclinical leaks (cut surface leaks after LDLT, minor leaks from anastomotic site not apparent on drain)<sup>[24]</sup>. Minor bile leaks may have minimal derangement of LFT's, any fever with pain abdomen should raise a suspicion of bile leak. Any undiagnosed sepsis in post-operative setting, should raise the suspicion of bile leak and all efforts should be made to diagnose it.

Biliary strictures usually present later than leaks but within first year after transplant<sup>[23]</sup>. The most common

**Table 2 Overview of factors contributing to biliary complications**

Ref.	Year	Factor	Inference
Dalgic <i>et al</i> <sup>[16]</sup>	2005	Corner sparing sutures	Decreased incidence of complications
Castaldo <i>et al</i> <sup>[17]</sup>	2007	Continuous <i>vs</i> interrupted sutures	No difference in two techniques
Soejima <i>et al</i> <sup>[18]</sup>	2008	Hilar dissection to preserve blood supply	Decreased incidence of complications
Lin <i>et al</i> <sup>[19]</sup>	2009	Microsurgical biliary reconstruction	Decreased incidence of complications
Kim <i>et al</i> <sup>[12]</sup>	2010	Telescopic reconstruction of bile duct	Decreased incidence of complications
Chok <i>et al</i> <sup>[20]</sup>	2011	CIT and acute cellular rejection	Higher biliary complications with increased CIT Acute cellular rejection predicted biliary strictures
Horster <i>et al</i> <sup>[21]</sup>	2013	HCV infection as etiology	Higher incidence of biliary complications in patients with HCV infection and higher viral load
Wadhawan <i>et al</i> <sup>[14]</sup>	2013	Type of anastomosis	Higher incidence of biliary complications in double duct and cystic duct anastomosis
Mathur <i>et al</i> <sup>[22]</sup>	2015	Internal biliary stenting	No difference in complications with or without stenting
Vij <i>et al</i> <sup>[15]</sup>	2015	Corner sparing sutures Bile duct mucosal eversion	Decreased incidence of biliary complications

CIT: Cold ischemia time; HCV: Hepatitis C virus.



**Figure 1 Types of biliary anastomoses and corresponding biliary reconstructions**<sup>[54]</sup>. A: Single duct anastomosis; B: Double duct - minimum distance between two donor ducts, requires ductoplasty with recipient CBD; C: Double duct - two donor duct are far away, requires two separate duct anastomosis or a hepaticojejunostomy; D: Single duct to duct reconstruction; E: Double duct to duct reconstruction using right and left hepatic ducts; F: Double duct to duct reconstruction using cystic and CHD; G: Mixed type using duct to duct and hepaticojejunostomy. CHD: Common hepatic duct; CBD: Common bile duct.

presenting symptoms of stricture is itching with or without jaundice. LFT reveal a cholestatic pattern; bilirubin rise may be late in the course after LDLT. Less commonly, cholangitis may be the presentation of a biliary stricture. The first investigation in such cases is ultrasound of the abdomen. The presence of ductal dilatation has a high positive predictive value for the diagnosis of a stricture<sup>[25]</sup>. However, ductal dilatation is not prominent in many cases after LDLT. Absence of ductal dilatation has been previously reported to be an unreliable indicator of adequate biliary drainage<sup>[26]</sup>. It has been shown that donor bile ducts do not respond to the distal obstruction by same extent of dilatation as the non transplant liver<sup>[27]</sup>. MRCP has a sensitivity and specificity of 85%-90% in diagnosing biliary strictures after transplant<sup>[28,29]</sup>. The phenomenon of limited dilatation of donor ducts further underestimates the diagnosis of strictures on MRCP imaging. Specific criteria have

been proposed for diagnosis on MRCP imaging<sup>[30]</sup>. The variables that need to be studied in MRCP include type of anastomosis, length of stricture, length of common stump proximal to the anastomosis and differential diameters of recipient and donor ducts, *etc.* These help in the diagnosis as well as planning of endoscopic treatment.

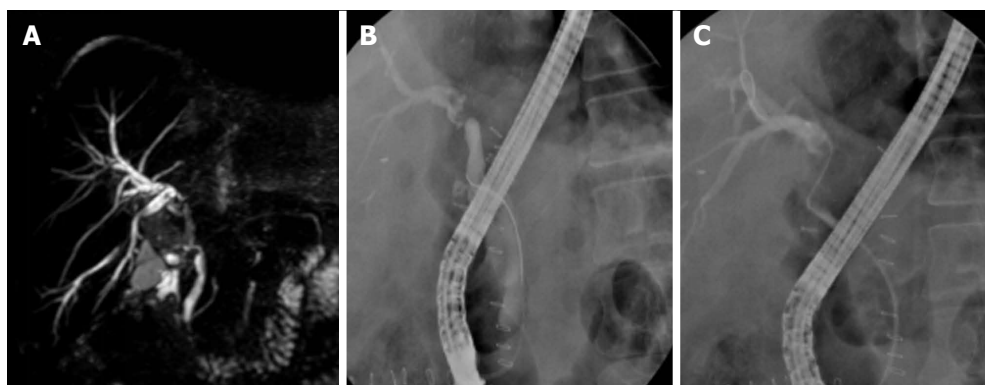
Acute cellular rejection is an important differential when we have graft dysfunction. In fact rejection has been shown to be associated with stricture<sup>[21]</sup>. In our own experience, in the presence of ductal dilatation patients should be first taken for biliary decompression and if graft dysfunction persists, they should be treated for rejection. In the absence of dilatation, a liver biopsy may help in diagnosing the predominant cause of graft dysfunction.

HBS has been used in diagnosing the biliary obstruction with variable results<sup>[31,32]</sup>. It has a high positive predictive value but low sensitivity and specificity. Hence it is not widely used in the diagnosis of strictures.

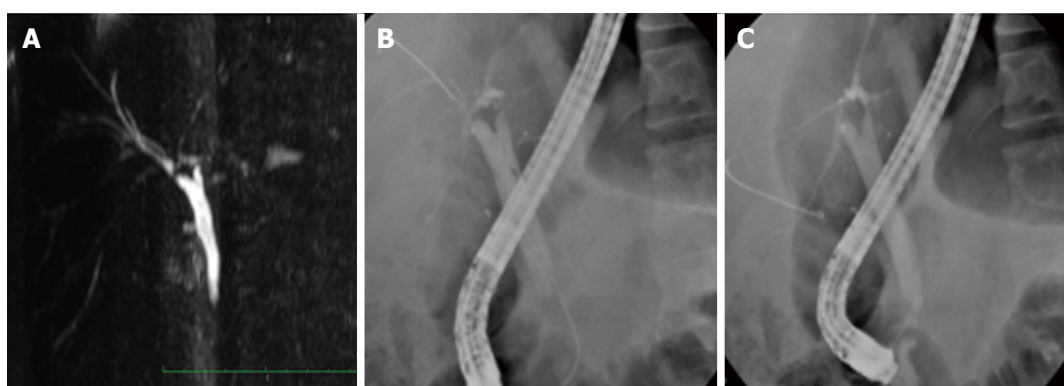
Despite the fact that diagnostic ERCP is on its way out, it still remains an important modality to diagnose and confirm suspected biliary strictures after transplant. Sometimes in doubtful cases, a direct cholangiography [ERCP, percutaneous transhepatic cholangiography (PTC)] is required for the diagnosis. Thus direct cholangiography is the gold standard not only in establishing the diagnosis but also in allowing therapeutic intervention in the same setting. ERCP being less invasive with lower complication rates, is the modality of choice and is preferred over PTC<sup>[2,14]</sup>.

## MANAGEMENT

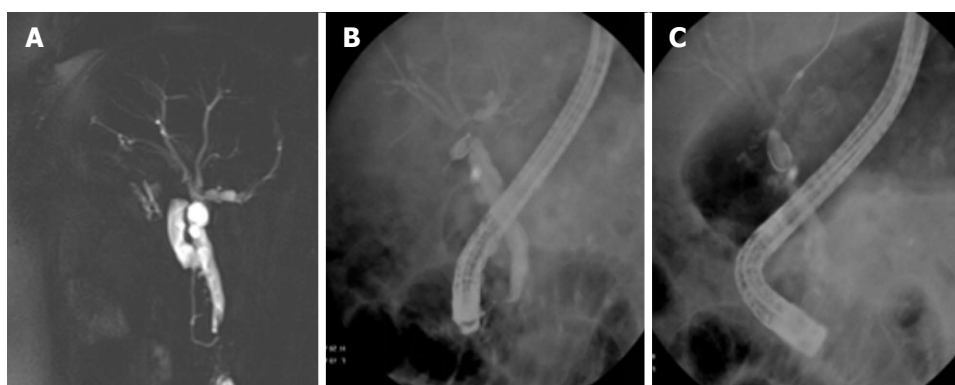
Biliary strictures can be managed by either endoscopic access (ERCP) or by percutaneous access (PTBD). All over the world, ERCP is the treatment of choice for management of biliary strictures after LDLT and is preferred over PTBD. Only one trial has compared the two modalities head to head<sup>[33]</sup>. The results of this study showed similar success and complication rates for



**Figure 2 Anastomotic stricture - single duct anastomosis.** A: Magnetic resonance cholangiopancreatography shows stricture at the anastomotic site of a single duct anastomosis; B: Endoscopic retrograde cholangiopancreatography (ERCP) in the same patient shows the stricture; C: ERCP in same patient shows guide wire negotiated across the stricture.



**Figure 3 Anastomotic stricture - double duct anastomosis.** A: Magnetic resonance cholangiopancreatography image shows stricture across both RASD as well as RPSD ductal anastomosis; B: Endoscopic retrograde cholangiopancreatography (ERCP) image shows guide wire negotiated across RPSD in this patient; C: ERCP image shows guidewire negotiated across RASD in this patient.



**Figure 4 Anastomotic stricture - ductoplasty.** A: Magnetic resonance cholangiopancreatography image of a ductoplasty of RASD and RPSD to common hepatic duct; B: Endoscopic retrograde cholangiopancreatography (ERCP) image shows stricture at ductoplasty site; C: ERCP image shows guide wire across one ductal system.

both approach. However, the number of interventions required was higher in the percutaneous arm. Despite sparse comparative data, ERCP is the preferred approach with PTBD being reserved for rescue in cases of failed ERCP/stenting. PTBD is considered more invasive, with a higher incidence of complications like hemorrhage, bile leak from entry site and need to keep an external stent that is liable to be displaced inadvertently.

#### **Definitions of stricture and endoscopic outcomes**

Classical definition of anastomotic biliary stricture on cholangiography is a dominant narrowing at the anastomotic site without effective drainage of the contrast material<sup>[34]</sup>. However, the diagnosis of stricture is nowadays made on MR cholangiography rather than direct cholangiography. The parameters to be studied on MRCP examination include the presence and location of



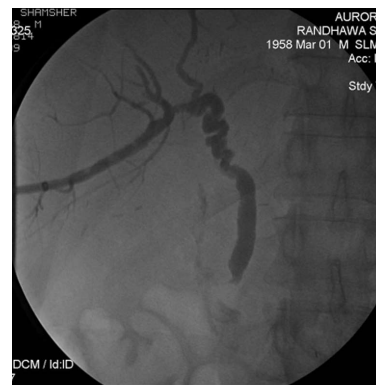
**Table 3 Definitions**

Term	Definition
Anastomotic biliary stricture	ERCP/PTC - Dominant narrowing at the anastomotic site without effective drainage of the contrast material MRCP - More than 50% reduction in anastomotic diameter compared to intrahepatic duct
Successful initial endoscopic outcome	Stricture negotiated with stent with continuous improvement in liver functions
Successful long-term endoscopic outcome	Persistent patency of the anastomotic site on cholangiography after stent removal (anastomotic site > 80% of intrahepatic ductal diameter)
Initial endoscopic treatment failure	Inability to negotiate the stricture on ERCP
Endoscopic treatment failure	Persistence of the stricture after 12 mo of therapy
Persistent ABS	Visible stricture on cholangiography after stent removal, measuring less than 80% of the diameter of the intrahepatic duct or hindering effective drainage of contrast medium
Recurrence of stricture	Biochemical derangement with ERCP documented recurrence of stricture after initial success

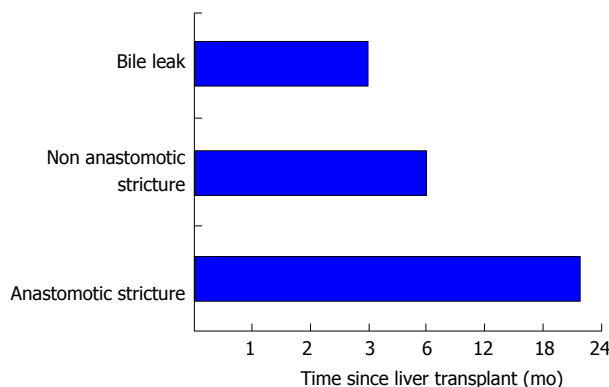
ERCP: Endoscopic retrograde cholangiopancreatography; PTC: Percutaneous transhepatic cholangiography; ABS: Anastomotic biliary strictures; MRCP: Magnetic resonance cholangiopancreatography.



**Figure 5** Anastomotic stricture - cystic duct anastomosis (endoscopic retrograde cholangiopancreatography failed, patient underwent percutaneous transhepatic biliary drainage).



**Figure 6** Cystic duct anastomosis after dilatation. This patient developed stricture again and underwent a hepaticojejunostomy.



**Figure 7** Timeline of biliary complications after transplant.

any strictures, upstream duct dilatation, the diameter of the ducts proximal to the anastomotic site (donor duct), distal to the anastomotic site (recipient duct) (Table 3).

ABS is diagnosed when the diameter of the anastomosis is less than 50% of the proximal (donor) bile duct<sup>[35]</sup>. If ABS is diagnosed, the length and diameter of the stricture is recorded. Also to be noted is the size discrepancy as well as angulation between donor and recipient ducts<sup>[34]</sup>. The percent stenosis is calculated as the difference between the donor duct diameter and the stricture diameter, divided by the donor duct

diameter. In case of multiple duct anastomoses, details of each anastomosis have to be recorded as it has implications on number of stents to be placed. Also in case of a single duct anastomosis, the possibility of stricture extending intrahepatic is to be considered (this can convert a single duct anastomosis similar to double duct thus mandating more than one stent). Although more than 50% change in diameter of anastomosis to intrahepatic (donor duct) is taken as suggestive of stricture, this has not been validated in any of the trials. There are no studies comparing the relative diameters in asymptomatic individuals compared to those with biochemical derangements.

Successful initial endoscopic outcome is defined as the continuous improvement in LFT. Successful long-term endoscopic outcome refers to persistent patency of the anastomotic site on cholangiography after stent removal. The biliary anastomosis is considered patent on cholangiography when the narrowest diameter at the anastomosis is greater than 80% of the upstream intrahepatic (donor) duct diameter, and spontaneous emptying of contrast medium is seen on fluoroscopy<sup>[2]</sup>. Initial endoscopic treatment failure is defined as inability to negotiate the stricture on ERCP<sup>[14]</sup>. Endoscopic treatment failure is defined as persistence of the stricture after 12 mo of therapy. A persistent ABS is defined as a visible stricture on cholangiography after stent

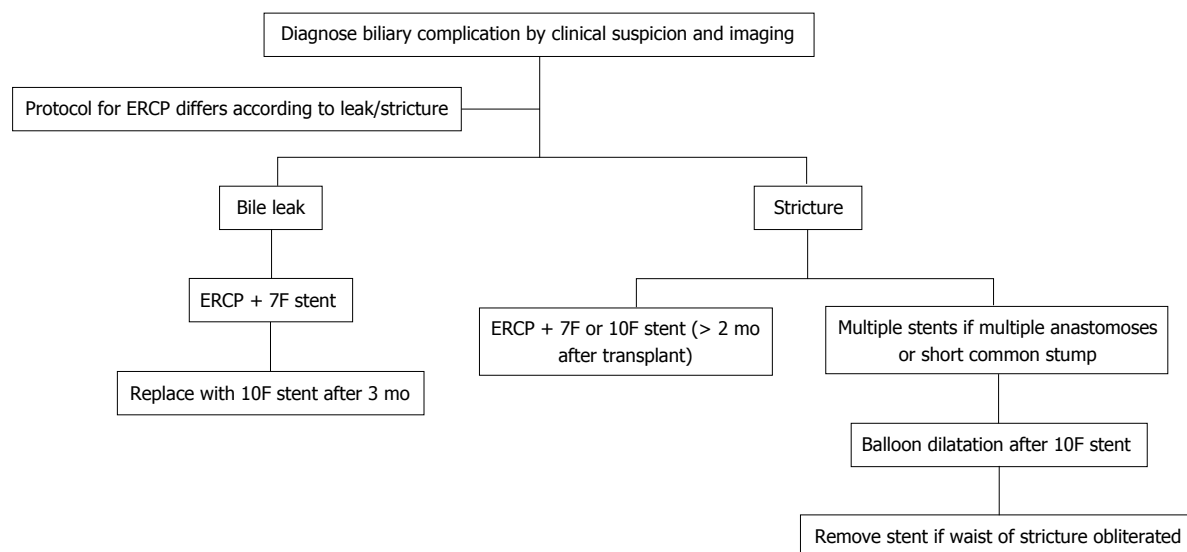


Figure 8 Protocol for endoscopic intervention (please see text also). ERCP: Endoscopic retrograde cholangiopancreatography.

removal, measuring less than 80% of the diameter of the intrahepatic duct or hindering effective drainage of contrast medium. Recurrence of stricture is defined as biochemical derangement with ERCP documented recurrence of stricture after initial success.

### Timing of intervention

There is no data available on the timing of intervention after development of biliary stricture. There is a fear of disrupting the anastomosis if ERCP is done early (first few weeks). However, there is no published data to substantiate that fear. In our own center, we tend to delay ERCP for at least 3 wk. Also we feel that early biliary leaks may merit surgery than ERCP.

The success rate also depends on the time gap between the transplant to the presentation of biliary stricture. We have found that strictures that present early have a higher rate of successful outcome after ERCP. This is probably explained by the fact that early strictures are often soft involving short segment and hence are easily negotiable on ERCP. Those presenting late (generally in such cases there is a lag period between onset of symptoms and time of presentation) often have very tight strictures, making the negotiation of stricture difficult. The rate of salvage PTBD as well requirement of surgical intervention is higher in these cases.

### Protocol of endoscopic intervention

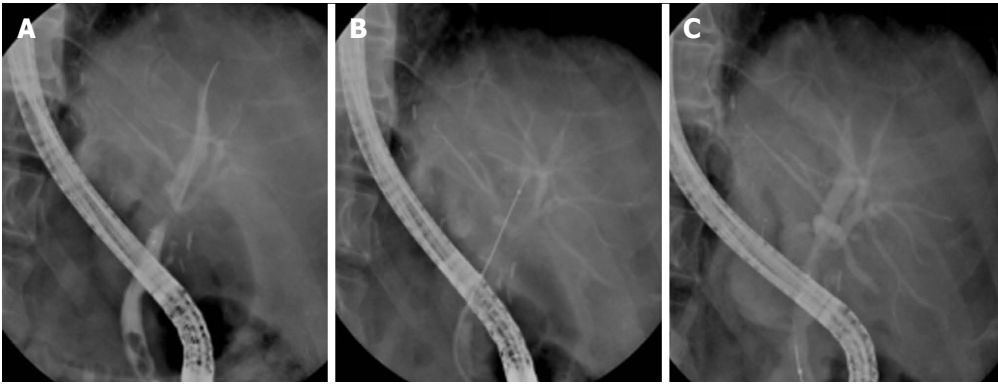
The intervention protocols vary between institutions. Most centers would only stent the stricture after initial sphincterotomy at the first ERCP. Balloon dilatation is usually done in subsequent ERCP's. But there are institutions where balloon dilatation is carried out at the first ERCP itself<sup>[36]</sup>. Usually after the initial ERCP, stents are replaced every 3 mo with larger stents. Stents placed for longer time are more likely to get blocked predisposing to cholangitis<sup>[37-39]</sup>. Use of multiple

stents has shown better long-term success than single stents<sup>[40,41]</sup>.

There are 4 published trials comparing stenting alone vs stenting and balloon dilatation. Three of these are in post DDLT biliary strictures and only one was in LDLT patients<sup>[42]</sup>. This trial showed better long-term outcomes with a combination of both strategies compared to either alone.

The protocol we follow at our center is described as follows (Figure 8)<sup>[14]</sup>. The initial stenting is done with 7F/10F plastic stent depending on the timing of presentation after transplant. We use 7F stents initially for those with biliary leak in addition to stricture, and in those presenting very early after transplant (within 2 mo). Patients presenting after 2 mo of transplant usually undergo either a 10F stent (single duct anastomosis) or two 7F stents (double duct anastomosis). We always place stents across all anastomoses even if only one has a stricture as we feel stenting only one duct may block the other biliary system leading to cholangitis. We use the same strategy of stenting both anterior and posterior duct in a single duct anastomosis if the common duct of donor is small. We do not use balloon dilatation in first ERCP for the fear of anastomotic disruption. The stents are usually exchanged after 3 mo. We perform balloon dilatation with 6 mm or 10 mm biliary dilatation balloon (depending on the size of intrahepatic ducts) during second ERCP. The stents are removed if the waist of stricture is completely obliterated. Each patient requires about 2-3 stent exchanges over 6-12 mo. This is quite less than what is seen in other cases of benign biliary strictures (Iatrogenic post cholecystectomy and strictures associated with chronic pancreatitis). This could be due to the fact that these patients are on immunosuppression and thus do not have significant fibrosis.

The success rate of ERCP in post LDLT biliary strictures has been reported between 60%-75%<sup>[9,14,41-45]</sup>.



**Figure 9 Balloon dilatation of biliary stricture.** A: Endoscopic retrograde cholangiopancreatography (ERCP) images show stricture at the anastomotic site; B: ERCP image showing balloon dilatation of the stricture; C: Successful obliteration of the waist of stricture after balloon dilatation.

This is lower than reported success rate for post DDLT strictures (80%-90%)<sup>[3]</sup>. The reasons for lower success in LDLT strictures are multiple and will be discussed in detail in technical challenges section.

There are reports of use of covered self-expandable metal stents (SEMS) in treatment of biliary leaks and strictures after transplant. However, most of the data is in post DDLT strictures<sup>[46,47]</sup>. The smaller size of donor liver ducts as well as very short common duct stump and discrepancy between recipient and donor duct size make it unsuitable for use in LDLT strictures. Moreover, using a fully covered stent in LDLT strictures will compromise the patency of the contralateral duct. We do not use SEMS in post LDLT strictures.

### Technical challenges

The ERCP procedure is much more challenging in post LDLT compared to DDLT recipient. The anastomosis is much higher and peripheral making the access difficult<sup>[44]</sup>. There is also a size discrepancy between donor and recipient ducts adding to the difficulty. The role of ischemia element at the anastomotic site often leads to the stricture extending intrahepatic, hence often converting single duct anastomosis akin to double duct anastomosis (separation of anterior and posterior segments of the donor liver)<sup>[48]</sup>. The hypertrophy of the partial liver in LDLT often creates a sharp angulation between donor and recipient ducts. This angulation when complicated by a stricture often leads to a very difficult situation less amenable to successful endoscopic treatment<sup>[49]</sup>. Kyoto group has described a similar anomaly as crane neck deformity, in which the biliary anastomosis is located at a point that is far below the highest portion of the recipient duct<sup>[43]</sup>. This is particularly difficult to negotiate with ERCP, but salvage PTBD is often successful in such cases.

A peculiar problem arises when strictures are associated with leaks also. In this scenario, the guide wire repeatedly slips preferentially into the leak area without negotiating the stricture (path of least resistance). In such cases also, ERCP is often unsuccessful and PTBD is required.

Newer techniques like cholangioscopy (spyglass)

have been described in LDLT for traversing difficult strictures<sup>[50,51]</sup>. However in our limited experience of three cases, we did not find it of any additional benefit. We found that limited visibility and steering ability of the currently available devices is the major problem hindering the usefulness. With the improving technology and introduction of better cholangioscopes, this may help in negotiating difficult strictures.

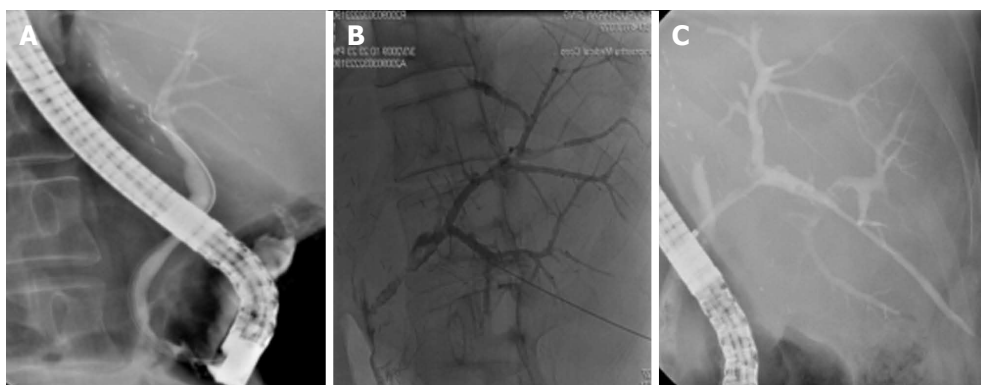
Another novel technique using magnets to traverse difficult biliary strictures after LDLT has been described<sup>[52,53]</sup>. This was initially described in LDLT from Korea by Jang *et al*<sup>[52]</sup>. Subsequently, a through the scope magnet has been used by turkey group with very good results. A similar technique with use of EUS-ERCP interface has also been successfully used to repair biliary anastomosis after LDLT<sup>[54]</sup>. Ersoz *et al*<sup>[55]</sup> described a novel technique using standard balloon to negotiate S shaped difficult strictures. With further refinement of these technique, it may help prevent surgery in difficult to negotiate biliary strictures after LDLT.

### Long-term outcomes

Long-term data after removal of stents is sparse. The only published paper which discusses long-term outcome, reported a stent free status in 42.5% of patients at a median follow-up of 33 mo<sup>[38]</sup>. In our own experience, 90% of the patients after balloon dilatation are free of stents at a median of 1 year after initial ERCP (Figure 9). The recurrence rate after stent removal is around 20% at a median follow-up of 30 mo after last balloon dilatation (unpublished data). Most patients who have recurrence of stricture after balloon dilatation are successfully treated by repeat ERCP only<sup>[33]</sup>.

### Failure of endoscopic treatment

The failure rate of endoscopic management in LDLT strictures varies from 25%-40% in various studies. The reasons for higher failure rate compared to DDLT have been discussed in technical considerations section. Patients who fail ERCP are usually successfully managed by PTBD. We at our center always do a rendezvous ERC procedure after a successful PTBD (Figure 10)<sup>[14]</sup>. Stenting *via* PTC route has been described but is not



**Figure 10 Rendezvous procedure.** A: Endoscopic retrograde cholangiopancreatography opacified only RASD; B: RPTBD accessed via percutaneous transhepatic biliary drainage; C: Rendezvous procedure being performed.

widely practiced as it entails dilatation of liver tract of PTBD<sup>[33]</sup>. In our series the technical success rate of ERCP was 75%, majority of the failures were managed by PTBD (combined success rate of 91%). However, in small number of patients, where both ERCP and PTBD fail (about 9%), surgical intervention in the form of hepaticojejunostomy is required<sup>[14]</sup>.

### Complications

Complication rates after ERCP have been variably reported between 10%-24%<sup>[14,32,39-44]</sup>. Due to altered duodenal anatomy (upper abdominal surgery), the approach to the papilla becomes difficult as after any other upper abdominal surgery. The incidence of complications including pancreatitis rates are similar as in non-transplant ERCP's. We have seen proximal migration of plastic stents in significant number of patients. Removal of these becomes quite difficult. To avoid that, we have started using single pigtail stents.

### Biliary complications and graft survival

Most of the biliary strictures are now managed successfully with non-surgical approach (ERCP or PTBD). The success rate of these interventions is very high with minimum morbidity and almost no mortality. At least two trials have analyzed the effect of biliary complications on graft survival in LDLT<sup>[14,37]</sup>. Both concluded that there is no effect of biliary complications on patient or graft survival. However, both these trials had analyzed strictures in relation to mortality. We believe that if the data on bile leaks is analyzed separately, the results may be different as bile leaks predispose to sepsis and graft dysfunction early after liver transplant.

### Future directions

Biliary strictures are the commonest complication of liver transplant both OLT and LDLT. Despite that there is no consensus on numerous management issues in it. We need more evidence to show what is the best protocol, i.e., only balloon or balloon dilatation plus stent, how many stents, for how long. Natural history of treated biliary strictures needs to be further studied. Newer

devices to facilitate difficult stricture cannulation during endoscopy need to be developed. Digital spyglass may be one such modality. Any bad/favorable prognostic signs for endoscopic treatment need to be defined. Above all more effort is required to refine the surgical techniques to avoid these strictures.

## CONCLUSION

Biliary complications are common after LDLT, strictures seen more commonly than leaks. With refining surgical skills and better understanding of factors predisposing to biliary strictures, the incidence of biliary complications is decreasing. ERCP is the first line modality of treatment of post LDLT biliary strictures with a technical success rate of 75%-80%. Most of ERCP failures are successfully handled by PTBD. A minority of patients may require surgical correction. ERCP for post LDLT strictures is technically more challenging than routine ERCP's as well post DDLT strictures ERCP's. With the introduction of new generation cholangioscopes, ERCP success rate may increase, obviating the need for PTBD and surgery in the management.

## REFERENCES

- 1 **Busuttil RW**, Farmer DG, Yersiz H, Hiatt JR, McDiarmid SV, Goldstein LI, Saab S, Han S, Durazo F, Weaver M, Cao C, Chen T, Lipshutz GS, Holt C, Gordon S, Gornbein J, Amersi F, Ghobrial RM. Analysis of long-term outcomes of 3200 liver transplantations over two decades: a single-center experience. *Ann Surg* 2005; **241**: 905-916; discussion 916-918 [PMID: 15912040 DOI: 10.1097/01.sla.0000164077.77912.98]
- 2 **Nacif LS**, Bernardo WM, Bernardo L, Andraus W, Torres L, Chaib E, D'Albuquerque LC, Maluf-Filho F. Endoscopic treatment of post-liver transplantation anastomotic biliary stricture: systematic review and meta-analysis. *Arq Gastroenterol* 2004; **51**: 240-249 [PMID: 25296086 DOI: 10.1590/S0004-28032014000300014]
- 3 **Sharma S**, Gurakar A, Jabbour N. Biliary strictures following liver transplantation: past, present and preventive strategies. *Liver Transpl* 2008; **14**: 759-769 [PMID: 18508368 DOI: 10.1002/lt.21509]
- 4 **Sugawara Y**, Sano K, Kaneko J, Akamatsu N, Kishi Y, Kokudo N, Makuuchi M. Duct-to-duct biliary reconstruction for living donor liver transplantation: experience of 92 cases. *Transplant Proc*



- 2003; **35**: 2981-2982 [PMID: 14697955 DOI: 10.1016/j.transproceed.2003.10.046]
- 5 **Gondolesi GE**, Varotti G, Florman SS, Muñoz L, Fishbein TM, Emre SH, Schwartz ME, Miller C. Biliary complications in 96 consecutive right lobe living donor transplant recipients. *Transplantation* 2004; **77**: 1842-1848 [PMID: 15223901 DOI: 10.1097/01.TP.0000123077.78702.0C]
- 6 **Lee KW**, Joh JW, Kim SJ, Choi SH, Heo JS, Lee HH, Park JW, Lee SK. High hilar dissection: new technique to reduce biliary complication in living donor liver transplantation. *Liver Transpl* 2004; **10**: 1158-1162 [PMID: 15350008 DOI: 10.1002/lt.20230]
- 7 **Liu CL**, Lo CM, Chan SC, Fan ST. Safety of duct-to-duct biliary reconstruction in right-lobe live-donor liver transplantation without biliary drainage. *Transplantation* 2004; **77**: 726-732 [PMID: 15021836 DOI: 10.1097/01.TP.0000116604.89083.2F]
- 8 **Soejima Y**, Taketomi A, Yoshizumi T, Uchiyama H, Harada N, Ijichi H, Yonemura Y, Ikeda T, Shimada M, Maehara Y. Biliary strictures in living donor liver transplantation: incidence, management, and technical evolution. *Liver Transpl* 2006; **12**: 979-986 [PMID: 16721777 DOI: 10.1002/lt.20740]
- 9 **Shah SA**, Grant DR, McGilvray ID, Greig PD, Selzner M, Lilly LB, Girgrah N, Levy GA, Cattral MS. Biliary strictures in 130 consecutive right lobe living donor liver transplant recipients: results of a Western center. *Am J Transplant* 2007; **7**: 161-167 [PMID: 17227565 DOI: 10.1111/j.1600-6143.2006.01601.x]
- 10 **Mita A**, Hashikura Y, Masuda Y, Ohno Y, Urata K, Nakazawa Y, Ikegami T, Terada M, Yamamoto H, Miyagawa S. Nonsurgical policy for treatment of bilioenteric anastomotic stricture after living donor liver transplantation. *Transpl Int* 2008; **21**: 320-327 [PMID: 18069923 DOI: 10.1111/j.1432-2277.2007.00609.x]
- 11 **Marubashi S**, Dono K, Nagano H, Kobayashi S, Takeda Y, Umeshita K, Monden M, Doki Y, Mori M. Biliary reconstruction in living donor liver transplantation: technical invention and risk factor analysis for anastomotic stricture. *Transplantation* 2009; **88**: 1123-1130 [PMID: 19898209 DOI: 10.1097/TP.0b013e3181ba184a]
- 12 **Kim SH**, Lee KW, Kim YK, Cho SY, Han SS, Park SJ. Tailored telescopic reconstruction of the bile duct in living donor liver transplantation. *Liver Transpl* 2010; **16**: 1069-1074 [PMID: 20818745 DOI: 10.1002/lt.22116]
- 13 **Mizuno S**, Inoue H, Tanemura A, Murata Y, Kuriyama N, Azumi Y, Kishiwada M, Usui M, Sakurai H, Tabata M, Yamada R, Yamamoto N, Sugimoto K, Shiraki K, Takei Y, Isaji S. Biliary complications in 108 consecutive recipients with duct-to-duct biliary reconstruction in living-donor liver transplantation. *Transplant Proc* 2014; **46**: 850-855 [PMID: 24767364 DOI: 10.1016/j.transproceed.2013.11.035]
- 14 **Wadhawan M**, Kumar A, Gupta S, Goyal N, Shandil R, Taneja S, Sibal A. Post-transplant biliary complications: an analysis from a predominantly living donor liver transplant center. *J Gastroenterol Hepatol* 2013; **28**: 1056-1060 [PMID: 23432435 DOI: 10.1111/jgh.12169]
- 15 **Vij V**, Makki K, Chorasiya VK, Sood G, Singhal A, Dargan P. Targeting the Achilles' heel of adult living donor liver transplant: Corner-sparing sutures with mucosal eversion technique of biliary anastomosis. *Liver Transpl* 2016; **22**: 14-23 [PMID: 26390361 DOI: 10.1002/lt.24343]
- 16 **Dalgic A**, Moray G, Emiroglu R, Sozen H, Karakayali H, Boyacioglu S, Bilgin N, Haberal M. Duct-to-duct biliary anastomosis with a "corner-saving suture" technique in living-related liver transplantation. *Transplant Proc* 2005; **37**: 3137-3140 [PMID: 16213329 DOI: 10.1016/j.transproceed.2005.08.046]
- 17 **Castaldo ET**, Pinson CW, Feurer ID, Wright JK, Gorden DL, Kelly BS, Chari RS. Continuous versus interrupted suture for end-to-end biliary anastomosis during liver transplantation gives equal results. *Liver Transpl* 2007; **13**: 234-238 [PMID: 17256781 DOI: 10.1002/lt.20986]
- 18 **Soejima Y**, Fukuhara T, Morita K, Yoshizumi T, Ikegami T, Yamashita Y, Sugimachi K, Taketomi A, Maehara Y. A simple hilar dissection technique preserving maximum blood supply to the bile duct in living donor liver transplantation. *Transplantation* 2008; **86**: 1468-1469 [PMID: 19034019 DOI: 10.1097/TP.0b013e318188d4dc]
- 19 **Lin TS**, Concejero AM, Chen CL, Chiang YC, Wang CC, Wang SH, Liu YW, Yang CH, Yong CC, Jawan B, Cheng YF. Routine microsurgical biliary reconstruction decreases early anastomotic complications in living donor liver transplantation. *Liver Transpl* 2009; **15**: 1766-1775 [PMID: 19938121 DOI: 10.1002/lt.21947]
- 20 **Chok KS**, Chan SC, Cheung TT, Sharr WW, Chan AC, Lo CM, Fan ST. Bile duct anastomotic stricture after adult-to-adult right lobe living donor liver transplantation. *Liver Transpl* 2011; **17**: 47-52 [PMID: 21254344 DOI: 10.1002/lt.22188]
- 21 **Horster S**, Bäuerlein FJ, Mandel P, Raziorrouh B, Hopf C, Stemmler HJ, Guba M, Angele M, Stangl M, Rentsch M, Frey L, Kaspar M, Kaczmarek I, Eberle J, Nickel T, Gruener N, Zachoval R, Diepolder H. Influence of hepatitis C virus infection and high virus serum load on biliary complications in liver transplantation. *Transpl Infect Dis* 2013; **15**: 306-313 [PMID: 23489913 DOI: 10.1111/tid.12069]
- 22 **Mathur AK**, Nadig SN, Kingman S, Lee D, Kinkade K, Sonnenday CJ, Welling TH. Internal biliary stenting during orthotopic liver transplantation: anastomotic complications, post-transplant biliary interventions, and survival. *Clin Transplant* 2015; **29**: 327-335 [PMID: 25604635 DOI: 10.1111/ctr.12518]
- 23 **Ayoub WS**, Esquivel CO, Martin P. Biliary complications following liver transplantation. *Dig Dis Sci* 2010; **55**: 1540-1546 [PMID: 20411422 DOI: 10.1007/s10620-010-1217-2]
- 24 **Young SA**, Sfakianakis GN, Pyrsopoulos N, Nishida S. Hepatobiliary scintigraphy in liver transplant patients: the "blind end sign" and its differentiation from bile leak. *Clin Nucl Med* 2003; **28**: 638-642 [PMID: 12897647]
- 25 **Kok T**, Van der Sluis A, Klein JP, Van der Jagt EJ, Peeters PM, Slooff MJ, Bijleveld CM, Haagsma EB. Ultrasound and cholangiography for the diagnosis of biliary complications after orthotopic liver transplantation: a comparative study. *J Clin Ultrasound* 1996; **24**: 103-115 [PMID: 8838298]
- 26 **St Peter S**, Rodriguez-Davalos MI, Rodriguez-Luna HM, Harrison EM, Moss AA, Mulligan DC. Significance of proximal biliary dilatation in patients with anastomotic strictures after liver transplantation. *Dig Dis Sci* 2004; **49**: 1207-1211 [PMID: 15387348 DOI: 10.1023/B:DDAS.0000037814.96308.7a]
- 27 **Venu M**, Brown RD, Lepe R, Berkes J, Cotler SJ, Benedetti E, Testa G, Venu RP. Laboratory diagnosis and nonoperative management of biliary complications in living donor liver transplant patients. *J Clin Gastroenterol* 2007; **41**: 501-506 [PMID: 17450034 DOI: 10.1097/01.mcg.0000247986.95053.2a]
- 28 **Fulcher AS**, Turner MA. Orthotopic liver transplantation: evaluation with MR cholangiography. *Radiology* 1999; **211**: 715-722 [PMID: 10352596 DOI: 10.1148/radiology.211.3.r99jn17715]
- 29 **Kitazono MT**, Qayyum A, Yeh BM, Chard PS, Ostroff JW, Coakley FV. Magnetic resonance cholangiography of biliary strictures after liver transplantation: a prospective double-blind study. *J Magn Reson Imaging* 2007; **25**: 1168-1173 [PMID: 17520726 DOI: 10.1002/jmri.20927]
- 30 **Linhares MM**, Gonzalez AM, Goldman SM, Coelho RD, Sato NY, Moura RM, Silva MH, Lanzoni VP, Salzedas A, Serra CB, Succi T, D'Ippolito G, Szejnfeld J, Triviño T. Magnetic resonance cholangiography in the diagnosis of biliary complications after orthotopic liver transplantation. *Transplant Proc* 2004; **36**: 947-948 [PMID: 15194328 DOI: 10.1016/j.transproceed.2004.04.005]
- 31 **Kim YJ**, Lee KT, Jo YC, Lee KH, Lee JK, Joh JW, Kwon CH. Hepatobiliary scintigraphy for detecting biliary strictures after living donor liver transplantation. *World J Gastroenterol* 2011; **17**: 2626-2631 [PMID: 21677831 DOI: 10.3748/wjg.v17.i21.2626]
- 32 **Kurzawinski TR**, Selves L, Farouk M, Dooley J, Hilson A, Buscombe JR, Burroughs A, Rolles K, Davidson BR. Prospective study of hepatobiliary scintigraphy and endoscopic cholangiography for the detection of early biliary complications after orthotopic liver transplantation. *Br J Surg* 1997; **84**: 620-623 [PMID: 9171746 DOI: 10.1046/j.1365-2168.1997.02653.x]

- 33 **Lee SH**, Ryu JK, Woo SM, Park JK, Yoo JW, Kim YT, Yoon YB, Suh KS, Yi NJ, Lee JM, Han JK. Optimal interventional treatment and long-term outcomes for biliary stricture after liver transplantation. *Clin Transplant* 2008; **22**: 484-493 [PMID: 18318735 DOI: 10.1111/j.1399-0012.2008.00813.x]
- 34 **Beltrán MM**, Marugán RB, Oton E, Blesa C, Nuño J. Accuracy of magnetic resonance cholangiography in the evaluation of late biliary complications after orthotopic liver transplantation. *Transplant Proc* 2005; **37**: 3924-3925 [PMID: 16386586 DOI: 10.1016/j.transproceed.2005.10.044]
- 35 **Pasha SF**, Harrison ME, Das A, Nguyen CC, Vargas HE, Balan V, Byrne TJ, Douglas DD, Mulligan DC. Endoscopic treatment of anastomotic biliary strictures after deceased donor liver transplantation: outcomes after maximal stent therapy. *Gastrointest Endosc* 2007; **66**: 44-51 [PMID: 17591473 DOI: 10.1016/j.gie.2007.02.017]
- 36 **Hsieh TH**, Mekeel KL, Crowell MD, Nguyen CC, Das A, Aqel BA, Carey EJ, Byrne TJ, Vargas HE, Douglas DD, Mulligan DC, Harrison ME. Endoscopic treatment of anastomotic biliary strictures after living donor liver transplantation: outcomes after maximal stent therapy. *Gastrointest Endosc* 2013; **77**: 47-54 [PMID: 23062758 DOI: 10.1016/j.gie.2012.08.034]
- 37 **Rizk RS**, McVicar JP, Emond MJ, Rohrmann CA, Kowdley KV, Perkins J, Carithers RL, Kimmey MB. Endoscopic management of biliary strictures in liver transplant recipients: effect on patient and graft survival. *Gastrointest Endosc* 1998; **47**: 128-135 [PMID: 9512276 DOI: 10.1016/S0016-5107(98)70344-X]
- 38 **Morelli J**, Mulcahy HE, Willner IR, Cunningham JT, Draganov P. Long-term outcomes for patients with post-liver transplant anastomotic biliary strictures treated by endoscopic stent placement. *Gastrointest Endosc* 2003; **58**: 374-379 [PMID: 14528211 DOI: 10.1067/S0016-5107(03)00011-7]
- 39 **Schwartz DA**, Petersen BT, Poterucha JJ, Gostout CJ. Endoscopic therapy of anastomotic bile duct strictures occurring after liver transplantation. *Gastrointest Endosc* 2000; **51**: 169-174 [PMID: 10650259 DOI: 10.1016/S0016-5107(00)70413-5]
- 40 **Chang JH**, Lee IS, Choi JY, Yoon SK, Kim DG, You YK, Chun HJ, Lee DK, Choi MG, Chung IS. Biliary Stricture after Adult Right-Lobe Living-Donor Liver Transplantation with Duct-to-Duct Anastomosis: Long-Term Outcome and Its Related Factors after Endoscopic Treatment. *Gut Liver* 2010; **4**: 226-233 [PMID: 20559526 DOI: 10.5009/gnl.2010.4.2.226]
- 41 **Tashiro H**, Itamoto T, Sasaki T, Ohdan H, Fudaba Y, Amano H, Fukuda S, Nakahara H, Ishiyama K, Ohshita A, Kohashi T, Mitsuta H, Chayama K, Asahara T. Biliary complications after duct-to-duct biliary reconstruction in living-donor liver transplantation: causes and treatment. *World J Surg* 2007; **31**: 2222-2229 [PMID: 17885788 DOI: 10.1007/s00268-007-9217-x]
- 42 **Park JS**, Kim MH, Lee SK, Seo DW, Lee SS, Han J, Min YI, Hwang S, Park KM, Lee YJ, Lee SG, Sung KB. Efficacy of endoscopic and percutaneous treatments for biliary complications after cadaveric and living donor liver transplantation. *Gastrointest Endosc* 2003; **57**: 78-85 [PMID: 12518136 DOI: 10.1067/mge.2003.11]
- 43 **Hisatsune H**, Yazumi S, Egawa H, Asada M, Hasegawa K, Kodama Y, Okazaki K, Itoh K, Takakuwa H, Tanaka K, Chiba T. Endoscopic management of biliary strictures after duct-to-duct biliary reconstruction in right-lobe living-donor liver transplantation. *Transplantation* 2003; **76**: 810-815 [PMID: 14501859 DOI: 10.1097/01.TP.0000083224.00756.8F]
- 44 **Tsujino T**, Isayama H, Sugawara Y, Sasaki T, Kogure H, Nakai Y, Yamamoto N, Sasahira N, Yamashiki N, Tada M, Yoshida H, Kokudo N, Kawabe T, Makuuchi M, Omata M. Endoscopic management of biliary complications after adult living donor liver transplantation. *Am J Gastroenterol* 2006; **101**: 2230-2236 [PMID: 16952286 DOI: 10.1111/j.1572-0241.2006.00797.x]
- 45 **Kao D**, Zepeda-Gomez S, Tandon P, Bain VG. Managing the post-liver transplantation anastomotic biliary stricture: multiple plastic versus metal stents: a systematic review. *Gastrointest Endosc* 2013; **77**: 679-691 [PMID: 23473000 DOI: 10.1016/j.gie.2013.01.015]
- 46 **Martins FP**, Phillips M, Gaidhane MR, Schmitt T, Kahaleh M. Biliary leak in post-liver-transplant patients: is there any place for metal stent? *HPB Surg* 2012; **2012**: 684172 [PMID: 22619479]
- 47 **Kaffes A**, Griffin S, Vaughan R, James M, Chua T, Tee H, Dinesen L, Corte C, Gill R. A randomized trial of a fully covered self-expandable metallic stent versus plastic stents in anastomotic biliary strictures after liver transplantation. *Therap Adv Gastroenterol* 2014; **7**: 64-71 [PMID: 24587819 DOI: 10.1177/1756283X13503614]
- 48 **Zoeft T**, Maldonado de Dechêne EJ, Dechêne A, Malágo M, Beckebaum S, Paul A, Gerken G, Hilgard P. Optimized endoscopic treatment of ischemic-type biliary lesions after liver transplantation. *Gastrointest Endosc* 2012; **76**: 556-563 [PMID: 22898414 DOI: 10.1016/j.gie.2012.04.474]
- 49 **Yazumi S**, Yoshimoto T, Hisatsune H, Hasegawa K, Kida M, Tada S, Uenoyama Y, Yamauchi J, Shio S, Kasahara M, Ogawa K, Egawa H, Tanaka K, Chiba T. Endoscopic treatment of biliary complications after right-lobe living-donor liver transplantation with duct-to-duct biliary anastomosis. *J Hepatobiliary Pancreat Surg* 2006; **13**: 502-510 [PMID: 17139423 DOI: 10.1007/s00534-005-1084-y]
- 50 **Parsi MA**, Guardino J, Vargo JJ. Peroral cholangioscopy-guided stricture therapy in living donor liver transplantation. *Liver Transpl* 2009; **15**: 263-265 [PMID: 19177445 DOI: 10.1002/lt.21584]
- 51 **Yazumi S**, Chiba T. Biliary complications after a right-lobe living donor liver transplantation. *J Gastroenterol* 2005; **40**: 861-865 [PMID: 16211341 DOI: 10.1007/s00535-005-1698-5]
- 52 **Jang SI**, Kim JH, Won JY, Lee KH, Kim HW, You JW, Itoi T, Lee D. Magnetic compression anastomosis is useful in biliary anastomotic strictures after living donor liver transplantation. *Gastrointest Endosc* 2011; **74**: 1040-1048 [PMID: 21855872 DOI: 10.1016/j.gie.2011.06.026]
- 53 **Parlak E**, Küçükay F, Köksal AŞ, Eminler AT, Uslan Mİ, Yılmaz S. Recanalization of complete anastomotic biliary obstruction after living donor related liver transplantation with a novel through-the-scope magnet. *Liver Transpl* 2015; **21**: 711-712 [PMID: 25641753 DOI: 10.1002/lt.24084]
- 54 **Perez-Miranda M**, Aleman N, de la Serna Higuera C, Gil-Simon P, Perez-Saborido B, Sanchez-Antolin G. Magnetic compression anastomosis through EUS-guided choledochoduodenostomy to repair a disconnected bile duct in orthotopic liver transplantation. *Gastrointest Endosc* 2014; **80**: 520-521 [PMID: 25127949 DOI: 10.1016/j.gie.2014.06.042]
- 55 **Ersoz G**, Tekin F, Ozutemiz O, Tekesin O. A novel technique for biliary strictures that cannot be passed with a guide wire. *Endoscopy* 2007; **39** Suppl 1: E332 [PMID: 18273782 DOI: 10.1055/s-2007-966559]

P- Reviewer: Gassler N, Tekin F S- Editor: Kong JX

L- Editor: A E- Editor: Liu SQ



## Hepatocellular carcinoma: Review of disease and tumor biomarkers

Jin Un Kim, Mohamed I F Shariff, Mary M E Crossey, Maria Gomez-Romero, Elaine Holmes, I Jane Cox, Haddy K S Fye, Ramou Njie, Simon D Taylor-Robinson

Jin Un Kim, Mohamed I F Shariff, Mary M E Crossey, Maria Gomez-Romero, Simon D Taylor-Robinson, Division of Digestive Health, Department of Surgery and Cancer, Imperial College London, London W2 1NY, United Kingdom

Elaine Holmes, Division of Computational Medicine, Department of Surgery and Cancer, Imperial College London, London W2 1NY, United Kingdom

I Jane Cox, the Foundation for Liver Research, Institute of Hepatology, London WC1E 6HX, United Kingdom

Haddy K S Fye, Ramou Njie, MRC Gambia, Fajara 273, The Gambia

**Author contributions:** The subject matter for the review was conceived and overseen by Holmes E, Cox IJ and Taylor-Robinson SD; Crossey MME, Fye HKS, Njie R and Holmes E were responsible for work on the essential biomarker development techniques reported in this review; the paper was written primarily by Kim JU, Shariff MIF and Taylor-Robinson SD; all authors contributed to the writing of the manuscript and approved the final version.

**Conflict-of-interest statement:** Authors declare no conflict of interests for this review.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Jin Un Kim, BSc, Division of Digestive Health, Department of Surgery and Cancer, Imperial College London, St Mary's Campus, South Wharf Road, London W2 1NY, United Kingdom. [juk11@ic.ac.uk](mailto:juk11@ic.ac.uk)  
 Telephone: +44-207-8866454  
 Fax: +44-207-7249369

Received: January 22, 2016  
 Peer-review started: January 23, 2016  
 First decision: February 22, 2016  
 Revised: March 2, 2016  
 Accepted: March 14, 2016  
 Article in press: March 16, 2016  
 Published online: April 8, 2016

### Abstract

Hepatocellular carcinoma (HCC) is a common malignancy and now the second commonest global cause of cancer death. HCC tumorigenesis is relatively silent and patients experience late symptomatic presentation. As the option for curative treatments is limited to early stage cancers, diagnosis in non-symptomatic individuals is crucial. International guidelines advise regular surveillance of high-risk populations but the current tools lack sufficient sensitivity for early stage tumors on the background of a cirrhotic nodular liver. A number of novel biomarkers have now been suggested in the literature, which may reinforce the current surveillance methods. In addition, recent metabolomic and proteomic discoveries have established specific metabolite expressions in HCC, according to Warburg's phenomenon of altered energy metabolism. With clinical validation, a simple and non-invasive test from the serum or urine may be performed to diagnose HCC, particularly benefiting low resource regions where the burden of HCC is highest.

**Key words:** Hepatocellular carcinoma; Biomarker; Metabonomics; Warburg hypothesis; Serum; Plasma; Urine

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Many independent authors have utilized

quantitative techniques, such as  $^1\text{H}$  nuclear magnetic resonance and mass spectrometry to discover novel biomarkers to aid early diagnosis, following the removal of alpha fetoprotein from international surveillance guidelines. However, relatively little effort has been directed to translate these findings to the clinical setting. hepatocellular carcinoma (HCC) is a global issue and the vast majority of the burden is placed upon resource-limited regions, where presentations are late and management techniques for advanced tumors are unavailable. Early identification through a simple serum or urinary investigation, therefore, may be a pivotal step in addressing the global burden of HCC.

Kim JU, Shariff MIF, Crossey MME, Gomez-Romero M, Holmes E, Cox IJ, Fye HKS, Njie R, Taylor-Robinson SD. Hepatocellular carcinoma: Review of disease and tumor biomarkers. *World J Hepatol* 2016; 8(10): 471-484 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i10/471.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i10.471>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth commonest malignancy and arises most frequently in patients with cirrhosis<sup>[1]</sup>. The global distribution of HCC is disproportionate, being most common in areas where chronic hepatitis B virus (HBV) infection is highly prevalent (Figure 1). However, HCC is an increasing problem in the western world, due to migration from HBV-endemic regions, hepatitis C virus (HCV) infection, alcoholic cirrhosis and non-alcoholic steatohepatitis, related to the obesity epidemics<sup>[2,3]</sup> (Figure 2).

Curative treatments, such as hepatic resection and orthotopic liver transplant, offer good prognosis, but are limited to early HCC<sup>[4]</sup>. In developing countries, medical advice is often sought late, resulting in delayed, end-stage presentation. More than two-thirds of HCC patients in the developed world are diagnosed at advanced stages<sup>[5]</sup>. The high global incidence and late presentation of HCC make it the second global cause of cancer-related mortality with 1.6 million global deaths, annually<sup>[6]</sup>. The key and as yet, unmet need is to identify small tumors, amenable to curable treatments, in an otherwise nodular cirrhotic liver parenchyma.

Improved surveillance of populations at-risk by adding a sensitive biomarker investigation to complement current imaging studies has the potential to detect tumors at an early stage, when curative interventions can be implemented. Furthermore, designing a simple and accessible investigative test for a set of HCC biomarkers may not only improve diagnosis and management of liver cancer, but pioneer proteomic or metabonomic diagnosis for other diseases in developing countries, where technical and human resources are limited.

## PATHOGENIC MECHANISMS WITH METABOLIC IMPLICATIONS

### *Altered tumor metabolism*

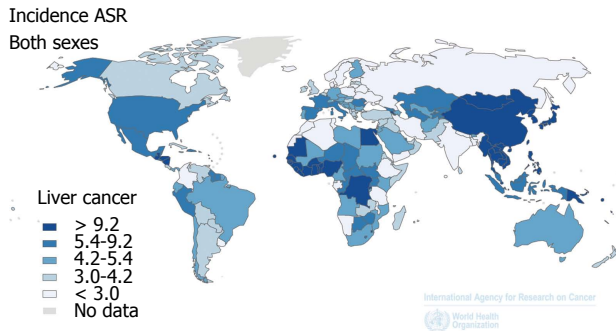
There is increasing evidence that altered metabolism in tumor cells is both a cause and effect of carcinogenesis. Tumor cells require increased amounts of energy and substrates for *de novo* synthesis of nucleotides, lipids, and proteins for rapid proliferation. Otto Warburg, in the 1920s, pioneered the theory of altered tumor metabolism. Recent evidence both supports and disputes his original conclusions.

### *“Warburg effect” and glycolysis*

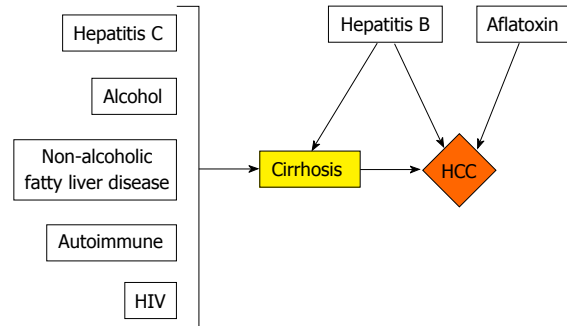
In 1924, Warburg, through placing a section of rat carcinoma in nitrogen-saturated Ringer’s solution (to simulate anaerobic conditions), observed that the tumor could be transplanted to a live donor if sugar was included in the Ringer’s solution, but not if the solution was left plain<sup>[7]</sup>. Following this work, Warburg discovered that even in the presence of oxygen, cancer cells preferentially metabolize glucose by glycolysis as oppose to oxidative phosphorylation, a vastly more inefficient route for energy production. He hypothesized that the increase in glycolysis under normal oxygen conditions arose from a deficiency in the mitochondrial oxidative phosphorylation<sup>[8]</sup> (Figure 3). He thus established that tumor cells take up glucose at high rates to fuel heightened glycolysis. Indeed, it is upon this basis that tumors can be identified with glucose-labeled positron emission tomography<sup>[9]</sup>. Glycolysis generates adenosine triphosphate (ATP) with lower efficiency, but at a faster rate than oxidative phosphorylation, which may be of benefit for rapidly dividing cells. The role of mitochondria in tumor cells is contentious. Primary defects in oxidative phosphorylation (which occurs within the mitochondrial membrane) have been invoked to explain the Warburg phenomenon because tumor mitochondria are often small, lack cristae and are deficient in the  $\beta$ -F1 subunit of the ATPase<sup>[10,11]</sup>. However, many groups have demonstrated that tumor cell mitochondria are actually functional and even Warburg admitted that despite their high glycolysis rate, oxygen consumption by cancer cells is not diminished<sup>[12]</sup>. Furthermore, HCC is a highly vascular tumor that, certainly in the early stages, is likely to be adequately supplied with oxygenated blood. Importantly, glycolysis also provides intermediates for the pentose phosphate pathway and subsequent biosynthesis of nucleic acids. Which of these functions heightened glycolysis serves is, as yet, unresolved.

There is now some consensus that the major role of heightened glycolysis in tumor cells is to provide substrates to the pentose phosphate pathway for nucleotide synthesis, rather than energy provision in the form of ATP<sup>[12,13]</sup>. In essence, the tumor is maximizing production of cellular constituents for proliferation at the expense of energy production.

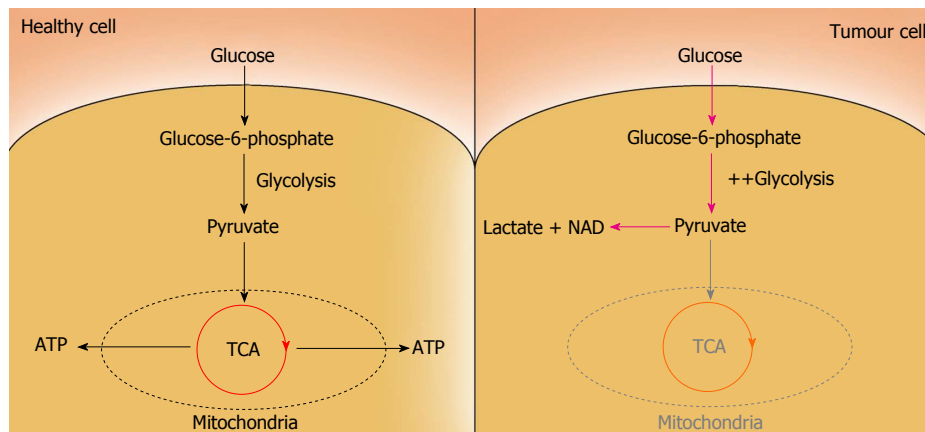




**Figure 1** Global incidence of hepatocellular carcinoma. Sourced from GLOBOCAN 2012.



**Figure 2** Independent risk factors of cirrhosis and hepatocellular carcinoma. HIV: Human immunodeficiency virus; HCC: Hepatocellular carcinoma.



**Figure 3** Warburg theory of heightened glycolysis in tumor cells. TCA: Tri-carboxylic acid cycle; ATP: Adenosine triphosphate; NAD: Nicotinamide adenine dinucleotide.

## MOLECULAR EFFECTORS AND TUMOUR METABOLISM

Several oncogenes and tumor suppressor genes have been implicated in altered tumor metabolism. Sequential mutations are common in HCC and two effectors in particular, hypoxia inducible factor 1 (HIF 1) and p53, may be responsible for some of the metabolic changes arising in HCC.

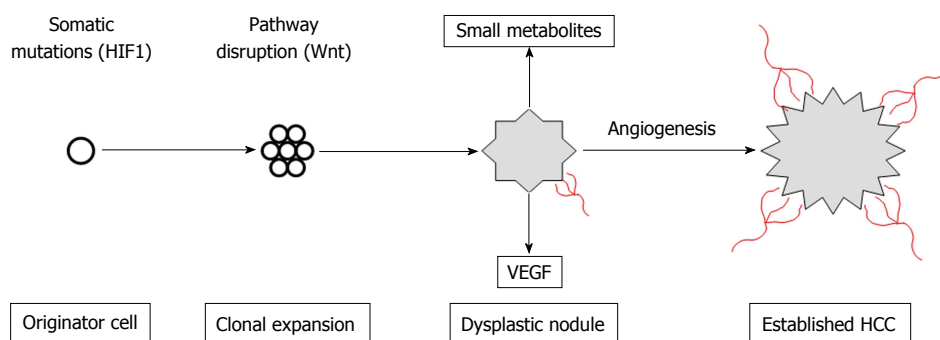
### HIF

HIF 1 is a heterodimeric protein complex transcription factor that is activated by hypoxic, inflammatory, metabolic and oxidative stress<sup>[10,12,14]</sup>. The HIF 1 heterodimeric complex (HIF 1 $\alpha$  + HIF 1 $\beta$ ) is stabilized at low oxygen levels, but degraded by the proteasome in normoxic conditions. The HIF 1 heterodimer stimulates glycolysis by increasing the expression of pro-glycolytic uptake enzymes and transport molecules, such as glucose transporter 1 (GLUT 1) and hexokinase<sup>[12]</sup>. HIF 1 $\beta$  deficient hepatoma cells grown as solid tumors in mice were found to have reduced rates of growth and glycolytic intermediates compared to wild type hepatoma cells<sup>[15]</sup>. It would therefore appear that HIF 1 may play a central role in the Warburg model. However, HIF 1 is only stable in hypoxic conditions and Warburg's model describes heightened glycolysis in normoxic conditions.

Only a minority of cancers display aberrant HIF 1 function in normoxia, such as renal cell carcinoma<sup>[16]</sup>. The role of HIF 1 in HCC is still under investigation but a number of recent studies, mostly in animal models, have observed high HIF 1 activity and its downstream counterparts, such as GLUT1, in hepatoma cells<sup>[17-19]</sup>. Recent studies have also identified association between HIF 1 and the prognosis of HCC, where HIF 1 $\alpha$  levels have been found to be significantly raised in HCC, compared to benign liver disease<sup>[19]</sup>. Furthermore, it appears that HIF 1 inhibition may be a potential target of therapeutic benefit in HCC by down-regulating its role in tumorigenesis. There have been several proposals to incorporate HIF 1 inhibition as adjunct to the current treatment pathways, but further investigations are required before its clinical application<sup>[20]</sup>.

### p53

Tumor suppressor genes, such as p53, have also been implicated in alterations in metabolism. Inactivation of p53 can cause the Warburg phenomenon. p53 positively regulates the expression of the protein synthesis of cytochrome C oxidase 2, which is required for the assembly of the oxidative phosphorylation enzyme, cytochrome C oxidase<sup>[21]</sup> and also negatively regulates phosphoglycerate mutase, a key glycolytic enzyme<sup>[22]</sup>. In addition, p53 transcriptionally activates TP53-induced



**Figure 4** “Angiogenic switch” in hepatocellular carcinoma. VEGF: Vascular endothelial growth factor; HCC: Hepatocellular carcinoma; HIF 1: Hypoxia inducible factor 1.

glycolysis and apoptosis regulator an inhibitor of phosphofructokinase activity which in turn lowers the level of fructose 1,6-bisphosphate which acts as an allosteric activator of glycolytic enzymes<sup>[23]</sup>.

These examples illustrate the evidence that genetic alteration through tumor-driven mutation can affect metabolism. It is likely that many genes and proteins are involved in altered tumor metabolism, with a few taking a lead role.

## METABOLITE EFFECTS ON CARCINOGENESIS

Metabolites can affect carcinogenesis and may not be mere by-products of cellular reactions. Lactate, thought to be a “waste” product of glycolysis, may be such a signal. Lactate may stimulate HIF 1 independently of hypoxia<sup>[24]</sup> and may condition the tumor environment and suppress anticancer immune effectors<sup>[10,25,26]</sup>. HIF 1 can also be stimulated by the buildup of tricarboxylic acid (TCA) cycle intermediates, fumarate and succinate. This is evidenced through tumorigenic germline mutations of TCA cycle enzymes fumarate hydratase and succinate dehydrogenase, resulting in an accumulation of fumarate and succinate which competitively inhibit the  $\alpha$ -ketoglutarate-dependent HIF 1 $\alpha$  prolyl hydroxylase, the enzyme that targets HIF 1 for destruction<sup>[27]</sup>. Through high-throughput liquid-and-gas-chromatography-based mass spectrometry of urine and plasma from patients with prostate carcinoma, Sreekumar *et al.*<sup>[28]</sup> identified sarcosine, a metabolite derivative of glycine, as a marker of the cancer. Furthermore, exogenous addition of sarcosine to tumor cells, or knockdown of sarcosine degrading enzymes, caused a shift of benign prostatic cells into a malignant phenotype.

## OTHER PATHOGENIC MECHANISMS

Genetic profiling studies of HCC tissue have shown several genes to be disrupted through somatic mutations, chromosomal disruption and epigenetic aberration through methylation abnormalities including *p53*, *Rb1*,  *$\beta$ -catenin*, *CMYC* and *survivin*. The Wnt- $\beta$  catenin pathway is the most commonly disrupted pathway,

usually as a result of mutations in *CTNNB1*, *AXIN1* genes, *CDH1* epigenetic silencing and changes in expression of Wnt receptors from the Frizzles family<sup>[29]</sup>. Activation of the pathway induces translocation of  $\beta$ -catenin into the nucleus where it regulates specific oncogenes such as *CMYC* and *CCND1*. An initial somatic mutation in an oncogene or tumor suppressor gene is likely to generate a clonal expansion of cells which then have the potential, through further “proliferation advantageous” mutations and chromosomal disruptions, to develop into pre-neoplastic lesions. These lesions, often < 1 cm, have been identified in patients with cirrhosis and have been sub-classified into low or high grade dysplastic nodules<sup>[30]</sup>. The former carry a low risk and the latter a very high risk, of malignant transformation.

### “Angiogenic switch”

Dysplastic nodules are often hypoechoic on ultrasound imaging and derive their blood supply from the portal vein. These nodules may, less frequently, appear as either hyperechoic or isoechoic. Established HCC displays typical arterial phase uptake on contrast imaging. At a critical point, an “angiogenic switch” is activated which stimulates arterial neo-vascularization of the nodule and development of an established HCC (Figure 4). Japanese groups have identified this as a critical moment before which total cure with resection is likely and after which prognosis deteriorates rapidly<sup>[31]</sup>. Certain factors may contribute to “neo-angiogenesis” of HCCs. Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) have been implicated as angiogenesis modulators. HCC cell lines may produce VEGF by themselves and increased concentration of VEGF in the serum of patients with HCC has been correlated with outcome after surgical resection<sup>[32,33]</sup>. HIF 1, a factor commonly expressed in HCC and heavily influential upon cellular metabolism, has been shown to induce expression of VEGF. A number of oncogenes have also been implicated in angiogenesis such as *ras* and *myc*<sup>[34]</sup>.

It has been shown that chemotherapeutics active against HCC such as the multikinase inhibitor, sorafenib, exert their effects through inhibition of pro-angiogenic factors such as VEGF and PDGF, establishing neo-

**Table 1 Comparison of nuclear magnetic resonance and mass spectrometry**

Variable	NMR	MS
Sensitivity	Lower than MS (nanomolar)	Higher than NMR (picomolar)
Sample degradation	No	Yes
Reproducibility	High	Moderate
Metabolite identification	Well categorized	Labor intensive

NMR: Nuclear magnetic resonance; MS: Mass spectrometry.

angiogenesis as a major therapeutic target in HCC<sup>[30]</sup>. With the onset of neo-angiogenesis, there is likely to be a rapid change in the metabolism of tumor cells and also the surrounding stroma<sup>[35]</sup>. The importance of the interaction between tumor and stromal cells is becoming increasingly recognized. Vizan *et al.*<sup>[36]</sup>, studied the metabolic adaption of endothelial cells, to stimulation by VEGF and fibroblast growth factor. Glycogen synthesis, the pentose cycle and glycolytic pathways were shown to be essential for endothelial cell proliferation and inhibition of these pathways decreased endothelial cell viability and migration<sup>[36]</sup>. The interaction of cellular metabolism and neo-angiogenesis is therefore crucial to tumor development.

## CURRENT SURVEILLANCE AND DIAGNOSIS

HCC is likely to originate from hepatic stem cells<sup>[37]</sup>, with internal and external stimuli, such as viral DNA integration, inflammation and fibrosis, likely inducing alterations in tumor originator cells leading to apoptosis, cell proliferation, dysplasia and eventually, neoplasia<sup>[34]</sup>. The global alteration of metabolites that arise during, or as a consequence of tumorigenesis, then, may measure both the presence and the severity of disease.

Unfortunately, HCC surveillance lacks reliable biomarkers. Serum alpha fetoprotein (AFP) historically has been the most used biomarker. However, not all HCCs secrete AFP. Furthermore, it may be elevated in chronic liver disease in the absence of HCC<sup>[38]</sup>, and its use is no longer recommended by international authorities. Ultrasonography (US) at 6 monthly intervals is the currently recommended screening and surveillance modality for patients with established liver cirrhosis<sup>[39]</sup>. Diagnosis is based on the fact that HCCs are highly arterialized, in contrast to the remainder of the liver. The most recent American Association for the Study of Liver Disease guidelines require the presence of features typical of HCC (arterial hypervascularity and venous phase washout) in just one imaging modality for lesions > 1 cm<sup>[39]</sup>. Previous guidelines suggested that diagnosis was made by the confirmation of two contrast-enhanced imaging modalities (contrast-enhanced ultrasound, computed tomography or magnetic resonance imaging) with characteristic features or one imaging modality suggestive of HCC with an AFP level of > 400 ng/mL<sup>[40]</sup>.

Diagnostic imaging techniques for HCC require a combination of equipment availability, infrastructural support and technicians to perform and interpret the results, which unsurprisingly, are limited in the majority of developing regions with high HCC burden. Alternative solutions to HCC diagnosis, therefore, are urgently required, as AFP measurement lacks sensitivity and specificity. An acceptable alternative requires the diagnostics to be quick, inexpensive, accessible and adequately sensitive and specific to the disease. Blood and urine tests are extremely simple methods of investigation, which are widely utilized in developing regions. For example, designing a urine dipstick test that can quantify and score the severity of HCC from a set of candidate biomarkers may significantly reduce cancer-related morbidity and mortality, and revolutionize the surveillance process in developing regions.

## METABOLIC PROFILING TO FIND BIOMARKERS

Metabolic profiling is a general term encompassing "metabonomics", which is the study of global metabolic responses to physiological, drug and disease stimuli<sup>[41]</sup> and "metabolomics", which aims to characterize and quantify all the small molecules in biofluid samples<sup>[42]</sup>. The most commonly used methods of metabolite characterization are proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectroscopy and mass spectrometry (MS). These techniques are complimentary and each has advantages and disadvantages (Table 1). Sensitivity of MS is high, with some forms of gas chromatography (GC)-MS reaching femtomolar levels, but samples are degraded during the run and metabolite identification can be challenging<sup>[43,44]</sup>. Nuclear magnetic resonance spectroscopy displays lower sensitivity (nano to millimolar), but samples remain intact and NMR spectral profiles have been extensively categorized making metabolite identification more straightforward<sup>[37-39]</sup>.

## PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Nuclear magnetic resonance is based on the behavior of nuclei subjected to a magnetic field. Hydrogen is the most abundant element in living organisms and using high power magnetic fields of *in vitro* samples, high-resolution metabolic NMR spectra can be obtained with clearly defined metabolite peaks of small mobile molecules (< 2 kDa). Comprehensive metabolic profiles have been generated from biofluids, including urine<sup>[45,46]</sup>, serum<sup>[47-50]</sup>, bile<sup>[51]</sup> and intact tissue<sup>[52]</sup>.

## MASS SPECTROMETRY

Mass spectrometry has been utilized for metabolic profiling since the 1970s<sup>[53]</sup>. Metabolites, or their constituent fragments, are detected and distinguished

by their molecular weight and ionic charge. Owing to their complex nature, biological fluids require separation prior to mass spectrometric analysis to achieve detection of as many metabolites as possible. The most common separation methods are GC or liquid chromatography (LC). Gas chromatography requires extensive sample pre-treatment and derivatization steps. In contrast, LC requires minimal sample preparation and is immediately amenable to biofluid analysis. Ultra performance LC utilizes separation columns with much smaller particle size packing material (1.4–1.7  $\mu\text{m}$ ) than traditional columns, permitting the injection of liquids at pressures exceeding 10000 psi, thus allowing for improved metabolite resolution. Once ionized, the particles are detected usually by a time-of-flight analyzer, which allows the detection of analytes over the range of  $m/z$  50–1000 Da.

## CLINICAL APPLICATION OF BIOMARKERS

The development and progression of HCC underscores complex molecular and metabolic interactions, involving several stages of disease over a prolonged period of time. A single reliable biomarker to assess both presence and severity of disease, such as it was for AFP, is likely to be unfeasible in this setting. Therefore, a panel that reliably assesses HCC tumorigenesis from a selection of candidate biomarkers may be better suited to tackle the situation. The candidate biomarkers must show adequate sensitivity and specificity by validation-based experiments, and demonstrate diagnostic synergism when individual biomarker results are combined. Such a new biomarker panel must then be assessed in comparison studies for the current diagnostic methods, such as US and biopsy, for different disease states of HCC, and its utility in surveillance protocols must then be considered, particularly in the developing world context. Biomarkers are also heterogeneous in their quantification and analysis, as different equipment and techniques are utilized. This practical issue must be addressed with thorough cost-benefit analyses that compare biomarker analysis to the local investigative methods.

## SERUM MARKERS OF HCC

### Serum AFP

Serum AFP is the most widely used marker of HCC. It is a fetal glycoprotein, which is synthesized *in utero* by the embryonic liver, cells of the vitelline sac and the fetal intestinal tract. Serum AFP is usually undetectable in healthy adults<sup>[54]</sup>. The production of AFP by HCC cells has been seen as confirmation that the tumor arises from hepatic stem cells as a form of maturation arrest, akin to an embryonic state<sup>[55]</sup>. Not all HCCs secrete AFP and its diagnostic accuracy is variable. A meta-analysis of AFP for HCC surveillance found that it displayed a sensitivity of 39% to 65% and a specificity of 76% to 94% for tumor diagnosis<sup>[56]</sup>. The cut-off level of AFP was important in determining the diagnostic power.

A cut-off of 20 ng/mL resulted in a sensitivity of 64% and specificity of 91%<sup>[57]</sup>, while a cut-off of 400 ng/mL resulted in a sensitivity of 17% and specificity of 99%<sup>[58]</sup>. Values of over 400 ng/mL are generally considered diagnostic of HCC, although only about 20% of patients with HCC display values this high. Furthermore, patients with chronic viral hepatitis may display a raised AFP during viral flares without the presence of HCC. In a study of 290 Chinese patients with chronic HBV, 44 were found to have elevated serum AFP levels (> 20 ng/mL) and only six (13%) had HCC. The remaining 38 had elevated serum AFP, either due to viral flares or due to unknown causes<sup>[59]</sup>. Trevisani *et al.*<sup>[58]</sup> also observed that an AFP elevation in non-infected patients could be more indicative of HCC when compared to infected patients.

### Lens culinaris agglutinin-reactive AFP

Lens culinaris agglutinin-reactive AFP (AFP-L3) is a glycoform variant of AFP and is expressed as a percentage of the total AFP level. It can be detected in the serum of approximately one third of patients with small HCCs (< 3 cm) where cut-off levels of 10% to 15% are used. At higher cut-off levels of > 15%, AFP-L3 displays a sensitivity of 75% to 96.9% and specificity of 90% to 92%<sup>[60,61]</sup>. The usefulness of this marker is limited as studies have only been conducted in East Asian populations in whom AFP levels are already raised.

### Des gamma carboxyprothrombin

Des gamma carboxyprothrombin (DCP) is an abnormal prothrombin protein and is also known as prothrombin induced by vitamin K absence II. It is produced as a result of an acquired defect in the post-translational carboxylation of the prothrombin precursor in malignant cells, the gene responsible being gamma-carboxylase<sup>[62]</sup>. In several large studies, serum DCP was found to display poor diagnostic sensitivity (48% to 62%), but good specificity (81% to 98%) for HCC<sup>[62,63]</sup>. A study comparing the performance characteristics of AFP, DCP and lens culinaris agglutinin-reactive AFP in the diagnosis of HCC observed that DCP was significantly better than the other markers in differentiating HCC from cirrhosis, with a sensitivity of 86% and a specificity of 93%<sup>[64]</sup>. There are conflicting reports, however, with a study by Nakamura *et al.*<sup>[65]</sup> reporting that the efficacy of DCP was lower than that of AFP in the diagnosis of small tumors, although higher than AFP for large tumors.

### Alpha-L-fucosidase

Alpha-L-fucosidase (AFU) is a glycosidase found in cellular lysosomes and increased activity is found in the serum of patients with HCC. Studies of its diagnostic accuracy have displayed high sensitivity (82%) and specificity (70.7%–85.4%)<sup>[66–68]</sup>. A comparative study of AFP and AFU in an Egyptian cohort found AFU to have a higher sensitivity (81.8% vs 68.2%) but lower specificity (55% vs 75%) with a combined AFP + AFU sensitivity of 88.6%<sup>[69]</sup>. Unfortunately, AFU has been



**Table 2 Diagnostic performance of serum markers of hepatocellular carcinoma**

Serum marker	Sensitivity	Specificity
AFP	39%-65%	79%-94%
AFP-L3	75%-97%	90%-92%
DCP	48%-62%	81%-98%
AFU	82%	71%-85%
AFP-L3 + DCP	85%	98%

AFP: Alpha fetoprotein; AFP-L3: Lens culinaris agglutinin-reactive AFP; DCP: Des-gamma-carboxy prothrombin; AFU: Alpha-L-fucosidase.

found to be elevated in other tumors and is therefore not specific to HCC. The diagnostic performance of these serum markers is outlined in Table 2.

### Glypican-3

Glypican-3 (GPC3) is a heparin sulfate proteoglycan and has been shown to be capable of promoting the proliferation of tumor cells by modulating Wnt pathways and affecting cellular adhesion. As a tumor marker, GPC3 expression has been shown to be elevated in HCC tissue and in serum of 40% to 53% of patients with HCC<sup>[69]</sup>.

### Vascular endothelial growth factor

VEGF is a homodimeric cytokine associated with tumor neovascularization. HCC is often diagnosed by imaging evidence of a highly vascularized mass in the liver, and HCC patients have been shown to have increased expressions of VEGF compared to those with normal liver tissues<sup>[70]</sup>. Furthermore, two previous studies have shown mortality in HCC increases with over-expression of VEGF<sup>[71,72]</sup>.

### Interleukin-8

Interleukin-8 (IL-8) is a multifunctional CXC chemokine, which may exert numerous effects on tumor proliferation, angiogenesis and migration. High serum IL-8 has been indicated in HCC patients compared to healthy controls, and its levels correlate to tumor size, absence of tumor capsule, presence of venous invasion, advanced pathological tumor-node-metastasis staging, and poorer disease-free survival<sup>[73,74]</sup>.

### Transforming growth factor-beta 1

Transforming growth factor-beta 1 (TGF- $\beta$ 1) is a negative autocrine growth factor that regulates cell proliferation and differentiation. Comparison studies against AFP (200 ng/mL) have shown TGF- $\beta$ 1 to have higher sensitivity at 68% (800 pg/mL cut-off), and a specificity of 95%<sup>[75]</sup>. Raised TGF- $\beta$ 1 also detected 23% of HCC patients with normal serum AFP<sup>[76]</sup>.

### Tumor-specific growth factor

Tumor-specific growth factor (TSGF) is released by malignant tumors, and has been shown to correlate with tumor growth and surrounding vascularization.

Therefore, it is reasonable to suggest that TSGF could be a potential biomarker that may be used for HCC grading in populations around the world. TSGF has been approved for use by the Chinese government following study results that showed a sensitivity of 82% in HCC diagnosis at the cut-off of 62 U/mL<sup>[77]</sup>.

### Squamous cell carcinoma antigen

Squamous cell carcinoma antigen is part of a family of serine protease inhibitors, or serpins, and has been utilized to diagnose a variety of squamous cell carcinomas<sup>[78]</sup>. It has also been found to have a diagnostic role in HCC, where the sensitivity and specificity were 77.6% and 84%, respectively<sup>[79]</sup>.

### Heat shock proteins

Another potential biomarker for HCC are heat shock proteins (HSP), which are cellular molecules that are expressed under non-specific stress stimuli, including carcinogenesis<sup>[80]</sup>. In particular, HSP70 has been identified as a potentially sensitive marker to differentiate early HCC from precancerous lesions<sup>[81]</sup>.

### Serum metabolites

Metabolic profiling using proteomic techniques mentioned above, such as *in vitro* proton <sup>1</sup>H NMR spectroscopy<sup>[49,82-85]</sup> and MS<sup>[85-99]</sup> have been incorporated to identify a specific metabolic pattern that may be utilized for identifying HCC. Lysophosphatidylcholines (LPC) have been reported in several studies to be significantly decreased in HCC sera compared to healthy controls<sup>[88,89,91-93,96-98]</sup>. LPCs have been described in endothelial cell migration<sup>[100]</sup>, which may contribute to the hypervascularized state in HCC. Two LPCs in particular, LPC 16:0 and LPC 18:0, were significantly altered in HCC compared to cirrhotic patients<sup>[91-93,97]</sup>. Morita *et al.*<sup>[101]</sup> confirmed the overexpression of LPC acyltransferase 1 (LPCAT1) which converts LPC C16:0 to phosphatidylcholine 18:1. The up-regulation of LPCAT1 could be the reason for the reduction in LPC C16:0. A careful interpretation is required, as expression of LPC species has been found to be significantly different between hepatic compensation and decompensation. Free fatty acid (FFA) species have been markedly different in HCC groups compared with control groups, but study results have been conflicting, perhaps due to patient heterogeneity regarding age, gender, ethnicity, diets and existing comorbidities<sup>[91,93-95,97,98,102]</sup>. The European Prospective Investigation into Cancer and Nutrition study additionally described an extensive interaction between HCC and modifiable lifestyle factors in a large European cohort<sup>[85]</sup>, and FFA levels have been linked to the severity of liver disease and disease etiology<sup>[103]</sup>. FFA species that have been identified include FFA C16:0, C18:0, C20:4 and C24:1.

Metabolites of energy production were broadly altered in HCC, particularly concerning products of beta-oxidation and other alternative metabolic pathways<sup>[49,82-84]</sup>. This may point to Warburg's phenomenon in HCC tumor-

rigenesis, where a shift of oxidative glucose metabolism to anaerobic glycolysis takes place to contribute a higher rate of energy production in tumor cells<sup>[8]</sup>. The increase in very low density lipoprotein, as seen in Gao *et al.*<sup>[49]</sup> study, may explain the global lipid mobilization for the lipolytic pathway. Studies have also identified a rise in ketone bodies, such as acetone and beta-hydroxybutyrate, which are formed as by-products of beta-oxidation<sup>[84]</sup>. Furthermore, components of the normal TCA cycle such as 2-oxoglutarate, succinate and glycerol also were significantly altered in HCC groups against controls<sup>[49,102,103]</sup>. The elevation of 2-oxoglutarate may be a consequence from a decreased mitochondrial respiration. Overall, the observed effect of reduced TCA, increased beta-oxidation and increased ketone bodies suggest a heightened alternative metabolic response in tumorigenesis.

Elevated levels of serum bile acids, such as glycochenodeoxycholic acid, glycocholic acid, deoxycholic acid and cholic acid, have long been recognized in many hepatobiliary diseases<sup>[104]</sup>. A study by Chen *et al.*<sup>[105]</sup> identified cirrhotic patients have significantly higher levels of bile acids than those without. Interestingly, levels are significantly different even when comparing compensated against decompensated cirrhosis. It is no surprise that HCC metabonomic studies have identified elevated bile acids in HCC patients when compared to the healthy population<sup>[91-94,96-98,102]</sup>. Bile acids may have a role in tumorigenesis, as reports have described their involvement in glucidic metabolism and acting as signaling molecules<sup>[106,107]</sup>. However, the studies have not controlled for possible confounding factors such as the compensation/decompensation profile, or the prandial state of patients, where certain bile acids are elevated after food intake<sup>[108]</sup>, and therefore, bile acids would not be suitable HCC biomarkers until specific studies are performed to address this issue.

## URINARY MARKERS OF HCC

For a urinary biomarker to be widely applicable three central attributes are necessary. First, the biomarker, if produced pre-renally, needs to be small enough and of the correct ionic charge to be filtered by the renal glomerulus and not re-absorbed by the tubules. Therefore, it has to be roughly less than 20 kDa in atomic weight. Second, the marker should be specific to the cancer in question and not secondary to the effects of cancer on general physiology. Finally, the marker should be secreted in adequate amounts for accurate, repeatable detection in early disease. Large, complex proteins are unlikely to enter the urinary stream, so are not candidates for urinary biomarkers.

### Nucleosides

Studies in the 1970s observed elevated levels of the methylated purines 7-methylguanine, 1-methylguanine, *N*-dimethylguanine, 1-methylhypoxanthine and adenine in the urine of patients with HCC. In 1976, it was found

that urine levels of cyclic guanosine 3':5' monophosphate (cGMP) were elevated in rats with transplanted liver and renal tumors<sup>[109]</sup>. In 1982, Dusheiko *et al.*<sup>[110]</sup>, found parallels in human studies, observing elevated urinary cGMP levels in patients with HCC. In the same study, cGMP was also elevated in the urine of patients with liver disease and other non-HCC tumors, reducing the specificity of the marker considerably.

In 1986, Tamura *et al.*<sup>[111]</sup> observed that urinary levels of pseudouridine, a C-glycoside isomer of the nucleoside uridine, to be elevated in patients with HCC. When combined with serum AFP, sensitivity for HCC detection was 83%. Disappointingly, this marker was also non-specific and found to be similarly elevated in patients with other malignancies such as non-Hodgkin's lymphoma. In a Taiwanese patient study, it was observed that the urinary nucleosides adenosine, cytidine and inosine were elevated in patients with HCC<sup>[112]</sup>. When combined with serum AFP, sensitivity for tumor diagnosis was 80%. The study was flawed in that controls consisted of healthy patients with no liver disease and ideally the finding should have been confirmed in comparison to a group of patients with cirrhosis.

### TGF $\alpha$ and $\beta$

TGF $\alpha$  and  $\beta$  have both been detected in the urine of patients with HCC. The first report was from 1990, observing elevated TGF $\alpha$  levels in urine<sup>[113]</sup>. In 1991, a TGF-related protein was found in HCC patient urine and this was confirmed as TGF $\beta$ 1 in 1997 by the same group<sup>[114,115]</sup>. In these studies, TGF $\beta$ 1 correlated with prognosis and survival. A functional link was attractive as TGFs are known to stimulate non-transformed cells reversibly to grow as colonies *in vitro*.

### Neopterin

In 1998, a study performed in Japan found neopterin, a protein now known to be released from macrophages following inflammatory stimulation, to be elevated in the urine of patients with advanced HCC<sup>[116,117]</sup>. Similar to other potential markers, neopterin has since been shown to be elevated in a number of malignancies and pro-inflammatory conditions such as human immunodeficiency virus related disease, reducing its validity as a specific marker for HCC<sup>[118]</sup>.

### Polyamines

The polyamines, organic compounds containing two or more amine groups, include putrescine, spermine, and spermidine. Their exact cellular role is unclear but they are required for cellular proliferation. Putrescine acts on *S*-adenosylmethionine (SAME), a methylating molecule, to produce spermine which in turn acts on further SAME molecules to produce spermidine<sup>[119]</sup>. Antonello *et al.*<sup>[120]</sup> reported increased urinary levels of free and acetylated polyamines using HCC patients compared to healthy controls and patients with cirrhosis, although the sensitivity of these markers was found not to be high enough for early tumor detection.

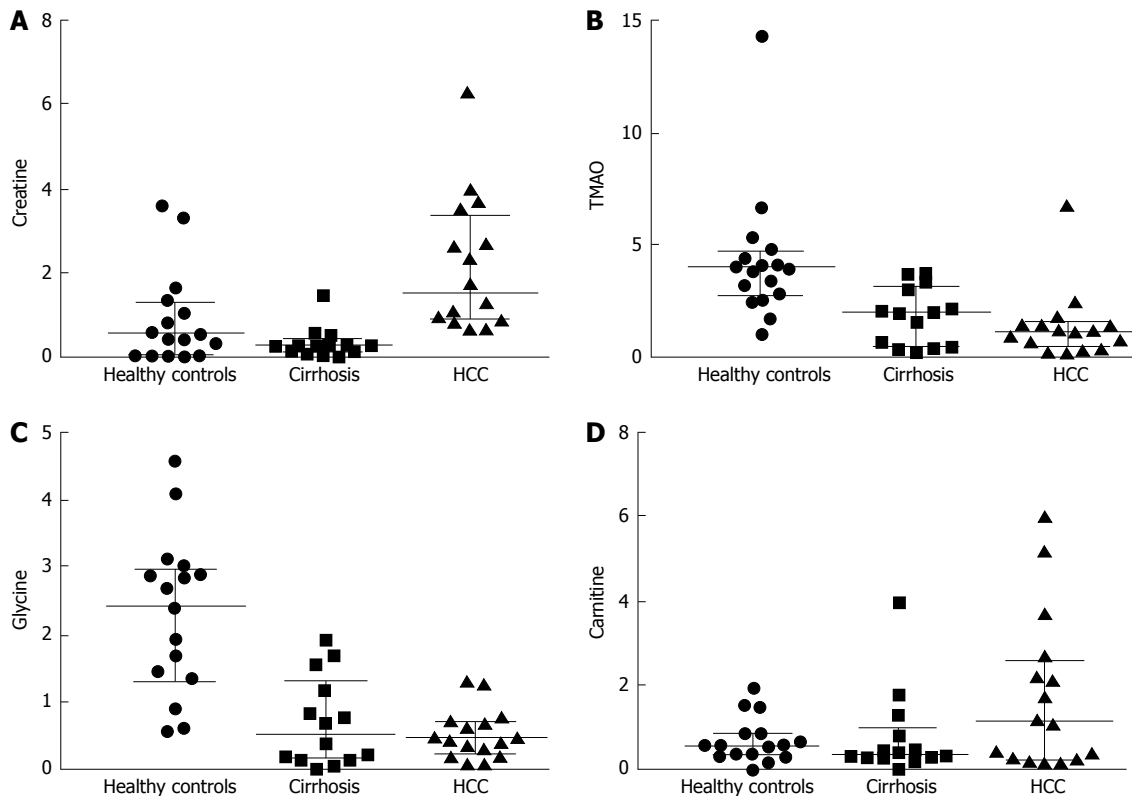


Figure 5 Univariate analysis of discriminatory urinary variables from an Egyptian cohort, comparing values from healthy controls, cirrhosis group and hepatocellular carcinoma group. Discriminatory variable A: Creatine; B: Trimethylamine N-oxide (TMAO); C: Glycine; D: Carnitine. Adapted from Shariff *et al*<sup>[125]</sup>. HCC: Hepatocellular carcinoma.

### Urinary trypsin inhibitor

Urinary trypsin inhibitor (UTI) is a 25 kDa protein thought to be produced by hepatocytes. In 2004, an enzyme-linked immunosorbent assay-based study observed that urinary UTI was elevated in patients with HCC, albeit not significantly when compared to patients with cirrhosis<sup>[121]</sup>. Follow-up studies have found correlations with severity of liver disease and patient prognosis in general, but not specifically with HCC<sup>[122]</sup>.

### Soluble urinary metabolites

Recently, Chen *et al*<sup>[102]</sup> analyzed the serum and urine from 82 patients with HCC and compared these profiles to patients with benign liver tumors and healthy volunteers. Forty three serum and 31 urine metabolites were differentially present in samples of patients with HCC. These included bile acids, free fatty acids, inosine and histidine.

Wu *et al*<sup>[103]</sup> reported a urinary GC-MS study of 20 HCC patients which identified a marker panel of 18 metabolites discriminating HCC and healthy Chinese controls. This panel included octanedioic acid, glycine and hypoxanthine. In the same year, Chen *et al*<sup>[123]</sup> utilized mass spectroscopy techniques with hydrophilic interaction chromatography and reverse phase liquid chromatography in a comparison of 21 urine samples of patients with HCC to 24 healthy volunteer samples. In this set, hypoxanthine, creatinine, betaine, carnitine, acetylcarnitine, leucylproline and phenylacetylglutamine

were altered between groups.

The most recent studies of urinary HCC metabolites to date have been performed within the African populations in Nigeria, Egypt and Gambia<sup>[124-126]</sup>. These studies compared the profiles of HCC with cohorts with cirrhosis without HCC, and healthy control, allowing further differentiation and insight into the metabolic difference in HCC tumorigenesis (Figure 5). Urinary creatinine was lowered in all three African cohorts. Urinary creatinine excretion has been associated with muscle mass<sup>[127]</sup>, and the results seen in the studies may reflect cancer cachexia rather than a specific marker for HCC.

Urinary carnitine levels were also elevated in HCC compared to cirrhosis in all three African groups. Carnitine is a hydrophilic compound, mainly absorbed from the diet and in part synthesized by the body. It is an essential compound for mitochondrial transport of long-chain fatty acids from the cytosol for beta-oxidation. Well-functioning kidneys efficiently reabsorb carnitine, a high urinary level inferring excess carnitine ingestion, biosynthesis or poor reabsorption. Increased urinary acylcarnitines have previously been reported in specific FFA oxidation disturbances and after intense exercise<sup>[128]</sup>. In the context of HCC, Shariff *et al*<sup>[125]</sup> hypothesized its elevation may be explained by increased metabolic activity and high cell-turnover, causing carnitine overproduction to fuel beta-oxidation and rapid energy production<sup>[127]</sup>.

Urinary creatine levels were significantly elevated in

the Egyptian cohort with HCC, but were non-significantly elevated in the Nigerian compared to the respective cirrhosis groups<sup>[124,125]</sup>. Creatine is a nitrogenous organic acid, synthesized mainly in the liver by its constituent parts arginine, glycine and methionine. It has a direct function in cellular energy transport, interacting directly with ATP to produce phosphocreatine and adenosine diphosphate. It is likely that the heightened cell turnover increases cellular energy transport demand, and subsequently raises creatine levels.

Dimethylglycine (DMG), choline, and trimethylamine-N-oxide (TMAO) are metabolites involved in choline intermediary metabolism. Urinary DMG and choline were elevated but a lower concentration of TMAO was noted in the Gambian population. Overexpression of choline has been well established in a series of different tumors. TMAO is typically formed by bacterial degradation of choline, it is likely that this alteration reflects dysregulation of intestinal microbiota, as suggested by Ladeb *et al.*<sup>[126]</sup>. The metabolic alterations that have been observed may be explained by the Warburg phenomenon and its preferential glucose metabolism *via* anaerobic glycolysis.

Urinary glycine levels were reduced in the Egyptian population, but have been unreported in the other studies<sup>[125]</sup>. Glycine's normal cell function involves the methylation of DNA. Its reduction in HCC may be explained by the widely noted phenomenon of hypomethylation within the tumorigenic process. In addition, the Nigerian and Egyptian studies have seen an increase in creatine, as mentioned above. Glycine is a molecular constituent of creatine, which is upregulated in the high cell turnover environment of HCC, which may also explain the decline in glycine observed from the Egyptian study<sup>[124,125]</sup>.

## CONCLUSION

This review provides an overview of HCC pathogenesis and from it, a large selection of potential biomarkers that correlate to the complex molecular and metabolic interaction in its tumorigenesis. HCC is a significant global health issue, which primarily affects countries where there is an infrastructural limitation on community-based surveillance for early disease, and therapeutic options in later stages of tumor presentation. Various diagnostic techniques that have been successfully utilized in developed countries, such as US surveillance, cannot be introduced in resource-limited regions where their application is fundamentally unsuitable. In the current absence of a simple and effective diagnostic investigation in those regions, we highlight the need for research progression in designing clinical diagnostic techniques that may be cheaply and effectively administered. In particular, we emphasize the potential of metabolomics identification of candidate metabolites through the development of a simple urine dipstick, which may be easily performed even in the lowest-income settings.

In considering biomarker application, there must be a careful and a realistic consideration as to the hetero-

geneous metabolic profiles of varying ethnic groups. It is unlikely that a single panel of metabolites that have adequate sensitivity and specificity in the developed population would be appropriate for the developing world population. Previous research has shown that there are clear racial differences in the diagnostic value of AFP, where a minority of Asian, Eurapoid, and Hispanic patients with HCV-related HCC had a normal AFP (18%), close to half the African American patients had a normal AFP level (43%), and furthermore, there was an observed difference between underlying etiology of liver disease, where HCV-related HCC had a stronger association with raised AFP, compared to HBV-related HCC<sup>[129]</sup>. The clear etiological, dietary, genetic and environmental factors that differ between populations suggest the need for specific metabolomic studies, or at least validation studies, in the very regions of the world where better diagnostics or screening tools are required.

To address the pressing issue of identifying novel biomarkers that are sensitive, practically applied, and ethnically specific, the most recent African urinary studies may present the most relevant biomarkers, which can be translated to a simple urine dipstick test<sup>[124-126]</sup>. The significant metabolites include urinary creatine, carnitine and creatinine, among others. Again, these metabolites reflect the molecular changes that happen as part of Warburg's hypothesis of altered energy metabolism. The close fit of the results to the hypothesis should encourage researchers to study the molecular pathway closer in relation to HCC.

In conclusion, success in the field of proteomics and metabolomics will ultimately depend on its clinical application, and this requires a greater emphasis on validation-based experiments of early HCC identification.

## REFERENCES

- 1 **El-Serag HB.** Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]
- 2 **Taylor-Robinson SD,** Foster GR, Arora S, Hargreaves S, Thomas HC. Increase in primary liver cancer in the UK, 1979-94. *Lancet* 1997; **350**: 1142-1143 [PMID: 9343506]
- 3 **El-Serag HB,** Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576 [PMID: 17570226]
- 4 **Llovet JM,** Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-338 [PMID: 10518312 DOI: 10.1055/s-2007-1007122]
- 5 **Stravitz RT,** Heuman DM, Chand N, Sterling RK, Shiffman ML, Luketic VA, Sanyal AJ, Habib A, Mihai AA, Giles HC, Maluf DG, Cotterell AH, Posner MP, Fisher RA. Surveillance for hepatocellular carcinoma in patients with cirrhosis improves outcome. *Am J Med* 2008; **121**: 119-126 [PMID: 18261500 DOI: 10.1016/j.amjmed.2007.09.020]
- 6 **International Agency for Research on Cancer.** World cancer report 2014. Geneva: WHO, 2014
- 7 **Warburg O,** Posener K, Negelein E. Ueber den stoffwechsel der tumoren. *Biochem Z* 1924; **152**: 319-344
- 8 **Warburg O.** [The effect of hydrogen peroxide on cancer cells and on embryonic cells]. *Acta Unio Int Contra Cancrum* 1958; **14**: 55-57 [PMID: 13533023]
- 9 **Ariff B,** Lloyd CR, Khan S, Shariff M, Thillainayagam AV, Bansi



- DS, Khan SA, Taylor-Robinson SD, Lim AK. Imaging of liver cancer. *World J Gastroenterol* 2009; **15**: 1289-1300 [PMID: 19294758 DOI: 10.3748/wjg.15.1289]
- 10 **Kroemer G**, Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. *Cancer Cell* 2008; **13**: 472-482 [PMID: 18538731 DOI: 10.1016/j.ccr.2008.05.005]
  - 11 **López-Ríos F**, Sánchez-Aragó M, García-García E, Ortega AD, Berrendero JR, Pozo-Rodríguez F, López-Encuentra A, Ballestín C, Cuezva JM. Loss of the mitochondrial bioenergetic capacity underlies the glucose avidity of carcinomas. *Cancer Res* 2007; **67**: 9013-9017 [PMID: 17909002]
  - 12 **Weinberg F**, Chandel NS. Mitochondrial metabolism and cancer. *Ann N Y Acad Sci* 2009; **1177**: 66-73 [PMID: 19845608 DOI: 10.1111/j.1749-6632.2009.05039.x]
  - 13 **Vander Heiden MG**, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009; **324**: 1029-1033 [PMID: 19460998 DOI: 10.1126/science.1160809]
  - 14 **Harris AL**. Hypoxia--a key regulatory factor in tumour growth. *Nat Rev Cancer* 2002; **2**: 38-47 [PMID: 11902584 DOI: 10.1038/nrc704]
  - 15 **Griffiths JR**, McSheehy PM, Robinson SP, Troy H, Chung YL, Leek RD, Williams KJ, Stratford IJ, Harris AL, Stubbs M. Metabolic changes detected by in vivo magnetic resonance studies of HEPA-1 wild-type tumors and tumors deficient in hypoxia-inducible factor-1beta (HIF-1beta): evidence of an anabolic role for the HIF-1 pathway. *Cancer Res* 2002; **62**: 688-695 [PMID: 11830521]
  - 16 **Semenza GL**. HIF-1 mediates the Warburg effect in clear cell renal carcinoma. *J Bioenerg Biomembr* 2007; **39**: 231-234 [PMID: 17551816 DOI: 10.1007/s10863-007-9081-2]
  - 17 **Amann T**, Maegdefrau U, Hartmann A, Agaimy A, Marienhagen J, Weiss TS, Stoeltzing O, Warnecke C, Schölmerich J, Oefner PJ, Kreutz M, Bosserhoff AK, Hellerbrand C. GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis. *Am J Pathol* 2009; **174**: 1544-1552 [PMID: 19286567 DOI: 10.2353/ajpath.2009.080596]
  - 18 **Wang W**, Xu GL, Jia WD, Wang ZH, Li JS, Ma JL, Ge YS, Xie SX, Yu JH. Expression and correlation of hypoxia-inducible factor-1alpha, vascular endothelial growth factor and microvessel density in experimental rat hepatocarcinogenesis. *J Int Med Res* 2009; **37**: 417-425 [PMID: 19383236 DOI: 10.1177/147323000903700217]
  - 19 **Yao DF**, Jiang H, Yao M, Li YM, Gu WJ, Shen YC, Qiu LW, Wu W, Wu XH, Sai WL. Quantitative analysis of hepatic hypoxia-inducible factor-1alpha and its abnormal gene expression during the formation of hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 407-413 [PMID: 19666411]
  - 20 **Luo D**, Wang Z, Wu J, Jiang C, Wu J. The role of hypoxia inducible factor-1 in hepatocellular carcinoma. *Biomed Res Int* 2014; **2014**: 409272 [PMID: 25101278 DOI: 10.1155/2014/409272]
  - 21 **Matoba S**, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurler PJ, Bunz F, Hwang PM. p53 regulates mitochondrial respiration. *Science* 2006; **312**: 1650-1653 [PMID: 16728594 DOI: 10.1126/science.1126863]
  - 22 **Kondoh H**, Leonart ME, Gil J, Wang J, Degan P, Peters G, Martinez D, Carnero A, Beach D. Glycolytic enzymes can modulate cellular life span. *Cancer Res* 2005; **65**: 177-185 [PMID: 15665293]
  - 23 **Bensaad K**, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R, Gottlieb E, Vousden KH. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell* 2006; **126**: 107-120 [PMID: 16839880 DOI: 10.1016/j.cell.2006.05.036]
  - 24 **Hsu PP**, Sabatini DM. Cancer cell metabolism: Warburg and beyond. *Cell* 2008; **134**: 703-707 [PMID: 18775299 DOI: 10.1016/j.cell.2008.08.021]
  - 25 **Koukourakis MI**, Giatromanolaki A, Harris AL, Sivridis E. Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: a metabolic survival role for tumor-associated stroma. *Cancer Res* 2006; **66**: 632-637 [PMID: 16423989 DOI: 10.1158/0008-5472.CAN-05-3260]
  - 26 **Swietach P**, Vaughan-Jones RD, Harris AL. Regulation of tumor pH and the role of carbonic anhydrase 9. *Cancer Metastasis Rev* 2007; **26**: 299-310 [PMID: 17415526 DOI: 10.1007/s10555-007-9064-0]
  - 27 **Gottlieb E**, Tomlinson IP. Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat Rev Cancer* 2005; **5**: 857-866 [PMID: 16327764]
  - 28 **Sreekumar A**, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, Laxman B, Mehra R, Lonigro RJ, Li Y, Nyati MK, Ahsan A, Kalyana-Sundaram S, Han B, Cao X, Byun J, Omenn GS, Ghosh D, Pennathur S, Alexander DC, Berger A, Shuster JR, Wei JT, Varambally S, Beecher C, Chinnaiyan AM. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* 2009; **457**: 910-914 [PMID: 19212411 DOI: 10.1038/nature07762]
  - 29 **Mínguez B**, Tovar V, Chiang D, Villanueva A, Llovet JM. Pathogenesis of hepatocellular carcinoma and molecular therapies. *Curr Opin Gastroenterol* 2009; **25**: 186-194 [PMID: 19387255 DOI: 10.1097/MOG.0b013e32832962a1]
  - 30 **Fernández M**, Semela D, Bruix J, Colle I, Pinzani M, Bosch J. Angiogenesis in liver disease. *J Hepatol* 2009; **50**: 604-620 [PMID: 19157625 DOI: 10.1016/j.jhep.2008.12.011]
  - 31 **Takayama T**, Makuuchi M, Hirohashi S, Sakamoto M, Yamamoto J, Shimada K, Kosuge T, Okada S, Takayasu K, Yamasaki S. Early hepatocellular carcinoma as an entity with a high rate of surgical cure. *Hepatology* 1998; **28**: 1241-1246 [PMID: 9794907 DOI: 10.1002/hep.510280511]
  - 32 **Armengol C**, Tarafa G, Boix L, Solé M, Queralt R, Costa D, Bachs O, Bruix J, Capellà G. Orthotopic implantation of human hepatocellular carcinoma in mice: analysis of tumor progression and establishment of the BCLC-9 cell line. *Clin Cancer Res* 2004; **10**: 2150-2157 [PMID: 15041736 DOI: 10.1158/1078-0432.CCR-03-1028]
  - 33 **Poon RT**, Ho JW, Tong CS, Lau C, Ng IO, Fan ST. Prognostic significance of serum vascular endothelial growth factor and endostatin in patients with hepatocellular carcinoma. *Br J Surg* 2004; **91**: 1354-1360 [PMID: 15376182 DOI: 10.1002/bjs.4594]
  - 34 **Vogelstein B**, Kinzler KW. Cancer genes and the pathways they control. *Nat Med* 2004; **10**: 789-799 [DOI: 10.1038/nm1087]
  - 35 **Fraisl P**, Baes M, Carmeliet P. Hungry for blood vessels: linking metabolism and angiogenesis. *Dev Cell* 2008; **14**: 313-314 [PMID: 18331707 DOI: 10.1016/j.devcel.2008.02.009]
  - 36 **Vizán P**, Sánchez-Tena S, Alcarraz-Vizán G, Soler M, Messegue R, Pujol MD, Lee WN, Cascante M. Characterization of the metabolic changes underlying growth factor angiogenic activation: identification of new potential therapeutic targets. *Carcinogenesis* 2009; **30**: 946-952 [PMID: 19369582 DOI: 10.1093/carcin/bgp083]
  - 37 **Yamashita T**, Wang XW. Cancer stem cells in the development of liver cancer. *J Clin Invest* 2013; **123**: 1911-1918 [PMID: 23635789 DOI: 10.1172/JCI66024]
  - 38 **Lok AS**, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, Morgan TR, Kim HY, Lee WM, Bonkovsky HL, Dienstag JL. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. *Gastroenterology* 2010; **138**: 493-502 [PMID: 19852963 DOI: 10.1053/j.gastro.2009.10.031]
  - 39 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
  - 40 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
  - 41 **Nicholson JK**, Lindon JC. Systems biology: Metabonomics. *Nature* 2008; **455**: 1054-1056 [PMID: 18948945 DOI: 10.1038/4551054a]
  - 42 **Fiehn O**. Combining genomics, metabolome analysis, and biochemical modelling to understand metabolic networks. *Comp Funct Genomics* 2001; **2**: 155-168 [PMID: 18628911 DOI: 10.1002/cfg.82]
  - 43 **Want EJ**, Cravatt BF, Siuzdak G. The expanding role of mass spectrometry in metabolite profiling and characterization. *Chem-biochem* 2005; **6**: 1941-1951 [PMID: 16206229 DOI: 10.1002/cbic.200500151]
  - 44 **Want EJ**, Nordström A, Morita H, Siuzdak G. From exogenous

- to endogenous: the inevitable imprint of mass spectrometry in metabolomics. *J Proteome Res* 2007; **6**: 459-468 [PMID: 17269703 DOI: 10.1021/pr060505]
- 45 **Holmes E**, Loo RL, Stampler J, Bictash M, Yap IK, Chan Q, Ebbels T, De Iorio M, Brown IJ, Veselkov KA, Daviglus ML, Kesteloot H, Ueshima H, Zhao L, Nicholson JK, Elliott P. Human metabolic phenotype diversity and its association with diet and blood pressure. *Nature* 2008; **453**: 396-400 [PMID: 18425110 DOI: 10.1038/nature06882]
  - 46 **Williams HR**, Cox IJ, Walker DG, North BV, Patel VM, Marshall SE, Jewell DP, Ghosh S, Thomas HJ, Teare JP, Jakobovits S, Zeki S, Welsh KI, Taylor-Robinson SD, Orchard TR. Characterization of inflammatory bowel disease with urinary metabolic profiling. *Am J Gastroenterol* 2009; **104**: 1435-1444 [PMID: 19491857 DOI: 10.1038/ajg.2009.175]
  - 47 **Bertini I**, Calabrò A, De Carli V, Luchinat C, Nepi S, Porfiro B, Renzi D, Saccenti E, Tenori L. The metabonomic signature of celiac disease. *J Proteome Res* 2009; **8**: 170-177 [PMID: 19072164 DOI: 10.1021/pr800548z]
  - 48 **Gao H**, Dong B, Liu X, Xuan H, Huang Y, Lin D. Metabonomic profiling of renal cell carcinoma: high-resolution proton nuclear magnetic resonance spectroscopy of human serum with multivariate data analysis. *Anal Chim Acta* 2008; **624**: 269-277 [PMID: 18706333 DOI: 10.1016/j.aca.2008.06.051]
  - 49 **Gao H**, Lu Q, Liu X, Cong H, Zhao L, Wang H, Lin D. Application of 1H NMR-based metabonomics in the study of metabolic profiling of human hepatocellular carcinoma and liver cirrhosis. *Cancer Sci* 2009; **100**: 782-785 [PMID: 19469021 DOI: 10.1111/j.1349-7006.2009.01086.x]
  - 50 **Nicholson JK**, Foxall PJ, Spraul M, Farrant RD, Lindon JC. 750 MHz 1H and 1H-13C NMR spectroscopy of human blood plasma. *Anal Chem* 1995; **67**: 793-811 [PMID: 7762816 DOI: 10.1021/ac00101a004]
  - 51 **Khan SA**, Cox IJ, Thillainayagam AV, Bansal DS, Thomas HC, Taylor-Robinson SD. Proton and phosphorus-31 nuclear magnetic resonance spectroscopy of human bile in hepatopancreaticobiliary cancer. *Eur J Gastroenterol Hepatol* 2005; **17**: 733-738 [PMID: 15947550]
  - 52 **Yang Y**, Li C, Nie X, Feng X, Chen W, Yue Y, Tang H, Deng F. Metabonomic studies of human hepatocellular carcinoma using high-resolution magic-angle spinning 1H NMR spectroscopy in conjunction with multivariate data analysis. *J Proteome Res* 2007; **6**: 2605-2614 [PMID: 17564425 DOI: 10.1021/pr070063h]
  - 53 **Pauling L**, Robinson AB, Teranishi R, Cary P. Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. *Proc Natl Acad Sci USA* 1971; **68**: 2374-2376 [PMID: 5289873 DOI: 10.1073/pnas.68.10.2374]
  - 54 **Gomaa AI**, Khan SA, Leen EL, Waked I, Taylor-Robinson SD. Diagnosis of hepatocellular carcinoma. *World J Gastroenterol* 2009; **15**: 1301-1314 [PMID: 19294759 DOI: 10.3748/wjg.15.1301]
  - 55 **Sell S**. Alpha-fetoprotein, stem cells and cancer: how study of the production of alpha-fetoprotein during chemical hepatocarcinogenesis led to reaffirmation of the stem cell theory of cancer. *Tumour Biol* 2008; **29**: 161-180 [PMID: 18612221 DOI: 10.1159/000143402]
  - 56 **Daniele B**, Bencivenga A, Megna AS, Tinessa V. Alpha-fetoprotein and ultrasonography screening for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S108-S112 [PMID: 15508073 DOI: 10.1053/j.gastro.2004.09.023]
  - 57 **Sherman M**, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology* 1995; **22**: 432-438 [PMID: 7543434 DOI: 10.1002/hep.1840220210]
  - 58 **Trevisani F**, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, Domenicali M, De Notariis S, Roda E, Bernardi M. Serum  $\alpha$ -fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol* 2001; **34**: 570-575 [DOI: 10.1016/S0168-8278(00)00053-2]
  - 59 **Lok AS**, Lai C.  $\alpha$ -fetoprotein monitoring in chinese patients with chronic hepatitis B virus infection: Role in the early detection of hepatocellular carcinoma. *Hepatology* 1989; **9**: 110-115 [DOI: 10.1002/hep.1840090119]
  - 60 **Khien VV**, Mao HV, Chinh TT, Ha PT, Bang MH, Lac BV, Hop TV, Tuan NA, Don LV, Taketa K, Satomura S. Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology-proven hepatocellular carcinoma. *Int J Biol Markers* 2001; **16**: 105-111 [PMID: 11471892]
  - 61 **Kumada T**, Nakano S, Takeda I, Kiriyaama S, Sone Y, Hayashi K, Katoh H, Endoh T, Sassa T, Satomura S. Clinical utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in small hepatocellular carcinoma: special reference to imaging diagnosis. *J Hepatol* 1999; **30**: 125-130 [PMID: 9927159]
  - 62 **Grizzi F**, Franceschini B, Hamrick C, Frezza EE, Cobos E, Chiriva-Internati M. Usefulness of cancer-testis antigens as biomarkers for the diagnosis and treatment of hepatocellular carcinoma. *J Transl Med* 2007; **5**: 3 [PMID: 17244360]
  - 63 **Marrero JA**, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, Lok AS. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. *Hepatology* 2003; **37**: 1114-1121 [PMID: 12717392 DOI: 10.1053/jhep.2003.50195]
  - 64 **Volk ML**, Hernandez JC, Su GL, Lok AS, Marrero JA. Risk factors for hepatocellular carcinoma may impair the performance of biomarkers: a comparison of AFP, DCP, and AFP-L3. *Cancer Biomark* 2007; **3**: 79-87 [PMID: 17522429]
  - 65 **Nakamura S**, Nouse K, Sakaguchi K, Ito YM, Ohashi Y, Kobayashi Y, Toshikuni N, Tanaka H, Miyake Y, Matsumoto E, Shiratori Y. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. *Am J Gastroenterol* 2006; **101**: 2038-2043 [PMID: 16848811]
  - 66 **Ishizuka H**, Nakayama T, Matsuoka S, Gotoh I, Ogawa M, Suzuki K, Tanaka N, Tsubaki K, Ohkubo H, Arakawa Y, Okano T. Prediction of the development of hepato-cellular-carcinoma in patients with liver cirrhosis by the serial determinations of serum alpha-L-fucosidase activity. *Intern Med* 1999; **38**: 927-931 [PMID: 10628928 DOI: 10.2169/internalmedicine.38.927]
  - 67 **Tangkijvanich P**, Tosukhowong P, Bunyongyod P, Lertmaharit S, Hanvivatvong O, Kullavanijaya P, Poovorawan Y. Alpha-L-fucosidase as a serum marker of hepatocellular carcinoma in Thailand. *Southeast Asian J Trop Med Public Health* 1999; **30**: 110-114 [PMID: 10695798]
  - 68 **Wang JJ**, Cao EH. Rapid kinetic rate assay of the serum alpha-L-fucosidase in patients with hepatocellular carcinoma by using a novel substrate. *Clin Chim Acta* 2004; **347**: 103-109 [PMID: 15313147 DOI: 10.1016/j.cccn.2004.04.007]
  - 69 **el-Houseini ME**, Mohammed MS, Elshemey WM, Hussein TD, Desouky OS, Elsayed AA. Enhanced detection of hepatocellular carcinoma. *Cancer Control* 2005; **12**: 248-253 [PMID: 16258497]
  - 70 **Moon WS**, Rhyu KH, Kang MJ, Lee DG, Yu HC, Yeum JH, Koh GY, Tarnawski AS. Overexpression of VEGF and angiopoietin 2: a key to high vascularity of hepatocellular carcinoma? *Mod Pathol* 2003; **16**: 552-557 [PMID: 12808060 DOI: 10.1097/01.MP.0000071841.17900.69]
  - 71 **Liu Z**, Yan L, Xiang T, Jiang L, Yang B. Expression of vascular endothelial growth factor and matrix metalloproteinase-2 correlates with the invasion and metastasis of hepatocellular carcinoma. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi* 2003; **20**: 249-250, 254 [PMID: 12856590]
  - 72 **Huang GW**, Yang LY, Lu WQ. Expression of hypoxia-inducible factor 1alpha and vascular endothelial growth factor in hepatocellular carcinoma: Impact on neovascularization and survival. *World J Gastroenterol* 2005; **11**: 1705-1708 [PMID: 15786555 DOI: 10.3748/wjg.v11.i11.1705]
  - 73 **Ren Y**, Poon RT, Tsui HT, Chen WH, Li Z, Lau C, Yu WC, Fan ST. Interleukin-8 serum levels in patients with hepatocellular carcinoma: correlations with clinicopathological features and prognosis. *Clin Cancer Res* 2003; **9**: 5996-6001 [PMID: 14676125]
  - 74 **Akiba J**, Yano H, Ogasawara S, Higaki K, Kojiro M. Expression

- and function of interleukin-8 in human hepatocellular carcinoma. *Int J Oncol* 2001; **18**: 257-264 [PMID: 11172590 DOI: 10.3892/ijo.18.2.257]
- 75 **Song BC**, Chung YH, Kim JA, Choi WB, Suh DD, Pyo SI, Shin JW, Lee HC, Lee YS, Suh DJ. Transforming growth factor-beta1 as a useful serologic marker of small hepatocellular carcinoma. *Cancer* 2002; **94**: 175-180 [PMID: 11815974 DOI: 10.1002/cncr.10170]
  - 76 **Sacco R**, Leuci D, Tortorella C, Fiore G, Marinosci F, Schiraldi O, Antonaci S. Transforming growth factor beta1 and soluble Fas serum levels in hepatocellular carcinoma. *Cytokine* 2000; **12**: 811-814 [PMID: 10843770 DOI: 10.1006/cyto.1999.0650]
  - 77 **Zhou L**, Liu J, Luo F. Serum tumor markers for detection of hepatocellular carcinoma. *World J Gastroenterol* 2006; **12**: 1175-1181 [PMID: 16534867]
  - 78 **Murakami A**, Fukushima C, Yositori K, Sueoka K, Nawata S, Fujimoto M, Nakamura K, Sugino N. Tumor-related protein, the squamous cell carcinoma antigen binds to the intracellular protein carbonyl reductase. *Int J Oncol* 2010; **36**: 1395-1400 [PMID: 20428762 DOI: 10.3892/ijo\_00000624]
  - 79 **Hussein MM**, Ibrahim AA, Abdella HM, Montasser IF, Hassan MI. Evaluation of serum squamous cell carcinoma antigen as a novel biomarker for diagnosis of hepatocellular carcinoma in Egyptian patients. *Indian J Cancer* 2008; **45**: 167-172 [PMID: 19112206 DOI: 10.4103/0019-509X.44666]
  - 80 **Abu El Makarem M**. An overview of biomarkers for the diagnosis of hepatocellular carcinoma. *Hepat Mon* 2012; **12**: e6122 [PMID: 23162601 DOI: 10.5812/hepatmon.6122]
  - 81 **Chuma M**, Sakamoto M, Yamazaki K, Ohta T, Ohki M, Asaka M, Hirohashi S. Expression profiling in multistage hepatocarcinogenesis: identification of HSP70 as a molecular marker of early hepatocellular carcinoma. *Hepatology* 2003; **37**: 198-207 [PMID: 12500205 DOI: 10.1053/jhep.2003.50022]
  - 82 **Nahon P**, Amathieu R, Triba MN, Bouchemal N, Nault JC, Ziol M, Seror O, Dhonneur G, Trinchet JC, Beaugrand M, Le Moyec L. Identification of serum proton NMR metabolomic fingerprints associated with hepatocellular carcinoma in patients with alcoholic cirrhosis. *Clin Cancer Res* 2012; **18**: 6714-6722 [PMID: 23136190 DOI: 10.1158/1078-0432.CCR-12-1099]
  - 83 **Wei S**, Suryani Y, Gowda GA, Skill N, Maluccio M, Raftery D. Differentiating hepatocellular carcinoma from hepatitis C using metabolite profiling. *Metabolites* 2012; **2**: 701-716 [PMID: 24957758 DOI: 10.3390/metabo2040701]
  - 84 **Liu Y**, Hong Z, Tan G, Dong X, Yang G, Zhao L, Chen X, Zhu Z, Lou Z, Qian B, Zhang G, Chai Y. NMR and LC/MS-based global metabolomics to identify serum biomarkers differentiating hepatocellular carcinoma from liver cirrhosis. *Int J Cancer* 2014; **135**: 658-668 [PMID: 24382646 DOI: 10.1002/ijc.28706]
  - 85 **Assi N**, Fages A, Vineis P, Chadeau-Hyam M, Stepien M, Duarte-Salles T, Byrnes G, Boumaza H, Knüppel S, Kühn T, Palli D, Bamia C, Boshuizen H, Bonet C, Overvad K, Johansson M, Travis R, Gunter MJ, Lund E, Dossus L, Elena-Herrmann B, Riboli E, Jenab M, Viallon V, Ferrari P. A statistical framework to model the meeting-in-the-middle principle using metabolomic data: application to hepatocellular carcinoma in the EPIC study. *Mutagenesis* 2015; **30**: 743-753 [PMID: 26130468]
  - 86 **Baniasadi H**, Gowda GA, Gu H, Zeng A, Zhuang S, Skill N, Maluccio M, Raftery D. Targeted metabolic profiling of hepatocellular carcinoma and hepatitis C using LC-MS/MS. *Electrophoresis* 2013; **34**: 2910-2917 [PMID: 23856972 DOI: 10.1002/elps.201300029]
  - 87 **Chen F**, Xue J, Zhou L, Wu S, Chen Z. Identification of serum biomarkers of hepatocarcinoma through liquid chromatography/mass spectrometry-based metabolomic method. *Anal Bioanal Chem* 2011; **401**: 1899-1904 [PMID: 21833635 DOI: 10.1007/s00216-011-5245-3]
  - 88 **Chen S**, Kong H, Lu X, Li Y, Yin P, Zeng Z, Xu G. Pseudotargeted metabolomics method and its application in serum biomarker discovery for hepatocellular carcinoma based on ultra high-performance liquid chromatography/triple quadrupole mass spectrometry. *Anal Chem* 2013; **85**: 8326-8333 [PMID: 23889541 DOI: 10.1021/ac4016787]
  - 89 **Chen S**, Yin P, Zhao X, Xing W, Hu C, Zhou L, Xu G. Serum lipid profiling of patients with chronic hepatitis B, cirrhosis, and hepatocellular carcinoma by ultra fast LC/IT-TOF MS. *Electrophoresis* 2013; **34**: 2848-2856 [PMID: 24228263 DOI: 10.1002/elps.201200629]
  - 90 **Huang Q**, Tan Y, Yin P, Ye G, Gao P, Lu X, Wang H, Xu G. Metabolic characterization of hepatocellular carcinoma using nontargeted tissue metabolomics. *Cancer Res* 2013; **73**: 4992-5002 [PMID: 23824744 DOI: 10.1158/0008-5472.CAN-13-0308]
  - 91 **Patterson AD**, Maurhofer O, Beyoglu D, Lanz C, Krausz KW, Pabst T, Gonzalez FJ, Dufour JF, Idle JR. Aberrant lipid metabolism in hepatocellular carcinoma revealed by plasma metabolomics and lipid profiling. *Cancer Res* 2011; **71**: 6590-6600 [PMID: 21900402 DOI: 10.1158/0008-5472.CAN-11-0885]
  - 92 **Ressom HW**, Xiao JF, Tuli L, Varghese RS, Zhou B, Tsai TH, Ranjbar MR, Zhao Y, Wang J, Di Poto C, Cheema AK, Tadesse MG, Goldman R, Shetty K. Utilization of metabolomics to identify serum biomarkers for hepatocellular carcinoma in patients with liver cirrhosis. *Anal Chim Acta* 2012; **743**: 90-100 [PMID: 22882828 DOI: 10.1016/j.aca.2012.07.013]
  - 93 **Wang B**, Chen D, Chen Y, Hu Z, Cao M, Xie Q, Chen Y, Xu J, Zheng S, Li L. Metabonomic profiles discriminate hepatocellular carcinoma from liver cirrhosis by ultraperformance liquid chromatography-mass spectrometry. *J Proteome Res* 2012; **11**: 1217-1227 [PMID: 22200553 DOI: 10.1021/pr2009252]
  - 94 **Xiao JF**, Varghese RS, Zhou B, Nezami Ranjbar MR, Zhao Y, Tsai TH, Di Poto C, Wang J, Goerlitz D, Luo Y, Cheema AK, Sarhan N, Soliman H, Tadesse MG, Ziada DH, Ransom HW. LC-MS based serum metabolomics for identification of hepatocellular carcinoma biomarkers in Egyptian cohort. *J Proteome Res* 2012; **11**: 5914-5923 [PMID: 23078175 DOI: 10.1021/pr300673x]
  - 95 **Xue R**, Lin Z, Deng C, Dong L, Liu T, Wang J, Shen X. A serum metabolomic investigation on hepatocellular carcinoma patients by chemical derivatization followed by gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 2008; **22**: 3061-3068 [PMID: 18767022 DOI: 10.1002/rcm.3708]
  - 96 **Yin P**, Wan D, Zhao C, Chen J, Zhao X, Wang W, Lu X, Yang S, Gu J, Xu G. A metabonomic study of hepatitis B-induced liver cirrhosis and hepatocellular carcinoma by using RP-LC and HILIC coupled with mass spectrometry. *Mol Biosyst* 2009; **5**: 868-876 [PMID: 19603122 DOI: 10.1039/b820224a]
  - 97 **Zhou L**, Ding L, Yin P, Lu X, Wang X, Niu J, Gao P, Xu G. Serum metabolic profiling study of hepatocellular carcinoma infected with hepatitis B or hepatitis C virus by using liquid chromatography-mass spectrometry. *J Proteome Res* 2012; **11**: 5433-5442 [PMID: 22946841 DOI: 10.1021/pr300683a]
  - 98 **Zhou L**, Wang Q, Yin P, Xing W, Wu Z, Chen S, Lu X, Zhang Y, Lin X, Xu G. Serum metabolomics reveals the deregulation of fatty acids metabolism in hepatocellular carcinoma and chronic liver diseases. *Anal Bioanal Chem* 2012; **403**: 203-213 [PMID: 22349331 DOI: 10.1007/s00216-012-5782-4]
  - 99 **Shang S**, Plymoth A, Ge S, Feng Z, Rosen HR, Sangrajang S, Hainaut P, Marrero JA, Beretta L. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. *Hepatology* 2012; **55**: 483-490 [DOI: 10.1002/hep.24703]
  - 100 **Linkous AG**, Yazlovitskaya EM, Hallahan DE. Cytosolic phospholipase A2 and lysophospholipids in tumor angiogenesis. *J Natl Cancer Inst* 2010; **102**: 1398-1412 [PMID: 20729478 DOI: 10.1093/jnci/djq290]
  - 101 **Morita Y**, Sakaguchi T, Ikegami K, Goto-Inoue N, Hayasaka T, Hang VT, Tanaka H, Harada T, Shibasaki Y, Suzuki A, Fukumoto K, Inaba K, Murakami M, Setou M, Konno H. Lysophosphatidylcholine acyltransferase 1 altered phospholipid composition and regulated hepatoma progression. *J Hepatol* 2013; **59**: 292-299 [PMID: 23567080 DOI: 10.1016/j.jhep.2013.02.030]
  - 102 **Chen T**, Xie G, Wang X, Fan J, Qiu Y, Zheng X, Qi X, Cao Y, Su M, Wang X, Xu LX, Yen Y, Liu P, Jia W. Serum and urine metabolite profiling reveals potential biomarkers of human hepatocellular carcinoma. *Mol Cell Proteomics* 2011; **10**: M110.004945 [PMID: 21518826 DOI: 10.1074/mcp.M110.004945]
  - 103 **Wu H**, Xue R, Dong L, Liu T, Deng C, Zeng H, Shen X. Metabolomic profiling of human urine in hepatocellular carcinoma



- patients using gas chromatography/mass spectrometry. *Anal Chim Acta* 2009; **648**: 98-104 [PMID: 19616694 DOI: 10.1016/j.aca.2009.06.033]
- 104 **Neale G**, Lewis B, Weaver V, Panveliwalla D. Serum bile acids in liver disease. *Gut* 1971; **12**: 145-152 [PMID: 5548561 DOI: 10.1136/gut.12.2.145]
  - 105 **Chen Y**, Xu Z, Kong H, Chen N, Chen J, Zhou L, Wang F, Dong Y, Zheng S, Chen Z. Differences between the metabolic profiles of decompensated and compensated cirrhosis patients with Hepatitis B virus infections under high-performance liquid chromatography-mass spectrometry. *Metabolomics* 2012; **8**: 845-853 [DOI: 10.1007/s11306-011-0379-z]
  - 106 **Thomas C**, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov* 2008; **7**: 678-693 [PMID: 18670431 DOI: 10.1038/nrd2619]
  - 107 **Baptissart M**, Vega A, Maqdasy S, Caira F, Baron S, Lobaccaro JM, Volle DH. Bile acids: from digestion to cancers. *Biochimie* 2013; **95**: 504-517 [PMID: 22766017 DOI: 10.1016/j.biochi.2012.06.022]
  - 108 **LaRusso NF**, Hoffman NE, Korman MG, Hofmann AF, Cowen AE. Determinants of fasting and postprandial serum bile acid levels in healthy man. *Am J Dig Dis* 1978; **23**: 385-391 [PMID: 677089 DOI: 10.1007/BF01072919]
  - 109 **Criss WE**, Murad F. Urinary excretion of cyclic guanosine 3':5'-monophosphate and cyclic adenosine 3':5'-monophosphate in rats bearing transplantable liver and kidney tumors. *Cancer Res* 1976; **36**: 1714-1716 [PMID: 178429]
  - 110 **Dusheiko GM**, Levin J, Kew MC. Cyclic nucleotides in biological fluids in hepatocellular carcinoma. *Cancer* 1981; **47**: 113-118 [PMID: 6257369]
  - 111 **Tamura S**, Amuro Y, Nakano T, Fujii J, Moriwaki Y, Yamamoto T, Hada T, Higashino K. Urinary excretion of pseudouridine in patients with hepatocellular carcinoma. *Cancer* 1986; **57**: 1571-1575 [PMID: 2418945]
  - 112 **Jeng LB**, Lo WY, Hsu WY, Lin WD, Lin CT, Lai CC, Tsai FJ. Analysis of urinary nucleosides as helper tumor markers in hepatocellular carcinoma diagnosis. *Rapid Commun Mass Spectrom* 2009; **23**: 1543-1549 [PMID: 19399767 DOI: 10.1002/rcm.4034]
  - 113 **Katoh M**, Inagaki H, Kurosawa-Ohsawa K, Katsuura M, Tanaka S. Detection of transforming growth factor alpha in human urine and plasma. *Biochem Biophys Res Commun* 1990; **167**: 1065-1072 [PMID: 2157422]
  - 114 **Chuang LY**, Tsai JH, Yeh YC, Chang CC, Yeh HW, Guh JY, Tsai JF. Epidermal growth factor-related transforming growth factors in the urine of patients with hepatocellular carcinoma. *Hepatology* 1991; **13**: 1112-1116 [PMID: 1646759]
  - 115 **Tsai JF**, Jeng JE, Chuang LY, Yang ML, Ho MS, Chang WY, Hsieh MY, Lin ZY, Tsai JH. Clinical evaluation of urinary transforming growth factor-beta1 and serum alpha-fetoprotein as tumour markers of hepatocellular carcinoma. *Br J Cancer* 1997; **75**: 1460-1466 [PMID: 9166938 DOI: 10.1038/bjc.1997.250]
  - 116 **Daito K**, Suou T, Kawasaki H. Clinical significance of serum and urinary neopterin levels in patients with various liver diseases. *Am J Gastroenterol* 1992; **87**: 471-476 [PMID: 1313206]
  - 117 **Kawasaki H**, Watanabe H, Yamada S, Watanabe K, Suyama A. Prognostic significance of urinary neopterin levels in patients with hepatocellular carcinoma. *Tohoku J Exp Med* 1988; **155**: 311-318 [PMID: 2852855 DOI: 10.1620/tjem.155.311]
  - 118 **Sucher R**, Schroecksnadel K, Weiss G, Margreiter R, Fuchs D, Brandacher G. Neopterin, a prognostic marker in human malignancies. *Cancer Lett* 2010; **287**: 13-22 [PMID: 19500901 DOI: 10.1016/j.canlet.2009.05.008]
  - 119 **Wishart DS**, Tzur D, Knox C, Eisner R, Guo AC, Young N, Cheng D, Jewell K, Arndt D, Sawhney S, Fung C, Nikolai L, Lewis M, Coutouly MA, Forsythe I, Tang P, Shrivastava S, Jeroncic K, Stothard P, Amegbey G, Block D, Hau DD, Wagner J, Miniaci J, Clements M, Gebremedhin M, Guo N, Zhang Y, Duggan GE, Macinnis GD, Weljie AM, Dowlatabadi R, Bamforth F, Clive D, Greiner R, Li L, Marrie T, Sykes BD, Vogel HJ, Querengesser L. HMDB: the Human Metabolome Database. *Nucleic Acids Res* 2007; **35**: D521-D526 [PMID: 17202168]
  - 120 **Antonello S**, Auletta M, Magri P, Pardo F. Urinary excretion of free and acetylated polyamines in hepatocellular carcinoma. *Int J Biol Markers* 1998; **13**: 92-97 [PMID: 9803357]
  - 121 **Lin SD**, Endo R, Kuroda H, Kondo K, Miura Y, Takikawa Y, Kato A, Suzuki K. Plasma and urine levels of urinary trypsin inhibitor in patients with chronic liver diseases and hepatocellular carcinoma. *J Gastroenterol Hepatol* 2004; **19**: 327-332 [PMID: 14748881]
  - 122 **Kikuchi I**, Uchinami H, Nanjo H, Hashimoto M, Nakajima A, Kume M, Mencin A, Yamamoto Y. Clinical and prognostic significance of urinary trypsin inhibitor in patients with hepatocellular carcinoma after hepatectomy. *Ann Surg Oncol* 2009; **16**: 2805-2817 [PMID: 19636634 DOI: 10.1245/s10434-009-0622-2]
  - 123 **Chen J**, Wang W, Lv S, Yin P, Zhao X, Lu X, Zhang F, Xu G. Metabonomics study of liver cancer based on ultra performance liquid chromatography coupled to mass spectrometry with HILIC and RPLC separations. *Anal Chim Acta* 2009; **650**: 3-9 [PMID: 19720165 DOI: 10.1016/j.aca.2009.03.039]
  - 124 **Shariff MI**, Ladep NG, Cox IJ, Williams HR, Okeke E, Malu A, Thillainayagam AV, Crossey MM, Khan SA, Thomas HC, Taylor-Robinson SD. Characterization of urinary biomarkers of hepatocellular carcinoma using magnetic resonance spectroscopy in a Nigerian population. *J Proteome Res* 2010; **9**: 1096-1103 [PMID: 19968328 DOI: 10.1021/pr901058t]
  - 125 **Shariff MI**, Gomaa AI, Cox IJ, Patel M, Williams HR, Crossey MM, Thillainayagam AV, Thomas HC, Waked I, Khan SA, Taylor-Robinson SD. Urinary metabolic biomarkers of hepatocellular carcinoma in an Egyptian population: a validation study. *J Proteome Res* 2011; **10**: 1828-1836 [PMID: 21275434 DOI: 10.1021/pr101096f]
  - 126 **Ladep NG**, Dona AC, Lewis MR, Crossey MM, Lemoine M, Okeke E, Shimakawa Y, Duguru M, Njai HF, Fye HK, Taal M, Chetwood J, Kasstan B, Khan SA, Garside DA, Wijeyesekera A, Thillainayagam AV, Banwat E, Thurst MR, Nicholson JK, Njie R, Holmes E, Taylor-Robinson SD. Discovery and validation of urinary metabolites for the diagnosis of hepatocellular carcinoma in West Africans. *Hepatology* 2014; **60**: 1291-1301 [PMID: 24923488 DOI: 10.1002/hep.27264]
  - 127 **Oterdoom LH**, Gansevoort RT, Schouten JP, de Jong PE, Gans RO, Bakker SJ. Urinary creatinine excretion, an indirect measure of muscle mass, is an independent predictor of cardiovascular disease and mortality in the general population. *Atherosclerosis* 2009; **207**: 534-540 [PMID: 19535078 DOI: 10.1016/j.atherosclerosis.2009.05.010]
  - 128 **Flanagan JL**, Simmons PA, Vehige J, Willcox MD, Garrett Q. Role of carnitine in disease. *Nutr Metab (Lond)* 2010; **7**: 30 [PMID: 20398344 DOI: 10.1186/1743-7075-7-30]
  - 129 **Nguyen MH**, Garcia RT, Simpson PW, Wright TL, Keefe EB. Racial differences in effectiveness of alpha-fetoprotein for diagnosis of hepatocellular carcinoma in hepatitis C virus cirrhosis. *Hepatology* 2002; **36**: 410-417 [PMID: 12143050]

P- Reviewer: Hashimoto N, Pompili M, Zhong JH  
S- Editor: Qi Y L- Editor: A E- Editor: Liu SQ





## Host nucleotide polymorphism in hepatitis B virus-associated hepatocellular carcinoma

Shilu Mathew, Hany Abdel-Hafiz, Abbas Raza, Kaneez Fatima, Ishtiaq Qadri

Shilu Mathew, Center of Excellence in Genomic Medicine Research, King Abdul Aziz University, Jeddah 21589, Saudi Arabia

Hany Abdel-Hafiz, University of Colorado Denver AMC, Aurora, CO 80045, United States

Abbas Raza, Department of Immunobiology, University of Vermont, Burlington, VT 05405, United States

Kaneez Fatima, IQ-Institute of Infection and Immunity, Lahore 54000, Pakistan

Ishtiaq Qadri, King Fahd Medical Research Center, King Abdul Aziz University, Jeddah 21589, Saudi Arabia

Author contributions: All authors contributed to this manuscript.

Supported by The STACK-Large grant 162-34 to Ishtiaq Qadri; IQ Foundation.

Conflict-of-interest statement: All authors disclose no conflict of interest.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Ishtiaq Qadri, PhD, Professor, King Fahd Medical Research Center, King Abdul Aziz University, PO Box 80216 Jeddah 21589, Saudi Arabia. [ishtiaq80262@yahoo.com](mailto:ishtiaq80262@yahoo.com)  
 Telephone: +966-12-6400000  
 Fax: +966-12-6952067

Received: September 12, 2015  
 Peer-review started: September 15, 2015  
 First decision: November 13, 2015  
 Revised: December 4, 2015

Accepted: March 7, 2016

Article in press: March 9, 2016

Published online: April 8, 2016

### Abstract

Hepatocellular carcinoma (HCC) is etiologically linked with hepatitis B virus (HBV) and is the leading cause of death amongst 80% of HBV patients. Among HBV affected patients, genetic factors are also involved in modifying the risk factors of HCC. However, the genetic factors that regulate progression to HCC still remain to be determined. In this review, we discuss several single nucleotide polymorphisms (SNPs) which were reportedly associated with increased or reduced risk of HCC occurrence in patients with chronic HBV infection such as cyclooxygenase (COX)-2 expression specifically at COX-2 -1195G/A in Chinese, Turkish and Egyptian populations, tumor necrosis factor  $\alpha$  and the three most commonly studied SNPs: PAT-/+, Lys939Gln (A33512C, rs2228001) and Ala499Val (C21151T, rs2228000). In genome-wide association studies, strong associations have also been found at loci 1p36.22, 11q22.3, 6p21 (rs1419881, rs3997872, rs7453920 and rs7768538), 8p12 (rs2275959 and rs37821974) and 22q11.21. The genes implicated in these studies include *HLA-DQB2*, *HLA-DQA1*, *TCF19*, *HLA-C*, *UBE2L3*, *LTL*, *FDX1*, *MICA*, *UBE4B* and *PG*. The SNPs found to be associated with the above-mentioned genes still require validation in association studies in order to be considered good prognostic candidates for HCC. Screening of these polymorphisms is very beneficial in clinical experiments to stratify the higher or lower risk for HCC and may help in designing effective and efficient HCC surveillance programs for chronic HBV-infected patients if further genetic vulnerabilities are detected.

**Key words:** Hepatitis B virus; Hepatocellular carcinoma; Subtypes; Genetic polymorphism; Liver cirrhosis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In this review, we discuss various common associations between hepatitis B virus (HBV) and host polymorphisms. These single nucleotide polymorphisms which have been found to be associated with various genes still require validation in association studies in order to be considered good prognostic candidates for hepatocellular carcinoma (HCC). Screening of these polymorphisms is very beneficial in clinical experiments to stratify the higher or lower risk for HCC and may help in designing effective and efficient HCC surveillance programs for chronic HBV-infected patients if further genetic vulnerabilities are detected.

Mathew S, Abdel-Hafiz H, Raza A, Fatima K, Qadri I. Host nucleotide polymorphism in hepatitis B virus-associated hepatocellular carcinoma. *World J Hepatol* 2016; 8(10): 485-498 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i10/485.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i10.485>

## HEPATITIS B VIRUS

Hepatitis B virus (HBV) infection is the third most common cause of cancer-related deaths in relation to hepatocellular carcinoma (HCC) with a high incidence in Asian countries. HCC is responsible for approximately 660000 deaths worldwide each year and 85%-90% of these deaths are due to primary liver cancers<sup>[1]</sup>. It is recognized that these cancers are mainly due to HBV infection with 60% of HCC cases seropositive for this virus<sup>[2]</sup>. Many risk factors including viral factors (e.g., genomic mutations, genotypes, HBV-DNA levels), host factors and unhealthy lifestyles all contribute to the development of liver diseases<sup>[3]</sup>.

Both epigenetic and genetic factors play a role in the malignant transformation of liver cells<sup>[4]</sup>. Multiple cellular signaling genes are enhanced by the incorporation of HBV into the host's genome which promotes transactivation of HBx protein<sup>[5]</sup>. This process activates/inactivates suppressor genes (e.g., *p53*), oncogenic genes (e.g., *c-fos* and *c-myc*), induces loss of heterozygosity and activates transcriptional factors [e.g., nuclear factor kappa-B (NF-κB) and AP-1]<sup>[6]</sup>.

However, underlying disease and the duration of severity vary significantly between each phase. Moreover, clinical progression varies between patients. Liver injuries in patients with HBV infection are thought to be the outcome of the host's immune responses against HBV. For example, cytotoxic T lymphocyte-mediated, an HLA-class I antigen-restricted, response to the HBV antigen expressed on hepatocytes results in necrosis and apoptosis<sup>[7]</sup>.

Several genome wide association studies have identified candidate single nucleotide polymorphisms

(SNPs) by comparing the SNPs present in HCC patients and those present in asymptomatic HBV carriers<sup>[8]</sup>. Therefore, to specifically evaluate genetic factors, it is vital that the controls and patients are well matched regarding these factors to identify the correct SNP. The results of many studies suggest that several SNPs are associated with HBV clearance and persistent infection. Functional analyses are necessary to confirm these results<sup>[6,7]</sup>. In this review, we discuss several SNPs which are reportedly associated with increased or reduced risk of HCC occurrence in patients with chronic HBV infection<sup>[9]</sup>.

## INFLAMMATORY GENETIC POLYMORPHISM

It has been reported previously that SNPs can affect disease progression after HBV infection. Cytokines, such as tumor necrosis factor-α (TNFα) and interleukin (IL)-10, have a significant role in regulating viral infection. Genetic variation of these cytokines is linked with the outcome of HBV infection<sup>[10-16]</sup>.

Several studies have shown that genetic polymorphisms in multiple genes such as *TP53*<sup>[17]</sup>, *IL-6*<sup>[18]</sup>, and DNA repair genes<sup>[19]</sup>, are associated with the development of chronic HBC infection, progression of the infection and increased risk of HCC. These may serve as biomarkers in identifying HCC risk<sup>[20]</sup>. However, these studies were predominantly performed in HBV-positive populations or populations with a high infection rate.

Genetic variation in tumor suppressor genes or oncogenes is capable of altering gene function and, consequently, may contribute to the development of cancer. Significant research has been conducted to investigate the association between polymorphisms in tumor suppressor genes and oncogenes and the risk of HCC; however, the results are controversial.

## ASSOCIATIONS BETWEEN HBV AND THE HOST POLYMORPHISM

### Cyclooxygenase-2

Cyclooxygenase-2 (COX-2) is involved in many cellular functions, including inflammation, inhibition of apoptosis, carcinogenesis, angiogenesis, invasion and metastasis<sup>[21,22]</sup>. COX-2 is overexpressed in many cancers including HCC, indicating that there is an association between COX-2 expression and the development of cancer<sup>[23,24]</sup>. Selective COX-2 inhibitors have been shown to suppress the growth of HCC cells *in vitro* and *in vivo*<sup>[25]</sup>. A polymorphism in the promoter region of the COX-2 gene could functionally upregulate the transcriptional activity of COX-2, indicating a possible mechanism by which COX-2 may contribute to genetic susceptibility to HCC<sup>[21]</sup>. Several studies have reported that COX-2 point mutations including -1195G/A, -765G/C and +8473T/C were correlated with liver diseases and

HBV-related HCC<sup>[26]</sup>. COX-2-765G/C is related to the risk of skin, esophageal, colorectal, breast and gastric cancers<sup>[27-29]</sup>. With regard to HCC, contradictory and inconclusive results were found. Some studies have reported a correlation between COX-2-765G/C and HBV-related HCC risk<sup>[30-32]</sup>, but other studies reported that no such correlation exists<sup>[26,33,34]</sup>. It has been reported that these inconsistent results were possibly due to limited sample sizes and ethnic variation in those studies. COX-2 + 8473T/C is associated with oral and breast cancers<sup>[35,36]</sup>, but is not associated with HCC<sup>[37]</sup>. A recent meta-analysis by Chen *et al.*<sup>[26]</sup> on Chinese, Turkish and Egyptian populations, concluded that COX-2-1195G/A may be associated with HCC risk, but not COX-2-765G/C or COX-2 + 847T/C.

### ***IL-1alpha and 1beta***

IL-1 $\alpha$  is a potent pro-inflammatory cytokine and has many different biological functions, including cell survival, proliferation, and anti-apoptosis<sup>[38,39]</sup>. IL-1 $\beta$  is also reported to inhibit interferon-induced antiviral activity<sup>[40]</sup> and is assumed to be closely associated with the pathogenesis of chronic hepatitis C. Several polymorphisms of the *IL-1* gene that are thought to affect IL-1 $\beta$  production have been reported<sup>[41]</sup>. -31T SNPs of IL-1 $\beta$  have been shown to enhance IL-1 $\beta$  transcriptional activity<sup>[42]</sup> and several studies reported that -511C/-31T is a risk factor for the development of cancer and liver diseases<sup>[43-45]</sup>. Wang *et al.*<sup>[41]</sup> showed that IL-1 $\beta$ -31 polymorphism was associated with HCC, after controlling for other confounding clinical parameters.

### ***E-cadherin (CDH1)***

E-cadherin is a transmembrane protein that mediates cell-cell adhesion and is expressed in most normal epithelial cells. Downregulation of E-cadherin may lead to a loss of E-cadherin-mediated adhesion, resulting in increased susceptibility to tumor development and is associated with poor prognosis in various carcinomas including HCC<sup>[45-52]</sup>. In addition, HBV and HCV reduce E-cadherin expression and promote tumor recurrence in HCC patients. One of the mechanisms that have been proposed for reduced E-cadherin expression is SNPs in the promoter region of the *CDH1* gene. CDH1-160 C/A and -347G/GA polymorphisms result in the downregulation of E-cadherin protein and is associated with cancer susceptibility<sup>[53]</sup>. Several studies demonstrated that CDH1-347 SNPs are significantly associated with HCC risk<sup>[52,54-57]</sup>. However, the correlation between CDH1-160 SNPs showed conflicting results. Some studies<sup>[58,59]</sup> have shown that CDH1-160 SNP carriers have an increased risk of prostate and bladder cancer, while others showed that it was not associated with the development of prostate, HCC, colorectal or gastric cancer<sup>[60]</sup>.

### ***Peroxisome proliferator-activated receptor gamma***

Peroxisome proliferator-activated receptor gamma

(PPAR $\gamma$ ) is a hormone receptor, present in adipose tissue and plays a critical role in the regulation of fatty acid storage and glucose metabolism<sup>[61]</sup>. PPAR $\gamma$  has been shown to be associated with type 2 diabetes mellitus (T2DM)<sup>[62]</sup>. PPAR $\gamma$  contains two isoforms, PPAR $\gamma$ 1 and PPAR $\gamma$ 2 and several variants in the *PPAR $\gamma$*  gene have been identified<sup>[63]</sup>. The A allele of PPAR $\gamma$ 2 is associated with a significant decrease in the development of T2DM<sup>[64]</sup>. The relationship between PPAR and HCC is not clear. Although experimental studies have shown that PPAR may have a role in HCC<sup>[65,66]</sup>, the implications of these findings are unclear. Koytak *et al.*<sup>[66]</sup> investigated the effect of the PPAR $\alpha$  L162V polymorphism on clinical outcome in a patient with HCC caused by hepatitis viruses. They concluded that there was a relationship between the PPAR $\alpha$  L162V polymorphism and HBV-induced HCC and was associated with advanced HCC. This polymorphism was shown to enhance PPAR $\alpha$  transcriptional activity and is associated with lipid abnormalities and an increased body mass index<sup>[67-70]</sup>.

### ***TNF $\alpha$ -inducible protein 3***

TNF $\alpha$ -inducible protein 3 (TNF $\alpha$ IP3), a cytoplasmic zinc finger protein with ubiquitin-modifying activity, has been shown to inhibit NF- $\kappa$ B activity and TNF-mediated apoptosis<sup>[71-74]</sup>. TNF $\alpha$ IP3 polymorphisms have been linked to inflammatory, autoimmune and malignant diseases. A recent study reported that there was no association between TNF $\alpha$ IP3 rs2230926 polymorphism and susceptibility to chronic HBV infection or the progression of HBV-related diseases<sup>[75]</sup>.

### ***Cytotoxic T lymphocyte-associated factor 4***

Cytotoxic T lymphocyte-associated factor 4 (CTLA-4) is a protein receptor expressed in T cells and it functions as a negative regulator of the immune system. Several *CTLA-4* gene polymorphisms have been identified including -318C>T, A49G and CT60<sup>[76]</sup>. CTLA-4 polymorphisms are associated with several autoimmune diseases, including thyroid and liver diseases<sup>[77,78]</sup>. It has been shown that SNPs in CTLA-4 may be associated with HBV progression and viral persistence<sup>[79]</sup>. CTLA-4 SNPs can be used as a marker for predicting treatment outcome in chronic HCV-infected patients<sup>[80-82]</sup>.

### ***TNF $\alpha$***

TNF $\alpha$  is a multifunctional cytokine that regulates the inflammatory reaction and has an important role in the development and progression of a number of diseases, including liver disease<sup>[83,84]</sup>. It has been suggested that genetic polymorphisms of TNF $\alpha$  may contribute to the pathogenesis of liver diseases, infectious diseases and inflammatory disorders<sup>[43,85]</sup>. For example, TNF $\alpha$  SNPs affect TNF $\alpha$  production leading to a greater risk of HCC. The polymorphism at site -1031T/C, -863C/A, -857C/T, -376, -308G/A and -238G/A of the TNF $\alpha$  promoter is associated with the outcome of HBV infection and disease progression<sup>[86-89]</sup>.

### IL-10

IL-10 is an important anti-inflammatory cytokine produced in macrophages. Three SNPs in the *IL-10* gene promoter, at -1082, -819 and -592, are associated with IL-10 production and secretion by peripheral blood monocytes. It has been shown that IL-10-592 A/C polymorphism was associated with susceptibility to HBV infection<sup>[90]</sup>.

### Glutathione S-transferases

The glutathione S-transferases (GSTs) enzymes play an important role in maintaining the cellular defense mechanism against the effects of reactive oxygen species and various exogenous toxins, and have been shown to be overexpressed in several cancers<sup>[91,92]</sup>. Deletion polymorphism of *GST* genes results in diminished enzyme activity leading to the insufficient defense of cells from metabolites and free radicals, elevated concentration of endogenous mutagens and a high risk of various tumors, including HCC<sup>[93-96]</sup>. GSTs polymorphisms have been shown to be associated with colorectal cancer, lung cancer, squamous cell carcinoma of the head and neck, HBV-related HCC, and various urogenital and gastrointestinal disorders<sup>[97-99]</sup>. For example, meta-analyses have shown that GSTM1, GSP1 and GSTT1 are associated with an increased risk of HCC<sup>[100,101]</sup>.

### Epidermal growth factor

Epidermal growth factor (EGF) and its respective receptor (EGFR) signaling are important regulators of proliferation and the pathogenesis of many human carcinomas<sup>[102,103]</sup>. Upon ligand binding, the two EGFR domains undergo trans-autophosphorylation at specific tyrosine residues<sup>[104]</sup>. These phosphotyrosines are recognized by Src homology 2 domain containing proteins<sup>[105]</sup> and activate a diverse signaling network that includes the RAS/extracellular signal-regulated kinase pathway<sup>[106]</sup>, the phosphatidylinositol 3-kinase pathway<sup>[107]</sup> and the Janus kinase/Signal transducer and activator of transcription pathway<sup>[108]</sup>.

Activation of EGF has also been shown to be required for hepatocyte growth during liver regeneration<sup>[109]</sup>. In addition, many viruses such as Epstein Barr virus and HBV can tweak EGF receptor expression in their favor<sup>[110-112]</sup>. The role of EGF polymorphism has been explored in numerous meta-analyses<sup>[113-116]</sup> and was shown to be highly associated with susceptibility to HCC<sup>[117]</sup>. Prominent among these is the EGF + 61A > G transversion (rs4444903) which was shown to regulate expression of the *EGF* gene<sup>[118,119]</sup>. This SNP is found in the 5' untranslated regions of the *EGF* gene and was shown in cell lines to enhance the stability of EGF mRNA<sup>[119]</sup>. The G/G allele is associated with higher serum levels of EGF compared with the A/A allele<sup>[119,120]</sup>. Numerous follow-up studies have validated the positive association between this G/G and G/A genotype with HCC in diverse genetic populations<sup>[117,121-123]</sup> and thus can be considered a good prognostic marker for the

genetically susceptible population.

### Murine double minute 2

Murine double minute 2 (MDM2) is a ubiquitin ligase that controls the turnover rate of an important tumor suppressor, p53, which is deleted or mutated in 50% of all human tumors<sup>[124]</sup>. P53 is also referred to as the guardian of the genome because it can activate DNA repair pathways<sup>[125]</sup>, arrest cell cycle at the G1/S regulation checkpoint<sup>[126]</sup> or initiate apoptosis if the damage cannot be repaired<sup>[127]</sup>. All these important networks converge in the active form of p53, which is kept in check by MDM2. The addition of ubiquitin subunits to critical lysine residues transfers the active p53 to 26S proteasome for degradation along with MDM2<sup>[128,129]</sup>. In addition, the binding of MDM2 can block p53-mediated transactivation functions<sup>[130]</sup>. The activity of MDM2 protein is equally important in regulating this DNA repair-cell cycle-apoptosis nexus and variation in the expression levels of this protein was shown to have serious consequences in cells or organisms<sup>[131]</sup>. Bond *et al.*<sup>[132]</sup> showed that the SNP 309T > G (rs 2279744) located in the promoter region of MDM2 can enhance the transcriptional levels of this protein and subsequent perturbation of p53 functions in the cell. This T > G mutation is thought to generate a binding site on the MDM2 promoter for Sp1 transcription factor<sup>[133]</sup> and thus enhances the levels of MDM2 protein in the cell.

The positive association between this SNP 309T > G (rs 2279744) in the *MDM2* gene and HCC was shown by numerous ethnic-based studies<sup>[134-136]</sup> and meta-analyses<sup>[137,138]</sup>. This epidemiological finding together with functional assays of MDM2 levels point to the relevance of MDM2 SNP 309T > G polymorphism as an important player in susceptibility to HCC development.

### T cell immunoglobulin mucin-3

T cell immunoglobulin mucin-3 (TIM3) negatively regulates the autoimmune and allergic responses and has been linked to T cell dysfunction associated with HBV-related HCC<sup>[139]</sup>. The 280 aa mature TIM3 is selectively expressed on CD4<sup>+</sup> Th1 and CD8<sup>+</sup> Tc1 cells, but not on CD4<sup>+</sup> Th2 cells<sup>[140]</sup>. It interacts with its ligand galectin-9 and drives death Th1 T cells<sup>[141,142]</sup>. Blocking TIM3-mediated signaling restores dysfunctional CD4 and CD8<sup>+</sup> T cell-specific adaptive immune responses<sup>[143]</sup>. TIM3 is upregulated on CD4 and CD8<sup>+</sup> T cells in chronic HBV infected individuals<sup>[144]</sup>.

Numerous potential SNPs (-1541C/T, -1516G/T, -882C/T, -574G/T and +4259T/G) in TIM3 have been tested for their association with chronic HBV and HCC<sup>[145]</sup>. TIM3-1516 G/T (rs10053538) polymorphism has been shown to predispose individuals to cirrhosis and/or HCC<sup>[146,147]</sup>. One study reported that TIM3 SNPs do not have a functional effect<sup>[148]</sup>, whereas others have reported a significant effect of these TIM3 polymorphic variants<sup>[149]</sup>. Further studies are needed to determine the functional relevance of this polymorphism.



**Xeroderma pigmentosum complementation group C**

Xeroderma pigmentosum complementation group C (XPC) protein along with seven other core members (ERCC1, XPA, XPB, XPD, XPE, XPF and XPG) constitutes the nucleotide excision repair pathway (NER). This pathway is required for the repair of DNA damage including pyrimidine dimers, photo products, chemical adducts and cross-links<sup>[150,151]</sup>. XPC requires an association with HR23B in order to recognize damaged DNA<sup>[152]</sup>. The protein HR23B is a human homolog of *Saccharomyces cerevisiae* RAD23 and binding of XPC-HR23B to a DNA lesion unwinds the helix<sup>[153]</sup>. The XPA protein can then bind and the whole repair machinery of the NER can be recruited onto the damaged base.

Many studies have investigated the association between XPC sequence variants and cancer risk<sup>[154-158]</sup>. The three most commonly studied SNPs in the literature are: PAT-/+<sup>[159]</sup>, Lys939Gln (A33512C, rs2228001)<sup>[155]</sup> and Ala499Val (C21151T, rs2228000)<sup>[160]</sup>. The poly (AT) insertion/deletion polymorphism (PAT) is located on intron 9 and has been shown to be linked to head and neck cancer risk<sup>[161]</sup> and to lung cancer<sup>[162]</sup>, but no studies have found an association with HCC risk. The XPC codon Lys939Gln alleles, on the other hand, significantly increased HCC risk<sup>[163,164]</sup>. The Ala499Val variant homozygous genotype is a risk factor for bladder cancer<sup>[158]</sup>, but has not been studied for HCC.

**IL-16**

IL-16 is a pro-inflammatory cytokine and was initially called lymphocyte chemoattractant factor<sup>[165]</sup>. It can activate a diverse set of immune cells such as CD4<sup>+</sup> T cells, monocytes, macrophages, eosinophils and dendritic cells<sup>[166-169]</sup>. In addition to inducing activation and chemotaxis of immune cells, IL-16 can upregulate the IL-2 receptor<sup>[170]</sup> and HLA-DR4 expression<sup>[171]</sup>. Upon CD4 receptor binding, IL-16 signaling increases intracellular calcium and inositol triphosphate, and translocation of protein kinase C from the cytosol to the plasma membrane<sup>[172,173]</sup>. Moreover, IL-16 can stimulate the production of further pro-inflammatory mediators including IL-1 $\beta$ , IL-6, IL-15 and TNF $\alpha$ , *e.g.*, by monocytes<sup>[174]</sup> thereby initiating and/or sustaining the inflammatory response.

Genetic polymorphisms in IL-16 have recently been reported and shown to affect susceptibility to a range of cancers including colorectal, gastric and prostate cancer and nasopharyngeal carcinoma<sup>[175-178]</sup>. Data regarding HCC and IL-16 polymorphisms are scarce in the literature and only two studies were found to have assessed three SNPs (rs11556218T > G, rs4778889T > C, and rs4072111C > T)<sup>[179]</sup>. In the study by Li *et al.*<sup>[180]</sup>, no association with HCC was found for all three SNPs (rs11556218T/G *P* = 0.511, rs4072111C/T *P* = 0.308 and rs4778889T/C *P* = 0.070). The other study by Thomas *et al.*<sup>[178]</sup> did not include HCC patients. However, this study did include chronic hepatitis B patients who showed a positive association between rs11556218T

> G, a negative association between rs4778889T > C and a positive association between rs4072111C > T polymorphisms and patient susceptibility to chronic hepatitis B infection<sup>[179]</sup>.

**Genome-wide association studies**

Numerous genome-wide association studies (GWAS) have been carried out with chronic HBV and HCC patients to identify novel susceptible loci contributing to disease<sup>[6,181-186]</sup>. Of these, strong associations were found at 1p36.22, 11q22.3, 6p21 (rs1419881, rs3997872, rs7453920 and rs7768538), 8p12 (rs2275959 and rs37821974) and 22q11.21. The genes implicated in these studies include HLA-DQB2, HLA-DQA1, transcription factor 19 (TCF19), HLA-C, ubiquitin-conjugating enzyme E2 (UBE2L3), LTL, ferredoxin 1 (FDX1), MICA, UBE4B and PG.

HLA-DQ is an MHC class II cell surface receptor found on antigen presenting cells, whereas HLA-C is an MHC class I receptor expressed by all cells. TCF19, as the name suggests, is an important transcription factor during cell cycle G1/S transition<sup>[187]</sup>. UBE2L3 is a typical E2 ligase that accepts ubiquitin from the E1 complex and transfers it to targeted proteins<sup>[188]</sup>. Leukocyte telomere length (LTL) has been associated with the risk of developing many malignancies<sup>[189]</sup> and LTL-related SNPs are potential targets for such GWAS studies. FDX1 is a gene that codes for a small iron-sulfur protein that transfers electrons from NADPH through ferredoxin reductase to mitochondrial cytochrome P450<sup>[190]</sup>. In addition, it is involved in steroid, vitamin D, and bile acid metabolism<sup>[191]</sup>.

These SNPs found to be associated with the above-mentioned genes still require validation in association studies in order to be considered good prognostic candidates for HCC.

**Tumor growth factor beta**

Tumor growth factor beta (TGF $\beta$ ) is a tumor suppressor gene located on chromosome 19q13.1-13.39. The protein TGF $\beta$  is involved in pleiotropic biological processes such as cell growth<sup>[192]</sup>, differentiation<sup>[193]</sup>, extracellular matrix synthesis<sup>[194]</sup>, hematopoiesis<sup>[195]</sup>, angiogenesis<sup>[196]</sup>, and cellular apoptosis<sup>[197]</sup>. TGF $\beta$ 1 is one of TGF $\beta$  isoforms and is upregulated in HCC tissues correlating with the carcinogenesis and prognosis of HCC<sup>[198,199]</sup>. TGF $\beta$ 1 also suppresses HBV replication by reducing hepatocyte nuclear factor-4- $\alpha$ <sup>[200]</sup>. Thus, the relevance of this cytokine and its single nucleotide polymorphism in HBV-associated HCC is of paramount importance.

Seven TGF $\beta$ 1 polymorphisms have been described in the literature, of which three lie in the upstream region of the gene at positions -988C > A, -800G > A, and -509C > T, one insertion in a nontranslated region at position +72C, two in exon 1 (Leu10Pro and Arg25Pro); and 1 in exon 5 (Thr263Ile)<sup>[201]</sup>. Numerous studies have investigated the association between these

**Table 1** List of polymorphic genes and their contribution to hepatocellular carcinoma

Polymorphism	Genotype	Significance	Ref.
COX-2	-1195G > A	$P < 0.00$ <sup>[26]</sup>	He <i>et al</i> <sup>[33]</sup>
	-765G > C	$P < 0.05$ <sup>[31]</sup> and $0.41$ <sup>[26]</sup>	Chen <i>et al</i> <sup>[26]</sup>
	+8473T > C	$P = 0.83$ <sup>[26]</sup>	
IL-1 $\alpha$ , $\beta$	511C > T	$P = 0.02$ <sup>[41]</sup>	Wang <i>et al</i> <sup>[41]</sup>
	-31C > T	$P = 0.02$ <sup>[41]</sup>	
CDH1	-347G > A	$P = 0.171$ <sup>[209]</sup> and $< 0.05$ <sup>[60]</sup>	Li <i>et al</i> <sup>[209]</sup> , Chien <i>et al</i> <sup>[60]</sup>
PPAR $\gamma$	L162V	$P = 0.071$ <sup>[66]</sup>	Koytak <i>et al</i> <sup>[66]</sup>
TNFAIP3	F127C	$P = 0.15$ <sup>[75]</sup>	Zhang <i>et al</i> <sup>[75]</sup>
TNF $\alpha$	-1031T/C	$P = 0.85$ <sup>[86]</sup>	Wei <i>et al</i> <sup>[86]</sup>
	-863C/A	$P = 0.006$ <sup>[86]</sup>	
	-857C/T	$P = 0.09$ <sup>[86]</sup>	
	-308G/A	$P = 0.046$ <sup>[86]</sup>	
	-238G/A	$P = 0.003$ <sup>[86]</sup>	
GST	GSTM1 + GSTT1	$P = 0.001$ <sup>[210]</sup>	Liu <i>et al</i> <sup>[210]</sup>
EGF	+61A > G	$P < 0.001$ <sup>[117]</sup>	Jiang <i>et al</i> <sup>[117]</sup>
MDM2	309G > T	$P = 0.001$ <sup>[133]</sup>	Ezzikouri <i>et al</i> <sup>[133]</sup>
TIM3	-1516G > T	$P = 0.001$ <sup>[146]</sup>	Li <i>et al</i> <sup>[146]</sup>
XPC	K939Q	$P = 0.001$ <sup>[163]</sup>	Long <i>et al</i> <sup>[163]</sup>
1p36.22, 11q22.3, 6p21, 8p12 22q11.21	Include genes HLA-DQB2, HLA-DQA1, TCF19, HLA-C, UBE2L3, LTL, FDX1, MICA, UBE4B and PG	$P = 1.7 \times 10^{-18}$ $P = 4.3 \times 10^{-8}$ $P = 0.0266$ $P = 0.0067$ $P = 1.71 \times 10^{-12}$	Al-Qahtani <i>et al</i> <sup>[181]</sup>
TGF $\beta$ 1	-509C > T	$P = 0.01$ <sup>[206]</sup> and $0.318$ <sup>[207]</sup>	Qi <i>et al</i> <sup>[206]</sup>
	R25P	$P = 0.472$ <sup>[207]</sup>	Hosseini Razavi <i>et al</i> <sup>[207]</sup>
	L10P	$P < 0.02$ <sup>[208]</sup>	Kim <i>et al</i> <sup>[208]</sup>

COX-2: Cyclooxygenase-2; IL-1 $\alpha$ ,  $\beta$ : Interleukin-1 $\alpha$ ,  $\beta$ ; CDH1: Cadherin 1; PPAR $\gamma$ : Peroxisome proliferator-activated receptor  $\gamma$ ; TNFAIP3: Tumor necrosis factor alpha-induced protein 3; TNF $\alpha$ : Tumor necrosis factor  $\alpha$ ; GST: Glutathione S transferase; EGF: Epidermal growth factor; MDM2: Mouse double minute 2 homolog; TIM3: T-cell immunoglobulin 3; XPC: Xeroderma pigmentosum; TGF $\beta$ 1: Transforming growth factor beta 1.

SNPs and HCC<sup>[202-205]</sup>. There are contrasting reports with some studies reporting a positive association between -509C > T (rs1800469) and HCC risk<sup>[206]</sup>, whereas another study reported a weak or no association<sup>[204]</sup>. In addition, the Arg25Pro change at +915G/C (rs1800471) was not correlated with HCC risk<sup>[207]</sup>. The mutation in codon 10 (Leu > Pro) was very strongly correlated with HCC according to one study<sup>[208]</sup>. There is still limited information regarding other polymorphisms of TGF $\beta$ 1 and further studies are required to draw firm conclusions on their association with HCC. Table 1 lists the polymorphic genes and their contribution to HCC.

## DISCUSSION

In this article, we discuss the association between the HBV genotype and its mutations in the development of liver cancer and the possibility that individuals with inherited genetic mutations have a hereditary predisposition for HBV-related HCC. Such individuals can inherit a germ-line mutation in one allele of the gene; somatic mutation of the second allele facilitates tumor progression. Although the inherited germ-line mutation may not be adequate to affect tumor development, it is likely that HBV proteins also induce many alterations in the genome. Analysis of the whole transcriptome in these individuals with genetic predisposition would be a useful indicator. It is now well understood that host genetic differences significantly influence susceptibility

and resistance to HBV infection and the development of liver cancer, thus it is important to identify these genotype-phenotype associations for better treatment of the disease (Figure 1). Genome-wide sequencing studies have identified numerous germline mutations associated with liver cancer predisposition and large numbers of somatic alterations. It is difficult to assess the difference between background and HBV-related mutations as HBV infection plays an important role in the development of host genetic mutations, due to impairment in the DNA repair process. To elucidate the role of HBV-related genetic variations, researchers have used traditional biological methods to identify genetic mutations. More recently, advanced techniques such as next generation sequencing technology have been used to identify key mutations involved in the development of HCC. Important HCC-associated mutations have been found in key regulatory genes including COX-2, IL-1 $\alpha$  and  $\beta$ , E-cadherin (CDH1), PPAR $\gamma$ , TNF $\alpha$ IP3, CTLA-4, TNF $\alpha$ , IL-10, GSTM1/GSTT1 Deletion Oxidative stress, EGF, MDM2, TIM3), XPC, IL-16, TGF $\beta$ , 1p36.22, 11q22.3, 6p21, 8p12 and 22q11.21 candidate SNPs in GWAS. The association between each locus and the outcome of liver disease is discussed in detail in this article.

Based on these findings, we predict that advanced sequence analysis of host genome will provide us with a better understanding of the viral and host genetic factors involved in the development of HCC. Further studies are needed to evaluate and understand the role



## REFERENCES

- 1 **El-Serag HB.** Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]
- 2 **Lai CL,** Ratzliff V, Yuen MF, Poynard T. Viral hepatitis B. *Lancet* 2003; **362**: 2089-2094 [PMID: 14697813 DOI: 10.1016/s0140-6736(03)15108-2]
- 3 **Yim HJ,** Lok AS. Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. *Hepatology* 2006; **43**: S173-S181 [PMID: 16447285 DOI: 10.1002/hep.20956]
- 4 **Sherman M.** Hepatocellular carcinoma: epidemiology, surveillance, and diagnosis. *Semin Liver Dis* 2010; **30**: 3-16 [PMID: 20175029 DOI: 10.1055/s-0030-1247128]
- 5 **Paterlini-Bréchet P,** Saigo K, Murakami Y, Chami M, Gozuacik D, Mugnier C, Lagorce D, Bréchet C. Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene. *Oncogene* 2003; **22**: 3911-3916 [PMID: 12813464 DOI: 10.1038/sj.onc.1206492]
- 6 **Kamatani Y,** Wattanapokayakit S, Ochi H, Kawaguchi T, Takahashi A, Hosono N, Kubo M, Tsunoda T, Kamatani N, Kumada H, Puseenam A, Sura T, Daigo Y, Chayama K, Chantravita W, Nakamura Y, Matsuda K. A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. *Nat Genet* 2009; **41**: 591-595 [PMID: 19349983 DOI: 10.1038/ng.348]
- 7 **Liaw YF.** Hepatitis flares and hepatitis B e antigen seroconversion: implication in anti-hepatitis B virus therapy. *J Gastroenterol Hepatol* 2003; **18**: 246-252 [PMID: 12603523 DOI: 10.1046/j.1440-1746.2003.02976.x]
- 8 **Sokal EM,** Paganelli M, Wirth S, Socha P, Vajro P, Lacaille F, Kelly D, Mieli-Vergani G. Management of chronic hepatitis B in childhood: ESPGHAN clinical practice guidelines: consensus of an expert panel on behalf of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition. *J Hepatol* 2013; **59**: 814-829 [PMID: 23707367 DOI: 10.1016/j.jhep.2013.05.016]
- 9 **Cheng HR,** Liu CJ, Tseng TC, Su TH, Yang HI, Chen CJ, Kao JH. Host genetic factors affecting spontaneous HBsAg seroclearance in chronic hepatitis B patients. *PLoS One* 2013; **8**: e53008 [PMID: 23326374 DOI: 10.1371/journal.pone.0053008]
- 10 **Cheong JY,** Cho SW, Hwang IL, Yoon SK, Lee JH, Park CS, Lee JE, Hahm KB, Kim JH. Association between chronic hepatitis B virus infection and interleukin-10, tumor necrosis factor- $\alpha$  gene promoter polymorphisms on disease progression in patients chronically infected with hepatitis B virus. *Am J Gastroenterol* 2002; **97**: 2086-2092 [PMID: 12190181 DOI: 10.1111/j.1572-0241.2002.05926.x]
- 11 **Du T,** Guo XH, Zhu XL, Li JH, Lu LP, Gao JR, Gou CY, Li Z, Liu Y, Li H. Association of TNF- $\alpha$  promoter polymorphisms with the outcomes of hepatitis B virus infection in Chinese Han population. *J Viral Hepat* 2006; **13**: 618-624 [PMID: 16907849 DOI: 10.1111/j.1365-2893.2006.00731.x]
- 12 **Wu JF,** Wu TC, Chen CH, Ni YH, Chen HL, Hsu HY, Chang MH. Serum levels of interleukin-10 and interleukin-12 predict early, spontaneous hepatitis B virus e antigen seroconversion. *Gastroenterology* 2010; **138**: 165-172.e1-3 [PMID: 19782084 DOI: 10.1053/j.gastro.2009.09.018]
- 13 **Wu JF,** Ni YH, Lin YT, Lee TJ, Hsu SH, Chen HL, Tsuei DJ, Hsu HY, Chang MH. Human interleukin-10 genotypes are associated with different precore/core gene mutation patterns in children with chronic hepatitis B virus infection. *J Pediatr* 2011; **158**: 808-813 [PMID: 21168854 DOI: 10.1016/j.jpeds.2010.11.015]
- 14 **Xia Q,** Zhou L, Liu D, Chen Z, Chen F. Relationship between TNF- $\alpha$  gene promoter polymorphisms and outcomes of hepatitis B virus infections: a meta-analysis. *PLoS One* 2011; **6**: e19606 [PMID: 21572952 DOI: 10.1371/journal.pone.0019606]
- 15 **Chatzidakis V,** Kouroumalis E, Galanakis E. Hepatitis B virus acquisition and pathogenesis in childhood: host genetic determinants. *J Pediatr Gastroenterol Nutr* 2011; **52**: 3-8 [PMID: 21119536 DOI: 10.1097/MPG.0b013e3181fb0cb9]
- 16 **Ortiz-Cuaran S,** Villar S, Gouas D, Ferro G, Plymoth A, Kluhuprema T, Kalalak A, Sangrajang S, Friesen MD, Groopman JD, Hainaut P. Association between HBx status, aflatoxin-induced R249S TP53 mutation and risk of hepatocellular carcinoma in a case-control study from Thailand. *Cancer Lett* 2013; **331**: 46-51 [PMID: 23200676 DOI: 10.1016/j.canlet.2012.11.012]
- 17 **Giannitrapani L,** Soresi M, Giacalone A, Campagna ME, Marasà M, Cervello M, Marasà S, Montalto G. IL-6 -174G/C polymorphism and IL-6 serum levels in patients with liver cirrhosis and hepatocellular carcinoma. *OMICS* 2011; **15**: 183-186 [PMID: 21329460 DOI: 10.1089/omi.2010.0093]
- 18 **Gulnaz A,** Sayyed AH, Amin F, Khan Au, Aslam MA, Shaikh RS, Ali M. Association of XRCC1, XRCC3, and XPD genetic polymorphism with an increased risk of hepatocellular carcinoma because of the hepatitis B and C virus. *Eur J Gastroenterol Hepatol* 2013; **25**: 166-179 [PMID: 23044807 DOI: 10.1097/

- MEG.0b013e328359a775]
- 20 **Su C**, Lin Y, Niu J, Cai L. Association between polymorphisms in tumor suppressor genes and oncogenes and risk of hepatocellular carcinoma: a case-control study in an HCC epidemic area within the Han Chinese population. *Med Oncol* 2014; **31**: 356 [PMID: 25412941 DOI: 10.1007/s12032-014-0356-2]
  - 21 **Wu H**, Wu X, Wan G, Zhang S. Associations between Cox-2 rs20417 and rs5275 polymorphisms and the risk of hepatocellular carcinoma: a meta analysis. *Int J Clin Exp Pathol* 2014; **7**: 6898-6905 [PMID: 25400773]
  - 22 **Miyashita M**, Ito T, Sakaki M, Kajiwar A, Nozawa H, Hiroishi K, Kobayashi M, Kumada H, Imawari M. Genetic polymorphism in cyclooxygenase-2 promoter affects hepatic inflammation and fibrosis in patients with chronic hepatitis C. *J Viral Hepat* 2012; **19**: 608-614 [PMID: 22863264 DOI: 10.1111/j.1365-2893.2011.01580.x]
  - 23 **Rouzer CA**, Marnett LJ. Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chem Rev* 2011; **111**: 5899-5921 [PMID: 21923193 DOI: 10.1021/cr2002799]
  - 24 **Pazhang Y**, Ahmadian S, Javadifar N, Shafiezhadeh M. COX-2 and survivin reduction may play a role in berberine-induced apoptosis in human ductal breast epithelial tumor cell line. *Tumour Biol* 2012; **33**: 207-214 [PMID: 22081376 DOI: 10.1007/s13277-011-0263-5]
  - 25 **Yin J**, Liu B, Li B, Liu Z, Xie X, Lv Z, Gao S, Guang J. The cyclooxygenase-2 inhibitor celecoxib attenuates hepatocellular carcinoma growth and c-Met expression in an orthotopic mouse model. *Oncol Res* 2011; **19**: 131-139 [PMID: 21473289 DOI: 10.3727/096504011X12935427587803]
  - 26 **Chen Z**, Zhu J, Huang C, Lian F, Wu G, Zhao Y. The association between three cyclooxygenase-2 polymorphisms and hepatocellular carcinoma risk: a meta-analysis. *PLoS One* 2015; **10**: e0118251 [PMID: 25730260 DOI: 10.1371/journal.pone.0118251]
  - 27 **Aubin F**, Courivaud C, Bamoulid J, Loupy A, Deschamps M, Ferrand C, Le Corre D, Tiberghien P, Chalopin JM, Legendre C, Thervet E, Humbert P, Saas P, Ducloux D. Influence of cyclooxygenase-2 (COX-2) gene promoter polymorphism at position -765 on skin cancer after renal transplantation. *J Invest Dermatol* 2010; **130**: 2134-2136 [PMID: 20445548 DOI: 10.1038/jid.2010.116]
  - 28 **Ben Nasr H**, Chahed K, Bouaouina N, Chouchane L. PTGS2 (COX-2) -765 G>C functional promoter polymorphism and its association with risk and lymph node metastasis in nasopharyngeal carcinoma. *Mol Biol Rep* 2009; **36**: 193-200 [PMID: 17968676 DOI: 10.1007/s11033-007-9166-3]
  - 29 **Sitarz R**, Leguit RJ, de Leng WW, Polak M, Morsink FM, Bakker O, Maciejewski R, Offerhaus GJ, Milne AN. The COX-2 promoter polymorphism -765 G>C is associated with early-onset, conventional and stump gastric cancers. *Mod Pathol* 2008; **21**: 685-690 [PMID: 18311113 DOI: 10.1038/modpathol.2008.36]
  - 30 **Xu DK**, Zhang XM, Zhao P, Cai JC, Zhao D, Tan W, Guo YL, Lin DX. [Association between single nucleotide polymorphisms in the promoter of cyclooxygenase COX-2 gene and hereditary susceptibility to pancreatic cancer]. *Zhonghua Yi Xue Za Zhi* 2008; **88**: 1961-1965 [PMID: 19062735]
  - 31 **He J**, Zhang Q, Ren Z, Li Y, Li X, Zhou W, Zhang H, Meng W, Yan J, He W. Cyclooxygenase-2 -765 G/C polymorphisms and susceptibility to hepatitis B-related liver cancer in Han Chinese population. *Mol Biol Rep* 2012; **39**: 4163-4168 [PMID: 21800055 DOI: 10.1007/s11033-011-1199-y]
  - 32 **Akkız H**, Bayram S, Bekar A, Akgöllü E, Ülger Y. Functional polymorphisms of cyclooxygenase-2 gene and risk for hepatocellular carcinoma. *Mol Cell Biochem* 2011; **347**: 201-208 [PMID: 21042835 DOI: 10.1007/s11010-010-0629-9]
  - 33 **Gharib AF**, Karam RA, Abd El Rahman TM, Elsayy WH. COX-2 polymorphisms -765G→C and -1195A→G and hepatocellular carcinoma risk. *Gene* 2014; **543**: 234-236 [PMID: 24720952 DOI: 10.1016/j.gene.2014.04.014]
  - 34 **Chang WS**, Yang MD, Tsai CW, Cheng LH, Jeng LB, Lo WC, Lin CH, Huang CY, Bau DT. Association of cyclooxygenase 2 single-nucleotide polymorphisms and hepatocellular carcinoma in Taiwan. *Chin J Physiol* 2012; **55**: 1-7 [PMID: 22242948 DOI: 10.4077/CJP.2012.AMM056]
  - 35 **Langsenlehner U**, Yazdani-Biuki B, Eder T, Renner W, Wascher TC, Paulweber B, Weitzer W, Samonigg H, Krippel P. The cyclooxygenase-2 (PTGS2) 8473T>C polymorphism is associated with breast cancer risk. *Clin Cancer Res* 2006; **12**: 1392-1394 [PMID: 16489098 DOI: 10.1158/1078-0432.CCR-05-2055]
  - 36 **Upadhyay R**, Jain M, Kumar S, Ghoshal UC, Mittal B. Functional polymorphisms of cyclooxygenase-2 (COX-2) gene and risk for esophageal squamous cell carcinoma. *Mutat Res* 2009; **663**: 52-59 [PMID: 19428370 DOI: 10.1016/j.mrfmmm.2009.01.007]
  - 37 **Pan F**, Tian J, Pan Y, Zhang Y. Lack of association of the cyclooxygenase 8473 T>C polymorphism with lung cancer: evidence from 9841 subjects. *Asian Pac J Cancer Prev* 2011; **12**: 1941-1945 [PMID: 22292629]
  - 38 **Tilg H**, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. *N Engl J Med* 2000; **343**: 1467-1476 [PMID: 11078773 DOI: 10.1056/NEJM200011163432007]
  - 39 **Roshak AK**, Jackson JR, McGough K, Chabot-Fletcher M, Mochan E, Marshall LA. Manipulation of distinct NFkappaB proteins alters interleukin-1beta-induced human rheumatoid synovial fibroblast prostaglandin E2 formation. *J Biol Chem* 1996; **271**: 31496-31501 [PMID: 8940164 DOI: 10.1074/jbc.271.49.31496]
  - 40 **Tian Z**, Shen X, Feng H, Gao B. IL-1 beta attenuates IFN-alpha beta-induced antiviral activity and STAT1 activation in the liver: involvement of proteasome-dependent pathway. *J Immunol* 2000; **165**: 3959-3965 [PMID: 11034404 DOI: 10.4049/jimmunol.165.7.3959]
  - 41 **Wang Y**, Kato N, Hoshida Y, Yoshida H, Taniguchi H, Goto T, Moriyama M, Otsuka M, Shiina S, Shiratori Y, Ito Y, Omata M. Interleukin-1beta gene polymorphisms associated with hepatocellular carcinoma in hepatitis C virus infection. *Hepatology* 2003; **37**: 65-71 [PMID: 12500190 DOI: 10.1053/jhep.2003.50017]
  - 42 **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; **404**: 398-402 [PMID: 10746728 DOI: 10.1038/35006081]
  - 43 **Roy N**, Mukhopadhyay I, Das K, Pandit P, Majumder PP, Santra A, Datta S, Banerjee S, Chowdhury A. Genetic variants of TNFα, IL10, IL1β, CTLA4 and TGFβ1 modulate the indices of alcohol-induced liver injury in East Indian population. *Gene* 2012; **509**: 178-188 [PMID: 22902304 DOI: 10.1016/j.gene.2012.07.077]
  - 44 **Takamatsu M**, Yamauchi M, Maezawa Y, Saito S, Maeyama S, Uchikoshi T. Genetic polymorphisms of interleukin-1beta in association with the development of alcoholic liver disease in Japanese patients. *Am J Gastroenterol* 2000; **95**: 1305-1311 [PMID: 10811344 DOI: 10.1111/j.1572-0241.2000.02030.x]
  - 45 **Endo K**, Ueda T, Ueyama J, Ohta T, Terada T. Immunoreactive E-cadherin, alpha-catenin, beta-catenin, and gamma-catenin proteins in hepatocellular carcinoma: relationships with tumor grade, clinicopathologic parameters, and patients' survival. *Hum Pathol* 2000; **31**: 558-565 [PMID: 10836294 DOI: 10.1053/hp.2000.6683]
  - 46 **Huang GT**, Lee HS, Chen CH, Sheu JC, Chiou LL, Chen DS. Correlation of E-cadherin expression and recurrence of hepatocellular carcinoma. *Hepatogastroenterology* 1999; **46**: 1923-1927 [PMID: 10430370]
  - 47 **Conacci-Sorrell M**, Zhurinsky J, Ben-Ze'ev A. The cadherin-catenin adhesion system in signaling and cancer. *J Clin Invest* 2002; **109**: 987-991 [PMID: 11956233 DOI: 10.1172/JCI0215429]
  - 48 **Valizadeh A**, Karayiannakis AJ, el-Hariry I, Kmiot W, Pignatelli M. Expression of E-cadherin-associated molecules (alpha-, beta-, and gamma-catenins and p120) in colorectal polyps. *Am J Pathol* 1997; **150**: 1977-1984 [PMID: 9176391]
  - 49 **Shiozaki H**, Tahara H, Oka H, Miyata M, Kobayashi K, Tamura S, Iihara K, Doki Y, Hirano S, Takeichi M. Expression of



- immunoreactive E-cadherin adhesion molecules in human cancers. *Am J Pathol* 1991; **139**: 17-23 [PMID: 1713020]
- 50 **Pignatelli M**, Ansari TW, Gunter P, Liu D, Hirano S, Takeichi M, Klöppel G, Lemoine NR. Loss of membranous E-cadherin expression in pancreatic cancer: correlation with lymph node metastasis, high grade, and advanced stage. *J Pathol* 1994; **174**: 243-248 [PMID: 7884585 DOI: 10.1002/path.1711740403]
  - 51 **Bringuier PP**, Umbas R, Schaafsma HE, Karthaus HF, Debruyne FM, Schalken JA. Decreased E-cadherin immunoreactivity correlates with poor survival in patients with bladder tumors. *Cancer Res* 1993; **53**: 3241-3245 [PMID: 8324734]
  - 52 **Umbas R**, Schalken JA, Aalders TW, Carter BS, Karthaus HF, Schaafsma HE, Debruyne FM, Isaacs WB. Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high-grade prostate cancer. *Cancer Res* 1992; **52**: 5104-5109 [PMID: 1516067]
  - 53 **Lee HH**, Uen YH, Tian YF, Sun CS, Sheu MJ, Kuo HT, Koay LB, Lin CY, Tzeng CC, Cheng CJ, Tang LY, Tsai SL, Wang AH. Wnt-1 protein as a prognostic biomarker for hepatitis B-related and hepatitis C-related hepatocellular carcinoma after surgery. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1562-1569 [PMID: 19423534 DOI: 10.1158/1055-9965.EPI-09-0039]
  - 54 **Carter BS**, Ewing CM, Ward WS, Treiger BF, Aalders TW, Schalken JA, Epstein JI, Isaacs WB. Allelic loss of chromosomes 16q and 10q in human prostate cancer. *Proc Natl Acad Sci USA* 1990; **87**: 8751-8755 [PMID: 1978938 DOI: 10.1073/pnas.87.22.8751]
  - 55 **Cleton-Jansen AM**, Moerland EW, Kuipers-Dijkshoorn NJ, Callen DF, Sutherland GR, Hansen B, Devilee P, Cornelisse CJ. At least two different regions are involved in allelic imbalance on chromosome arm 16q in breast cancer. *Genes Chromosomes Cancer* 1994; **9**: 101-107 [PMID: 7513539 DOI: 10.1002/gcc.2870090205]
  - 56 **Ribeiro-Filho LA**, Franks J, Sasaki M, Shiina H, Li LC, Nojima D, Arap S, Carroll P, Enokida H, Nakagawa M, Yonezawa S, Dahiya R. CpG hypermethylation of promoter region and inactivation of E-cadherin gene in human bladder cancer. *Mol Carcinog* 2002; **34**: 187-198 [PMID: 12203370 DOI: 10.1002/mc.10064]
  - 57 **Matsumura T**, Makino R, Mitamura K. Frequent down-regulation of E-cadherin by genetic and epigenetic changes in the malignant progression of hepatocellular carcinomas. *Clin Cancer Res* 2001; **7**: 594-599 [PMID: 11297254]
  - 58 **Zhang X**, Ma X, Zhu QG, Li LC, Chen Z, Ye ZQ. Association between a C/A single nucleotide polymorphism of the E-cadherin gene promoter and transitional cell carcinoma of the bladder. *J Urol* 2003; **170**: 1379-1382 [PMID: 14501773 DOI: 10.1097/01.ju.0000084297.43710.e9]
  - 59 **Verhage BA**, van Houwelingen K, Ruijter TE, Kiemeny LA, Schalken JA. Single-nucleotide polymorphism in the E-cadherin gene promoter modifies the risk of prostate cancer. *Int J Cancer* 2002; **100**: 683-685 [PMID: 12209606 DOI: 10.1002/ijc.10541]
  - 60 **Chien MH**, Yeh KT, Li YC, Hsieh YH, Lin CH, Weng MS, Kuo WH, Yang SF. Effects of E-cadherin (CDH1) gene promoter polymorphisms on the risk and clinicopathological development of hepatocellular carcinoma. *J Surg Oncol* 2011; **104**: 299-304 [PMID: 21462191 DOI: 10.1002/jso.21929]
  - 61 **Seemple RK**, Chatterjee VK, O'Rahilly S. PPAR gamma and human metabolic disease. *J Clin Invest* 2006; **116**: 581-589 [PMID: 16511590 DOI: 10.1172/JCI28003]
  - 62 **Gouda HN**, Sagoo GS, Harding AH, Yates J, Sandhu MS, Higgins JP. The association between the peroxisome proliferator-activated receptor-gamma2 (PPARG2) Pro12Ala gene variant and type 2 diabetes mellitus: a HuGE review and meta-analysis. *Am J Epidemiol* 2010; **171**: 645-655 [PMID: 20179158 DOI: 10.1093/aje/kwp450]
  - 63 **Tönjes A**, Stumvoll M. The role of the Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma in diabetes risk. *Curr Opin Clin Nutr Metab Care* 2007; **10**: 410-414 [PMID: 17563457 DOI: 10.1097/MCO.0b013e3281e389d9]
  - 64 **Huguenin GV**, Rosa G. The Ala allele in the PPAR-gamma2 gene is associated with reduced risk of type 2 diabetes mellitus in Caucasians and improved insulin sensitivity in overweight subjects. *Br J Nutr* 2010; **104**: 488-497 [PMID: 20420754 DOI: 10.1017/S0007114510000851]
  - 65 **Gonzalez FJ**. The peroxisome proliferator-activated receptor alpha (PPARalpha): role in hepatocarcinogenesis. *Mol Cell Endocrinol* 2002; **193**: 71-79 [PMID: 12161004 DOI: 10.1016/S0303-7207(02)00098-9]
  - 66 **Koytak ES**, Mizrak D, Bektaş M, Verdi H, Arslan Ergül A, Idilman R, Cinar K, Yurdaydin C, Ersöz S, Karayalçın K, Uzunalımoğlu O, Bozkaya H. PPAR-alpha L162V polymorphism in human hepatocellular carcinoma. *Turk J Gastroenterol* 2008; **19**: 245-249 [PMID: 19119483]
  - 67 **Flavell DM**, Pineda Torra I, Jamshidi Y, Evans D, Diamond JR, Elkeles RS, Bujac SR, Miller G, Talmud PJ, Staels B, Humphries SE. Variation in the PPARalpha gene is associated with altered function in vitro and plasma lipid concentrations in Type II diabetic subjects. *Diabetologia* 2000; **43**: 673-680 [PMID: 10855543 DOI: 10.1007/s001250051357]
  - 68 **Vohl MC**, Lepage P, Gaudet D, Brewer CG, Bétard C, Perron P, Houde G, Cellier C, Faith JM, Després JP, Morgan K, Hudson TJ. Molecular scanning of the human PPARa gene: association of the L162v mutation with hyperapobetalipoproteinemia. *J Lipid Res* 2000; **41**: 945-952 [PMID: 10828087]
  - 69 **Tai ES**, Demissie S, Cupples LA, Corella D, Wilson PW, Schaefer EJ, Ordovas JM. Association between the PPARA L162V polymorphism and plasma lipid levels: the Framingham Offspring Study. *Arterioscler Thromb Vasc Biol* 2002; **22**: 805-810 [PMID: 12006394 DOI: 10.1161/01.ATV.0000012302.11991.42]
  - 70 **Robitaille J**, Brouillette C, Houde A, Lemieux S, Périus L, Tchernof A, Gaudet D, Vohl MC. Association between the PPARalpha-L162V polymorphism and components of the metabolic syndrome. *J Hum Genet* 2004; **49**: 482-489 [PMID: 15309680 DOI: 10.1007/s10038-004-0177-9]
  - 71 **Jäättelä M**, Mouritzen H, Elling F, Bastholm L. A20 zinc finger protein inhibits TNF and IL-1 signaling. *J Immunol* 1996; **156**: 1166-1173 [PMID: 8557994]
  - 72 **Lee EG**, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* 2000; **289**: 2350-2354 [PMID: 11009421 DOI: 10.1126/science.289.5488.2350]
  - 73 **Boone DL**, Turer EE, Lee EG, Ahmad RC, Wheeler MT, Tsui C, Hurley P, Chien M, Chai S, Hitotsumatsu O, McNally E, Pickart C, Ma A. The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. *Nat Immunol* 2004; **5**: 1052-1060 [PMID: 15334086 DOI: 10.1038/ni1110]
  - 74 **Hitotsumatsu O**, Ahmad RC, Tavares R, Wang M, Philpott D, Turer EE, Lee BL, Shiffin N, Advincula R, Malynn BA, Werts C, Ma A. The ubiquitin-editing enzyme A20 restricts nucleotide-binding oligomerization domain containing 2-triggered signals. *Immunity* 2008; **28**: 381-390 [PMID: 18342009 DOI: 10.1016/j.immuni.2008.02.002]
  - 75 **Zhang P**, Li N, Zhu Q, Li F, Yang C, Zeng X, Lv Y, Zhou Z, Han Q, Liu Z. Association between TNFAIP3 nonsynonymous single-nucleotide polymorphism rs2230926 and chronic hepatitis B virus infection in a Chinese Han population. *Virology* 2015; **12**: 33 [PMID: 25890346 DOI: 10.1186/s12985-015-0268-6]
  - 76 **Danilovic DL**, Mendes-Correa MC, Lima EU, Zambrini H, K Barros R, Marui S. Correlations of CTLA-4 gene polymorphisms and hepatitis C chronic infection. *Liver Int* 2012; **32**: 803-808 [PMID: 22136395 DOI: 10.1111/j.1478-3231.2011.02694.x]
  - 77 **Tomer Y**, Davies TF. Searching for the autoimmune thyroid disease susceptibility genes: from gene mapping to gene function. *Endocr Rev* 2003; **24**: 694-717 [PMID: 14570752 DOI: 10.1210/er.2002-0030]
  - 78 **Kristiansen OP**, Larsen ZM, Pociot F. CTLA-4 in autoimmune diseases--a general susceptibility gene to autoimmunity? *Genes Immun* 2000; **1**: 170-184 [PMID: 11196709 DOI: 10.1038/sj.gen.6363655]
  - 79 **Chen M**, Chang Y, Tang F, Xie QH, Li J, Yang H, He XX, Lin JS. Influence of cytotoxic T lymphocyte-associated antigen 4 polymorphisms on the outcomes of hepatitis B virus infection. *Mol*

- Med Rep* 2014; **9**: 645-652 [PMID: 24270470]
- 80 **Yee LJ**, Perez KA, Tang J, van Leeuwen DJ, Kaslow RA. Association of CTLA4 polymorphisms with sustained response to interferon and ribavirin therapy for chronic hepatitis C virus infection. *J Infect Dis* 2003; **187**: 1264-1271 [PMID: 12696006 DOI: 10.1086/374561]
- 81 **Schott E**, Witt H, Hinrichsen H, Neumann K, Weich V, Bergk A, Halangk J, Müller T, Tinjala S, Puhl G, Neuhaus P, Wiedenmann B, Berg T. Gender-dependent association of CTLA4 polymorphisms with resolution of hepatitis C virus infection. *J Hepatol* 2007; **46**: 372-380 [PMID: 17150279 DOI: 10.1016/j.jhep.2006.09.011]
- 82 **Nischalke HD**, Vogel M, Mauss S, Baumgarten A, Lutz T, Danta M, Naumann U, Coenen M, Sauerbruch T, Rockstroh JK, Spengler U, Nattermann J. The cytotoxic lymphocyte antigen 4 polymorphisms affect response to hepatitis C virus-specific therapy in HIV(+) patients with acute and chronic hepatitis C virus co-infection. *AIDS* 2010; **24**: 2001-2007 [PMID: 20588168 DOI: 10.1097/QAD.0b013e32833bedc8]
- 83 **O'Shea RS**, Dasarathy S, McCullough AJ. Alcoholic liver disease. *Am J Gastroenterol* 2010; **105**: 14-32; quiz 33 [PMID: 19904248 DOI: 10.1038/ajg.2009.593]
- 84 **Schuppan D**, Afdhal NH. Liver cirrhosis. *Lancet* 2008; **371**: 838-851 [PMID: 18328931 DOI: 10.1016/S0140-6736(08)60383-9]
- 85 **Rosen HR**, Lentz JJ, Rose SL, Rabkin J, Corless CL, Taylor K, Chou S. Donor polymorphism of tumor necrosis factor gene: relationship with variable severity of hepatitis C recurrence after liver transplantation. *Transplantation* 1999; **68**: 1898-1902 [PMID: 10628771 DOI: 10.1097/00007890-199912270-00014]
- 86 **Wei Y**, Liu F, Li B, Chen X, Ma Y, Yan L, Wen T, Xu M, Wang W, Yang J. Polymorphisms of tumor necrosis factor-alpha and hepatocellular carcinoma risk: a HuGE systematic review and meta-analysis. *Dig Dis Sci* 2011; **56**: 2227-2236 [PMID: 21336601 DOI: 10.1007/s10620-011-1617-y]
- 87 **Wilson AG**, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997; **94**: 3195-3199 [PMID: 9096369 DOI: 10.1073/pnas.94.7.3195]
- 88 **Machado MV**, Martins A, Almeida R, Marques-Vidal P, Gonçalves MS, Camilo ME, Cortez-Pinto H. Does the simultaneous tumor necrosis factor receptor 2, tumor necrosis factor promoter gene polymorphism represent a higher risk for alcoholic liver disease? *Eur J Gastroenterol Hepatol* 2009; **21**: 201-205 [PMID: 19212208 DOI: 10.1097/MEG.0b013e32831016e0]
- 89 **Cookson S**, Constantini PK, Clare M, Underhill JA, Bernal W, Czaja AJ, Donaldson PT. Frequency and nature of cytokine gene polymorphisms in type 1 autoimmune hepatitis. *Hepatology* 1999; **30**: 851-856 [PMID: 10498633 DOI: 10.1002/hep.510300412]
- 90 **Grove J**, Daly AK, Bassendine MF, Gilvarry E, Day CP. Interleukin 10 promoter region polymorphisms and susceptibility to advanced alcoholic liver disease. *Gut* 2000; **46**: 540-545 [PMID: 10716685 DOI: 10.1136/gut.46.4.540]
- 91 **Strange RC**, Spiteri MA, Ramachandran S, Fryer AA. Glutathione-S-transferase family of enzymes. *Mutat Res* 2001; **482**: 21-26 [PMID: 11535245 DOI: 10.1016/S0027-5107(01)00206-8]
- 92 **Mohammadzadeh GS**, Nasseri Moghadam S, Rasaei MJ, Zaree AB, Mahmoodzadeh H, Allameh A. Measurement of glutathione S-transferase and its class-pi in plasma and tissue biopsies obtained after laparoscopy and endoscopy from subjects with esophagus and gastric cancer. *Clin Biochem* 2003; **36**: 283-288 [PMID: 12810157 DOI: 10.1016/S0009-9120(03)00012-2]
- 93 **Parl FF**. Glutathione S-transferase genotypes and cancer risk. *Cancer Lett* 2005; **221**: 123-129 [PMID: 15808397 DOI: 10.1016/j.canlet.2004.06.016]
- 94 **McIlwain CC**, Townsend DM, Tew KD. Glutathione S-transferase polymorphisms: cancer incidence and therapy. *Oncogene* 2006; **25**: 1639-1648 [PMID: 16550164 DOI: 10.1038/sj.onc.1209373]
- 95 **Hayes JD**, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology* 2000; **61**: 154-166 [PMID: 10971201 DOI: 10.1159/000028396]
- 96 **Sun L**, Xi B, Yu L, Gao XC, Shi DJ, Yan YK, Xu DJ, Han Q, Wang C. Association of glutathione S-transferases polymorphisms (GSTM1 and GSTT1) with senile cataract: a meta-analysis. *Invest Ophthalmol Vis Sci* 2010; **51**: 6381-6386 [PMID: 20574021 DOI: 10.1167/iovs.10-5815]
- 97 **Chen SY**, Wang LY, Lun RM, Tsai WY, Lee PH, Lee CS, Ahsan H, Zhang YJ, Chen CJ, Santella RM. Polycyclic aromatic hydrocarbon-DNA adducts in liver tissues of hepatocellular carcinoma patients and controls. *Int J Cancer* 2002; **99**: 14-21 [PMID: 11948486 DOI: 10.1002/ijc.10291]
- 98 **Zhong S**, Tang MW, Yeo W, Liu C, Lo YM, Johnson PJ. Silencing of GSTP1 gene by CpG island DNA hypermethylation in HBV-associated hepatocellular carcinomas. *Clin Cancer Res* 2002; **8**: 1087-1092 [PMID: 11948118]
- 99 **Yu MW**, Yang SY, Pan JJ, Lin CL, Liu CJ, Liaw YF, Lin SM, Chen PJ, Lee SD, Chen CJ. Polymorphisms in XRCC1 and glutathione S-transferase genes and hepatitis B-related hepatocellular carcinoma. *J Natl Cancer Inst* 2003; **95**: 1485-1488 [PMID: 14519756 DOI: 10.1093/jnci/djg051]
- 100 **Yu L**, Wang CY, Xi B, Sun L, Wang RQ, Yan YK, Zhu LY. GST polymorphisms are associated with hepatocellular carcinoma risk in Chinese population. *World J Gastroenterol* 2011; **17**: 3248-3256 [PMID: 21912475]
- 101 **Wang B**, Huang G, Wang D, Li A, Xu Z, Dong R, Zhang D, Zhou W. Null genotypes of GSTM1 and GSTT1 contribute to hepatocellular carcinoma risk: evidence from an updated meta-analysis. *J Hepatol* 2010; **53**: 508-518 [PMID: 20561699 DOI: 10.1016/j.jhep.2010.03.026]
- 102 **Normanno N**, Bianco C, De Luca A, Maiello MR, Salomon DS. Target-based agents against ErbB receptors and their ligands: a novel approach to cancer treatment. *Endocr Relat Cancer* 2003; **10**: 1-21 [PMID: 12653668 DOI: 10.1677/erc.0.0100001]
- 103 **Abd El-Rehim DM**, Pinder SE, Paish CE, Bell JA, Rampaul RS, Blamey RW, Robertson JF, Nicholson RI, Ellis IO. Expression and co-expression of the members of the epidermal growth factor receptor (EGFR) family in invasive breast carcinoma. *Br J Cancer* 2004; **91**: 1532-1542 [PMID: 15480434 DOI: 10.1038/sj.bjc.6602184]
- 104 **Böni-Schnetzler M**, Pilch PF. Mechanism of epidermal growth factor receptor autophosphorylation and high-affinity binding. *Proc Natl Acad Sci USA* 1987; **84**: 7832-7836 [PMID: 3500470 DOI: 10.1073/pnas.84.22.7832]
- 105 **Rotin D**, Margolis B, Mohammadi M, Daly RJ, Daum G, Li N, Fischer EH, Burgess WH, Ullrich A, Schlessinger J. SH2 domains prevent tyrosine dephosphorylation of the EGF receptor: identification of Tyr992 as the high-affinity binding site for SH2 domains of phospholipase C gamma. *EMBO J* 1992; **11**: 559-567 [PMID: 1537335]
- 106 **Lowenstein EJ**, Daly RJ, Batzer AG, Li W, Margolis B, Lammers R, Ullrich A, Skolnik EY, Bar-Sagi D, Schlessinger J. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* 1992; **70**: 431-442 [PMID: 1322798 DOI: 10.1016/0092-8674(92)90167-B]
- 107 **Zhang Y**, Wang L, Zhang M, Jin M, Bai C, Wang X. Potential mechanism of interleukin-8 production from lung cancer cells: an involvement of EGF-EGFR-PI3K-Akt-Erk pathway. *J Cell Physiol* 2012; **227**: 35-43 [PMID: 21412767 DOI: 10.1002/jcp.22722]
- 108 **Aaronson DS**, Horvath CM. A road map for those who don't know JAK-STAT. *Science* 2002; **296**: 1653-1655 [PMID: 12040185 DOI: 10.1126/science.1071545]
- 109 **Kiso S**, Kawata S, Tamura S, Inui Y, Yoshida Y, Sawai Y, Umeki S, Ito N, Yamada A, Miyagawa J, Higashiyama S, Iwakawa T, Saito M, Taniguchi N, Matsuzawa Y, Kohno K. Liver regeneration in heparin-binding EGF-like growth factor transgenic mice after partial hepatectomy. *Gastroenterology* 2003; **124**: 701-707 [PMID: 12612909 DOI: 10.1053/gast.2003.50097]
- 110 **Kung CP**, Meckes DG, Raab-Traub N. Epstein-Barr virus LMP1 activates EGFR, STAT3, and ERK through effects on PKCdelta. *J Virol* 2011; **85**: 4399-4408 [PMID: 21307189 DOI: 10.1128/JVI.01703-10]

- 111 **Miyaki M**, Sato C, Sakai K, Konishi M, Tanaka K, Muraoka M, Kikuchi-Yanoshita R, Nadaoka Y, Kanda H, Kitagawa T. Malignant transformation and EGFR activation of immortalized mouse liver epithelial cells caused by HBV enhancer-X from a human hepatocellular carcinoma. *Int J Cancer* 2000; **85**: 518-522 [PMID: 10699924]
- 112 **Chen YJ**, Chien PH, Chen WS, Chien YF, Hsu YY, Wang LY, Chen JY, Lin CW, Huang TC, Yu YL, Huang WC. Hepatitis B Virus-Encoded X Protein Downregulates EGFR Expression via Inducing MicroRNA-7 in Hepatocellular Carcinoma Cells. *Evid Based Complement Alternat Med* 2013; **2013**: 682380 [PMID: 23840262 DOI: 10.1155/2013/682380]
- 113 **Chaleshi V**, Haghighi MM, Javadi GR, Fatemi SR, Vahedi M, Zali MR. The effect of 5'untranslated region polymorphism in EGF gene, rs4444903, on colorectal cancer. *Gastroenterol Hepatol Bed Bench* 2013; **6**: 129-135 [PMID: 24834259]
- 114 **Peng Q**, Li S, Qin X, Lao X, Chen Z, Zhang X, Chen J. EGF +61A/G polymorphism contributes to increased gastric cancer risk: evidence from a meta-analysis. *Cancer Cell Int* 2014; **14**: 134 [PMID: 25729328 DOI: 10.1186/s12935-014-0134-4]
- 115 **Li YL**, Tian Z, Zhao L, Zhang CL. Association between the EGF rs4444903 polymorphism and liver cancer susceptibility: a meta-analysis and meta-regression. *Genet Mol Res* 2014; **13**: 8066-8079 [PMID: 25299191 DOI: 10.4238/2014.October.7.1]
- 116 **Hu M**, Shi H, Xu Z, Liu W. Association between epidermal growth factor gene rs4444903 polymorphism and risk of glioma. *Tumour Biol* 2013; **34**: 1879-1885 [PMID: 23645212 DOI: 10.1007/s13277-013-0730-2]
- 117 **Jiang G**, Yu K, Shao L, Yu X, Hu C, Qian P, Xie H, Li J, Zheng J, Zheng S. Association between epidermal growth factor gene +61A/G polymorphism and the risk of hepatocellular carcinoma: a meta-analysis based on 16 studies. *BMC Cancer* 2015; **15**: 314 [PMID: 25927412 DOI: 10.1186/s12885-015-1318-6]
- 118 **Shahbazi M**, Pravica V, Nasreen N, Fakhoury H, Fryer AA, Strange RC, Hutchinson PE, Osborne JE, Lear JT, Smith AG, Hutchinson IV. Association between functional polymorphism in EGF gene and malignant melanoma. *Lancet* 2002; **359**: 397-401 [PMID: 11844511 DOI: 10.1016/S0140-6736(02)07600-6]
- 119 **Tanabe KK**, Lemoine A, Finkelstein DM, Kawasaki H, Fujii T, Chung RT, Lauwers GY, Kulu Y, Muzikansky A, Kuruppu D, Lanuti M, Goodwin JM, Azoulay D, Fuchs BC. Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. *JAMA* 2008; **299**: 53-60 [PMID: 18167406 DOI: 10.1001/jama.2007.65]
- 120 **Yuan JM**, Fan Y, Ognjanovic S, Wang R, Van Den Berg D, Govindarajan S, Yu MC. Genetic polymorphisms of epidermal growth factor in relation to risk of hepatocellular carcinoma: two case-control studies. *BMC Gastroenterol* 2013; **13**: 32 [PMID: 23419149 DOI: 10.1186/1471-230X-13-32]
- 121 **Suenaga M**, Yamada S, Fujii T, Fuchs BC, Okumura N, Kanda M, Kobayashi D, Tanaka C, Nakayama G, Sugimoto H, Koike M, Nomoto S, Fujiwara M, Takeda S, Hayashi K, Tanabe KK, Goto H, Kodera Y. A functional polymorphism in the epidermal growth factor gene predicts hepatocellular carcinoma risk in Japanese hepatitis C patients. *Onco Targets Ther* 2013; **6**: 1805-1812 [PMID: 24363559 DOI: 10.2147/OTT.S53625]
- 122 **Abbas E**, Shaker O, Abd El Aziz G, Ramadan H, Esmat G. Epidermal growth factor gene polymorphism 61A/G in patients with chronic liver disease for early detection of hepatocellular carcinoma: a pilot study. *Eur J Gastroenterol Hepatol* 2012; **24**: 458-463 [PMID: 22293333 DOI: 10.1097/meg.0b013e3283508d45]
- 123 **Zhong JH**, You XM, Gong WF, Ma L, Zhang Y, Mo QG, Wu LC, Xiao J, Li LQ. Epidermal growth factor gene polymorphism and risk of hepatocellular carcinoma: a meta-analysis. *PLoS One* 2012; **7**: e32159 [PMID: 22403631 DOI: 10.1371/journal.pone.0032159]
- 124 **Rivlin N**, Brosh R, Oren M, Rotter V. Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis. *Genes Cancer* 2011; **2**: 466-474 [PMID: 21779514 DOI: 10.1177/1947601911408889]
- 125 **Meek DW**. The p53 response to DNA damage. *DNA Repair* (Amst) 2004; **3**: 1049-1056 [PMID: 15279792 DOI: 10.1016/j.dnarep.2004.03.027]
- 126 **Pellegata NS**, Antoniono RJ, Redpath JL, Stanbridge EJ. DNA damage and p53-mediated cell cycle arrest: a reevaluation. *Proc Natl Acad Sci USA* 1996; **93**: 15209-15214 [PMID: 8986789 DOI: 10.1073/pnas.93.26.15209]
- 127 **Soengas MS**, Alarcón RM, Yoshida H, Giaccia AJ, Hakem R, Mak TW, Lowe SW. Apaf-1 and caspase-9 in p53-dependent apoptosis and tumor inhibition. *Science* 1999; **284**: 156-159 [PMID: 10102818 DOI: 10.1126/science.284.5411.156]
- 128 **Moll UM**, Petrenko O. The MDM2-p53 interaction. *Mol Cancer Res* 2003; **1**: 1001-1008 [PMID: 14707283]
- 129 **Rodriguez MS**, Desterro JM, Lain S, Lane DP, Hay RT. Multiple C-terminal lysine residues target p53 for ubiquitin-proteasome-mediated degradation. *Mol Cell Biol* 2000; **20**: 8458-8467 [PMID: 11046142 DOI: 10.1128/MCB.20.22.8458-8467.2000]
- 130 **Haupt Y**, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature* 1997; **387**: 296-299 [PMID: 9153395 DOI: 10.1038/387296a0]
- 131 **Bond GL**, Hu W, Levine AJ. MDM2 is a central node in the p53 pathway: 12 years and counting. *Curr Cancer Drug Targets* 2005; **5**: 3-8 [PMID: 15720184 DOI: 10.2174/1568009053332627]
- 132 **Bond GL**, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G, Levine AJ. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* 2004; **119**: 591-602 [PMID: 15550242 DOI: 10.1016/j.cell.2004.11.022]
- 133 **Ezzikouri S**, El Feydi AE, Afifi R, El Kihal L, Benazzouz M, Hassar M, Marchio A, Pineau P, Benjelloun S. MDM2 SNP309T>G polymorphism and risk of hepatocellular carcinoma: a case-control analysis in a Moroccan population. *Cancer Detect Prev* 2009; **32**: 380-385 [PMID: 19233569 DOI: 10.1016/j.cdp.2009.01.003]
- 134 **Di Vuolo V**, Buonaguro L, Izzo F, Losito S, Botti G, Buonaguro FM, Tornesello ML. TP53 and MDM2 gene polymorphisms and risk of hepatocellular carcinoma among Italian patients. *Infect Agent Cancer* 2011; **6**: 13 [PMID: 21843334 DOI: 10.1186/1750-9378-6-13]
- 135 **Dharel N**, Kato N, Muroyama R, Moriyama M, Shao RX, Kawabe T, Omata M. MDM2 promoter SNP309 is associated with the risk of hepatocellular carcinoma in patients with chronic hepatitis C. *Clin Cancer Res* 2006; **12**: 4867-4871 [PMID: 16914573]
- 136 **Yoon YJ**, Chang HY, Ahn SH, Kim JK, Park YK, Kang DR, Park JY, Myoung SM, Kim do Y, Chon CY, Han KH. MDM2 and p53 polymorphisms are associated with the development of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *Carcinogenesis* 2008; **29**: 1192-1196 [PMID: 18390844 DOI: 10.1093/carcin/bgn090]
- 137 **Liu GY**, Jiang DK, Shen SQ, Yu L. MDM2 SNP309T>G polymorphism with hepatocellular carcinoma risk: a meta-analysis. *Arch Med Res* 2011; **42**: 149-155 [PMID: 21565629 DOI: 10.1016/j.arcmed.2011.02.002]
- 138 **Peng Q**, Lao X, Chen Z, Lai H, Deng Y, Wang J, Mo C, Sui J, Wu J, Zhai L, Yang S, Qin X, Li S. TP53 and MDM2 gene polymorphisms, gene-gene interaction, and hepatocellular carcinoma risk: evidence from an updated meta-analysis. *PLoS One* 2013; **8**: e82773 [PMID: 24376578 DOI: 10.1371/journal.pone.0082773]
- 139 **Li H**, Wu K, Tao K, Chen L, Zheng Q, Lu X, Liu J, Shi L, Liu C, Wang G, Zou W. Tim-3/galectin-9 signaling pathway mediates T-cell dysfunction and predicts poor prognosis in patients with hepatitis B virus-associated hepatocellular carcinoma. *Hepatology* 2012; **56**: 1342-1351 [PMID: 22505239 DOI: 10.1002/hep.25777]
- 140 **Monney L**, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, Manning S, Greenfield EA, Coyle AJ, Sobel RA, Freeman GJ, Kuchroo VK. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature* 2002; **415**: 536-541 [PMID: 11823861 DOI: 10.1038/415536a]
- 141 **Zhu C**, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, Zheng XX, Strom TB, Kuchroo VK. The Tim-3 ligand galectin-9



- negatively regulates T helper type 1 immunity. *Nat Immunol* 2005; **6**: 1245-1252 [PMID: 16286920 DOI: 10.1038/ni1271]
- 142 **Sánchez-Fueyo A**, Tian J, Picarella D, Domenig C, Zheng XX, Sabatos CA, Manlongat N, Bender O, Kamradt T, Kuchroo VK, Gutiérrez-Ramos JC, Coyle AJ, Strom TB. Tim-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance. *Nat Immunol* 2003; **4**: 1093-1101 [PMID: 14556005 DOI: 10.1038/ni987]
- 143 **Golden-Mason L**, Palmer BE, Kassam N, Townshend-Bulson L, Livingston S, McMahon BJ, Castelblanco N, Kuchroo V, Gretsch DR, Rosen HR. Negative immune regulator Tim-3 is overexpressed on T cells in hepatitis C virus infection and its blockade rescues dysfunctional CD4+ and CD8+ T cells. *J Virol* 2009; **83**: 9122-9130 [PMID: 19587053 DOI: 10.1128/JVI.00639-09]
- 144 **Wu W**, Shi Y, Li J, Chen F, Chen Z, Zheng M. Tim-3 expression on peripheral T cell subsets correlates with disease progression in hepatitis B infection. *Virol J* 2011; **8**: 113 [PMID: 21392402 DOI: 10.1186/1743-422X-8-113]
- 145 **Li Z**, Liu Z, Zhang G, Han Q, Li N, Zhu Q, Lv Y, Chen J, Xing F, Wang Y, Li F. TIM3 gene polymorphisms in patients with chronic hepatitis B virus infection: impact on disease susceptibility and hepatocellular carcinoma traits. *Tissue Antigens* 2012; **80**: 151-157 [PMID: 22587604 DOI: 10.1111/j.1399-0039.2012.01898.x]
- 146 **Li Z**, Li N, Zhu Q, Zhang G, Han Q, Zhang P, Xun M, Wang Y, Zeng X, Yang C, Liu Z. Genetic variations of PD1 and TIM3 are differentially and interactively associated with the development of cirrhosis and HCC in patients with chronic HBV infection. *Infect Genet Evol* 2013; **14**: 240-246 [PMID: 23291409 DOI: 10.1016/j.meegid.2012.12.008]
- 147 **Wang L**, Zhao C, Peng Q, Shi J, Gu G. Expression levels of CD28, CTLA-4, PD-1 and Tim-3 as novel indicators of T-cell immune function in patients with chronic hepatitis B virus infection. *Biomed Rep* 2014; **2**: 270-274 [PMID: 24649109]
- 148 **Zhang J**, Daley D, Akhabir L, Stefanowicz D, Chan-Yeung M, Becker AB, Laprise C, Paré PD, Sandford AJ. Lack of association of TIM3 polymorphisms and allergic phenotypes. *BMC Med Genet* 2009; **62**: 62 [PMID: 19566956 DOI: 10.1186/1471-2350-10-62]
- 149 **DeKruyff RH**, Bu X, Ballesteros A, Santiago C, Chim YL, Lee HH, Karisola P, Pichavant M, Kaplan GG, Umetsu DT, Freeman GJ, Casasnovas JM. T cell/transmembrane, Ig, and mucin-3 allelic variants differentially recognize phosphatidylserine and mediate phagocytosis of apoptotic cells. *J Immunol* 2010; **184**: 1918-1930 [PMID: 20083673 DOI: 10.4049/jimmunol.0903059]
- 150 **Sugasawa K**, Ng JM, Masutani C, Iwai S, van der Spek PJ, Eker AP, Hanaoka F, Bootsma D, Hoeijmakers JH. Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. *Mol Cell* 1998; **2**: 223-232 [PMID: 9734359 DOI: 10.1016/S1097-2765(00)80132-X]
- 151 **de Laat WL**, Jaspers NG, Hoeijmakers JH. Molecular mechanism of nucleotide excision repair. *Genes Dev* 1999; **13**: 768-785 [PMID: 10197977 DOI: 10.1101/gad.13.7.768]
- 152 **Thoma BS**, Vasquez KM. Critical DNA damage recognition functions of XPC-hHR23B and XPA-RPA in nucleotide excision repair. *Mol Carcinog* 2003; **38**: 1-13 [PMID: 12949838 DOI: 10.1002/mc.10143]
- 153 **Rouillon C**, White MF. The XBP-Bax1 helicase-nuclease complex unwinds and cleaves DNA: implications for eukaryal and archaeal nucleotide excision repair. *J Biol Chem* 2010; **285**: 11013-11022 [PMID: 20139443 DOI: 10.1074/jbc.M109.094763]
- 154 **Hollander MC**, Philburn RT, Patterson AD, Velasco-Miguel S, Friedberg EC, Linnola RI, Fornace AJ. Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis. *Proc Natl Acad Sci USA* 2005; **102**: 13200-13205 [PMID: 16141330 DOI: 10.1073/pnas.0503133102]
- 155 **Hu Z**, Wang Y, Wang X, Liang G, Miao X, Xu Y, Tan W, Wei Q, Lin D, Shen H. DNA repair gene XPC genotypes/haplotypes and risk of lung cancer in a Chinese population. *Int J Cancer* 2005; **115**: 478-483 [PMID: 15700316]
- 156 **Vogel U**, Overvad K, Wallin H, Tjønneland A, Nexø BA, Raaschou-Nielsen O. Combinations of polymorphisms in XPD, XPC and XPA in relation to risk of lung cancer. *Cancer Lett* 2005; **222**: 67-74 [PMID: 15837542 DOI: 10.1016/j.canlet.2004.11.016]
- 157 **Zhu Y**, Lai M, Yang H, Lin J, Huang M, Grossman HB, Dinney CP, Wu X. Genotypes, haplotypes and diplotypes of XPC and risk of bladder cancer. *Carcinogenesis* 2007; **28**: 698-703 [PMID: 17052994 DOI: 10.1093/carcin/bgl201]
- 158 **Qiu L**, Wang Z, Shi X, Wang Z. Associations between XPC polymorphisms and risk of cancers: A meta-analysis. *Eur J Cancer* 2008; **44**: 2241-2253 [PMID: 18771913 DOI: 10.1016/j.ejca.2008.06.024]
- 159 **Khan SG**, Metter EJ, Tarone RE, Bohr VA, Grossman L, Hedayati M, Bale SJ, Emmert S, Kraemer KH. A new xeroderma pigmentosum group C poly(AT) insertion/deletion polymorphism. *Carcinogenesis* 2000; **21**: 1821-1825 [PMID: 11023539 DOI: 10.1093/carcin/21.10.1821]
- 160 **Khan SG**, Muniz-Medina V, Shahnavi T, Baker CC, Inui H, Ueda T, Emmert S, Schneider TD, Kraemer KH. The human XPC DNA repair gene: arrangement, splice site information content and influence of a single nucleotide polymorphism in a splice acceptor site on alternative splicing and function. *Nucleic Acids Res* 2002; **30**: 3624-3631 [PMID: 12177305 DOI: 10.1093/nar/gkf469]
- 161 **Zhang D**, Chen C, Fu X, Gu S, Mao Y, Xie Y, Huang Y, Li Y. A meta-analysis of DNA repair gene XPC polymorphisms and cancer risk. *J Hum Genet* 2008; **53**: 18-33 [PMID: 18097734 DOI: 10.1007/s10038-007-0215-5]
- 162 **Jin B**, Dong Y, Zhang X, Wang H, Han B. Association of XPC polymorphisms and lung cancer risk: a meta-analysis. *PLoS One* 2014; **9**: e93937 [PMID: 24736739 DOI: 10.1371/journal.pone.0093937]
- 163 **Long XD**, Ma Y, Zhou YF, Ma AM, Fu GH. Polymorphism in xeroderma pigmentosum complementation group C codon 939 and aflatoxin B1-related hepatocellular carcinoma in the Guangxi population. *Hepatology* 2010; **52**: 1301-1309 [PMID: 20658464 DOI: 10.1002/hep.23807]
- 164 **Yao JG**, Huang XY, Long XD. Interaction of DNA repair gene polymorphisms and aflatoxin B1 in the risk of hepatocellular carcinoma. *Int J Clin Exp Pathol* 2014; **7**: 6231-6244 [PMID: 25337275]
- 165 **Cruikshank W**, Center DM. Modulation of lymphocyte migration by human lymphokines. II. Purification of a lymphotactic factor (LCF). *J Immunol* 1982; **128**: 2569-2574 [PMID: 7042841]
- 166 **Ferland C**, Flamand N, Davoine F, Chakir J, Lavolette M. IL-16 activates plasminogen-plasmin system and promotes human eosinophil migration into extracellular matrix via CCR3-chemokine-mediated signaling and by modulating CD4 eosinophil expression. *J Immunol* 2004; **173**: 4417-4424 [PMID: 15383572 DOI: 10.4049/jimmunol.173.7.4417]
- 167 **Bandeira-Melo C**, Sugiyama K, Woods LJ, Phoofolo M, Center DM, Cruikshank WW, Weller PF. IL-16 promotes leukotriene C(4) and IL-4 release from human eosinophils via CD4- and autocrine CCR3-chemokine-mediated signaling. *J Immunol* 2002; **168**: 4756-4763 [PMID: 11971026 DOI: 10.4049/jimmunol.168.9.4756]
- 168 **Liu Y**, Cruikshank WW, O'Loughlin T, O'Reilly P, Center DM, Kornfeld H. Identification of a CD4 domain required for interleukin-16 binding and lymphocyte activation. *J Biol Chem* 1999; **274**: 23387-23395 [PMID: 10438516 DOI: 10.1074/jbc.274.33.23387]
- 169 **Krautwald S**. IL-16 activates the SAPK signaling pathway in CD4+ macrophages. *J Immunol* 1998; **160**: 5874-5879 [PMID: 9637499]
- 170 **Cruikshank WW**, Greenstein JL, Theodore AC, Center DM. Lymphocyte chemoattractant factor induces CD4-dependent intracytoplasmic signaling in lymphocytes. *J Immunol* 1991; **146**: 2928-2934 [PMID: 1673145]
- 171 **Cruikshank WW**, Berman JS, Theodore AC, Bernardo J, Center DM. Lymphokine activation of T4+ T lymphocytes and monocytes. *J Immunol* 1987; **138**: 3817-3823 [PMID: 3108375]
- 172 **Parada NA**, Cruikshank WW, Danis HL, Ryan TC, Center DM. IL-16- and other CD4 ligand-induced migration is dependent upon protein kinase C. *Cell Immunol* 1996; **168**: 100-106 [PMID:



- 8599832 DOI: 10.1006/cimm.1996.0054]
- 173 **Ryan TC**, Cruikshank WW, Kornfeld H, Collins TL, Center DM. The CD4-associated tyrosine kinase p56lck is required for lymphocyte chemoattractant factor-induced T lymphocyte migration. *J Biol Chem* 1995; **270**: 17081-17086 [PMID: 7615501 DOI: 10.1074/jbc.270.29.17081]
  - 174 **Mathy NL**, Scheuer W, Lanzendörfer M, Honold K, Ambrosius D, Norley S, Kurth R. Interleukin-16 stimulates the expression and production of pro-inflammatory cytokines by human monocytes. *Immunology* 2000; **100**: 63-69 [PMID: 10809960 DOI: 10.1046/j.1365-2567.2000.00997.x]
  - 175 **Gao LB**, Liang WB, Xue H, Rao L, Pan XM, Lv ML, Bai P, Fang WL, Liu J, Liao M, Zhang L. Genetic polymorphism of interleukin-16 and risk of nasopharyngeal carcinoma. *Clin Chim Acta* 2009; **409**: 132-135 [PMID: 19758567 DOI: 10.1016/j.cca.2009.09.017]
  - 176 **Gao LB**, Rao L, Wang YY, Liang WB, Li C, Xue H, Zhou B, Sun H, Li Y, Lv ML, Du XJ, Zhang L. The association of interleukin-16 polymorphisms with IL-16 serum levels and risk of colorectal and gastric cancer. *Carcinogenesis* 2009; **30**: 295-299 [PMID: 19073878 DOI: 10.1093/carcin/bgn281]
  - 177 **Qin X**, Peng Q, Lao X, Chen Z, Lu Y, Lao X, Mo C, Sui J, Wu J, Zhai L, Yang S, Li S, Zhao J. The association of interleukin-16 gene polymorphisms with IL-16 serum levels and risk of nasopharyngeal carcinoma in a Chinese population. *Tumour Biol* 2014; **35**: 1917-1924 [PMID: 24101193 DOI: 10.1007/s13277-013-1257-2]
  - 178 **Thomas G**, Jacobs KB, Yeager M, Kraft P, Wacholder S, Orr N, Yu K, Chatterjee N, Welch R, Hutchinson A, Crenshaw A, Cancel-Tassin G, Staats BJ, Wang Z, Gonzalez-Bosquet J, Fang J, Deng X, Berndt SI, Calle EE, Feigelson HS, Thun MJ, Rodriguez C, Albanes D, Virtamo J, Weinstein S, Schumacher FR, Giovannucci E, Willett WC, Cussenot O, Valeri A, Andriole GL, Crawford ED, Tucker M, Gerhard DS, Fraumeni JF, Hoover R, Hayes RB, Hunter DJ, Chanock SJ. Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet* 2008; **40**: 310-315 [PMID: 18264096 DOI: 10.1038/ng.91]
  - 179 **Romani S**, Hosseini SM, Mohebbi SR, Kazemian S, Derakhshani S, Khanyaghma M, Azimzadeh P, Sharifian A, Zali MR. Interleukin-16 gene polymorphisms are considerable host genetic factors for patients' susceptibility to chronic hepatitis B infection. *Hepat Res Treat* 2014; **2014**: 790753 [PMID: 25692036 DOI: 10.1155/2014/790753]
  - 180 **Li S**, Deng Y, Chen ZP, Huang S, Liao XC, Lin LW, Li H, Peng T, Qin X, Zhao JM. Genetic polymorphism of interleukin-16 influences susceptibility to HBV-related hepatocellular carcinoma in a Chinese population. *Infect Genet Evol* 2011; **11**: 2083-2088 [PMID: 22019522 DOI: 10.1016/j.meegid.2011.09.025]
  - 181 **Al-Qahtani A**, Khalak HG, Alkuraya FS, Al-hamoudi W, Alswat K, Al Balwi MA, Al Abdulkareem I, Sanai FM, Abdo AA. Genome-wide association study of chronic hepatitis B virus infection reveals a novel candidate risk allele on 11q22.3. *J Med Genet* 2013; **50**: 725-732 [PMID: 24065354 DOI: 10.1136/jmedgenet-2013-101724]
  - 182 **Chan KY**, Wong CM, Kwan JS, Lee JM, Cheung KW, Yuen MF, Lai CL, Poon RT, Sham PC, Ng IO. Genome-wide association study of hepatocellular carcinoma in Southern Chinese patients with chronic hepatitis B virus infection. *PLoS One* 2011; **6**: e28798 [PMID: 22174901 DOI: 10.1371/journal.pone.0028798]
  - 183 **Chen K**, Shi W, Xin Z, Wang H, Zhu X, Wu X, Li Z, Li H, Liu Y. Replication of genome wide association studies on hepatocellular carcinoma susceptibility loci in a Chinese population. *PLoS One* 2013; **8**: e77315 [PMID: 24204805 DOI: 10.1371/journal.pone.0077315]
  - 184 **Hu Z**, Liu Y, Zhai X, Dai J, Jin G, Wang L, Zhu L, Yang Y, Liu J, Chu M, Wen J, Xie K, Du G, Wang Q, Zhou Y, Cao M, Liu L, He Y, Wang Y, Zhou G, Jia W, Lu J, Li S, Liu J, Yang H, Shi Y, Zhou W, Shen H. New loci associated with chronic hepatitis B virus infection in Han Chinese. *Nat Genet* 2013; **45**: 1499-1503 [PMID: 24162738 DOI: 10.1038/ng.2809]
  - 185 **Chang SW**, Fann CS, Su WH, Wang YC, Weng CC, Yu CJ, Hsu CL, Hsieh AR, Chien RN, Chu CM, Tai DI. A genome-wide association study on chronic HBV infection and its clinical progression in male Han-Taiwanese. *PLoS One* 2014; **9**: e99724 [PMID: 24940741 DOI: 10.1371/journal.pone.0099724]
  - 186 **Pan W**, Cheng G, Xing H, Shi J, Lu C, Wei J, Li L, Zhou C, Yuan Q, Zhou L, Yang M. Leukocyte telomere length-related rs621559 and rs398652 genetic variants influence risk of HBV-related hepatocellular carcinoma. *PLoS One* 2014; **9**: e110863 [PMID: 25365256 DOI: 10.1371/journal.pone.0110863]
  - 187 **Krautkramer KA**, Linnemann AK, Fontaine DA, Whillock AL, Harris TW, Schleis GJ, Truchan NA, Marty-Santos L, Lavine JA, Cleaver O, Kimple ME, Davis DB. Tcf19 is a novel islet factor necessary for proliferation and survival in the INS-1  $\beta$ -cell line. *Am J Physiol Endocrinol Metab* 2013; **305**: E600-E610 [PMID: 23860123 DOI: 10.1152/ajpendo.00147.2013]
  - 188 **Hoeller D**, Hecker CM, Wagner S, Rogov V, Dötsch V, Dikic I. E3-independent monoubiquitination of ubiquitin-binding proteins. *Mol Cell* 2007; **26**: 891-898 [PMID: 17588522 DOI: 10.1016/j.molcel.2007.05.014]
  - 189 **Xing J**, Ajani JA, Chen M, Izzo J, Lin J, Chen Z, Gu J, Wu X. Constitutive short telomere length of chromosome 17p and 12q but not 11q and 2p is associated with an increased risk for esophageal cancer. *Cancer Prev Res (Phila)* 2009; **2**: 459-465 [PMID: 19401529 DOI: 10.1158/1940-6207.CAPR-08-0227]
  - 190 **Imamichi Y**, Mizutani T, Ju Y, Matsumura T, Kawabe S, Kanno M, Yazawa T, Miyamoto K. Transcriptional regulation of human ferredoxin 1 in ovarian granulosa cells. *Mol Cell Endocrinol* 2013; **370**: 1-10 [PMID: 23435367 DOI: 10.1016/j.mce.2013.02.012]
  - 191 **Sheftel AD**, Stehling O, Pierik AJ, Elsässer HP, Mühlenhoff U, Webert H, Hobler A, Hannemann F, Bernhardt R, Lill R. Humans possess two mitochondrial ferredoxins, Fdx1 and Fdx2, with distinct roles in steroidogenesis, heme, and Fe/S cluster biosynthesis. *Proc Natl Acad Sci USA* 2010; **107**: 11775-11780 [PMID: 20547883 DOI: 10.1073/pnas.1004250107]
  - 192 **Huang SS**, Huang JS. TGF- $\beta$  control of cell proliferation. *J Cell Biochem* 2005; **96**: 447-462 [PMID: 16088940 DOI: 10.1002/jcb.20558]
  - 193 **Massagué J**, Xi Q. TGF- $\beta$  control of stem cell differentiation genes. *FEBS Lett* 2012; **586**: 1953-1958 [PMID: 22710171 DOI: 10.1016/j.febslet.2012.03.023]
  - 194 **Sethi A**, Mao W, Wordinger RJ, Clark AF. Transforming growth factor-beta induces extracellular matrix protein cross-linking lysyl oxidase (LOX) genes in human trabecular meshwork cells. *Invest Ophthalmol Vis Sci* 2011; **52**: 5240-5250 [PMID: 21546528 DOI: 10.1167/jovs.11-7287]
  - 195 **Larsson J**, Blank U, Helgadóttir H, Björnsson JM, Ehinger M, Goumans MJ, Fan X, Levéen P, Karlsson S. TGF- $\beta$  signaling-deficient hematopoietic stem cells have normal self-renewal and regenerative ability in vivo despite increased proliferative capacity in vitro. *Blood* 2003; **102**: 3129-3135 [PMID: 12842983 DOI: 10.1182/blood-2003-04-1300]
  - 196 **Ferrari G**, Cook BD, Terushkin V, Pintucci G, Mignatti P. Transforming growth factor-beta 1 (TGF- $\beta$ 1) induces angiogenesis through vascular endothelial growth factor (VEGF)-mediated apoptosis. *J Cell Physiol* 2009; **219**: 449-458 [PMID: 19180561 DOI: 10.1002/jcp.21706]
  - 197 **Sledzińska A**, Hemmers S, Mair F, Gorka O, Ruland J, Fairbairn L, Nissler A, Müller W, Waisman A, Becher B, Buch T. TGF- $\beta$  signalling is required for CD4+ T cell homeostasis but dispensable for regulatory T cell function. *PLoS Biol* 2013; **11**: e1001674 [PMID: 24115907 DOI: 10.1371/journal.pbio.1001674]
  - 198 **Okumoto K**, Hattori E, Tamura K, Kiso S, Watanabe H, Saito K, Saito T, Togashi H, Kawata S. Possible contribution of circulating transforming growth factor-beta1 to immunity and prognosis in unresectable hepatocellular carcinoma. *Liver Int* 2004; **24**: 21-28 [PMID: 15101997 DOI: 10.1111/j.1478-3231.2004.00882.x]
  - 199 **Sacco R**, Leuci D, Tortorella C, Fiore G, Marinucci F, Schiraldi O, Antonaci S. Transforming growth factor beta1 and soluble Fas serum levels in hepatocellular carcinoma. *Cytokine* 2000; **12**: 811-814 [PMID: 10843770 DOI: 10.1006/cyto.1999.0650]

- 200 **Hong MH**, Chou YC, Wu YC, Tsai KN, Hu CP, Jeng KS, Chen ML, Chang C. Transforming growth factor- $\beta$ 1 suppresses hepatitis B virus replication by the reduction of hepatocyte nuclear factor-4 $\alpha$  expression. *PLoS One* 2012; **7**: e30360 [PMID: 22276183 DOI: 10.1371/journal.pone.0030360]
- 201 **Cambien F**, Ricard S, Troesch A, Mallet C, Générénaz L, Evans A, Arveiler D, Luc G, Ruidavets JB, Poirier O. Polymorphisms of the transforming growth factor-beta 1 gene in relation to myocardial infarction and blood pressure. The Etude Cas-Témoin de l'Infarctus du Myocarde (ECTIM) Study. *Hypertension* 1996; **28**: 881-887 [PMID: 8901839 DOI: 10.1161/01.HYP.28.5.881]
- 202 **Ben-Ari Z**, Mor E, Papo O, Kfir B, Sulkes J, Tambur AR, Tur-Kaspa R, Klein T. Cytokine gene polymorphisms in patients infected with hepatitis B virus. *Am J Gastroenterol* 2003; **98**: 144-150 [PMID: 12526950 DOI: 10.1111/j.1572-0241.2003.07179.x]
- 203 **Kwon OS**, Song SH, Ju KT, Chung MG, Park DK, Kim SS, Kim YS, Koo YS, Kim YK, Choi DJ, Kim JH, Hwang YJ, Byun KS, Lee CH. [Polymorphism in codons 10 and 25 of the transforming growth factor-beta1 gene in Korean population and in patients with liver cirrhosis and hepatocellular carcinoma]. *Korean J Gastroenterol* 2003; **42**: 212-219 [PMID: 14532743]
- 204 **Migita K**, Miyazoe S, Maeda Y, Daikoku M, Abiru S, Ueki T, Yano K, Nagaoka S, Matsumoto T, Nakao K, Hamasaki K, Yatsuhashi H, Ishibashi H, Eguchi K. Cytokine gene polymorphisms in Japanese patients with hepatitis B virus infection--association between TGF-beta1 polymorphisms and hepatocellular carcinoma. *J Hepatol* 2005; **42**: 505-510 [PMID: 15763337 DOI: 10.1016/j.jhep.2004.11.026]
- 205 **Shi HZ**, Ren P, Lu QJ, Niedrgethmn M, Wu GY. Association between EGF, TGF- $\beta$ 1 and TNF- $\alpha$  gene polymorphisms and hepatocellular carcinoma. *Asian Pac J Cancer Prev* 2012; **13**: 6217-6220 [PMID: 23464434 DOI: 10.7314/APJCP.2012.13.12.6217]
- 206 **Qi P**, Chen YM, Wang H, Fang M, Ji Q, Zhao YP, Sun XJ, Liu Y, Gao CF. -509C>T polymorphism in the TGF-beta1 gene promoter, impact on the hepatocellular carcinoma risk in Chinese patients with chronic hepatitis B virus infection. *Cancer Immunol Immunother* 2009; **58**: 1433-1440 [PMID: 19169878 DOI: 10.1007/s00262-009-0660-4]
- 207 **Hosseini Razavi A**, Azimzadeh P, Mohebbi SR, Hosseini SM, Romani S, Khanyaghma M, Hatami Y, Sharifian A, Zali MR. Lack of Association Between Transforming Growth Factor Beta 1 -509C/T and +915G/C Polymorphisms and Chronic Hepatitis B in Iranian Patients. *Hepat Mon* 2014; **14**: e13100 [PMID: 24748892]
- 208 **Kim YJ**, Lee HS, Im JP, Min BH, Kim HD, Jeong JB, Yoon JH, Kim CY, Kim MS, Kim JY, Jung JH, Kim LH, Park BL, Shin HD. Association of transforming growth factor-beta1 gene polymorphisms with a hepatocellular carcinoma risk in patients with chronic hepatitis B virus infection. *Exp Mol Med* 2003; **35**: 196-202 [PMID: 12858019 DOI: 10.1038/emmm.2003.27]
- 209 **Li XD**, Wu LM, Xie HY, Xu X, Zhou L, Liang TB, Wang WL, Shen Y, Zhang M, Zheng SS. No association exists between E-cadherin gene polymorphism and tumor recurrence in patients with hepatocellular carcinoma after transplantation. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 254-258 [PMID: 17548247]
- 210 **Liu K**, Zhang L, Lin X, Chen L, Shi H, Magaye R, Zou B, Zhao J. Association of GST genetic polymorphisms with the susceptibility to hepatocellular carcinoma (HCC) in Chinese population evaluated by an updated systematic meta-analysis. *PLoS One* 2013; **8**: e57043 [PMID: 23437305 DOI: 10.1371/journal.pone.0057043]

**P- Reviewer:** Chung YH, Vaughan G **S- Editor:** Wang JL  
**L- Editor:** Webster JR **E- Editor:** Liu SQ



Basic Study

## Metabolomics studies identify novel diagnostic and prognostic indicators in patients with alcoholic hepatitis

Mona Ascha, Zeneng Wang, Mustafa S Ascha, Raed Dweik, Nizar N Zein, David Grove, J Mark Brown, Stephanie Marshall, Rocio Lopez, Ibrahim A Hanouneh

Mona Ascha, Mustafa S Ascha, Nizar N Zein, Department of Gastroenterology and Hepatology, Cleveland Clinic, Cleveland, OH 44195, United States

Zeneng Wang, J Mark Brown, Stephanie Marshall, Department of Cellular and Molecular Medicine, Cleveland Clinic, Cleveland, OH 44195, United States

Raed Dweik, David Grove, Department of Pulmonary, Allergy, and Critical Care Medicine/Respiratory Institute, Cleveland Clinic, Cleveland, OH 44195, United States

Rocio Lopez, Department of Quantitative Health Science, Cleveland Clinic, Cleveland, OH 44195, United States

Ibrahim A Hanouneh, Minnesota Gastroenterology, Minneapolis, Minnesota, PA 55414, United States

**Author contributions:** Ascha M, Ascha MS and Hanouneh IA performed the writing and critical revision of the manuscript; Wang Z, Dweik R, Grove D, Brown JM and Marshall S performed the majority of data collection; Zein NN and Hanouneh IA conceived and implemented the design of the project; Lopez R performed the statistical analysis.

**Supported by** In part by NIH grant R01 HL122283 (Brown JM).

**Institutional review board statement:** The study was reviewed and approved by the Cleveland Clinic Foundation Institutional Review Board.

**Institutional animal care and use committee statement:** No animals were involved in this study.

**Conflict-of-interest statement:** The authors have no conflict of interest to report.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at [mx256@case.edu](mailto:mx256@case.edu). Participants gave informed consent for data sharing.

**Open-Access:** This article is an open-access article which was

selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Ibrahim A Hanouneh, MD, Minnesota Gastroenterology, P.O. Box 14909, Minneapolis, Minnesota, PA 55414, United States. [ibrahim.hanouneh@mngastro.com](mailto:ibrahim.hanouneh@mngastro.com)  
**Telephone:** +1-612-8711145  
**Fax:** +1-612-8705491

**Received:** September 12, 2015  
**Peer-review started:** September 16, 2015  
**First decision:** October 28, 2015  
**Revised:** January 22, 2016  
**Accepted:** March 9, 2016  
**Article in press:** March 14, 2016  
**Published online:** April 8, 2016

### Abstract

**AIM:** To identify plasma analytes using metabolomics that correlate with the diagnosis and severity of liver disease in patients with alcoholic hepatitis (AH).

**METHODS:** We prospectively recruited patients with cirrhosis from AH ( $n = 23$ ) and those with cirrhosis with acute decompensation (AD) from etiologies other than alcohol ( $n = 25$ ). We used mass spectrometry to identify 29 metabolic compounds in plasma samples from fasted subjects. A receiver operating characteristics analysis was performed to assess the utility of biomarkers in distinguishing acute AH from alcoholic cirrhosis. Logistic regression analysis was performed to build a predictive model for AH based on clinical characteristics. A survival analysis was used to construct Kaplan Meier curves

evaluating transplant-free survival.

**RESULTS:** A comparison of model for end-stage liver disease (MELD)-adjusted metabolomics levels between cirrhosis patients who had AD or AH showed that patients with AH had significantly higher levels of betaine, and lower creatinine, phenylalanine, homocitrulline, citrulline, tyrosine, octenoyl-carnitine, and symmetric dimethylarginine. When considering combined levels, betaine and citrulline were highly accurate predictors for differentiation between AH and AD (area under receiver operating characteristics curve = 0.84). The plasma levels of carnitine [0.54 (0.18, 0.91);  $P = 0.005$ ], homocitrulline [0.66 (0.34, 0.99);  $P < 0.001$ ] and pentanoyl-carnitine [0.53 (0.16, 0.90);  $P = 0.007$ ] correlated with MELD scores in patients diagnosed with AH. Increased levels of many biomarkers (carnitine  $P = 0.005$ , butyrobetaine  $P = 0.32$ , homocitrulline  $P = 0.002$ , leucine  $P = 0.027$ , valine  $P = 0.024$ , phenylalanine  $P = 0.037$ , tyrosine  $P = 0.012$ , acetyl-carnitine  $P = 0.006$ , propionyl-carnitine  $P = 0.03$ , butyryl-carnitine  $P = 0.03$ , trimethyl-lisine  $P = 0.034$ , pentanoyl-carnitine  $P = 0.03$ , hexanoyl-carnitine  $P = 0.026$ ) were associated with increased mortality in patients with AH.

**CONCLUSION:** Metabolomics plasma analyte levels might be used to diagnose of AH or help predict patient prognoses.

**Key words:** Metabolomics; Biomarkers; Liver disease; Model for end-stage liver disease; Cirrhosis; Alcoholic hepatitis; Liver biopsy

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The model for end-stage liver disease score, which is commonly used to predict outcomes in patients who have liver disease, is far from perfect. We report results from a study that uses metabolomics biomarkers as a means for assessing diagnosis and prognosis in patients who have liver disease. Plasma analytes from fasted subjects have provided information regarding 3 and 6 mo transplant free survival. This study is one of the first to employ the novel metabolomics approach as it relates to patient outcomes. These results can pave the way for future research that can enhance the way we assess patients with liver disease.

Ascha M, Wang Z, Ascha MS, Dweik R, Zein NN, Grove D, Brown JM, Marshall S, Lopez R, Hanounieh IA. Metabolomics studies identify novel diagnostic and prognostic indicators in patients with alcoholic hepatitis. *World J Hepatol* 2016; 8(10): 499-508 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i10/499.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i10.499>

## INTRODUCTION

Generally, clinical assessment is sufficient to generate

a diagnosis of alcoholic hepatitis (AH). However, sole dependence on clinical signs and symptoms is not specific, and further confirmation is usually needed. Thus, the gold standard for the diagnosis of AH is liver biopsy. Liver biopsy is considered an expensive and invasive procedure, and 1%-5% of patients require post-procedural hospitalization<sup>[1]</sup>. In addition, sampling error and inter-observer variability contribute to the limitations of liver biopsy as a procedure<sup>[1]</sup>. Therefore, it behooves practitioners to utilize alternative non-invasive tools to diagnose AH. Hanounieh *et al*<sup>[1]</sup> have shown promise in the possibility of analyzing volatile compounds in breath samples as a useful diagnostic test in patients with AH. Consequently, a rapid, non-invasive, accurate, and precise test would greatly benefit AH diagnosis.

Furthermore, prognosis of AH is determined by several scoring systems, including the model for end-stage liver disease (MELD), which is primarily based on serum lab values and is one of the chief parameters in evaluation of long-term outcome and qualification for liver transplant. While the MELD score can detect short-term survival in patients with AH with good accuracy, its prediction of long-term survival is still debated<sup>[2]</sup>. Palaniyappan *et al*<sup>[2]</sup> evaluated several scoring systems and their ability to predict long-term outcome of AH and concluded that all scoring systems were uniformly poor in predicting long term survival beyond six months. In addition, the cut-off value for the MELD score in detecting severe AH has not been agreed upon, with various studies employing different values<sup>[2]</sup>. Therefore, MELD score may not accurately reflect the risk of death in some groups of patients with liver disease such as AH awaiting liver transplantation.

Metabolomics was originally defined as the detailed qualitative and quantitative analysis of the metabolites present in complex biological samples<sup>[3]</sup>. Metabolites are both the intermediate and end result of all the biological processes taking place in a cell, tissue, or organism, thereby serving as the most proximal reporters of the body's response to a disease process or drug therapy<sup>[4]</sup>. By identifying and quantifying metabolites, one can gather a picture of the genetic variations and environmental influences (such as diet, lifestyle, drug use, and toxicological exposure) in a biological specimen. In more recent years physicians have been exploring the potential of metabolite profiling in providing diagnostic and prognostic information for many diseases, such as AH. For example, Rachakonda *et al*<sup>[5]</sup> demonstrated *via* metabolomics profiling that specific biomarkers could be used to determine disease prognosis in patients with severe AH. Thus, the potential of utilizing biomarkers in diagnosis of liver disease, assessing liver disease severity, and determining long-term survival in patients with AH is worth investigating; further exploration is warranted as there is limited information on this subject. Herein, we used a targeted metabolomics approach to identify plasma analytes that may provide improved diagnostic and prognostic value in patients with alcoholic hepatitis and end-stage liver disease.



## MATERIALS AND METHODS

### Patients

We recruited patients with liver cirrhosis awaiting liver transplantation from a single tertiary care center. The study population was divided between those with AH with cirrhosis ( $n = 25$ ) and those with acute decompensated (AD) cirrhosis from etiologies other than alcohol ( $n = 23$ ). The diagnosis of AH with cirrhosis was based on clinical and laboratory features: A patient with a history of heavy alcohol use, exclusion of other causes of liver disease, elevated aspartate aminotransferase that remained under  $< 300$  IU/mL, a ratio of aspartate aminotransferase (AST) level to alanine aminotransferase (ALT) level that is  $> 2$ , total serum bilirubin level of  $> 5$  mg/dL, an elevated international normalized ratio, and neutrophilia. Significant alcohol intake was defined as a consumption of  $> 2$  drinks daily or  $> 6$  drinks daily on weekends for the past 5 years. We used the definition of the American Association for the Study of Liver Disease guidelines of what constitutes a standard drink: 12 g of alcohol with range 9.3-13.2 g.

The diagnosis of liver cirrhosis was based on the histologic features of cirrhosis on liver biopsy and/or a composite of clinical signs and findings of cirrhosis provided by laboratory tests, endoscopy, and radiologic imaging. AH was defined by the acute development of one major complication of liver disease including acute kidney injury, ascites, encephalopathy, or gastrointestinal hemorrhage secondary to gastrointestinal varices or portal hypertensive gastropathy and enteropathy. Hepatic encephalopathy was assessed by a single individual using Conn score and asterix grade. Acute kidney injury was defined as an abrupt (arbitrarily set at 48 h) reduction in kidney function manifested by an absolute increase in serum creatinine of 0.3 mg/dL or more, equivalent to a percentage increase in serum creatinine of 50% or more (1.5-fold from baseline)<sup>[6]</sup>.

Among patients with acute decompensated liver cirrhosis, only those who remained abstinent from alcohol use for at least 6 mo before admission were included, whereas all patients with AH were (by definition) actively abusing alcohol before admission. The data was collected at the time of diagnosis and admission with alcoholic hepatitis - subjects were not drinking alcohol following admission. We also excluded all individuals with ongoing tobacco use. Patients with liver cancer or other malignancies were excluded, as were those with prior history of transplantation.

### Data collection

Mass spectrometry identified and measured 29 metabolomics compounds related to amino acid and intermediary metabolism in plasma samples from fasted subjects. Samples and associated clinical data were collected from fasting subjects undergoing community health screens. All subjects gave written informed consent and the Institutional Review Board of the Cleve-

land Clinic approved all study protocols.

### Quantification of plasma analytes by liquid chromatography/mass spectrometry/mass spectrometry

Stable isotope dilution liquid chromatography/mass spectrometry (MS)/MS was used to quantify plasma analytes. Four volumes of methanol containing isotope-labeled internal standards was added to one volume of plasma for protein precipitation. After centrifugation, supernatant was analyzed by injection onto a silica column that was interfaced with an atmospheric pressure ionization 4000 Q-TRAP mass spectrometer (AB SCIEX, Framingham, MA)<sup>[7]</sup>. A discontinuous gradient was generated to resolve analytes by mixing 0.1% propanoic acid in water with 0.1% acetic acid in methanol<sup>[7]</sup>. Analytes and the isotope-labeled internal standards were monitored in positive multiple reaction monitoring MS mode using characteristic precursor-product ion transitions (Table 1). Parameters for ion monitoring were optimized for each analyte. Various concentrations of analytes were spiked into a control plasma sample to prepare calibration curves for quantification of analytes.

### Statistical analysis

Data are presented as mean  $\pm$  SD, median (25<sup>th</sup>, 75<sup>th</sup> percentiles) or  $n$  (%). Univariable analysis was performed to compare clinical characteristics and biomarker levels between the two groups. Analysis of variance or the non-parametric Kruskal-Wallis test were used to assess differences in continuous variables and Pearson's  $\chi^2$  tests or Fisher's exact tests were used for categorical factors. Analysis of covariance was used to assess differences in biomarker levels while adjusting for MELD; the logarithm of each compound was modeled as the outcome variable with group and MELD as the independent variables. Receiver operating characteristics (ROC) analysis was performed to assess the utility of biomarkers in distinguishing acute alcoholic hepatitis from alcoholic cirrhosis; the area under the ROC curves [area under receiver operating characteristics curve (AUC)] and corresponding 95%CI are presented.

We used various statistical analyses to compare clinical characteristics and plasma levels of compounds among groups and to test the correlation between levels of compounds and severity of liver disease. Correlations between 0.0-0.3 are considered low, between 0.3-0.5 are considered moderate, and between 0.5-0.7 are considered high, and between 0.7-1.0 are considered very high. Spearman's correlation coefficients were also used to assess correlations between biomarkers and severity of liver disease for each group separately. Finally, logistic regression analysis was performed to build a predictive model for AH.

Lastly, a survival analysis was done to evaluate transplant-free survival. Kaplan-Meier product-limit estimates were used to assess transplant-free survival. Follow-up time was defined as time from sample collection to death and subjects were censored at time of

**Table 1** Characteristic precursor-product transitions

	Name	Precursor	Product
Analytes	Trimethylamine N-oxide	76	58
	Choline	104	60
	Betaine	118	59
	Valine	118	72
	Leucine	86	43
	Isoleucine	86	56
	Ornithine	133	70
	Crotonobetaine	144	59
	Butyrobetaine	146	60
	Lysine	147	84
	Methyl-lysine	161	84
	Carnitine	162	60
	Phenylalanine	166	120
	Arginine	175	70
	Citrulline	176	70
	Tyrosine	182	136
	Methyl-arginine	189	70
	Symmetric dimethyl-arginine	203	70
	Asymmetric dimethylarginine	203	70
	Acetyl-carnitine	204	85
	Propionyl-carnitine	218	85
	Butyryl-carnitine	232	85
	Pentanoyl-carnitine	246	85
	Hexanoyl-carnitine	260	85
	Octenoyl-carnitine	286	85
Internal standard	Trimethylamine N-oxide-d <sub>9</sub>	85	66
	Choline-trimethyl-d <sub>9</sub>	113	69
	Betaine-trimethyl-d <sub>9</sub>	127	68
	Valine- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N <sub>1</sub>	124	77
	Leucine- <sup>13</sup> C <sub>6</sub> , <sup>15</sup> N <sub>1</sub>	139	92
	Ornithine 3, 3, 4, 4, 5, 5-d <sub>6</sub>	139	76
	Crotonobetaine-trimethyl-d <sub>9</sub>	153	68
	Butyrobetaine-trimethyl-d <sub>9</sub>	155	69
	Lysine-u- <sup>13</sup> C <sub>6</sub> , <sup>15</sup> N <sub>2</sub>	155	90
	Phenylalanine- <sup>13</sup> C <sub>6</sub>	172	126
	Citrulline 2, 3, 4, 5-d <sub>4</sub>	180	74
	Arginine- <sup>13</sup> C <sub>6</sub>	181	74
	Tyrosine-u- <sup>13</sup> C <sub>9</sub> , <sup>15</sup> N <sub>1</sub>	192	145
	Asymmetric dimethylarginine	210	77
	2, 3, 3, 4, 4, 5, 5-d <sub>7</sub>		
	Acetyl-carnitine-d <sub>3</sub>	207	85
	Propionyl-carnitine-d <sub>3</sub>	221	85
	butyryl-carnitine-d <sub>3</sub>	235	85
	Pentanoyl-carnitine-d <sub>3</sub>	246	85
	Hexanoyl-carnitine-d <sub>3</sub>	263	85

orthotopic liver transplantation (OLT), if applicable, or last follow-up visit. Cox regression was used to assess associations between biomarker levels and transplant-free survival. In addition, inverse probability of censoring weighting estimation of cumulative/dynamic time-dependent ROC curve was used to assess the role of novel biomarkers in prediction of 3 and 6-mo LT-free survival<sup>[8,9]</sup>. Each marker was compared to the MELD score and markers with AUC of at least 0.70 were further assessed to see if any of these improved prediction of survival in combination with MELD. A  $P < 0.05$  was considered statistically significant. A 95%CI encompassing 0.5 was considered to indicate no significant predictive value. SAS (version 9.2, the SAS Institute, Cary, NC) and R (version 3.0.3, the R Foundation for Statistical Computing) were used to perform all analyses. The statistical methods of this study were reviewed by

Rocio Lopez from the Cleveland Clinic Foundation.

## RESULTS

### Baseline characteristics

Table 2 presents a summary of patient demographic and clinical characteristics. A total of 45 subjects were included in the analysis. The average age was  $53 \pm 10$  years, 54% were male, and 75% were Caucasian. The mean MELD score was  $18.0 \pm 9.3$ . MELD score was comparable between subjects with AH and those with AD.

### Metabolomics biomarkers of alcoholic hepatitis

Table 3 presents a summary of MELD-adjusted biomarker levels in the two study groups. Betaine, creatinine, homocitrulline and citrulline, tyrosine, phenylalanine, octenoyl carnitine, and symmetric dimethylarginine (SDMA) were significantly higher in patients with AH compared to those with AD.

Table 4 presents AUC data using ROC analysis, where values greater than 0.7 are strongly predictive for differentiation between AH and AD. Citrulline, betaine, and tyrosine were all notable for their values in differentiating AH from AD. Using a combination of citrulline and betaine provided the greatest AUC, at 0.835 with a 95%CI between 0.747 and 0.978. Other significant biomarkers include homocitrulline, SDMA, octenoyl-carnitine, creatinine, and phenylalanine. The remaining biomarkers were insignificant.

Table 5 presents the correlations between biomarkers and liver disease severity for alcoholic hepatitis. There was moderate to strong correlation between several biomarkers and both MELD and Maddrey's scores. Correlations between 0.0-0.3 are considered trivial/low, 0.3-0.5 are considered moderate, 0.5-0.7 are considered high and 0.7-1.0 are considered very high/strong.

The objective of this study was to detect patterns in biomarkers or hypothesis generation. In addition, adjustments for multiple comparisons are typically somewhat conservative and it would be possible to miss many potential associations that should be further explored. Holm-Bonferroni adjustment is quite conservative when the number of tests is large or the tests are not independent<sup>[10]</sup>. Despite this, we performed the Holm-Bonferroni adjustment to provide a more complete set of data (Table 6). In this case only citrulline, phenylalanine, and homocitrulline remain significantly different between the groups.

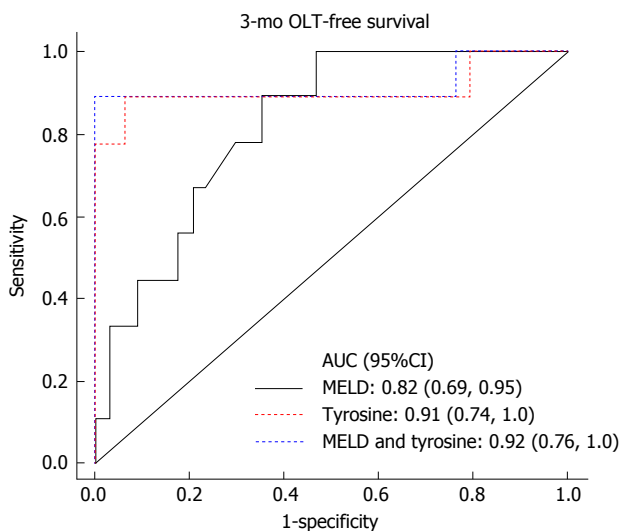
### Metabolomics biomarkers of severity of liver disease

Patients were followed over 12.5 (P25, P75: 4.3, 14.1) mo during which three subjects received a liver transplant and a total of 24% of subjects expired. As seen in Figure 1, tyrosine was strongly associated with transplant-free survival outcome in patients with liver cirrhosis [AUC for 3-mo OLT-free survival AUC = 0.91 (0.74-1.0)]. Combined MELD scores and tyrosine levels provided the best accuracy for 3-mo transplant-

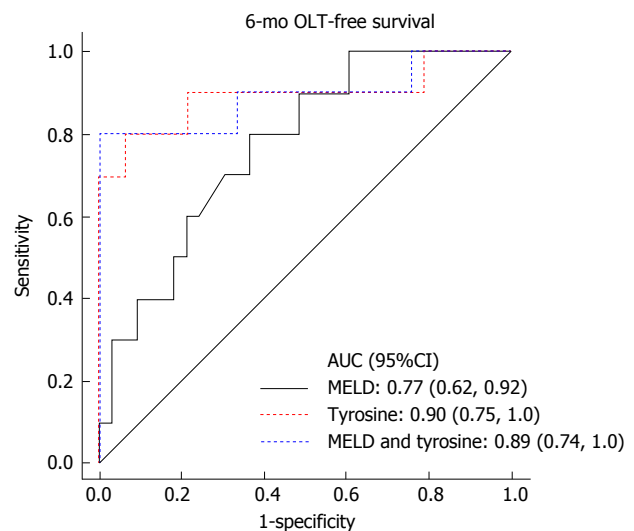
**Table 2 Patient characteristics**

Factor	Cirrhosis with acute decompensation from etiologies other than alcohol ( <i>n</i> = 23)		Alcoholic hepatitis ( <i>n</i> = 25)		<i>P</i> -value
	<i>n</i>	Summary	<i>n</i>	Summary	
Age (yr)	14	53.8 ± 9.8	20	51.5 ± 10.4	0.51 <sup>1</sup>
Male	21	13 (61.9)	23	10 (43.5)	0.22 <sup>3</sup>
Caucasian	10	10 (100.0)	19	16 (84.2)	0.53 <sup>4</sup>
AST	23	40.0 (33.0, 75.0)	25	138.0 (88.0, 161.0)	< 0.001 <sup>2,b</sup>
ALT	23	21.0 (15.0, 30.0)	25	51.0 (42.0, 71.0)	< 0.001 <sup>2,b</sup>
Bilirubin	23	3.8 (1.4, 6.0)	25	9.4 (6.8, 21.7)	0.005 <sup>2,b</sup>
Albumin	23	2.8 ± 0.66	25	2.8 ± 0.68	0.86 <sup>1</sup>
INR	23	1.5 ± 0.41	25	1.7 ± 0.59	0.16 <sup>1</sup>
PT	23	16.4 ± 4.5	25	19.1 ± 6.1	0.095 <sup>1</sup>
Creatinine	23	0.94 (0.74, 1.5)	25	0.72 (0.57, 1.04)	0.062 <sup>2</sup>
MELD score	23	16.4 ± 8.8	25	20.5 ± 10.0	0.14 <sup>1</sup>
Maddrey's score	16	22.2 (16.0, 37.0)	17	43.5 (34.0, 60.6)	0.028 <sup>2,a</sup>
Ascites	23		25		0.14 <sup>2</sup>
None		9 (39.1)		14 (56.0)	
Small		4 (17.4)		6 (24.0)	
Large		9 (39.1)		4 (16.0)	
Severe		1 (4.3)		1 (4.0)	
HE	23		25		0.94 <sup>2</sup>
None		2 (8.7)		6 (24.0)	
Mild		12 (52.2)		7 (28.0)	
Severe		9 (39.1)		12 (48.0)	
Steroids	23	2 (8.7)	25	13 (52.0)	0.001 <sup>3,b</sup>
Trental	23	3 (13.0)	25	9 (36.0)	0.067 <sup>3</sup>
OLT	23	2 (8.7)	25	1 (4.0)	0.60 <sup>4</sup>
Deceased	23	6 (26.1)	25	8 (32.0)	0.65 <sup>3</sup>

*P*-values were calculated using the test corresponding to superscript characters: <sup>1</sup>ANOVA; <sup>2</sup>Kruskal-Wallis test; <sup>3</sup>Pearson's  $\chi^2$  test; <sup>4</sup>Fisher's exact test. <sup>a</sup>*P* < 0.05 and <sup>b</sup>*P* < 0.01. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; MELD: Model for end-stage liver disease; OLT: Orthotopic liver transplantation; PT: Prothrombin time; INR: International normalized ratio; HE: Hepatic encephalopathy.



**Figure 1 Tyrosine predicts 3-mo liver transplant-free survival in patients with end-stage liver disease.** Results are presented as AUC (P25, P75). AUC: Area under receiver operating characteristics curve; OLT: Orthotopic liver transplantation; MELD: Model for end-stage liver disease.



**Figure 2 Tyrosine predicts 6-mo liver transplant-free survival in patients with end-stage liver disease.** AUC: Area under receiver operating characteristics curve; OLT: Orthotopic liver transplantation; MELD: Model for end-stage liver disease.

free survival AUC = 0.92 (0.76-1.0). Evidently these biomarkers can be used to predict OLT-free survival with reasonable sensitivity and specificity.

Figure 2 shows the same analysis with similar results, except for 6-mo OLT-free survival. MELD provided an AUC of 0.77, tyrosine provided an AUC of 0.90, and MELD and tyrosine together provided an AUC of 0.89.

Tyrosine alone as well as tyrosine in combination with MELD provided better AUC values than MELD alone, suggesting its utility in predicting OLT-free survival.

A multivariable Cox regression analysis was used to adjust for MELD, the most important predictor of mortality, and tyrosine remained significantly associated with mortality [HR = 1.02 (1.01, 1.04) for a one unit

**Table 3** Model for end-stage liver disease-adjusted average biomarker levels

Biomarker (μmol/L)	Cirrhosis with acute decompensation from etiologies other than alcohol (n = 23)	Alcoholic hepatitis (n = 25)	P-value
Choline	5.8 (4.7, 7.2)	7.0 (5.7, 8.6)	0.22
TMAO	0.74 (0.34, 1.6)	0.87 (0.42, 1.8)	0.76
Carnitine	37.2 (32.1, 43.1)	32.6 (28.3, 37.5)	0.2
Betaine	83.6 (64.7, 108.2)	134.0 (104.7, 171.5)	0.012 <sup>a</sup>
Butyrobetaine	1.6 (1.3, 1.8)	1.7 (1.4, 2.0)	0.46
Crotonobetaine	0.12 (0.10, 0.15)	0.14 (0.12, 0.18)	0.35
Creatinine	92.0 (75.0, 112.9)	59.3 (48.8, 72.1)	0.003 <sup>b</sup>
Ornithine	71.6 (59.1, 86.9)	62.0 (51.5, 74.6)	0.29
Lysine	131.3 (112.7, 153.1)	134.8 (116.4, 156.2)	0.81
Methyl-lysine	4.0 (2.9, 5.6)	3.4 (2.5, 4.7)	0.46
Argine	61.7 (52.0, 73.2)	61.8 (52.4, 72.8)	0.99
Citrulline	40.2 (33.2, 48.6)	23.7 (19.7, 28.5)	< 0.001 <sup>b</sup>
MMA	0.24 (0.20, 0.27)	0.21 (0.19, 0.24)	0.32
Homocitrulline	0.73 (0.55, 0.97)	0.37 (0.28, 0.48)	0.001 <sup>b</sup>
Leucine	52.8 (44.0, 63.4)	48.8 (40.9, 58.1)	0.54
Iso-leucine	27.1 (21.7, 33.8)	28.0 (22.6, 34.6)	0.83
Valine	115.4 (100.4, 132.7)	101.8 (89.1, 116.4)	0.2
Phenylalanine	90.4 (78.0, 104.7)	60.2 (52.3, 69.3)	< 0.001 <sup>b</sup>
Tyrosine	166.0 (126.8, 217.3)	107.4 (83.0, 139.1)	0.025 <sup>a</sup>
Acetyl-carnitine	17.8 (14.9, 21.1)	16.9 (14.3, 20.0)	0.69
Propionyl-carnitine	1.02 (0.76, 1.4)	1.2 (0.88, 1.5)	0.5
Butyryl-carnitine	2.0 (1.6, 2.5)	2.2 (1.8, 2.7)	0.58
Trimethyl-Lysine	1.00 (0.81, 1.2)	1.1 (0.92, 1.4)	0.42
SDMA	0.81 (0.65, 1.02)	0.58 (0.47, 0.72)	0.042 <sup>a</sup>
Dimethyl-Lysine	0.92 (0.72, 1.2)	0.74 (0.59, 0.94)	0.22
ADMA	0.90 (0.78, 1.03)	0.82 (0.72, 0.93)	0.32
Pentanoyl-carnitine	0.25 (0.20, 0.31)	0.27 (0.22, 0.34)	0.48
Hexanoyl-carnitine	0.69 (0.56, 0.85)	0.61 (0.50, 0.74)	0.38
Octenoyl-carnitine	0.05 (0.02, 0.12)	0.01 (0.00, 0.02)	0.009 <sup>b</sup>

Values presented as mean (95%CI) and P-values obtained from analysis of covariance. The natural logarithm of each biomarker was modeled as the outcome variable with disease group and MELD as the independent variables. <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01. ADMA: Asymmetric dimethylarginine; SDMA: Symmetric dimethylarginine; MMA: Monomethylarginine; TMAO: Trimethylamine N-oxide.

increase in tyrosine; *P* = 0.002]. In Figures 1 and 2 it can also be seen that tyrosine performs better than MELD for prediction of 3- and 6-mo mortality, and the combination of MELD and tyrosine (in a multivariable analysis) performs more or less the same as the compound by itself.

Time-dependent ROC analysis (Table 7) shows that phenylalanine [AUC = 0.77 (0.56, 0.97)], carnitine [AUC = 0.73 (0.53, 0.93)], asymmetric dimethylarginine (ADMA) [AUC = 0.72 (0.49, 0.96)] and monomethylarginine (MMA) [AUC = 0.71 (0.47, 0.94)] all provide excellent predictive value for transplant-free survival in patients with liver cirrhosis, but there was no evidence to suggest that they were significantly better than MELD.

## DISCUSSION

The purpose of this study was two-fold. First, the utility of metabolomics as an un-invasive diagnostic tool for AH was assessed. The diagnosis of AH is usually a clinical one, based on severe liver dysfunction in the context of excessive alcohol consumption, excluding other causes of acute and chronic liver disease (CLD)<sup>[1]</sup>. However, this method of diagnosis is not steadfast, as some studies that have included a liver biopsy in all patients with clinically suspected AH have shown histologic confirmation in only

70%-80% of patients<sup>[1]</sup>. Thus, liver biopsy remains the gold standard in diagnosing AH patients; however it is invasive, expensive, and burdensome for the patient. The utilization of metabolic biomarkers as an alternative, objective, un-invasive diagnostic tool is promising.

Our results demonstrated that AH patients have a specific metabolome that can be employed for diagnostic purposes. AH patients had higher levels of betaine, and lower levels of creatinine, citrulline, homocitrulline, tyrosine, phenylalanine, octenoyl-carnitine, and SDMA. Most importantly, betaine and citrulline provided excellent prediction accuracy in distinguishing AH from AD. Figure 3 shows the sensitivity and specificity of citrulline and betaine for diagnosis and acute decompensation from non-alcohol-related etiologies. Alcohol consumption in patients with alcoholic liver disease results in bacterial overgrowth and increases gut permeability and translocation of bacteria-derived lipopolysaccharides from the gut to the liver<sup>[1]</sup>. This could explain the altered levels of amino acids in these patients.

Betaine is a molecule involved in transmethylation reactions in biological systems. S-adenosylmethionine (SAM), a critical methylating agent, is crucial to maintaining the integrity of the liver. One important function of SAM is its conversion of phosphatidylethanolamine to phosphatidylcholine, the latter of which constitutes lipoproteins involved in transporting fat away from the



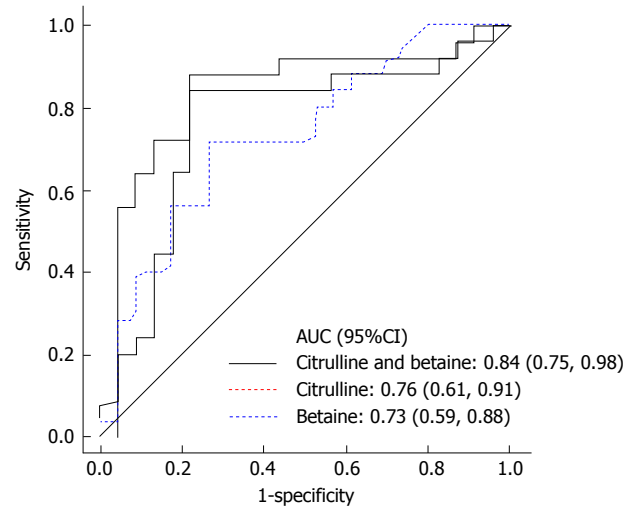
**Table 4** Utility of biomarkers in differentiating cirrhosis with alcoholic hepatitis from cirrhosis with acute decompensation from etiologies other than alcohol: Receiver operating characteristics analysis

Biomarker	AUC (95%CI)
Citrulline and betaine	0.835 (0.747, 0.978)
Betaine and phenylalanine	0.810 (0.684, 0.937)
Citrulline and phenylalanine	0.758 (0.609, 0.907)
Citrulline	0.758 (0.610, 0.907)
Betaine	0.732 (0.588, 0.877)
Phenylalanine	0.715 (0.567, 0.863)
Crotonobetaine	0.663 (0.498, 0.827)
Tyrosine	0.650 (0.484, 0.817)
Butyrobetaine	0.649 (0.489, 0.808)
Creatinine	0.645 (0.486, 0.805)
Octenoyl-carnitine	0.631 (0.488, 0.774)
Propionyl-carnitine	0.620 (0.453, 0.787)
Homocitrulline	0.618 (0.457, 0.779)
Trimethyl-lysine	0.616 (0.447, 0.784)
Butyryl-carnitine	0.615 (0.451, 0.778)
Pentanoyl-carnitine	0.613 (0.455, 0.771)
Choline	0.597 (0.432, 0.762)
Valine	0.588 (0.424, 0.752)
Lysine	0.559 (0.393, 0.725)
Methyl-lysine	0.552 (0.383, 0.721)
Iso-leucine	0.548 (0.379, 0.716)
Acetyl-carnitine	0.543 (0.375, 0.710)
Hexanoyl-carnitine	0.529 (0.362, 0.696)
TMAO	0.521 (0.347, 0.695)
Argine	0.507 (0.339, 0.675)
ADMA	0.481 (0.314, 0.647)
Leucine	0.477 (0.309, 0.644)
Carnitine	0.470 (0.301, 0.638)
MMA	0.453 (0.286, 0.620)
SDMA	0.447 (0.277, 0.617)
Dimethyl-lysine	0.417 (0.243, 0.592)
Ornithine	0.351 (0.186, 0.517)

AUC: Area under receiver operating characteristics curve; ADMA: Asymmetric dimethylarginine; SDMA: Symmetric dimethylarginine; MMA: Monomethylarginine; TMAO: Trimethylamine N-oxide.

liver, thereby preventing hepatic fat infiltration and subsequent liver injury<sup>[11]</sup>. Betaine plays a significant role in this pathway as a methylating agent in the liver. Betaine transfers a methyl group to homocysteine *via* betaine-homocysteine methyl transferase (BHMT) in order to form methionine, which then goes on to form SAM and methylate biological molecules to protect the liver. Thus, betaine is protective against harmful fatty deposits in the liver due to alcohol abuse. While acute alcohol ingestion induces BHMT activity so that SAM levels can remain physiologically normal, chronic alcohol abuse leads to diminished SAM levels due to exhaustion of this system<sup>[11]</sup>. Consequently, this lead to increased betaine levels in the serum of AH patients, as the hepatocytes cannot compensate and regenerate SAM *via* the BHMT pathway. Furthermore, other studies have shown that dietary supplementation with betaine generated increased SAM in the liver and protected against ethanol-induced steatosis in rats<sup>[12]</sup>. However, with chronic alcohol abuse and dysfunction of the BHMT pathway, betaine cannot be metabolized.

Citrulline, in particular, is a biomarker of intestinal

**Figure 3** Citrulline and betaine serve as diagnostic biomarkers in patients with alcoholic hepatitis. AUC: Area under receiver operating characteristics curve.

functionality<sup>[13]</sup>. Consequently, changes in intestinal flora due to liver disease can lead to imbalances in citrulline. Furthermore, it has been demonstrated that portal hypertension secondary to cirrhosis stimulates nitric oxide synthase (NOS)<sup>[14]</sup>. NOS converts arginine and oxygen into citrulline and nitric oxide (NO), resulting in vasodilation and increased blood flow. Pârvu *et al*<sup>[13]</sup> found that CLD patients had an increased serum citrulline concentration, indicating increased NO production to counter the mechanisms of portal hypertension<sup>[1]</sup>. Therefore, it makes sense that acute exacerbations of liver function seen in AH patients would deplete citrulline stores in an effort to produce NO and increase blood flow to an acute on chronic liver injury.

The second goal of this study was to assess the utility of metabolomics as a marker for the prognosis of liver disease. One particularly noteworthy result is the association between tyrosine and transplant-free survival outcome in patients with liver cirrhosis. The MELD score and tyrosine level, considered together, provided the greatest sensitivity and specificity for predicting 3-mo transplant-free survival. Tyrosine and phenylalanine are aromatic amino acids whose metabolism can become impaired as a consequence of liver injury, as the enzymes that metabolize these compounds are produced by the liver. Concentrations of aromatic amino acids are increased in patients with chronic liver disease who experience an acute inflammatory event such as acute alcoholic hepatitis, gastrointestinal bleeding, infection, or encephalopathy<sup>[15]</sup>. Liver cirrhosis with a superimposed liver injury will lead to systemic inflammation resulting in elevated tyrosine levels. Systemic inflammation in the context of acute on chronic liver failure exacerbates the patient's health through the release of various pro-inflammatory cytokines<sup>[15]</sup>. Therefore, plasma tyrosine levels can be used to estimate the degree and severity of this inflammation, and provide novel information on prognosis and outcome.

**Table 5** Correlations between biomarkers and model for end-stage liver disease and Maddrey's score in patients with alcoholic hepatitis

Biomarker	Alcoholic hepatitis			
	MELD		Maddrey's score	
	rho (95%CI)	P-value	rho (95%CI)	P-value
Choline	0.28 (-0.13, 0.69)	0.18	0.02 (-0.53, 0.57)	0.95
TMAO	-0.25 (-0.67, 0.17)	0.23	0.29 (-0.24, 0.82)	0.26
Carnitine	0.54 (0.18, 0.91)	0.005 <sup>b</sup>	0.48 (-0.01, 0.96)	0.054
Betaine	0.24 (-0.18, 0.65)	0.26	-0.00 (-0.55, 0.55)	0.99
Butyrobetaine	0.30 (-0.11, 0.71)	0.15	0.29 (-0.24, 0.81)	0.26
Crotonobetaine	0.07 (-0.36, 0.50)	0.74	-0.03 (-0.58, 0.52)	0.91
Creatinine	0.44 (0.05, 0.83)	0.027 <sup>a</sup>	0.48 (-0.00, 0.96)	0.052
Ornithine	0.28 (-0.13, 0.70)	0.17	0.26 (-0.27, 0.79)	0.32
Lysine	0.37 (-0.03, 0.77)	0.068	0.27 (-0.25, 0.80)	0.29
Methyl-lysine	0.09 (-0.34, 0.52)	0.67	-0.01 (-0.56, 0.54)	0.96
Argine	0.00 (-0.43, 0.44)	0.98	0.42 (-0.08, 0.92)	0.095
Citrulline	0.09 (-0.34, 0.52)	0.66	0.05 (-0.50, 0.60)	0.86
MMA	0.34 (-0.07, 0.74)	0.098	0.39 (-0.11, 0.90)	0.12
Homocitrulline	0.66 (0.34, 0.99)	< 0.001 <sup>b</sup>	0.59 (0.14, 1.00)	0.014 <sup>a</sup>
Leucine	0.03 (-0.40, 0.46)	0.89	0.50 (0.02, 0.97)	0.043
Iso-leucine	0.11 (-0.32, 0.54)	0.59	0.29 (-0.24, 0.82)	0.26
Valine	0.20 (-0.22, 0.62)	0.34	0.54 (0.08, 1.00)	0.025 <sup>a</sup>
Phenylalanine	0.34 (-0.06, 0.75)	0.092	0.56 (0.11, 1.00)	0.018 <sup>a</sup>
Tyrosine	0.30 (-0.11, 0.71)	0.14	0.44 (-0.05, 0.94)	0.074
Acetyl-carnitine	0.49 (0.11, 0.86)	0.014 <sup>a</sup>	0.50 (0.03, 0.98)	0.04 <sup>a</sup>
Propionyl-carnitine	0.40 (0.01, 0.80)	0.046 <sup>a</sup>	0.28 (-0.25, 0.81)	0.28
Butyryl-carnitine	0.48 (0.10, 0.86)	0.016 <sup>a</sup>	0.25 (-0.29, 0.78)	0.34
Trimethyl-lysine	0.48 (0.11, 0.86)	0.014 <sup>a</sup>	0.38 (-0.13, 0.89)	0.14
SDMA	0.38 (-0.02, 0.78)	0.064	0.55 (0.09, 1.00)	0.023 <sup>a</sup>
Dimethyl-lysine	0.31 (-0.10, 0.72)	0.13	0.18 (-0.37, 0.72)	0.5
ADMA	0.42 (0.03, 0.81)	0.037 <sup>a</sup>	0.60 (0.16, 1.00)	0.011 <sup>a</sup>
Pentanoyl-carnitine	0.53 (0.16, 0.90)	0.007 <sup>b</sup>	0.31 (-0.22, 0.83)	0.23
Hexanoyl-carnitine	0.49 (0.12, 0.87)	0.013 <sup>a</sup>	0.40 (-0.11, 0.90)	0.11
Octenoyl-carnitine	0.45 (0.07, 0.84)	0.024 <sup>a</sup>	0.23 (-0.30, 0.77)	0.37

Values presented as mean (95%CI) and *P*-values obtained from analysis of covariance. The natural logarithm of each biomarker was modeled as the outcome variable with disease group and MELD as the independent variables. A superscript of a indicates <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01. rho: Spearman's correlation; MELD: Model for end-stage liver disease; ADMA: Asymmetric dimethylarginine; SDMA: Symmetric dimethylarginine; MMA: Monomethylarginine; TMAO: Trimethylamine N-oxide.

Other analytes such as phenylalanine, carnitine, ADMA, and MMA were all found to be accurate predictors of transplant-free survival in patients with liver cirrhosis. It should be noted that there was no evidence to suggest that tyrosine, phenylalanine, carnitine, ADMA, and MMA were significantly better than MELD. However, despite the lack of statistical significance, in terms of the AUC even a small jump of 0.01-0.02 is promising as it denotes clinical significance. This study was an exploratory analysis designed to assess usefulness of metabolites and given the small sample size of 45 patients, no strong conclusions can be reached. However, the promising results of this study indicate the need for future studies with larger sample sizes to evaluate and corroborate the usefulness of these analytes in predicting transplant-free survival. Future studies can also explore more complex combinations of metabolites in predicting OLT-free survival. More data can help determine if plasma analytes are superior or inferior to the MELD score, or if they should be used in combination with the MELD score as a prognostic tool.

Limitations of this study include the small number

of patients in the sample size. Furthermore, no power calculations were done to determine optimum sample size. Given no power calculations and a small sample size, we are only capable of generating sufficient power for large differences and the false negative rate may be high. Further research must validate the findings from this study utilizing larger patient populations. Another limitation was the lack of control group in this study. A control group is an essential part of any experiment that seeks to find a significant difference among populations. While this project had no control group, other research has corroborated the results from this study with control groups<sup>[7]</sup>. Lastly, this study was limited in that liver biopsy was not performed in all patients to confirm the diagnosis of AH; it was only performed in a subset of patients. One final limitation of this study is the lack of biopsy confirmation of AH as a diagnosis. Since liver biopsy is considered the gold standard in diagnosing AH, it cannot be said with absolute certainty that all patients were diagnosed with AH. Further research in this area might involve standardized biopsy evaluation alongside metabolomic correlations to liver disease.

**Table 6** Model for end-stage liver disease-adjusted average biomarker levels

Biomarker ( $\mu\text{mol/L}$ )	Alcoholic cirrhosis ( $n = 23$ )	Alcoholic hepatitis ( $n = 25$ )	Holm-bonferroni corrected $P$ -value
Citrulline	40.2 (33.2, 48.6)	23.7 (19.7, 28.5)	0.009 <sup>b</sup>
Phenylalanine	90.4 (78.0, 104.7)	60.2 (52.3, 69.3)	0.009 <sup>b</sup>
Homocitrulline	0.73 (0.55, 0.97)	0.37 (0.28, 0.48)	0.029 <sup>a</sup>
Creatinine	92.0 (75.0, 112.9)	59.3 (48.8, 72.1)	0.087
Octenoyl-carnitine	0.05 (0.02, 0.12)	0.01 (0.00, 0.02)	0.26
Betaine	83.6 (64.7, 108.2)	134.0 (104.7, 171.5)	0.35
Tyrosine	166.0 (126.8, 217.3)	107.4 (83.0, 139.1)	0.73
SDMA	0.81 (0.65, 1.02)	0.58 (0.47, 0.72)	0.99
Carnitine	37.2 (32.1, 43.1)	32.6 (28.3, 37.5)	0.99
Valine	115.4 (100.4, 132.7)	101.8 (89.1, 116.4)	0.99
Choline	5.8 (4.7, 7.2)	7.0 (5.7, 8.6)	0.99
Dimethyl-lysine	0.92 (0.72, 1.2)	0.74 (0.59, 0.94)	0.99
Ornithine	71.6 (59.1, 86.9)	62.0 (51.5, 74.6)	0.99
MMA	0.24 (0.20, 0.27)	0.21 (0.19, 0.24)	0.99
ADMA	0.90 (0.78, 1.03)	0.82 (0.72, 0.93)	0.99
Crotonobetaine	0.12 (0.10, 0.15)	0.14 (0.12, 0.18)	0.99
Hexanoyl-carnitine	0.69 (0.56, 0.85)	0.61 (0.50, 0.74)	0.99
Trimethyl-lysine	1.00 (0.81, 1.2)	1.1 (0.92, 1.4)	0.99
Butyrobetaine	1.6 (1.3, 1.8)	1.7 (1.4, 2.0)	0.99
Methyl-lysine	4.0 (2.9, 5.6)	3.4 (2.5, 4.7)	0.99
Pentanoyl-carnitine	0.25 (0.20, 0.31)	0.27 (0.22, 0.34)	0.99
Propionyl-carnitine	1.02 (0.76, 1.4)	1.2 (0.88, 1.5)	0.99
Leucine	52.8 (44.0, 63.4)	48.8 (40.9, 58.1)	0.99
Butyryl-carnitine	2.0 (1.6, 2.5)	2.2 (1.8, 2.7)	0.99
Acetyl-carnitine	17.8 (14.9, 21.1)	16.9 (14.3, 20.0)	0.99
TMAO	0.74 (0.34, 1.6)	0.87 (0.42, 1.8)	0.99
Lysine	131.3 (112.7, 153.1)	134.8 (116.4, 156.2)	0.99
Iso-leucine	27.1 (21.7, 33.8)	28.0 (22.6, 34.6)	0.99
Argine	61.7 (52.0, 73.2)	61.8 (52.4, 72.8)	0.99

Values presented as mean (95%CI) and  $P$ -values obtained from analysis of covariance. The natural logarithm of each biomarker was modeled as the outcome variable with disease group and MELD as the independent variables. A superscript of a indicates <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ . ADMA: Asymmetric dimethylarginine; SDMA: Symmetric dimethylarginine; MMA: Monomethylarginine; TMAO: Trimethylamine N-oxide.

**Table 7** Utility of biomarkers in predicting transplant-free survival

Biomarker ( $\mu\text{mol/L}$ )	3-mo survival AUC (95%CI)	6-mo survival AUC (95%CI)
MELD	0.82 (0.69, 0.95)	0.77 (0.62, 0.92)
Tyrosine	0.91 (0.74, 1.0)	0.89 (0.74, 1.0)
Phenylalanine	0.77 (0.56, 0.97)	0.79 (0.61, 0.98)
Carnitine	0.73 (0.53, 0.93)	0.74 (0.56, 0.93)
ADMA	0.72 (0.49, 0.96)	0.70 (0.48, 0.92)
MMA	0.71 (0.47, 0.94)	0.70 (0.49, 0.91)

AUC: Area under receiver operating characteristics curve; MELD: Model for end-stage liver disease; ADMA: Asymmetric dimethylarginine; MMA: Monomethylarginine.

This is the first study that profiles plasma metabolites in patients with AH and CLD. Investigation of the human metabolome in disease states can be very useful in generating diagnostic markers and understanding the pathophysiology of those disease states. However, this study is limited in its relatively small sample size. Future larger studies are needed to confirm the diagnostic value of biomarkers in AH and CLD.

Metabolomics plasma analyte levels could help diagnose AH and determine the prognosis of patients

with liver cirrhosis awaiting liver transplantation. Specifically, combined citrulline and betaine plasma levels yield a highly sensitive and specific discriminatory test of AH vs AD. Tyrosine, in combination with MELD score, provides even greater sensitivity and specificity for predicting 3 mo OLT-free survival than the MELD score on its own.

In conclusion, metabolomics plasma analyte levels could aid in diagnosing AH or in determining potential patient prognosis.

## COMMENTS

### Background

Liver biopsy remains the gold standard for the diagnosis of alcoholic hepatitis (AH). Herein, the authors use a novel metabolomics approach to identify plasma analytes that may correlate with the diagnosis of AH and the severity of liver disease in patients with AH.

### Research frontiers

Metabolomics represents the analysis of metabolites present in biological samples. By identifying and quantifying metabolites, one can gather a picture of the genetic variations and environmental influences (such as diet, lifestyle, drug use, and toxicological exposure) in a biological specimen. The authors use metabolomics to assess prognostic and diagnostic factors in patients with liver disease with the hopes of developing more accurate measures of patient

outcomes.

### Innovations and breakthroughs

In this study, several metabolites were found to be associated with survival in patients with liver disease.

### Applications

These findings could potentially be used to develop more robust measures to provide a diagnosis and prognosis in patients with liver disease. The model for end-stage liver disease score and liver biopsy, which are currently used, are imperfect; a less invasive and more accurate measure is needed.

### Peer-review

This is a very important paper and presents impact on health system. It is very well elaborated.

## REFERENCES

- 1 **Hanounch IA**, Zein NN, Cikach F, Dababneh L, Grove D, Alkhouri N, Lopez R, Dweik RA. The breathprints in patients with liver disease identify novel breath biomarkers in alcoholic hepatitis. *Clin Gastroenterol Hepatol* 2014; **12**: 516-523 [PMID: 24036050 DOI: 10.1016/j.cgh.2013.08.048]
- 2 **Palaniyappan N**, Subramanian V, Ramappa V, Ryder SD, Kaye P, Aithal GP. The utility of scoring systems in predicting early and late mortality in alcoholic hepatitis: whose score is it anyway? *Int J Hepatol* 2012; **2012**: 624675 [PMID: 22988517 DOI: 10.1155/2012/624675]
- 3 **Dumas ME**, Davidovic L. Metabolic Profiling and Phenotyping of Central Nervous System Diseases: Metabolites Bring Insights into Brain Dysfunctions. *J Neuroimmune Pharmacol* 2015; **10**: 402-424 [PMID: 25616565 DOI: 10.1007/s11481-014-9578-5]
- 4 **Lewis GD**. The emerging role of metabolomics in the development of biomarkers for pulmonary hypertension and other cardiovascular diseases (2013 Grover Conference series). *Pulm Circ* 2014; **4**: 417-423 [PMID: 25621155 DOI: 10.1086/677369]
- 5 **Rachakonda V**, Gabbert C, Raina A, Bell LN, Cooper S, Malik S, Behari J. Serum metabolomic profiling in acute alcoholic hepatitis identifies multiple dysregulated pathways. *PLoS One* 2014; **9**: e113860 [PMID: 25461442 DOI: 10.1371/journal.pone.0113860]
- 6 **Angeli P**, Gines P, Wong F, Bernardi M, Boyer TD, Gerbes A, Moreau R, Jalan R, Sarin SK, Piano S, Moore K, Lee SS, Durand F, Salerno F, Caraceni P, Kim WR, Arroyo V, Garcia-Tsao G. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. *Gut* 2015; **64**: 531-537 [PMID: 25631669 DOI: 10.1136/gutjnl-2014-308874]
- 7 **Wang Z**, Levison BS, Hazen JE, Donahue L, Li XM, Hazen SL. Measurement of trimethylamine-N-oxide by stable isotope dilution liquid chromatography tandem mass spectrometry. *Anal Biochem* 2014; **455**: 35-40 [PMID: 24704102 DOI: 10.1016/j.ab.2014.03.016]
- 8 **Hung H**, Chiang C. Estimation methods for time-dependent AUC with survival data. *Can J Stat* 2010; **38**: 8-26 [DOI: 10.1002/cjs.10046]
- 9 **Blanche P**, Dartigues JF, Jacqmin-Gadda H. Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks. *Stat Med* 2013; **32**: 5381-5397 [PMID: 24027076 DOI: 10.1002/sim.5958]
- 10 **Barak AJ**, Beckenhauer HC, Tuma DJ. Betaine, ethanol, and the liver: a review. *Alcohol* 1996; **13**: 395-398 [PMID: 8836329 DOI: 10.1016/0741-8329(96)00030-4]
- 11 **Barak AJ**, Beckenhauer HC, Badakhsh S, Tuma DJ. The effect of betaine in reversing alcoholic steatosis. *Alcohol Clin Exp Res* 1997; **21**: 1100-1102 [PMID: 9309323 DOI: 10.1111/j.1530-0277.1997.tb04259.x]
- 12 **Crenn P**, Coudray-Lucas C, Thuillier F, Cynober L, Messing B. Postabsorptive plasma citrulline concentration is a marker of absorptive enterocyte mass and intestinal failure in humans. *Gastroenterology* 2000; **119**: 1496-1505 [PMID: 11113071 DOI: 10.1053/gast.2000.20227]
- 13 **Părvu AE**, Negrean V, Pleșca-Manea L, Cosma A, Drăghici A, Uifălean A, Moldovan R. Nitric oxide in patients with chronic liver diseases. *Rom J Gastroenterol* 2005; **14**: 225-230 [PMID: 16200231]
- 14 **Amathieu R**, Triba MN, Nahon P, Bouchemal N, Kamoun W, Haouache H, Trinchet JC, Savarin P, Le Moyec L, Dhonneur G. Serum 1H-NMR metabolomic fingerprints of acute-on-chronic liver failure in intensive care unit patients with alcoholic cirrhosis. *PLoS One* 2014; **9**: e89230 [PMID: 24586615 DOI: 10.1371/journal.pone.0089230]
- 15 **Rothman KJ**. No adjustments are needed for multiple comparisons. *Epidemiology* 1990; **1**: 43-46 [PMID: 2081237 DOI: 10.1097/00001648-199001000-00010]

**P- Reviewer:** da Silva NMO, Fan L **S- Editor:** Gong XM  
**L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 April 18; 8(11): 509-532





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verducci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



## Contents

Three issues per month Volume 8 Number 11 April 18, 2016

### MINIREVIEWS

- 509 Extension for Community Health Outcomes-hepatitis C: Small steps carve big footprints in the allocation of scarce resources for hepatitis C virus treatment to remote developing areas

*Tahan V, Almashhrawi A, Kahveci AM, Mutrux R, Ibdah JA*

- 513 Hepatic resection beyond barcelona clinic liver cancer indication: When and how

*Garancini M, Pinotti E, Nespoli S, Romano F, Gianotti L, Giardini V*

### ORIGINAL ARTICLE

#### Retrospective Study

- 520 Predictors of mortality after transjugular portosystemic shunt

*Ascha M, Abuqayyas S, Hanouneh I, Alkukhun L, Sands M, Dweik RA, Tonelli AR*

### CASE REPORT

- 530 Management of pregnancy in Crigler Najjar syndrome type 2

*Chaubal AN, Patel R, Choksi D, Shah K, Ingle M, Sawant P*

## Contents

*World Journal of Hepatology*  
Volume 8 Number 11 April 18, 2016

### ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Feng Wu, MD, PhD, Professor, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, Oxford OX3 7LE, United Kingdom

### AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

### FLYLEAF

I-IV Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
April 18, 2016

#### COPYRIGHT

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

#### SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

#### INSTRUCTIONS TO AUTHORS

Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjgnet.com/bpg/g_info_20160116143427.htm)

#### ONLINE SUBMISSION

<http://www.wjgnet.com/esps/>



## Extension for Community Health Outcomes-hepatitis C: Small steps carve big footprints in the allocation of scarce resources for hepatitis C virus treatment to remote developing areas

Veysel Tahan, Ashraf Almashhrawi, Ali M Kahveci, Rachel Mutrux, Jamal A Ibdah

Veysel Tahan, Ashraf Almashhrawi, Ali M Kahveci, Jamal A Ibdah, Division of Gastroenterology and Hepatology, University of Missouri, Columbia, MO 65201, United States

Rachel Mutrux, Missouri Telehealth Network and Missouri Health IT Assistance Center, Columbia, MO 65201 United States

**Author contributions:** Tahan V, Almashhrawi A and Kahveci AM performed the majority of the writing, prepared the figure; Mutrux R performed data accusation and writing; Ibdah JA provided the input in writing the paper, designed the outline with outer authors and coordinated the writing of the paper.

**Conflict-of-interest statement:** There is no conflict of interest associated with any of the authors contributed their efforts in this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Veysel Tahan, MD, Assistant Professor, Division of Gastroenterology and Hepatology, University of Missouri, 1 Hospital Dr, Columbia, MO 65201, United States. [tahanv@health.missouri.edu](mailto:tahanv@health.missouri.edu)  
Telephone: +1-573-8846044  
Fax: +1-573-8844595

Received: February 2, 2016  
Peer-review started: February 2, 2016  
First decision: March 1, 2016  
Revised: March 7, 2016  
Accepted: March 24, 2016  
Article in press: March 25, 2016  
Published online: April 18, 2016

### Abstract

Hepatitis C virus (HCV) infection is still a major health problem throughout the world. HCV patients living in rural areas are less fortunate than their counterparts residing in populous urbanized regions. The lack of medical resources and properly trained medical personnel in rural regions make it especially burdensome for HCV patients seeking treatment. Dr. Sanjeev Arora at the University of New Mexico Health Sciences Center took initiative to resolve the issue at hand by developing a model named Project Extension for Community Health Outcomes (ECHO). ECHO connects primary care providers (PCPs), usually family medicine physicians, in local communities with specialists. ECHO providers test the efficacy of treatment given using the ECHO model vs that at academic medical centers. The ECHO model has produced promising results such that the sustained virologic response rates for both types of sites were near-equivalent. Show Me ECHO was adapted from Project ECHO to train PCPs in Missouri and equip them with the tools and skills to properly treat and diagnose HCV in a timely manner. This healthcare model can be implemented for treating other common infections and chronic diseases. Telemedicine is the direction healthcare is headed for the next several decades. It has potential to be applied in developing countries to alleviate agony and despair resulting from limited resources and lack of access to expert medical care.

**Key words:** Hepatitis C; Treatment; Community; Health care; Outcome; Rural; Primary care; Extension for Community Health Outcomes

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The American Association for the Study of Liver

Diseases recommends Project Extension for Community Health Outcomes (ECHO). Project ECHO aims to move the knowledge not the patients. By bringing expertise to primary care physicians, patients from rural and underserved communities will benefit by alleviating the struggle associated with travel and appointment delays. The framework of this project can be used to manage other diseases that require specialty physician care that may not be feasible. Telemedicine represents the future of healthcare, its success will substantially reshape the healthcare delivery in developing countries and is pivotal for geographically isolated and underserved populations.

Tahan V, Almashhrawi A, Kahveci AM, Mutrux R, Ibdah JA. Extension for Community Health Outcomes-hepatitis C: Small steps carve big footprints in the allocation of scarce resources for hepatitis C virus treatment to remote developing areas. *World J Hepatol* 2016; 8(11): 509-512 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i11/509.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i11.509>

## INTRODUCTION

Hepatitis C virus (HCV) infection and its complications are still a major health problem throughout the world. There are roughly 4 million persons with a seropositive test for HCV in the United States, many of whom are even not aware of the disease and many of those who know are in a medical quandary with regards on how to access treatment<sup>[1]</sup>. Incredible progress has been achieved regarding HCV treatment in the past decade, highlighted by an increase in HCV cure rates.

Access to proper HCV treatment and care has truly become a hurdle for millions of patients housed in rural settings because of the uneven distribution of trained medical personnel and resources to select urbanized cities. As a result, patients in rural and remote areas often find themselves at the mercy of HCV because of the shortage of medical resources at their disposal. Oftentimes, these patients have no other option but visit nearby primary care providers (PCPs) in small clinics. However, PCPs are only equipped to address basic healthcare needs which mean HCV patients are subject to subpar treatment at best<sup>[2,3]</sup>.

## WHAT IS PROJECT EXTENSION FOR COMMUNITY HEALTHCARE OUTCOMES?

The University of New Mexico Health Sciences Center (UNMHSC) launched the Extension for Community Healthcare Outcomes (ECHO) model in 2003 in response to the external circumstances that burdened many rural HCV patients from successfully being treated. Dr. Sanjeev Arora, a distinguished Professor of Medicine, Division of Gastroenterology, at the UNMHSC, is the

director and founder of Project ECHO. He pioneered a new high-speed approach for providing expert healthcare to patients. Dr. Arora was distraught with the reality that there is a prevalent shortage of resources; there are thousands of HCV patients needing quality care and treatment. He chose to be proactive to instill necessary change that was long overdue. Coupled with the rapid technological advancements of the time, telemedicine progressed and ECHO was born<sup>[3,4]</sup> and has been proven to be effective<sup>[5-7]</sup>.

The purpose of the ECHO model is to establish a working network of PCPs, psychiatrists, pharmacists, infectious disease specialists, and other healthcare professionals that can collaborate and exchange information such as patient lab results and treatment plans (Figure 1). Through a video conferencing platform, the teams are given an opportunity to inform each other of their own personal experiences and challenges to better serve the interests of HCV patients in the long run. These sessions, called Knowledge Networks, allow PCPs to acquire the critical skills necessary in treating geographically isolated HCV patients<sup>[8]</sup>.

## HIT OR MISS?

Arora *et al*<sup>[8]</sup> set up an experimental model to test HCV treatment efficacy of their newly inaugurated ECHO program. They hypothesized that any success they have had with treating patients at academic medical centers would be mirrored in remote clinics employing the ECHO model. The parameter used to measure efficacy of treatment is sustained virologic response (SVR). After a patient completes therapy, he/she is evaluated for a period of 6 mo. If the HCV does not reappear during this time, the patient has achieved SVR<sup>[8]</sup>.

Originally, 519 patients enrolled in the study from both the ECHO sites and University HCV clinics. Of these 519, 407 remained relevant for the overall SVR rates in the study. HCV patients who got at least one dose of HCV treatment were included in the analysis. Any patient without follow-up data was considered as treatment failure<sup>[5]</sup>. The patient count was 261 and 146 at the ECHO sites and University HCV clinics, respectively. Some more patients were also discontinued from the study for not meeting specified health targets. The overall SVR was 152/261 (58.2%) and 84/146 (57.5%) for ECHO sites and the University of New Mexico HCV clinic, respectively. Therefore, the magnitude of this success supports what Arora *et al*<sup>[8]</sup> had hypothesized early on about HCV treatment using the ECHO model. As a result, the number of ECHO sites drastically increased to around 300 nowadays<sup>[8]</sup>. Currently, each center is collecting the SVR data on new and more effective interferon free HCV treatment to compare the outcomes.

## NEW MEXICO TO MISSOURI

Missouri residents amount to a little over 6 million



Figure 1 Extension for Community Health Outcomes Model. ECHO: Extension for Community Health Outcomes.

people. About one-fourth of all Missourians inhabit rural areas of the state<sup>[9]</sup>. Missouri residents that belong to the underserved areas of the state are oftentimes disconnected from their specialty care providers because of geographic barriers. As mentioned before, the pool of health care resources is often concentrated in large metropolitan cities, which subsequently attracts many specialty care providers to these populous areas<sup>[3]</sup>. According to the Bureau of Health Professions, about 20% to 25% people in Missouri live in a rural community. However, the percentage of physicians caring for these communities are roughly 9% of all physicians in Missouri with a notable shortage of specialists<sup>[10]</sup>.

## TARGETS OF SHOW ME ECHO MODEL

Show Me ECHO, an adaptation of the University of New Mexico School of Medicine's ECHO model, was instigated with a similar purpose: To promote accessible and affordable quality care for HCV patients in disadvantaged underserved and rural populations in Missouri with an aim to move the knowledge not the patients<sup>[6]</sup>.

Show Me ECHO model in Missouri also echoes developing PCPs expertise. By educating and empowering PCPs, it will be possible to screen, diagnose, and treat HCV in a timely fashion in remote and underserved areas. The use of telemedicine surely has that potential to bridge the gap between specialists and PCPs through an exchange of knowledge and treatment protocols which can improve the patient experience for generations to come. In addition to all of this, a health surveillance system is essential to ensure fluid interactions between patients and healthcare providers. One of Show Me ECHO project goals is to create a link between the Missouri Department of Health, Senior Services, and the Missouri Primary Care Association so that HCV cases are accurately recorded. Thus, a sound health surveillance system across Missouri will advance the timeliness of diagnosis and treatment.

## IMPLICATIONS OF ECHO

The American Association for the Study of Liver Diseases recommends Project ECHO because of its success in treating HCV patients amid the adversities experienced initially by both patients and healthcare professionals<sup>[7]</sup>. Indeed, the benefits of Project ECHO

are paramount and not limited to successful SVR rates. By bringing expertise to PCPs, patients from rural and underserved communities will benefit by alleviating the struggle associated with travel and appointment delays. Additionally, community-based health centers, rather than university clinics, are usually more suitable for rural HCV patients because PCPs are often more cognizant of their local community. Visiting the same PCP for HCV treatment reduces tensions between both patients and healthcare providers, allowing for optimal coordination in a familiar setting<sup>[8]</sup>. Without Project ECHO, many HCV treatments would have been stymied. Show Me ECHO is on track as well to produce promising results.

ECHO model is a great Segway into healthcare in developing countries. The possibilities for introducing Project ECHO in those countries are immense because healthcare is presumably hindered by the lack of appropriate expert medical care. Telemedicine can leverage the aid and significantly alleviate the patients' sufferings.

Project ECHO is a great template for the medical field to actively embrace because of its potential to allocate specialized care needed for disadvantaged HCV patients in developing countries. The framework of this project can be used to manage other diseases that require specialty physician care that may not be feasible. Telemedicine represents the future of healthcare, its success will substantially reshape the health care delivery in developing countries and is pivotal for geographically isolated and underserved populations.

## REFERENCES

- 1 CDC-Viral Hepatitis Statistics and Surveillance. Available from: URL: <http://www.cdc.gov/HEPATITIS/Statistics/index.htm>
- 2 Arora S, Thornton K, Komaromy M, Kalishman S, Katzman J, Duhigg D. Demonopolizing medical knowledge. *Acad Med* 2014; **89**: 30-32 [PMID: 24280860 DOI: 10.1097/ACM.0000000000000051]
- 3 Volk ML, Tocco R, Saini S, Lok AS. Public health impact of antiviral therapy for hepatitis C in the United States. *Hepatology* 2009; **50**: 1750-1755 [PMID: 19824079 DOI: 10.1002/hep.23220]
- 4 University of New Mexico. Available from: URL: <http://echo.unm.edu/about-echo/our-story/>
- 5 Arora S, Thornton K, Murata G, Deming P, Kalishman S, Dion D, Parish B, Burke T, Pak W, Dunkelberg J, Kistin M, Brown J, Jenkuskus S, Komaromy M, Qualls C. Outcomes of treatment for hepatitis C virus infection by primary care providers. *N Engl J Med* 2011; **364**: 2199-2207 [PMID: 21631316 DOI: 10.1056/NEJMoa1009370]
- 6 Missouri Telehealth Network. ECHO: Show Me ECHO. Available

- from: URL: <http://medicine.missouri.edu/telehealth/echo.html>
- 7 **AASLD.** HCV Guideline 2015. Available from: URL: <http://www.hcvguidelines.org/full-report/hcv-testing-and-linkage-care>
- 8 **Arora S**, Kalishman S, Thornton K, Dion D, Murata G, Deming P, Parish B, Brown J, Komaromy M, Colleran K, Bankhurst A, Katzman J, Harkins M, Curet L, Cosgrove E, Pak W. Expanding access to hepatitis C virus treatment--Extension for Community Healthcare Outcomes (ECHO) project: disruptive innovation in specialty care. *Hepatology* 2010; **52**: 1124-1133 [PMID: 20607688 DOI: 10.1002/hep.23802]
- 9 **USDA.** Economic Research Service: State fact Sheets. Available from: URL: <http://www.ers.usda.gov/data-products/state-fact-sheets/state-data.aspx?reportPath=/StateFactSheets/StateFactSheet&StateFIPS=21>
- 10 **Rosenblatt RA**, Hart LG. Physicians and rural America. *West J Med* 2000; **173**: 348-351 [PMID: 11069878]

**P- Reviewer:** Cao GW, Jin B, Rezaee-Zavareh MS  
**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Liu SQ





## Hepatic resection beyond barcelona clinic liver cancer indication: When and how

Mattia Garancini, Enrico Pinotti, Stefano Nespoli, Fabrizio Romano, Luca Gianotti, Vittorio Giardini

Mattia Garancini, Enrico Pinotti, Stefano Nespoli, Fabrizio Romano, Luca Gianotti, Vittorio Giardini, Department of Surgery, Hepatobiliopancreatic Unit, San Gerardo Hospital, University of Milano Bicocca, 20900 Monza, Italy

**Author contributions:** All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting, critical revision, editing, and final approval of the final version.

**Conflict-of-interest statement:** All authors have no potential conflicts of interest and no financial support to declare.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Mattia Garancini, MD, Department of Surgery, Hepatobiliopancreatic Unit, San Gerardo Hospital, University of Milano Bicocca, Via Pergolesi 33, 20900 Monza, Italy. [mattia\\_garancini@yahoo.it](mailto:mattia_garancini@yahoo.it)  
 Telephone: +39-039-2339783  
 Fax: +39-039-2339783

Received: September 11, 2015  
 Peer-review started: September 11, 2015  
 First decision: October 27, 2015  
 Revised: February 18, 2016  
 Accepted: March 24, 2016  
 Article in press: March 25, 2016  
 Published online: April 18, 2016

### Abstract

Hepatocellular carcinoma (HCC) is the main common primary tumour of the liver and it is usually associated with cirrhosis. The barcelona clinic liver cancer (BCLC)

classification has been approved as guidance for HCC treatment algorithms by the European Association for the Study of Liver and the American Association for the Study of Liver Disease. According to this algorithm, hepatic resection should be performed only in patients with small single tumours of 2-3 cm without signs of portal hypertension (PHT) or hyperbilirubinemia. BCLC classification has been criticised and many studies have shown that multiple tumors and large tumors, as wide as those with macrovascular infiltration and PHT, could benefit from liver resection. Consequently, treatment guidelines should be revised and patients with intermediate/advanced stage HCC, when technically resectable, should receive the opportunity to be treated with radical surgical treatment. Nevertheless, the surgical treatment of HCC on cirrhosis is complex: The goal to be oncologically radical has always to be balanced with the necessity to minimize organ damage. The aim of this review was to analyze when and how liver resection could be indicated beyond BCLC indication. In particular, the role of multidisciplinary approach to assure a proper indication, of the intraoperative ultrasound for intraoperative restaging and resection guidance and of laparoscopy to minimize surgical trauma have been enhanced.

**Key words:** Hepatocellular carcinoma; Liver surgery; Hepatic resection; Multiple hepatocellular carcinoma; Cirrhosis; Barcelona clinic liver cancer; Multidisciplinary approach; Intraoperative ultrasound; Laparoscopy; Portal hypertension

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** According to the barcelona clinic liver cancer (BCLC) classification liver resection should be performed only in patients with small single hepatocellular carcinoma of 2-3 cm without signs of portal hypertension (PHT). Nevertheless, many studies have shown that patients with multiple and large hepatocellular carcinoma

noma, as like as those with macrovascular infiltration and PHT, could benefit from liver resection. Consequently BCLC algorithm should be updated and revised. The aim of this review was to analyze when and how liver resection could be indicated beyond BCLC indications. In this perspective, the role of multidisciplinary approach, of intraoperative ultrasound and of laparoscopy have been enhanced.

Garancini M, Pinotti E, Nespoli S, Romano F, Gianotti L, Giardini V. Hepatic resection beyond barcelona clinic liver cancer indication: When and how. *World J Hepatol* 2016; 8(11): 513-519 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i11/513.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i11.513>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the main common primary tumour of the liver, representing approximately 85%-90% of primary hepatic malignancies; it is ranked as the fifth and seventh most common cancer respectively in males and females, and represents the third leading cause of neoplasm-related deaths worldwide<sup>[1,2]</sup>. HCC is usually associated with cirrhosis, whose major causes could be identified in viral and alcoholic liver disease, although recent epidemiological data highlighted the increasing etiological role of obesity, diabetes and metabolic syndrome in liver oncogenesis<sup>[3]</sup>. The treatment of HCC set on cirrhosis is complex: The aim to be oncologically radical has always to be balanced with the necessity to minimize organ damage. In this sense liver transplantation is considered the gold standard treatment, because offers the possibility to treat simultaneously the liver cancer and the damaged organ; on the other hand organ shortage led to the development of restricted indication to liver transplantation, addressing many patients to receive local therapies<sup>[4]</sup>. In literature, several HCC staging systems based on tumour's features and liver function have been developed and proposed to guide the therapeutic decisions in such patients; among all the barcelona clinic liver cancer (BCLC) classification (Figure 1) has been approved as guidance for HCC treatment algorithms by the European Association for the Study of Liver (EASL) and the American Association for the Study of Liver Disease (AASLD), combining independent prognostic predictors like the background liver status, patient's performance status and tumor morphological features, and showing a reliable capacity to categorize patients with different prognosis in order to provide recommendations regarding therapeutic options. The BCLC flow chart recommends curative treatments for HCC in very early- or early-stage (stage 0-A), trans-arterial chemoembolization for intermediate-stage disease (stage B), sorafenib administration for advanced stage HCC (stage C), and supportive care for end stage HCC (stage D)<sup>[5,6]</sup>. BCLC indication to liver resection seems to be markedly limiting. On contrary, other

authors have shown that surgical resection can offer good short- and long-term outcomes even in presence of portal hypertension (PHT), multinodular disease, large nodules or even HCC with macrovascular invasion<sup>[7-9]</sup>; thus the BCLC classification has been criticised because some patients who may benefit from surgical treatment are excluded from curative resection and these findings have encouraged many experts to disregard the EASL/AASLD therapeutic recommendations<sup>[6-8]</sup>.

The aim of this review was to analyze when and how liver resection could be indicated beyond BCLC indications.

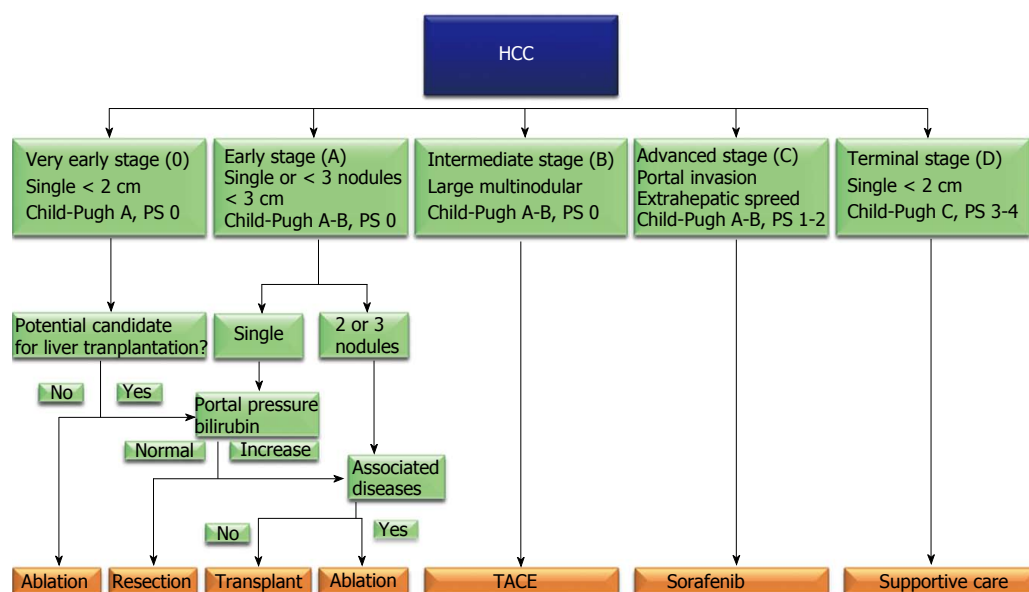
## DISCUSSION

### **Liver resection beyond BCLC classification: When?**

Historically hepatic resection has been performed with caution to HCC patients because of concerns about morbidity and mortality rates. However, recent improvements in surgical technique and perioperative care have improved hepatic resections outcomes with consequent extension of indications to surgical procedures. In this sense some high volume surgical liver unit recently reported hospital mortality less than 2%<sup>[10]</sup>. According to BCLC algorithm, liver resection would be indicated only in patients with single tumours of 2-3 cm in diameter without PHT or increased bilirubinemia<sup>[6]</sup> (Figure 1). BCLC classification has been criticised because it excludes many patients who could benefit from curative resection. PHT, large tumor size, multifocal presentation and vascular invasion are well recognized risk factors for post-operative morbidity and mortality and for poor long-term prognosis, but should not be considered contraindication to surgical treatments.

### **LARGE HCC (> 5 cm)**

BCLC algorithm suggests hepatic resection only in presence of small HCC (< 5 cm). On contrary, several authors have recently reported that liver resection can offer good short- and long-term outcomes even in patients with HCC > 5 cm. Main concerns related to restricted surgical indication in patients with large HCC take into account the increased rates of presence of satellite nodules, increased rate of distant metastases and of vascular invasion those are related to increasing tumour size and represent important prognostic factors for poor survival. Furthermore patients with large HCC (> 5 cm) may necessitate a major hepatectomy, which is considered a high-risk procedure especially in HCC set on cirrhosis<sup>[11,12]</sup>. Anyway radical liver resection can be considered a valuable option in patients with large HCC<sup>[13]</sup>. Large surgical series recently published reported a significant rate (up to 36%) of large HCC surgically treated with good results<sup>[14]</sup>. In this sense, it's remarkable that in literature there are many studies reporting cases of liver resection for HCC > 8-10 cm with good results even considering the poor prognostic results of the main alternative for such BCLC stage B HCC represented



**Figure 1** Barcelona clinic liver cancer algorithm for treatment of hepatocellular carcinoma<sup>[9]</sup>. HCC: Hepatocellular carcinoma; TACE: Transarterial chemo-embolization.

by transarterial chemo-embolization (TACE). Zhong *et al.*<sup>[15]</sup> comparing liver resection to TACE in a wide cohort of patients with large HCC in BCLC B stage (mean size 8.8 cm) showed that tumor resection offers better 5-year overall survival than TACE (41% and 18%, respectively). Proper identification and multidisciplinary discussion of risk factors for surgical morbidity and mortality in such patients (including presence of vascular invasion, cirrhosis, high level of alpha-fetoprotein and the presence of multiple lesions<sup>[16]</sup>) is critical for patients' selection and to obtain good outcomes.

## MULTIPLE HCC

Treatment guidelines do not recommend hepatic resection for multifocal HCC. Liver resection in presence of multiple HCC is still controversial; anyway it has been recognized a survival benefit for patients with a number of HCC  $\leq 3$  and lesions less than 3 cm in diameter (according to Milan criteria)<sup>[17]</sup>. Multifocal presentation is well recognized independent prognostic factor for early recurrence and poor prognosis<sup>[18]</sup>. Anyway recent prospective studies showed that hepatic resection in patients with BCLC stage B HCC is well tolerated and related to a low mortality rate, acceptable morbidity and significant survival benefits<sup>[4]</sup>. Surgical resection yielded better results than TACE in patients with multiple HCCs of the same stage; Zhong *et al.*<sup>[19]</sup> analyzed outcomes of patients with more and less of 3 HCC tumors who underwent liver resection or TACE. Survival was significantly higher in the surgery subgroup at 1 year (90% vs 59%), 3 years (52% vs 11%), and 5 years (33% vs 6%).

## PORTAL VEIN HYPERTENSION

PHT is considered a contraindication for liver resection

according to the EASL and AASLD published guidelines for HCC management. PHT may increase the risk of peri-operative haemorrhage, impair liver regeneration, and increase the risk of liver failure. Recent advances in surgical techniques and peri-operative care for patients with cirrhosis have reduced the number of cirrhosis-related complications and deaths. Several authors demonstrated that patients with and without PHT had similar morbidity (28%-39% vs 21%-32.2%) and 90-d mortality (2%-2.1% vs 3.1%-6%). The overall survival at 1, 3 and 5 years is similar or slightly longer in patients without portal vein hypertension (respectively 85%-96%, 67%-80% and 50%-65%) compared to patients with PHT (respectively 83%-90%, 59%-67% and 45%-48%), these results appear significant and encouraging, considering that liver resection, with exclusion of liver transplantation, represent the best choice of radical cure<sup>[20-22]</sup>. Patients with PHT should be carefully selected for surgery, but PHT should not be considered a contraindication to liver resection.

## MACROVASCULAR INFILTRATION

According to the EASL and AASLD guidelines for management of HCC, patients with macrovascular infiltration are considered in advanced stage (stage C) and should be treated only with chemotherapy (Sorafenib). Presence of macrovascular invasion is related to an increased risk of metastases and is a well known predictor of poor survival<sup>[23]</sup>. The median survival for untreated patients with macrovascular portal or major hepatic vein infiltration is 3-5 mo and median survival for such patients treated with sorafenib is 6 mo<sup>[18,24]</sup>. Selected patients with macrovascular infiltration who underwent liver resection for HCC can achieve longer overall survival, 46%-49% and 11.2%-38% respectively

at 3 and 5 years with acceptable morbidity and mortality rate (under 3%-5%)<sup>[13,14,25]</sup>. Consequently the surgical resection should be considered when planning the treatment's strategy for such patients and formally included together with other treatment modalities for the cure of BCLC stage C patients.

#### ***Liver resection beyond BCLC classification: How?***

Patients suffering from cirrhosis are at increased risk of developing significant postoperative complications including ascites, lung infection or pleural effusion, transient encephalopathy, kidney failure, portal vein thrombosis and bleeding due to primary haemostasis dysfunction<sup>[26,27]</sup>. In order to reduce mortality and morbidity after liver surgery in patients with cirrhosis, surgeons have developed meticulous selection criteria to guide surgical indication in such patients. For all these reasons the decision to submit a cirrhotic patient to a liver resection is complex. It is of paramount importance that pre-operative evaluation of cirrhotic patients with hepatocellular carcinoma would be performed by a multidisciplinary team, in order to match different point of view and possible therapeutic approach. Furthermore, some technical aspects of liver resection should be enhanced discussing the approach to patients with an advanced stage HCC.

### **THE ROLE OF MULTIDISCIPLINARY APPROACH**

HCC has different presentations those are compounded by the status of liver disease, and the multiple treatment options available make choosing the first line of treatment for a given patient a difficult task. Management of HCC patients should be undertaken by a multidisciplinary team including all the specialties those have a role in the treatment of such patients; if this kind of approach should represent the standard for the treatment of every patient with HCC, the importance of the sharing of indication in disagreement with BCLC algorithm is even increased. Studies those have shown a decrease in morbidity and mortality after liver resection for HCC also showed the importance of a multidisciplinary approach<sup>[28-31]</sup>. Patients in intermediate/advanced stages should be carefully selected for the best treatment according to the stage of the disease, to the presence of cirrhosis, to the age of patient, to general condition and comorbidity. A team of surgeon, oncologist, hepatologist, radiologist and interventional radiologist, anesthesiologist and pathologist should evaluate the best treatment for each of these patients, in order to perform a tailored treatment that could include more than one approach. BCLC indication to liver resection should be less restrictive; on the other hand the importance of patients' selection must be considered and great efforts are needed to establish selection criteria to be included in the treatment algorithm.

### **THE ROLE OF INTRAOPERATIVE ULTRASOUND**

Intra-operative ultrasound (IOUS) is still considered the most accurate diagnostic technique for detecting focal liver lesions in hepatocellular carcinoma. The main advantages related to an extensive use of ultrasounds in liver surgery for HCC on cirrhosis concerns the intra-operative re-staging and the possibility to perform echo-guided surgical procedures. IOUS may detect additional nodules compared with pre-operative imaging in 33%-41% of patients undergoing liver resection for HCC<sup>[32,33]</sup>. The removal of new nodules after this early diagnosis may increase the BCLC stage of patients but also contribute to perform a more complete treatment and improve choice of cure. Moreover intra-operative echo guidance, allowing to perform a parenchyma-sparing anatomical hepatic surgery in respect of principles of oncologic radicality, is an invaluable tool to engage surgical procedure in intermediate/advanced-stage patients<sup>[34]</sup>. The use of ultrasound guidance is mandatory for planning the surgical strategy, decide the exact resection plane during the parenchymal transection in order to respect the surrounding vessels and biliary structures. Main concerns related to the restricted indication to liver surgery following BCLC indication regards the possibility of increased peri-operative mortality and morbidity and the poor chance of radical cure and prolonged survival in patients with intermediate/advanced HCC. IOUS, minimizing the extension of the parenchyma removed in respect of oncological radicality and offering a re-staging and a consequent more radical treatment, represents an invaluable tool in the perspective of expansion of surgical indication beyond BCLC recommendations. The extensive use of ultrasound in liver surgery together with technological improvements in recent years allowed *per se* an expansion of surgical indication in advanced HCC: The possibility to detect intra-operatively connecting veins between adjacent hepatic veins allows to perform radical limited liver resection even in patients with major hepatic vein invasion, in order to reduce the rate of major resection and its consequent increased morbidity and mortality<sup>[35,36]</sup>. Anatomic liver resection is usually performed because of HCC spreading along the nourishing portal venous branch and consequent growth of satellite nodules within the same anatomical segment. Thus, anatomic resection allows removal of the known tumor, as well as of potential undetectable satellite metastases; the advantages of anatomic resection can be maximized in particular in large HCC which are frequently surrounded by satellite lesions<sup>[37,38]</sup>. IOUS is of paramount importance to guide and assure an anatomic liver resection, either with traditional puncture technique of the portal branch feeding the tumor, either by means of recently introduced compression technique or other methods as trans-hepatic balloon catheter or CEIOUS portography combined with indigo carmine dye injection<sup>[39-43]</sup>. In a recent meta-analysis Chen *et*



*al*<sup>[44]</sup> analyzed outcomes of 833 patients underwent anatomic liver resection for HCC and 670 patients underwent non-anatomic resection for the same disease. The surgical margin *per se* does not represent a main aspect, because an anatomic resection (segmentectomy or sub-segmentectomy) can be considered adequate even in presence of a narrow margin; the advantages related to limited anatomic resection can be maximized to perform multiple limited resection in multiple HCC, in order to assure local radical tumor removal with a parenchyma sparing policy<sup>[44]</sup>. There are several methods up to now available to perform an anatomic (segmental or subsegmental) US-guided liver resection: Puncture technique proposed by Makuuchi *et al*<sup>[40]</sup>, insertion of a balloon catheter transhepatically to occlude the feeding portal branch<sup>[41]</sup> and ultrasound-guided finger compression technique<sup>[43]</sup>.

## THE ROLE OF LAPAROSCOPY

Laparoscopic surgery for liver tumors requires skilled surgeons and specific technological instruments. Moreover its indications have not been still clearly defined; for such reasons it has not been widely performed even if its employment is progressively expanding. The main concern about the use of laparoscopic technique for malignancies is the risk of inadequate tumor resection; positive margin is a well known prognostic factor for poor survival in surgery for HCC, in this perspective intra-operative ultrasound should be considered an indispensable tool to achieve a safe and effective liver resection. Anyway according to several meta-analyses comparing open vs laparoscopic liver resections for HCC, laparoscopic liver resection is considered a safe procedure with comparable overall and recurrence-free survival rates<sup>[45-47]</sup>. Laparoscopic approach might improve the postoperative course of cirrhotic patients, because limited mobilization of the liver reduces parenchymal trauma, nonexposure of intestinal viscera restricts fluid requirements and decreased the formation of ascites<sup>[26,48]</sup>. In a recent study Kanazawa *et al*<sup>[26]</sup> compared outcomes of cirrhotic patients underwent laparoscopic and laparotomic liver resection; the two groups was not different by age, sex, stage of cirrhosis, number and size of lesions. In this study the incidence of intractable ascites was significantly higher in the laparotomy group than in the laparoscopy group (71% vs 11%). Furthermore the use of laparoscopy in cirrhotic patients may allow the preservation of wall portosystemic shunts, and in some cases the integrity of round ligament, which can contain collateral vessels. This can result in a lower increase of post-operative PHT and risk of bleeding<sup>[26]</sup>. Laparoscopic liver resection is associated with less total and major morbidity, shorter hospital stay and lower rate of post-operative early readmissions or number of outpatient clinic appointments compared with open counterpart<sup>[49,50]</sup>. The above mentioned advantages of laparoscopic approach can be crucial in the perspective of expansion

of surgical indication to patients with HCC beyond BCLC indications. In particular patients with PHT or multinodular disease can benefit of a minimally invasive treatment, which can include also combined laparoscopic resection and ablation of multiple HCC in the perspective of a tailored treatment<sup>[51,52]</sup>. Surgical resection has shown better results in terms of disease free and overall long term survival compared to laparoscopic ablation. Nevertheless, in the treatment of HCC not suitable for liver transplantation or not eligible for resection because of severe PHT and not manageable by percutaneous approach for tumor size or location, laparoscopic ablation should be considered as a valuable choice since it proved to be a safe and effective technique, as it permits to treat lesions with low-morbidity-rate<sup>[51]</sup>. Laparoscopic approach is also useful to avoid unnecessary laparotomy in patients who show unresectable not previously diagnosed lesions (36% of patients with HCC with surgical indication)<sup>[53]</sup>. Several authors showed that laparoscopic liver resections can be technically performed regardless tumor size and location, but important reviews have recognized that it can be considered more safe and feasible for lesions located on left lateral (segments II and III) and anterior right (III, VI) segments<sup>[45-47]</sup>. For this reason the position of lesions is an essential element to establishing the indication to surgical resection for advanced HCC. In order to minimize postoperative complications in fragile patients, the possibility of laparoscopic liver resections is a decisive element in the decision-making process of the best treatment of patients with HCC.

## CONCLUSION

According to BCLC classification hepatic resection should be performed only in patients with small single tumours of 2-3 cm without signs of PHT or hyperbilirubinemia. By contrast many studies have shown that surgical resection can lead to good short and long-term survival in patients with PHT and with multinodular, large or macrovascular invasive HCC. The treatment of these patients is complex, surgery should only be performed in selected patients and a multidisciplinary team is necessary to choose the best treatment for each patient. Intra-operative ultrasound and laparoscopy are necessary tools in a modern liver unit, especially for cirrhotic patients.

BCLC indication should be expanded and redefined: BCLC algorithm should take into account the survival benefit of surgical resection in selected patients with HCC in B-C stages and should discuss the invaluable role of IOUS and the potential role of laparoscopy with the aim to standardize the surgical management. After the recognition that ablative treatment in HCC  $\leq 2$  cm offer the same survival benefit than surgical removal and consequently can be considered the treatment of choice for such patients<sup>[54]</sup>, it should be recognized that surgical treatment offers the best choice of prolonged survival even to selected patients with intermediate/advanced

stage HCC.

## REFERENCES

- 1 **Lafaro KJ**, Demirjian AN, Pawlik TM. Epidemiology of hepatocellular carcinoma. *Surg Oncol Clin N Am* 2015; **24**: 1-17 [PMID: 25444466 DOI: 10.1016/j.soc.2014.09.001]
- 2 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J; EASL Panel of Experts on HCC. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607 DOI: 10.1016/S0168-8278(01)00130-1]
- 3 **Singal AG**, El-Serag HB. Hepatocellular Carcinoma From Epidemiology to Prevention: Translating Knowledge into Practice. *Clin Gastroenterol Hepatol* 2015; **13**: 2140-2151 [PMID: 26284591 DOI: 10.1016/j.cgh.2015.08.014]
- 4 **Torzilli G**, Donadon M, Marconi M, Palmisano A, Del Fabbro D, Spinelli A, Botea F, Montorsi M. Hepatectomy for stage B and stage C hepatocellular carcinoma in the Barcelona Clinic Liver Cancer classification: results of a prospective analysis. *Arch Surg* 2008; **143**: 1082-1090 [PMID: 19015467 DOI: 10.1001/archsurg.143.11.1082]
- 5 **Choi C**, Choi GH, Kim TH, Tanaka M, Meng MB, Seong J. Multimodality Management for Barcelona Clinic Liver Cancer Stage C Hepatocellular Carcinoma. *Liver Cancer* 2014; **3**: 405-416 [PMID: 26280002 DOI: 10.1159/000343861]
- 6 **Schlachterman A**, Craft WW, Hilgenfeldt E, Mitra A, Cabrera R. Current and future treatments for hepatocellular carcinoma. *World J Gastroenterol* 2015; **21**: 8478-8491 [PMID: 26229392 DOI: 10.3748/wjg.v21.i28.8478]
- 7 **Guglielmi A**, Ruzzenente A, Conci S, Valdegamberi A, Vitali M, Bertuzzo F, De Angelis M, Mantovani G, Iacono C. Hepatocellular carcinoma: surgical perspectives beyond the barcelona clinic liver cancer recommendations. *World J Gastroenterol* 2014; **20**: 7525-7533 [PMID: 24976693 DOI: 10.3748/wjg.v20.i24.7525]
- 8 **Ho MC**, Huang GT, Tsang YM, Lee PH, Chen DS, Sheu JC, Chen CH. Liver resection improves the survival of patients with multiple hepatocellular carcinomas. *Ann Surg Oncol* 2009; **16**: 848-855 [PMID: 19159983 DOI: 10.1245/s10434-008-0282-7]
- 9 **Forner A**, Gilabert M, Bruix J, Raoul JL. Treatment of intermediate-stage hepatocellular carcinoma. *Nat Rev Clin Oncol* 2014; **11**: 525-535 [PMID: 25091611 DOI: 10.1038/nrclinonc.2014.122]
- 10 **Zhou Y**, Lei X, Wu L, Wu X, Xu D, Li B. Outcomes of hepatectomy for noncirrhotic hepatocellular carcinoma: a systematic review. *Surg Oncol* 2014; **23**: 236-242 [PMID: 25465529 DOI: 10.1016/j.suronc.2014.11.001]
- 11 **Poon RT**, Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Extended hepatic resection for hepatocellular carcinoma in patients with cirrhosis: is it justified? *Ann Surg* 2002; **236**: 602-611 [PMID: 12409666 DOI: 10.1097/01.SLA.0000033038.38956.5E]
- 12 **Schroeder RA**, Marroquin CE, Bute BP, Khuri S, Henderson WG, Kuo PC. Predictive indices of morbidity and mortality after liver resection. *Ann Surg* 2006; **243**: 373-379 [PMID: 16495703 DOI: 10.1097/01.sla.0000201483.95911.08]
- 13 **Zhang ZM**, Guo JX, Zhang ZC, Jiang N, Zhang ZY, Pan LJ. Therapeutic options for intermediate-advanced hepatocellular carcinoma. *World J Gastroenterol* 2011; **17**: 1685-1689 [PMID: 21483627 DOI: 10.3748/wjg.v17.i13.1685]
- 14 **Torzilli G**, Belghiti J, Kokudo N, Takayama T, Capussotti L, Nuzzo G, Vauthey JN, Choti MA, De Santibanes E, Donadon M, Morenghi E, Makuuchi M. A snapshot of the effective indications and results of surgery for hepatocellular carcinoma in tertiary referral centers: is it adherent to the EASL/AASLD recommendations?: an observational study of the HCC East-West study group. *Ann Surg* 2013; **257**: 929-937 [PMID: 23426336 DOI: 10.1097/SLA.0b013e31828329b8]
- 15 **Zhong JH**, Xiang BD, Gong WF, Ke Y, Mo QG, Ma L, Liu X, Li LQ. Comparison of long-term survival of patients with BCLC stage B hepatocellular carcinoma after liver resection or transarterial chemoembolization. *PLoS One* 2013; **8**: e68193 [PMID: 23874536 DOI: 10.1371/journal.pone.0068193]
- 16 **Tsoufas G**, Mekras A, Agorastou P, Kiskinis D. Surgical treatment for large hepatocellular carcinoma: does size matter? *ANZ J Surg* 2012; **82**: 510-517 [PMID: 22548726 DOI: 10.1111/j.1445-2197.2012.06079]
- 17 **Ruzzenente A**, Guglielmi A, Sandri M, Campagnaro T, Valdegamberi A, Conci S, Bagante F, Turcato G, D'Onofrio M, Iacono C. Surgical resection versus local ablation for HCC on cirrhosis: results from a propensity case-matched study. *J Gastrointest Surg* 2012; **16**: 301-311; discussion 311 [PMID: 22095524 DOI: 10.1007/s11605-011-1745-x]
- 18 **Llovet JM**, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, Brú C, Rodés J, Bruix J. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. *Hepatology* 1999; **29**: 62-67 [PMID: 9862851 DOI: 10.1002/hep.510290145]
- 19 **Zhong JH**, Ke Y, Gong WF, Xiang BD, Ma L, Ye XP, Peng T, Xie GS, Li LQ. Hepatic resection associated with good survival for selected patients with intermediate and advanced-stage hepatocellular carcinoma. *Ann Surg* 2014; **260**: 329-340 [PMID: 24096763 DOI: 10.1097/SLA.0000000000000236]
- 20 **Zhong JH**, Li H, Xiao N, Ye XP, Ke Y, Wang YY, Ma L, Chen J, You XM, Zhang ZY, Lu SD, Li LQ. Hepatic resection is safe and effective for patients with hepatocellular carcinoma and portal hypertension. *PLoS One* 2014; **9**: e108755 [PMID: 25268959 DOI: 10.1371/journal.pone.0108755]
- 21 **Santambrogio R**, Kluger MD, Costa M, Belli A, Barabino M, Laurent A, Opoche E, Azoulay D, Cherqui D. Hepatic resection for hepatocellular carcinoma in patients with Child-Pugh's A cirrhosis: is clinical evidence of portal hypertension a contraindication? *HPB (Oxford)* 2013; **15**: 78-84 [PMID: 23216782 DOI: 10.1111/j.1477-2574.2012.00594.x]
- 22 **He W**, Zeng Q, Zheng Y, Chen M, Shen J, Qiu J, Chen M, Zou R, Liao Y, Li Q, Wu X, Li B, Yuan Y. The role of clinically significant portal hypertension in hepatic resection for hepatocellular carcinoma patients: a propensity score matching analysis. *BMC Cancer* 2015; **15**: 263 [PMID: 25886495 DOI: 10.1186/s12885-015-1280-3]
- 23 **Hirokawa F**, Hayashi M, Miyamoto Y, Asakuma M, Shimizu T, Komeda K, Inoue Y, Uchiyama K. Predictors of poor prognosis by recurrence patterns after curative hepatectomy for hepatocellular carcinoma in Child-Pugh classification A. *Hepatogastroenterology* 2015; **62**: 164-168 [PMID: 25911889]
- 24 **Wang Y**, Yuan L, Ge RL, Sun Y, Wei G. Survival benefit of surgical treatment for hepatocellular carcinoma with inferior vena cava/right atrium tumor thrombus: results of a retrospective cohort study. *Ann Surg Oncol* 2013; **20**: 914-922 [PMID: 22956071 DOI: 10.1245/s10434-012-2646-2]
- 25 **Chok KS**, Cheung TT, Chan SC, Poon RT, Fan ST, Lo CM. Surgical outcomes in hepatocellular carcinoma patients with portal vein tumor thrombosis. *World J Surg* 2014; **38**: 490-496 [PMID: 24132826 DOI: 10.1007/s00268-013-2290-4]
- 26 **Kanazawa A**, Tsukamoto T, Shimizu S, Kodai S, Yamazoe S, Yamamoto S, Kubo S. Impact of laparoscopic liver resection for hepatocellular carcinoma with F4-liver cirrhosis. *Surg Endosc* 2013; **27**: 2592-2597 [PMID: 23392977]
- 27 **Violi F**, Leo R, Vezza E, Basili S, Cordova C, Balsano F. Bleeding time in patients with cirrhosis: relation with degree of liver failure and clotting abnormalities. C.A.L.C. Group. Coagulation Abnormalities in Cirrhosis Study Group. *J Hepatol* 1994; **20**: 531-536 [PMID: 8051393]
- 28 **Lencioni R**, Chen XP, Dagher L, Venook AP. Treatment of intermediate/advanced hepatocellular carcinoma in the clinic: how can outcomes be improved? *Oncologist* 2010; **15** Suppl 4: 42-52 [PMID: 21115580 DOI: 10.1634/theoncologist.2010-S4-42]
- 29 **Gish RG**, Lencioni R, Di Bisceglie AM, Raoul JL, Mazzaferro V. Role of the multidisciplinary team in the diagnosis and treatment of hepatocellular carcinoma. *Expert Rev Gastroenterol Hepatol* 2012; **6**: 173-185 [PMID: 22375523 DOI: 10.1586/egh.11.105]

- 30 **Gomaa AI**, Waked I. Recent advances in multidisciplinary management of hepatocellular carcinoma. *World J Hepatol* 2015; **7**: 673-687 [PMID: 25866604 DOI: 10.4254/wjh.v7.i4.673]
- 31 **Gaba RC**, Kallwitz ER, Parvinian A, Bui JT, Von Roenn NM, Berkes JL, Cotler SJ. Imaging surveillance and multidisciplinary review improves curative therapy access and survival in HCC patients. *Ann Hepatol* 2013; **12**: 766-773 [PMID: 24018494]
- 32 **Torzilli G**, Palmisano A, Del Fabbro D, Marconi M, Donadon M, Spinelli A, Bianchi PP, Montorsi M. Contrast-enhanced intraoperative ultrasonography during surgery for hepatocellular carcinoma in liver cirrhosis: is it useful or useless? A prospective cohort study of our experience. *Ann Surg Oncol* 2007; **14**: 1347-1355 [PMID: 17253105 DOI: 10.1245/s10434-006-9278-3]
- 33 **Wu H**, Lu Q, Luo Y, He XL, Zeng Y. Application of contrast-enhanced intraoperative ultrasonography in the decision-making about hepatocellular carcinoma operation. *World J Gastroenterol* 2010; **16**: 508-512 [PMID: 20101780 DOI: 10.3748/wjg.v16.i4.508]
- 34 **Torzilli G**, Montorsi M, Donadon M, Palmisano A, Del Fabbro D, Gambetti A, Olivari N, Makuuchi M. "Radical but conservative" is the main goal for ultrasonography-guided liver resection: prospective validation of this approach. *J Am Coll Surg* 2005; **201**: 517-528 [PMID: 16183489 DOI: 10.1016/j.jamcollsurg.2005.04.026]
- 35 **Torzilli G**, Palmisano A, Procopio F, Cimino M, Botea F, Donadon M, Del Fabbro D, Montorsi M. A new systematic small for size resection for liver tumors invading the middle hepatic vein at its caval confluence: mini-mesohepatectomy. *Ann Surg* 2010; **251**: 33-39 [PMID: 19858707 DOI: 10.1097/SLA.0b013e3181b61db9]
- 36 **Torzilli G**, Garancini M, Donadon M, Cimino M, Procopio F, Montorsi M. Intraoperative ultrasonographic detection of communicating veins between adjacent hepatic veins during hepatectomy for tumours at the hepatocaval confluence. *Br J Surg* 2010; **97**: 1867-1873 [PMID: 20799289 DOI: 10.1002/bjs.7230]
- 37 **Slotta JE**, Kollmar O, Ellenrieder V, Ghadimi BM, Homayounfar K. Hepatocellular carcinoma: Surgeon's view on latest findings and future perspectives. *World J Hepatol* 2015; **7**: 1168-1183 [PMID: 26019733 DOI: 10.4254/wjh.v7.i9.1168]
- 38 **Zhou Y**, Xu D, Wu L, Li B. Meta-analysis of anatomic resection versus nonanatomic resection for hepatocellular carcinoma. *Langenbecks Arch Surg* 2011; **396**: 1109-1117 [PMID: 21476060 DOI: 10.1007/s00423-011-0784-9]
- 39 **Makuuchi M**, Hasegawa H, Yamazaki S. Intraoperative ultrasonic examination for hepatectomy. *Ultrasound Med Biol* 1983; Suppl 2: 493-497 [PMID: 6100712]
- 40 **Makuuchi M**, Hasegawa H, Yamazaki S. Ultrasonically guided subsegmentectomy. *Surg Gynecol Obstet* 1985; **161**: 346-350 [PMID: 2996162]
- 41 **Shimamura Y**, Gunvén P, Takenaka Y, Shimizu H, Akimoto H, Shima Y, Arima K, Takahashi A, Kitaya T, Matsuyama T. Selective portal branch occlusion by balloon catheter during liver resection. *Surgery* 1986; **100**: 938-941 [PMID: 3022413]
- 42 **Park YS**, Lee CH, Park PJ, Kim KA, Park CM. Intraoperative contrast-enhanced sonographic portography combined with indigo carmine dye injection for anatomic liver resection in hepatocellular carcinoma: a new technique. *J Ultrasound Med* 2014; **33**: 1287-1291 [PMID: 24958416 DOI: 10.7863/ultra.33.7.1287]
- 43 **Torzilli G**, Procopio F, Cimino M, Del Fabbro D, Palmisano A, Donadon M, Montorsi M. Anatomical segmental and subsegmental resection of the liver for hepatocellular carcinoma: a new approach by means of ultrasound-guided vessel compression. *Ann Surg* 2010; **251**: 229-235 [PMID: 19838106 DOI: 10.1097/SLA.0b013e3181b7fdcd]
- 44 **Chen J**, Huang K, Wu J, Zhu H, Shi Y, Wang Y, Zhao G. Survival after anatomic resection versus nonanatomic resection for hepatocellular carcinoma: a meta-analysis. *Dig Dis Sci* 2011; **56**: 1626-1633 [PMID: 21082347 DOI: 10.1007/s10620-010-1482-0]
- 45 **Mirnezami R**, Mirnezami AH, Chandrakumaran K, Abu Hilal M, Pearce NW, Primrose JN, Sutcliffe RP. Short- and long-term outcomes after laparoscopic and open hepatic resection: systematic review and meta-analysis. *HPB (Oxford)* 2011; **13**: 295-308 [PMID: 21492329 DOI: 10.1111/j.1477-2574.2011.00295.x]
- 46 **Nguyen KT**, Gamblin TC, Geller DA. World review of laparoscopic liver resection-2,804 patients. *Ann Surg* 2009; **250**: 831-841 [PMID: 19801936 DOI: 10.1097/SLA.0b013e3181b0c4df]
- 47 **Buell JF**, Cherqui D, Geller DA, O'Rourke N, Iannitti D, Dagher I, Koffron AJ, Thomas M, Gayet B, Han HS, Wakabayashi G, Belli G, Kaneko H, Ker CG, Scatton O, Laurent A, Abdalla EK, Chaudhury P, Dutson E, Gamblin C, D'Angelica M, Nagorney D, Testa G, Labow D, Manas D, Poon RT, Nelson H, Martin R, Clary B, Pinson WC, Martinie J, Vauthey JN, Goldstein R, Roayaie S, Barlet D, Espot J, Abecassis M, Rees M, Fong Y, McMasters KM, Broelsch C, Busuttil R, Belghiti J, Strasberg S, Chari RS. The international position on laparoscopic liver surgery: The Louisville Statement, 2008. *Ann Surg* 2009; **250**: 825-830 [PMID: 19916210]
- 48 **Santambrogio R**, Aldrighetti L, Barabino M, Pulitanò C, Costa M, Montorsi M, Ferla G, Opocher E. Laparoscopic liver resections for hepatocellular carcinoma. Is it a feasible option for patients with liver cirrhosis? *Langenbecks Arch Surg* 2009; **394**: 255-264 [PMID: 18553101 DOI: 10.1007/s00423-008-0349-8]
- 49 **Xiong JJ**, Altaf K, Javed MA, Huang W, Mukherjee R, Mai G, Sutton R, Liu XB, Hu WM. Meta-analysis of laparoscopic vs open liver resection for hepatocellular carcinoma. *World J Gastroenterol* 2012; **18**: 6657-6668 [PMID: 23236242 DOI: 10.3748/wjg.v18.i45.6657]
- 50 **Slim A**, Garancini M, Di Sandro S, Mangoni I, Lauterio A, Giacomoni A, De Carlis L. Laparoscopic versus open liver surgery: a single center analysis of post-operative in-hospital and post-discharge results. *Langenbecks Arch Surg* 2012; **397**: 1305-1311 [PMID: 22918605 DOI: 10.1007/s00423-012-0992-y]
- 51 **Santambrogio R**, Barabino M, Bruno S, Costa M, Ceretti AP, Angiolini MR, Zuin M, Meloni F, Opocher E. Long-term outcome of laparoscopic ablation therapies for unresectable hepatocellular carcinoma: a single European center experience of 426 patients. *Surg Endosc* 2015; Epub ahead of print [PMID: 26275555 DOI: 10.1007/s00464-015-4468-3]
- 52 **Santambrogio R**, Opocher E, Zuin M, Selmi C, Bertolini E, Costa M, Conti M, Montorsi M. Surgical resection versus laparoscopic radiofrequency ablation in patients with hepatocellular carcinoma and Child-Pugh class a liver cirrhosis. *Ann Surg Oncol* 2009; **16**: 3289-3298 [PMID: 19727960 DOI: 10.1245/s10434-009-0678-z]
- 53 **Lai EC**, Tang CN, Ha JP, Tsui DK, Li MK. The evolving influence of laparoscopy and laparoscopic ultrasonography on patients with hepatocellular carcinoma. *Am J Surg* 2008; **196**: 736-740 [PMID: 18558389 DOI: 10.1016/j.amjsurg.2007.08.073]
- 54 **Livraghi T**, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, Rossi S. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology* 2008; **47**: 82-89 [PMID: 18008357 DOI: 10.1002/hep.21933]

**P- Reviewer:** Chuang WL, Penkova-Radicheva MP, Wang GY

**S- Editor:** Gong XM **L- Editor:** A **E- Editor:** Liu SQ



Retrospective Study

## Predictors of mortality after transjugular portosystemic shunt

Mona Ascha, Sami Abuqayyas, Ibrahim Hanouneh, Laith Alkukhun, Mark Sands, Raed A Dweik, Adriano R Tonelli

Mona Ascha, Department of Gastroenterology and Hepatology, Cleveland Clinic, Cleveland, OH 44195, United States

Sami Abuqayyas, Laith Alkukhun, Department of Internal Medicine, Cleveland Clinic, Cleveland, OH 44195, United States

Ibrahim Hanouneh, Minnesota Gastroenterology, Minneapolis, MN 55114, United States

Mark Sands, Department of Diagnostic Radiology, Cleveland Clinic, Cleveland, OH 44195, United States

Raed A Dweik, Adriano R Tonelli, Department of Pulmonary, Allergy and Critical Care Medicine, Respiratory Institute, Cleveland Clinic, Cleveland, OH 44195, United States

**Author contributions:** Ascha M participated in writing and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted; Abuqayyas S participated in interpretation of the results, writing and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted; Hanouneh I interpretation of the results, writing and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted; Alkukhun L participated in the data collection, interpretation of the results and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted; Sands M participated in the interpretation of the results and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted; Dweik RA participated in the conception of the study, interpretation of the results and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted; Tonelli AR participated in the conception, design of the study, data analysis, interpretation of the results, writing and critical revision of the manuscript for important intellectual content and final approval of the manuscript submitted.

**Institutional review board statement:** The study was reviewed and approved by the Cleveland Clinic Foundation Institutional Review Board.

**Informed consent statement:** Written informed consent was

waived for study participants.

**Conflict-of-interest statement:** None of the authors have significant conflicts of interest with any companies or organization whose products or services may be discussed in this article.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at [tonella@ccf.org](mailto:tonella@ccf.org). Participants gave informed consent for data sharing.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Adriano R Tonelli, MD, Department of Pulmonary, Allergy and Critical Care Medicine, Respiratory Institute, Cleveland Clinic, 9500 Euclid Avenue A-90, Cleveland, OH 44195, United States. [tonella@ccf.org](mailto:tonella@ccf.org)  
Telephone: +1-216-4440812  
Fax: +1-216-4456024

Received: December 22, 2015

Peer-review started: December 23, 2015

First decision: January 15, 2016

Revised: January 21, 2016

Accepted: March 14, 2016

Article in press: March 16, 2016

Published online: April 18, 2016

### Abstract

**AIM:** To investigate if echocardiographic and hemodynamic determinations obtained at the time of transjugular intrahepatic portosystemic shunt (TIPS) can provide prognostic information that will enhance risk



stratification of patients.

**METHODS:** We reviewed medical records of 467 patients who underwent TIPS between July 2003 and December 2011 at our institution. We recorded information regarding patient demographics, underlying liver disease, indication for TIPS, baseline laboratory values, hemodynamic determinations at the time of TIPS, and echocardiographic measurements both before and after TIPS. We recorded patient comorbidities that may affect hemodynamic and echocardiographic determinations. We also calculated Model for End-stage Liver Disease (MELD) score and Child Turcotte Pugh (CTP) class. The following pre- and post-TIPS echocardiographic determinations were recorded: Left ventricular ejection fraction, right ventricular (RV) systolic pressure, subjective RV dilation, and subjective RV function. We recorded the following hemodynamic measurements: Right atrial (RA) pressure before and after TIPS, inferior vena cava pressure before and after TIPS, free hepatic vein pressure, portal vein pressure before and after TIPS, and hepatic venous pressure gradient (HVPG).

**RESULTS:** We reviewed 418 patients with portal hypertension undergoing TIPS. RA pressure increased by a mean  $\pm$  SD of  $4.8 \pm 3.9$  mmHg ( $P < 0.001$ ), HVPG decreased by  $6.8 \pm 3.5$  mmHg ( $P < 0.001$ ). In multivariate linear regression analysis, a higher MELD score, lower platelet count, splenectomy and a higher portal vein pressure were independent predictors of higher RA pressure ( $R = 0.55$ ). Three variables predicted 3-mo mortality after TIPS in a multivariate analysis: Age, MELD score, and CTP grade C. Change in the RA pressure after TIPS predicted long-term mortality (per 1 mmHg change, HR = 1.03, 95%CI: 1.01-1.06,  $P < 0.012$ ).

**CONCLUSION:** RA pressure increased immediately after TIPS particularly in patients with worse liver function, portal hypertension, emergent TIPS placement and history of splenectomy. The increase in RA pressure after TIPS was associated with increased mortality. Age, splenectomy, MELD score and CTP grade were independent predictors of long-term mortality after TIPS.

**Key words:** Transjugular intrahepatic portosystemic shunt; Transjugular portosystemic shunts; Right atrial pressure; Outcomes; Mortality

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Transjugular intrahepatic portosystemic shunt (TIPS) is a procedure accompanied by morbidity and mortality. We hypothesize that echocardiographic and hemodynamic determinations obtained at the time of TIPS can provide prognostic information that will enhance risk stratification of patients. We measured echocardiographic and hemodynamic variables before

and immediately after the TIPS procedure in a large cohort of patients at our institution. Our findings corroborate previous literature stating that right atrial pressure increased after TIPS. Our study demonstrates several predictors of long-term mortality after TIPS, such as age, splenectomy, and Model for End-stage Liver Disease score; this data can help assess the risk for patients undergoing TIPS.

Ascha M, Abuqayyas S, Hanouneh I, Alkukhun L, Sands M, Dweik RA, Tonelli AR. Predictors of mortality after transjugular portosystemic shunt. *World J Hepatol* 2016; 8(11): 520-529 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i11/520.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i11.520>

## INTRODUCTION

Transjugular intrahepatic portosystemic shunt (TIPS) is a procedure performed to treat complications of portal hypertension such as bleeding esophageal varices, refractory ascites and hepatic hydrothorax<sup>[1-3]</sup>. The placement of a covered stent creates an anastomosis between the hypertensive portal vein and the inferior vena cava *via* the hepatic vein; this non-surgically decompresses the portal pressure. Although TIPS is minimally invasive, patients with advanced liver disease-particularly those with comorbidities-can have complications related to the procedure. The Model of End-stage Liver Disease (MELD) score was originally conceived to determine survival outcomes in patients receiving TIPS. In their original study, Malinchoc *et al*<sup>[4]</sup> created a model utilizing serum bilirubin, serum creatinine, international normalized ratio (INR), and cause of underlying liver disease, all of which were used to predict three-month mortality in patients undergoing TIPS. In today's practice, the MELD score is primarily used to determine the extent of liver failure and subsequent placement on organ transplant waiting lists in addition to predicting risk and mortality of TIPS placement. However, there remains a limited amount of data available that can ascertain which variables convey a higher risk of complications from TIPS.

TIPS is a procedure that should be employed meticulously, as it can be accompanied by morbidity and mortality. Existing literature has elucidated variables that are traditionally associated with a poor outcome after TIPS, which include increasing age, male gender, high Child-Turcotte-Pugh (CTP) score, high MELD score, urgent placement of TIPS for uncontrolled variceal hemorrhage, renal dysfunction, ascites, and pre-existing hepatic encephalopathy<sup>[5-10]</sup>. However, there is a dearth of studies assessing the prognostic value of echocardiographic and hemodynamic determinations at the time of TIPS.

Liver cirrhosis is characterized by a hyperdynamic circulation, with an increased cardiac preload and a decreased cardiac afterload; this pre-existing hemody-

dynamic stress in cirrhotic patients may be worsened after TIPS placement. After TIPS placement, there is a rapid increase in blood flow from the splanchnic circulation to both the right heart and pulmonary circulation<sup>[11-13]</sup>. This increase in volume can precipitate right ventricular (RV) failure and pulmonary hypertension<sup>[13,14]</sup>. The pulmonary pressures may increase, particularly if the vasculature cannot vasodilate to accommodate the increase in cardiac output. In addition, TIPS permits more direct delivery of vasoactive and neurohumoral mediators, which are normally cleared by the liver, to the pulmonary circulation<sup>[5,14]</sup>. This higher load of vasoactive mediators may increase the RV afterload<sup>[14]</sup>. Due to these hemodynamic changes, it has been recommended that the TIPS procedure be considered with caution in patients with limited cardiac reserve<sup>[11,14]</sup>. While there are no clinical studies that identify a single RA pressure measurement that constitutes an absolute threshold above which TIPS should not be performed, intervention should be reconsidered or performed cautiously when right atrial (RA) pressure is greater than 20 mmHg; furthermore, a pulmonary arterial pressure greater than 45 mmHg may contraindicate TIPS placement.

Evidently, TIPS is not suitable for every patient that presents with portal hypertension, and contraindications must be ruled out prior to stent placement. Further research is indispensable to optimizing patient selection in order to achieve maximum survival benefits. We hypothesize that the echocardiographic and hemodynamic determinations obtained at the time of TIPS can provide prognostic information that will enhance risk stratification of patients for this procedure. We particularly sought to assess whether RA pressure could provide prognostic information, given that a higher RA pressure may reflect a higher intravascular volume and a degree of systolic/diastolic RV dysfunction, conditions that could worsen after TIPS placement. We tested our hypothesis in a large number of patients who underwent TIPS placement at the Cleveland Clinic.

## MATERIALS AND METHODS

This retrospective study received approval from the Cleveland Clinic Institutional Review Board (study number: 12-579). Written informed consent was waived. We reviewed the medical records of 467 patients who underwent TIPS placement between July 2003 and December 2011. Patients were identified using the billing codes for TIPS. Subjects were excluded from the analyses if they underwent liver transplantation before TIPS, TIPS placement was unsuccessful, or if the initial TIPS procedure was performed at an outside facility. Forty-nine patients met exclusion criteria and were thus excluded from analysis.

We recorded information regarding patient demographics, underlying liver disease, indication for TIPS, baseline laboratory values (albumin, bilirubin, INR, creatinine, and platelets), hemodynamic determinations at the time of TIPS, and echocardiographic measure-

ments both before and after TIPS. We also recorded patient comorbidities that may affect hemodynamic and echocardiographic determinations, including arterial hypertension, cardiac heart failure, heart valvular disease, chronic obstructive pulmonary disease, interstitial lung disease, scleroderma, splenectomy, sarcoidosis, sleep apnea, hypothyroidism, chronic kidney disease on hemodialysis, human immunodeficiency virus status, and cocaine use. In addition, we calculated MELD score and CTP class.

We recorded the following pre-TIPS echocardiographic determinations: Left ventricular ejection fraction (LVEF), RV systolic pressure (RVSP), subjective RV dilation, and subjective RV function. We recorded the following hemodynamic measurements: RA pressure before and after TIPS, inferior vena cava pressure before and after TIPS, free hepatic vein pressure, portal vein pressure before and after TIPS, and hepatic venous pressure gradient (HVPG). RV and pulmonary artery pressures were not routinely measured. It should be noted that all variables collected after TIPS were measured immediately after the procedure was performed. We also collected information regarding the type, diameter and length of the stent placed.

We recorded the following post-TIPS echocardiographic determinations: LVEF, RVSP, subjective RV dilation and function.

### TIPS technique

The TIPS procedure was performed according to previously described techniques, with modifications as needed<sup>[15]</sup>. After instillation of local anesthesia, the internal jugular vein was cannulated under direct ultrasound guidance and an introducer was placed. Through the introducer, an angled catheter was advanced into the RA and the pressure was recorded. The catheter was then maneuvered into the right hepatic vein. A long sheath was advanced to the proximal right hepatic vein, followed by a Fogarty balloon. Wedged and free hepatic vein pressures are not routinely collected during the TIPS procedure; these values are usually known from prior transjugular liver biopsy procedures performed on these patients. Carbon dioxide was injected as the contrast agent while digital images were obtained in an attempt to opacify the portal venous system. A parenchymal tract was created from the right hepatic vein to the right portal vein using a sheathed modified Colapinto needle. Alternatively, the TIPS procedure was performed with the modified Rosch-Uchida set. The kit used depends on the preference of the physician performing the procedure. A catheter was advanced into the portal vein and an initial pressure measurement was obtained. Nonionic contrast material was injected in order to display the anatomy and confirm the entry site. The parenchymal tract was then dilated and a stent was placed with the goal of obtaining an HVPG < 12 mmHg for patients with GI bleeding. There is no defined HVPG for patients with ascites; too low of a gradient puts these patients at risk for hepatic encephalopathy. At our institution, we aim to obtain an

**Table 1** Baseline characteristics of the patient cohort

Demographics	<i>n</i> (%) or mean $\pm$ SD
<i>n</i>	418
Age (yr)	55.8 $\pm$ 11.6
Male gender	242 (57.9)
Etiologies of portal hypertension	
NASH cirrhosis	132 (31.6)
Alcohol induced liver disease	105 (25.1)
HCV	105 (25.1)
Primary sclerosing cholangitis	16 (3.8)
Primary biliary cirrhosis	11 (2.6)
Others <sup>1</sup>	49 (11.7)
Patient comorbidities	
Systemic hypertension	155 (37.1)
Hypothyroidism	50 (12.0)
COPD/ILD	34 (8.1)
Sleep apnea	20 (4.8)
Cardiac heart failure	18 (4.3)
Chronic kidney disease on hemodialysis	17 (4.1)
Valvular heart disease	17 (4.1)
Sarcoidosis	5 (1.2)
Splenectomy	5 (1.2)
Scleroderma	4 (1.0)
Cocaine use	4 (1.0)
HIV	1 (0.2)
Indications for TIPS	
GI bleeding	182 (43.5)
Refractory ascites	157 (37.6)
Hepatic hydrothorax	51 (12.2)
Others <sup>2</sup>	28 (6.7)
Basic laboratory parameters	
Serum albumin (g/dL)	2.9 $\pm$ 0.7
Serum bilirubin (mg/dL)	3.0 $\pm$ 5.4
INR	1.3 $\pm$ 0.4
Serum creatinine (mg/dL)	1.3 $\pm$ 1.1
Platelets (K/ $\mu$ L)	115.3 $\pm$ 77.6

<sup>1</sup>Other etiologies of portal hypertension include hepatitis B virus, autoimmune hepatitis, alpha-1 anti-trypsin deficiency, Budd Chiari syndrome, hemochromatosis, Wilson's disease, sarcoidosis, cystic fibrosis, biliary atresia, portal vein thrombosis, nodular regenerative hyperplasia, veno-occlusive disease and Caroli's disease; <sup>2</sup>Other indications for TIPS include hepatorenal syndrome, portal hypertensive gastropathy, superior mesenteric vein thrombosis, splenomegaly and the need to decrease the portal pressure prior to a surgical intervention. COPD: Chronic obstructive pulmonary disease; HIV: Human immunodeficiency virus; ILD: Interstitial lung disease; HCV: Hepatitis C virus; TIPS: Transjugular portosystemic shunt; INR: International normalized ratio.

HVPG around 7-8 mmHg, but this is not absolute and is adjusted to the clinical circumstances such as LFTs and the presence of encephalopathy pre-TIPS. The majority of our TIPS procedures are performed for control of ascites. A final angiogram was used to confirm good flow through the TIPS. In addition, the RA pressure and portal vein pressure were measured again immediately after the TIPS procedure was completed.

### Statistical analysis

Means and SD are provided for continuous variables, while numbers of patients with percentages are given for categorical variables. Hemodynamic variables before and after TIPS were compared using paired *t*-test. Binary logistic regression was used to identify variables that predict 3-mo mortality and results are reported as odds

ratio with 95%CI. We evaluated the association between RA pressure and other variables with univariate linear regression. We tested the relationship between survival and variables of interest with Cox proportional-hazards modeling adjusted for age and gender. The start point for the analysis was the date of the TIPS and the end of follow-up was marked by the patient's death or the end of study in December 2011. Patients were censored at the time of orthotopic liver transplant (OLT). Factors associated with survival in the univariate analysis (*P* value < 0.05) were entered into a multivariate model (forward selection). Results are expressed as hazard ratios (HRs) with the corresponding 95%CI. Predictors with HRs > 1 are associated with a higher risk for the outcome tested. We constructed receiver operating characteristic (ROC) curves to determine the sensitivity and specificity of different cutoffs of RA pressure and estimated RVSP for discriminating patients who died during follow-up. All *P* values reported are two tailed and *P*-values < 0.05 were considered significant. The statistical analyses were performed using SPSS version 17 (SPSS, Inc, Chicago, IL) and MedCalc, version 14.12.0 (Ostend, Belgium). The statistical methods of this study were reviewed by Dr. Adriano Tonelli from the Cleveland Clinic Foundation.

## RESULTS

### Patient characteristics

We included 418 patients with portal hypertension in the study. The mean  $\pm$  SD age was 55.8  $\pm$  11.6 years and 242 patients (57.9%) were male. The primary causes of portal hypertension were cryptogenic and non-alcoholic steatohepatitis (NASH) induced cirrhosis [*n* = 132 (31.6%)], alcohol induced liver disease [*n* = 105 (25.1%)] and hepatitis C virus [*n* = 105 (25.1%)]. Less common etiologies for portal hypertension and comorbidities are listed in Table 1.

Indications for TIPS included gastrointestinal bleeding [*n* = 182 (43.5%)], refractory ascites [*n* = 157 (37.6%)], hepatic hydrothorax [*n* = 51 (12.2%)] and other causes [*n* = 28 (6.7%)] (Table 1). A total of 113 (27.8%) TIPS procedures were done emergently. Laboratory evaluations of patients before TIPS (*n* = 416) are as follows (mean  $\pm$  SD): Serum albumin (g/dL) 2.9  $\pm$  0.7, serum bilirubin (mg/dL) 3.0  $\pm$  5.4, INR 1.3  $\pm$  0.4, serum creatinine (mg/dL) 1.3  $\pm$  1.1, platelets (K/ $\mu$ L) 115.3  $\pm$  77.6. MELD score pre-TIPS revealed a mean  $\pm$  SD of 13.3  $\pm$  6.9. Meanwhile, CTP classes A, B and C were present in 46 (11.5%), 224 (55.9%) and 131 patients (31.3%), respectively. The mean  $\pm$  SD diameter of the stent placed was 9.9  $\pm$  1 mm.

### Echocardiographic and invasive hemodynamic determinations

Among the 301 patients who had echocardiography data available, 224 patients (74%) had estimates of the RVSP, which demonstrated a mean  $\pm$  SD of 31.9  $\pm$  10.9 mmHg. Only 11 patients (3.7%) out of 294 in whom

**Table 2** Multivariate linear regression model with right atrial pressure as dependent variable

Model	$\beta$	Standard error	P-value
Constant	-2.21	1.01	0.03 <sup>a</sup>
MELD score	0.19	0.03	< 0.001 <sup>b</sup>
Platelet count (K/ $\mu$ L)	-0.01	0.01	0.004 <sup>b</sup>
Portal vein pressure (mmHg)	0.30	0.03	< 0.001 <sup>b</sup>
Splenectomy	4.69	1.97	0.02

R = 0.55, R<sup>2</sup> = 0.3, adjusted R<sup>2</sup> = 0.29. <sup>a</sup>P < 0.05; <sup>b</sup>P < 0.01. MELD: Model for End stage Liver Disease.

the RV function was evaluated had mild or moderate RV dysfunction prior to TIPS placement. There were several notable hemodynamic changes that occurred immediately after TIPS. The RA pressure increased by a mean  $\pm$  SD of 4.8  $\pm$  3.9 mmHg ( $P$  < 0.001). The HVPG decreased by a mean  $\pm$  SD of 6.8  $\pm$  3.5 mmHg ( $P$  < 0.001). However, this was at the expense of a reduction in the portal vein pressure, which decreased by a mean  $\pm$  SD of 11.7  $\pm$  5.6 mmHg ( $P$  < 0.001). Finally, the RVSP measured by echocardiography ( $n$  = 109) increased by 7.4  $\pm$  2.6 mmHg ( $P$  < 0.001).

#### Factors associated with RA pressure before TIPS

We found several factors to be associated with elevated RA pressure prior to TIPS placement, including MELD score ( $R$  = 0.36,  $P$  < 0.001), serum bilirubin ( $R$  = 0.29,  $P$  < 0.001), INR ( $R$  = 0.26,  $P$  < 0.001), serum creatinine ( $R$  = 0.26,  $P$  < 0.001), platelets ( $R$  = -0.18,  $P$  < 0.001) and portal vein pressure ( $R$  = 0.46,  $P$  < 0.001). Elevated RA Pressure before TIPS is directly related to severity of the patient's underlying liver disease. Moreover, splenectomy ( $R$  = 0.11,  $P$  = 0.03) and emergent TIPS placement ( $R$  = 0.24,  $P$  < 0.001) were associated with higher RA pressure in univariate linear regression analysis. RA pressure was not significantly different among the major etiologies of portal hypertension (ETOH, chronic hepatitis, NASH or others). In patients in whom TIPS were placed for acute variceal bleeding the RA before TIPS was higher (7.8  $\pm$  5.9 mmHg) than TIPS placed for ascites (6.0  $\pm$  3.9 mmHg,  $P$  = 0.001). Similarly, RA pressure after TIPS was higher in patients who received TIPS for acute variceal bleeding (12.4  $\pm$  6.5 mmHg vs 10.9  $\pm$  4.0 mmHg,  $P$  = 0.01) instead of ascites.

In multivariate linear regression analysis, a higher MELD score, lower platelet count, splenectomy and a higher portal vein pressure were independent predictors of higher RA pressure ( $R$  = 0.55,  $P$  < 0.001) (Table 2). Adding the etiology of portal hypertension and/or the reason for TIPS as variables did not affect the model. RA pressure was not found to be associated with RVSP or RV function obtained with echocardiography.

#### Predictors of 3-mo survival after TIPS

A total of 97 (24.7%) patients died within the 3-mo period after TIPS. Twenty-six patients underwent OLT during this time frame and were excluded from this

**Table 3** Univariate predictors of three-month survival

Variables	OR	95%CI	P
Age (per 1 yr)	1.03	1.01-1.06	0.003 <sup>b</sup>
CKD on HD (yes)	5.93	1.94-18.16	0.002 <sup>b</sup>
MELD (per unit change)	1.15	1.10-1.19	< 0.001 <sup>b</sup>
CTP B (compared to A)	4.56	1.06-19.67	0.04 <sup>a</sup>
CTP C (compare to A)	13.90	3.21-60.20	< 0.001 <sup>b</sup>
RVSP (per mmHg)	1.03	1.00-1.06	0.02 <sup>a</sup>
Emergent placement (yes)	2.56	1.57-4.19	< 0.001 <sup>b</sup>
RA pressure before TIPS (per 1 mmHg)	1.1	1.05-1.15	< 0.001 <sup>b</sup>
Portal vein pressure before TIPS (per 1 mmHg)	1.04	1.01-1.08	0.02 <sup>a</sup>
RA pressure after TIPS (per 1 mmHg)	1.07	1.03-1.12	0.002 <sup>b</sup>
Portal vein pressure after TIPS (per 1 mmHg)	1.06	1.02-1.10	0.006 <sup>b</sup>

<sup>a</sup>P < 0.05; <sup>b</sup>P < 0.01. CKD: Chronic kidney disease; CTP: Child Turcotte Pugh; HD: Hemodialysis; MELD: Model for End stage Liver Disease; OR: Odds ratio; RA: Right atrium; RVSP: Right ventricular systolic pressure; TIPS: Transjugular portosystemic pressure.

analysis. Table 3 shows variables that predicted mortality at 3-mo in a univariate binary logistic regression. Etiology of portal hypertension and reason for TIPS were not significant predictors of this outcome. Only three variables remained predictors of 3-mo mortality after TIPS in a multivariate binary analysis. These included age (per 1 year, OR = 1.04, 95%CI: 1.02-1.08,  $P$  = 0.003), MELD score (per 1 unit, OR = 1.14, 95%CI: 1.08-1.19,  $P$  < 0.001) and CTP grade C (compared to A, OR = 4.75, 95%CI: 1.02-22.17,  $P$  < 0.001).

#### Predictors of long-term survival after TIPS

Patients were followed for a median (interquartile range) of 26.7 (2-45) mon. A total of 68 (16.3%) patients underwent OLT after TIPS. Of the remaining patients, 261 (74.6%) patients died before transplantation during follow-up. Median survival after TIPS was 26 mo (95%CI: 17-33) (Figure 1). Table 4 shows several variables that predicted long-term mortality post TIPS in a univariate Cox survival analysis. Etiology of portal hypertension or reason for TIPS was non-significant predictors of long-term mortality in our cohort. Table 5 shows significant predictors of long-term mortality after TIPS (age, splenectomy, MELD score, CTP groups B and C) according to a multivariate analysis that excluded echocardiographic parameters. When echocardiographic parameters were factored into the model, MELD score (per 1 unit, HR = 1.05, 95%CI: 1.02-1.08,  $P$  < 0.001) and RV function (per increase in 1 degree of severity, HR = 2.24, 95%CI: 1.34-3.74,  $P$  < 0.002) were significant predictors of long-term mortality. Of the hemodynamic determinations studied, only the change in the RA pressure after TIPS predicted long-term mortality (per 1 mmHg change, HR = 1.03, 95%CI: 1.01-1.06,  $P$  < 0.012).

#### Receiver operating characteristic analysis for RA pressure and RVSP

We also constructed ROC curves using the classification



**Table 4 Predictors of long term mortality in univariate Cox survival analysis**

Variables	HR	95%CI	P
Age (per 1 yr)	1.02	1.01-1.03	0.001 <sup>b</sup>
HCV (ETOH reference)	1.45	1.03-2.04	0.03 <sup>a</sup>
Splenectomy (yes)	3.32	1.36-8.10	0.008 <sup>b</sup>
CHF (yes)	1.32	1.01-1.74	0.04 <sup>a</sup>
Hypothyroidism (yes)	1.20	1.00-1.42	0.04 <sup>a</sup>
CKD on HD (yes)	1.56	1.17-2.06	0.002 <sup>b</sup>
Portal vein pressure before TIPS (per 1 mmHg)	1.03	1.01-1.04	0.008 <sup>b</sup>
RA pressure before TIPS (per 1 mmHg)	1.03	1.01-1.06	0.01 <sup>a</sup>
RA pressure after TIPS (per 1 mmHg)	1.03	1.00-1.05	0.02 <sup>a</sup>
MELD score (per 1 unit)	1.07	1.05-1.09	< 0.001 <sup>b</sup>
Albumin (per 1 mg/dL)	0.70	0.58-0.84	< 0.001 <sup>b</sup>
Billirubin (per 1 mg/dL)	1.05	1.03-1.07	< 0.001 <sup>b</sup>
INR (per 1 unit change)	1.30	1.07-1.60	0.01 <sup>a</sup>
Creatinine (per 1 mg/dL)	1.26	1.14-1.39	< 0.001 <sup>b</sup>
CTP category B (reference A)	1.98	1.24-3.18	0.004 <sup>b</sup>
CTP category C (reference A)	3.02	1.85-4.94	< 0.001 <sup>b</sup>
EF echocardiogram pre TIPS (per 1% increase)	0.98	0.95-1.00	0.04 <sup>a</sup>
RVSP pre TIPS (per 1 mmHg increase)	1.02	1.01-1.04	0.005 <sup>b</sup>
Moderate RV dysfunction (normal RV reference)	2.84	1.18-6.85	0.02 <sup>a</sup>
EF post TIPS (per 1% increase)	0.98	0.96-1.00	0.03 <sup>a</sup>
RVSP post TIPS (per 1 mmHg increment)	1.02	1.01-1.04	0.01 <sup>a</sup>

<sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ . CHF: Congestive heart failure; CKD: Chronic kidney disease; CTP: Child turcotte pugh; EF: Ejection fraction; HCV: Hepatitis C virus; HD: Hemodialysis; HR: Hazard ratio; MELD: Model for End stage Liver Disease; RA: Right atrium; RV: Right ventricle; RVSP: Right ventricular systolic pressure; TIPS: Transjugular intrahepatic portosystemic shunt; INR: International normalized ratio; ETOH: Alcoholic etiology.

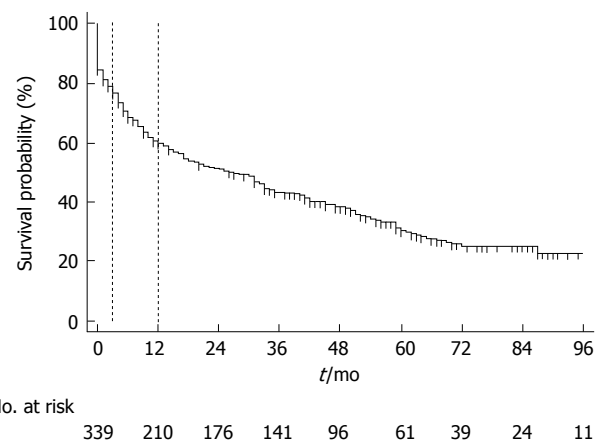
variables mortality at 3-mo (excluding liver transplant) and mortality or liver transplant at one year. The first variable tested was RA pressure measure by right heart catheterization immediately before TIPS. The area under the curve (AUC) for 3-mo mortality was 0.63 (95%CI: 0.58-0.68,  $P < 0.001$ ) (Figure 2A) with an optimal cut-off by Youden index of  $> 9$  mmHg (sensitivity of 41.3%, specificity of 80.5%). In addition, RA pressure  $> 14$  mmHg had a sensitivity of 14.1% and specificity of 95.7%. The AUC for mortality or liver transplant at one year was 0.58 (95%CI: 0.53-0.64,  $P = 0.009$ ) and a RA pressure of  $> 9$  mmHg showed a sensitivity of 18.1% and specificity of 66.4% in predicting this outcome.

An ROC curve testing a second variable, RVSP estimated by echocardiography, was also constructed. The AUC for 3-mo mortality was 0.60 (95%CI: 0.53-0.67,  $P = 0.04$ ) (Figure 2B) with a Youden index of  $> 29$  mmHg (sensitivity of 67.4 % and specificity of 50.6%). At a cut-off  $> 40$  mmHg, the sensitivity was 28.3% and specificity was 88.0%. Moreover, at a cut-off  $> 50$  mmHg, the sensitivity was 10.9% and specificity was 95.6% for predicting 3-mo mortality. The AUC predicting mortality or liver transplant at one year for RVSP was 0.58 (0.51-0.65,  $P = 0.047$ ) with a Youden index of  $> 29$  mmHg (sensitivity of 41.8% and specificity of 42.0%). At a cut-off  $> 40$  mmHg, the sensitivity was 10.0% and specificity was 79.0% and

**Table 5 Multivariate Cox survival analysis (without including hocardiographic arameters)**

Variables	HR	95%CI	P
Age (per 1 yr)	1.02	1.01-1.03	0.004 <sup>b</sup>
Splenectomy (yes)	3.06	1.24-7.52	0.02 <sup>a</sup>
MELD score (per 1 unit)	1.05	1.03-1.08	< 0.001 <sup>b</sup>
CTP B (compared to A)	1.99	1.20-3.32	0.008 <sup>b</sup>
CTP C (compared to A)	2.45	1.41-4.26	0.001 <sup>b</sup>

<sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ . CTP: Child turcotte pugh; HR: Hazard ratio; MELD: Model for End-stage Liver Disease.



**Figure 1 Survival after transjugular portosystemic shunts.** Kaplan-Meier survival analysis censored by liver transplantation. Markers are shown at 3 and 12 mo.

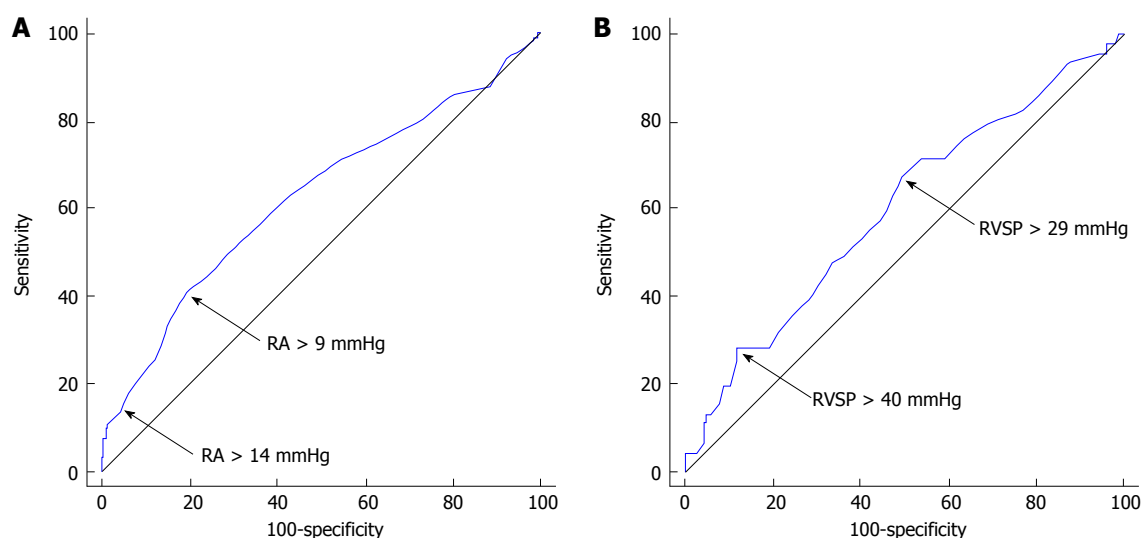
at a cut-off  $> 50$  mmHg, the sensitivity was 3.60% and specificity was 91.4%.

A Kaplan-Meier analysis was performed, and three-month survival after TIPS based on RA pressure  $> 9$  mmHg vs  $\leq 9$  mmHg and estimated RVSP pressure  $> 40$  mmHg vs  $\leq 40$  mmHg is presented in Figure 3.

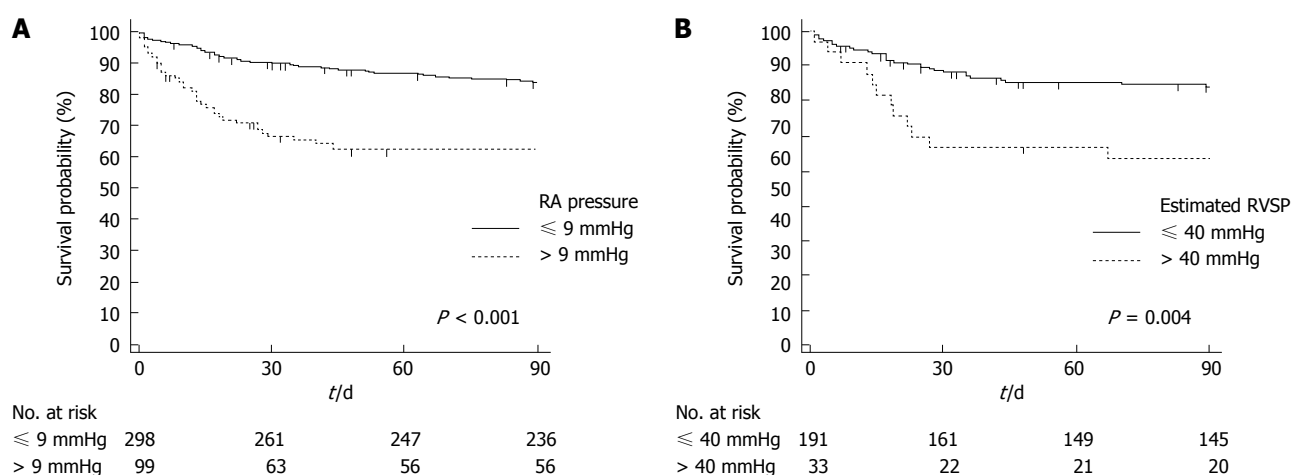
## DISCUSSION

We analyzed a large cohort of patients with portal hypertension who underwent TIPS for a variety of reasons, chiefly to assess hemodynamic variables before and after TIPS as potential predictors for mortality. We found an increase in RA pressure and a decrease in portal vein pressure after TIPS. The increase in RA pressure immediately after TIPS was associated with worsening liver function, portal hypertension, emergent TIPS placement and history of splenectomy. Of all the hemodynamic variables measured at the time TIPS, the increase in RA pressure after TIPS was associated with increased long-term mortality and a RA pressure  $> 9$  mmHg before TIPS predicted 3- and 12-mo mortality with specificity of 81% and 66%, respectively. It is important to note that in our models, the etiology of portal hypertension and the reasons for placing TIPS had no impact on short- or long-term survival.

Other studies examined the hemodynamic changes before and after TIPS. Kovács *et al*<sup>[16]</sup> assessed the short-



**Figure 2 Receiver operating characteristic curves for three-month mortality.** We tested the variables right atrium pressure before TIPS (A) and estimated RVSP by echocardiography pre TIPS (B). RVSP: Right ventricular systolic pressure; TIPS: Transjugular portosystemic shunt; RA: Right atrium.



**Figure 3 Kaplan-Meier analysis of three-month survival after transjugular portosystemic shunts.** A: Stratified by RA pressure > 9 mmHg vs ≤ 9 mmHg; B: Stratified by estimated RVSP pressure > 40 mmHg vs ≤ 40 mmHg. The separation in survival curves in both panels is particularly noted during the first month. *P* values are provided by log-rank test. RVSP: Right ventricular systolic pressure; RA: Right atrium.

term hemodynamic and cardiac magnetic resonance imaging changes after TIPS in 11 patients with liver cirrhosis and intractable esophageal varices or refractory ascites. They concluded that the amount of shunted blood after TIPS was more than the preload reserve of the right and left ventricle, and this was manifested by the significant increase of the pulmonary capillary wedge pressure and persistent enlargement of the left and right atria. Van der Linden *et al.*<sup>[14]</sup> studied the short and mid-term hemodynamic changes after TIPS in 16 sedated biopsy proven cirrhotic patients. They noted an increase in the mean pulmonary artery pressure (PAP), cardiac index, and RA pressure after TIPS. After a transient balloon occlusion of the shunt, they measured these hemodynamics variables once again. Interestingly, all hemodynamic determinations returned to baseline except for the mean PAP, which remained significantly elevated. This hemodynamic change persisted after one month, suggesting that the increase in pulmonary

pressure after TIPS is not only due to volume overload but also due to neurohumoral changes. This is consistent with findings from previous studies that evaluated the hemodynamic changes after TIPS placement<sup>[11,17]</sup>.

Azoulay *et al.*<sup>[11]</sup> investigated 12 cirrhotic patients who underwent the TIPS procedure due to refractory ascites or refractory esophageal variceal bleeding. Hemodynamics were measured before TIPS, at 30 min, and one month after TIPS. Significant changes recorded included the decrease in the HVPG from  $15 \pm 3$  to  $7 \pm 3$  mmHg at 30 min after TIPS, and the subsequent decrease to  $8 \pm 3$  mmHg at one month. The cardiac index increased from  $4.5 \pm 1.3$  to  $5.7 \pm 1.5$  at 30 min after TIPS and subsequently to  $7.4 \pm 1.4$  L/(min·m<sup>2</sup>) at one month. Colombato *et al.*<sup>[17]</sup> studied in 15 cirrhotic patients the systemic, splanchnic and pulmonary hemodynamics before TIPS, at 15-30 min and at two months after TIPS. Immediately after TIPS, the cardiac index increased by 32% and at two months the increase

was attenuated but remained significantly elevated. In our study, the only hemodynamic variable measured that significantly predicted long-term mortality after TIPS was RA pressure. It is worth noting that our cohort was much larger: 418 patients in our study vs 15 patients in Colombato *et al.*<sup>[17]</sup>'s study.

Mortality after TIPS continues to be elevated despite better selection of patients and improvements in the technical aspects of the procedure. In fact, the overall 30-d mortality ranges between 3% and 44%; meanwhile the one year mortality varies from 11% to 58%. The mortality is greater in high risk patients, in whom it can be as high as 90% within a few weeks after the procedure<sup>[5,6,18,19]</sup>. In our study, we observed a 3-mo and 12-mo mortality after TIPS (censored by liver transplant) of 23.6% and 40.3%, respectively. Given this high mortality rate, it is advantageous to identify predictors of short- and long-term mortality in patients considered for TIPS. Variables previously reported to adversely impact outcomes after TIPS include age<sup>[19,20]</sup>, gender, need for emergent TIPS, encephalopathy<sup>[6]</sup>, ascites, variceal hemorrhage<sup>[6]</sup>, CTP class C<sup>[10,20]</sup>, MELD score, bilirubin > 3<sup>[6,21]</sup>, INR<sup>[21]</sup>, creatinine, alanine aminotransferase > 100 IU/L<sup>[6]</sup>, sodium level<sup>[10]</sup>, albumin<sup>[21]</sup> and portosystemic gradient.

Our study yielded results that corroborate the aforementioned literature. In our study, both echocardiographic (RVSP) and hemodynamic (RA and portal vein pressures both before and after TIPS) variables were predictors of 3-mo survival after TIPS. However, the effect of these determinations became non-significant when adjusting for age, MELD score and CTP grade. Interestingly, a large number of variables impacted long-term survival. Those with independent value included age, MELD score, CTP grade, splenectomy and RV function. We also noted that the higher MELD score and CTP grade impacts adversely the short and long-term prognosis after TIPS. These findings have been described in the literature as predictors of short and long term mortality after TIPS creation in cirrhotic patients<sup>[5,10,20]</sup>. Parvinian *et al.*<sup>[19]</sup> evaluated the specificity of RA pressure in predicting mortality after TIPS at 30- and 90-d in a series of 125 patients. They demonstrated 30-d mortality of 18% and 90-d mortality of 28%. According to univariate analysis, baseline RA pressure and final RA pressure were significantly associated with survival at 30- and 90-d, in addition to Child-Pugh score and MELD score. As in our study, multivariate analysis did not include RA pressure as an independent predictor of mortality at 90-d, supporting these results in a large patient cohort.

In this study, we particularly focused on the prognostic importance of hemodynamic and echocardiographic determinations. Patients with advanced cirrhosis and portal hypertension can develop cardiomyopathy with left ventricular diastolic dysfunction<sup>[21]</sup>, hyperdynamic state, volume overload, and less commonly portopulmonary hypertension; these conditions can be aggravated with the insertion of TIPS<sup>[14,17,22-24]</sup>. The placement of TIPS rapidly increases the RV preload and afterload, which

can lead to overt heart failure, pulmonary hypertension and death<sup>[11,14,16,22-37]</sup>. RA pressure obtained before TIPS could be of value in clinical practice; physicians may elect to abort a TIPS procedure based on this hemodynamic parameter.

Our study has limitations that include the: (1) retrospective collection of data; (2) the lack of data on cardiac output, pulmonary artery and pulmonary capillary wedge pressures which are determinations not routinely obtained at the time of TIPS; and (3) echocardiographic determinations were not done at the time of TIPS. The nature of our patient cohort also poses some limitations. Despite these limitations this study presents data on a large number of TIPS procedures performed during the course of eight years. It describes factors that affect short (3-mo) and long-term prognosis. Most importantly, we found that an important factor with predictive value is RA pressure, which increases after TIPS most prominently in patients with more severe liver disease.

RA pressure increased immediately after TIPS particularly in patients with worse liver function, portal hypertension, emergent TIPS placement and history of splenectomy. The increase in RA pressure after TIPS was associated with increased mortality. Age, splenectomy, MELD score and CTP grade were independent predictors of long-term mortality after TIPS.

## ACKNOWLEDGMENTS

We would like to thank the interventional radiology laboratory personnel for their outstanding work. We are indebted to Jennie Newman licensed practical nurse for her invaluable assistance in this project.

## COMMENTS

### Background

Transjugular intrahepatic portosystemic shunt (TIPS) is a procedure that can be accompanied by morbidity and mortality. There is a lack of studies assessing the prognostic value of echocardiographic and hemodynamic determinations at the time of TIPS. The hypothesis that the echocardiographic and hemodynamic determinations obtained at the time of TIPS can provide prognostic information that will enhance risk stratification of patients for this procedure.

### Research frontiers

Risk stratification of patients with liver disease who are undergoing TIPS is imperfect. It is evident that a number of hemodynamic changes occur after the procedure; their effect on patient outcomes still warrants investigation. The authors examine echocardiographic and hemodynamic variables in this cohort of patients in order to glean information regarding survival and outcomes in patients undergoing TIPS. Furthermore, through a multivariate analysis they also investigate other variables that may significantly influence patient outcomes. This will help to optimize patient benefit from the TIPS procedure.

### Innovations and breakthroughs

In their study, they found that right atrial pressure increased immediately after TIPS particularly in patients with worse liver function, portal hypertension, emergent TIPS placement and history of splenectomy. The increase in right atrial pressure after TIPS was associated with increased mortality. Age, splenectomy, Model of End-stage Liver Disease score and Child Turcotte Pugh

grade were independent predictors of long-term mortality after TIPS.

## Applications

These findings could be used to enhance patient selection for TIPS.

## Peer-review

This retrospective study is important clinical value to select the patients for TIPS and evaluate the prognosis for patients who underwent TIPS placement.

## REFERENCES

- Rössle M, Haag K, Ochs A, Sellinger M, Nöldge G, Perarnau JM, Berger E, Blum U, Gabelmann A, Hauenstein K. The transjugular intrahepatic portosystemic stent-shunt procedure for variceal bleeding. *N Engl J Med* 1994; **330**: 165-171 [PMID: 8264738 DOI: 10.1056/NEJM199401203300303]
- Ochs A, Rössle M, Haag K, Hauenstein KH, Deibert P, Siegertetter V, Huonker M, Langer M, Blum HE. The transjugular intrahepatic portosystemic stent-shunt procedure for refractory ascites. *N Engl J Med* 1995; **332**: 1192-1197 [PMID: 7700312 DOI: 10.1056/NEJM199505043321803]
- Gordon FD, Anastopoulos HT, Crenshaw W, Gilchrist B, McEniff N, Falchuk KR, LoCicero J, Lewis WD, Jenkins RL, Trey C. The successful treatment of symptomatic, refractory hepatic hydrothorax with transjugular intrahepatic portosystemic shunt. *Hepatology* 1997; **25**: 1366-1369 [PMID: 9185754 DOI: 10.1002/hep.510250611]
- Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871 [PMID: 10733541 DOI: 10.1053/he.2000.5852]
- Garcia-Pagán JC, Heydtmann M, Raffa S, Plessier A, Murad S, Fabris F, Vizzini G, Gonzales Abraldes J, Olliff S, Nicolini A, Luca A, Primignani M, Janssen HL, Valla D, Elias E, Bosch J. TIPS for Budd-Chiari syndrome: long-term results and prognostic factors in 124 patients. *Gastroenterology* 2008; **135**: 808-815 [PMID: 18621047 DOI: 10.1053/j.gastro.2008.05.051]
- Chalasani N, Clark WS, Martin LG, Kamean J, Khan MA, Patel NH, Boyer TD. Determinants of mortality in patients with advanced cirrhosis after transjugular intrahepatic portosystemic shunting. *Gastroenterology* 2000; **118**: 138-144 [PMID: 10611162 DOI: 10.1016/S0016-5085(00)70422-7]
- Russo MW, Jacques PF, Mauro M, Odell P, Brown RS. Predictors of mortality and stenosis after transjugular intrahepatic portosystemic shunt. *Liver Transpl* 2002; **8**: 271-277 [PMID: 11910573 DOI: 10.1053/jlts.2002.31653]
- Rajan DK, Haskal ZJ, Clark TW. Serum bilirubin and early mortality after transjugular intrahepatic portosystemic shunts: results of a multivariate analysis. *J Vasc Interv Radiol* 2002; **13**: 155-161 [PMID: 11830621 DOI: 10.1016/S1051-0443(07)61932-0]
- Patch D, Nikolopoulou V, McCormick A, Dick R, Armonis A, Wannamethee G, Burroughs A. Factors related to early mortality after transjugular intrahepatic portosystemic shunt for failed endoscopic therapy in acute variceal bleeding. *J Hepatol* 1998; **28**: 454-460 [PMID: 9551684 DOI: 10.1016/S0168-8278(98)80320-6]
- Jalan R, Elton RA, Redhead DN, Finlayson ND, Hayes PC. Analysis of prognostic variables in the prediction of mortality, shunt failure, variceal rebleeding and encephalopathy following the transjugular intrahepatic portosystemic stent-shunt for variceal haemorrhage. *J Hepatol* 1995; **23**: 123-128 [PMID: 7499782 DOI: 10.1016/0168-8278(95)80325-4]
- Azoulay D, Castaing D, Dennison A, Martino W, Eyraud D, Bismuth H. Transjugular intrahepatic portosystemic shunt worsens the hyperdynamic circulatory state of the cirrhotic patient: preliminary report of a prospective study. *Hepatology* 1994; **19**: 129-132 [PMID: 8276348 DOI: 10.1002/hep.1840190121]
- Blendis L, Wong F. The hyperdynamic circulation in cirrhosis: an overview. *Pharmacol Ther* 2001; **89**: 221-231 [PMID: 11516477 DOI: 10.1016/S0163-7258(01)00124-3]
- Huonker M, Schumacher YO, Ochs A, Sorichter S, Keul J, Rössle M. Cardiac function and haemodynamics in alcoholic cirrhosis and effects of the transjugular intrahepatic portosystemic stent shunt. *Gut* 1999; **44**: 743-748 [PMID: 10205217 DOI: 10.1136/gut.44.5.743]
- Van der Linden P, Le Moine O, Ghysels M, Ortíz M, Devière J. Pulmonary hypertension after transjugular intrahepatic portosystemic shunt: effects on right ventricular function. *Hepatology* 1996; **23**: 982-987 [PMID: 8621179 DOI: 10.1002/hep.510230507]
- Perarnau JM, Le Gouge A, Nicolas C, d'Alteroche L, Borentain P, Saliba F, Minello A, Anty R, Chagneau-Derrode C, Bernard PH, Abergel A, Ollivier-Hourmand I, Gournay J, Ayoub J, Gaborit C, Rusch E, Giraudeau B. Covered vs. uncovered stents for transjugular intrahepatic portosystemic shunt: a randomized controlled trial. *J Hepatol* 2014; **60**: 962-968 [PMID: 24480619 DOI: 10.1016/j.jhep.2014.01.015]
- Kovács A, Schepke M, Heller J, Schild HH, Flacke S. Short-term effects of transjugular intrahepatic shunt on cardiac function assessed by cardiac MRI: preliminary results. *Cardiovasc Intervent Radiol* 2010; **33**: 290-296 [PMID: 19730936 DOI: 10.1007/s00270-009-9696-2]
- Colombato LA, Spahr L, Martinet JP, Dufresne MP, Lafortune M, Fenyes D, Pomier-Layrargues G. Haemodynamic adaptation two months after transjugular intrahepatic portosystemic shunt (TIPS) in cirrhotic patients. *Gut* 1996; **39**: 600-604 [PMID: 8944572 DOI: 10.1136/gut.39.4.600]
- Harrod-Kim P, Saad WE, Waldman D. Predictors of early mortality after transjugular intrahepatic portosystemic shunt creation for the treatment of refractory ascites. *J Vasc Interv Radiol* 2006; **17**: 1605-1610 [PMID: 17057001 DOI: 10.1097/01.RVI.0000240651.38289.4B]
- Parvinian A, Shah KD, Couture PM, Minocha J, Knuttinen MG, Bui JT, Gaba RC. Older patient age may predict early mortality after transjugular intrahepatic portosystemic shunt creation in individuals at intermediate risk. *J Vasc Interv Radiol* 2013; **24**: 941-946 [PMID: 23707226 DOI: 10.1016/j.jvir.2013.03.018]
- Williams D, Waugh R, Gallagher N, Perkins K, Dilworth P, Duggan A, Selby W. Mortality and rebleeding following Transjugular Intrahepatic Portosystemic Stent Shunt for variceal haemorrhage. *J Gastroenterol Hepatol* 1998; **13**: 163-169 [PMID: 10221818 DOI: 10.1111/j.1440-1746.1998.tb00632.x]
- Tyburski JG, Noorily MJ, Wilson RF. Prognostic factors with the use of the transjugular intrahepatic portosystemic shunt for bleeding varices. *Arch Surg* 1997; **132**: 626-630; discussion 630-632 [PMID: 9197855 DOI: 10.1001/archsurg.1997.01430300068014]
- Møller S, Henriksen JH. Cardiovascular complications of cirrhosis. *Postgrad Med J* 2009; **85**: 44-54 [PMID: 19240290 DOI: 10.1136/gut.2006.112177]
- Pozzi M, Carugo S, Boari G, Pecci V, de Ceglie S, Maggiolini S, Bolla GB, Roffi L, Failla M, Grassi G, Giannattasio C, Mancina G. Evidence of functional and structural cardiac abnormalities in cirrhotic patients with and without ascites. *Hepatology* 1997; **26**: 1131-1137 [PMID: 9362352 DOI: 10.1002/hep.510260507]
- Finucci G, Desideri A, Sacerdoti D, Bolognesi M, Merkel C, Angeli P, Gatta A. Left ventricular diastolic function in liver cirrhosis. *Scand J Gastroenterol* 1996; **31**: 279-284 [PMID: 8833359 DOI: 10.3109/00365529609004879]
- Salerno F, Cazzaniga M, Pagnozzi G, Cirello I, Nicolini A, Meregaglia D, Burdick L. Humoral and cardiac effects of TIPS in cirrhotic patients with different "effective" blood volume. *Hepatology* 2003; **38**: 1370-1377 [PMID: 14647047 DOI: 10.1016/j.jhep.2003.09.030]
- Rabie R, Cazzaniga M, Salerno F, Wong F. The effect of cirrhotic cardiomyopathy on the post-TIPS outcome of patients treated for complications of portal hypertension. *Hepatology* 2006; **44** (Suppl 1): 444A
- Rabie RN, Cazzaniga M, Salerno F, Wong F. The use of E/A ratio as a predictor of outcome in cirrhotic patients treated with transjugular intrahepatic portosystemic shunt. *Am J Gastroenterol* 2009; **104**: 2458-2466 [PMID: 19532126 DOI: 10.1038/ajg.2009.321]
- Lee SS, Liu H. Cardiovascular determinants of survival in cirr-



- hosis. *Gut* 2007; **56**: 746-748 [PMID: 17519479 DOI: 10.1136/gut.2006.112169]
- 29 **Rodríguez-Laiz JM**, Bañares R, Echenagusia A, Casado M, Camuñez F, Pérez-Roldán F, de Diego A, Cos E, Clemente G. Effects of transjugular intrahepatic portosystemic shunt (TIPS) on splanchnic and systemic hemodynamics, and hepatic function in patients with portal hypertension. Preliminary results. *Dig Dis Sci* 1995; **40**: 2121-2127 [PMID: 7587778 DOI: 10.1007/BF02208995]
  - 30 **Cazzaniga M**, Salerno F, Pagnozzi G, Dionigi E, Visentin S, Cirello I, Meregaglia D, Nicolini A. Diastolic dysfunction is associated with poor survival in patients with cirrhosis with transjugular intrahepatic portosystemic shunt. *Gut* 2007; **56**: 869-875 [PMID: 17135305 DOI: 10.1136/gut.2006.102467]
  - 31 **Willoughby PH**, Beers RA, Murphy KD. Pulmonary edema after transjugular intrahepatic portosystemic shunt. *Anesth Analg* 1996; **82**: 895-896 [PMID: 8615536 DOI: 10.1213/00000539-199604000-00066]
  - 32 **Braverman AC**, Steiner MA, Picus D, White H. High-output congestive heart failure following transjugular intrahepatic portal-systemic shunting. *Chest* 1995; **107**: 1467-1469 [PMID: 7750353 DOI: 10.1378/chest.107.5.1467]
  - 33 **Modock J**. Acute pulmonary hypertension after transjugular intra-hepatic portosystemic shunt: a potentially deadly but commonly forgotten complication. *Gastroenterol Nurs* 2014; **37**: 33-8; quiz 39-40 [PMID: 24476830 DOI: 10.1097/SGA.000000000000016]
  - 34 **Merli M**, Valeriano V, Funaro S, Attili AF, Masini A, Efrati C, De CS, Riggio O. Modifications of cardiac function in cirrhotic patients treated with transjugular intrahepatic portosystemic shunt (TIPS). *Am J Gastroenterol* 2002; **97**: 142-148 [PMID: 11808939 DOI: 10.1111/j.1572-0241.2002.05438.x]
  - 35 **Salerno F**, Merli M, Cazzaniga M, Valeriano V, Rossi P, Lovaria A, Meregaglia D, Nicolini A, Lubatti L, Riggio O. MELD score is better than Child-Pugh score in predicting 3-month survival of patients undergoing transjugular intrahepatic portosystemic shunt. *J Hepatol* 2002; **36**: 494-500 [PMID: 11943420 DOI: 10.1016/S0168-8278(01)00309-9]
  - 36 **Gaba RC**, Khiatani VL, Knuttinen MG, Omene BO, Carrillo TC, Bui JT, Owens CA. Comprehensive review of TIPS technical complications and how to avoid them. *AJR Am J Roentgenol* 2011; **196**: 675-685 [PMID: 21343513 DOI: 10.2214/AJR.10.4819]
  - 37 **Parvinian A**, Bui JT, Knuttinen MG, Minocha J, Gaba RC. Right atrial pressure may impact early survival of patients undergoing transjugular intrahepatic portosystemic shunt creation. *Ann Hepatol* 2014; **13**: 411-419 [PMID: 24927612]

**P- Reviewer:** Minicis SD, Qin JM, Wong GLH **S- Editor:** Qiu S  
**L- Editor:** A **E- Editor:** Liu SQ



## Management of pregnancy in Crigler Najjar syndrome type 2

Alisha Nitin Chaubal, Ruchir Patel, Dhaval Choksi, Kaivan Shah, Meghraj Ingle, Prabha Sawant

Alisha Nitin Chaubal, Ruchir Patel, Dhaval Choksi, Kaivan Shah, Meghraj Ingle, Prabha Sawant, Department of Gastroenterology, LTMG Hospital, Mumbai 400022, India

**Author contributions:** Chaubal AN wrote the case report; Chaubal AN, Patel R, Choksi D, Shah K, Ingle M and Sawant P managed the case.

**Institutional review board statement:** The institutional review board of LTMG hospital have reviewed and accepted the case report.

**Informed consent statement:** I am aware that my clinical problem is being reported without revealing my identity and I have no objections to the same. I have been explained in detail the procedure for the same and will not hold anyone responsible for the outcome.

**Conflict-of-interest statement:** The authors do not hold any conflict of interest with reviewers.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Alisha Nitin Chaubal, Registrar, Department of Gastroenterology, LTMGH Hospital, Dr. Babasaheb Ambedkar Road, Sion West, Mumbai 400022, India. [alishachaubal@gmail.com](mailto:alishachaubal@gmail.com)  
Telephone: +91-022-24063088  
Fax: +91-022-24044154

Received: October 18, 2015

Peer-review started: November 12, 2015

First decision: January 4, 2016

Revised: February 22, 2016

Accepted: March 9, 2016

Article in press: March 14, 2016

Published online: April 18, 2016

### Abstract

Crigler Najjar syndrome is associated with indirect hyperbilirubinemia due to a deficiency of enzyme Uridine Di Phospho Glucuronosyl Transferase (UDPGT). Presented here is a case of a female in the first trimester of pregnancy, who was diagnosed to have type 2 Crigler Najjar syndrome. We also discuss the management of this rare disease especially in pregnancy. Unconjugated bilirubin can cross the placental barrier causing neurological damage in the newborn. Patient was carefully monitored during pregnancy and treatment with phenobarbitone in low doses was adjusted such that the serum bilirubin levels were below 10 mg/dL. Crigler Najjar syndrome being rare needs to be diagnosed early in pregnancy to avoid adverse fetal outcomes. Phenobarbitone being an inducer of enzyme UDPGT is used as the first line of treatment and is not teratogenic in the low doses used. Treatment protocol followed was on the basis of previous reported cases and successful perinatal outcome was achieved.

**Key words:** Crigler Najjar type 2; Phenobarbitone; Folic acid; Pregnancy; Kernicterus

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Crigler Najjar syndrome type 2 is a rare disorder causing indirect hyperbilirubinemia. In pregnancy placental crossing of unconjugated bilirubin can cause high bilirubin levels in the fetus with low Uridine Di Phospho Glucuronosyl Transferase activity causing permanent neurological impairment in the newborn. Hence timely diagnosis and treatment with low dose phenobarbitone is required.

Chaubal AN, Patel R, Choksi D, Shah K, Ingle M, Sawant P. Management of pregnancy in Crigler Najjar syndrome type 2. *World J Hepatol* 2016; 8(11): 530-532 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i11/530.htm> DOI:

## INTRODUCTION

Crigler Najjar type 2 is a rare condition with an incidence of 1 per 1000000 births. There is no predilection to any race or sex. Being an autosomal recessive disorder consanguineous marriage is a risk factor. Uridine Di Phospho Glucuronosyl Transferase (UDPGT) level in the liver is less than 10% of normal. The serum bilirubin ranges from 3-20 mg/dL. Patients usually present with jaundice in the first year of life but can sometimes occur even in the third decade. Acute increase in bilirubin levels can occur during fasting or illness. DNA analysis of *UDPGT* gene shows mutation in exon 1  $\times$  1-5. Expression analysis of the gene shows residual activity<sup>[1]</sup>. Greater than 25% fall in bilirubin levels after treatment with phenobarbitone distinguishes it from Crigler Najjar type 1.

Levels of bilirubin can be elevated due to the stress of pregnancy. The placenta is an ineffective barrier for unconjugated bilirubin and can result in high bilirubin levels in the neonate causing kernicterus and sometimes even death<sup>[2]</sup>.

Proper identification of the condition and timely treatment with phenobarbitone can avoid morbidity and mortality in the neonate<sup>[3]</sup>.

## CASE REPORT

A female patient of age 24 years had a history of jaundice since childhood. She came to us in her first trimester of pregnancy because her jaundice had increased since the previous 2 wk. Patient had unconjugated hyperbilirubinemia with normal liver enzymes. Tests for viral hepatitis, autoimmune liver disease and Wilson's disease were negative. Abdominal ultrasound including a selective hepatobiliary scan did not show any abnormalities. The *UDT1A1* gene was studied for the TATA sequence. The result was negative; Gilbert's syndrome was thus ruled out. Total bilirubin at 12 wk of gestation was 6.85 mg/dL with indirect bilirubin being 6.14 mg/dL and albumin of 4 g/dL. Her liver enzymes were normal. A dose of 30 mg/d of phenobarbitone was started for the patient. Her serum bilirubin and albumin levels were measured at weekly intervals for the first month and then monthly. Her liver enzymes were also measured simultaneously. We diagnosed the patient to be a case of Crigler Najjar type 2 based on: (1) history of hyperbilirubinemia since childhood; and (2) response to phenobarbitone. A congenital anomaly scan at 20 wk showed no fetal abnormalities. Through her pregnancy, bilirubin levels were maintained in the range of 4 to 8 mg/dL. Figure 1 shows readings of the patient's bilirubin levels taken throughout pregnancy. Total bilirubin was measured at 4.92 mg/dL at time of delivery, which was completed at the normal full term. At the same time, indirect bilirubin was 3.78 mg/dL. Bilirubin levels in the

neonate were normal. As a result, no treatment of any form was required.

## DISCUSSION

Crigler Najjar syndrome is a rare autosomal recessive condition with an incidence of 1 in 1000000 births. Pregnancy in Crigler Najjar syndrome type 2 has been reported only in 6 cases so far (type 1-4 type 2-6 cases)<sup>[4]</sup>.

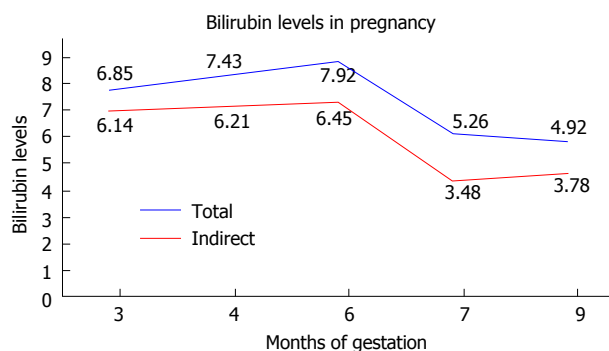
Our patient was a case of Crigler Najjar type 2 where serum bilirubin usually does not exceed 10 mg/dL. However pregnancy being a stressful condition the bilirubin levels can increase to more than 10 mg/dL. We had monitored the patient's bilirubin levels, serum albumin and liver enzymes at monthly intervals.

Crigler-Najjar disease type 2 seems to pose no unique maternal risk during pregnancy. The fetus seems to be resistant to elevated maternal unconjugated bilirubin, but the neonate may require therapy for hyperbilirubinemia<sup>[5]</sup>. Unconjugated bilirubin crosses the placental barrier to cause high levels of bilirubin in the fetus resulting in neurological damage or even death<sup>[6]</sup>. There is no fixed level of bilirubin at which neurological damage occurs but a proposed level above 10 mg/dL has been suggested<sup>[4]</sup>. In a study by Holstein *et al*<sup>[7]</sup>, maternal bilirubin levels between 4.2 and 8.9 maintained by treatment with phototherapy/phenobarbitone resulted in a normal neonate.

Pinkie *et al*<sup>[8]</sup> observed that a maternal bilirubin of 10.8 mg/dL at delivery necessitated treatment with exchange transfusions and phototherapy. We had started our patient on low dose phenobarbitone (30 mg daily) and we were able to maintain bilirubin levels less than 10 mg/dL. Phenobarbitone is known to be teratogenic causing facial dysmorphism and mental retardation. However this is seen only at high doses of 750-1500 mg/d and has not been observed at enzyme inducing doses of 60 mg/d<sup>[3]</sup>.

It has been recommended that an acute increase in bilirubin should be treated with phototherapy and albumin. If patient is already on phototherapy then duration of phototherapy should be increased to 24 h. If neurological toxicity develops then plasmapheresis should be done. However our patient had maintained bilirubin levels with phenobarbitone alone.

The newborn was born without jaundice and did not require any treatment. Bilirubin levels over 10 mg/dL is an indication for phototherapy in term infants without risk factors 4 mg/dL in infants with high risk for kernicterus (preterm, low birth weight)<sup>[9]</sup>. A follow-up of the infant is required for at least 18 mo<sup>[10]</sup>. A study by Taylor *et al*<sup>[11]</sup> showed that an untreated maternal level of 20 mg/dL resulted in a normal infant at birth but the child developed quadriplegia at 18 mo of age. Hence we propose that the standard guidelines for the management of pregnancy in Crigler najjar syndrome type 2 should be followed<sup>[9]</sup>: (1) Genetic counselling before becoming pregnant; (2) Folic acid at a dose of 10 mg during pregnancy; (3) Maternal bilirubin serum



**Figure 1** Monitoring of bilirubin levels throughout pregnancy.

levels should be below 10 mg/dL; (4) Phenobarbitone at low dose of 60 mg/d; (5) Avoid drugs that increase unbound, unconjugated bilirubin like sulfonamides, salicylates, furosemide, ampicillin, and ceftriaxone; and (6) Neurologic follow-up of the newborn including hearing disorders (brainstem evoked potentials).

## COMMENTS

### Case characteristics

Pregnant female with jaundice.

### Clinical diagnosis

Icterus with negative abdominal findings.

### Differential diagnosis

Viral hepatitis, auto-immune hepatitis, Wilson's disease, hyperbilirubinemias, biliary pathology.

### Laboratory diagnosis

Indirect hyperbilirubinemia with normal liver enzymes.

### Imaging diagnosis

Normal hepatobiliary scan.

### Treatment

Phenobarbitone 30-60 mg once daily.

### Experiences and lessons

Suspicion for indirect hyperbilirubinemias for patients presenting with jaundice

and management of Crigler Najjar syndrome in pregnancy with low dose Phenobarbitone.

## Peer-review

Short, clear and well written manuscript. A rare disease that should be of interest to the Journal readers.

## REFERENCES

- Kadacol A**, Ghosh SS, Sappal BS, Sharma G, Chowdhury JR, Chowdhury NR. Genetic lesions of bilirubin uridine-diphosphoglucuronate glucuronosyltransferase (UGT1A1) causing Crigler-Najjar and Gilbert syndromes: correlation of genotype to phenotype. *Hum Mutat* 2000; **16**: 297-306 [PMID: 11013440]
- Raimondi F**, Capasso L, Migliaro F, Romano A, Paludetto R. Prenatal exposure to conjugated bilirubin. *Pediatrics* 2006; **118**: 2265 [PMID: 17079608]
- Kjaer D**, Horvath-Puhó E, Christensen J, Vestergaard M, Czeizel AE, Sørensen HT, Olsen J. Use of phenytoin, phenobarbital, or diazepam during pregnancy and risk of congenital abnormalities: a case-time-control study. *Pharmacoepidemiol Drug Saf* 2007; **16**: 181-188 [PMID: 16941718]
- Passuello V**, Puhl AG, Wirth S, Steiner E, Skala C, Koelbl H, Kohlschmidt N. Pregnancy outcome in maternal Crigler-Najjar syndrome type II: a case report and systematic review of the literature. *Fetal Diagn Ther* 2009; **26**: 121-126 [PMID: 19752526 DOI: 10.1159/000238122]
- Smith JF**, Baker JM. Crigler-Najjar disease in pregnancy. *Obstet Gynecol* 1994; **84**: 670-672 [PMID: 9205443]
- Serrano MA**, Bayón JE, Pascolo L, Tiribelli C, Ostrow JD, Gonzalez-Gallego J, Marin JJ. Evidence for carrier-mediated transport of unconjugated bilirubin across plasma membrane vesicles from human placental trophoblast. *Placenta* 2002; **23**: 527-535 [PMID: 12175967]
- Holstein A**, Plaschke A, Lohse P, Egberts EH. Successful photo- and phenobarbital therapy during pregnancy in a woman with Crigler-Najjar syndrome type II. *Scand J Gastroenterol* 2005; **40**: 1124-1126 [PMID: 16211719]
- Pinke S**, Renu A, Bharati M. Crigler-Najjar syndrome with pregnancy. *J Obstet Gynecol India* 2005; **55**: 270-271
- Wilson JH**, Sinaasappel M, Lotgering FK, Langendonk JG. Recommendations for pregnancies in patients with crigler-najjar syndrome. *JIMD Rep* 2013; **7**: 59-62 [PMID: 23430496 DOI: 10.1007/8904\_2012\_142]
- Shapiro SM**. Definition of the clinical spectrum of kernicterus and bilirubin-induced neurologic dysfunction (BIND). *J Perinatol* 2005; **25**: 54-59 [PMID: 15578034]
- Taylor WG**, Walkinshaw SA, Farquharson RG, Fisk RA, Gilmore IT. Pregnancy in Crigler-Najjar syndrome. Case report. *Br J Obstet Gynaecol* 1991; **98**: 1290-1291 [PMID: 1777465]

**P- Reviewer:** Morini S, Rovas L, Younis JS **S- Editor:** Ji FF

**L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 April 28; 8(12): 533-572





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



## Contents

Three issues per month Volume 8 Number 12 April 28, 2016

### TOPIC HIGHLIGHT

- 533 Incidence, risk factors and outcomes of *de novo* malignancies post liver transplantation  
*Mukthinuthalapati PK, Gotur R, Ghabril M*

### REVIEW

- 545 Hepatitis C virus and neurological damage  
*Mathew S, Faheem M, Ibrahim SM, Iqbal W, Rauff B, Fatima K, Qadri I*

### MINIREVIEWS

- 557 Antiviral therapy for hepatitis C: Has anything changed for pregnant/lactating women?  
*Spera AM, Kamal Eldin T, Tosone G, Orlando R*

### ORIGINAL ARTICLE

#### Retrospective Study

- 566 Predictors of fifty days in-hospital mortality in decompensated cirrhosis patients with spontaneous bacterial peritonitis  
*Bal CK, Daman R, Bhatia V*

## Contents

*World Journal of Hepatology*  
Volume 8 Number 12 April 28, 2016

### ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Dr. Jih Ru Hwu, BSc, FRSC, PhD, President, Academic Fellow, Editor, Professor, Research Fellow, Department of Chemistry, National Tsing Hua University, Hsinchu 30013, Taiwan

### AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

### FLYLEAF

I-IV Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
April 28, 2016

#### COPYRIGHT

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

#### SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

#### INSTRUCTIONS TO AUTHORS

Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjgnet.com/bpg/g_info_20160116143427.htm)

#### ONLINE SUBMISSION

<http://www.wjgnet.com/esps/>



2016 Liver Transplantation: Global view

## Incidence, risk factors and outcomes of *de novo* malignancies post liver transplantation

Pavan Kedar Mukthinuthalapati, Raghavender Gotur, Marwan Ghabril

Pavan Kedar Mukthinuthalapati, Raghavender Gotur, Marwan Ghabril, Division of Gastroenterology and Hepatology, Indiana University School of Medicine, Indianapolis, IN 46202, United States

**Author contributions:** Mukthinuthalapati PK interpretation of literature, drafting and final approval of the article; Gotur R interpretation of literature, drafting and final approval of the article; Ghabril M interpretation of literature, drafting and final approval of the article.

**Conflict-of-interest statement:** All the authors have no conflicts to report.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Marwan Ghabril, MD, Associate Professor of Medicine, Division of Gastroenterology and Hepatology, Indiana University School of Medicine, 702 Rotary Circle 225, Indianapolis, IN 46202, United States. [mghabril@iu.edu](mailto:mghabril@iu.edu)  
 Telephone: +1-317-2781630  
 Fax: +1-317-2786870

Received: January 9, 2016  
 Peer-review started: January 10, 2016  
 First decision: February 22, 2016  
 Revised: March 8, 2016  
 Accepted: April 5, 2016  
 Article in press: April 6, 2016  
 Published online: April 28, 2016

### Abstract

Liver transplantation (LT) is associated with a 2 to

7 fold higher, age and gender adjusted, risk of *de novo* malignancy. The overall incidence of *de novo* malignancy post LT ranges from 2.2% to 26%, and 5 and 10 years incidence rates are estimated at 10% to 14.6% and 20% to 32%, respectively. The main risk factors for *de novo* malignancy include immunosuppression with impaired immunosurveillance, and a number of patient factors which include; age, latent oncogenic viral infections, tobacco and alcohol use history, and underlying liver disease. The most common cancers after LT are non-melanoma skin cancers, accounting for approximately 37% of *de novo* malignancies, with a noted increase in the ratio of squamous to basal cell cancers. While these types of skin cancer do not impact patient survival, post-transplant lymphoproliferative disorders and solid organ cancer, accounting for 25% and 48% of malignancies, are associated with increased mortality. Patients developing these types of cancer are diagnosed at more advanced stages, and their cancers behave more aggressively compared with the general population. Patients undergoing LT for primary sclerosing cholangitis (particularly with inflammatory bowel disease) and alcoholic liver disease have high rates of malignancies compared with patients undergoing LT for other indications. These populations are at particular risk for gastrointestinal and aerodigestive cancers respectively. Counseling smoking cessation, skin protection from sun exposure and routine clinical follow-up are the current approach in practice. There are no standardized surveillance protocol, but available data suggests that regimented surveillance strategies are needed and capable of yielding cancer diagnosis at earlier stages with better resulting survival. Evidence-based strategies are needed to guide optimal surveillance and safe minimization of immunosuppression.

**Key words:** Liver transplant; Immunosuppression; Risk; Outcomes; Malignancy

© The Author(s) 2016. Published by Baishideng Publishing

Group Inc. All rights reserved.

**Core tip:** The risk of new cancers is significantly increased after liver transplantation (LT), and is driven by patient factors, oncogenic viruses and lifelong immunosuppression. *De novo* malignancy is a major risk factor for mortality after LT, equaling the risk of cardiovascular disease or infectious diseases. The risk of *de novo* malignancies may be reduced by attention to patient risk factors and minimization of immunosuppression when possible. Ultimately rigorous surveillance is needed to allow for early diagnosis and attenuation of mortality risk.

Mukthinuthalapati PK, Gotur R, Ghabril M. Incidence, risk factors and outcomes of *de novo* malignancies post liver transplantation. *World J Hepatol* 2016; 8(12): 533-544 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i12/533.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i12.533>

## INTRODUCTION

Liver transplantation (LT) is the definitive therapy for decompensated end-stage liver disease regardless of etiology. During the past 2 decades, the outcomes of LT have steadily improved as a result of more widespread expertise, better surgical techniques and more effective and better tolerated immunosuppressive agents. The growing number of LT recipients and improving survival rates place particular importance on the factors that jeopardize long term survival. Inherent to this population is the need for lifelong immunosuppression, which is associated with some broad categories of risk for morbidity and mortality. These include infection, cardiovascular risks, renal injury and cancer. When studied in patients surviving the early post LT period, *de novo* malignancy emerges as the leading category of immunosuppression associated long term mortality risk, accounting for approximately 21% to 25% of deaths<sup>[1,2]</sup>. This review summarizes current knowledge of *de novo* malignancy post LT including; epidemiology, pathogenesis, disease burden, clinical implications, preventive and surveillance considerations, while emphasizing risk factors and outcomes.

## INCIDENCE

Multiple studies report widely varying incidence rates of *de novo* malignancy post LT, along with considerable variations in associated risks, cancer types and outcomes. The incidence of *de novo* malignancies in relatively large cohorts (subjectively defined as more than 150 patients) is summarized in Table 1, the last row of which contains the means of the respective variables. These include single center experiences<sup>[3-7]</sup>, registry based studies<sup>[8-11]</sup>, and the majority are retrospective with few exceptions<sup>[12]</sup>. Variability in *de novo* malignancy

incidence rates reflect actual differences (based on differing cohort characteristics and risks) and artificial differences (based on differing methodologies and study design). The factors impacting actual differences in cancers types and their incidence may include age, gender, racial and geographical considerations, as well as the predominant underlying liver diseases and their associated comorbidities. Whereas artificial heterogeneity may be less apparent, yet could arise from variability in the: (1) definitions of *de novo* malignancy, *e.g.*, not all include non-melanoma skin cancers; (2) designated time threshold for of exclusion of cancers that are likely pre-existing before LT; (3) method of identification of malignancies, *e.g.*, in-center chart review vs utilization of cancer registries; (4) surveillance protocols and frequency of clinical follow up at study centers (critical for in-center reporting of cancer cases); (5) duration of follow up post LT since cancer incidence increases with time<sup>[8,13]</sup>; and (6) in the case of standardized incidence ratio (SIR) calculations, the control population used and type of cancers captured by the respective registries. In this review, we have described incidence rates of cancers and as well as the SIR where possible, as it allows age and gender adjusted risk analysis. SIR is calculated as the ratio of observed incidence in a cohort to the expected incidence in the population (hence has no unit).

Cancer registry data used to calculate expected age and gender adjusted incidence rates for SIR estimation doesn't capture non-melanoma skin cancers (NMSCs). Therefore SIR analyses succinctly reflect the risk of more life-threatening types of cancer. Interestingly, purely registry based analyses yield higher SIR values for *de novo* malignancy post LT, ranging from 2.2 to 4.9<sup>[10,14-16]</sup>, than 1.4 to 3.1<sup>[2,9,11,17,18]</sup> of single and multi-center studies. The reasons for this are unclear but could reflect differing approaches to immunosuppression given the reporting bias for higher transplant volume centers.

## RISK FACTORS FOR *DE NOVO* MALIGNANCY

The risk factors for the development of *de novo* malignancy after LT are not fully understood, but it is likely that patient, transplant and environmental factors interact to shape that risk.

### *Immunosuppression related risk*

Over the past few decades, a better understanding of the role of the immune system in preventing malignancy in immunocompetent individuals helped establish the concept of immunosurveillance<sup>[19]</sup>. Transplant recipients receive lifelong immunosuppression with chronic impairment of immunosurveillance, which promotes proliferation and survival of malignant cellular clones. Though immunosuppressive drug dose intensity likely contributes to cancer risk, the evidence for this is

Table 1 Summary of study characteristics and reported incidence of de novo malignancy post liver transplantation in large series

Ref.	Year published	Country of study center	Study period	No. of liver transplant recipients	<sup>4</sup> Duration of follow-up (yr)	<sup>4</sup> Age at transplant in patients with de novo malignancy (yr)	Proportion of males with de novo malignancy	<sup>4</sup> Interval to de novo malignancy (yr)	Overall incidence of de novo malignancy (number of patients)	5/10/15 and 20 yr incidence of de novo malignancy	Estimated overall risk relative to control population
Jonas <i>et al.</i> <sup>[28]</sup>	1997	Germany	1988-1994	458	4.2	46 ± 14	48%	3.6	7.2% (33)	14.6%/-/-/-	-
Jain <i>et al.</i> <sup>[3]</sup>	1998	United States	1996-2006	1000	6.5 ± 1	Approximately 56	77%	3	5.7% (57)	-	SIR calculated for specific cancer types
Kelly <i>et al.</i> <sup>[25]</sup>	1998	United Kingdom	1988-1996	888	-	Approximately 52	46%	2 ± 1.5	<sup>2</sup> 4.4% (29)	-	-
Galve <i>et al.</i> <sup>[16]</sup>	1999	Spain	1984-1997	1827	-	-	-	2.5 ± 1.8	3.8% (70)	-	-
Haagsma <i>et al.</i> <sup>[6]</sup>	2001	The Netherlands	1979-1996	174	5.1	Approximately 49	29%	5.9	12% (21)	6%/20%/55%/-	RR = 4.3 (95%CI: 2.4-7.1)
Sanchez <i>et al.</i> <sup>[5]</sup>	2002	United States	1985-1999	1421	5.5 ± 3.7	50 ± 12	55%	-	8.8% (125)	-	-
Saigal <i>et al.</i> <sup>[4]</sup>	2003	United Kingdom	1988-1999	1140	-	51.5	70%	3.8 ± 2.8	2.6% (30)	-	-
Beniloch <i>et al.</i> <sup>[60]</sup>	2004	Spain	1991-2001	772	4.3	50	59%	3.5	5.3% (41) <sup>1</sup>	-	-
Oo <i>et al.</i> <sup>[65]</sup>	2005	United Kingdom	1982-2004	1778	6.5	-	43%	-	7.9% (141)	-	SIR = 2.1 (95%CI: 1.7-2.2)
Herrero <i>et al.</i> <sup>[7]</sup>	2005	Spain	1990-2001	187	5.5	-	-	-	26% (49)	25%/39%/-/-	RR = 2.9 (95%CI: 1.6-5.0)
Yao <i>et al.</i> <sup>[60]</sup>	2006	United States	1988-2000	1043	6.7	53.2	52%	-	4.8% (50)	-	-
Aberg <i>et al.</i> <sup>[9]</sup>	2008	Finland	1982-2005	540	6.3	-	53%	5.1	6.7% (36) <sup>3</sup>	5%/13%/-/-16%	<sup>3</sup> SIR = 2.6 (95%CI: 1.8-3.5)
Jiang <i>et al.</i> <sup>[10]</sup>	2008	Canada	1983-1998	2034	-	-	53%	3.5 ± 2.8	5.5% (113) <sup>1</sup>	2%/8.6%/-/-	<sup>1</sup> SIR = 2.5 (95%CI: 2.1-3.0)
Watt <i>et al.</i> <sup>[12]</sup>	2009	United States	1990-1994	798	10	-	60% <sup>1</sup>	-	21.4% (171)	12%/22%/-/-	-
Finkenstedt <i>et al.</i> <sup>[18]</sup>	2009	Austria	1982-2007	779	4.1	-	-	4.4	12.3% (96)	10%/24%/32%/42%	<sup>1</sup> SIR = 1.9 (95%CI: 1.5-2.4)
Baccarani <i>et al.</i> <sup>[17]</sup>	2010	Italy	1991-2005	417	6.7	-	74%	4.2	10.3% (43) <sup>1</sup>	-	<sup>1</sup> SIR = 2.6 (95%CI: 1.9-3.6)
Tjon <i>et al.</i> <sup>[27]</sup>	2010	Denmark	198-2007	85	5	-	-	-	3% (50)	10%/19%/34%/-	SIR = 2.2 (95%CI: 1.6-2.8)
Park <i>et al.</i> <sup>[67]</sup>	2012	South Korea	1998-2008	1952	3.5 ± 2.8	56	79%	3.4 ± 2.4	2.3% (44) <sup>1</sup>	-	<sup>1</sup> RR = 7.7 for men and 7.3 for women
Chatrath <i>et al.</i> <sup>[21]</sup>	2013	United States	1997-2004	534	5.7 ± 3.2	53 ± 12	67%	4 ± 2.2	13.7% (73)	12%/25%/-/-	<sup>1</sup> SIR = 3.1 (95%CI: 2.9-3.2)
Wimmer <i>et al.</i> <sup>[24]</sup>	2013	Germany	1985-2007	609	5.2	53 ± 10	73%	5.7	11.5% (70)	10%/26%/35%/-	-
Ettore <i>et al.</i> <sup>[11]</sup>	2014	Italy	1990-2008	1675	5.2	-	-	3.2	5.9% (98) <sup>1</sup>	-	<sup>1</sup> SIR = 1.4 (95%CI: 1.2-1.7)
Yu <i>et al.</i> <sup>[69]</sup>	2014	China	2005-2011	569	3.5 ± 2.2	-	76%	-	3.2% (17)	-	-
Antinucci <i>et al.</i> <sup>[17]</sup>	2015	Argentina	2006-2014	168	-	67 ± 7	75%	1.3	7.5% (12)	-	-
Sanaei <i>et al.</i> <sup>[68]</sup>	2015	Iran	1992-2012	1700	-	34 ± 10	63%	5.5	2.2% (38)	-	-
Overall means				940	5.5	52	61%	3.8	8.10%	11%/22%/39%/29%	3.0

<sup>1</sup>Excluding non-melanoma skin cancers; <sup>2</sup>Excluding post-transplant lymphoproliferative disorder; <sup>3</sup>Excluding basal cell skin cancer; <sup>4</sup>Median or mean ± SD. SIR: Standardized incidence ratio.

indirect. Comparative studies indicate a lower SIR for *de novo* malignancies in LT (2.2) recipients compared with heart (2.5) or lung (3.6) recipients who typically require higher intensity of immunosuppression<sup>[16,20]</sup>. A higher rate of hepatocellular carcinoma recurrence has been described with higher trough levels of the calcineurin inhibitors (CNI), tacrolimus and cyclosporine, particularly in the early post LT period<sup>[21,22]</sup>. Calcineurin inhibitors inhibit T-lymphocyte cell mediated immunity, and may also increase the risk of malignancy by increasing synthesis of growth factors, *e.g.*, transforming growth factor- $\beta$ , interleukin-6 and vascular endothelial growth factor in tumor cells, and impair DNA repair, thereby enhancing tumor growth, metastasis and angiogenesis<sup>[23]</sup>. The duration of immunosuppression also likely increases risk of malignancy, with increased incidence reported in recipients who were immunosuppressed before LT<sup>[9]</sup>.

The choice of immunosuppressive drug is a potentially modifiable cancer risk factor. Cyclosporine initially, and tacrolimus subsequently, have been and remain the mainstay of long term immunosuppression in LT over the last few decades. Even though some studies have shown higher carcinogenic risk with tacrolimus<sup>[7,24]</sup>, and others with cyclosporine based protocols<sup>[2,25-27]</sup>, there is no accepted cancer risk advantage for either agent<sup>[3,28]</sup>. A more practical concern in choice of immunosuppressant relates to the class of mammalian target of rapamycin (mTOR) inhibitors, sirolimus and everolimus, though these agents are used mainly in renal sparing regimens. The putative

**Table 2** A listing on known oncogenic viruses and the malignancies associated with them

Oncogenic virus	Associated malignancy
EBV	PTLD
Human papilloma virus	Cervical, skin, oropharynx, anal
Human T-cell lymphotropic virus type 1	Adult T cell leukemia
Kaposi's sarcoma-associated herpesvirus	KS, primary effusion lymphoma, castleman's disease
HBV	HCC
HCV	HCC, PTLD <sup>1</sup>

<sup>1</sup>Role controversial. HCC: Hepatocellular carcinoma; EBV: Epstein-Barr virus; PTLD: Post transplant lymphoproliferative disorder; KS: Kaposi's sarcoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

anti-proliferative properties of mTOR inhibition include inhibition of cellular growth, proliferation, metabolism and angiogenesis<sup>[29]</sup>. Though there is no prospective randomized controlled study data currently, a number of retrospective studies have described lower rates of hepatocellular carcinoma (HCC) recurrence<sup>[30,31]</sup>, and *de novo* malignancies<sup>[32,33]</sup> with mTOR inhibitors post LT and renal transplantation<sup>[34]</sup>. A meta-analysis of retrospective studies has shown that mTOR inhibitor, sirolimus, is of value in preventing recurrence and increasing survival in those transplanted for HCC<sup>[35]</sup>.

The post LT cancer risk related to anti-metabolites has been described for azathioprine in one study, with an odds ratio (OR = 3.8, 95%CI: 1.7-8.6,  $P = 0.004$ )<sup>[36]</sup>. Whereas mycophenolate mofetil has been shown to have anti-tumor properties in animal studies<sup>[37]</sup>, and was associated with a trend towards lower risk of non-skin *de novo* malignancies post renal transplant in a large United States, and European/Canadian registry based study<sup>[38]</sup>. In a recent study of solid organ transplant, mycophenolate mofetil use was associated with lower risk of proximal colon cancer<sup>[39]</sup>.

Immunosuppression induction with anti-lymphocyte antibodies or anti-thymocyte globulin was associated with increased of skin cancer in one study<sup>[9]</sup>, however that risk was not seen in larger series using anti-thymocyte globulin induction<sup>[2,28]</sup>. Rejection episodes also did not alter the risk of malignancy in LT recipients<sup>[5,6,12,40]</sup>. These data suggest that higher levels of immunosuppression in the short term do not increase the long term risk of cancer.

Immunosuppression also increases the cancer risk related to latent oncogenic virus infections (Table 2)<sup>[41]</sup>. Oncogenic virus associated tumors may be more immunogenic than those related to other factors, and may regress once immunosuppression is stopped or minimized<sup>[42]</sup>. This provides the rationale for a decrease in immunosuppression as the first line intervention for some virus related cancers, such post-transplant lymphoproliferative disorder (PTLD), particularly when associated with Epstein-barr virus (EBV) viremia<sup>[43]</sup>.

### Recipient related factors

The association of specific patient factors with cancer risk are organized and elaborated on below.

**Age:** Advanced age is a well described risk factor for *de novo* malignancy<sup>[2,7,8,12,44,45]</sup>, although this is not a universal finding<sup>[25,28]</sup>. This suggests that other factors may supersede age in cancer risk, though some caveats are notable with the extremes of age. For example the SIR for early PTLD was high (18.1) in pediatric LT recipients in one study<sup>[9]</sup>, with a similar observation in another study<sup>[10]</sup>. In another study, LT recipients older than 60 had > 2 fold higher 5-year incidence of new cancers (> 40%) compared to younger LT recipients (< 20%), largely due to non-skin cancers, with significantly higher cancer related mortality<sup>[46]</sup>.

**Gender and race:** There is conflicting data on the relative risk of *de novo* malignancy according to gender, with slightly higher SIR of cancers in females in one registry study<sup>[14]</sup>, and in males in another<sup>[45]</sup>, limiting any meaningful conclusion. Although skin cancer risk would be expected to differ according to race, there is limited data of cancer risk in relation to race. Non-Caucasian race was associated with a higher hazard ratio (HR = 2.5, 95%CI: 1.3-4.3) for non-skin cancers in one study, but the small size of that subgroup was limiting<sup>[2]</sup>.

**Indication for LT:** Patients who receive LT for certain indications are more prone to some malignancies. Patients with primary sclerosing cholangitis (PSC) in a United States multicenter prospective observational study had the highest cumulative incidence of non-skin cancer of 5.5%, 10.4%, and 21.9% at 1, 5, and 10 years, respectively<sup>[12]</sup>. Patients with PSC and inflammatory bowel disease (IBD) and an intact colon at the time of LT were at increased risk of gastrointestinal (colon) malignancy (HR = 2.34, 95%CI: 1.02-5.38)<sup>[12]</sup>, which may not be surprising given the association of PSC and IBD with colon cancer risk. However, patients with PSC also exhibited an increased risk for PTLD, skin malignancies and solid organ malignancies<sup>[12]</sup>. A high cancer risk for LT recipients with PSC was also observed in an Italian study, though cancer types were not specified<sup>[47]</sup>. The reasons for generalized cancer risk are unclear, but may reflect immunosuppression before LT, and possibly vitamin D deficiency which may promote malignancy<sup>[48]</sup>.

**Alcohol use history and smoking:** Many studies have described the carcinogenic properties of alcohol and smoking in immunocompetent individuals<sup>[49,50]</sup>. Alcoholic liver disease (ALD) is associated with increased cancer risk post LT<sup>[7,12,36,45,51-54]</sup>. Synergy between the carcinogenic effects of alcohol and smoking is well described<sup>[55,56]</sup>. Smokers were more likely to have alcoholic liver disease than non-smokers (35% vs 13%,



**Table 3** A summary of ranges of reported overall incidence rates and standardized incidence ratios of a number of cancer types following liver transplantation<sup>[2-5,7-12,14-16,28,36,47,66,110,118-120]</sup>

	Incidence (%)	SIR
Skin cancers		
Represent 24%-54% of all cancers, average 37%		
Overall (non-melanoma)	0.9-11.6	2.1-70, average 24
Squamous cell cancer	0.6-15.3	Not reported
Basal cell cancer	0.6-10.6	Not reported
KS	0.2-1.4	128-144
Melanoma	0.2-3.9	4.4
Post-transplant lymphoproliferative disorders		
Represent 4%-57% of all cancers, average 25%		
Overall	0.5-2.9	3.9-21, average 12
Hodgkin's lymphoma	0.001-0.4	8.2-8.9
Non-Hodgkin's lymphoma	0.8-3.7	3.5-37.3
Solid organ cancers		
Represent 24%-75% of all cancers, average 48%		
Overall	1.4-7.5	1.4-3.1, average 2.3
Lip	1.8	14-24.8
Oropharyngeal	1.7-1.9	7-10
Lung	0.6-2.4	1.4-2.0
Stomach	0.2-0.7	0.5-3.7
Colorectal	0.5-1.1	1.4-4.9
Breast (in females)	0.2-0.6	0.6-1.6 <sup>1</sup>
Cervix (in females)	0.7-1.4	1.3-5.7
Prostate (in males)	0.2-1.8	0.6-1.6 <sup>1</sup>

<sup>1</sup>The SIR was not found to be significantly higher for transplant recipients compared with the reference population. Of note, studies often reported either incidence rate or SIR, but rarely both values. SIR: Standardized incidence ratio; KS: Kaposi's sarcoma.

$P = 0.008$ ) in one study<sup>[56]</sup>, and patients transplanted for ALD were more likely to be smokers (82% vs 45%,  $P = 0.001$ ) and smoked more cigarettes per day ( $27 \pm 15$  vs  $16 \pm 11$ ,  $P = 0.001$ ) in another<sup>[54]</sup>. A United Kingdom registry study reported a higher SIR (3.16) of *de novo* malignancy for ALD compared to all other LT indications (1.99)<sup>[45]</sup>. In the immunocompetent population, there is evidence that the increased risk of cancer due to alcohol abuse could be reversed by abstinence<sup>[57]</sup>. However, this effect may be delayed by a more than a decade<sup>[58]</sup>, with cancer risk carried through post LT.

**History of cancer prior to LT:** A history of cancer prior to LT was not associated with its recurrence after LT<sup>[8,25]</sup>. However, LT for HCC has been associated with an increased risk of *de novo* malignancy<sup>[7,44]</sup>. An increased incidence of non-skin cancers in patients with a history of non-liver cancer prior to LT (30.8% vs 8.3%,  $P = 0.001$ ) has also been described, where it was additionally an independent predictor of non-skin *de novo* malignancies (HR = 2.5, 95%CI: 1.3-4.9,  $P = 0.005$ )<sup>[2]</sup>. This association is supported by data from renal transplantation studies<sup>[34,59,60]</sup>. Therefore, a prior history of cancer may reflect a patient's composite (genetic and epigenetic) risk of malignancy.

## SITE SPECIFIC *DE NOVO* MALIGNANCIES

The risk of *de novo* malignancy is variable across a range of tumor types, as reported by cancer registry

studies. These cancers are commonly grouped according to three broad categories including; skin cancers, PTLD and solid organ cancers. The risks of specific tumors post LT are summarized in Table 3.

### Skin cancers

Skin malignancy, typically NMSC, is the most common malignancy after LT<sup>[2,7,9,12,40,61]</sup>. These include squamous cell cancer (SCC), basal cell cancer (BCC) and Kaposi's sarcoma (KS). Ultraviolet radiation is an important risk factor in the pathogenesis of skin malignancies, and exerts a field cancerization mutagenic effect in exposed areas of the skin<sup>[62-65]</sup>. In a prospective study of LT recipients with comprehensive dermatology follow-up, only total pre transplant sun burden and skin characteristics were found to be the risk factors for NMSC<sup>[66]</sup>. The relative risk of cutaneous malignancies in this cohort was found to be 20 fold higher than the general population. Conversely studies from Iran, South Korea and China described no to very low incidence rates of skin cancer, likely due to the prevalent skin types<sup>[67-69]</sup>. In organ transplant recipients, SCC is more common than BCC, in contrast to the general population<sup>[44,70]</sup>. Additionally, while SCC and BCC are easily surveyed and resected, SCC can behave more aggressively in LT recipients<sup>[70,71]</sup>. In general though, LT recipients with SCC and BCC have similar survival to patients not developing *de novo* malignancies post LT<sup>[2,40]</sup>.

Immunosuppression with CNIs and azathioprine is a significant risk factor for NMSC<sup>[72-76]</sup>, but it is likely

the degree of immunosuppression that represents the main risk rather than the choice of agent<sup>[62,77,78]</sup>. However, there is mounting evidence that mTOR inhibitors have protective effect against NMSC due to their aforementioned anti-proliferative properties<sup>[72,77]</sup>, especially in renal transplant recipients. In a randomized trial, converting renal transplant recipients with NMSC from CNI to sirolimus based immunosuppression was associated with a reduced risk of subsequent NMSC (relative risk 0.56, 95%CI: 0.32-0.98) and longer recurrence free interval (15 mo vs 7 mo,  $P = 0.02$ )<sup>[79]</sup>. However, similar evidence in LT recipients is currently lacking.

Kaposi's sarcoma is related to human herpes virus-8 (HHV-8) and occurs only in immunocompromised individuals. The incidence of KS after LT reflects the prevalence of HHV-8 (also known as Kaposi's sarcoma-associated herpes virus), with high rates reported in the Mediterranean region<sup>[80,81]</sup>. Not surprisingly the highest rates and SIR (commonly > 100) for KS post LT are reported in Italian transplant series<sup>[11,17,47]</sup>.

### Post-transplant lymphoproliferative disorders

The term PTLD encompasses a broad spectrum of lymphoproliferative disorders observed in the immunocompromised solid organ transplant recipients. It is the second most common malignancy in LT recipients, and is notable in its wide age distribution, extending to the very young<sup>[14]</sup>. The rate of PTLD is lower in the liver compared to other solid organ recipients<sup>[82]</sup>, likely due to lower immunosuppression levels needed to prevent liver allograft rejection, and possibly a smaller number of donor lymphocytes in the graft<sup>[83]</sup>. The other factor driving PTLD risk is EBV infection, with associated PTLD generally occurring earlier, in the first 12 to 18 mo, after LT and involving younger patients<sup>[82,84]</sup>. Infection with EBV and immunosuppression appear to play crucial roles in the pathogenesis of PTLD. EBV mismatch between donor and recipient of LT increases the risk of PTLD by 70 fold<sup>[85,86]</sup>. Primary infection with EBV after LT also increases the risk significantly<sup>[87]</sup>. Primary EBV or latent (of virus within B cells) infection can stimulate B cell proliferation and transformation<sup>[88]</sup>. EBV associated PTLD occurs three times more frequently in pediatric patients<sup>[87,89]</sup>. This is likely a reflection of the EBV negative status of pediatric recipient, whereas EBV infects 90% of the adults worldwide<sup>[90]</sup>.

Another important phenotype of PTLD develops later post LT in the absence of EBV infection involves older recipients and carries a worse prognosis<sup>[82]</sup>. The pathogenesis of EBV negative PTLD is uncertain<sup>[91]</sup>, but some risk factors were described in a study of 480 adult LT recipient PTLD in France, where 16 developed PTLD<sup>[92]</sup>. These were age above 50, LT for hepatitis C virus (HCV) or alcoholic cirrhosis, and the use of anti-lymphocyte antibodies such as muromonab, the latter reported by others<sup>[82,87]</sup>. The use of anti-thymocyte globulins in LT for HCV cirrhosis augmented PTLD risk in another study (27% for HCV vs 6.4% for non-HCV

cases,  $P = 0.08$ )<sup>[93]</sup>. When compared to lymphomas in the immunocompetent population, PTLD are more likely to exhibit extra-nodal involvement, high-grade and poor outcomes<sup>[94]</sup>. Factors which confer a poor prognosis with PTLD are; high grade or stage at diagnosis<sup>[43]</sup>, T cell disease<sup>[95]</sup>, central nervous system and bone marrow involvement<sup>[96,97]</sup>, poor performance status<sup>[98]</sup>, higher number of extra-nodal sites<sup>[98]</sup>, and EBV negative disease<sup>[43,85,99]</sup>.

### Solid organ cancers

Like PTLD, this category of *de novo* malignancy carries significant risk of mortality post LT, but is a term loosely used to group a wide range of tumor types and organ involved. Some characteristics of risk are evident in relation to subgroups of solid organ cancers, including aerodigestive, gastrointestinal, genitourinary and gynecologic systems.

### Aerodigestive cancers

Aerodigestive cancers are associated with smoking and alcohol use, and arise from the tissues of the aerodigestive tract, which include the respiratory tract and the upper part of the digestive tract (including the lips, mouth, tongue, nose, throat, vocal cords, and part of the esophagus and windpipe). These are largely reported as head and neck cancers and lung cancer post LT.

A meta-analysis of studies examining head and neck cancer after LT found an overall SIR of 3.8 (95%CI: 2.7-4.9)<sup>[100]</sup>. They develop at mean post LT intervals that range from 34 to 61 mo<sup>[3-5,92,101]</sup>. Liver transplant recipients with a history of tobacco use and ALD are at high risk for developing head and neck cancers<sup>[7,12,102]</sup>, and in some studies only developed in patients with a history of ALD<sup>[6,103]</sup>.

In a large study encompassing all solid organ transplants in the United States, the SIR for lung cancer after LT was found to be 1.95 (95%CI: 1.74-2.19)<sup>[14]</sup>. Lung cancer develops at mean post LT intervals ranging from 42 to 50 mo<sup>[3,5,28,61,101]</sup>. The main risk factors for lung cancer, similar to the general population, in LT recipients was smoking<sup>[2,7,12,54]</sup>. Those transplanted for ALD also had increased risk of lung cancer compared to those transplanted for other causes (4.3% vs 0.7%,  $P < 0.001$ ), though tobacco use which prevalent in this population may confound these observations<sup>[7,12,54]</sup>. Post LT lung cancer is commonly diagnosed in advanced stages<sup>[3,5,54]</sup>, suggesting the need for diligent surveillance programs in the high risk population (smokers and those transplanted for ALD). It remains unclear how long tobacco and alcohol related cancer risk persist following cessation.

### Gastrointestinal cancers

The most common gastrointestinal cancer seen in solid organ transplant recipients is colon cancer<sup>[14]</sup>. The SIR for colon cancer in LT recipients ranges from 1.4 to as high 27.3 in subsets of high risk patients with PSC<sup>[16,17,45]</sup>.

Patients receiving LT for PSC are at particularly high risk for colon cancer, due to the association with IBD<sup>[12,45,104-106]</sup>. In the study by Watt *et al.*<sup>[12]</sup> PSC alone (HR = 1.9,  $P = 0.12$ ) was not a risk factor for gastrointestinal malignancy, whereas patients with PSC, IBD and intact colons had a significant cancer risk (HR = 3.51, 95%CI: 1.48-8.36,  $P = 0.005$ ). Colon cancer was more common in LT recipients with ulcerative colitis (SIR = 27.3 vs 3.5), than those without it, particularly in patients older than 40 (SIR = 4.8 vs 1 in younger patients)<sup>[45]</sup>. Longer duration of IBD and more extensive colonic involvement increase the risk for colorectal cancer in LT recipients with PSC<sup>[104-106]</sup>. Colorectal cancer develops at a younger age in LT recipients compared with the general population, and has a worse prognosis<sup>[107,108]</sup>. A relatively high incidence of colon and stomach cancer have been reported in a South Korean study<sup>[67]</sup>, with otherwise relatively low (2.2%-2.3%) *de novo* malignancy incidence rates reported in East Asian studies<sup>[67,69]</sup>.

### Genitourinary and gynecologic cancers

Registry studies indicate an increased SIR of some (cervical, vulvar, bladder and kidney) but not all genitourinary or gender-specific (breast, prostate, uterine, ovarian) cancers following solid organ transplant<sup>[10,14-17,45]</sup>. In the largest of these, there was a slightly lower SIR for breast and prostate cancer in transplant recipients<sup>[14]</sup>. Cervical cancer risk was significantly elevated in one series (SIR = 30.7)<sup>[17]</sup>, and other human papilloma virus related cancers (vulvar, vaginal, anal, penile) all appear to have higher SIR (range 2.4-7.6) relative to the general population<sup>[14]</sup>. Bladder cancer risk is increased in a number of studies, with a range of SIR value from 1.5 to 2.4<sup>[14-16]</sup>, and were noted to develop late (10 years) post LT in one cohort<sup>[47]</sup>.

## SURVIVAL AFTER *DE NOVO* NON-SKIN CANCERS

In a comparison of patients from a solid organ transplant cancer registry with a general population from the Surveillance, Epidemiology, and End Results database, transplant patients were more likely to be diagnosed with American Joint Commission on Cancer stage > 2 cancers, and worse cancer-specific survival<sup>[109]</sup>. The relative risk of cancer-related mortality compared to the general population was 2.9 (95%CI: 1.59-5.11)<sup>[7]</sup>. In a large single center study *de novo* malignancy, excluding NMSC, was a leading category of mortality risk (14.2%), along with infections (15%), disease recurrence (13%) and cardiovascular (9%) complications<sup>[2]</sup>. Patient survival rates at 1.3 and 5 years after diagnosis of *de novo* malignancy were 55%, 36%, and 27% compared with 100%, 100% and 67% for patients with only NMSC,  $P = 0.001$ , respectively<sup>[2]</sup>. Similarly, *de novo* malignancy excluding NMSC was associated with an increased risk of mortality [HR = 4.9 (95%CI: 1.67-14.2),  $P = 0.003$ ] in another large series<sup>[40]</sup>, and probability of death after

diagnosis was 40% at 1 year, and 55% at 5 years, respectively<sup>[12]</sup>.

There is considerable variability in reported survival after PTLD, with median survival as low as 2 mo (95%CI: 0.3-3.5 mo) in one study<sup>[36]</sup>, likely as a result of heterogeneity in risk characteristics of PTLD<sup>[95]</sup>. Longer median survival intervals (27 mo to 35 mo) are noted in other LT series<sup>[12,14]</sup>, with reported 1 and 5 year survival rates of 56% and 46%, respectively<sup>[82]</sup>. Pediatric LT recipients with PTLD appear to have better outcomes, with median survival of 8.2 years and reported 10 years survival rates of 59%<sup>[85,96]</sup>, and no reported mortality in some series<sup>[94]</sup>. Advanced stage, Burkitt or Burkitt-like PTLD, and c-myc translocations indicated poor prognosis and short survival in pediatric PTLD<sup>[96]</sup>.

The reported site-specific cancer survival rates for the aforementioned solid organ cancer categories are: Oropharyngeal cancer 1 and 5 year survival of 43% to 78% and 56% respectively, lung cancer 1 and 5 year survival of 41% to 43% and 16% respectively, gastrointestinal cancers 1 and 5 year survival of 67% to 80% and 52% respectively, and genitourinary cancers 1 and 5 year survival of 79%-100% and 71% respectively<sup>[3,12]</sup>.

## SURVEILLANCE

The increased risk and mortality associated with *de novo* malignancies underlines the need for surveillance strategies to detect tumors at earlier stages, allow more effective treatments, and improve survival. However, there are no standardized surveillance protocols for LT recipients at present. Routine follow up visits alone were only capable of detecting 12% of the non-skin cancers in one series, and annual visits resulted in identifying half of all malignancies in another<sup>[8,9]</sup>. Poor compliance with surveillance protocols was also cited as a limitation in a study where active surveillance identified only 3 of 28 non-skin cancers<sup>[7]</sup>. These data further highlight the need for regimented surveillance strategies in this regard.

In a compelling study, the incidence and outcome of *de novo* malignancy were compared before and after institution of an intensified surveillance protocol which included: Annual chest and abdominal computerized tomography (CT), urological, gynecological (pap smear and mammography) and dermatological examination, and colonoscopies every 5 years<sup>[18]</sup>. With a historical surveillance program consisting of annual chest radiographs and abdominal ultrasounds serving as the reference comparator, the detection rate for *de novo* malignancies increased from 4.9% to 13% with intensified surveillance ( $P = 0.001$ ), fewer tumors were diagnosed at stage III or IV (46% vs 75%), and median survival following a diagnosis of non-skin cancer increased from 1.2 to 3.3 years ( $P = 0.001$ )<sup>[18]</sup>.

At another center, a similarly multifaceted surveillance protocol that included: (1) urinalysis, chest radiographs and abdominal ultrasounds performed every

6 mo in the first year post LT and annually thereafter; (2) mammography every two years; (3) colonoscopy every 7-10 years if no adenomas were detected; and (4) in patients with smoking history, an annual otolaryngological evaluation and low dose CT of the chest after 2006<sup>[110]</sup>. Patients that were diagnosed with *de novo* malignancy through active surveillance had better survival (all were alive after 25 mo of follow up) compared with patients diagnosed with symptomatic disease or incidentally (median survival of 13.5 mo) ( $P = 0.002$ )<sup>[110]</sup>. The use of annual low dose chest CT in LT recipients with more than 10 pack years of cumulative smoking history led to a diagnosis of early stage lung cancer in 12% of patients<sup>[111]</sup>.

Additionally, special populations amongst LT recipient and the specialized surveillance strategies that are or may be warranted for them include those with: (1) underlying PSC and IBD, or IBD alone of more than 8-10 years duration with annual surveillance colonoscopy; (2) a history of human papilloma virus infection with annual pap smear in females, and annual genital and anal pap/scraping in both genders; and (3) patients from the Mediterranean region with testing of HHV-8 titers due to increased prevalence and association with risk of KS<sup>[112]</sup>.

## PREVENTATIVE MEASURES

Smoking is a major risk factor for cancer, especially nasopharyngeal cancers and lung cancer, as well cardiovascular disease related mortality<sup>[56]</sup>, and smoking cessation should be counseled as early as possible. Regular application of broad spectrum sunscreen (SPF > 50, with high-UVA absorption) over sun-exposed areas in solid organ transplant recipients, in conjunction with counseling of excessive sun exposure avoidance, reduced the risk of actinic keratosis, invasive SCC and BCC from developing in a prospective case control study in solid organ transplant recipients<sup>[113]</sup>. Protective clothing has also been shown to protect against UV radiation<sup>[114]</sup>. The minimization of immunosuppression without risking graft rejection is limited by the lack of accurate markers of over or under immunosuppression, but would likely to attenuate the risk of *de novo* malignancy in LT recipient. There is also insufficient evidence to guide the routine use of mTOR inhibitors in at risk patients, but those studies are ongoing<sup>[115-120]</sup>.

## CONCLUSION

Liver transplant recipients are at increased risk of cancer when compared to the general population, and the most commonly encountered cancers are NMSC, PTLT, and aerodigestive. They are due mainly due to the effects of immunosuppression and latent oncogenic viruses prevalent in the population. Important risk factors for development of *de novo* malignancy include age, degree of immunosuppression, history of smoking and alcohol abuse and transplantation for PSC and ALD. *De novo* malignancies, excluding NMSC, represent a major risk

category for post LT mortality. There are no standardized surveillance protocols for *de novo* malignancy post LT, but available evidence supports adoption of some consistent surveillance strategies. Minimization of immunosuppression and attention and counseling related to other risk factors in LT recipients may reduce an individual's risk of developing cancers post LT, but more evidence is needed to optimize care.

## REFERENCES

- 1 **Pruthi J**, Medkiff KA, Esrason KT, Donovan JA, Yoshida EM, Erb SR, Steinbrecher UP, Fong TL. Analysis of causes of death in liver transplant recipients who survived more than 3 years. *Liver Transpl* 2001; **7**: 811-815 [PMID: 11552217 DOI: 10.1053/jlts.2001.27084]
- 2 **Chattrath H**, Berman K, Vuppalanchi R, Slaven J, Kwo P, Tector AJ, Chalasani N, Ghabril M. De novo malignancy post-liver transplantation: a single center, population controlled study. *Clin Transplant* 2013; **27**: 582-590 [PMID: 23808800 DOI: 10.1111/ctr.12171]
- 3 **Jain AB**, Yee LD, Nalesnik MA, Youk A, Marsh G, Reyes J, Zak M, Rakela J, Irish W, Fung JJ. Comparative incidence of de novo nonlymphoid malignancies after liver transplantation under tacrolimus using surveillance epidemiologic end result data. *Transplantation* 1998; **66**: 1193-1200 [PMID: 9825817 DOI: 10.1097/00007890-199811150-00014]
- 4 **Saigal S**, Norris S, Muiesan P, Rela M, Heaton N, O'Grady J. Evidence of differential risk for posttransplantation malignancy based on pretransplantation cause in patients undergoing liver transplantation. *Liver Transpl* 2002; **8**: 482-487 [PMID: 12004349 DOI: 10.1053/jlts.2002.32977]
- 5 **Sanchez EQ**, Marubashi S, Jung G, Levy MF, Goldstein RM, Molmenti EP, Fasola CG, Gonwa TA, Jennings LW, Brooks BK, Klintmalm GB. De novo tumors after liver transplantation: a single-institution experience. *Liver Transpl* 2002; **8**: 285-291 [PMID: 11910575 DOI: 10.1053/jlts.2002.29350]
- 6 **Schmilovitz-Weiss H**, Mor E, Sulkes J, Bar-Nathan N, Shaharabani E, Melzer E, Tur-Kaspa R, Ben-Ari Z. De novo tumors after liver transplantation: a single-center experience. *Transplant Proc* 2003; **35**: 665-666 [PMID: 12644086 DOI: 10.1016/S0041-1345(03)00089-7]
- 7 **Herrero JI**, Lorenzo M, Quiroga J, Sangro B, Pardo F, Rotellar F, Alvarez-Cienfuegos J, Prieto J. De Novo neoplasia after liver transplantation: an analysis of risk factors and influence on survival. *Liver Transpl* 2005; **11**: 89-97 [PMID: 15690541 DOI: 10.1002/lt.20319]
- 8 **Haagsma EB**, Hagens VE, Schaapveld M, van den Berg AP, de Vries EG, Klompmaier IJ, Slooff MJ, Jansen PL. Increased cancer risk after liver transplantation: a population-based study. *J Hepatol* 2001; **34**: 84-91 [PMID: 11211912 DOI: 10.1016/S0168-8278(00)00077-5]
- 9 **Aberg F**, Pukkala E, Höckerstedt K, Sankila R, Isoniemi H. Risk of malignant neoplasms after liver transplantation: a population-based study. *Liver Transpl* 2008; **14**: 1428-1436 [PMID: 18825704 DOI: 10.1002/lt.21475]
- 10 **Jiang Y**, Villeneuve PJ, Fenton SS, Schaubel DE, Lilly L, Mao Y. Liver transplantation and subsequent risk of cancer: findings from a Canadian cohort study. *Liver Transpl* 2008; **14**: 1588-1597 [PMID: 18975293 DOI: 10.1002/lt.21554]
- 11 **Ettorre GM**, Piselli P, Galatioto L, Rendina M, Nudo F, Sforza D, Miglioresi L, Fantola G, Cimaglia C, Vennarecci G, Vizzini GB, Di Leo A, Rossi M, Tisone G, Zamboni F, Santoro R, Agresta A, Puro V, Serraino D. De novo malignancies following liver transplantation: results from a multicentric study in central and southern Italy, 1990-2008. *Transplant Proc* 2013; **45**: 2729-2732 [PMID: 24034034 DOI: 10.1016/j.transproceed.2013.07.050]
- 12 **Watt KD**, Pedersen RA, Kremers WK, Heimbach JK, Sanchez W, Gores GJ. Long-term probability of and mortality from de novo



- malignancy after liver transplantation. *Gastroenterology* 2009; **137**: 2010-2017 [PMID: 19766646 DOI: 10.1053/j.gastro.2009.08.070]
- 13 **Penn I.** Cancer in the immunosuppressed organ recipient. *Transplant Proc* 1991; **23**: 1771-1772 [PMID: 2053149]
  - 14 **Engels EA, Pfeiffer RM, Fraumeni JF, Kasiske BL, Israni AK, Snyder JJ, Wolfe RA, Goodrich NP, Bayakly AR, Clarke CA, Copeland G, Finch JL, Fleissner ML, Goodman MT, Kahn A, Koch L, Lynch CF, Madeleine MM, Pawlish K, Rao C, Williams MA, Castenson D, Curry M, Parsons R, Fant G, Lin M.** Spectrum of cancer risk among US solid organ transplant recipients. *JAMA* 2011; **306**: 1891-1901 [PMID: 22045767 DOI: 10.1001/jama.2011.1592]
  - 15 **Adami J, Gäbel H, Lindelöf B, Ekström K, Rydh B, Glimelius B, Ekbom A, Adami HO, Granath F.** Cancer risk following organ transplantation: a nationwide cohort study in Sweden. *Br J Cancer* 2003; **89**: 1221-1227 [PMID: 14520450 DOI: 10.1038/sj.bjc.6601219]
  - 16 **Collett D, Mumford L, Banner NR, Neuberger J, Watson C.** Comparison of the incidence of malignancy in recipients of different types of organ: a UK Registry audit. *Am J Transplant* 2010; **10**: 1889-1896 [PMID: 20659094 DOI: 10.1111/j.1600-6143.2010.02766.x]
  - 17 **Baccarani U, Piselli P, Serraino D, Adani GL, Lorenzin D, Gambato M, Buda A, Zanusi G, Vitale A, De Paoli A, Cimaglia C, Bresadola V, Toniutto P, Risaliti A, Cillo U, Bresadola F, Burra P.** Comparison of de novo tumours after liver transplantation with incidence rates from Italian cancer registries. *Dig Liver Dis* 2010; **42**: 55-60 [PMID: 19497797 DOI: 10.1016/j.dld.2009.04.017]
  - 18 **Finkenstedt A, Graziadei IW, Oberaigner W, Hilbe W, Nachbaur K, Mark W, Margreiter R, Vogel W.** Extensive surveillance promotes early diagnosis and improved survival of de novo malignancies in liver transplant recipients. *Am J Transplant* 2009; **9**: 2355-2361 [PMID: 19663894 DOI: 10.1111/j.1600-6143.2009.02766.x]
  - 19 **Dunn GP, Old LJ, Schreiber RD.** The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* 2004; **21**: 137-148 [PMID: 15308095 DOI: 10.1016/j.immuni.2004.07.017]
  - 20 **Na R, Grulich AE, Meagher NS, McCaughan GW, Keogh AM, Vajdic CM.** Comparison of de novo cancer incidence in Australian liver, heart and lung transplant recipients. *Am J Transplant* 2013; **13**: 174-183 [PMID: 23094788 DOI: 10.1111/j.1600-6143.2012.04302.x]
  - 21 **Vivarelli M, Cucchetti A, La Barba G, Ravaioli M, Del Gaudio M, Lauro A, Grazi GL, Pinna AD.** Liver transplantation for hepatocellular carcinoma under calcineurin inhibitors: reassessment of risk factors for tumor recurrence. *Ann Surg* 2008; **248**: 857-862 [PMID: 18948815 DOI: 10.1097/SLA.0b013e3181896278]
  - 22 **Rodríguez-Perálvarez M, Tsochatzis E, Naveas MC, Pieri G, García-Caparrós C, O'Beirne J, Poyato-González A, Ferrín-Sánchez G, Montero-Álvarez JL, Patch D, Thorburn D, Briceño J, De la Mata M, Burroughs AK.** Reduced exposure to calcineurin inhibitors early after liver transplantation prevents recurrence of hepatocellular carcinoma. *J Hepatol* 2013; **59**: 1193-1199 [PMID: 23867318 DOI: 10.1016/j.jhep.2013.07.012]
  - 23 **Weischer M, Röcken M, Berneburg M.** Calcineurin inhibitors and rapamycin: cancer protection or promotion? *Exp Dermatol* 2007; **16**: 385-393 [PMID: 17437481 DOI: 10.1111/j.1600-0625.2007.00555.x]
  - 24 **Wimmer CD, Angele MK, Schwarz B, Pratschke S, Rentsch M, Khandoga A, Guba M, Jauch KW, Bruns C, Graeb C.** Impact of cyclosporine versus tacrolimus on the incidence of de novo malignancy following liver transplantation: a single center experience with 609 patients. *Transpl Int* 2013; **26**: 999-1006 [PMID: 23952102 DOI: 10.1111/tri.12165]
  - 25 **Kelly DM, Emre S, Guy SR, Miller CM, Schwartz ME, Sheiner PA.** Liver transplant recipients are not at increased risk for nonlymphoid solid organ tumors. *Cancer* 1998; **83**: 1237-1243 [DOI: 10.1002/(SICI)1097-0142(19980915)83:6<1237::AID-CNCR25>3.0.CO;2-5]
  - 26 **Marqués Medina E, Jiménez Romero C, Gómez de la Cámara A, Rota Bernal A, Manrique Municio A, Moreno González E.** Malignancy after liver transplantation: cumulative risk for development. *Transplant Proc* 2009; **41**: 2447-2449 [PMID: 19715947 DOI: 10.1016/j.transproceed.2009.06.153]
  - 27 **Tjøn AS, Sint Nicolaas J, Kwekkeboom J, de Man RA, Kazemier G, Tilanus HW, Hansen BE, van der Laan LJ, Tha-In T, Metselaar HJ.** Increased incidence of early de novo cancer in liver graft recipients treated with cyclosporine: an association with C2 monitoring and recipient age. *Liver Transpl* 2010; **16**: 837-846 [PMID: 20583092 DOI: 10.1002/lt.22064]
  - 28 **Jonas S, Rayes N, Neumann U, Neuhaus R, Bechstein WO, Guckelberger O, Tullius SG, Serke S, Neuhaus P.** De novo malignancies after liver transplantation using tacrolimus-based protocols or cyclosporine-based quadruple immunosuppression with an interleukin-2 receptor antibody or antithymocyte globulin. *Cancer* 1997; **80**: 1141-1150 [PMID: 9305716 DOI: 10.1002/(SICI)1097-0142(19970915)80]
  - 29 **Geissler EK, Schlitt HJ, Thomas G.** mTOR, cancer and transplantation. *Am J Transplant* 2008; **8**: 2212-2218 [PMID: 18785960 DOI: 10.1111/j.1600-6143.2008.02391.x]
  - 30 **Vivarelli M, Dazzi A, Zanello M, Cucchetti A, Cescon M, Ravaioli M, Del Gaudio M, Lauro A, Grazi GL, Pinna AD.** Effect of different immunosuppressive schedules on recurrence-free survival after liver transplantation for hepatocellular carcinoma. *Transplantation* 2010; **89**: 227-231 [PMID: 20098287 DOI: 10.1097/TP.0b013e3181c3c540]
  - 31 **Chinnakotla S, Davis GL, Vasani S, Kim P, Tomiyama K, Sanchez E, Onaca N, Goldstein R, Levy M, Klintmalm GB.** Impact of sirolimus on the recurrence of hepatocellular carcinoma after liver transplantation. *Liver Transpl* 2009; **15**: 1834-1842 [PMID: 19938137 DOI: 10.1002/lt.21953]
  - 32 **Bilbao I, Sapisochin G, Dopazo C, Lazaro JL, Pou L, Castells L, Caralt M, Blanco L, Gantxegi A, Margarit C, Charco R.** Indications and management of everolimus after liver transplantation. *Transplant Proc* 2009; **41**: 2172-2176 [PMID: 19715864 DOI: 10.1016/j.transproceed.2009.06.087]
  - 33 **Jiménez-Romero C, Manrique A, Marqués E, Calvo J, Sesma AG, Cambra F, Abradelo M, Sterup RM, Olivares S, Justo I, Colina F, Moreno E.** Switching to sirolimus monotherapy for de novo tumors after liver transplantation. A preliminary experience. *Hepatogastroenterology* 2011; **58**: 115-121 [PMID: 21510297]
  - 34 **Kauffman HM, Cherikh WS, Cheng Y, Hanto DW, Kahan BD.** Maintenance immunosuppression with target-of-rapamycin inhibitors is associated with a reduced incidence of de novo malignancies. *Transplantation* 2005; **80**: 883-889 [PMID: 16249734 DOI: 10.1097/01.TP.0000184006.43152.8D]
  - 35 **Menon KV, Hakeem AR, Heaton ND.** Meta-analysis: recurrence and survival following the use of sirolimus in liver transplantation for hepatocellular carcinoma. *Aliment Pharmacol Ther* 2013; **37**: 411-419 [PMID: 23278125 DOI: 10.1111/apt.12185]
  - 36 **Benlloch S, Berenguer M, Prieto M, Moreno R, San Juan F, Rayón M, Mir J, Segura A, Berenguer J.** De novo internal neoplasms after liver transplantation: increased risk and aggressive behavior in recent years? *Am J Transplant* 2004; **4**: 596-604 [PMID: 15023152 DOI: 10.1111/j.1600-6143.2004.00380.x]
  - 37 **Tressler RJ, Garvin LJ, Slate DL.** Anti-tumor activity of mycophenolate mofetil against human and mouse tumors in vivo. *Int J Cancer* 1994; **57**: 568-573 [PMID: 8181860 DOI: 10.1002/ijc.2910570421]
  - 38 **Robson R, Cecka JM, Opelz G, Budde M, Sacks S.** Prospective registry-based observational cohort study of the long-term risk of malignancies in renal transplant patients treated with mycophenolate mofetil. *Am J Transplant* 2005; **5**: 2954-2960 [PMID: 16303010 DOI: 10.1111/j.1600-6143.2005.01125.x]
  - 39 **Safaeian M, Robbins HA, Berndt SI, Lynch CF, Fraumeni JF, Engels EA.** Risk of Colorectal Cancer After Solid Organ Transplantation in the United States. *Am J Transplant* 2016; **16**: 960-967 [PMID: 26731613 DOI: 10.1111/ajt.13549]
  - 40 **Yao FY, Gautam M, Palese C, Rebres R, Terrault N, Roberts JP, Peters MG.** De novo malignancies following liver transplantation: a case-control study with long-term follow-up. *Clin Transplant* 2006; **20**: 617-623 [PMID: 16968488 DOI: 10.1111/j.1399-0012.2006.00527.x]

- 41 **Schulz TF.** Cancer and viral infections in immunocompromised individuals. *Int J Cancer* 2009; **125**: 1755-1763 [PMID: 19588503 DOI: 10.1002/ijc.24741]
- 42 **Piselli P,** Busnach G, Fratino L, Citterio F, Ettore GM, De Paoli P, Serrano D. De novo malignancies after organ transplantation: focus on viral infections. *Curr Mol Med* 2013; **13**: 1217-1227 [PMID: 23278452 DOI: 10.2174/15665240113139990041]
- 43 **Parker A,** Bowles K, Bradley JA, Emery V, Featherstone C, Gupta G, Marcus R, Parameshwar J, Ramsay A, Newstead C. Diagnosis of post-transplant lymphoproliferative disorder in solid organ transplant recipients - BCSH and BTS Guidelines. *Br J Haematol* 2010; **149**: 675-692 [PMID: 20408847 DOI: 10.1111/j.1365-2141.20]
- 44 **Xiol X,** Guardiola J, Menendez S, Lama C, Figueras J, Marcoval J, Serrano T, Botargues JM, Mañer M, Rota R. Risk factors for development of de novo neoplasia after liver transplantation. *Liver Transpl* 2001; **7**: 971-975 [PMID: 11699033 DOI: 10.1053/jlts.2001.28744]
- 45 **Oo YH,** Gunson BK, Lancashire RJ, Cheng KK, Neuberger JM. Incidence of cancers following orthotopic liver transplantation in a single center: comparison with national cancer incidence rates for England and Wales. *Transplantation* 2005; **80**: 759-764 [PMID: 16210962 DOI: 10.1097/01.TP.0000173775.16579.18]
- 46 **Herrero JI,** Lucena JF, Quiroga J, Sangro B, Pardo F, Rotellar F, Álvarez-Cienfuegos J, Prieto J. Liver transplant recipients older than 60 years have lower survival and higher incidence of malignancy. *Am J Transplant* 2003; **3**: 1407-1412 [PMID: 14525602]
- 47 **Maggi U,** Consonni D, Manini MA, Gatti S, Cuccaro F, Donato F, Conte G, Bertazzi PA, Rossi G. Early and late de novo tumors after liver transplantation in adults: the late onset of bladder tumors in men. *PLoS One* 2013; **8**: e65238 [PMID: 23785414 DOI: 10.1371/journal.pone.0065238]
- 48 **Holick MF.** High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* 2006; **81**: 353-373 [PMID: 16529140 DOI: 10.4065/81.3.353]
- 49 **Seitz HK,** Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. *Nat Rev Cancer* 2007; **7**: 599-612 [PMID: 17646865 DOI: 10.1038/nrc2191]
- 50 **Carbone D.** Smoking and cancer. *Am J Med* 1992; **93**: 13S-17S [PMID: 1496998 DOI: 10.1016/0002-9343(92)90621-H]
- 51 **Dumortier J,** Guillaud O, Adham M, Boucaud C, Delafosse B, Bouffard Y, Paliard P, Scoazec JY, Boillot O. Negative impact of de novo malignancies rather than alcohol relapse on survival after liver transplantation for alcoholic cirrhosis: a retrospective analysis of 305 patients in a single center. *Am J Gastroenterol* 2007; **102**: 1032-1041 [PMID: 17313502 DOI: 10.1111/j.1572-0241.2007.01079.x]
- 52 **Jiménez C,** Marqués E, Loinaz C, Romano DR, Gómez R, Meneu JC, Hernández-Vallejo G, Alonso O, Abradelo M, García I, Moreno E. Upper aerodigestive tract and lung tumors after liver transplantation. *Transplant Proc* 2003; **35**: 1900-1901 [PMID: 12962840 DOI: 10.1016/S0041-1345(03)00641-9]
- 53 **Zanus G,** Carraro A, Vitale A, Gringeri E, D'Amico F, Valmasoni M, D'Amico FE, Brolese A, Boccagni P, Neri D, Srsen N, Burra P, Feltracco P, Bonsignore P, Scopelliti M, Cillo U. Alcohol abuse and de novo tumors in liver transplantation. *Transplant Proc* 2009; **41**: 1310-1312 [PMID: 19460548 DOI: 10.1016/j.transproceed.2009.03.055]
- 54 **Jiménez C,** Rodríguez D, Marqués E, Loinaz C, Alonso O, Hernández-Vallejo G, Marín L, Rodríguez F, García I, Moreno E. De novo tumors after orthotopic liver transplantation. *Transplant Proc* 2002; **34**: 297-298 [PMID: 11959293 DOI: 10.1016/S0041-1345(01)02770-1]
- 55 **Mashberg A,** Boffetta P, Winkelmann R, Garfinkel L. Tobacco smoking, alcohol drinking, and cancer of the oral cavity and oropharynx among U.S. veterans. *Cancer* 1993; **72**: 1369-1375 [PMID: 8339227 DOI: 10.1002/1097-0142(19930815)72]
- 56 **Leithead JA,** Ferguson JW, Hayes PC. Smoking-related morbidity and mortality following liver transplantation. *Liver Transpl* 2008; **14**: 1159-1164 [PMID: 18668649 DOI: 10.1002/lt.21471]
- 57 **Castelli E,** Hrelia P, Maffei F, Fimognari C, Foschi FG, Caputo F, Cantelli-Forti G, Stefanini GF, Gasbarrini G. Indicators of genetic damage in alcoholics: reversibility after alcohol abstinence. *Hepato-gastroenterology* 1999; **46**: 1664-1668 [PMID: 10430317]
- 58 **Castellsagué X,** Muñoz N, De Stefani E, Vitoria CG, Quintana MJ, Castelletto R, Rolón PA. Smoking and drinking cessation and risk of esophageal cancer (Spain). *Cancer Causes Control* 2000; **11**: 813-818 [PMID: 11075870 DOI: 10.1023/A:1008984922453]
- 59 **Danpanich E,** Kasiske BL. Risk factors for cancer in renal transplant recipients. *Transplantation* 1999; **68**: 1859-1864 [PMID: 10628765 DOI: 10.1097/00007890-199912270-00008]
- 60 **Webster AC,** Craig JC, Simpson JM, Jones MP, Chapman JR. Identifying high risk groups and quantifying absolute risk of cancer after kidney transplantation: a cohort study of 15,183 recipients. *Am J Transplant* 2007; **7**: 2140-2151 [PMID: 17640312 DOI: 10.1111/j.1600-6143.2007.01908.x]
- 61 **Frezza EE,** Fung JJ, van Thiel DH. Non-lymphoid cancer after liver transplantation. *Hepato-gastroenterology* 1997; **44**: 1172-1181 [PMID: 9261620]
- 62 **Bouwes Bavinck JN,** Claas FH, Hardie DR, Green A, Vermeer BJ, Hardie IR. Relation between HLA antigens and skin cancer in renal transplant recipients in Queensland, Australia. *J Invest Dermatol* 1997; **108**: 708-711 [PMID: 9129219 DOI: 10.1111/1523-1747.ep12292086]
- 63 **Hofbauer GF,** Bouwes Bavinck JN, Euvrard S. Organ transplantation and skin cancer: basic problems and new perspectives. *Exp Dermatol* 2010; **19**: 473-482 [PMID: 20482618 DOI: 10.1111/j.1600-0625.20]
- 64 **Braakhuis BJ,** Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res* 2003; **63**: 1727-1730 [PMID: 12702551]
- 65 **Jonason AS,** Kunala S, Price GJ, Restifo RJ, Spinelli HM, Persing JA, Leffell DJ, Tarone RE, Brash DE. Frequent clones of p53-mutated keratinocytes in normal human skin. *Proc Natl Acad Sci USA* 1996; **93**: 14025-14029 [PMID: 8943054]
- 66 **Herrero JI,** España A, Quiroga J, Sangro B, Pardo F, Álvarez-Cienfuegos J, Prieto J. Nonmelanoma skin cancer after liver transplantation. Study of risk factors. *Liver Transpl* 2005; **11**: 1100-1106 [PMID: 16123952 DOI: 10.1002/lt.20525]
- 67 **Park HW,** Hwang S, Ahn CS, Kim KH, Moon DB, Ha TY, Song GW, Jung DH, Park GC, Namgoong JM, Yoon SY, Park CS, Park YH, Lee HJ, Lee SG. De novo malignancies after liver transplantation: incidence comparison with the Korean cancer registry. *Transplant Proc* 2012; **44**: 802-805 [PMID: 22483500 DOI: 10.1016/j.transproceed.2012.01.027]
- 68 **Sanaei AK,** Aliakbarian M, Kazemi K, Nikeghbalian S, Shamsa-eefar A, Mehdi SH, Bahreini A, Dehghani SM, Geramizadeh B, Malekhosseini SA. De novo malignancy after liver transplant. *Exp Clin Transplant* 2015; **13**: 163-166 [PMID: 24844266 DOI: 10.6002/ect.2013.0135]
- 69 **Yu S,** Gao F, Yu J, Yan S, Wu J, Zhang M, Wang W, Zheng S. De novo cancers following liver transplantation: a single center experience in China. *PLoS One* 2014; **9**: e85651 [PMID: 24475047 DOI: 10.1371/journal.pone.0085651]
- 70 **Euvrard S,** Kanitakis J, Claudy A. Skin cancers after organ transplantation. *N Engl J Med* 2003; **348**: 1681-1691 [PMID: 12711744 DOI: 10.1056/NEJMra022137]
- 71 **Otley CC.** Organization of a specialty clinic to optimize the care of organ transplant recipients at risk for skin cancer. *Dermatol Surg* 2000; **26**: 709-712 [PMID: 10886290 DOI: 10.1046/j.1524-4725.2000.00091.x]
- 72 **Euvrard S,** Ulrich C, Lefrançois N. Immunosuppressants and skin cancer in transplant patients: focus on rapamycin. *Dermatol Surg* 2004; **30**: 628-633 [PMID: 15061847 DOI: 10.1111/j.1524-4725.2004.30148.x]
- 73 **Guba M,** Graeb C, Jauch KW, Geissler EK. Pro- and anti-cancer effects of immunosuppressive agents used in organ transplantation. *Transplantation* 2004; **77**: 1777-1782 [PMID: 15223891 DOI: 10.1097/01.TP.0000120181.89206.54]

- 74 **Hojo M**, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, Shimbo T, Suthanthiran M. Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature* 1999; **397**: 530-534 [PMID: 10028970 DOI: 10.1038/17401]
- 75 **de Graaf YG**, Rebel H, Elghalbzouri A, Cramers P, Nellen RG, Willemze R, Bouwes Bavinck JN, de Gruijl FR. More epidermal p53 patches adjacent to skin carcinomas in renal transplant recipients than in immunocompetent patients: the role of azathioprine. *Exp Dermatol* 2008; **17**: 349-355 [PMID: 17979968 DOI: 10.1111/j.1600-0625.2007.00651.x]
- 76 **O'Donovan P**, Perrett CM, Zhang X, Montaner B, Xu YZ, Harwood CA, McGregor JM, Walker SL, Hanaoka F, Karran P. Azathioprine and UVA light generate mutagenic oxidative DNA damage. *Science* 2005; **309**: 1871-1874 [PMID: 16166520 DOI: 10.1126/science.1114233]
- 77 **Duncan FJ**, Wulff BC, Tober KL, Ferketich AK, Martin J, Thomas-Ahner JM, Allen SD, Kusewitt DF, Oberyzy TM, Vanbuskirk AM. Clinically relevant immunosuppressants influence UVB-induced tumor size through effects on inflammation and angiogenesis. *Am J Transplant* 2007; **7**: 2693-2703 [PMID: 17941958 DOI: 10.1111/j.1600-6143.2007.02004.x]
- 78 **Tessari G**, Naldi L, Boschiero L, Nacchia F, Fior F, Forni A, Rugiu C, Faggian G, Sassi F, Gotti E, Fiocchi R, Talamini G, Girolomoni G. Incidence and clinical predictors of a subsequent nonmelanoma skin cancer in solid organ transplant recipients with a first nonmelanoma skin cancer: a multicenter cohort study. *Arch Dermatol* 2010; **146**: 294-299 [PMID: 20231501 DOI: 10.1001/archdermatol.2009.377]
- 79 **Euvrard S**, Morelon E, Rostaing L, Goffin E, Brocard A, Tromme I, Broeders N, del Marmol V, Chatelet V, Domp Martin A, Kessler M, Serra AL, Hofbauer GF, Pouteil-Noble C, Campistol JM, Kanitakis J, Roux AS, Decullier E, Dantal J. Sirolimus and secondary skin-cancer prevention in kidney transplantation. *N Engl J Med* 2012; **367**: 329-339 [PMID: 22830463 DOI: 10.1056/NEJMoa1204166]
- 80 **Serraino D**, Piselli P, Scognamiglio P. Viral infections and cancer: epidemiological aspects. *J Biol Regul Homeost Agents* 2001; **15**: 224-228 [PMID: 11693428]
- 81 **Dukers NH**, Rezza G. Human herpesvirus 8 epidemiology: what we do and do not know. *AIDS* 2003; **17**: 1717-1730 [PMID: 12891058 DOI: 10.1097/01.aids.0000076337.42412.86]
- 82 **Kremers WK**, Devarbhavi HC, Wiesner RH, Krom RAF, Macon WR, Habermann TM. Post-Transplant Lymphoproliferative Disorders Following Liver Transplantation: Incidence, Risk Factors and Survival. *Am J Transplantat* 2006; **6** (5p1): 1017-1024 [DOI: 10.1111/j.1600-6143.2006.01294.x]
- 83 **LaCasce AS**. Post-transplant lymphoproliferative disorders. *Oncologist* 2006; **11**: 674-680 [PMID: 16794246 DOI: 10.1634/theoncologist.11-6-674]
- 84 **Taylor AL**, Marcus R, Bradley JA. Post-transplant lymphoproliferative disorders (PTLD) after solid organ transplantation. *Crit Rev Oncol Hematol* 2005; **56**: 155-167 [PMID: 15979320]
- 85 **Knight JS**, Tsodikov A, Cibrik DM, Ross CW, Kaminski MS, Blayney DW. Lymphoma after solid organ transplantation: risk, response to therapy, and survival at a transplantation center. *J Clin Oncol* 2009; **27**: 3354-3362 [PMID: 19451438 DOI: 10.1200/jco.2008.20.0857]
- 86 **Walker RC**, Marshall WF, Strickler JG, Wiesner RH, Velosa JA, Habermann TM, McGregor CG, Paya CV. Pretransplantation assessment of the risk of lymphoproliferative disorder. *Clin Infect Dis* 1995; **20**: 1346-1353 [PMID: 7620022 DOI: 10.1093/clinids/20.5.1346]
- 87 **Newell KA**, Alonso EM, Whittington PF, Bruce DS, Millis JM, Piper JB, Woodle ES, Kelly SM, Koeppen H, Hart J, Rubin CM, Thistlethwaite JR. Posttransplant lymphoproliferative disease in pediatric liver transplantation. Interplay between primary Epstein-Barr virus infection and immunosuppression. *Transplantation* 1996; **62**: 370-375 [PMID: 8779685 DOI: 10.1097/00007890-199608150-00012]
- 88 **Saha A**, Robertson ES. Epstein-Barr virus-associated B-cell lymphomas: pathogenesis and clinical outcomes. *Clin Cancer Res* 2011; **17**: 3056-3063 [PMID: 21372216 DOI: 10.1158/1078-0432.ccr-10-2578]
- 89 **Collins MH**, Montone KT, Leahey AM, Hodinka RL, Salhany KE, Belchis DA, Tomaszewski JE. Autopsy pathology of pediatric posttransplant lymphoproliferative disorder. *Pediatrics* 2001; **107**: E89 [PMID: 11389287 DOI: 10.1542/peds.107.6.e89]
- 90 **Niederman JC**, Evans AS, Subrahmanyam L, McCollum RW. Prevalence, incidence and persistence of EB virus antibody in young adults. *N Engl J Med* 1970; **282**: 361-365 [PMID: 4312365 DOI: 10.1056/nejm197002122820704]
- 91 **Dotti G**, Fiocchi R, Motta T, Gamba A, Gotti E, Gridelli B, Borleri G, Manzoni C, Viero P, Remuzzi G, Barbui T, Rambaldi A. Epstein-Barr virus-negative lymphoproliferative disorders in long-term survivors after heart, kidney, and liver transplant. *Transplantation* 2000; **69**: 827-833 [PMID: 10755535 DOI: 10.1097/00007890-200003150-00027]
- 92 **Duvoux C**, Pageaux GP, Vanlemmens C, Roudot-Thoraval F, Vincens-Rolland AL, Hézode C, Gaulard P, Miguet JP, Larrey D, Dhumeaux D, Cherqui D. Risk factors for lymphoproliferative disorders after liver transplantation in adults: an analysis of 480 patients. *Transplantation* 2002; **74**: 1103-1109 [PMID: 12438954 DOI: 10.1097/00007890-200210270-00008]
- 93 **Hézode C**, Duvoux C, Germanidis G, Roudot-Thoraval F, Vincens AL, Gaulard P, Cherqui D, Pawlotsky JM, Dhumeaux D. Role of hepatitis C virus in lymphoproliferative disorders after liver transplantation. *Hepatology* 1999; **30**: 775-778 [PMID: 10462385 DOI: 10.1002/hep.510300314]
- 94 **Fernández MC**, Bes D, De Dávila M, López S, Cambaceres C, Dip M, Inventarza O. Post-transplant lymphoproliferative disorder after pediatric liver transplantation: characteristics and outcome. *Pediatr Transplant* 2009; **13**: 307-310 [PMID: 18346039 DOI: 10.1111/j.1399-3046.2008.00914.x]
- 95 **Leblond V**, Dhedin N, Mamzer Bruneel MF, Choquet S, Hermine O, Porcher R, Nguyen Quoc S, Davi F, Charlotte F, Dorent R, Barrou B, Vernant JP, Raphael M, Levy V. Identification of prognostic factors in 61 patients with posttransplantation lymphoproliferative disorders. *J Clin Oncol* 2001; **19**: 772-778 [PMID: 11157030]
- 96 **Maecker B**, Jack T, Zimmermann M, Abdul-Khalik H, Burdelski M, Fuchs A, Hoyer P, Koepf S, Kraemer U, Laube GF, Müller-Wiefel DE, Netz H, Pohl M, Toenshoff B, Wagner HJ, Wallot M, Welte K, Melter M, Offner G, Klein C. CNS or bone marrow involvement as risk factors for poor survival in post-transplantation lymphoproliferative disorders in children after solid organ transplantation. *J Clin Oncol* 2007; **25**: 4902-4908 [PMID: 17971586 DOI: 10.1200/jco.2006]
- 97 **Evens AM**, David KA, Helenowski I, Nelson B, Kaufman D, Kircher SM, Gimelfarb A, Hattersley E, Mauro LA, Jovanovic B, Chadburn A, Stiff P, Winter JN, Mehta J, Van Besien K, Gregory S, Gordon LI, Shammo JM, Smith SE, Smith SM. Multicenter analysis of 80 solid organ transplantation recipients with post-transplantation lymphoproliferative disease: outcomes and prognostic factors in the modern era. *J Clin Oncol* 2010; **28**: 1038-1046 [PMID: 20085936 DOI: 10.1200/jco.2009.25.4961]
- 98 **Richendollar BG**, Tsao RE, Elson P, Jin T, Steinle R, Pohlman B, Hsi ED. Predictors of outcome in post-transplant lymphoproliferative disorder: an evaluation of tumor infiltrating lymphocytes in the context of clinical factors. *Leuk Lymphoma* 2009; **50**: 2005-2012 [PMID: 19860626 DOI: 10.3109/10428190903315713]
- 99 **Zimmermann H**, Oschlies I, Fink S, Pott C, Neumayer HH, Lehmkuhl H, Hauser IA, Dreyling M, Kneba M, Gärtner B, Anagnostopoulos I, Riess H, Klapper W, Trappe RU. Plasmablastic posttransplant lymphoma: cytogenetic aberrations and lack of Epstein-Barr virus association linked with poor outcome in the prospective German Posttransplant Lymphoproliferative Disorder Registry. *Transplantation* 2012; **93**: 543-550 [PMID: 22234349 DOI: 10.1097/TP.0b013e318242162d]
- 100 **Liu Q**, Yan L, Xu C, Gu A, Zhao P, Jiang ZY. Increased incidence of head and neck cancer in liver transplant recipients: a meta-analysis. *BMC Cancer* 2014; **14**: 776 [PMID: 25338638 DOI: 10.1186/s12957-014-0776-7]



- 10.1186/1471-2407-14-776]
- 101 **Chak E**, Saab S. Risk factors and incidence of de novo malignancy in liver transplant recipients: a systematic review. *Liver Int* 2010; **30**: 1247-1258 [PMID: 20602682 DOI: 10.1111/j.1478-3231.20]
- 102 **Herrero JI**, Pardo F, D'Avola D, Alegre F, Rotellar F, Iñarrairaegui M, Martí P, Sangro B, Quiroga J. Risk factors of lung, head and neck, esophageal, and kidney and urinary tract carcinomas after liver transplantation: the effect of smoking withdrawal. *Liver Transpl* 2011; **17**: 402-408 [PMID: 21445923 DOI: 10.1002/lt.22247]
- 103 **Vallejo GH**, Romero CJ, de Vicente JC. Incidence and risk factors for cancer after liver transplantation. *Crit Rev Oncol Hematol* 2005; **56**: 87-99 [PMID: 15979889 DOI: 10.1016/j.critrevonc.2004.12.011]
- 104 **Bleday R**, Lee E, Jessurun J, Heine J, Wong WD. Increased risk of early colorectal neoplasms after hepatic transplant in patients with inflammatory bowel disease. *Dis Colon Rectum* 1993; **36**: 908-912 [PMID: 8404380 DOI: 10.1007/BF02050624]
- 105 **Fabia R**, Levy MF, Testa G, Obiekwe S, Goldstein RM, Husberg BS, Gonwa TA, Klintmalm GB. Colon carcinoma in patients undergoing liver transplantation. *Am J Surg* 1998; **176**: 265-269 [PMID: 9776156 DOI: 10.1016/S0002-9610(98)00141-X]
- 106 **Vera A**, Gunson BK, Ussatoff V, Nightingale P, Candinas D, Radley S, Mayer A, Buckels JA, McMaster P, Neuberger J, Mirza DF. Colorectal cancer in patients with inflammatory bowel disease after liver transplantation for primary sclerosing cholangitis. *Transplantation* 2003; **75**: 1983-1988 [PMID: 12829898 DOI: 10.1097/01.tp.0000058744.34965.38]
- 107 **Buell JF**, Papaconstantinou HT, Skalow B, Hanaway MJ, Alloway RR, Woodle ES. De novo colorectal cancer: five-year survival is markedly lower in transplant recipients compared with the general population. *Transplant Proc* 2005; **37**: 960-961 [PMID: 15848590 DOI: 10.1016/j.transproceed.2004.12.122]
- 108 **Johnson EE**, Levenson GE, Pirsch JD, Heise CP. A 30-year analysis of colorectal adenocarcinoma in transplant recipients and proposal for altered screening. *J Gastrointest Surg* 2007; **11**: 272-279 [PMID: 17458597 DOI: 10.1007/s11605-007-0084-4]
- 109 **Miao Y**, Everly JJ, Gross TG, Tevar AD, First MR, Alloway RR, Woodle ES. De novo cancers arising in organ transplant recipients are associated with adverse outcomes compared with the general population. *Transplantation* 2009; **87**: 1347-1359 [PMID: 19424035 DOI: 10.1097/TP.0b013e3181a238f6]
- 110 **Herrero JI**, Alegre F, Quiroga J, Pardo F, Iñarrairaegui M, Sangro B, Rotellar F, Montiel C, Prieto J. Usefulness of a program of neoplasia surveillance in liver transplantation. A preliminary report. *Clin Transplant* 2009; **23**: 532-536 [PMID: 19681977 DOI: 10.1111/j.1399-0012.2008.00927.x]
- 111 **Herrero JI**, Bastarrika G, D'Avola D, Montes U, Pueyo J, Iñarrairaegui M, Pardo F, Quiroga J, Zulueta J. Lung cancer screening with low-radiation dose computed tomography after liver transplantation. *Ann Transplant* 2013; **18**: 587-592 [PMID: 24165787 DOI: 10.12659/aot.884021]
- 112 **Farge D**, Lebbé C, Marjanovic Z, Tuppin P, Mouquet C, Peraldi MN, Lang P, Hiesse C, Antoine C, Legendre C, Bedrossian J, Gagnadoux MF, Loirat C, Pellet C, Sheldon J, Golmard JL, Agbalika F, Schulz TF. Human herpes virus-8 and other risk factors for Kaposi's sarcoma in kidney transplant recipients. Groupe Cooperatif de Transplantation d'Ile de France (GCIF). *Transplantation* 1999; **67**: 1236-1242 [PMID: 10342315 DOI: 10.1097/00007890-199905150-00007]
- 113 **Ulrich C**, Jürgensen JS, Degen A, Hackethal M, Ulrich M, Patel MJ, Eberle J, Terhorst D, Sterry W, Stockfleth E. Prevention of non-melanoma skin cancer in organ transplant patients by regular use of a sunscreen: a 24 months, prospective, case-control study. *Br J Dermatol* 2009; **161** Suppl 3: 78-84 [PMID: 19775361 DOI: 10.1111/j.1365-2133.2009.09453.x]
- 114 **Aguilera J**, de Gálvez MV, Sánchez-Roldán C, Herrera-Ceballos E. New advances in protection against solar ultraviolet radiation in textiles for summer clothing. *Photochem Photobiol* 2014; **90**: 1199-1206 [PMID: 24861801 DOI: 10.1111/php.12292]
- 115 **Schnitzbauer AA**, Zuelke C, Graeb C, Rochon J, Bilbao I, Burra P, de Jong KP, Duvoux C, Kneteman NM, Adam R, Bechstein WO, Becker T, Beckebaum S, Chazouillères O, Cillo U, Colledan M, Fändrich F, Gugenheim J, Hauss JP, Heise M, Hidalgo E, Jamieson N, Königsrainer A, Lamby PE, Lerut JP, Mäkitalo H, Margreiter R, Mazzaferro V, Mutzbauer I, Otto G, Pageaux GP, Pinna AD, Pirenne J, Rizell M, Rossi G, Rostaing L, Roy A, Turrión VS, Schmidt J, Troisi RI, van Hoek B, Valente U, Wolf P, Wolters H, Mirza DF, Scholz T, Steininger R, Soderdahl G, Strasser SI, Jauch KW, Neuhaus P, Schlitt HJ, Geissler EK. A prospective randomised, open-labeled, trial comparing sirolimus-containing versus mTOR-inhibitor-free immunosuppression in patients undergoing liver transplantation for hepatocellular carcinoma. *BMC Cancer* 2010; **10**: 190 [PMID: 20459775 DOI: 10.1186/1471-2407-10-190]
- 116 **Galve ML**, Cuervas-Mons V, Figueras J, Herrero I, Mata M, Clemente G, Prieto M, Margarit C, Bernardos A, Casafont F. Incidence and outcome of de novo malignancies after liver transplantation. *Transplant Proc* 1999; **31**: 1275-1277 [PMID: 10083569 DOI: 10.1016/S0041-1345(98)01994-0]
- 117 **Antinucci F**, Anders M, Orozco F, Mella J, Cobos M, McCormack L, Mastai R. [De novo malignant tumors following liver transplantation. A single-center experience in Argentina]. *Medicina (B Aires)* 2015; **75**: 18-22 [PMID: 25637895]
- 118 **Na R**, Grulich AE, Meagher NS, McCaughan GW, Keogh AM, Vajdic CM. De novo cancer-related death in Australian liver and cardiothoracic transplant recipients. *Am J Transplant* 2013; **13**: 1296-1304 [PMID: 23464511 DOI: 10.1111/ajt.12192]
- 119 **Ducroux E**, Boillot O, Ocampo MA, Decullier E, Roux A, Dumortier J, Kanitakis J, Jullien D, Euvrard S. Skin cancers after liver transplantation: retrospective single-center study on 371 recipients. *Transplantation* 2014; **98**: 335-340 [PMID: 24621534 DOI: 10.1097/TP.0000000000000051]
- 120 **Mithoefer AB**, Supran S, Freeman RB. Risk factors associated with the development of skin cancer after liver transplantation. *Liver Transpl* 2002; **8**: 939-944 [PMID: 12360438 DOI: 10.1053/jlts.2002.35551]

**P- Reviewer:** Dehghani SM, Keller F, Kin T **S- Editor:** Qi Y

**L- Editor:** A **E- Editor:** Liu SQ





## Hepatitis C virus and neurological damage

Shilu Mathew, Muhammed Faheem, Sara M Ibrahim, Waqas Iqbal, Bisma Rauff, Kaneez Fatima, Ishtiaq Qadri

Shilu Mathew, Muhammed Faheem, Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah 21589, Saudi Arabia

Muhammed Faheem, Department of Biosciences, Faculty of Sciences, COMSATS Institute of Information Technology, Islamabad 45550, Pakistan

Sara M Ibrahim, Waqas Iqbal, Department of Biochemistry, King Abdulaziz University, Jeddah 21589, Saudi Arabia

Bisma Rauff, Westmead Millennium Institute for Medical Research, University of Sydney, Sydney, NSW 2006, Australia

Kaneez Fatima, IQ Institute of Infection and Immunity, Lahore 54000, Pakistan

Ishtiaq Qadri, King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Saudi Arabia

**Author contributions:** Mathew S and Faheem M contributed equally to this manuscript; Qadri I conceived and designed the topic; Mathew S, Faheem M, Ibrahim SM, Iqbal W and Rauff B contributed to materials and wrote the paper; Faheem M, Ibrahim SM, Iqbal W, Rauff B, Fatima K and Qadri I contributed to proof reading of the manuscript.

**Supported by** KACST large R and D grant to Ishtiaq Qadri (#162-34).

**Conflict-of-interest statement:** Authors declare no conflict of interests for this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Ishtiaq Qadri, Professor, King Fahd Medical Research Center, King Abdulaziz University, P.O. Box 80200, Jeddah 21589, Saudi Arabia. [ishtiaq80262@yahoo.com](mailto:ishtiaq80262@yahoo.com)

Received: August 27, 2015

Peer-review started: August 31, 2015

First decision: October 8, 2015

Revised: March 19, 2016

Accepted: April 7, 2016

Article in press: April 11, 2016

Published online: April 28, 2016

### Abstract

Chronic hepatitis C virus (HCV) infection exhibits a wide range of extrahepatic complications, affecting various organs in the human body. Numerous HCV patients suffer neurological manifestations, ranging from cognitive impairment to peripheral neuropathy. Overexpression of the host immune response leads to the production of immune complexes, cryoglobulins, as well as auto-antibodies, which is a major pathogenic mechanism responsible for nervous system dysfunction. Alternatively circulating inflammatory cytokines and chemokines and HCV replication in neurons is another factor that severely affects the nervous system. Furthermore, HCV infection causes both sensory and motor peripheral neuropathy in the mixed cryoglobulinemia as well as known as an important risk aspect for stroke. These extrahepatic manifestations are the reason behind underlying hepatic encephalopathy and chronic liver disease. The brain is an apt location for HCV replication, where the HCV virus may directly wield neurotoxicity. Other mechanisms that takes place by chronic HCV infection due the pathogenesis of neuropsychiatric disorders includes derangement of metabolic pathways of infected cells, autoimmune disorders, systemic or cerebral inflammation and alterations in neurotransmitter circuits. HCV and its pathogenic role is suggested by enhancement of psychiatric and neurological symptoms in patients attaining a sustained virologic response followed by treatment with interferon; however, further studies are required to fully assess the impact of HCV infection and its specific antiviral targets associated with neuropsychiatric disorders.

**Key words:** Hepatitis C virus; Neuro disorders; Blood brain barrier; Nervous system; Inflammation

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** There is high prevalence rate of neuropsychiatric ailments with respect to patients infected with chronic hepatitis C virus (HCV). Brain inflammatory disorders, cerebrovascular disease peripheral neuropathy, psychiatric disturbs and cognitive symptoms are the complex clinical signs which occurs when infected by chronic HCV infection. HCV prompts psychiatric and neurological symptoms through numerous pathways with imprecise mechanisms, which includes neurotransmitter and metabolic pathway imbalance, immune-mediated responses and direct brain neurotoxicity inflammation. Awareness of HCV-associated neuropsychiatric disorders and its pathogenic mechanisms is vital to understand the clinical manifestations and to introduce an applicable treatment.

Mathew S, Faheem M, Ibrahim SM, Iqbal W, Rauff B, Fatima K, Qadri I. Hepatitis C virus and neurological damage. *World J Hepatol* 2016; 8(12): 545-556 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i12/545.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i12.545>

## INTRODUCTION

Hepatitis C virus (HCV) with a prevalence rate of 2.8% globally, affects around 185 million people<sup>[1]</sup>. Targeting mainly liver parenchymal cells (hepatocytes), HCV causes severe hepatitis, cirrhosis that could lead to hepatocellular carcinoma if left unattended. It may affect other organs too<sup>[2]</sup>. The association of hepatitis with insulin resistance and diabetes type 2 is well documented. Nevertheless it has also been correlated to various other organs like eye, gut, kidney, thyroid and cardiovascular complications leading to rheumatic diseases, neuropathology, and dermatological complications<sup>[3-8]</sup>. Hence, it could be considered a systemic complication, due to its ability to use cellular machinery for replication regardless of the organ<sup>[9]</sup>. How HCV is correlated with extrahepatic complications is poorly understood. Nevertheless, chronic infections are characterized by hepatic and systemic inflammation through activation of several signaling pathways and through their effect of release of various cytokines and augmented oxidative stress<sup>[10]</sup>. HCV infection could directly or indirectly cause systemic inflammations by inducing immunological response to the disease and metabolic imbalance.

The correlation of HCV disease covers a wide spectrum of clinical manifestations therefore it necessitates the importance of understanding the disease in this context, clinically, to overcome these manifestations in a more robust way. It is reported that chronic HCV infection

has been associated with neurological as well as psychiatric conditions in upto about 50% of the cases<sup>[11]</sup>. Major HCV related neurological ailments comprise of autoimmune disorders, cerebrovascular events, myelitis, encephalopathy, encephalomyelitis, and cognitive impairment; psychiatric disorders include, anxiety depression, and fatigue<sup>[12,13]</sup>. All the aforementioned complications that are manifested by HCV infection, regardless of the severity of disease<sup>[14]</sup>. There seems to be a lack of knowledge about how hepatitis is linked to numerous complications though it's certain that the brain can be a suitable site for viral replication<sup>[15]</sup>. Nevertheless sequence analysis of HCV residing in liver and brain show variability suggesting an evolutionary path that a virus may embark on to be able to infest the central nervous system (CNS)<sup>[14,16]</sup>.

## CNS INVOLVEMENT IN HCV INFECTION

HCV infection has been correlated to numerous neurological disorders ranging from meningeal to encephalic inflammation and leukoencephalitis<sup>[17,18]</sup>.

Clinically a wide range of complications have been associated with HCV infection, ranging from encephalomyelitis to loss of neurons though sphincter impairment, spastic quadriparesis and sensory dominate the clinically know complication<sup>[17]</sup>. Finding the viral genome in brain during postmortem signifies the correlation of disease with neurological pathology. HCV related transverse myelitis and neuronal malfunction has been well documented<sup>[19-22]</sup>.

Severe demyelination in addition to the infiltration of parenchyma and perivascular T cells has been linked to HCV infection by examining spinal cord biopsy. The commencement of the disease is indicated by acute partial transverse and myelopathy transverse myelitis, or else by sensory ataxia or spastic paraplegia. Recurrence and multi-segmental spinal involvement are commonly reported. A patient with no sign of virus but positive for anti-HCV antibodies postulates an immune mediated response leading to neurological complications.

Chronic HCV has also been associated with severe encephalomyelitis<sup>[23,24]</sup>. Magnetic resonance imaging (MRI) reports point toward CNS injuries in the cerebral and cerebellar white matter. Clinically, dysfunctional psychomotors, consciousness, hemianopsia, urinary retention, hemiparesis and other neurological defects are documented. HCV has been proposed to prompt demyelination *via* an immune-mediated response. These findings proposed that in cases with acute disseminated encephalomyelitis the likelihood of HCV infection increases<sup>[11]</sup>.

## NEUROPHYSIOLOGICAL SYMPTOMS

Around 50% HCV infection patients complain of neuropsychiatric symptoms, brain fog, fatigue, and also show quality of life impairment upto some extent, regardless liver disease severity<sup>[25]</sup>. During the onset of the disease

HCV patients report complications like, fatigue, malaise, maintaining attention and forgetfulness. In a study on 37 HCV infected patients without other complications by McAndrews *et al.*<sup>[26]</sup>, verbal learning impairment and lack of attention were observed. A correlation of cognitive impairment and fatigue with HCV infection was observed in half of the patients observed in a study conducted by Weissenborn *et al.*<sup>[27]</sup>, comparing neuropsychological functioning of HCV positive patients with normal liver function; though in another study by Montagnese *et al.*<sup>[28]</sup>, an exceptionally high incidence of fast ( $\beta$ -dominated) electroencephalograms was documented.

## REPLICATION OF HCV IN BRAIN

HCV, though primarily infecting the liver, is frequently associated with CNS abnormalities<sup>[27]</sup>. Neurocognitive defects in chronic HCV infection independent of hepatic encephalopathy is increasingly reported in several studies<sup>[10,26,29]</sup>. It is however unclear if the CNS itself supports the viral replication. A recent study has shown the expression of HCV receptors in the brain microvascular endothelial cells. Interestingly, the microvascular endothelia are the only cells in the neuronal pool to bear the receptors for HCV<sup>[30]</sup>. Microvascular endothelial cells, that form integral components of blood brain barrier (BBB), are thus assumed to play critical role in the transit of HCV into CNS<sup>[30]</sup>.

Quantification of HCV RNA in the brain, liver and plasma have shown a 1000-10000 fold lower load in brain compared to the liver. The HCV RNA was detected in a minimum of one region of the brain of four HCV infected subjects, independent of human immunodeficiency virus (HIV) co-infection status. The viral RNA quantities from the brain and liver - however significantly varied between clinical samples, which may be due to a higher postmortem interval resulting in the degradation of RNA in some sample<sup>[30]</sup>. The E1 and 5' untranslated region sequences of HCV also varies between the liver, brain and plasma, further reinforcing the hypothesis of HCV replication and involvement in the brain<sup>[31-33]</sup>.

Visualizing the hepatocytes expressing HCV antigen is difficult due to the low cellular viral<sup>[34,35]</sup>. Based on the relatively low HCV RNA content in brain to the liver, detection of HCV antigen in the brain is extremely challenging, and existing imaging methodologies are not sensitive enough to detect the cells of CNS that are infected by the virus<sup>[29]</sup>.

Prior studies have shown the presence of HCV RNA in microglia and astrocytes that were also isolated by laser capture microdissection<sup>[36,37]</sup>. Another study has shown that two independently derived brain endothelial cell lines (hCMEC/D3 and HBMEC) facilitate the entry and replication of the virus. Antibodies specific for CD81, SR-BI, and claudin-1 inhibited the infection, demonstrating a common receptor dependent entry pathway for hepatocytes and hepatoma-derived cell lines<sup>[30,38,39]</sup>. All these studies have shown that the viral entry may not

be limited to hepatocytes. mRNA and protein profile database have shown the expression of CD81, SR-BI, and claudin-1 in epithelial and endothelial cells derived from various tissues<sup>[40,41]</sup> strongly suggesting that HCV infection may be supported by extrahepatically<sup>[29]</sup>. Besides, the entry of HCV into the brain endothelial cells, its replication has also been observed. The HCV infected hCMEC/D3 cells release lower level of virus that can potentially infect hepatoma cells, thereby spreading infection which was CD81 dependent.

Studies have also shown that ApoE plays important role in the infection of brain endothelial cell<sup>[42,43]</sup>. This is evident by the neutralization of HCV infection in hCME/D3 cells by ApoE antibodies, while only partially neutralized Huh 7 further, underlining its role in exacerbation of infection in the hCME/D3 cells<sup>[30]</sup>.

The tight junction between endothelial cells forming the BBB restricts the exchange of substances between the blood and CNS. Moreover, the receptor-mediated efflux transport systems further restrict the entry of hydrophilic molecules into the brain<sup>[44]</sup>. The presence of multidrug resistance proteins such as the P glycoprotein in the BBB provide a protective niche for the replication of virus by restricting the access of antiviral drugs in patients treated for HCV infection<sup>[45]</sup>. Studies have also shown the inhibition of HCV replication through antiviral agents targeting NS3 protease and NS5B polymerase enzymes *in vitro*<sup>[30]</sup>.

Disruption of BBB result in enhanced access of pathogens such as the HIV and west Nile virus into the CNS<sup>[46,47]</sup>. Infection of hCMEC/D3 is associated with enhanced HCV RNA and antigen expression as confirmed by TUNEL staining with increased permeability to FD-70 a paracellular permeability marker.

In conclusion, it was observed that the brain microvascular endothelium, expressing the major viral receptors essentially contribute to the CNS infection of HCV. Specific brain endothelial cell lines have been identified to support the entry and replication of HCV in the brain that may be controlled by antibodies specific for HCV receptor-such as interferon and antiviral agents.

Low level release of virus by HCV-infected hCMEC/D3 cells with cytotoxic properties supports a model in which the BBB provides - an ideal extrahepatic environment for infection, implying a direct role of HCV to induce neuropathology<sup>[30]</sup>.

## MECHANISMS CONTRIBUTING TO NEUROLOGICAL DYSFUNCTION

HCV could lead to various CNS complications ranging from cerebrovascular events to autoimmune syndromes. Acute cerebrovascular events which includes transient ischemic attacks, ischemic stroke and lacunar syndromes have been reported in patients suffering with HCV<sup>[13,48,49]</sup>. Occurrence of occlusive vasculopathy as well the vasculitis is also the well-known events<sup>[50,51]</sup>. Isolated CNS vasculitis could lead to the narrowing of

cerebral arteries<sup>[52]</sup>. In few of the patients, the CNS ischemic changes might be possible in the setting of an anti-phospholipid associated syndrome or it might be associated with the anti-neutrophil cytoplasmic antibodies<sup>[53]</sup>. A recent study has shown the HCV-metabolic syndrome association with an evidence that HCV infection is a great risk factor for an enhanced thickness in the carotid wall and plaque formation, thus is a major contributory factor of cerebrovascular mortality specifically in the patients who have higher levels of HCV-RNA<sup>[54]</sup>.

Encephalopathic syndromes that have been clinically characterized by confusion, altered consciousness, cognitive impairment, dysphagia, and dysarthria are linked with the diffuse involvement of white matter in HCV patients with cryoglobulins and/or circulating anticardiolipin antibodies. The patients suffering with these syndromes have also shown small lesions in the sub-cortical regions and periventricular white matter. Additionally, alterations in severe and diffuse infra and supratentorial white matter that could cause vasculitis have been observed in patients with coincidental systemic vasculitis. Another study has shown that a CNS vasculitis-induced ischemic damage in a patient that also suffering with mixed cryoglobulins (MC), peripheral neuropathy, and relapsing multiinfarct encephalopathy<sup>[55]</sup>. The neuropathological examination of this patient has shown multiple ischemic lesions (0.5-3 mm in diameter) in the white matter of cerebral hemispheres, cerebellum, parenchymal infiltration, and an accumulation of the lymphocytes around small vessels. Further study has also shown the incidence of vasculitis-induced ischemic changes in a patient that was suffering with chronic HCV, MC, and sensory neuropathy<sup>[56]</sup>.

Besides the encephalopathic syndromes, cognitive decline that has been clinically characterized by an impaired attention, visual constructive, and spatial functions have been associated with an enhanced occurrence of periventricular white matter high intensity signals (WMHISs) on T2-weighted MRI<sup>[56]</sup>. The patients have shown a relationship between CG level and number of impaired cognitive functions whereas no correlation was observed with systemic manifestations of CG, including peripheral neuropathy. A variation in the WMHIS has shown vessel disease that could lead to chronic hypo-perfusion of white matter and local alterations of blood-brain barrier<sup>[57]</sup>. Spectrum of CNS syndromes encountered in HCV patients is not restricted to the foregoing vasculitic and vasculopathic forms but also causes inflammatory disorders such as an acute encephalitis, meningoradiculitis and encephalomyelitis. Studies have also shown the patients suffering with leuko-encephalitis and perivascular T-cell infiltration in association with HCV genome<sup>[18]</sup> or fatal progressive encephalomyelitic syndromes<sup>[17]</sup>. Another study has shown a patient suffering with an acute disseminated encephalomyelitis, an autoimmune post-infectious CNS disease that has been developed after HCV-infection

which supports the role of cellular immune-mediated mechanisms in CNS complications of HCV infection<sup>[24]</sup>.

Most of the patients with chronic HCV-infection complain of fatigue, poor memory and impaired concentration. Fatigue, mood alterations and cognitive dysfunction has shown a disturbed social and physical activity of the patients. Few of the HCV patients with severe fatigue also complain of sleep disturbances, restless leg syndrome, muscle and joint pain, and depression. A recent study of 53 HCV-positive patients with neuropsychiatric has shown an increase choline and myo-inositol concentrations in the basal ganglia and white matter, and an increase in the concentration of creatinine, N-acetyl-aspartate (NAA), and N-acetyl-aspartyl-glutamate in basal ganglia<sup>[58]</sup>, these findings are consistent with the HCV-induced chronic cellular inflammation. Another study revealed an increased ratio of choline/creatine (Cho/Cr) in the basal ganglia as well as the frontal white matter of HCV infected patients through magnetic resonance spectroscopy<sup>[59]</sup>. Further findings have shown<sup>[60]</sup> a lower level of NAA/Cr ratio in the frontal grey matter of HCV-patients without any change in the Cho/Cr ratio. Both findings have suggested the occurrence of an increased cell membrane turnover and reduced neuronal function<sup>[27]</sup>. Use of ondansetron which is a competitive antagonist of serotonin receptors has ameliorated fatigue in HCV infected patient. Also, the placebo controlled randomized study of thirty six HCV infected patients have shown an improved fatigue and depression scores with ondansetron<sup>[61]</sup>. These studies have shown an important role of serotonergic pathway dysfunction that causes fatigue, reduced level of serum tryptophan, and a reduced synthesis of serotonin<sup>[62,63]</sup>. Moreover, findings of fifteen HCV infected patients reporting neuropsychiatric symptoms was carried out through different neuropsychological tests including 18F-fluoro-desoxy-glucose, serotonin, and positron emission tomography. The results have shown significant decrease in striatal and midbrain dopamine availability and decrease metabolism in limbic, parietal, frontal, and temporal cortices. These findings further confirmed significant role for defective dopaminergic transmission in causing cognitive impairment in the HCV<sup>[64]</sup>. The HCV infection has also been linked with myopathy and a few cases of non-inflammatory and inflammatory myopathies were reported. The clinical features of HCV associated myopathies ranging from progressive weakness to relapsing forms, mild increase in muscle enzymes, and moderate weakness. In non-inflammatory myopathies, pathological features include vacuolar changes<sup>[65]</sup> and necrotizing myopathy<sup>[66]</sup> with slow or progressive proximal weakness, and selective atrophy of type 2 fibers in relapsing myopathy. Study has showed the oxidative mitochondrial damage in a patient with severe ptosis, generalized weakness, diplopia and respiratory involvement, and ultra-structural changes of mitochondrial shape, and cristae<sup>[67]</sup>. Additional findings have showed that HCV promotes tumor necrosis factor -



mediated apoptosis in myocytes<sup>[68]</sup>.

## CRYOGLOBULINEMIA

Cryoglobulins are immunoglobulins in nature. They are able to clump together at temperatures below 37 °C<sup>[69,70]</sup> causing organ damage chiefly through two main pathways, vascular sludging (Hyperviscosity syndrome, associated with type I cryoglobulinaemia) or immune-mediated (Vasculitis, associated with mixed cryoglobulinemia)<sup>[70]</sup>. Causes of cryoglobulinaemia range from, infections, autoimmune disorders and malignancy though the main culprit is HCV<sup>[70]</sup>. Our understanding of cryoglobulinaemia advocate successive antiviral therapy in conjunction with targeted therapy rather than following a monotherapeutic<sup>[71,72]</sup>.

While our understanding of the disease progression still has loop holes, chronic immune stimulation/lymph proliferation due to increased cryoglobulins production, formation of complex by cryoglobulins or their antigens and their inadequate clearance are considered to be three main causative factors of cryoglobulinemia.

## TYPES OF CRYOGLOBULINEMIA

Type I cryoglobulinemia is characterized by monoclonal globulins produced during lymphoproliferative disease. These Igs precipitate during exposure to cold, leading to inflammatory vasculitis and vessel obstruction.

Types II and III cryoglobulinemia is associated with increased production of cryoglobulins by proliferative B-cells clones<sup>[72,73]</sup>. Chronic HCV infection may trigger hyperactivation of B-cells causing infection *via* CD81, a cell surface protein<sup>[74,75]</sup>.

## HCV IN CRYOGLOBULINEMIA

Detection of HCV in 1989<sup>[76]</sup> significantly changed the course of scientific research from fundamental to HCV-oriented cryoglobulinaemia<sup>[77]</sup>. Ferri *et al*<sup>[78]</sup> detected circulating HCV-RNA in almost 90% of subjects with mixed cryoglobulinemia, though subsequent analyses by other groups discovered wider ecological. HBV is found to be associated with mixed cryoglobulinemia whereas HCV is primarily correlated with type II cryoglobulinaemia<sup>[79]</sup>. HIV infected patients have low percentage (7%-17%) of cryoglobulinemia but rises to almost 65% in patients coinfecting with HCV<sup>[78]</sup> that can be reduced by anti-retroviral therapies<sup>[79]</sup>. Apart from viral infections the disease has also been associated with a wide range of other infectious.

HCV infection is an important model to understand the mechanisms that lie behind cryoglobulinaemic etiology. HCV lympho-tropism is the first step in B-cell hyper proliferation, regardless of cryoglobulinaemia<sup>[78,80]</sup>. E2 an HCV envelope protein interacts with the major extracellular loop of tetraspanin CD81, a signaling protein expressed by hepatocytes, B and T lymphocytes<sup>[74]</sup>. This interaction purportedly triggers prolonged

B-cell stimulation<sup>[81]</sup>. B-cell clones are found in peripheral blood, bone marrow, and liver HCV patients, predominantly those with type II cryoglobulinaemia. These B-cell clones produce monoclonal IgM with an idiotype, WA that works as a cross-linker, this binds to immunoglobulins directed towards HCV core protein. Precipitates from HCV-related cryoglobulinaemia patients comprise of HCV core proteins and RNA, this means the immune system forms cryoglobulins during chronic HCV infection<sup>[82]</sup>.

## PERIPHERAL NEUROPATHY

Peripheral neuropathy (PN) is a complication secondary to large number of common diseases such as diabetes, thyroid disorders, renal failure, vitamin deficiency and treatments, including viral infection. Degradation of sensory or motor axons commonly occurs which disrupts the effective communication between the central and peripheral nervous system<sup>[83]</sup>. Clinically, PN manifests itself as motor impairment largely resulting in weakness, sensory defects such as numbness, paresthesia, hyperalgesia/allodynia and pain or more severe autonomic dysfunction leading to organ failures. Patients may present with multiple symptoms of varying severity, making it highly heterogeneous, which in turn depends on the underlying trigger<sup>[84]</sup>. Degeneration of axon, vascular occlusion and inflammation<sup>[84]</sup> with perivascular trafficking of mononuclear cells are essential pathologic features of the debilitating condition<sup>[85]</sup>. Demyelization and absence of axonal fascicular differentiation is also reported.

Forty percent to seventy-five percent patients positive for HCV, present with symptomatic PN, being more prominent with HCV associated cryoglobulinemia (CG)<sup>[86]</sup>. Although presence of serum cryoglobulins is a prognostic marker of severe manifestation of PN, symptoms may be reported even in its absence, underlying a direct role of the virus in precipitating the disease<sup>[87]</sup>. Yoon *et al*<sup>[88]</sup> reported 43.5% prevalence of PN in the absence of CG based on clinical and electrophysiological examination of HCV infected patients.

Involvement of peripheral nervous system in HCV infection is variable depending upon age, duration of infection, CG and other comorbidities<sup>[12,88]</sup>. Twenty-six percent to eighty-six percent HCV patients positive for CG present with clinical/electrophysiological PN. Pathogenesis of HCV associated PN is indirect and mostly inflammatory, as the virus itself does not invade the nerve and muscle tissues. Mechanisms proposed to explain the neurologic manifestation of the virus include the vascular deposition of HCV RNA containing CG, direct viral invasion and perivascular inflammation<sup>[89]</sup>.

HCV associated neurologic impairments range from sensory axonopathy to mononeuritis multiplex. Sensory or motor impairment of one or more distal nerves is most frequent, that tends to become symmetric causing loss of sensation and weakness<sup>[90]</sup>. Prevalence of sensory and motor neuropathy with HCV was found to be

9% and 10% respectively<sup>[91]</sup>. Sensory predominant symmetrical polyneuropathy involves perivascular infiltration of lymphocytes and monocytes of small sized vessels. Mononeuritis multiplex, involving one or two non-contiguous nerves, has a more systemic effect and involves inflammation of medium sized vessels with myriad of inflammatory cells accompanied with vascular necrosis that is asymmetric<sup>[92]</sup>. Asymmetrical sensory neuropathies may be large or small fiber. Demyelinating polyneuropathy and polyradiculoneuropathy is less frequently encountered with HCV infection. Pure motor neuropathies and autonomic neuropathies are rare in HCV<sup>[12]</sup>.

Abd El-Kader *et al.*<sup>[93]</sup> estimated the prevalence of PN in patients with HCV related liver disorders based on complete neurologic examination and nerve conduction study<sup>[93]</sup>. Of the 50 subjects included in the study, 22% had sensory abnormalities, 18% had motor impairment while 10% had a combination of both. Furthermore, PN was found unrelated to serum vitamin B12 levels and severity of disease. Distal sensory loss of pain and reflexes may observe on neurologic examination in otherwise asymptomatic patients.

Biasiotta *et al.*<sup>[90]</sup> characterized the clinical and neurological features of HCV related neuropathies. Sixty-eight percent (47 out of 69) of patients were diagnosed with peripheral neuropathy with 45 exhibiting a predominantly sensory, distal symmetric polyneuropathy while 2 showing mononeuropathy multiplex. Thermal pain sensitivity was specifically linked to pure small fiber neuropathy while sensory abnormalities observed in both mononeuropathy multiplex and mixed fiber, distal symmetric polyneuropathy.

PN can be considered a manifestation of HCV induced CG<sup>[86]</sup>. Abnormal immunoglobulin, reversibly precipitating at low temperatures, *i.e.*, 4 degrees, with marked rheumatoid factor activity are produced by B cells, form immune complexes that obstruct vessels and trigger vascular inflammation<sup>[90]</sup>. IgG and IgM are primarily implicated to precipitate HCV associated PN either by their direct binding to myelin inducing an erosive immune attack or as lymphocytic irritant within the vasa nervorum resulting in vasculitis. In one study antibodies against myelin associated glycoprotein were identified to induce the immune trigger<sup>[93]</sup>. Such demyelinating association is rare with an occurrence of 5 per 10000. Anti MAG neuropathy is clinically characterized with sensory ataxia with motor involvement and hand tremor intention involving large nerve fibers<sup>[94]</sup>.

## PSYCHIATRIC DISORDERS IN HCV-INFECTED PATIENTS

HCV like HIV is among the few known infections that cause psychiatric disorders<sup>[95]</sup>. Illicit drug injection (IDU) is a major factor for HCV infection<sup>[96]</sup>. IDU is common among patients who have personality disorders besides alcoholism, illicit sexual behavior and mood

disorders<sup>[97-99]</sup>. Alcohol is predominantly associated with increased prevalence of anti-HCV antibody. Synergy between alcohol and HCV aggravates liver disease and lessens the effectiveness of interferon (IFN) treatment. IFN $\alpha$  is increasingly being used to treat HCV because of its effectiveness though it can induce a variable number of psychiatric disorders like acute confusional state, depressive and agitated manic episode<sup>[100]</sup>. Up to 70% of HCV patients treated with IFN may develop depression<sup>[96]</sup>. This could be attributed to numerous pathophysiological complication that are associated with IFN treatment including distorted monoamine metabolism<sup>[96]</sup>, increase in apoptosis, BDNF reduction, and altered hypothalamus-pituitary-adrenal axis function<sup>[100]</sup>. In one study observing the neurological implication of IFN in conjunction with ribavirin treatment the authors found 1/4 of all the patients developed major depressive disorder (MDD). Higher interleukin 6 concentrations in serum, history of psychiatric condition, depression and low educational level considerably increases the incidence of MDD during antiviral therapy<sup>[101]</sup>. Symptoms of neuro-vegetative depression start to occur early during treatment though cognitive symptoms start in a span of 4 wk of IFN treatment<sup>[96]</sup>. Depression, anxiety, and cognitive impairments can be treated through serotonergic antidepressants, while neuro-vegetative symptoms like loss of appetite, fatigue, sexual impairment, and psychosomatic symptoms are not much responsive to treatment by SSRIs<sup>[102]</sup>. Hence serotonin-norepinephrine reuptake inhibitors, bupropion, methylphenidate or modafinil are used to address neuro-vegetative disorders<sup>[100]</sup>. Confusional state induced by IFN $\alpha$  is associated with psychomotor retardation, disorientation, Parkinsonism, and psychosis in addition to induction of manic disorder. In case of acute mania mood stabilization and antidepressants are administered<sup>[100]</sup>. HCV patients with MDD or bipolar symptoms are more prone to psychiatric disorders as compared to patients with no psychiatric illnesses during treatment with IFN<sup>[96]</sup>. It's not just IFN but HCV itself might be associated with mood disorder. This can be in part linked to factors such as high-risk behavior, stigma and drug abuse. Nevertheless, evidence suggests the association some HCV genotypes such as 3a are related to risk of depression<sup>[103]</sup>. Neuronal invasion by HCV is another factor that could lead to mental distortion<sup>[103-105]</sup>.

## ANTIVIRAL TREATMENT

HCV related CG is clinically challenging. Use of immunosuppressive agents such as glucocorticoids and cytotoxic drugs is not recommended to manage CG induced severe neuropathic pain, because of the ensuing viral infection. Interestingly, antiviral agents considerably improve symptoms, underlining pathogenic role of virus in precipitating the secondary symptoms. Targeting the underlying viral infection is thus a reasonable strategy to treat HCV associated CG symptoms, although the response produced would be slow. Additionally, anti-

ral therapy suppresses B cell proliferation in the bone marrow, thereby controlling CG in more than one way. However, achieving a sustained virologic response is critical for the success of these therapies<sup>[86]</sup>. Immunosuppression prior to induction of antiviral therapy can be considered in patients with severe symptoms to obtain a reasonable and timely therapeutic response. Ideally, a combination of immunosuppressive and an anti-viral agent is highly desirable that can directly act on the proliferating B cells while simultaneously wiping out the etiologic trigger, *i.e.*, the virus<sup>[106]</sup>.

IFN $\alpha$  is a cytokine produced by cells that primarily modulates the immune response during viral infections<sup>[83]</sup>. Interferon in combination with other drugs is an essential component of HCV therapy. Therapeutic utility of IFN lies in their ability to decrease virus replication rate, inhibit lymphocyte proliferation, Ig synthesis with enhanced immune complex competency and macrophage activity<sup>[86]</sup>.

IFN $\alpha$  monotherapy, although active against virus, was associated with heightened autoimmunity<sup>[107]</sup>. Thus, the IFN $\alpha$  itself is assumed to brew the pathogenic inflammatory environment for neuropathy *via* immune mediated myelin degradation and vessel occlusion causing nerve ischemia, in the absence of CG. Despite its increased high autoimmune titer, IFN $\alpha$  forms the core of HCV therapy. Peg IFN $\alpha$ -ribavirin is clinically, virological, immunologically superior to IFN $\alpha$ -ribavirin and is recommended in mild to severe cryovas with HCV. Moreover, it was associated with shorter duration of therapy, less frequent side effects and deaths producing, sustained virologic response in 60% patients<sup>[84]</sup>. Therapeutic success of the combination varies between 48%-88% depending upon the HCV genotype. Reduction of neuropathic pain in HCV positive patients was observed from 65.2% to 22.1% after peg IFN and ribavirin therapy<sup>[108]</sup>. Chronic inflammatory demyelinating polyneuropathy, reported in a minority of HCV infected population, was significantly corrected with IFN $\alpha$  and ribavirin therapy, although a few studies have classified it as a side effect of IFN $\alpha$ <sup>[109]</sup>. In that case, intravenous Ig administration and plasma exchange were effective for management of PN. The efficacy of ribavirin in reducing PN is attributed to its viral clearance, decreasing inflammation, circulating cryoglobulins and anti MAG antibodies<sup>[83]</sup>.

In patients not responding to antiviral therapy, addition of protease inhibitors telaprevir/boceprevir significantly enhanced the clinical outcome. Potency of the triple antiviral therapy was comprehensively described by Saadoun *et al.*<sup>[110]</sup>. In a cohort with genotype 1 HCV and CG. Patients randomly received either telaprevir/boceprevir with peg IFN $\alpha$ /ribavirin for 48 wk and were followed up to 6 mo after treatment. Of the 56.6% patients included in the study with peripheral neuropathy, 47% showed improvement after treatment as clinically assessed by the neuropathy total symptom score. The study further highlighted the clinic considerations for the success of the triple antiviral

therapy.

Successes of these trials have driven the direct use antiviral agents to reduce cryoglobulinemia and related symptoms associated with HCV. The NS3/4A inhibitor simeprevir and the NS5B inhibitor sofosbuvir have recently been approved, for their nearly absolute sustained virologic response 95% with minimum toxicities<sup>[106]</sup>. The magnified therapeutic response is due to their shortened courses of combination IFN free therapy.

Rituximab, a CD 20 monoclonal antibody, directly acts by arresting cryoglobulins production and its subsequent pathogenic cascade<sup>[108]</sup>. Rituximab monotherapy is thus highly relevant in treating cryovas emergencies such as neuropathic pain<sup>[111]</sup>. However its sole efficacy in reducing PN has not been satisfactorily assessed. In one study, 36% patients showed a subsidy in peripheral neuropath with rituximab administration.

Studies have reported higher efficacy and safety of rituximab against the conventional immunosuppressive agent's, *i.e.*, glucocorticoids, azathioprine and cyclophosphamide to treat cryovas. An early clinical remission of cryovas with rituximab therapy was reported in patients with HCV, who did not show improvement with previous antiviral treatment<sup>[111-113]</sup>. Patients with liver cirrhosis, not eligible for antiviral treatment, also showed improvement with rituximab therapy, with enhanced protidosynthetic and ascites activity of the liver.

Rituximab monotherapy effectively alleviated MC symptoms in about 71.4%-84% HCV patients<sup>[111,112]</sup>. Rituximab given in combination peg IFN $\alpha$ -ribavirin was evaluated in patients with severe HCV-cryo, resistant to IFN $\alpha$  combination therapy. Of the 16 patients enrolled, 15 showed marked clinical improvement with 10 complete responders. Efficacy and safety of peg IFN $\alpha$ /ribavirin with and without rituximab was evaluated in two separate studies. Rituximab with the antiviral regimen produced earlier clinical remission<sup>[114-116]</sup>.

Recently, low dose of interleukin 2 (IL2) has emerged as a promising approach based on the presence of defective regulatory T cells in HCV-cryo. CD4<sup>+</sup> CD25<sup>+</sup> Fox P3<sup>+</sup> T cells are assumed to be responsible for disease refractoriness, after the complete resolution of HCV and vasculitis<sup>[117,118]</sup>. These are regulatory cells that control the autoimmune response of the body and their deficiency during viral infection account for the expansion of holistic B cells. Efficacy of low dose IL2 in patients with refractory HCV-cryo was assessed in a prospective open labeled phase I / II a trial wherein IL2 increased the percentage of the regulatory T cells while decreasing the B cells<sup>[119-121]</sup>.

## DISCUSSION

Antiviral treatment should be the first line treatment for managing mild to moderate vascular and neurologic symptoms. Symptoms usually recede with an optimum sustained virologic response. Rituximab therapy should be opted for patients with severe exacerbations of secondary symptoms. Plasmapheresis may be required

before placing patients on the antiviral therapy. Patients on IFN therapy should be monitored as IFN therapy may aggravate the symptoms. Corticosteroids may be used for temporary relief of minor inflammatory pain. Immunosuppressant should be the last resort opted in patients not responding to antiviral treatment/refractory disease.

## CONCLUSION

Age and duration of HCV infection are the major clinical determinants of PN. Furthermore, it was found that duration of HCV infection and not the presence of cryoglobulins, was related to PN. Clinically, Neuropathic pain with HCV should be approached with a multimodal approach, with the prime objective being reducing the viral load, that automatically resolves secondary symptoms. Treatment should be strategized based on the severity of the disease and patients response. Addition of steroids, tricyclic antidepressants, local anesthetics and opioids may be required to the standard antiviral therapy, in case of acute pain attacks. Persistence or relapse of neurologic symptoms despite viral clearance may be indicative of other seeding conditions.

## REFERENCES

- Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]
- Zignego AL, Craxi A. Extrahepatic manifestations of hepatitis C virus infection. *Clin Liver Dis* 2008; **12**: 611-636, ix [PMID: 18625431 DOI: 10.1016/j.cld.2008.03.012]
- Adinolfi LE, Restivo L, Zampino R, Lonardo A, Loria P. Metabolic alterations and chronic hepatitis C: treatment strategies. *Expert Opin Pharmacother* 2011; **12**: 2215-2234 [PMID: 21883025 DOI: 10.1517/14656566.2011.597742]
- Adinolfi LE, Zampino R, Restivo L, Lonardo A, Guerrera B, Marrone A, Nascimbeni F, Florio A, Loria P. Chronic hepatitis C virus infection and atherosclerosis: clinical impact and mechanisms. *World J Gastroenterol* 2014; **20**: 3410-3417 [PMID: 24707124 DOI: 10.3748/wjg.v20.i13.3410]
- Antonelli A, Ferri C, Fallahi P, Ferrari SM, Ghinoi A, Rotondi M, Ferrannini E. Thyroid disorders in chronic hepatitis C virus infection. *Thyroid* 2006; **16**: 563-572 [PMID: 16839258 DOI: 10.1089/thy.2006.16.563]
- Durante-Mangoni E, Iardino P, Resse M, Cesaro G, Sica A, Farzati B, Ruggiero G, Adinolfi LE. Silent celiac disease in chronic hepatitis C: impact of interferon treatment on the disease onset and clinical outcome. *J Clin Gastroenterol* 2004; **38**: 901-905 [PMID: 15492610 DOI: 10.1097/00004836-200411000-00014]
- Johnson RJ, Gretch DR, Yamabe H, Hart J, Bacchi CE, Hartwell P, Couser WG, Corey L, Wener MH, Alpers CE. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. *N Engl J Med* 1993; **328**: 465-470 [PMID: 7678440 DOI: 10.1056/NEJM199302183280703]
- Nagao Y, Sata M, Noguchi S, Seno'o T, Kinoshita M, Kameyama T, Ueno T. Detection of hepatitis C virus RNA in oral lichen planus and oral cancer tissues. *J Oral Pathol Med* 2000; **29**: 259-266 [PMID: 10890556 DOI: 10.1034/j.1600-0714.2000.290604.x]
- Revie D, Salahuddin SZ. Human cell types important for hepatitis C virus replication in vivo and in vitro: old assertions and current evidence. *Viral J* 2011; **8**: 346 [PMID: 21745397 DOI: 10.1186/1743-422X-8-346]
- Zampino R, Marrone A, Restivo L, Guerrera B, Sellitto A, Rinaldi L, Romano C, Adinolfi LE. Chronic HCV infection and inflammation: Clinical impact on hepatic and extra-hepatic manifestations. *World J Hepatol* 2013; **5**: 528-540 [PMID: 24179612 DOI: 10.4254/wjh.v5.i10.528]
- Adinolfi LE, Nevola R, Lus G, Restivo L, Guerrera B, Romano C, Zampino R, Rinaldi L, Sellitto A, Giordano M, Marrone A. Chronic hepatitis C virus infection and neurological and psychiatric disorders: an overview. *World J Gastroenterol* 2015; **21**: 2269-2280 [PMID: 25741133 DOI: 10.3748/wjg.v21.i8.2269]
- Monaco S, Ferrari S, Gajofatto A, Zanusso G, Mariotto S. HCV-related nervous system disorders. *Clin Dev Immunol* 2012; **2012**: 236148 [PMID: 22899946 DOI: 10.1155/2012/236148]
- Origgi L, Vanoli M, Carbone A, Grasso M, Scorza R. Central nervous system involvement in patients with HCV-related cryoglobulinemia. *Am J Med Sci* 1998; **315**: 208-210 [PMID: 9519936 DOI: 10.1016/S0002-9629(15)40308-8]
- Fletcher NF, McKeating JA. Hepatitis C virus and the brain. *J Viral Hepat* 2012; **19**: 301-306 [PMID: 22497808 DOI: 10.1111/j.1365-2893.2012.01591.x]
- Radkowski M, Wilkinson J, Nowicki M, Adair D, Vargas H, Ingui C, Rakela J, Laskus T. Search for hepatitis C virus negative-strand RNA sequences and analysis of viral sequences in the central nervous system: evidence of replication. *J Virol* 2002; **76**: 600-608 [PMID: 11752151 DOI: 10.1128/JVI.76.2.600-608.2002]
- Forton DM, Karayiannis P, Mahmud N, Taylor-Robinson SD, Thomas HC. Identification of unique hepatitis C virus quasispecies in the central nervous system and comparative analysis of internal translational efficiency of brain, liver, and serum variants. *J Virol* 2004; **78**: 5170-5183 [PMID: 15113899 DOI: 10.1128/JVI.78.10.5170-5183.2004]
- Bolay H, Söylemezoğlu F, Nurlu G, Tuncer S, Varli K. PCR detected hepatitis C virus genome in the brain of a case with progressive encephalomyelitis with rigidity. *Clin Neurol Neurosurg* 1996; **98**: 305-308 [PMID: 9081776 DOI: 10.1016/0303-8467(96)00040-6]
- Seifert F, Struffert T, Hildebrandt M, Blümcke I, Brück W, Staykov D, Huttner HB, Hilz MJ, Schwab S, Bardutzky J. In vivo detection of hepatitis C virus (HCV) RNA in the brain in a case of encephalitis: evidence for HCV neuroinvasion. *Eur J Neurol* 2008; **15**: 214-218 [PMID: 18215154 DOI: 10.1111/j.1468-1331.2007.02044.x]
- Aktipi KM, Ravaglia S, Ceroni M, Nemni R, Debiaggi M, Bastianello S, Alfonsi E, Zardini E, Minoli L, Tavazzi E, Marchioni E. Severe recurrent myelitis in patients with hepatitis C virus infection. *Neurology* 2007; **68**: 468-469 [PMID: 17283325]
- De Carli DM, Pannebeker J, Pedro FL, Haygert CJ, Hertz E, Beck Mde O. Transverse myelitis associated to HCV infection. *Braz J Infect Dis* 2009; **13**: 147-152 [PMID: 20140361 DOI: 10.1590/S1413-86702009000200015]
- Grewal AK, Lopes MB, Berg CL, Bennett AK, Alves VA, Trugman JM. Recurrent demyelinating myelitis associated with hepatitis C viral infection. *J Neurol Sci* 2004; **224**: 101-106 [PMID: 15450779 DOI: 10.1016/j.jns.2004.06.013]
- Zandman-Goddard G, Levy Y, Weiss P, Shoenfeld Y, Langevitz P. Transverse myelitis associated with chronic hepatitis C. *Clin Exp Rheumatol* 2003; **21**: 111-113 [PMID: 12673901]
- Sim JE, Lee JB, Cho YN, Suh SH, Kim JK, Lee KY. A case of acute disseminated encephalomyelitis associated with hepatitis C virus infection. *Yonsei Med J* 2012; **53**: 856-858 [PMID: 22665357 DOI: 10.3349/ymj.2012.53.4.856]
- Sacconi S, Salviati L, Merelli E. Acute disseminated encephalomyelitis associated with hepatitis C virus infection. *Arch Neurol* 2001; **58**: 1679-1681 [PMID: 11594929 DOI: 10.1001/archneur.58.10.1679]
- Senzolo M, Schiff S, D'Aloiso CM, Crivellin C, Cholongitas E, Burra P, Montagnese S. Neuropsychological alterations in hepatitis C infection: the role of inflammation. *World J Gastroenterol* 2011; **17**: 3369-3374 [PMID: 21876628 DOI: 10.3748/wjg.v17.i29.3369]
- McAndrews MP, Farcnik K, Carlen P, Damyanovich A, Mrkonjic



- M, Jones S, Heathcote EJ. Prevalence and significance of neurocognitive dysfunction in hepatitis C in the absence of correlated risk factors. *Hepatology* 2005; **41**: 801-808 [PMID: 15793853 DOI: 10.1002/hep.20635]
- 27 **Weissenborn K**, Tryck AB, Heeren M, Worthmann H, Pflugrad H, Berding G, Bokemeyer M, Tillmann HL, Goldbecker A. Hepatitis C virus infection and the brain. *Metab Brain Dis* 2009; **24**: 197-210 [PMID: 19130196 DOI: 10.1007/s11011-008-9130-5]
  - 28 **Montagnese S**, Jackson C, Ennen JC, Krause J, Tillmann HL, Morgan MY, Weissenborn K. Evidence of central nervous system (CNS) involvement in patients with chronic hepatitis C (HCV) infection and minimal liver disease. *Hepatology* 2005; **42**: 429A
  - 29 **Forton DM**, Taylor-Robinson SD, Thomas HC. Central nervous system changes in hepatitis C virus infection. *Eur J Gastroenterol Hepatol* 2006; **18**: 333-338 [PMID: 16538103 DOI: 10.1097/00042737-200604000-00005]
  - 30 **Fletcher NF**, Yang JP, Farquhar MJ, Hu K, Davis C, He Q, Dowd K, Ray SC, Krieger SE, Neyts J, Baumert TF, Balfé P, McKeating JA, Wong-Staal F. Hepatitis C virus infection of neuroepithelioma cell lines. *Gastroenterology* 2010; **139**: 1365-1374 [PMID: 20538002 DOI: 10.1053/j.gastro.2010.06.008]
  - 31 **Fishman SL**, Murray JM, Eng FJ, Walewski JL, Morgello S, Branch AD. Molecular and bioinformatic evidence of hepatitis C virus evolution in brain. *J Infect Dis* 2008; **197**: 597-607 [PMID: 18275278 DOI: 10.1086/526519]
  - 32 **Fletcher NF**, Wilson GK, Murray J, Hu K, Lewis A, Reynolds GM, Stamataki Z, Meredith LW, Rowe IA, Luo G, Lopez-Ramirez MA, Baumert TF, Weksler B, Couraud PO, Kim KS, Romero IA, Jopling C, Morgello S, Balfé P, McKeating JA. Hepatitis C virus infects the endothelial cells of the blood-brain barrier. *Gastroenterology* 2012; **142**: 634-643.e6 [PMID: 22138189 DOI: 10.1053/j.gastro.2011.11.028]
  - 33 **Murray J**, Fishman SL, Ryan E, Eng FJ, Walewski JL, Branch AD, Morgello S. Clinicopathologic correlates of hepatitis C virus in brain: a pilot study. *J Neurovirol* 2008; **14**: 17-27 [PMID: 18300072 DOI: 10.1080/13550280701708427]
  - 34 **Lau DT**, Fish PM, Sinha M, Owen DM, Lemon SM, Gale M. Interferon regulatory factor-3 activation, hepatic interferon-stimulated gene expression, and immune cell infiltration in hepatitis C virus patients. *Hepatology* 2008; **47**: 799-809 [PMID: 18203148 DOI: 10.1002/hep.22076]
  - 35 **Liang Y**, Shilagard T, Xiao SY, Snyder N, Lau D, Cicalese L, Weiss H, Vargas G, Lemon SM. Visualizing hepatitis C virus infections in human liver by two-photon microscopy. *Gastroenterology* 2009; **137**: 1448-1458 [PMID: 19632233 DOI: 10.1053/j.gastro.2009.07.050]
  - 36 **Vivithanaporn P**, Maingat F, Lin LT, Na H, Richardson CD, Agrawal B, Cohen EA, Jhamandas JH, Power C. Hepatitis C virus core protein induces neuroimmune activation and potentiates Human Immunodeficiency Virus-1 neurotoxicity. *PLoS One* 2010; **5**: e12856 [PMID: 20877724 DOI: 10.1371/journal.pone.0012856]
  - 37 **Wilkinson J**, Radkowski M, Laskus T. Hepatitis C virus neuroinvasion: identification of infected cells. *J Virol* 2009; **83**: 1312-1319 [PMID: 19019968 DOI: 10.1128/JVI.01890-08]
  - 38 **Farquhar MJ**, McKeating JA. Primary hepatocytes as targets for hepatitis C virus replication. *J Viral Hepat* 2008; **15**: 849-854 [PMID: 19087224 DOI: 10.1111/j.1365-2893.2008.01051.x]
  - 39 **Ploss A**, Evans MJ, Gaysinskaya VA, Panis M, You H, de Jong YP, Rice CM. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 2009; **457**: 882-886 [PMID: 19182773 DOI: 10.1038/nature07684]
  - 40 **Berglund L**, Björling E, Oksvold P, Fagerberg L, Asplund A, Szgyarto CA, Persson A, Ottosson J, Wernérus H, Nilsson P, Lundberg E, Sivertsson A, Navani S, Wester K, Kampf C, Hober S, Pontén F, Uhlén M. A gene-centric Human Protein Atlas for expression profiles based on antibodies. *Mol Cell Proteomics* 2008; **7**: 2019-2027 [PMID: 18669619 DOI: 10.1074/mcp.R800013-MCP200]
  - 41 **Su AI**, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G, Cooke MP, Walker JR, Hogenesch JB. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci USA* 2004; **101**: 6062-6067 [PMID: 15075390 DOI: 10.1073/pnas.0400782101]
  - 42 **Zensi A**, Begley D, Pontikis C, Legros C, Mihoreanu L, Wagner S, Büchel C, von Briesen H, Kreuter J. Albumin nanoparticles targeted with Apo E enter the CNS by transcytosis and are delivered to neurones. *J Control Release* 2009; **137**: 78-86 [PMID: 19285109 DOI: 10.1016/j.jconrel.2009.03.002]
  - 43 **Hülsermann U**, Hoffmann MM, Massing U, Fricker G. Uptake of apolipoprotein E fragment coupled liposomes by cultured brain microvessel endothelial cells and intact brain capillaries. *J Drug Target* 2009; **17**: 610-618 [PMID: 19694613 DOI: 10.1080/10611860903105986]
  - 44 **Ballabh P**, Braun A, Nedergaard M. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis* 2004; **16**: 1-13 [PMID: 15207256 DOI: 10.1016/j.nbd.2003.12.016]
  - 45 **Varatharajan L**, Thomas SA. The transport of anti-HIV drugs across blood-CNS interfaces: summary of current knowledge and recommendations for further research. *Antiviral Res* 2009; **82**: A99-109 [PMID: 19176219 DOI: 10.1016/j.antiviral.2008.12.013]
  - 46 **Diamond MS**, Klein RS. West Nile virus: crossing the blood-brain barrier. *Nat Med* 2004; **10**: 1294-1295 [PMID: 15580248 DOI: 10.1038/nm1204-1294]
  - 47 **Kramer-Hämmerle S**, Rothenaigner I, Wolff H, Bell JE, Brack-Werner R. Cells of the central nervous system as targets and reservoirs of the human immunodeficiency virus. *Virus Res* 2005; **111**: 194-213 [PMID: 15885841 DOI: 10.1016/j.virusres.2005.04.009]
  - 48 **Petty GW**, Duffy J, Houston J. Cerebral ischemia in patients with hepatitis C virus infection and mixed cryoglobulinemia. *Mayo Clin Proc* 1996; **71**: 671-678 [PMID: 8656709 DOI: 10.4065/71.7.671]
  - 49 **Ramos-Casals M**, Robles A, Brito-Zerón P, Nardi N, Nicolás JM, Forns X, Plaza J, Yagüe J, Sánchez-Tapias JM, Font J. Life-threatening cryoglobulinemia: clinical and immunological characterization of 29 cases. *Semin Arthritis Rheum* 2006; **36**: 189-196 [PMID: 16996578 DOI: 10.1016/j.semarthrit.2006.08.005]
  - 50 **Arena MG**, Ferlazzo E, Bonanno D, Quattrocchi P, Ferlazzo B. Cerebral vasculitis in a patient with HCV-related type II mixed cryoglobulinemia. *J Investig Allergol Clin Immunol* 2003; **13**: 135-136 [PMID: 12968400]
  - 51 **Heckmann JG**, Kayser C, Heuss D, Manger B, Blum HE, Neundörfer B. Neurological manifestations of chronic hepatitis C. *J Neurol* 1999; **246**: 486-491 [PMID: 10431776 DOI: 10.1007/s004150050388]
  - 52 **Dawson TM**, Starkebaum G. Isolated central nervous system vasculitis associated with hepatitis C infection. *J Rheumatol* 1999; **26**: 2273-2276 [PMID: 10529155]
  - 53 **Malnick SD**, Abend Y, Evron E, Stoecker ZM. HCV hepatitis associated with anticardiolipin antibody and a cerebrovascular accident. Response to interferon therapy. *J Clin Gastroenterol* 1997; **24**: 40-42 [PMID: 9013350 DOI: 10.1097/00004836-199701000-00009]
  - 54 **Lee MH**, Yang HI, Wang CH, Jen CL, Yeh SH, Liu CJ, You SL, Chen WJ, Chen CJ. Hepatitis C virus infection and increased risk of cerebrovascular disease. *Stroke* 2010; **41**: 2894-2900 [PMID: 20966408 DOI: 10.1161/STROKEAHA.110.598136]
  - 55 **Serena M**, Biscaro R, Moretto G, Recchia E. Peripheral and central nervous system involvement in essential mixed cryoglobulinemia: a case report. *Clin Neuropathol* 1991; **10**: 177-180 [PMID: 1884525]
  - 56 **Buccoliero R**, Gambelli S, Sicurelli F, Malandrini A, Palmeri S, De Santis M, Stromillo ML, De Stefano N, Sperduto A, Musumeci SA, Federico A. Leukoencephalopathy as a rare complication of hepatitis C infection. *Neurol Sci* 2006; **27**: 360-363 [PMID: 17122948 DOI: 10.1007/s10072-006-0711-y]
  - 57 **Casato M**, Saadoun D, Marchetti A, Limal N, Picq C, Pantano P, Galanaud D, Ciani R, Duhaut P, Piette JC, Fiorilli M, Cacoub P. Central nervous system involvement in hepatitis C virus cryoglobulinemia vasculitis: a multicenter case-control study using magnetic resonance imaging and neuropsychological tests. *J*

- Rheumatol* 2005; **32**: 484-488 [PMID: 15742440]
- 58 **Bokemeyer M**, Ding XQ, Goldbecker A, Raab P, Heeren M, Arvanitis D, Tillmann HL, Lanfermann H, Weissenborn K. Evidence for neuroinflammation and neuroprotection in HCV infection-associated encephalopathy. *Gut* 2011; **60**: 370-377 [PMID: 20926642 DOI: 10.1136/gut.2010.217976]
  - 59 **Forton DM**, Allsop JM, Main J, Foster GR, Thomas HC, Taylor-Robinson SD. Evidence for a cerebral effect of the hepatitis C virus. *Lancet* 2001; **358**: 38-39 [PMID: 11454379 DOI: 10.1016/S0140-6736(00)05270-3]
  - 60 **Weissenborn K**, Krause J, Bokemeyer M, Hecker H, Schüler A, Ennen JC, Ahl B, Manns MP, Böker KW. Hepatitis C virus infection affects the brain-evidence from psychometric studies and magnetic resonance spectroscopy. *J Hepatol* 2004; **41**: 845-851 [PMID: 15519659 DOI: 10.1016/j.jhep.2004.07.022]
  - 61 **Piche T**, Vanbiervliet G, Cherikh F, Antoun Z, Huet PM, Gelsi E, Demarquay JF, Caroli-Bosc FX, Benzaken S, Rigault MC, Renou C, Rampal P, Tran A. Effect of ondansetron, a 5-HT<sub>3</sub> receptor antagonist, on fatigue in chronic hepatitis C: a randomised, double blind, placebo controlled study. *Gut* 2005; **54**: 1169-1173 [PMID: 16009690 DOI: 10.1136/gut.2004.055251]
  - 62 **Cozzi A**, Zignego AL, Carpendo R, Biagiotti T, Aldinucci A, Monti M, Giannini C, Rosselli M, Laffi G, Moroni F. Low serum tryptophan levels, reduced macrophage IDO activity and high frequency of psychopathology in HCV patients. *J Viral Hepat* 2006; **13**: 402-408 [PMID: 16842443 DOI: 10.1111/j.1365-2893.2005.00706.x]
  - 63 **Jones EM**, Gray-Keller M, Art JJ, Fettiplace R. The functional role of alternative splicing of Ca(2+)-activated K<sup>+</sup> channels in auditory hair cells. *Ann N Y Acad Sci* 1999; **868**: 379-385 [PMID: 10414307 DOI: 10.1111/j.1749-6632.1999.tb11299.x]
  - 64 **Heeren M**, Weissenborn K, Arvanitis D, Bokemeyer M, Goldbecker A, Tountopoulou A, Peschel T, Grosskreutz J, Hecker H, Buchert R, Berding G. Cerebral glucose utilisation in hepatitis C virus infection-associated encephalopathy. *J Cereb Blood Flow Metab* 2011; **31**: 2199-2208 [PMID: 21629258 DOI: 10.1038/jcbfm.2011.82]
  - 65 **Zoccollella S**, Serlenga L, Amati A, Lavalpe V, Minerva N, Agremoriz M, Toscano A, Lamberti P. A case of vacuolar myopathy during the course of chronic hepatitis C. *Funct Neurol* 2006; **21**: 167-169 [PMID: 17049137]
  - 66 **Satoh J**, Eguchi Y, Narukiyo T, Mizuta T, Kobayashi O, Kawai M, Nonaka I, Kuroda Y. Necrotizing myopathy in a patient with chronic hepatitis C virus infection: a case report and a review of the literature. *Intern Med* 2000; **39**: 176-181 [PMID: 10732841 DOI: 10.2169/internalmedicine.39.176]
  - 67 **Cortelli P**, Mandrioli J, Zeviani M, Lodi R, Prata C, Pecorari M, Orlando G, Guaraldi G. Mitochondrial complex III deficiency in a case of HCV related noninflammatory myopathy. *J Neurol* 2007; **254**: 1450-1452 [PMID: 17932705 DOI: 10.1007/s00415-007-0537-4]
  - 68 **Zhu N**, Khoshnan A, Schneider R, Matsumoto M, Dennert G, Ware C, Lai MM. Hepatitis C virus core protein binds to the cytoplasmic domain of tumor necrosis factor (TNF) receptor 1 and enhances TNF-induced apoptosis. *J Virol* 1998; **72**: 3691-3697 [PMID: 9557650]
  - 69 **Dammacco F**, Sansonno D, Piccoli C, Tucci FA, Racanelli V. The cryoglobulins: an overview. *Eur J Clin Invest* 2001; **31**: 628-638 [PMID: 11454019 DOI: 10.1046/j.1365-2362.2001.00824.x]
  - 70 **Ramos-Casals M**, Stone JH, Cid MC, Bosch X. The cryoglobulinaemias. *Lancet* 2012; **379**: 348-360 [PMID: 21868085 DOI: 10.1016/S0140-6736(11)60242-0]
  - 71 **Brouet JC**, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. A report of 86 cases. *Am J Med* 1974; **57**: 775-788 [PMID: 4216269 DOI: 10.1016/0002-9343(74)90852-3]
  - 72 **Ferraccioli GF**, De Vita S, Casatta L, Damato R, Pegoraro I, Bartoli E. Autoimmune connective tissue disease, chronic polyarthritides and B cell expansion: risks and perspectives with immunosuppressive drugs. *Clin Exp Rheumatol* 1996; **14** Suppl 14: S71-S80 [PMID: 8722204]
  - 73 **Ramos-Casals M**, Muñoz S, Medina F, Jara LJ, Rosas J, Calvo-Alen J, Brito-Zerón P, Forns X, Sánchez-Tapias JM. Systemic autoimmune diseases in patients with hepatitis C virus infection: characterization of 1020 cases (The HISPAMEC Registry). *J Rheumatol* 2009; **36**: 1442-1448 [PMID: 19369460 DOI: 10.3899/jrheum.080874]
  - 74 **Pileri P**, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, Abrignani S. Binding of hepatitis C virus to CD81. *Science* 1998; **282**: 938-941 [PMID: 9794763 DOI: 10.1126/science.282.5390.938]
  - 75 **Schott P**, Polzien F, Müller-Issberner A, Ramadori G, Hartmann H. In vitro reactivity of cryoglobulin IgM and IgG in hepatitis C virus-associated mixed cryoglobulinemia. *J Hepatol* 1998; **28**: 17-26 [PMID: 9537859 DOI: 10.1016/S0168-8278(98)80197-9]
  - 76 **Choo QL**, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**: 359-362 [PMID: 2523562 DOI: 10.1126/science.2523562]
  - 77 **Bambara LM**, Biasi D, Caramaschi P, Carletto A, Pacor ML. Cryoglobulinaemia and hepatitis C virus (HCV) infection. *Clin Exp Rheumatol* 1991; **9**: 96-97 [PMID: 1711427]
  - 78 **Ferri C**, Sebastiani M, Giuggioli D, Cazzato M, Longombardo G, Antonelli A, Puccini R, Michelassi C, Zignego AL. Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. *Semin Arthritis Rheum* 2004; **33**: 355-374 [PMID: 15190522]
  - 79 **Cohen P**, Roulot D, Ferrière F, Nguyen QT, Lortholary O, Jarrousse B, Dény P, Coste T, Robineau M, Guillemin L. Prevalence of cryoglobulins and hepatitis C virus infection in HIV-infected patients. *Clin Exp Rheumatol* 1997; **15**: 523-527 [PMID: 9307860]
  - 80 **Kosmas N**, Kontos A, Panayiotakopoulos G, Dimitrakopoulos A, Kordossis T. Decreased prevalence of mixed cryoglobulinemia in the HAART era among HIV-positive, HCV-negative patients. *J Med Virol* 2006; **78**: 1257-1261 [PMID: 16927287 DOI: 10.1002/jmv.20695]
  - 81 **Ferri C**, Antonelli A, Mascia MT, Sebastiani M, Fallahi P, Ferrari D, Pileri SA, Zignego AL. HCV-related autoimmune and neoplastic disorders: the HCV syndrome. *Dig Liver Dis* 2007; **39** Suppl 1: S13-S21 [PMID: 17936215 DOI: 10.1016/S1590-8658(07)80005-3]
  - 82 **Rosa D**, Saletti G, De Gregorio E, Zorat F, Comar C, D'Oro U, Nuti S, Houghton M, Barnaba V, Pozzato G, Abrignani S. Activation of naïve B lymphocytes via CD81, a pathogenetic mechanism for hepatitis C virus-associated B lymphocyte disorders. *Proc Natl Acad Sci USA* 2005; **102**: 18544-18549 [PMID: 16339892 DOI: 10.1073/pnas.0509402102]
  - 83 **Sansonno D**, Lauletta G, Nisi L, Gatti P, Pesola F, Pansini N, Dammacco F. Non-enveloped HCV core protein as constitutive antigen of cold-precipitable immune complexes in type II mixed cryoglobulinaemia. *Clin Exp Immunol* 2003; **133**: 275-282 [PMID: 12869035 DOI: 10.1046/j.1365-2249.2003.02204.x]
  - 84 **Cashman CR**, Höke A. Mechanisms of distal axonal degeneration in peripheral neuropathies. *Neurosci Lett* 2015; **596**: 33-50 [PMID: 25617478 DOI: 10.1016/j.neulet.2015.01.048]
  - 85 **Rosenthal E**, Cacoub P. Extrahepatic manifestations in chronic hepatitis C virus carriers. *Lupus* 2015; **24**: 469-482 [PMID: 25801890 DOI: 10.1177/0961203314556140]
  - 86 **Bonetti B**, Scardoni M, Monaco S, Rizzuto N, Scarpa A. Hepatitis C virus infection of peripheral nerves in type II cryoglobulinaemia. *Virchows Arch* 1999; **434**: 533-535 [PMID: 10394889 DOI: 10.1007/s004280050380]
  - 87 **Carvalho-Filho RJ**, Narciso-Schiavon JL, Tolentino LH, Schiavon LL, Ferraz ML, Silva AE. Central nervous system vasculitis and polyneuropathy as first manifestations of hepatitis C. *World J Gastroenterol* 2012; **18**: 188-191 [PMID: 22253526 DOI: 10.3748/wjg.v18.i2.188]
  - 88 **Yoon MS**, Obermann M, Dockweiler C, Assert R, Canbay A, Haag S, Gerken G, Diener HC, Katsarava Z. Sensory neuropathy in patients with cryoglobulin negative hepatitis-C infection.

- J Neurol* 2011; **258**: 80-88 [PMID: 20683606 DOI: 10.1007/s00415-010-5686-1]
- 89 **Nemni R**, Sanvito L, Quattrini A, Santuccio G, Camerlingo M, Canal N. Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia. *J Neurol Neurosurg Psychiatry* 2003; **74**: 1267-1271 [PMID: 12933932 DOI: 10.1136/jnnp.74.9.1267]
  - 90 **Biasiotto A**, Casato M, La Cesa S, Colantuono S, Di Stefano G, Leone C, Carlesimo M, Piroso S, Cruccu G, Truini A. Clinical, neurophysiological, and skin biopsy findings in peripheral neuropathy associated with hepatitis C virus-related cryoglobulinemia. *J Neurol* 2014; **261**: 725-731 [PMID: 24500496 DOI: 10.1007/s00415-014-7261-7]
  - 91 **Santoro L**, Manganelli F, Briani C, Giannini F, Benedetti L, Vitelli E, Mazzeo A, Beghi E. Prevalence and characteristics of peripheral neuropathy in hepatitis C virus population. *J Neurol Neurosurg Psychiatry* 2006; **77**: 626-629 [PMID: 16464900 DOI: 10.1136/jnnp.2005.081570]
  - 92 **Cacoub P**, Poynard T, Ghillani P, Charlotte F, Olivi M, Piette JC, Opolon P. Extrahepatic manifestations of chronic hepatitis C. MULTIVIRC Group. Multidepartment Virus C. *Arthritis Rheum* 1999; **42**: 2204-2212 [PMID: 10524695]
  - 93 **Abd El-Kader SM**, El-Den Ashmawy EM. Non-alcoholic fatty liver disease: The diagnosis and management. *World J Hepatol* 2015; **7**: 846-858 [PMID: 25937862 DOI: 10.4254/wjh.v7.i6.846]
  - 94 **Cacoub P**, Renou C, Rosenthal E, Cohen P, Loury I, Loustaud-Ratti V, Yamamoto AM, Camproux AC, Hausfater P, Musset L, Veyssier P, Raguin G, Piette JC. Extrahepatic manifestations associated with hepatitis C virus infection. A prospective multicenter study of 321 patients. The GERMIVIC. Groupe d'Etude et de Recherche en Medecine Interne et Maladies Infectieuses sur le Virus de l'Hépatite C. *Medicine (Baltimore)* 2000; **79**: 47-56 [PMID: 10670409 DOI: 10.1097/00005792-200001000-00005]
  - 95 **Yuki N**, Yoshioka A, Yasuda R, Ohmichi T, Oka N. Hepatitis C virus-associated neuropathy accompanied by eosinophilic vasculitis and granuloma formation. *Intern Med* 2014; **53**: 1187-1190 [PMID: 24881746 DOI: 10.2169/innermedicine.53.2060]
  - 96 **Mariotto S**, Ferrari S, Monaco S. HCV-related central and peripheral nervous system demyelinating disorders. *Inflamm Allergy Drug Targets* 2014; **13**: 299-304 [PMID: 25198705 DOI: 10.2174/1871528113666140908113841]
  - 97 **Latov N**. Diagnosis and treatment of chronic acquired demyelinating polyneuropathies. *Nat Rev Neurol* 2014; **10**: 435-446 [PMID: 24980070 DOI: 10.1038/nrneurol.2014.117]
  - 98 **Rifai MA**, Indest D, Loftis J, Hauser P. Psychiatric management of the hepatitis C patient. *Curr Treat Options Gastroenterol* 2006; **9**: 508-519 [PMID: 17081484 DOI: 10.1007/s11938-006-0007-6]
  - 99 **Schaefer M**, Capuron L, Friebe A, Diez-Quevedo C, Robaey G, Neri S, Foster GR, Kautz A, Forton D, Pariente CM. Hepatitis C infection, antiviral treatment and mental health: a European expert consensus statement. *J Hepatol* 2012; **57**: 1379-1390 [PMID: 22878466 DOI: 10.1016/j.jhep.2012.07.037]
  - 100 **Rifai MA**, Gleason OC, Sabouni D. Psychiatric care of the patient with hepatitis C: a review of the literature. *Prim Care Companion J Clin Psychiatry* 2010; **12**: pii: PCC.09r00877 [PMID: 21494349 DOI: 10.4088/PCC.09r00877whi]
  - 101 **McMahon JM**, Tortu S. A potential hidden source of hepatitis C infection among noninjecting drug users. *J Psychoactive Drugs* 2003; **35**: 455-460 [PMID: 14986874 DOI: 10.1080/02791072.2003.10400492]
  - 102 **el-Serag HB**, Kunik M, Richardson P, Rabeneck L. Psychiatric disorders among veterans with hepatitis C infection. *Gastroenterology* 2002; **123**: 476-482 [PMID: 12145801 DOI: 10.1053/gast.2002.34750]
  - 103 **Raison CL**, Demetrasvili M, Capuron L, Miller AH. Neuropsychiatric adverse effects of interferon-alpha: recognition and management. *CNS Drugs* 2005; **19**: 105-123 [PMID: 15697325]
  - 104 **Udina M**, Castellvi P, Moreno-España J, Navinés R, Valdés M, Forns X, Langohr K, Solà R, Vieta E, Martín-Santos R. Interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. *J Clin Psychiatry* 2012; **73**: 1128-1138 [PMID: 22967776 DOI: 10.4088/JCP.12r07694]
  - 105 **McNutt MD**, Liu S, Manatunga A, Royster EB, Raison CL, Woolwine BJ, Demetrasvili MF, Miller AH, Musselman DL. Neurobehavioral effects of interferon- $\alpha$  in patients with hepatitis-C: symptom dimensions and responsiveness to paroxetine. *Neuropsychopharmacology* 2012; **37**: 1444-1454 [PMID: 22353759 DOI: 10.1038/npp.2011.330]
  - 106 **Sheridan DA**, Price DA, Schmid ML, Toms GL, Donaldson P, Neely D, Bassendine MF. Apolipoprotein B-associated cholesterol is a determinant of treatment outcome in patients with chronic hepatitis C virus infection receiving anti-viral agents interferon-alpha and ribavirin. *Aliment Pharmacol Ther* 2009; **29**: 1282-1290 [PMID: 19392865 DOI: 10.1111/j.1365-2036.2009.04012.x]
  - 107 **Navinés R**, Castellvi P, Moreno-España J, Gimenez D, Udina M, Cañizares S, Diez-Quevedo C, Valdés M, Solà R, Martín-Santos R. Depressive and anxiety disorders in chronic hepatitis C patients: reliability and validity of the Patient Health Questionnaire. *J Affect Disord* 2012; **138**: 343-351 [PMID: 22326842 DOI: 10.1016/j.jad.2012.01.018]
  - 108 **Sockalingam S**, Abbey SE, Alosaimi F, Novak M. A review of sleep disturbance in hepatitis C. *J Clin Gastroenterol* 2010; **44**: 38-45 [PMID: 19730115 DOI: 10.1097/MCG.0b013e3181b314ea]
  - 109 **Cacoub P**, Terrier B, Saadoun D. Hepatitis C virus-induced vasculitis: therapeutic options. *Ann Rheum Dis* 2014; **73**: 24-30 [PMID: 23921995 DOI: 10.1136/annrheumdis-2013-203883]
  - 110 **Saadoun D**, Terrier B, Semoun O, Sene D, Maisonnobe T, Musset L, Amoura Z, Rigon MR, Cacoub P. Hepatitis C virus-associated polyarteritis nodosa. *Arthritis Care Res (Hoboken)* 2011; **63**: 427-435 [PMID: 20981809]
  - 111 **Jadali Z**. Autoimmune thyroid disorders in hepatitis C virus infection: Effect of interferon therapy. *Indian J Endocrinol Metab* 2013; **17**: 69-75 [PMID: 23776855 DOI: 10.4103/2230-8210.107856]
  - 112 **El Khayat HR**, Fouad YM, Ahmad EA, El Amin H, Ismael F, Rizk A. Hepatitis C virus (genotype 4)-associated mixed cryoglobulinemia vasculitis: effects of antiviral treatment. *Hepatol Int* 2012; **6**: 606-612 [PMID: 22020820 DOI: 10.1007/s12072-011-9303-x]
  - 113 **Fatima K**, Mathew S, Suhail M, Ali A, Damanhoury G, Azhar E, Qadri I. Docking studies of Pakistani HCV NS3 helicase: a possible antiviral drug target. *PLoS One* 2014; **9**: e106339 [PMID: 25188400]
  - 114 **Meriggioli MN**, Rowin J. Chronic inflammatory demyelinating polyneuropathy after treatment with interferon-alpha. *Muscle Nerve* 2000; **23**: 433-435 [PMID: 10679722]
  - 115 **Reyes-Gibby CC**, Wang J, Yeung SC, Shete S. Informative gene network for chemotherapy-induced peripheral neuropathy. *BioData Min* 2015; **8**: 24 [PMID: 26269716 DOI: 10.1186/s13040-015-0058-0]
  - 116 **Mathew S**, Faheem M, Archunan G, Ilyas M, Begum N, Jahangir S, Qadri I, Qahtani MA, Mathew S. In silico studies of medicinal compounds against hepatitis C capsid protein from north India. *Bioinform Biol Insights* 2014; **8**: 159-168 [PMID: 25002815]
  - 117 **Dammacco F**, Tucci FA, Lauletta G, Gatti P, De Re V, Conteduca V, Sansonno S, Russi S, Marigliò MA, Chironna M, Sansonno D. Pegylated interferon-alpha, ribavirin, and rituximab combined therapy of hepatitis C virus-related mixed cryoglobulinemia: a long-term study. *Blood* 2010; **116**: 343-353 [PMID: 20308602 DOI: 10.1182/blood-2009-10-245878]
  - 118 **De Vita S**, Quartuccio L, Isola M, Mazzaro C, Scaini P, Lenzi M, Campanini M, Naclerio C, Tavoni A, Pietrogrande M, Ferri C, Mascia MT, Masolini P, Zabotti A, Maset M, Roccatello D, Zignego AL, Pioltelli P, Gabrielli A, Filippini D, Perrella O, Migliaresi S, Galli M, Bombardieri S, Monti G. A randomized controlled trial of rituximab for the treatment of severe cryoglobulinemic vasculitis. *Arthritis Rheum* 2012; **64**: 843-853 [PMID: 22147661 DOI: 10.1002/art.34331]
  - 119 **Saadoun D**, Resche Rigon M, Sene D, Terrier B, Karras A, Perard L, Schoindre Y, Coppéré B, Blanc F, Musset L, Piette JC,

- Rosenzwajg M, Cacoub P. Rituximab plus Peg-interferon-alpha/ribavirin compared with Peg-interferon-alpha/ribavirin in hepatitis C-related mixed cryoglobulinemia. *Blood* 2010; **116**: 326-334; quiz 504-505 [PMID: 20439619 DOI: 10.1182/blood-2009-10-248518]
- 120 **Mathew S**, Ali A, Abdel-Hafiz H, Fatima K, Suhail M, Archunan G, Begum N, Jahangir S, Ilyas M, Chaudhary AG, Al Qahtani M, Mohamad Bazarah S, Qadri I. Biomarkers for virus-induced hepatocellular carcinoma (HCC). *Infect Genet Evol* 2014; **26**: 327-339 [PMID: 24956436]
- 121 **Mathew S**, Fatima K, Fatmi MQ, Archunan G, Ilyas M, Begum N, Azhar E, Damanhour G, Qadri I. Computational Docking Study of p7 Ion Channel from HCV Genotype 3 and Genotype 4 and Its Interaction with Natural Compounds. *PLoS One* 2015; **10**: e0126510 [PMID: 26030803]

**P- Reviewer:** Conti B, Narciso-Schiavon JL **S- Editor:** Kong JX  
**L- Editor:** A **E- Editor:** Liu SQ





## Antiviral therapy for hepatitis C: Has anything changed for pregnant/lactating women?

Anna Maria Spera, Tarek Kamal Eldin, Grazia Tosone, Raffaele Orlando

Anna Maria Spera, Tarek Kamal Eldin, Grazia Tosone, Raffaele Orlando, Department of Clinical Medicine and Surgery, Section of Infectious Diseases, University of Naples Federico II, 80131 Napoli, Italy

**Author contributions:** Spera AM designed and wrote the review; Kamal Eldin T analyzed pharmacological and clinical data; Tosone G and Orlando R overview the manuscript.

**Conflict-of-interest statement:** All authors declare any conflicting interests (including but not limited to commercial, personal, political, intellectual or religious interests). In addition, reviewers have not potential conflicting interests related to any particular paper they maybe are asked to review.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Anna Maria Spera, MD, Department of Clinical Medicine and Surgery, Section of Infectious Diseases, University of Naples Federico II, Via Sergio Pansini 5, 80131 Napoli, Italy. [annamariaspera@hotmail.it](mailto:annamariaspera@hotmail.it)  
 Telephone: +39-08-17463082  
 Fax: +39-08-17493094

Received: November 24, 2015  
 Peer-review started: November 25, 2015  
 First decision: December 28, 2015  
 Revised: February 9, 2016  
 Accepted: March 22, 2016  
 Article in press: March 23, 2016  
 Published online: April 28, 2016

### Abstract

Hepatitis C virus (HCV) affects about 3% of the world's

population, with the highest prevalence in individuals under 40. The prevalence in pregnant women varies with geographical distribution (highest in developing countries). Prevalence also increases in sub-populations of women at high risk for blood-transmitted infections. HCV infection in pregnancy represents a non-negligible problem. However, most of the past antiviral regimens cannot be routinely offered to pregnant or breastfeeding women because of their side effects. We briefly reviewed the issue of treatment of HCV infection in pregnant/breastfeeding women focusing on the effects of the new direct-acting antivirals on fertility, pregnancy and lactation in animal studies and on the potential risk for humans based on the pharmacokinetic properties of each drug. Currently, all new therapy regimens are contraindicated in this setting because of lack of sufficient safety information and adequate measures of contraception are still routinely recommended for female patients of childbearing potential.

**Key words:** Hepatitis C virus infection; Breastfeeding woman; Antiviral therapy; Pregnancy category; Direct-acting antivirals

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Until recently, the only drugs available for the treatment of hepatitis C virus infection had a well-documented teratogenic effect limiting their use in childbearing women. Recently, new generation drugs, designated the direct-acting antivirals have been approved. There are no studies available describing their effects on pregnant and lactating women. We here will try to analyze their pharmacokinetic properties and data from animal studies to try to predict their potential use pregnancy.

Spera AM, Kamal Eldin T, Tosone G, Orlando R. Antiviral therapy for hepatitis C: Has anything changed for pregnant/

lactating women? *World J Hepatol* 2016; 8(12): 557-565  
Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i12/557.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i12.557>

## INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health problem that affects more than 150 million people (about 3% of the world's population), most of whom are unaware of their infection<sup>[1,2]</sup>. The prevalence of HCV infection is between 0.5%-2% in most European countries and in the United States, in which 5-10 million and almost 4 million people, respectively, are affected, most of whom are in the fourth decade of life<sup>[1,3-5]</sup>. Differently, the prevalence of HCV infection exceeds 10% in some developing countries (especially in Africa, Asia and South America)<sup>[1]</sup>. One of the modes of HCV transmission is vertical transmission. Rates of vertical transmission of HCV infection range between 2%-10%<sup>[4]</sup>. Although HCV infection acquired at birth may resolve spontaneously, about 25000 to 50000 of children become chronically infected<sup>[4-7]</sup>. The prevalence of HCV infection in children is very low in Europe and the United States (0.05%-0.36%)<sup>[8]</sup>, and increases to between 1.8% and 5.8% in Egypt (which has the highest prevalence of pediatric HCV infection), Sub-Saharan Africa, Mongolia and the Amazon Basin<sup>[8]</sup>. Consequently, birth to an infected mother is one of most frequent routes of infection, and is comparable to injection drug use, unsafe medical practices and high-risk sexual practices<sup>[1]</sup>.

Acute HCV infection, asymptomatic in most cases, can progress to chronic hepatitis in more than half the patients. Chronic hepatitis C is associated with progression to fibrosis, which leads to liver cirrhosis in about 10% to 20% of patients within 20-30 years. Lastly, from about 1%-5% of cirrhotic patients can develop hepatocellular carcinoma each year<sup>[2]</sup>. Currently, HCV-related liver cirrhosis is the major cause of liver transplantation in developed countries<sup>[1,2]</sup>. Notwithstanding the decline in the number of cases of acute HCV infection, the burden of liver cirrhosis, hepatocellular carcinoma and HCV-related death remains high due to the existence of a reservoir of infected patients<sup>[1,3]</sup>.

The epidemiology of hepatitis C infection might change radically in the next few years thanks to antiviral therapies that result in viral clearance in terms of sustained virologic response (SVR), namely undetectable HCV RNA 12 wk (SVR12) or SVR24 after treatment completion. HCV infection is cured in more than 99% of patients who achieve an SVR. Generally, liver disease can be cured only in non-cirrhotic patients<sup>[2]</sup>.

The effect of maternal viremia on vertical transmission and on the rate of spontaneous resolution of the acquired infection among newborns is not well defined. However, mothers with undetectable plasma HCV RNA levels rarely transmit HCV by the vertical

route<sup>[4]</sup>. Therefore, it seems reasonable to assume that treatment to decrease viremia in pregnant women with chronic HCV may result in lower rates of vertical HCV transmission.

Until 2011, the standard-of-care therapy for HCV infection, which was based on the association of pegylated interferon (PEG-IFN) and Ribavirin, resulted in an SVR in only 40%-80% of patients depending on HCV genotype (lower for genotypes 1 and 4 than for genotypes 2 and 3)<sup>[2]</sup>. In 2011, new antiviral drugs, namely, direct-acting antivirals (DAAs), became available. These drugs act mainly by targeting the non-structural HCV proteases NS3-4A and NS5A or by inhibiting RNA-dependent RNA polymerase, and are thus referred to as protease inhibitors and inhibitors of HCV RNA-dependent RNA polymerase, respectively<sup>[2]</sup>. Each of these DAAs can be used as a component of combination regimens (with or without PEG-IFN and Ribavirin) and result in SVR rates as high as 60%-100%<sup>[2]</sup>. The SVR rate depends on the DAA used, the HCV genotype, pre-existing amino acid substitutions (that might confer resistance to some DAAs) and the severity of liver disease<sup>[2]</sup>. These new regimens could change both the epidemiology and the natural progression of hepatitis C.

Here we discuss the potential use, in pregnant and breastfeeding women, of the antiviral therapies (including DAAs) licensed for the treatment of chronic C hepatitis. We also examine the adverse effects of anti-HCV drugs on fertility, pregnancy and lactation (in particular, embryo toxic and teratogenic effects). In this context, no antiviral therapy has yet been approved for use in childbearing women, and therefore little is known about the effects of anti-HCV drugs on pregnancy and lactation in this population. Consequently, our discussion and conclusions are based principally on data derived from animal studies<sup>[9-15]</sup>.

## ANTIVIRAL THERAPY OF HEPATITIS C IN CHILDBEARING WOMEN

Hepatitis C infection in pregnant and breastfeeding women is not a negligible problem. About 1%-8% of pregnant women have markers of HCV infection, and the prevalence is lower in western/northern countries than in Eastern/Southern countries<sup>[11,15]</sup>. Since, HCV infection is usually asymptomatic, most infected women are unaware of their status and may be diagnosed with chronic C hepatitis incidentally when undergoing serological tests during pregnancy or before delivery. For example, in Italy, free-of-charge screening for HCV, HBV and the human immunodeficiency virus, is routinely offered to all pregnant women from the 33<sup>rd</sup> to the 37<sup>th</sup> week of gestation<sup>[16]</sup> and reveals many cases of previously undiagnosed chronic C hepatitis. Notably, the number of HCV-infected childbearing women is expected to increase with the increase in the migratory flow from developing countries to Western/Northern countries. As mentioned above, the vertical transmission

of HCV infection is now one of most frequent routes of transmission<sup>[1]</sup>. Consequently, eradication of the virus in pregnant women and women of childbearing age is the main target in the prevention and control of HCV infection<sup>[4]</sup>. Problems related to the treatment of HCV infection in pregnant and breastfeeding women are not rare. In a developed country such as the United States, pregnancy is the third most common contraindication to treatment and delayed treatment onset in about 2% of more than 45000 HCV-infected patients<sup>[17]</sup>. In addition, in the same study about 1.3% of women undergoing antiviral therapy for HCV became pregnant during therapy<sup>[17]</sup>. Thus, the problem is not only whether or not to start treatment in a pregnant and/or breastfeeding woman, but also how to manage a woman who becomes pregnant during antiviral therapy.

### ***The past of antiviral therapy: PEG-IFN/Ribavirin and PEG-IFN/Ribavirin plus first-generation DAAs***

For many years, the two cornerstones of the standard-of-care treatment of HCV infection were IFN and Ribavirin, both of which have side-effects and contraindications that limited their use in the setting of pregnant/breastfeeding patients<sup>[18,19]</sup>. IFN- $\alpha$  is a protein released in response to viral infections. It binds to specific receptors on the cell surface thereby promoting a complex cascade of protein-protein interactions that rapidly activate gene transcription. IFN-stimulated genes regulate many biologic effects (*i.e.*, inhibition of viral replication in infected cells, inhibition of cell proliferation and immunomodulation). The United States Food and Drug Administration (FDA) classified the first pharmacological formulation of IFN- $\alpha$  in Pregnancy Category C since the molecule had an abortifacient effect in animals (rhesus monkeys) during the early/middle fetal period of organogenesis and late fetal development<sup>[20,21]</sup>. The drug may also impair fertility; in fact, menstrual cycle irregularities, namely, prolonged or shortened menstrual periods and erratic bleeding, have been observed in nonhuman primates, and menstrual rhythm normalized upon treatment discontinuation<sup>[22]</sup>. Decreased serum estradiol and progesterone concentrations have been reported in women treated with human leukocyte IFN although no mutagenic effect or toxicity has been reported<sup>[22]</sup>. Given the species-specificity of IFN, effects in animals are unlikely to be predictive of those in humans<sup>[20,21]</sup>. Nevertheless, in clinical practice, IFN- $\alpha$  is widely used in most pregnant women affected by essential thrombocythemia to prevent or reduce the risk of thrombocythemia-related fetal loss<sup>[22]</sup>. The risk of major malformation, miscarriage, stillbirth or preterm delivery does not seem to be significantly higher in this setting than in the general population<sup>[22]</sup>.

Also the pegylated formulation of IFN- $\alpha$  (PEG-IFN- $\alpha$ ) should be assumed to have abortifacient potential despite the lack of well-controlled studies in pregnant women<sup>[23,24]</sup>. Apart from the potential risks for the fetus, a major concern is the risk of serious IFN-related adverse effects on the patient's psychological status,

namely exacerbation of postpartum depression<sup>[18]</sup>. Therefore, pregnant candidates for PEG-IFN treatment should undergo psychiatric evaluation. The degree of IFN excretion in human milk is unknown. However, given the potential risk of serious adverse reactions to the drug in nursing infants, IFN is contraindicated in children below the age of 2 years<sup>[20,21,23,24]</sup>. The decision whether to discontinue nursing and to initiate antiviral therapy depends solely on whether or not the progression of maternal liver disease must be immediately blocked. Given its low SVR rate (< 30%), PEG-IFN mono-therapy has been widely used in recent years in association with Ribavirin<sup>[25]</sup>. The combination of PEG-IFN and Ribavirin increased the SVR24 to 40% in North America and to 50% in western Europe in patients infected with HCV genotype 1<sup>[25]</sup>. Even better results were obtained in patients with genotypes 2, 3, 5 and 6: The best SVR was achieved in patients with genotype 2 (up to 80% SVR)<sup>[25]</sup>. The results of combined treatment in genotype 4 patients are the same as those obtained in genotype 1 patients or slightly better<sup>[25]</sup>.

Ribavirin is a guanosine analog nucleotide inhibitor that acts by interrupting viral RNA synthesis and viral mRNA capping. It is a prodrug that, when metabolized (into purine RNA nucleotides), interferes with RNA metabolism required for viral replication<sup>[26,27]</sup>. The mechanism underlying this effect is unknown. The FDA classified Ribavirin in Pregnancy Category X<sup>[26,27]</sup> because of its embryocidal and teratogenic effects in animals<sup>[26-28]</sup>. The fetal malformations reported in animal studies include abnormalities of the skull, palate, eye, jaw, limbs, skeleton and gastrointestinal tract<sup>[26-28]</sup>. Therefore, Ribavirin is absolutely contraindicated for both HCV-infected childbearing women and HCV-infected male partners of pregnant women unless they take effective contraceptive measures. In addition, since Ribavirin-induced spermatogenic abnormalities (cell toxicity, mutagenicity and a decreased epididymal sperm count) reverted only 4-8 mo after treatment withdrawal in all animal species studied<sup>[29,30]</sup>, women are advised to avoid pregnancy for at least 6 mo after partners of men taking Ribavirin treatment<sup>[26,27]</sup>.

Between 2003 and 2009, the United States Ribavirin Registry collected the data of 118 babies born to mothers exposed to the drug (49 direct and 69 indirect exposures) during pregnancy: Only six cases of birth defects were reported (torticollis, hypospadias, polydactyly, neonatal teeth, glucose-6-phosphate dehydrogenase deficiency, ventricular septal defect, and cyst of the fourth ventricle of the brain)<sup>[28]</sup>. Despite the low rate of birth defects, it seems reasonable not to encourage or support the use of Ribavirin in pregnant women, and, moreover, to recommend that women avoid pregnancy during Ribavirin treatment.

In conclusion, because of its low SVR rate unless combined with Ribavirin, PEG-IFN should not be administered in childbearing women even though it has not been reported to have abortifacient and/or teratogenic effects.

**Table 1** Main pharmacokinetic properties of the new direct-acting antivirals

Drug	Molecular weight	Effect of food on absorption	Cytochrome P450 enzymes interaction			Binding to plasma protein	Half-life
			Enzyme	Effect of the drug on the enzyme	Effect of the enzyme on the drug		
Sofosbuvir	529.45 Da	Increased absorption, slower rate	NO	None	None	85%	0.5 h/26 h <sup>1</sup>
Simeprevir	749.93 Da	Increased absorption, slower rate	CYP3A4	Inhibitor	Alter AUC	> 99.9%	10-41 h
Daclatasvir	738.98 Da	Decreased absorption <sup>2</sup>	CYP3A4	Weak inducer	Alter AUC	> 99%	12-15 h
Ledipasvir	888.9 Da	No effect	CYP3A4	Weak inducer	None	> 99.8%	47 h
Viekirax	Ombitasvir 894.1	Increased absorption	CYP3A4	Inhibitor	Alter AUC	99.9%	21-25 h
	Paritaprevir 765.8 Da					98.6%	5.5 h
	Ritonavir 720.9 Da					99%	4 h
Dasabuvir	493.5 Da	Increased absorption	CYP3A4	Inducer	None	99.5% (94.5% <sup>3</sup> )	6 h
			CYP2C8	None	Alter AUC		
			CYP3A4	None	Alter AUC <sup>3</sup>		
			CYP3A4	Inducer	None		

<sup>1</sup>GS-331007 metabolite; <sup>2</sup>Following a high-fat meal; <sup>3</sup>Dasabuvir M1 metabolite. AUC: Area under the curve; NO: Not metabolized by P450 enzymes; CYP: Cytochrome.

The year 2011 saw the advent of DAAs that target essential components of the HCV life cycle. The first-generation DAAs were the protease inhibitors boceprevir and telaprevir, which were indicated mainly for the treatment of chronic hepatitis C patients infected by genotype 1 virus. Boceprevir is an inhibitor of HCV NS3/4A protease, an enzyme required for the proteolytic cleavage of HCV-encoded polyprotein into mature forms of the non-structural proteins NS4A, NS4B, NS5A and NS5B. Telaprevir is an NS3-4A protease inhibitor that competes with NS5A/5B for its substrate-binding site. The FDA classified both these first-generation DAAs in Pregnancy Category B<sup>[31,32]</sup>. In fact, neither boceprevir nor telaprevir negatively affected fetal development in animals (mice, rats and rabbits). Consequently, in the absence of well-controlled human studies, "no evidence of risk in humans" has been supposed. Nevertheless, the major limitation to the use of these drugs is that they must be administered in association with PEG-IFN and Ribavirin as part of a triple-therapy regimen. Consequently, both boceprevir and telaprevir are contraindicated during pregnancy and adequate contraceptive measures are strongly recommended for both childbearing women and their male sexual partners throughout treatment duration and up to 6 mo after withdrawal<sup>[31,32]</sup>. Lastly, the excretion of protease inhibitors into human breast milk remains to be clarified; the levels of these drugs in the milk of lactating rats can be higher than those observed in maternal blood<sup>[31,32]</sup>.

### Second-generation DAAs

The second-generation DAAs, which became available in 2015, and their principal pharmacokinetic properties are listed in Table 1. Pharmacokinetic data are not complete for all second-generation DAAs. In the absence of data on their properties and effects on pregnant and lactating human females, clinicians can only try to predict the effect that pregnancy-associated physiological changes may have on the peak plasma dose, drug metabolism, and the ability of the drug to cross the placental barrier and/or enter into the mother's milk. Generally, drugs

that are more likely to cross the placenta are lipids or weak acids with a molecular weight below 500 Da, are poorly bound to plasma proteins and have a long half-life. The concentration of the drug in breast milk, and therefore its potential effect on the newborn, depends on dosage, rate of absorption in the maternal circulation, maternal drug metabolism and the time from drug administration to breastfeeding<sup>[33,34]</sup>. In the following section we will briefly review the data on the pharmacokinetics and teratogenicity in animals of the DAAs currently available to try to identify the ones that could potentially be used in childbearing women.

Sofosbuvir (Sovaldi®) is a pangenotypic nucleotide prodrug converted by hepatocytes into its active form that acts by competitively inhibiting the HCV NS5B polymerase active site and thus blocking viral RNA synthesis. It is indicated for the treatment of chronic hepatitis C as a component of a combination antiviral regimen. Neither the area under the curve (AUC) nor the product's absorption changes when the drug is taken with food, which suggests that the prolonged gastric emptying observed in pregnancy would not affect absorption of the drug or the time-to-peak plasma dose. Sofosbuvir is readily available after oral administration, and undergoes extensive first pass metabolism. Gender does not appear to significantly affect its pharmacokinetics<sup>[35]</sup>. Since P450 enzymes do not seem to be involved in metabolizing Sofosbuvir, increased activity of these enzymes in pregnancy is unlikely to affect its plasma concentration. On the other hand, Sofosbuvir has strong affinity for the P-glycoprotein efflux protein (Table 1). The drug is eliminated as GS-331007 in urine. The glomerular filtration rate usually increases during pregnancy and consequently renal drug elimination is generally greater than elimination in the non-pregnant state; however, it is unclear whether this process could alter the plasma concentration of Sofosbuvir to the point of requiring dose adjustment to attain a clinical response. Similarly, it is unclear whether the drug could cross the placental barrier. In studies conducted on animals (rats and rabbits), Sofosbuvir



**Table 2** Effect of new direct-acting antivirals in pregnancy and Food and Drug Administration Pregnancy Categories

Drug		Embryotoxicity and/or teratogenicity <sup>1</sup>	Dose-escalation <sup>2</sup>	Transfer across placenta	Transfer into milk	FDA Pregnancy Category <sup>3</sup>
Sofosbuvir		No	28-fold	Yes	Yes	B
Simeprevir		Yes	4-fold	Yes	Yes	C
Daclatasvir		Yes	4-fold	Yes	Yes	NA <sup>4</sup>
Ledipasvir		No	Maternal toxic doses	Yes	Yes	B
Viekirax	Ombitasvir	Yes	4-fold	Minimal	Yes	B
	Paritaprevir		32-fold			
	Ritonavir		8-fold			
Dasabuvir		No	48-fold	Minimal	Yes	B

<sup>1</sup>Based on animal studies; <sup>2</sup>Dose escalation above therapeutic dose; <sup>3</sup>FDA Pregnancy Category without association with ribavirin. NA: Not available; FDA: The Food and Drug Administration.

metabolites crossed the placenta and entered the milk of lactating animals. However, this process did not appear to significantly affect the viability or the development of embryos or fetuses<sup>[34,35]</sup>. Little is known regarding the use of Sofosbuvir in pregnant women. The outcomes of less than 300 pregnancies are mentioned in the product characteristics reports of the European Medical Agency, but no data about those outcomes are available on the Pubmed database. The FDA classified Sofosbuvir in Pregnancy Category B when used alone or with Ledipasvir, and in Pregnancy Category X when used in combination with Ribavirin. The latter combination is strongly contraindicated during pregnancy and adequate contraceptive measures are highly recommended for both childbearing women and their male sexual partners throughout treatment duration and up to 6 mo after treatment withdrawal (Table 2)<sup>[36]</sup>.

Simeprevir (Olysio®) is a specific NS3/4A HCV serine protease inhibitor that interrupts the processing of the HCV-encoded polypeptide thereby blocking the HCV viral life cycle. Simeprevir is considered a second-generation HCV protease inhibitor because its binding affinity and specificity for NS3/4A is higher than that of first-generation protease inhibitors that have a linear structure. It has been approved as part of combination regimens with PEG-IFN and Ribavirin or with Sofosbuvir for the treatment of chronic hepatitis C genotype 1 infection in adults. When Simeprevir is taken with food, its absorption is delayed so that its bioavailability reaches 62% (Table 1). It is therefore possible that the prolongation of gastric emptying observed in pregnancy may also affect absorption and the time-to-peak plasma dose of Simeprevir. After its absorption, Simeprevir undergoes first-pass metabolism by the P450 cytochrome enzymes, mainly the CYP3A4 system (Table 1). It is also a substrate of the P-glycoprotein drug transporters. Plasma levels of Simeprevir change significantly when administered with inducers or inhibitors of CYP3A4. Plasma exposure of simeprevir is greatly affected also by the state of the liver, and there may be an increase of up to 5-fold in the AUC depending on the degree of hepatic impairment. Therefore, the increased activity of the P450 enzymes in pregnancy, and the possible physiopathological changes that may

affect the liver of pregnant women may affect the plasma concentration of Simeprevir. Metabolites of Simeprevir are mainly eliminated *via* biliary excretion. Gender did not appear to have a clinically relevant role on the pharmacokinetics of Simeprevir.

As yet, there are no data concerning the passage of Simeprevir across the human placenta (Table 2), however, animal studies established that the drug is transferred across the placenta, and that it exerts teratogenic effects on the foetal skeletal system, namely supernumerary ribs and delayed ossification at exposures 4-fold higher than those observed at the recommended dose (Table 2). Moreover, Simeprevir can be excreted in the milk of lactating animals. The drug is classified in FDA Pregnancy Category C when administered alone, and Pregnancy Category X when used in combination with Ribavirin<sup>[37-39]</sup>.

Daclatasvir (Daklinza®) inhibits the NS5A protein (Table 1), and appears to act on viral replication, and on the assembly and secretion stages of the viral life cycle, thereby causing a rapid decline in both intra- and extracellular levels of HCV RNA. It is the first NS5A complex inhibitor approved for use in the European Union as part of combined regimens with Sofosbuvir, Ribavirin and PEG-IFN for the treatment of chronic HCV infection in adults. Oral clearance (CL/F) of Daclatasvir is significantly lower in women than in men<sup>[40]</sup>. However, this gender difference does not appear to be clinically relevant. It remains unclear whether the documented non-significant gender difference in oral clearance, and the expected changes in drug bioavailability and clearance in the pregnant state may, together, significantly affect Daclatasvir exposure in pregnant women.

Daclatasvir is a substrate of P-glycoprotein and is metabolized by the CYP3A4 enzyme (Table 1). Dose adjustments are recommended when it is administered with strong inducers of this class of cytochrome enzymes. It is therefore likely that the increased activity of the P450 enzymes in pregnancy would affect the plasma concentration of Daclatasvir.

Daclatasvir is primarily excreted unchanged through the biliary route. Overall, based on its chemical characteristics, it is unlikely that Daclatasvir could cross the materno-fetal circulation at therapeutic doses. However,

Daclatasvir was found to cross the placenta in a study conducted in rats and rabbits<sup>[40]</sup> (Table 2). In the latter study, there was a decrease in the gestational weight of mothers exposed to the drug. Daclatasvir exerted an embryotoxic and teratogenic effect at exposures 4-fold to 16-fold higher than the clinical AUC exposure, and the potential toxic exposure was exponentially greater with the increase in the animals' body surface area<sup>[40]</sup>. In other studies, Daclatasvir was excreted in the milk of lactating animals at concentrations 1.7- to 2-fold higher than maternal plasma concentrations<sup>[40,41-43]</sup>. Daclatasvir has recently received FDA approval for marketing in the United States. At the time of writing this article, it has not been included in a Pregnancy Category.

Ledipasvir is available in a combined formulation with Sofosbuvir called Harvoni. Harvoni is administered alone or in combination with Ribavirin in patients with chronic hepatitis C infection<sup>[2]</sup>. Ledipasvir acts on the replication, assembly and secretion phases of HCV by inhibiting HCV NS5A phosphoprotein<sup>[44]</sup>. Based on the limited data available, Ledipasvir acts only on genotypes 1, 3 and 4. It is gradually absorbed after oral administration; the AUC does not appear to be affected when the drug is administered with meals. Moreover, Ledipasvir does not appear to undergo significant first pass and/or pre-excretory metabolism and it is mainly excreted unchanged through the biliary route, in faeces. Like Sofosbuvir, Ledipasvir is not metabolized by the P450 enzymes. It is therefore unlikely that increased activity of these enzymes in pregnancy affects its plasma concentration. Slow oxidative metabolism of Ledipasvir into M19 has been demonstrated *in vivo*, although the mechanism underlying this process is unknown. However, it is not possible to make any assumption regarding changes in this particular metabolic route in pregnant women. Both the AUC and C-max of Ledipasvir appear to be greater in females than in males, but this difference has not been considered clinically significant by the regulating authorities<sup>[44]</sup> (Table 1).

Studies conducted with animals showed that Ledipasvir crosses the placenta and is excreted in the milk of lactating animals. In non pregnant animals, the number of corpora lutea and implantation sites were decreased with a 6-fold increase in exposure, while in pregnant animals the effects on offspring, *i.e.*, mainly alterations in body weight, were observed at a concentration 4-fold higher than the recommended clinical dosage<sup>[44]</sup>. The FDA categorized Ledipasvir in the Pregnancy Category B when used with Sofosbuvir without Ribavirin<sup>[42-44]</sup> (Table 2).

Viekirax<sup>®</sup> is a combination formulation composed of three pharmacologically active substances, namely Ombitasvir, Paritaprevir and Ritonavir. The combination acts on different steps of the HCV lifecycle: Ombitasvir inhibits HCV NS5A and Paritaprevir inhibits HCV NS3/4A, while Ritonavir, which does not directly affect HCV, acts as a booster of Paritaprevir through its inhibitory effect on CYP3A. Viekirax is indicated only in combination with Ribavirin and/or Dasabuvir (see below) for the treatment

of chronic hepatitis C in adults. The combination reaches T-max 4-5 h after oral administration and requires up to 12 d of dosing to reach steady state<sup>[45,46]</sup>. Exposures of the individual components are affected by drug-to-drug interactions, even with the other components of Viekirax and with Dasabuvir. Food also significantly affects Viekirax absorption. In fact, absorption of the drug is much lower when administered in the fasting state. All three components are highly-bound to plasma proteins and undergo extensive hepatic metabolism. Notably, Paritaprevir is predominantly metabolized by CYP3A4, and therefore requires boosting with Ritonavir, which is also metabolized by the same enzyme. The components of the combination have different half-lives: Ombitasvir has the longest half-life, around 21-25 h and is mainly excreted by the biliary route. Paritaprevir and Ritonavir have a mean half-life of 5.5 and 4 h, respectively and are excreted mainly in faeces with only a small proportion being eliminated renally (8.8% for Paritaprevir and 11.3% for Ritonavir). Since exposures of the three individual components of Viekirax do not seem to vary significantly irrespectively of the degree of renal impairment, therefore renal elimination does not appear to be significant. Exposure of all the three active components of Viekirax is related to gender. In fact, concentrations of Ombitasvir and Paritaprevir were found to be 0.5- and 1-fold higher, respectively in women<sup>[47]</sup>. Moreover, exposure of Ombitasvir was found to be related to body weights. Body weight also affects Ombitasvir exposure but not Paritaprevir exposure (Table 1).

Both Ombitasvir and Paritaprevir/Ritonavir caused malformations in the eyes and teeth of animals at exposures 4-fold higher than the AUC. In the case of Paritaprevir/Ritonavir, an exposure 32/8-fold higher than those observed at the recommended dose resulted in malformation in the offspring of animals, again involving the eyes. Passage of Ombitasvir and Paritaprevir metabolites in the milk of lactating animals, and to a lesser extent through the placenta, has been demonstrated, but no effect was observed in lactating pups. The FDA categorized Viekirax in Pregnancy Category B<sup>[45-47]</sup> (Table 2).

Dasabuvir (Exviera<sup>®</sup>) is a non-nucleoside inhibitor of the HCV RNA-dependent RNA polymerase. It is indicated for the treatment of chronic hepatitis C infection in adults only in combination with Viekirax, thereby forming the "Viekira pak". Dasabuvir reaches T-max 4-5 h after oral administration. Viekira pak reaches steady state after 12 d of dosing. Like Viekirax, Dasabuvir must be administered with food. In fact, taken with food, its exposure is 30% higher than in the fasting state. It is metabolised by the P450 enzymes, namely CYP2C8 and to a lesser extent by CYP3A. Its metabolites are mainly eliminated through the biliary route. Exposure is 30% higher in women than in men. Also Dasabuvir exposure is affected by body weight and by impairment of renal and hepatic functions, albeit not in a clinically significant way (Table 1).

At doses of Dasabuvir 48-fold higher than the maximum recommended dose, Dasabuvir did not cause any embryocidal and/or teratogenic effects in animals<sup>[47]</sup>. The drug was excreted in the milk of lactating animals probably by the breast cancer resistance protein efflux transporter of which Dasabuvir is a substrate. However, the drug did not affect nursing pups. The FDA categorized Dasabuvir in Pregnancy Category B<sup>[45,46,48]</sup> (Table 2).

## CONCLUSION

Given the lack of human studies, no DAA has yet been approved for use in pregnancy or during breast feeding. Consequently, we have reviewed the features of the DAAs approved for treatment of chronic HCV infection in adults in the attempt to identify the most promising candidates, in terms of pharmacokinetic profile and adverse effects, for use in pregnancy or during breast feeding. Sofosbuvir appears to have a favourable pharmacokinetic profile and animal studies indicate that it may be safe during pregnancy. Thus, Sofosbuvir, used in Ribavirin-free regimens, may become the drug of choice for women of childbearing age affected by HCV infection. On the contrary, Simeprevir is not suitable for use in pregnant or breast-feeding women, because its AUC and half-life are greatly affected by liver performance and by drug-drug interactions. Moreover, Simeprevir was associated with teratogenic effects in animals at doses only 4-fold higher than recommended doses. Ledipasvir has a highly favourable pharmacokinetic profile, and too moreover was safe in animal embryos and fetuses. Consequently, its combined formulation with Sofosbuvir (Harvoni), appears to be a good choice in women of child-bearing potential. Daclatasvir, based on its pharmacokinetic profile, appears to have a wide safety margin when used at therapeutic levels. It also appears that dosages may have to be increased in pregnant women. However, in contrast to its expected safety, it was found to cross the placenta and exert a teratogenic effect in animals. It is still awaiting FDA pregnancy categorization.

Although the Ombitasvir/Paritaprevir/Ritonavir combination is in FDA Pregnancy Category B, the pharmacokinetic profile of the individual components, namely absorption and affinity for P450 enzymes, suggest a potential for variability in AUC exposures with the physiological changes of pregnancy to the point that dose adjustment may be required. Furthermore, the components of the combination exerted a teratogenic effect on animals. Lastly, given its indication for use in combination with Ribavirin, it would not be suitable for women of childbearing potential. Dasabuvir is supposed to be relatively safe in pregnancy based on its pharmacokinetic profile and animal studies.

Until very recently infection with HCV genotype 2 would have posed a further treatment challenge in infected pregnant women, because all the recommended regimens for its treatment included Ribavirin. Even

though Italian authorities have very recently approved the use of Daclatasvir for the treatment of adult patients chronically infected by HCV genotype 2 in Ribavirin-free regimen associated with Sofosbuvir<sup>[49]</sup>, a safety data on pregnant women are lacking.

In conclusion, second-generation anti-HCV DAAs have revolutionized the standard of care and prognosis of patients suffering from chronic hepatitis C infection, however, childbearing women cannot benefit from this advance. As concluded by other authors<sup>[4]</sup>, despite promising safety profiles, there are no approved therapies to prevent vertical HCV transmission. Therefore the only achievable goal seems to be universal screening of fertile women to identify and treat those with HCV infection before they become pregnant.

Lastly, it would be useful to create a registry similar to the Ribavirin Pregnancy Registry in order to monitor the effect of the second-generation DAAs on women who become pregnant during therapy, in terms of outcome on both the mother and the product of conception.

## REFERENCES

- 1 **World Health Organization.** Diabetes. Fact sheet N°312. [Updated 2015 Jan; accessed 2015 Nov 18]. Available from: URL: <http://www.who.int/mediacentre/factsheets/fs312/en/index.html>
- 2 **European Association for Study of Liver.** EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol* 2015; **63**: 199-236 [PMID: 25911336 DOI: 10.1016/j.jhep.2015.03.025]
- 3 **World Health Organization.** Global Alert and Response (GAR). Hepatitis C virus. [Accessed 2015 Aug 5]. Available from: URL: <http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index4.html>
- 4 **Kanninen TT, Dieterich D, Ascutti S.** HCV vertical transmission in pregnancy: New horizons in the era of DAAs. *Hepatology* 2015; **62**: 1656-1658 [PMID: 26238474 DOI: 10.1002/hep.28032]
- 5 **American College of Obstetricians and Gynecologists.** ACOG Practice Bulletin No. 86: Viral hepatitis in pregnancy. *Obstet Gynecol* 2007; **110**: 941-956 [PMID: 17906043]
- 6 **Jhaveri R.** Diagnosis and management of hepatitis C virus-infected children. *Pediatr Infect Dis J* 2011; **30**: 983-985 [PMID: 21997662 DOI: 10.1097/INF.0b013e318236ac37]
- 7 **Dunkelberg JC, Berkley EM, Thiel KW, Leslie KK.** Hepatitis B and C in pregnancy: a review and recommendations for care. *J Perinatol* 2014; **34**: 882-891 [PMID: 25233195 DOI: 10.1038/jp.2014.167]
- 8 **Tosone G, Maraolo AE, Mascolo S, Palmiero G, Tambaro O, Orlando R.** Vertical hepatitis C virus transmission: Main questions and answers. *World J Hepatol* 2014; **6**: 538-548 [PMID: 25232447 DOI: 10.4254/wjh.v6.i8.538]
- 9 **Kim WR.** The burden of hepatitis C in the United States. *Hepatology* 2002; **36**: S30-S34 [PMID: 12407574 DOI: 10.1053/jhep.2002.36791]
- 10 **Smith BD, Morgan RL, Beckett GA, Falck-Ytter Y, Holtzman D, Ward JW.** Hepatitis C virus testing of persons born during 1945-1965: recommendations from the Centers for Disease Control and Prevention. *Ann Intern Med* 2012; **157**: 817-822 [PMID: 22910836 DOI: 10.7326/0003-4819-157-9-201211060-00529]
- 11 **Kopilović B, Poljak M, Seme K, Klavs I.** Hepatitis C virus infection among pregnant women in Slovenia: study on 31,849 samples obtained in four screening rounds during 1999, 2003, 2009 and 2013. *Euro Surveill* 2015; **20**: 21144 [PMID: 26062646 DOI: 10.2807/1560-7917.ES2015.20.22.21144]
- 12 **Yeung CY, Lee HC, Chan WT, Jiang CB, Chang SW, Chuang CK.** Vertical transmission of hepatitis C virus: Current knowledge and perspectives. *World J Hepatol* 2014; **6**: 643-651 [PMID: 25276280]

- DOI: 10.4254/wjh.v6.i9.643]
- 13 **El-Kamary SS**, Hashem M, Saleh DA, Ehab M, Sharaf SA, El-Mougy F, Abdelsalam L, Jhaveri R, Aboulnasr A, El-Ghazaly H. Reliability of risk-based screening for hepatitis C virus infection among pregnant women in Egypt. *J Infect* 2015; **70**: 512-519 [PMID: 25623176 DOI: 10.1016/j.jinf.2015.01.009]
  - 14 **Khamis HH**, Farghaly AG, Shatat HZ, El-Ghitany EM. Prevalence of hepatitis C virus infection among pregnant women in a rural district in Egypt. *Trop Doct* 2016; **46**: 21-27 [PMID: 25515736 DOI: 10.1177/0049475514561330]
  - 15 **Gasim GI**, Murad IA, Adam I. Hepatitis B and C virus infections among pregnant women in Arab and African countries. *J Infect Dev Ctries* 2013; **7**: 566-578 [PMID: 23949291 DOI: 10.3855/jidc.3243]
  - 16 *Gazzetta Ufficiale Repubblica Italiana*. Serie generale, 1998: 20
  - 17 **Talal AH**, LaFleur J, Hoop R, Pandya P, Martin P, Jacobson I, Han J, Korner EJ. Absolute and relative contraindications to pegylated-interferon or Ribavirin in the US general patient population with chronic hepatitis C: results from a US database of over 45 000 HCV-infected, evaluated patients. *Aliment Pharmacol Ther* 2013; **37**: 473-481 [PMID: 23289640 DOI: 10.1111/apt.12200]
  - 18 **Arshad M**, El-Kamary SS, Jhaveri R. Hepatitis C virus infection during pregnancy and the newborn period--are they opportunities for treatment? *J Viral Hepat* 2011; **18**: 229-236 [PMID: 21392169 DOI: 10.1111/j.1365-2893.2010.01413.x]
  - 19 **Valladares G**, Chacaltana A, Sjogren MH. The management of HCV-infected pregnant women. *Ann Hepatol* 2010; **9** Suppl: 92-97 [PMID: 20714003]
  - 20 **Food and Drug Administration**. U.S. National Library of Medicine Interferon Alfa. [Accessed 2015 Nov 18]. Available from: URL: <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=4c918b02-f158-4f7c-8ecc-fd49574ec228>
  - 21 **Food and Drug Administration**. U.S. National Library of Medicine. [Accessed 2015 Nov 18]. Available from: URL: <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=fd653d74-48ab-49e4-a42d-ec1cbc59badb>
  - 22 **Yazdani Brojeni P**, Matok I, Garcia Bournissen F, Koren G. A systematic review of the fetal safety of interferon alpha. *Reprod Toxicol* 2012; **33**: 265-268 [PMID: 22200624 DOI: 10.1016/j.reprotox.2011.11.003]
  - 23 **Food and Drug Administration**. U.S. National Library of Medicine PEGASYS-peginterferon alfa-2a PEGASYS-peginterferon alfa-2a injection, solution. [Accessed 2015 Nov 18]. Available from: URL: <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=de61685e-2b8c-4e22-84bb-869e13600440>
  - 24 **Food and Drug Administration**. U.S. National Library of Medicine PEGINTRON-peginterferon alfa-2b. [Accessed 2015 Nov 18]. Available from: URL: <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=0a8f3137-0e3a-4a60-a872-cb7d761b30e1>
  - 25 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]
  - 26 **Food and Drug Administration**. U.S. National Library of Medicine Ribavirin. [Accessed 2015 Nov 18]. Available from: URL: <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=d370635f-5530-4d42-a019-76b61639787>
  - 27 **Food and Drug Administration**. U.S. National Library of Medicine REBETOL- Ribavirin capsule. [Accessed 2015 Nov 18]. Available from: URL: <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=04d2b6f4-bd9b-4871-9527-92c81aa2d4d0>
  - 28 **Roberts SS**, Miller RK, Jones JK, Lindsay KL, Greene MF, Maddrey WC, Williams IT, Liu J, Spiegel RJ. The Ribavirin Pregnancy Registry: Findings after 5 years of enrollment, 2003-2009. *Birth Defects Res A Clin Mol Teratol* 2010; **88**: 551-559 [PMID: 20564430 DOI: 10.1002/bdra.20682]
  - 29 **Hofer H**, Donnerer J, Sator K, Staufer K, Scherzer TM, Dejado C, Sator M, Kessler H, Ferenci P. Seminal fluid Ribavirin level and functional semen parameters in patients with chronic hepatitis C on antiviral combination therapy. *J Hepatol* 2010; **52**: 812-816 [PMID: 20399525 DOI: 10.1016/j.jhep.2009.12.039]
  - 30 **Pecou S**, Moinard N, Walschaerts M, Pasquier C, Daudin M, Bujan L. Ribavirin and pegylated interferon treatment for hepatitis C was associated not only with semen alterations but also with sperm deoxyribonucleic acid fragmentation in humans. *Fertil Steril* 2009; **91**: 933.e17-933.e22 [PMID: 18930227 DOI: 10.1016/j.fertnstert.2008.07.1755]
  - 31 **Food and Drug Administration**. U.S. National Library of Medicine VICTRELIS- boceprevir capsule. [Accessed 2015 Nov 18]. Available from: URL: <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=ae879ebe-b620-4829-b6f8-74b58da1c771>
  - 32 **Food and Drug Administration**. U.S. National Library of Medicine, National Institutes of Health, DailyMed. Incivek (telaprevir) tablet, film coated. Vertex Pharmaceuticals. [Accessed 2015 Nov 18]. Available from: URL: <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=ed0e4f33-cf21-4fe3-918d-1d5b3a23eeec4>
  - 33 **American Academy of Pediatrics Committee on Drugs**. Transfer of drugs and other chemicals into human milk. *Pediatrics* 2001; **108**: 776-789 [PMID: 11533352]
  - 34 **Food and Drug Administration**. U.S. National Library of Medicine. National Center for Biotechnology Information. Compound Summary for CID 45375808 Sofosbuvir. [Accessed 2015 Nov 18]. Available from: URL: <http://pubchem.ncbi.nlm.nih.gov/compound/45375808>
  - 35 **Food and Drug Administration**. European Medicines Agency - Science Medicine Health. Sovaldi, INN-Sofosbuvir summary of product characteristics. [Accessed 2015 Nov 18]. Available from: URL: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_Product\\_Information/human/002798/WC500160597.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Product_Information/human/002798/WC500160597.pdf)
  - 36 **Food and Drug Administration**. U.S. Food and Drug Administration. Sovaldi highlights of prescribing information. [Accessed 2015 Nov 18]. Available from: URL: [http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2015/204671s004lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/204671s004lbl.pdf)
  - 37 **Food and Drug Administration**. U.S. National Library of Medicine. National Center for Biotechnology Information. Compound Summary for CID 57956385 Olysio. [Accessed 2015 Nov 18]. Available from: URL: <http://pubchem.ncbi.nlm.nih.gov/compound/57956385>
  - 38 **Food and Drug Administration**. U.S. Food and Drug Administration Olysio Highlights of prescribing information. [Accessed 2015 Nov 18]. Available from: URL: <http://www.janssentherapeutics.com/shared/product/olysio/prescribing-information.pdf>
  - 39 **European Medicines Agency - Science Medicine Health**. Olysio summary of product characteristics. [Accessed 2015 Nov 18]. Available from: URL: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_Product\\_Information/human/002777/WC500167867.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Product_Information/human/002777/WC500167867.pdf)
  - 40 **European Medicines Agency - Science Medicine Health**. Daklinza summary of product characteristics. [Accessed 2015 Nov 18]. Available from: URL: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_Product\\_Information/human/003768/WC500172848.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Product_Information/human/003768/WC500172848.pdf)
  - 41 **Food and Drug Administration**. U.S. National Library of Medicine. National Center for Biotechnology Information. Compound Summary for CID 25154714 Daclatasvir. [Accessed 2015 Nov 18]. Available from: URL: <http://pubchem.ncbi.nlm.nih.gov/compound/Daclatasvir>
  - 42 **Food and Drug Administration**. U.S. National Library of Medicine. National Center for Biotechnology Information. Compound Summary for CID 72734365 Sofosbuvir/Ledipasvir. [Accessed 2015 Nov 18]. Available from: URL: <http://pubchem.ncbi.nlm.nih.gov/compound/72734365>
  - 43 **Food and Drug Administration**. U.S. Food and Drug Administration. Harvoni highlights of prescribing information. [Accessed 2015 Nov 18]. Available from: URL: [http://www.gilead.com/media/Files/pdfs/medicines/liver-disease/harvoni/harvoni\\_pi.pdf](http://www.gilead.com/media/Files/pdfs/medicines/liver-disease/harvoni/harvoni_pi.pdf)
  - 44 **European Medicines Agency - Science Medicine Health**. Harvoni summary of product characteristics. [Accessed 2015 Nov 18]. Available from: URL: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_Product\\_Information/human/003850/WC500177995.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Product_Information/human/003850/WC500177995.pdf)



- 45 **Food and Drug Administration.** U.S. National Library of Medicine. National Center for Biotechnology Information. Compound Summary for CID 86291595 Viekira Pak. [Accessed 2015 Nov 18]. Available from: URL: <http://pubchem.ncbi.nlm.nih.gov/compound/86291595>
- 46 **Food and Drug Administration.** U.S. Food and Drug Administration. Viekira Pak highlights of prescribing information. [Accessed 2015 Nov 18]. Available from: URL: [http://www.rxabbvie.com/pdf/viekirapak\\_pi.pdf](http://www.rxabbvie.com/pdf/viekirapak_pi.pdf)
- 47 **European Medicines Agency - Science Medicine Health.** Viekirax summary of product characteristics. [Accessed 2015 Nov 18]. Available from: URL: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_\\_Product\\_Information/human/003839/WC500183997.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR__Product_Information/human/003839/WC500183997.pdf)
- 48 **Sulkowski MS,** Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, McPhee F, Sherman D, Hindes R, Symonds W, Pasquinelli C, Grasela DM. Daclatasvir plus Sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med* 2014; **370**: 211-221 [PMID: 24428467 DOI: 10.1056/NEJMoa1306218]
- 49 **Agenzia Italiana del Farmaco.** AIFA Unita Coordinamento Segreteria Organismi Collegiali Esiti Ufficio Ricerca e Sperimentazioni Cliniche CTS. Available from: URL: [http://www.agenziafarmaco.gov.it/sites/default/files/esiti\\_SPER\\_CTS\\_nov2015.pdf](http://www.agenziafarmaco.gov.it/sites/default/files/esiti_SPER_CTS_nov2015.pdf)

**P- Reviewer:** Jin B, Picardi A, Snyder N **S- Editor:** Qiu S  
**L- Editor:** A **E- Editor:** Liu SQ



## Retrospective Study

# Predictors of fifty days in-hospital mortality in decompensated cirrhosis patients with spontaneous bacterial peritonitis

Chinmaya Kumar Bal, Ripu Daman, Vikram Bhatia

Chinmaya Kumar Bal, Ripu Daman, Vikram Bhatia, Department of Hepatology, Institute of Liver and Biliary Science, New Delhi 110070, India

**Author contributions:** Bal CK contributed to conception and study design, acquisition of data, statistical analysis and interpretation of data, preparation of graphs and final approval of article; Daman R contributed to data collection, drafting and final approval of manuscript; Bhatia V contributed to conception and study design, drafting and the final approval of manuscript.

**Institutional review board statement:** This study was reviewed and approved by the Institutional Ethics Committee of Institute of Liver and Biliary Sciences, dated June 5<sup>th</sup> 2013.

**Informed consent statement:** The entire study participants, or their legal guardians, have provided written informed consent prior to study enrolment.

**Conflict-of-interest statement:** There is no conflict of interest in this study.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Chinmaya Kumar Bal, MBBS, Department of Hepatology, Institute of Liver and Biliary Science, Sector D1, Vasant Kunj, New Delhi 110070, India. [chinmaya.bal@gmail.com](mailto:chinmaya.bal@gmail.com)  
 Telephone: +91-11-46300000  
 Fax: +91-11-4630001

Received: January 12, 2016

Peer-review started: January 14, 2016

First decision: February 2, 2016

Revised: February 28, 2016

Accepted: April 7, 2016

Article in press: April 11, 2016

Published online: April 28, 2016

## Abstract

**AIM:** To determine the predictors of 50 d in-hospital mortality in decompensated cirrhosis patients with spontaneous bacterial peritonitis (SBP).

**METHODS:** Two hundred and eighteen patients admitted to an intensive care unit in a tertiary care hospital between June 2013 and June 2014 with the diagnosis of SBP (during hospitalization) and cirrhosis were retrospectively analysed. SBP was diagnosed by abdominal paracentesis in the presence of polymorphonuclear cell count  $\geq 250$  cells/mm<sup>3</sup> in the peritoneal fluid. Student's *t* test, multivariate logistic regression, cox proportional hazard ratio (HR), receiver operating characteristics (ROC) curves and Kaplan-Meier survival analysis were utilized for statistical analysis. Predictive abilities of several variables identified by multivariate analysis were compared using the area under ROC curve.  $P < 0.05$  were considered statistical significant.

**RESULTS:** The 50 d in-hospital mortality rate attributable to SBP is 43.11% ( $n = 94$ ). Median survival duration for those who died was 9 d. In univariate analysis acute kidney injury (AKI), hepatic encephalopathy, septic shock, serum bilirubin, international normalized ratio, aspartate transaminase, and model for end-stage liver disease - sodium (MELD-Na) were significantly associated with in - hospital mortality in patients with SBP ( $P \leq 0.001$ ). Multivariate cox

proportional regression analysis showed AKI (HR = 2.16, 95%CI: 1.36-3.42,  $P = 0.001$ ) septic shock (HR = 1.73, 95%CI: 1.05-2.83,  $P = 0.029$ ) MELD-Na (HR = 1.06, 95%CI: 1.02-1.09,  $P \leq 0.001$ ) was significantly associated with 50 d in-hospital mortality. The prognostic accuracy for AKI, MELD-Na and septic shock was 77%, 74% and 71% respectively associated with 50 d in-hospital mortality in SBP patients.

**CONCLUSION:** AKI, MELD-Na and septic shock were predictors of 50 d in-hospital mortality in decompensated cirrhosis patients with SBP.

**Key words:** Decompensated cirrhosis; Acute kidney injury; Model for end-stage liver disease sodium; Septic shock; Spontaneous bacterial peritonitis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Spontaneous bacterial peritonitis (SBP) is associated with poor prognosis especially with in-hospital patients. The mortality rate ranges from 20%-40%. The model for end-stage liver disease (MELD) has been suggested as a predictor of the in-hospital mortality in patients with SBP. However, the role of other predictors has not been established. The goal of this study is to identify other prognostic factors for mortality in decompensated cirrhotic patients with SBP and to evaluate the predictive power of acute kidney injury, MELD-sodium and septic shock.

Bal CK, Daman R, Bhatia V. Predictors of fifty days in-hospital mortality in decompensated cirrhosis patients with spontaneous bacterial peritonitis. *World J Hepatol* 2016; 8(12): 566-572 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i12/566.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i12.566>

## INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is defined as acute infection of ascitic fluid without any identifiable surgically treatable intra-abdominal source<sup>[1]</sup>. SBP is a major complication of decompensated cirrhosis with ascites<sup>[2]</sup>. The SBP is diagnosed by abdominal paracentesis with an elevated ascitic fluid neutrophil count ( $\geq 250$  cells/mm<sup>3</sup>) and/or a positive ascitic fluid culture. In up to 60% cases, gram-negative bacteria (*Escherichia coli* or *Klebsiella pneumonia*) are most prevalent organism involved<sup>[3]</sup>. In about 25% of the cases, gram-positive bacteria (mainly *Streptococcus species* and *Enterococci*) are involved<sup>[3]</sup>. The prevalence of SBP is up to 30% in hospitalized cirrhotic patients with ascites<sup>[4]</sup>. Despite intensive management, the in-hospital mortality remains between 20%-40%<sup>[5]</sup>. Model for end-stage liver disease (MELD) scores have been investigated with their predictive accuracy; however it is vulnerable to variations in laboratory measurements, making their utili-

zation in prediction of SBP related in-hospital mortality affected<sup>[6,7]</sup>. In addition, decompensated cirrhotics with major complication like SBP may have low MELD scores with high mortality<sup>[7]</sup>. Acute kidney injury (AKI) is common in patients with decompensated cirrhosis with ascites. AKI in cirrhosis was diagnosed by AKI network (AKIN) based on serum creatinine/urine output. AKI can be used to predict mortality in decompensated cirrhotic with ascites<sup>[8]</sup>. SBP-associated septic shock carries significant mortality in cirrhosis<sup>[9]</sup>. Thus this study aimed to have a comprehensive approach to determine possible prognostic factors predicting SBP related in-hospital mortality, compare the predictive power of AKI, MELD-sodium (MELD-Na), and septic shock and to identify the best cut-off point of MELD-Na scores to predict 50 d in-hospital mortality.

## MATERIALS AND METHODS

### Patients

Medical records of 218 adult patients admitted to hepatology intensive care unit (ICU) of the Institute of Liver and Biliary Sciences, New Delhi between June 2013 and June 2014 with the diagnosis of SBP (during hospitalization) and cirrhosis were reviewed. The study was approved by the Institutional Ethics Committee and the guidelines of Helsinki declaration were followed<sup>[10]</sup>. The Ethics Committee waived the requirement for the consent for data analysis.

The diagnosis of cirrhosis was based on clinical, laboratory and imaging findings. SBP was diagnosed by abdominal paracentesis in the presence of neutrophil count  $\geq 250$  cells/mm<sup>3</sup> in the ascitic fluid and the absence of the secondary features suggestive of secondary bacterial peritonitis<sup>[11]</sup>. We also required a positive culture.

Patient charts were retrospectively reviewed. Data include patient demographics, etiology, severity of liver disease, laboratory values, co-existing medical diagnoses (diabetes mellitus, hepatocellular carcinoma), medication use, organ failure, ascitic fluid analysis results, duration of ICU stay, and patient outcome. In the case of culture-positive infections, all microorganisms and their antibiotic susceptibility patterns were recorded. Most patients admitted to the ICU were referred after a variable antibiotic exposure; prior systemic or non-absorbable antibiotic data could not be collected.

The laboratory parameters [bilirubin, creatinine levels and international normalized ratio (INR)] at admission to intensive care unit were used to calculate MELD-Na score using the UNOS Internet site<sup>[12]</sup>. As a protocol, all patients admitted/transferred to the ICU with ascites, underwent an ascitic fluid analysis within 24 h of admission, in the absence of severe coagulopathy. Ascitic fluid was sent for albumin and cell count with differential and cultured by inoculation of 10 mL of ascitic fluid in blood culture bottles. Paired blood culture samples were also collected at admission in all patients. Antibiotic choice varied from patient to patient, and no standard first-line

drugs were used. The choice of antibiotic was decided based on previous antibiotic exposure of the patient before development of SBP, whether the patient was on quinolone prophylaxis, and physician discretion based on the perceived severity of patient illness. The antibiotic use was narrowed and modified as per the gram-stain and antibiotic sensitivity results. No patient underwent fluid restriction or hypertonic saline for management of dilution hyponatremia.

Renal dysfunction was defined by AKIN criteria<sup>[8]</sup> and managed by albumin infusions and intravenous terlipressin, with dose titrated as per response and tolerance. Intravenous albumin was used in all patients, with a minimal daily dose of 20 g and increased to up to 60 g/d<sup>[13]</sup>, titrated by clinical monitoring and hourly urine output. We did not stratify renal dysfunction into hepatorenal syndrome (HRS), and non-HRS. However, any cause of secondary renal dysfunction was actively investigated, with urine sediment, 24-h urine protein excretion, and bedside-renal ultrasound. All patients were evaluated daily by a nephrologist. Hepatic encephalopathy was treated with oral and rectal lactulose and rifaximin. No patient received neomycin. We suspected secondary peritonitis in patients with inadequate response to therapy, severe abdominal tenderness or when multiple organisms were identified in the ascitic fluid. These patients underwent a non-contrast computed tomography (CT) scan of abdomen.

American College of Chest Physicians/Society of Critical Care Medicine consensus conference criteria were used to diagnose septic shock<sup>[14]</sup>.

### Exclusion criteria

Patients with cirrhosis and ascites fluid polymorphonuclear cell (PMN) < 250 cells/mm<sup>3</sup>. Patients admitted from the community with SBP. Patients presented with ascites unrelated to cirrhosis. Patients with variceal haemorrhage advanced malignancy and human immunodeficiency virus.

### Statistical analysis

Stata version 14 for Windows was used for analysis. All the variables were normally distributed with equal variance. The continuous variables were described as mean  $\pm$  SD. The means of continuous variables were compared using student's *t* test. Categorical variables were described as proportions. The means of categorical variables were compared with logistics regression. Multivariate logistics regression was employed to analyse statistically significant variables. Cox proportional hazard model was used to analyse the hazard rates of the predictors adjusted by age and gender. The predictive accuracy of the prognostic variables like MELD-Na, AKI and septic shock was measured using receiver operating characteristics (ROC) curves. The best cut-off point for MELD-Na was created using acceptable sensitivity and specificity in the ROC analysis to determine 50 d in-hospital mortality risk. For each predictor variable, sensitivity, specificity, positive predictive value (PPV),

negative predictive values (NPV), positive likelihood ratio (+LR) and negative LR (-LR) were calculated to fit into the prognostic model. Two tailed *P* value < 0.05 was considered statistically significant. The power of the study was set at 80%. STROBE checklist for retrospective analysis was performed.

## RESULTS

Total of 218 patients with decompensated cirrhosis with ascites and SBP were included in the study. Two hundred and eleven (97%) patients were diagnosed with SBP for the first time and only 7 patients (0.03%) had previous episodes (more than once). The 50 d in-hospital mortality rate was 43.11% (*n* = 94). Median survival duration for those who died was 9 d. In univariate analysis AKI, hepatic encephalopathy, septic shock, total leucocyte count, serum bilirubin, INR, aspartate transaminase (SGOT), and MELD-Na were significantly associated with in-hospital mortality in patients with SBP (Table 1).

The baseline characteristics of the demographics, etiology, clinical and laboratory data is shown in Table 1. Mean age was 49.90  $\pm$  12.52 years and the male was predominant (83%). Most common etiology of liver cirrhosis was ethanol-induced (45.87%) followed by crypto/non-alcoholic fatty liver disease-NAFLD (28.9%). Hepatitis C virus related cirrhosis constitute only 11% in this study. A total of 109 subjects (50.0%) had hepatic encephalopathy with 59 deaths (62.77%), *P* = 0.001. Overall, 99 patients (45.11%) had AKI in hospitalized patients out of which 64 died (68.09%), *P* < 0.001. Compared with survivors the deceased had a higher proportion of septic shock (25.53% vs 3.23%), *P* < 0.001. Total leukocyte counts, bilirubin, INR, SGOT were significantly higher in the patients who died compared to the survivors. Mean MELD-Na score was higher among the deaths comparing to the survivors (30.59  $\pm$  6.62 vs 25.21  $\pm$  7.44) with statistical significance (*P* < 0.001). Child-Turcotte-Pugh (CTP) (B/C) score was not different among the groups. The mean CTP scores were high with mean 10.72 (SD: 1.82).

On multivariate regression analysis, AKI (*P* = 0.001), septic shock (*P* = 0.029), MELD-Na (*P* < 0.001) were found to be independent predictors of 50 d in-hospital mortality in patients with SBP (Table 2). Cox proportional hazard model showed the hazard ratio (HR) of AKI was 2.16 (95%CI: 1.36-3.42), septic shock (HR = 1.73, 95%CI: 1.05-2.83) and MELD-Na (HR = 1.1, 95%CI: 1.02-1.21). ROC curve for AKI, septic shock and MELD-Na had better prognostic accuracy for 50 d in-hospital mortality in patients with SBP (Figure 1). AKI had highest area under the curve (AUC) 0.77 (95%CI: 0.71-0.83), followed by MELD-Na (AUC: 0.74, 95%CI: 0.69-0.79), septic shock (AUC: 0.71, 95%CI: 0.65-0.77). Table 3 reports the sensitivity, specificity, PPV, NPV, +LR and -LR for these predictors. The cut off for MELD-Na derived from the ROC with the best ability to predict 50 d in-hospital mortality in decompensated cirrhotic patient with SBP was 28, with sensitivity 92.9%, specificity 60.3%, and NPV of 97.9%. The Kaplan-Meier



**Table 1** Baseline characteristics of the hospitalized patients with spontaneous bacterial peritonitis in decompensated cirrhosis

Variables	Overall (n = 218)	Survivors (n = 124)	Deaths (n = 94)	P value
Demographic data				
Age (yr) mean $\pm$ SD	49.90 $\pm$ 12.52	49.86 $\pm$ 13.37	49.96 $\pm$ 11.37	0.950
Male (%)	177 (81.19)	99 (79.84)	78 (82.98)	0.557
Etiology of cirrhosis (%)				
Ethanol	100 (45.87)	48 (38.71)	52 (55.32)	0.689
Crypto/NAFLD	63 (28.90)	38 (30.65)	25 (26.60)	0.104
HCV	23 (10.55)	16 (12.905)	7 (7.45)	0.068
Clinical data (%)				
Hepatocellular carcinoma	17 (7.80)	9 (7.26)	8 (8.51)	0.733
Diabetes	47 (21.56)	27 (21.77)	20 (21.28)	0.929
Acute kidney injury	99 (45.41)	35 (28.23)	64 (68.09)	< 0.001
Respiratory failure	10 (4.59)	6 (4.84)	4 (4.26)	0.978
Hepatic encephalopathy	109 (50.0)	50 (40.32)	59 (62.77)	0.001
Septic shock	28 (12.84)	4 (3.23)	24 (25.53)	< 0.001
Positive culture	48 (22.02)	21 (16.94)	27 (28.72)	0.038
Laboratory data <sup>1</sup> (mean $\pm$ SD)				
Ascitic neutrophil count (cells/mm <sup>3</sup> )	3346.07 $\pm$ 4700.60	3899.28 $\pm$ 5003.75	2616.30 $\pm$ 4182.81	0.040
Hemoglobin (g/dL)	9.42 $\pm$ 1.88	9.58 $\pm$ 1.77	9.21 $\pm$ 2.01	0.154
Platelet count (mmol/L)	128.24 $\pm$ 102.11	138.43 $\pm$ 111.25	115.03 $\pm$ 87.69	0.095
Leucocyte count (10 <sup>3</sup> / $\mu$ L)	13.30 $\pm$ 9.35	11.86 $\pm$ 8.65	15.17 $\pm$ 9.92	0.009
Sodium (mEq/L)	132.14 $\pm$ 7.69	132.50 $\pm$ 6.54	131.67 $\pm$ 9.01	0.454
Billirubin (mg/dL)	8.17 $\pm$ 8.81	5.85 $\pm$ 6.27	11.24 $\pm$ 10.61	< 0.001
Albumin (g/dL)	2.32 $\pm$ 0.50	2.35 $\pm$ 0.48	2.28 $\pm$ 0.52	0.250
INR	2.31 $\pm$ 1.11	2.09 $\pm$ 1.08	2.59 $\pm$ 1.08	0.001
AST (U/L)	59.66 $\pm$ 109.81	79.23 $\pm$ 98.71	171.3 $\pm$ 321.94	0.003
ALT (U/L)	59.66 $\pm$ 109.81	46.49 $\pm$ 72.93	77.04 $\pm$ 143.41	0.041
Urea (mg/dL)	70.31 $\pm$ 52.42	62.24 $\pm$ 48.23	80.94 $\pm$ 55.98	0.008
Creatinine (mg/dL)	1.67 $\pm$ 1.29	1.58 $\pm$ 1.39	1.80 $\pm$ 1.15	0.217
Scores (mean $\pm$ SD)				
CTP (B/C)	10.72 $\pm$ 1.82	10.50 $\pm$ 1.95	11.02 $\pm$ 1.60	0.034
MELD	24.79 $\pm$ 8.28	22.20 $\pm$ 7.59	28.20 $\pm$ 7.94	< 0.001
MELD-Na	27.53 $\pm$ 7.57	25.21 $\pm$ 7.44	30.59 $\pm$ 6.62	< 0.001

<sup>1</sup>Results obtained on the day of diagnosis of spontaneous bacterial peritonitis. MELD-Na: Model for end-stage liver disease-sodium; MELD: Model for end-stage liver disease; HCV: Hepatitis C virus; NAFLD: Non-alcoholic fatty liver disease; INR: International normalized ratio; AST: Aspartate aminotransferase; ALT: Alanineaminotransferase; CTP: Child-Turcotte-Pugh.

**Table 2** Cox proportional regression analysis of risk factors for spontaneous bacterial peritonitis related in-hospital related mortality

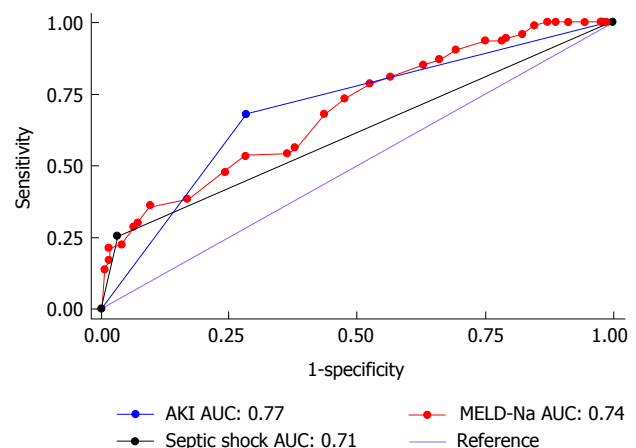
Variables	<sup>1</sup> HR (95%CI)	P value
AKI	2.16 (1.36-3.42)	0.001
Septic shock	1.73 (1.05-2.83)	0.029
MELD-Na	1.06 (1.02-1.09)	< 0.001

<sup>1</sup>Hazard ratio (HR) adjusted for age and gender. AKI: Acute kidney injury; MELD-Na: Model for end-stage liver disease-sodium.

**Table 3** Diagnostic accuracy of prognostic variables to predict spontaneous bacterial peritonitis related in-hospital mortality

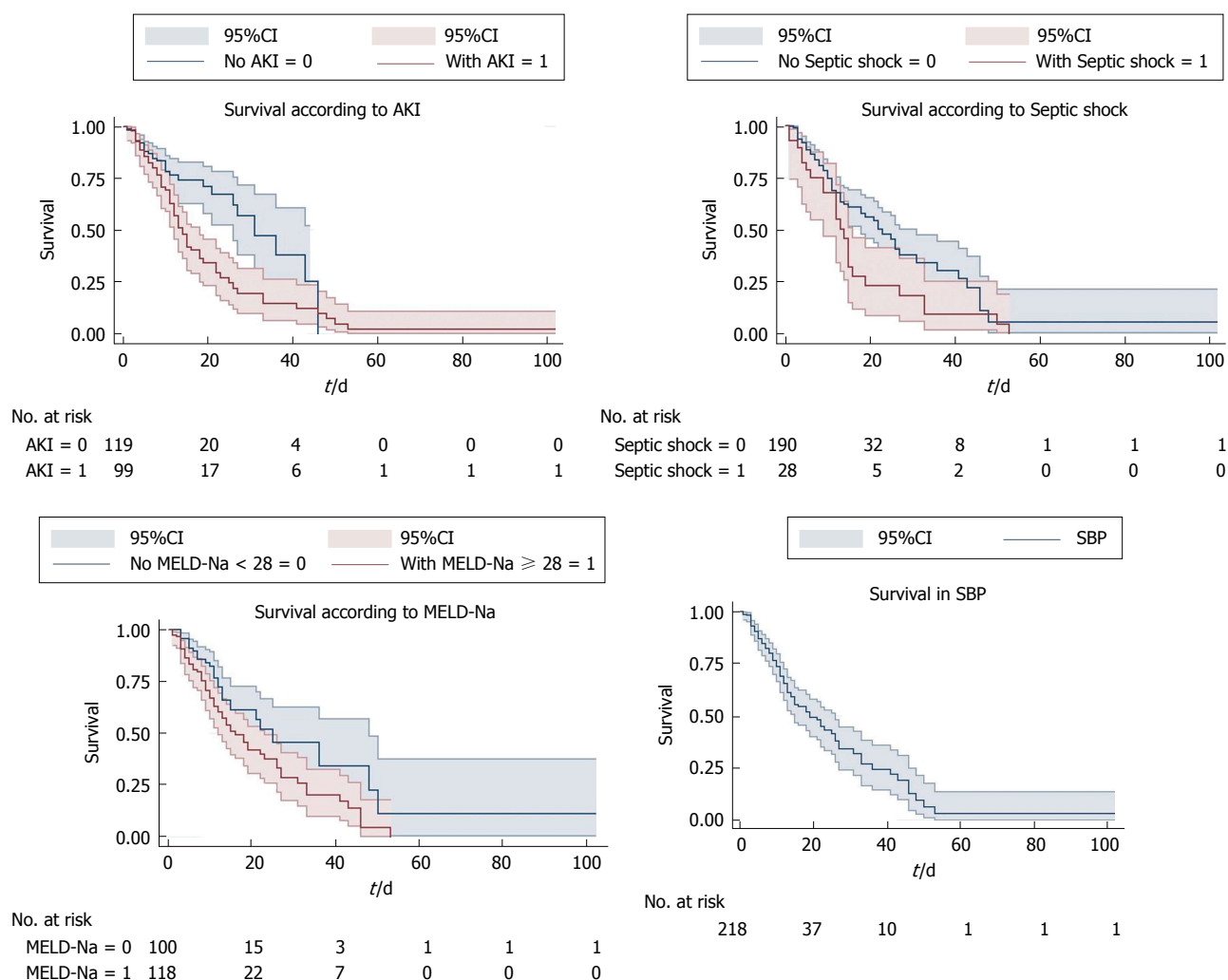
Predictors	Sensitivity	Specificity	PPV	NPV	+LR	-LR
AKI	64.6	74.8	68.1	71.8	2.56	0.47
Septic shock	85.7	63.2	25.5	96.8	2.33	0.23
MELD-Na(28) <sup>1</sup>	92.9	60.3	24.5	97.9	2.34	0.12

<sup>1</sup>Cut off score for MELD-Na. PPV: Positive predictive value; NPV: Negative predictive value; +LR: Positive likelihood ratio; -LR: Negative likelihood ratio; AKI: Acute kidney injury; MELD-Na: Model for end stage liver disease-sodium.



**Figure 1** Receiver operator characteristic curve for acute kidney injury, septic shock and model for end-stage liver disease - sodium had better prognostic accuracy for 50 d in-hospital mortality in patients with spontaneous bacterial peritonitis. AKI: Acute kidney injury; AUC: Area under the curve; MELD-Na: Model for end stage liver disease-sodium.

survival analysis was plotted for the 50-d survival in SBP patients along with individual prognostic variables like AKI, MELD-Na, and septic shock (Figure 2).



**Figure 2** Kaplan-Meier survival analysis was plotted for the 50-d survival in spontaneous bacterial peritonitis patients along with individual prognostic variables like acute kidney injury, model for end-stage liver disease - sodium, and septic shock. AKI: Acute kidney injury; MELD-Na: Model for end stage liver disease-sodium; SBP: Spontaneous bacterial peritonitis.

## DISCUSSION

The prevalence of SBP in outpatients has been reported to be 1.5%-3.5%<sup>[15]</sup>. Among in-patients the prevalence is around 10%<sup>[15]</sup>. Half of the episodes of SBP are acquired during hospitalization. In the present observational study SBP related 50 d in-hospital mortality in decompensated cirrhosis was 43%. Of total 94 cases, 93 patients with SBP (99%) died on or before 50<sup>th</sup> d of hospitalisation.

This study assessed different prognostic factors which can be used to predict mortality in hospitalized patient with SBP and corroborates that hepatic encephalopathy, total leukocyte count, serum bilirubin, SGOT, INR and child pugh score significantly associated with mortality<sup>[5,7,16]</sup>. The MELD score shows promise as a means for risk - stratifying patients with SBP including those waiting for liver transplantation<sup>[7]</sup>. Certain limitations of MELD model<sup>[17]</sup> prompted us to include MELD-Na as hyponatremia is a well-known predictor of death in cirrhotic patients. Isolated creatinine is inaccurate measurement of renal failure in decompensated

liver cirrhosis due to significant reduction in creatinine production in liver and muscle wasting<sup>[18]</sup>. We found AKI, MELD-Na and septic shock to be the important predictors of mortality. We did not incorporate other independent variables like total leukocyte count, serum bilirubin, INR since these were the components in the present predictive model like MELD-Na and septic shock.

In this study AKI has the single best predictive ability (AUC: 0.77) followed by MELD-Na (AUC: 0.74) and septic shock (AUC: 0.71). In addition, we identified MELD-Na cut-off 28 with sensitivity 92.9% and NPV of 97.9%. The hazard ratio of mortality for patients with AKI was significantly higher 2.16 (95%CI: 1.36-3.42) compared to septic shock (HR = 1.73, 95%CI: 1.05-2.83) and MELD-Na (HR = 1.06, 95%CI: 1.02-1.09). Kaplan-Meier survival analysis showed AKI, MELD-Na, and septic shock as predictors for the 50 d in-hospital mortality in decompensated patients with SBP. It can help in the further improvement of the quality of care of hospitalized SBP patients with reduction of their short-term mortality. The cut-off for MELD-Na can be applied to prioritize high-risk patients

upon hospital admission who would benefit by expectant management.

Diagnosis of SBP is based on the demonstration of an absolute number of PMNs in ascitic fluid equal to or greater than 250/mm<sup>3</sup>. However, the best specificity for diagnosis has been reported with a cut-off of 500 PMN/mm<sup>3</sup>. It is unclear whether a positive culture in the absence of elevated ascitic fluid PMN count (bacteriascites), requires antibiotic therapy. In these cases, some guidelines recommend antibiotic treatment only if the patient shows signs of infection<sup>[18]</sup>. Ascitic fluid culture is positive in 40% of all cases. The most common isolates include GNB, usually *Escherichia coli* and Gram-positive cocci (mainly *Streptococcus* species and *Enterococci*)<sup>[3]</sup>. Gram negative organism infections predominate in community acquired and gram-positive organisms in nosocomial infections<sup>[3]</sup>. Recommended first-line antibiotics for treatment of SBP include third generation cephalosporins (mainly Cefotaxime), Amoxicillin-Clavulanic acid, ciprofloxacin, and ofloxacin<sup>[19]</sup>, with an expected resolution rate of over 90%. These guidelines from the western medicine acknowledge the increasing problem of antibiotic resistance<sup>[20]</sup> and recommend coverage for resistant organisms if there is no evidence of infection resolution at repeat ascitic fluid analysis at 48 h. Resistant infections are usually caused by *Enterococcus faecium* and extended-spectrum  $\beta$ -lactamase-producing *Enterobacteriaceae*, which are resistant to the current recommended empirical antibiotic therapy<sup>[21]</sup>. These findings led to the suggestion that nosocomial SBP should be treated with carbapenems or with tigecycline<sup>[22]</sup>. We included only patients with hospital-acquired SBP because most of the present ICU admissions include transferred patients already hospitalised, with a variable but consistent antibiotic exposure. Only a minority of our patients are admitted directly from the community, and usually to the wards and not to the ICU. These patients would be expected to have a higher prevalence of resistant infections. A hospital-acquired infection was an independent predictor of death, likely due to a higher rate of multidrug resistance (resistance to third-generation cephalosporin)<sup>[23]</sup>.

This study has certain strengths and limitations. The results clearly show AKI has greater predictive ability than septic shock and MELD-Na as far as 50 d in-hospital mortality in SBP patient is concerned. This study did not account for the stages of ascites. We didn't stratify our patients according to different stages of AKI as per AKIN criteria. We didn't consider HRS into account in this study. We didn't evaluate the antibiotic resistance in SBP patients who are culture positive at the baseline. We included only nosocomial acquired SBP. Most of our patients presented with advanced decompensated liver cirrhosis at the time of SBP diagnosis. The advanced liver cirrhosis was assessed by lower serum albumin, high serum bilirubin and INR values. This study is a single centre study, these findings needed to be supplemented by multicentre prospective studies.

## COMMENTS

### Background

Spontaneous bacterial peritonitis (SBP) is associated with poor prognosis especially in-hospital patients. The mortality rate ranges from 20%-40%. The model for end-stage liver disease (MELD) has been suggested as a predictor of the in-hospital mortality in patients with SBP. The authors' goal is to identify other prognostic factors for mortality in decompensated cirrhotic patients with SBP and to evaluate the predictive power of acute kidney injury (AKI), MELD-sodium (MELD-Na) and septic shock to predict mortality.

### Research frontiers

The prognostic factors for mortality with SBP patients in liver cirrhosis are important in determining the management. MELD has been considered as an important predictive factor. But it's not clear about role of other prognostic factors.

### Innovations and breakthroughs

In this study, 50 d in-hospital mortality rate attributable to SBP is 43.11%. receiver operating characteristic (ROC) curve, Kaplan Meier survival analysis was useful tool in predicting 50 d in-hospital mortality in SBP with liver cirrhosis. Multivariate cox proportional regression analysis showed AKI [hazrd ratio (HR) = 2.16, 95%CI: 1.36-3.42,  $P = 0.001$ ] septic shock (HR = 1.73, 95%CI: 1.05-2.83,  $P = 0.029$ ) MELD-Na (HR = 1.06, 95%CI: 1.02-1.09,  $P \leq 0.001$ ) were significantly associated with 50 d in-hospital mortality. The prognostic accuracy for AKI, MELD-Na and septic shock was 77%, 74% and 71% respectively.

### Applications

Liver transplant is potentially only curative therapeutic option with long term result in patients with decompensated cirrhosis and SBP. The cost of liver transplant and the shortages of the liver donor is a point of concern. The findings of this study can be used as a strategic approach in advanced liver cirrhosis patients on hospital admission that would benefit from intensive management where liver transplant is not a plausible option. It can help in the further improvement of the quality of care of hospitalized SBP patients with reduction of their short-term mortality. The cut-off for MELD-Na can be used to stratify high-risk patients on hospital admission who would benefit by intensive management.

### Terminology

The ROC analysis is a graphical plot in statistical methods to create a cut off value for the predictors. The graph is plotted with true positive value against false positive value. The accuracy of cut off value is interpenetrated by the area under curve (AUC) in ROC curve. AUC = 1 is gold standard, 0.9-1 = excellent, 0.8-0.9 = good, 0.7-0.8 = fair, 0.6-0.7 = poor,  $\leq 0.5$  = fail. Kaplan-Meier survival curve is a time to event analysis of series of events over a period of time recorded in horizontal and declining horizontal steps. When a person withdraws from the study (censored), lost follow up, or died there will be a sudden drop in the curve. HR: It's a ratio of hazard rates. It is the probability of an event in the study group or population at a particular time. HRs are used in time to event analysis.

### Peer-review

This is a retrospective study to analyze predictors of 50 d in-hospital mortality in decompensated cirrhosis patients with spontaneous bacterial peritonitis. The authors review the medical records of 218 adults admitted with SBP in period of one year, to identify factors related to mortality. The article is very well described; it was properly planned and conducted.

## REFERENCES

- 1 Rimola A, García-Tsao G, Navasa M, Piddock LJ, Planas R, Bernard B, Inadomi JM. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. International Ascites Club. *J Hepatol* 2000; **32**: 142-153 [PMID: 10673079 DOI: 10.1016/S0168-8278(00)80201-9]

- 2 **Runyon BA.** Ascites and spontaneous bacterial peritonitis. In: Feldman M, Friedman LS, Sleisenger MH, editors. Sleisenger and Fordran's Gastrointestinal and Liver Disease, 8th ed. Philadelphia, PA: Saunders, 2006: 1935-1964
- 3 **Wiest R,** Krag A, Gerbes A. Spontaneous bacterial peritonitis: recent guidelines and beyond. *Gut* 2012; **61**: 297-310 [PMID: 22147550 DOI: 10.1136/gutjnl-2011-300779]
- 4 **Evans LT,** Kim WR, Poterucha JJ, Kamath PS. Spontaneous bacterial peritonitis in asymptomatic outpatients with cirrhotic ascites. *Hepatology* 2003; **37**: 897-901 [PMID: 12668984 DOI: 10.1053/jhep.2003.50119]
- 5 **Thuluvath PJ,** Morss S, Thompson R. Spontaneous bacterial peritonitis--in-hospital mortality, predictors of survival, and health care costs from 1988 to 1998. *Am J Gastroenterol* 2001; **96**: 1232-1236 [PMID: 11316175 DOI: 10.1111/j.1572-0241.2001.03708.x]
- 6 **Zhang QB,** Chen YT, Lian GD, Qian CC, Chen SJ, Huang KH. A combination of models for end-stage liver disease and cirrhosis-related complications to predict the prognosis of liver cirrhosis. *Clin Res Hepatol Gastroenterol* 2012; **36**: 583-591 [PMID: 22704816 DOI: 10.1016/j.clinre.2012.04.014]
- 7 **Nobre SR,** Cabral JE, Gomes JJ, Leitão MC. In-hospital mortality in spontaneous bacterial peritonitis: a new predictive model. *Eur J Gastroenterol Hepatol* 2008; **20**: 1176-1181 [PMID: 18941414 DOI: 10.1097/MEG.0b013e32830607a2]
- 8 **de Carvalho JR,** Villela-Nogueira CA, Luiz RR, Guzzo PL, da Silva Rosa JM, Rocha E, Moraes Coelho HS, de Mello Perez R. Acute kidney injury network criteria as a predictor of hospital mortality in cirrhotic patients with ascites. *J Clin Gastroenterol* 2012; **46**: e21-e26 [PMID: 21934526 DOI: 10.1097/MCG.0b013e32830607a2]
- 9 **Eduardo RP,** Margarida FS. Hepatorenal syndrome, septic shock and renal failure as mortality predictors in patients with spontaneous bacterial peritonitis. *GE J Port Gastroenterol* 2012; **19**: 278-283 [DOI: 10.1016/j.jpg.2012.09.002]
- 10 WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. Available from: <http://www.wma.net/en/30publications/10policies/b3/>
- 11 **Guarner C,** Soriano G. Spontaneous bacterial peritonitis. *Semin Liver Dis* 1997; **17**: 203-217 [PMID: 9308125 DOI: 10.1055/s-2007-1007198]
- 12 **United Network for Organ Sharing (UNOS).** Available from: URL: <http://www.unos.org/>
- 13 **Alves de Mattos A.** Current indications for the use of albumin in the treatment of cirrhosis. *Ann Hepatol* 2011; **10** Suppl 1: S15-S20 [PMID: 21566250]
- 14 **Bone RC,** Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; **101**: 1644-1655 [PMID: 1303622 DOI: 10.1378/chest.101.6.1644]
- 15 **Nousbaum JB,** Cadranet JF, Nahon P, Khac EN, Moreau R, Thévenot T, Silvain C, Bureau C, Nouel O, Pilette C, Paupard T, Vanbiervliet G, Oberti F, Davion T, Jouannaud V, Roche B, Bernard PH, Beaulieu S, Danne O, Thabut D, Chagneau-Derrode C, de Lédinghen V, Mathurin P, Pauwels A, Bronowicki JP, Habersetzer F, Abergel A, Audigier JC, Sapey T, Grangé JD, Tran A. Diagnostic accuracy of the Multistix 8 SG reagent strip in diagnosis of spontaneous bacterial peritonitis. *Hepatology* 2007; **45**: 1275-1281 [PMID: 17464969 DOI: 10.3748/wjg.v17.i9.1091]
- 16 **Terg R,** Gadano A, Cartier M, Casciato P, Lucero R, Muñoz A, Romero G, Levi D, Terg G, Miguez C, Abecasis R. Serum creatinine and bilirubin predict renal failure and mortality in patients with spontaneous bacterial peritonitis: a retrospective study. *Liver Int* 2009; **29**: 415-419 [PMID: 18803587 DOI: 10.1111/j.1478-3231.2008.01877.x]
- 17 **Gotthardt D,** Weiss KH, Baumgärtner M, Zahn A, Stremmel W, Schmidt J, Bruckner T, Sauer P. Limitations of the MELD score in predicting mortality or need for removal from waiting list in patients awaiting liver transplantation. *BMC Gastroenterol* 2009; **9**: 72 [PMID: 19778459 DOI: 10.1186/1471-230X-9-72]
- 18 **Runyon BA.** Management of adult patients with ascites due to cirrhosis: an update. *Hepatology* 2009; **49**: 2087-2107 [PMID: 19475696 DOI: 10.1002/hep.22853]
- 19 **European Association for the Study of the Liver.** EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]
- 20 **Cheong HS,** Kang CI, Lee JA, Moon SY, Joung MK, Chung DR, Koh KC, Lee NY, Song JH, Peck KR. Clinical significance and outcome of nosocomial acquisition of spontaneous bacterial peritonitis in patients with liver cirrhosis. *Clin Infect Dis* 2009; **48**: 1230-1236 [PMID: 19302016 DOI: 10.1086/597585]
- 21 **Ariza X,** Castellote J, Lora-Tamayo J, Girbau A, Salord S, Rota R, Ariza J, Xiol X. Risk factors for resistance to ceftriaxone and its impact on mortality in community, healthcare and nosocomial spontaneous bacterial peritonitis. *J Hepatol* 2012; **56**: 825-832 [PMID: 22173153 DOI: 10.1016/j.jhep.2011.11.010]
- 22 **Fernández J,** Acevedo J, Castro M, Garcia O, de Lope CR, Roca D, Pavesi M, Sola E, Moreira L, Silva A, Seva-Pereira T, Corradi F, Mensa J, Ginès P, Arroyo V. Prevalence and risk factors of infections by multiresistant bacteria in cirrhosis: a prospective study. *Hepatology* 2012; **55**: 1551-1561 [PMID: 22183941 DOI: 10.1002/hep.25532]
- 23 **Umgeleiter A,** Reindl W, Miedaner M, Schmid RM, Huber W. Failure of current antibiotic first-line regimens and mortality in hospitalized patients with spontaneous bacterial peritonitis. *Infection* 2009; **37**: 2-8 [PMID: 19169633 DOI: 10.1007/s15010-008-8060-9]

**P- Reviewer:** Haddad L, Tovo CV **S- Editor:** Qiu S

**L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 May 8; 8(13): 573-606





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**Stephen DH Malnick, *Rehovot***Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*

**Jordan**Kamal E Bani-Hani, *Zarqa***Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**Angela Peltec, *Chishinev***Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**CA Asabamaka Onyekwere, *Lagos***Pakistan**Bikha Ram Devrajani, *Jamshoro***Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**Jacek Zielinski, *Gdansk***Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**Reem Al Olaby, *Doha***Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**Ser Yee Lee, *Singapore***South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**REVIEW**

- 573 Hepatocellular carcinoma and the risk of occupational exposure  
*Rapisarda V, Loreto C, Malaguarnera M, Arditi A, Proiti M, Rigano G, Frazzetto E, Ruggeri MI, Malaguarnera G, Bertino N, Malaguarnera M, Catania VE, Di Carlo I, Toro A, Bertino E, Mangano D, Bertino G*

**MINIREVIEWS**

- 591 Innovative surgical approaches for hepatocellular carcinoma  
*Memeo R, de'Angelis N, de Blasi V, Cherkaoui Z, Brunetti O, Longo V, Piardi T, Sommacale D, Marescaux J, Mutter D, Pessaux P*

**ORIGINAL ARTICLE****Retrospective Cohort Study**

- 597 Risk factors for deterioration of long-term liver function after radiofrequency ablation therapy  
*Honda K, Seike M, Oribe J, Endo M, Arakawa M, Syo H, Iwao M, Tokoro M, Nishimura J, Mori T, Yamashita T, Fukuchi S, Muro T, Murakami K*

**LETTERS TO THE EDITOR**

- 605 Antiviral therapy for hepatitis B virus-related hepatocellular carcinoma after surgery: A comment for moving forward  
*Zhong JH, Yang T, Xiang BD, Li LQ, Ma L*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Yong Zhang, MD, PhD, Associate Professor, Attending Doctor, Department of Surgical Oncology, First Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Jin-Lei Wang*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: editorialoffice@wjgnet.com  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
May 8, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjgnet.com/bpg/g_info_20160116143427.htm)

**ONLINE SUBMISSION**

<http://www.wjgnet.com/esps/>



## Hepatocellular carcinoma and the risk of occupational exposure

Venerando Rapisarda, Carla Loreto, Michele Malaguarnera, Annalisa Ardiri, Maria Proiti, Giuseppe Rigano, Evelise Frazzetto, Maria Irene Ruggeri, Giulia Malaguarnera, Nicoletta Bertino, Mariano Malaguarnera, Vito Emanuele Catania, Isidoro Di Carlo, Adriana Toro, Emanuele Bertino, Dario Mangano, Gaetano Bertino

Venerando Rapisarda, Dario Mangano, Occupational Medicine Unit, Department of Clinical and Experimental Medicine, University of Catania, 95123 Catania, Italy

Carla Loreto, Department of Biomedical Sciences, Human Anatomy and Histology Section, University of Catania, 95100 Catania, Italy

Michele Malaguarnera, Department of Biomedical Sciences, University of Catania, 95100 Catania, Italy

Annalisa Ardiri, Maria Proiti, Giuseppe Rigano, Evelise Frazzetto, Gaetano Bertino, Hepatology Unit, Department of Clinical and Experimental Medicine, University of Catania, 95123 Catania, Italy

Maria Irene Ruggeri, Internal Medicine Unit ARNAS Civic Hospital, 90142 Palermo, Italy

Giulia Malaguarnera, Mariano Malaguarnera, Vito Emanuele Catania, Research Centre "La Grande Senescenza", University of Catania, 95100 Catania, Italy

Nicoletta Bertino, Emanuele Bertino, Faculty of Pharmacy, University of Catania, 95123 Catania, Italy

Isidoro Di Carlo, Adriana Toro, Department of Surgical Sciences, Organ Transplantation and Advanced Technologies, University of Catania, 95100 Catania, Italy

**Author contributions:** All the authors contributed to this paper.

**Conflict-of-interest statement:** There are no potential conflicts of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and

the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Gaetano Bertino, Professor, Hepatology Unit, Department of Clinical and Experimental Medicine, University of Catania, Policlinic - Via S. Sofia n. 78, 95123 Catania, Italy. [gaetanobertinounict@libero.it](mailto:gaetanobertinounict@libero.it)  
 Telephone: +39-095-3781573  
 Fax: +39-095-3781572

**Received:** June 17, 2015

**Peer-review started:** June 19, 2015

**First decision:** August 10, 2015

**Revised:** April 1, 2016

**Accepted:** April 14, 2016

**Article in press:** April 18, 2016

**Published online:** May 8, 2016

### Abstract

Hepatocellular carcinoma (HCC) is the most common type of liver cancer. The main risk factors for HCC are alcoholism, hepatitis B virus, hepatitis C virus, nonalcoholic steatohepatitis, obesity, type 2 diabetes, cirrhosis, aflatoxin, hemochromatosis, Wilson's disease and hemophilia. Occupational exposure to chemicals is another risk factor for HCC. Often the relationship between occupational risk and HCC is unclear and the reports are fragmented and inconsistent. This review aims to summarize the current knowledge regarding the association of infective and non-infective occupational risk exposure and HCC in order to encourage further research and draw attention to this global occupational public health problem.

**Key words:** Hepatocellular carcinoma; Autophagy; Epigenetic events; Hepatitis B virus; Hepatitis C virus; Occupational exposure; Chemical agents; Mitophagy;

Arsenic; Cadmium

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatocellular carcinoma (HCC) is the fifth most common human cancer. This review summarizes current knowledge regarding the occupational risk factors of HCC. In particular, we underline not only the infective but also non-infective occupational risk exposure, including chemical agents and toxic metabolites which are a major cause of liver damage.

Rapisarda V, Loreto C, Malaguarnera M, Ardiri A, Proiti M, Rigano G, Frazzetto E, Ruggeri MI, Malaguarnera G, Bertino N, Malaguarnera M, Catania VE, Di Carlo I, Toro A, Bertino E, Mangano D, Bertino G. Hepatocellular carcinoma and the risk of occupational exposure. *World J Hepatol* 2016; 8(13): 573-590 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i13/573.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i13.573>

## INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) is increasing worldwide. There are geographical areas with a high prevalence, as in Asia and Africa, and death from HCC has increased in the United States and Europe<sup>[1-5]</sup>.

Aflatoxin<sup>[6]</sup>, alcohol intake<sup>[7]</sup>, hepatitis B virus (HBV)<sup>[4]</sup>, hepatitis C virus (HCV) infection<sup>[5]</sup> and oral contraception<sup>[8,9]</sup> are known risk factors for HCC, whereas cigarette smoke, anabolic steroids and insulin resistance are suspected to be contributing factors<sup>[10-16]</sup>.

The relationship between occupational risk and HCC is often unclear and the reports are fragmented and inconsistent<sup>[17-19]</sup>; however, it is very commonly reported that vinyl chloride monomer (VCM) induced angiosarcoma of the liver<sup>[20]</sup>.

HCC mortality, assessed by standardized mortality ratio, has been reported in different categories of workers: Building and chemical workers, painters, subjects exposed to solvents and workers in the textile industry have often been reported to be at high risk for HCC<sup>[21-30]</sup>. However, such studies have often failed to identify a single agent responsible for the heightened HCC risk. There have been few investigations of occupational exposure and liver cancer. A number of factors and confounders have precluded drawing firm conclusions<sup>[31]</sup>.

The possible associations between the risk of infection and non-infectious occupational hazards and HCC will be discussed, in the hope of drawing attention to this global public health problem.

## REVIEW METHOD

The PubMed, Scopus and Web of Science databases were searched using the following keywords: "HCC", "occupational exposure", "chemical agents", "arsenic",

"cadmium", "HBV", "HCV", "molecular hepatocarcinogenesis", "molecular immunological targets", "autophagy", "mitophagy" and "epigenetic events". Published data at the International Agency for Research on Cancer (IARC) were consulted.

## INFECTIVE RISK FACTORS FOR HCC

Infection is one of the main contributors to cancer development<sup>[32]</sup>. There are 11 biological agents classified as IARC group 1 carcinogens<sup>[33,34]</sup>. HBV, HCV and AFB1 are responsible for HCC development<sup>[35]</sup>. The vast majority of the global cancer burden attributable to infection occurs in less developed regions (Table 1).

## HEPATITIS INFECTIONS

Infection with HBV and HCV can be through parenteral or unapparent transmission<sup>[36-42]</sup>.

### Occupational exposure to hepatitis B

The risk of hepatitis from needlestick injury from an hepatitis B envelope antigen positive (HBsAg+) source is 22%-31%, whereas the risk of contracting clinical hepatitis from a needlestick injury involving an hepatitis B surface antigen positive (HBsAg+), eAg- source is 1%-6%. Post-exposure prophylaxis (PEP), including HBIG and the HBV vaccine, is believed to be 85%-95% effective. HBV vaccine or HBIG alone is thought to be 70%-75% effective<sup>[43-45]</sup>.

### Occupational exposure to hepatitis C

The risk of HCV transmission from percutaneous exposure is approximately 2%. HCV is rarely transmitted from mucous membrane exposure to blood (both documented cases have been when the source patient was human immunodeficiency virus/HCV co-infected) and it has never been documented following blood exposure to intact or non-intact skin. There is no known PEP for HCV exposure. According to a European case-control study, assessment of the risk of transmission after occupational HCV exposure should take into account the injury severity, device involved and the HCV RNA status of the source patient<sup>[46-50]</sup>.

## DEVELOPMENT OF HCC IN CHRONIC HBV INFECTION

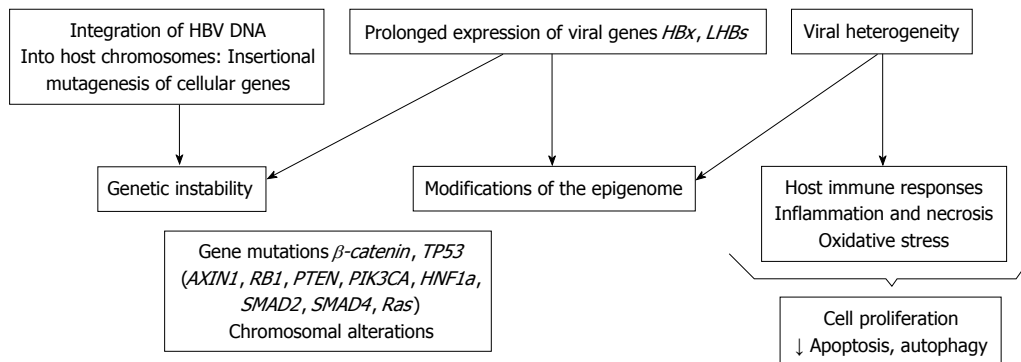
Chronic HBV infection has a causal role in HCC development<sup>[36]</sup> since it promotes carcinogenesis through liver injury (necrosis and inflammation) and cirrhosis development (fibrosis and regeneration)<sup>[41,43-45]</sup>. Moreover, HBV and HCV co-infection causes a higher than 50-fold risk compared to HCC<sup>[51-54]</sup>.

Risk factors for liver cancer in HBV patients include: (1) host-related risk factors: Older age, Asian ethnicity, male sex, alcohol intake and advanced liver disease<sup>[55-57]</sup>; (2) viral risk factors: HBV genotype C, mutations of pre-S, enhancer-H, core promoter, HCV or hepatitis

**Table 1** Hepatocellular carcinoma and occupational exposure to infective agents

Risk agent	CAS No.	Occupational exposure	IARC class
Infective risk			
HBV	-	Health care workers <sup>[4,38,41,44]</sup> , waste operators <sup>[38,44]</sup>	Group 1 <sup>[34]</sup>
HCV	-	Health care workers <sup>[38,39,61]</sup>	Group 1 <sup>[34]</sup>
Aflatoxin B1	1162-65-8	Paper mill and sugar factory; poultry production; rice mill; waste management; swine industry; agri-food industry; wheat handling; textile manufacturing <sup>[77,78,87-91,93,96]</sup>	Group 1 <sup>[76]</sup>

IARC: International Agency for Research on Cancer; CAS No.: Chemical abstract service number; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

**Figure 1** Pathogenesis of hepatitis B virus-related hepatocellular carcinoma. HBV: Hepatitis B virus.

Delta virus infection and PC/BCP HBV variants<sup>[45,58]</sup>; and (3) risk factors related to host-virus interaction: Cirrhosis, high HBV-DNA serum levels, prolonged HBeAg positivity, prolonged HBsAg positivity and high HBsAg serum levels<sup>[59-62]</sup>.

Lastly, the HCC risk factors in chronic HBV infection are different and the pathogenesis is characterized by the combined action of different alterations involving genetic, epigenetic and immunological factors<sup>[63-71]</sup> (Figure 1).

## DEVELOPMENT OF HCC IN CHRONIC HCV INFECTION

The mechanism by which HCV causes HCC is not wholly clear. It has been suggested that HCV proteins have direct oncogenic properties<sup>[5]</sup>. Chronic HCV infection leads to cirrhosis in 10%-20% of patients of whom 1%-5% develop liver cancer<sup>[5]</sup>. Central tumor suppressor genes and a number of proto-oncogenes, such as retinoblastoma tumor suppressor (*Rb*) and *P53*, have been suggested as targets of direct alteration by HCV proteins; the wnt/ $\beta$ -catenin and transforming growth factor- $\beta$  pathways may also be directly affected<sup>[5]</sup>.

Moreover, chronic infection, necrosis and cell regeneration, fibrosis and cirrhosis are, together with the direct mechanisms, the high risk factors for HCC. Finally, HBV or HCV chronic infection has immunomodulatory and immunosuppressive effects<sup>[71-73]</sup>.

## AFLATOXINS

The aflatoxins are metabolic products of certain fungi, *Aspergillus flavus* and *parasiticus* that develop in cereals

(maize), oilseeds (groundnuts) and dried fruit and are chemicals of the furanocoumarins type. To date, we have isolated 17 aflatoxins and 5 are relevant to dissemination and toxicity. High exposure concentrations cause acute hepatitis. Chronic exposure causes the development of liver cancer. This could be caused by the aflatoxin ability to determine the mutation of the p53 tumor suppressor gene, which in normal conditions induces the apoptosis processes<sup>[74-77]</sup>.

The risk of HCC increases when the exposure occurs in the presence of HBV infection, as occurs in the Chinese population<sup>[78-96]</sup>.

## NON-INFECTIVE RISK FACTORS FOR HEPATOCELLULAR CARCINOMA

A wide range of occupational activities may involve worker exposure to a variety of chemical agents. The liver is the main organ involved in metabolism and in toxicokinetics of a xenobiotic. However, it is frequently also a target organ because of its blood supply and the many metabolic and excretory processes in which it has a role. Adverse effects of chemical exposure involving the liver (hepatotoxicity) comprise hepatocellular damage, cholestatic injury, fatty liver, granulomatous disease, cirrhosis and malignancies, including HCC. A variety of chemicals comprising VCM, organic solvents, chlorinated pesticides and arsenic exert adverse effects on the liver<sup>[97]</sup> (Tables 2 and 3).

## VCM AND POLYVINYL CHLORIDE

VCM, chemical abstract service number (CAS No.

**Table 2 Hepatocellular carcinoma and occupational exposure to chemical agents**

Risk agent	CAS No.	Occupational exposure	IARC class
Non-infective risk			
VCM	75-01-4	Plastics, plumbing, cabling, house framing, waterproof clothing, medical devices and food packaging industry <sup>[98,99,102,103,105-108,111,112,114-120]</sup>	Group 1 <sup>[76]</sup>
TCE	79-01-6	Dry cleaning; paint stripping; metal degreasing; production of chlorinated chemical compounds; shoe manufacturing; aircraft/aerospace, electronics and printing industry <sup>[125,127]</sup>	Group 1 <sup>[129]</sup>
PCE	127-18-4	Dry cleaning; textile processing; metal degreasing <sup>[138]</sup>	Group 2A <sup>[129]</sup>
DDT	50-29-3	Farming industry <sup>[141,145]</sup>	Group 2B <sup>[148]</sup>
N-nitrosamines	35576-91-1	Plastic, rubber and pharmacological manufacturing; farming industry; metalworking; electrical component production and use; gasoline and lubricant additives, production and use <sup>[159-165]</sup>	Group 1 <sup>[160,161]</sup>
TCDD	1746-01-6	Waste management; paper mill; timber manufacturing; iron and steel manufacturing; electric power industry <sup>[175,179]</sup>	Group 1 <sup>[76]</sup>
PeCDF	57117-31-4	Cement and metalworking industry; chemical manufacturing <sup>[171,172,175]</sup>	Group 1 <sup>[76]</sup>
PCB	1336-36-3	Electrical industry, plastic and chemical industry; maintenance/repair technicians of PCB devices <sup>[175,186-190]</sup>	Group 1 <sup>[76,207]</sup>
PBB		Electronics recycling industry; maintenance/repair technicians of PBB devices <sup>[209-212]</sup>	Group 2A <sup>[207]</sup>
Chloral	75-87-6	Insecticides and herbicide production; polyurethane foam production and use <sup>[125,214,215]</sup>	Group 2A <sup>[216]</sup>
Chloral hydrate	302-17-0	Pharmaceutical producing; health care workers; laboratory research; water disinfection by chlorination <sup>[129,216]</sup>	Group 2A <sup>[216]</sup>
O-toluidine	95-53-4	Dye production and use; herbicide and pharmaceutical production; rubber industry; clinical laboratories <sup>[220-222,227,228]</sup>	Group 1 <sup>[76]</sup>
MOCA	101-14-4	Rubber and polyurethane industry <sup>[220,230-232]</sup>	Group 1 <sup>[76]</sup>
4-ABP	92-67-1	Rubber industry; dyes production <sup>[220,235-238]</sup>	Group 1 <sup>[76]</sup>
BZD and dyes metabolized to BZD	92-87-5	Dye production and use; clinical laboratories <sup>[220,247]</sup>	Group 1 <sup>[76]</sup>

<sup>1</sup>Not all of them are to be referred to group 1. VCM: Vinyl chloride monomer; TCE: Trichloroethylene; PCE: Perchloroethylene; DDT: 1,1,1-Trichloro-2,2-bis (p-chlorophenyl)-ethane; TCDD: 2,3,7,8-Tetrachlorodibenzo-p-dioxin; PeCDF: 2,3,4,7,8-Pentachlorodibenzofuran; PCB: Polychlorinated biphenyls; PBB: Polybrominated biphenyls; O-toluidine: Ortho-toluidine; MOCA: 4,4'-Methylene bis (2-chlorobenzeneamine); 4-ABP: 4-aminobiphenyl; BZD: Benzidine; IARC: International Agency for Research on Cancer; CAS No.: Chemical abstract service number.

**Table 3 Hepatocellular carcinoma and occupational exposure to metals**

Risk agent	CAS No.	Occupational exposure	IARC class
Non-infective risk			
As	7440-38-2	Timber manufacturing; pesticide use; As extraction industry; lead processing; pharmaceutical industry; glass industry; leather preservatives; antifouling paints; agrochemical production; microelectronics and optical industries; non-ferrous metal smelters; coal-fired power plants <sup>[254-258]</sup>	Group 1 <sup>[263]</sup>
Cd	7440-43-9	Cd mining; manufacturing of Cd-containing ores and products; Ni-Cd battery manufacturing, Cd alloy production <sup>[275,277,278]</sup>	Group 1 <sup>[263]</sup>

As: Arsenic; Cd: Cadmium; IARC: International Agency for Research on Cancer; CAS No.: Chemical abstract service number.

75-01-4), is a chlorinated organic compound. VCM is found in cigarette smoke and is mainly used in the production of polymer polyvinyl chloride (PVC). VCM is rapidly absorbed after inhalation and is primarily metabolized by the liver.

Since PVC is harmless in its polymeric form, workers handling the finished goods are not at risk of exposure. The risk phases are those in which the workers are in contact with the material when still in the monomeric state. Many epidemiological studies have demonstrated the high prevalence of exposure to VCM in those working with the chemical. Thiodiglycolic acid is the main VCM metabolite detected in the urine of occupationally exposed subjects.

It has been shown in both human and animal models that VCM is able to induce liver angiosarcoma and HCC<sup>[98-104]</sup>.

Maroni *et al.*<sup>[105]</sup> reported the hepatotoxicity of VCM and other studies have shown the capacity of VCM to

induce specific gene mutations in the liver<sup>[105-117]</sup>.

Various European and Italian studies have reported the apparent association between the amount and timing of exposure to VCM and development of HCC in those exposed<sup>[118-120]</sup>.

## ORGANIC SOLVENTS

Organic solvents are substances that contain carbon and are capable of dissolving or dispersing one or more other substances. Millions of workers are exposed to organic solvents contained in products such as varnishes, adhesives, glues, plastics, textiles, printing inks, agricultural products and pharmaceuticals.

Many organic solvents are recognized by NIOSH as carcinogens (carbon tetrachloride, benzene and trichloroethylene), reproductive hazards and neurotoxins. Among the organic solvents, trichloroethylene (TCE) and perchloroethylene (PCE) have been reported to be



capable of promoting cancer in humans<sup>[121,122]</sup>.

TCE (CAS 06/01/79) has been associated with a high prevalence of liver tumors in exposed workers. Although the hepatic metabolism of this solvent is known, the molecular alterations that cause liver cancer are not completely known<sup>[123-127]</sup>.

It is hypothesized that TCE may be involved in various mechanisms, such as the reduction of programmed cell apoptosis and the uncontrolled proliferation induced by peroxisome activated receptor (PPAR). In fact, it has been proved that TCE is able to bind PPAR<sup>[128-132]</sup>.

*RAD51* is a eukaryote gene. The protein encoded by this gene is a member of the RAD51 protein family which assists in the repair of DNA. TCE binds the *RAD51*, consequently alters the DNA repair and can cause a certain degree of genomic instability.

Finally, it was reported that TCE can cause hypomethylation of DNA and hyperexpression of oncogenes (e.g., *MYC* and *JUG*), responsible for uncontrolled cell proliferation<sup>[133-137]</sup>.

A high prevalence of liver cancer was found in animal models exposed to PCE (CAS 127-18-4)<sup>[138,139]</sup>.

Porru *et al.*<sup>[140]</sup> showed that, in workers chronically exposed to organic solvents (toluene and xylene), there is an increased risk of HCC and that the risk is time-dependent.

## PESTICIDES

Pesticides are widely used in agriculture to get the best quality products and appearance. Farmers and many workers in the agro-food chain are exposed to these substances as well as consumers who eat agricultural products that are not properly cleaned and decontaminated.

Among these substances, 1,1,1-trichloro-2,2-bis (p-chlorophenyl)-ethane (DDT) and its metabolite 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (DDE) have been extensively studied. DDT was used both in agriculture and for environmental disinfection until its use was later forbidden in both America and Europe because of its toxic effects on humans. However, in Africa and many parts of Asia it is currently used to control diseases delivered by an insect as a vector (e.g., Leishmaniasis, malaria).

In humans, DDT contamination occurs through contact with the skin, mucous membranes and inhalation. After DDT absorption, it is distributed to all organs and a portion will be stored in fatty tissues, especially if the exposure was massive<sup>[141-146]</sup>.

Many insecticides, including DDT, were reported to be responsible for leading the development of HCC<sup>[147-152]</sup>. This occurs through different mechanisms not yet completely understood. Moreover, DDT has an estrogenic effect, while DDE has anti-androgenic effects. DDT may also interfere with the *CYP3A1* gene involved in the inflammatory and immune responses in the liver.

Probably none of these mechanisms is individually able to result in HCC but the simultaneous presence of these alterations may lead to the development of liver cancer. Furthermore, the presence of important cofactors, such as HBV, HCV and AFB1, amplifies the risk in exposed populations<sup>[152-158]</sup>.

## N-NITROSAMINES

Nitrosamines are carcinogenic chemical compounds produced when nitrite, a preservative added to certain foods (fish, fish byproducts, certain types of meat, cheese products, beer), combines with amino acids in the stomach. Nitrosamines can be also found in latex products and tobacco smoke. Moreover, nitrosamines are produced in research laboratories, in rubber and tyre manufacturing processes and may be found as contaminants in the final rubber product. Some nitrosamines have been found to be effective for a variety of purposes, including antimicrobial (No. 11) or chemotherapeutic agents (Nos. 5 and 9) in conjunction with others, herbicides (Nos. 5 and 6), additives to soluble and synthetic metalworking fluids (No. 3), solvents or gasoline and lubricant additives (No. 4), antioxidants, stabilizers in plastics, fiber industry solvents and copolymer softeners, and to increase dielectric constants in condensers. Contamination can occur with skin contact and by ingestion and/or inhalation.

Nitrosamines are carcinogenic and are implicated in nasopharyngeal, esophageal, stomach, liver and urinary bladder cancers<sup>[159]</sup>.

From 1981 to 1991, the United States - National Toxicology Program conducted several investigations to characterize and assess the toxicological potential and carcinogenic activity of N-nitrosamines in laboratory animals (rats and mice). The results were reported in the second (1981) (N-nitrosamines: 2-7, 9-15) and sixth (1991) (N-nitrosamines: 1-8) annual report on carcinogens<sup>[159-163]</sup>.

In environmental surveys of some European rubber factories, de Vocht *et al.*<sup>[164]</sup> found the average N-nitrosamine levels well below the regulatory limits in force but high accidental exposures have still occurred. In fact, they detected high levels of urinary N-nitrosamines in exposed workers<sup>[162,164-166]</sup>. Recent studies have reported a correlation between exposure to N-nitrosamines and HCC which might be due to the shortening of telomeres among workers in the rubber industry. Telomeres are critical to maintaining the integrity of chromosomes and telomere length abnormalities are associated with carcinogenesis<sup>[163,165,167-169]</sup>.

## DIOXINS AND DIOXIN-LIKE COMPOUNDS

The dioxins and dioxin-like compounds are a class of heterocyclic organic compounds whose molecular structure fundamentally consists of a ring of six atoms, four carbon and two oxygen atoms; dioxin in the strict

sense is differently stable and comes in two different positional isomers. Commonly referred to dioxins are also compounds derived from furan, in particular dibenzofurans. Therefore, part of the dioxin-like compounds are polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans and among them, the most toxic is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). It has been shown that compounds of the family of dioxins are formed during the initial stage of the waste combustion when combustion generates gaseous HCl in the presence of catalysts, such as copper and iron. Organic chlorine, which is bound to organic compounds of polymers such as PVC, is mainly responsible for the formation of compounds belonging to the family of dioxins. Dioxins are generated even in the absence of combustion, for example in bleaching paper and tissues with chlorine.

About 90% of human dioxin, except for cases of exposure to specific sources such as industrial plants and incinerators, takes place through food (in particular the fat of animals exposed to dioxin) and not directly by air. The phenomenon of bioaccumulation is very important, *i.e.*, the possibility that dioxin enters into the human food chain from plants, through herbivores, carnivores and finally humans<sup>[170-176]</sup>. Dioxins are classified as definitely carcinogenic and are in the IARC group 1 carcinogenics for humans.

The National Institute for Occupational Safety and Health (NIOSH) has classified TCDD as an occupational carcinogen that can cause space-occupying liver lesions, both non-neoplastic and neoplastic, such as in HCC<sup>[177-181]</sup>.

Many studies have indicated that the carcinogenic capacity of TCDD may be due to the interaction between TCDD and the aryl hydrocarbon receptor (AhR). This receptor is implicated in several xenobiotic metabolisms but there is evidence that AhR is able to control other genes, some of which have a pro-oncogenic capacity<sup>[182-184]</sup>. The TCDD is an important AhR agonist and is therefore able to induce and enhance HCC development and diffusion<sup>[184]</sup>.

## POLYCHLORINATED BIPHENYLS

Polychlorinated biphenyls (PCB) are synthetic chlorinated aromatic hydrocarbons, chemically stable and therefore persistent environmental contaminants. The contamination occurs by skin contact or inhalation, which also allows the possibility of developing vapors for equipment containing PCB overheating<sup>[185]</sup>.

Studies in animal models have shown that these chemical compounds can cause chronic hepatitis as well as cancers, such as HCC and cholangiocarcinoma, especially if there is high exposure and a prolonged time. However, there is little data on liver injury in humans. In one case, exposure to olive oil accidentally contaminated with PCB resulted in death from hepatic cirrhosis. Other studies in workers exposed to the PCB have reported an increased incidence of liver tumors<sup>[185-188]</sup>.

Some possible mechanisms by which PCB can cause cancer have been assumed: Reactive oxygen species (ROS) is produced through the enzymatic oxidation or autooxidation of PCB; PCB determines the increased expression of genes responsible for inflammation and apoptosis in the liver; and PCB has "toxic" effects on certain genes, such as the loss of part of a chromosome and chromosome breakage<sup>[189-199]</sup>. ROS are also able to reduce telomerase activity which can determine telomere shortening. The contribution of all or part of these alterations may facilitate the onset of tumors and more specifically HCC<sup>[200-205]</sup>. At present we have no conclusive data on the relationship between PCBs and HCC and further studies will be needed to establish the causal link. However, the evidence reported by animal model studies have made it possible to classify PCB in IARC group 1<sup>[206,207]</sup>.

## POLYBROMINATED BIPHENYLS

Polybrominated biphenyls are polyhalogenated derivatives of a biphenyl core<sup>[208]</sup> that are chemically stable and therefore persistent environmental contaminants. Whereas they were widely used just a few years ago, they are now subject to restrictive rules that limit their use in the European Union (Restriction of Hazardous Substances Directive).

Contamination can occur through skin contact, inhalation and ingestion<sup>[209-212]</sup>. Based on data obtained from animal research, PDDs are considered potential human carcinogens and can result in hematological, digestive system and liver malignancies. The pathogenic mechanisms by which they can result in PDD cancer are similar to those described for PCB which allows them to be defined as "probably carcinogenic for humans" (group 2A)<sup>[207]</sup>.

## CHLORAL AND CHLORAL HYDRATE

Chloral (or trichloroacetaldehyde) is a chemical compound with the formula  $C_2HCl_3O$  and CAS (chemical abstracts service) 75-87-6. Chloral is produced by the chlorination of ethanol and is also produced as an intermediate in the synthesis of various products, for example DDT. Chloral is used for production of chloral hydrate (formula  $C_2H_3Cl_3O_2$  and CAS No. 302-17-0).

Chloral hydrate is an ingredient used in Hoyer's solution<sup>[213-216]</sup>. In mouse studies, oral administration of chloral in water induced liver nodules as well as hyperplastic nodules and HCC after 92 wk. Significant increases in HCC incidence were seen in treated mice surviving 104 wk<sup>[217,218]</sup>. Some studies indicate that chloral hydrate is able to produce genomic alterations, such as chromosomal aberrations, loss of cell apoptosis and rupture of the gap junction. There are limited studies on carcinogenicity in humans. However, thanks to evidence in animal studies, chloral and chloral hydrate are currently classified in group A2<sup>[216-219]</sup>.

## ORTHO-TOLUIDINE

Ortho-toluidine (O-toluidine) (CAS No. 95-53-4) is used in the chemical and rubber industry and is found in some colorants, herbicides and pesticides. O-toluidine can be an environmental contaminant if in the water used for irrigation of the cultivated fields. It has also been found in tobacco cigarettes. In animal models, O-toluidine caused bladder cancer and its exposure increased the incidence of HCC. Its carcinogenic power is probably due to the ability to determine the formation of DNA adducts, causing damage to the DNA structure. Therefore, O-toluidine is classified in group A<sup>[220-229]</sup>.

## 4,4'-METHYLENE BIS (2-CHLOROBENZENAMINE)

4,4'-Methylene bis (2-chlorobenzylamine) (MOCA) (CAS No. 101-14-4), used in the rubber industry, can be absorbed through the skin in workers, while population exposure occurs by ingestion of vegetables grown in contaminated soil. The ingestion or subcutaneous injection of MOCA in rats results in an increased incidence of HCC and lung cancer<sup>[230-232]</sup>. MOCA has a documented detrimental effect on the genome; in fact, it is able to determine chromatin alterations and deletions<sup>[76,233]</sup>. MOCA is classified in IARC group 1.

## 4-AMINOBIHENYL

4-aminobiphenyl (4-ABP) is used in the rubber industry as an antioxidant and a dye and is also found in cigarettes. It is classified in IARC group 1<sup>[76]</sup>. In rats, 4-ABP ingestion causes bladder cancer, angiosarcoma and HCC; subcutaneous or intraperitoneal exposure determines a high incidence of HCC<sup>[234]</sup>. The metabolism of 4-ABP determines the formation of N-hydroxyl ABP which is a mutagen. 4-ABP can form a DNA adduct. In human liver tissue, higher 4-ABP-DNA levels were observed in HCC cases compared with controls<sup>[235-241]</sup>. Although there was a dose-related increase in 4-ABP DNA (cigarettes smoked/day) and an association with mutant p53 protein expression in bladder cancers, there are currently no reports of p53 or other specific gene mutations caused by exposure to PAH or 4-ABP in HCC<sup>[242-244]</sup>.

## BENZIDINE AND DYES METABOLIZED TO BENZIDINE

In the past, benzidine (BZD) (CAS No. 92-87-5) and dyes metabolized to benzidine have been widely used in the production of dyes. Their use is currently banned in the United States and Europe. However, the use of products containing these substances may expose people to health risks<sup>[245-248]</sup>. Epidemiological data on the risk of tumors in humans are limited, but the ingestion of BZD in rats increases the incidence of HCC<sup>[249-252]</sup>.

BZD and dyes metabolized to BZD are classified in group 1 carcinogens<sup>[76]</sup>.

## ARSENIC

Arsenic (As) (CAS 7440-38-2) is widespread in nature and, combined with other elements, forms very toxic inorganic compounds that can pollute the water and contaminate the population. The workers in mechanical industries are exposed to the risk of illness from dyes, chemicals and glass<sup>[253-258]</sup>.

After oral intake and gastrointestinal absorption, it is metabolized in the liver where it is conjugated with glutathione and methylated<sup>[259,260]</sup>. The chronic exposure to small amounts produces chronic liver disease, cirrhosis and HCC.

In the 2004 IARC monograph, the result of inorganic As in HCC formation was called "limited". In contrast, more recent data from animal models have shown the possibility of a strong bond with liver tumor formation<sup>[261-268]</sup>.

Various carcinogenic mechanisms, genetic and epigenetic, have been proposed: DNA methylation, oxidative damage, genomic instability and reduction of programmed cell death<sup>[269-274]</sup>.

## CADMIUM

Cadmium (Cd) (CAS No. 7440-43-9) is a chemical element used as an anti-corrosion coating and a pigment. It is combined with lithium in rechargeable batteries and is also in cigarette tobacco. In fact, a cigarette contains about 2.0 µg Cd, of which 10.2% is transferred to the smoke<sup>[275]</sup>. Cd in the blood and body of smokers are typically double those found in non-smokers<sup>[276]</sup>. Burning municipal waste leads to inhalation of Cd. Workers in the metal and plastic product industry and workers involved in the construction of solar panels are exposed to Cd<sup>[277,278]</sup>.

In 2011, Cd production was estimated to be 600 metric tons in United States. Most of the Cd produced today is obtained from zinc and products recovered from spent Ni-Cd batteries. China, South Korea and Japan are the leading producers, followed by North America<sup>[278]</sup>. According to OSHA estimates, 300000 workers are exposed to Cd in the United States. Cd found in food and cigarette smoke accumulates in the liver, kidney and pancreas. Liver concentrations increase with age, peaking at 40-60 years.

Based on epidemiological data, the IARC states that there is no evidence of unequivocal carcinogenic effects of Cd<sup>[278-282]</sup>.

However, many animal studies have demonstrated the ability of Cd to determine various tumors, including HCC. This risk is dose and time-dependent and it is conditioned on the exposure mode. Oxidative stress, DNA methylation, the failure of DNA repair, activation of oncogenes, uncontrolled cell growth and the loss of apoptosis are among the mechanisms hypothesized by

researchers<sup>[283-286]</sup>. Interestingly, Sabolić *et al.*<sup>[287]</sup> have shown that the Cd can be internalized in the Kupffer cells which begin to produce cytokines, some of these are indicated as cofactors in the development of HCC.

Some studies have reported that chronic exposure to Cd increases the risk of tumors in humans<sup>[288-290]</sup>. However, large epidemiological studies are necessary to demonstrate whether long term Cd contamination is responsible for HCC development in humans, as in animal models.

## DISCUSSION

Workplace risk prevention and safety rely chiefly on eliminating the risk itself (primary prevention) and, when it is not technically feasible, measures have to be enacted to reduce risk to a minimum<sup>[291]</sup>.

When chemical agents are involved, primary prevention entails replacing a toxic agent with a non-toxic one. However, some mutagenic/carcinogenic agents can be produced in synthetic processes as intermediates or waste products<sup>[292]</sup>. As regards biological agents, it is critical to distinguish deliberate introduction of an agent into the working cycle, as in research centers, from the potential exposure resulting from its unwanted presence, as in the case of health care workers. Whereas the biological agent can be replaced in the former case, other measures have to be enacted in the latter<sup>[293]</sup>.

When risk assessment determines the existence of a healthy risk, adequate risk control systems have to be implemented. Such systems are divided into general and personal protection devices (PPD). The former include adoption of technical and procedural measures, for instance the reduction of environmental pollutants, whereas PPD largely consist of devices worn by workers (*e.g.*, masks, gloves), preventing direct contact with vapors, fumes and/or potentially contaminated material, *e.g.*, biological fluids<sup>[294]</sup>. Biological risk prevention may involve mandatory vaccine prophylaxis, as in the case of HBV infection. Moreover, the fast pace of advances in vaccine development and protection equipment and devices requires continuous re-assessment of workplace protection systems<sup>[295,296]</sup>.

In workplaces where risks are documented, safety procedures must be instituted in accordance with national guidelines. In case of flaws or deficiencies in such guidelines, those in charge of workplace safety are required to refer to the guidelines of internationally recognized organizations such as the Centers for Disease Control and Prevention, American Conference of Industrial Hygienists, NIOSH, *etc.*

The employer and occupational physician have key roles in preventing occupational risk and diseases. The occupational physician, besides carrying out biological monitoring and health surveillance (secondary prevention), is responsible for promoting workplace health<sup>[291]</sup>.

As regards HCC prevention, all exposed workers should have HBV vaccination. In addition, campaigns

against smoking and alcohol drinking should be organized, providing an explicit warning that these factors may contribute to the development of liver cancer<sup>[10-12,101]</sup>.

Development and progression of HCC is still not a completely known multistage process. Genetic, epigenetic and immunological factors probably contribute to the development of HCC<sup>[7,11,13,37,38,50,51,101,297,298]</sup>.

## CONCLUSION

In conclusion, the precancerous milieu of chronic liver disease is characterized by neo-angiogenesis, inflammation with ROS production and fibrosis. Synchronous events occurring in this setting also include hypoxia, oxidative stress, apoptosis, mitophagy and autophagy<sup>[299-302]</sup>.

Autophagy shows a double face in HCC. While autophagy helps to prevent tumorigenesis, it is also used by the cancer cells for survival against apoptosis by traditional chemotherapeutic drugs<sup>[303,304]</sup>. Initially, autophagy functions as a tumor suppressor and later, when HCC has developed, the autophagy may contribute to its growth<sup>[303,305]</sup>.

Microbes have evolved mechanisms to evade and exploit autophagy and both HBV and HCV use autophagy for their own survival<sup>[306]</sup>. Studies have shown that autophagy enhances viral replication at most steps of HBV replication and that autophagy proteins are likely to be factors for the initial steps of HCV replication<sup>[307,308]</sup>. In tumor cells with defects in apoptosis, autophagy allows prolonged survival.

## Future directions

All these mechanisms are still being studied in order to provide new therapeutic approaches to HCC<sup>[309]</sup>. Despite the progress achieved in understanding the cancer process and the impact of this knowledge on treatment, primary prevention remains the most effective approach to reduce cancer mortality in both developed and developing countries for the near future<sup>[9,37,38,50,51,56,309]</sup>.

## REFERENCES

- 1 **IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.** Biological agents. Volume 100 B. A review of human carcinogens. *IARC Monogr Eval Carcinog Risks Hum* 2012; **100**: 1-441 [PMID: 23189750]
- 2 **McGlynn KA,** Petrick JL, London WT. Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability. *Clin Liver Dis* 2015; **19**: 223-238 [PMID: 25921660 DOI: 10.1016/j.cld.2015.01.001]
- 3 **Wallace MC,** Preen D, Jeffrey GP, Adams LA. The evolving epidemiology of hepatocellular carcinoma: a global perspective. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 765-779 [PMID: 25827821 DOI: 10.1586/17474124.2015.1028363]
- 4 **Papatheodoridis GV,** Chan HL, Hansen BE, Janssen HL, Lampertico P. Risk of hepatocellular carcinoma in chronic hepatitis B: assessment and modification with current antiviral therapy. *J Hepatol* 2015; **62**: 956-967 [PMID: 25595883 DOI: 10.1016/j.jhep.2015.01.002]
- 5 **Lemon SM,** McGovern DR. Is hepatitis C virus carcinogenic? *Gastroenterology* 2012; **142**: 1274-1278 [PMID: 22537433 DOI: 10.1016/j.gastro.2012.05.001]



- 10.1053/j.gastro.2012.01.045]
- 6 **Saad-Hussein A**, Taha MM, Beshir S, Shahy EM, Shaheen W, Elhamshary M. Carcinogenic effects of aflatoxin B1 among wheat handlers. *Int J Occup Environ Health* 2014; **20**: 215-219 [PMID: 25000109 DOI: 10.1179/2049396714Y]
- 7 **Askgaard G**, Grønbaek M, Kjær MS, Tjønneland A, Tolstrup JS. Alcohol drinking pattern and risk of alcoholic liver cirrhosis: a prospective cohort study. *J Hepatol* 2015; **62**: 1061-1067 [PMID: 25634330 DOI: 10.1016/j.jhep.2014.12.005]
- 8 **Bassuk SS**, Manson JE. Oral contraceptives and menopausal hormone therapy: relative and attributable risks of cardiovascular disease, cancer, and other health outcomes. *Ann Epidemiol* 2015; **25**: 193-200 [PMID: 25534509 DOI: 10.1016/j.annepidem.2014.11.004]
- 9 **Bertino G**, Demma S, Ardiri A, Toro A, Calvagno Gs, Malaguarnera G, Bertino N, Malaguarnera M, Malaguarnera M, Di Carlo I. Focal nodular hyperplasia from the surgery to the follow-up. Change of therapeutic approach. *Acta Medica Mediterranea* 2014; **30**: 1329-1336
- 10 **Lv Y**, Liu C, Wei T, Zhang JF, Liu XM, Zhang XF. Cigarette smoking increases risk of early morbidity after hepatic resection in patients with hepatocellular carcinoma. *Eur J Surg Oncol* 2015; **41**: 513-519 [PMID: 25656703 DOI: 10.1016/j.ejso.2015.01.015]
- 11 **Purohit V**, Rapaka R, Kwon OS, Song BJ. Roles of alcohol and tobacco exposure in the development of hepatocellular carcinoma. *Life Sci* 2013; **92**: 3-9 [PMID: 23123447 DOI: 10.1016/j.lfs.2012.10.009]
- 12 **Hsieh YH**, Chang WS, Tsai CW, Tsai JP, Hsu CM, Jeng LB, Bau DT. DNA double-strand break repair gene XRCC7 genotypes were associated with hepatocellular carcinoma risk in Taiwanese males and alcohol drinkers. *Tumour Biol* 2015; **36**: 4101-4106 [PMID: 25944161 DOI: 10.1007/s13277-014-2934-5]
- 13 **Loomba R**, Yang HI, Su J, Brenner D, Barrett-Connor E, Iloeje U, Chen CJ. Synergism between obesity and alcohol in increasing the risk of hepatocellular carcinoma: a prospective cohort study. *Am J Epidemiol* 2013; **177**: 333-342 [PMID: 23355498 DOI: 10.1093/aje/kws252]
- 14 **Bertino G**, Ardiri AM, Ali FT, Boemi PM, Cilio D, Di Prima P, Fisichella A, Ierna D, Neri S, Pulvirenti D, Urso G, Mauceri B, Valenti M, Bruno CM. Obesity and related diseases: an epidemiologic study in eastern Sicily. *Minerva Gastroenterol Dietol* 2006; **52**: 379-385 [PMID: 17108868]
- 15 **Hardt A**, Stippel D, Odenthal M, Hölscher AH, Dienes HP, Drebbler U. Development of hepatocellular carcinoma associated with anabolic androgenic steroid abuse in a young bodybuilder: a case report. *Case Rep Pathol* 2012; **2012**: 195607 [PMID: 22934212 DOI: 10.1155/2012/195607]
- 16 **Toro A**, Mahfouz AE, Ardiri A, Malaguarnera M, Malaguarnera G, Loria F, Bertino G, Di Carlo I. What is changing in indications and treatment of hepatic hemangiomas. A review. *Ann Hepatol* 2014; **13**: 327-339 [PMID: 24927603]
- 17 **Vinci M**, Malaguarnera L, Pistone G. RS3PE and ovarian cancer. *Ann Rheum Dis* 2001; **60**: 429-431 [PMID: 11284457 DOI: 10.1136/ard.60.4.429b]
- 18 **Czarnecki LA**, Moberly AH, Turkel DJ, Rubinstein T, Pottackal J, Rosenthal MC, McCandlish EF, Buckley B, McGann JP. Functional rehabilitation of cadmium-induced neurotoxicity despite persistent peripheral pathophysiology in the olfactory system. *Toxicol Sci* 2012; **126**: 534-544 [PMID: 22287023 DOI: 10.1093/toxsci/kfs030]
- 19 **Yang CJ**, Lin JL, Lin-Tan DT, Weng CH, Hsu CW, Lee SY, Lee SH, Chang CM, Lin WR, Yen TH. Spectrum of toxic hepatitis following intentional paraquat ingestion: analysis of 187 cases. *Liver Int* 2012; **32**: 1400-1406 [PMID: 22672665 DOI: 10.1111/j.1478-3231.2012.02829.x]
- 20 **Sherman M**. Vinyl chloride and the liver. *J Hepatol* 2009; **51**: 1074-1081 [PMID: 19836850 DOI: 10.1016/j.jhep.2009.09.012]
- 21 **Wong O**, Morgan RW, Kheifets L, Larson SR, Whorton MD. Mortality among members of a heavy construction equipment operators union with potential exposure to diesel exhaust emissions. *Br J Ind Med* 1985; **42**: 435-448 [PMID: 2410010 DOI: 10.1136/oem.42.7.435]
- 22 **Jansson C**, Alderling M, Hogstedt C, Gustavsson P. Mortality among Swedish chimney sweeps (1952-2006): an extended cohort study. *Occup Environ Med* 2012; **69**: 41-47 [PMID: 21705462 DOI: 10.1136/oem.2010.064246]
- 23 **Ward EM**, Fajen JM, Ruder AM, Rinsky RA, Halperin WE, Fessler-Flesch CA. Mortality study of workers in 1,3-butadiene production units identified from a chemical workers cohort. *Environ Health Perspect* 1995; **103**: 598-603 [PMID: 7556014 DOI: 10.1289/ehp.95103598]
- 24 **Malaguarnera M**, Vacante M, Russo C, Gargante MP, Giordano M, Bertino G, Neri S, Malaguarnera M, Galvano F, Li Volti G. Rosuvastatin reduces nonalcoholic fatty liver disease in patients with chronic hepatitis C treated with  $\alpha$ -interferon and ribavirin: Rosuvastatin reduces NAFLD in HCV patients. *Hepat Mon* 2011; **11**: 92-98 [PMID: 22087124]
- 25 **Steenland K**, Palu S. Cohort mortality study of 57,000 painters and other union members: a 15 year update. *Occup Environ Med* 1999; **56**: 315-321 [PMID: 10472305 DOI: 10.1136/oem.56.5.315]
- 26 **Chen R**, Seaton A. A meta-analysis of mortality among workers exposed to organic solvents. *Occup Med (Lond)* 1996; **46**: 337-344 [PMID: 8918147 DOI: 10.1093/occmed/46.5.337]
- 27 **Gibbs GW**, Amsel J, Soden K. A cohort mortality study of cellulose triacetate-fiber workers exposed to methylene chloride. *J Occup Environ Med* 1996; **38**: 693-697 [PMID: 8823660 DOI: 10.1097/00043764-199607000-00012]
- 28 **Chow WH**, McLaughlin JK, Zheng W, Blot WJ, Gao YT. Occupational risks for primary liver cancer in Shanghai, China. *Am J Ind Med* 1993; **24**: 93-100 [PMID: 8352295 DOI: 10.1002/ajim.4700240109]
- 29 **Toro A**, Ardiri A, Mannino M, Arcerito MC, Mannino G, Palermo F, Bertino G, Di Carlo I. Effect of pre- and post-treatment  $\alpha$ -fetoprotein levels and tumor size on survival of patients with hepatocellular carcinoma treated by resection, transarterial chemoembolization or radiofrequency ablation: a retrospective study. *BMC Surg* 2014; **14**: 40 [PMID: 24993566 DOI: 10.1186/1471-2482-14-40]
- 30 **Heinemann K**, Willich SN, Heinemann LA, DoMinh T, Möhner M, Heuchert GE. Occupational exposure and liver cancer in women: results of the Multicentre International Liver Tumour Study (MILTS). *Occup Med (Lond)* 2000; **50**: 422-429 [PMID: 10994245 DOI: 10.1093/occmed/50.6.422]
- 31 **Chang CK**, Astrakianakis G, Thomas DB, Seixas NS, Ray RM, Gao DL, Wernli KJ, Fitzgibbons ED, Vaughan TL, Checkoway H. Occupational exposures and risks of liver cancer among Shanghai female textile workers—a case-cohort study. *Int J Epidemiol* 2006; **35**: 361-369 [PMID: 16373377 DOI: 10.1093/ije/dyi282]
- 32 **Oh JK**, Weiderpass E. Infection and cancer: global distribution and burden of diseases. *Ann Glob Health* 2014; **80**: 384-392 [PMID: 25512154 DOI: 10.1016/j.aogh.2014.09.013]
- 33 **Bouvard V**, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Coglian V. A review of human carcinogens—Part B: biological agents. *Lancet Oncol* 2009; **10**: 321-322 [PMID: 19350698 DOI: 10.1016/S1470-2045(09)70096-8]
- 34 **Coutlée F**, Franco EL. Infectious agents. *IARC Sci Publ* 2011; **(163)**: 175-187 [PMID: 22997862]
- 35 **Kew MC**. Aflatoxins as a cause of hepatocellular carcinoma. *J Gastrointest Liver Dis* 2013; **22**: 305-310 [PMID: 24078988]
- 36 **Carr BI**, Guerra V, Steel JL, Lu SN. A comparison of patients with hepatitis B- or hepatitis C-based advanced-stage hepatocellular carcinoma. *Semin Oncol* 2015; **42**: 309-315 [PMID: 25843735 DOI: 10.1053/j.seminoncol.2014.12.019]
- 37 **Stroffolini T**, Spadaro A, Di Marco V, Scifo G, Russello M, Montalto G, Bertino G, Surace L, Caroleo B, Foti G, Portelli V, Madonia S, Sapienza M, Cosco L, Frugiele P, Galdieri A, Brandolino N, Siciliano R, Bruno S, Almasio PL. Current practice of chronic hepatitis B treatment in Southern Italy. *Eur J Intern Med* 2012; **23**: e124-e127 [PMID: 22726382 DOI: 10.1016/j.ejim.2012.03.018]

- 38 **Askarian M**, Yadollahi M, Kuochak F, Danaei M, Vakili V, Momeni M. Precautions for health care workers to avoid hepatitis B and C virus infection. *Int J Occup Environ Med* 2011; **2**: 191-198 [PMID: 23022838]
- 39 **Hajarizadeh B**, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 553-562 [PMID: 23817321 DOI: 10.1038/nrgastro.2013.107]
- 40 **Bosques-Padilla FJ**, Vázquez-Elizondo G, Villaseñor-Todd A, Garza-González E, Gonzalez-Gonzalez JA, Maldonado-Garza HJ. Hepatitis C virus infection in health-care settings: medical and ethical implications. *Ann Hepatol* 2010; **9** Suppl: 132-140 [PMID: 20714010]
- 41 **Sinn DH**, Lee J, Goo J, Kim K, Gwak GY, Paik YH, Choi MS, Lee JH, Koh KC, Yoo BC, Paik SW. Hepatocellular carcinoma risk in chronic hepatitis B virus-infected compensated cirrhosis patients with low viral load. *Hepatology* 2015; **62**: 694-701 [PMID: 25963803 DOI: 10.1002/hep.27889]
- 42 **Grosso G**, Mistretta A, Marventano S, Ferranti R, Mauro L, Cunsolo R, Proietti L, Malaguarnera M. Long-term persistence of seroprotection by hepatitis B vaccination in healthcare workers of southern Italy. *Hepat Mon* 2012; **12**: e6025 [PMID: 23087756 DOI: 10.5812/hepatmon.6025]
- 43 **Yang HI**, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, Wang LY, Lu SN, You SL, Chen DS, Liaw YF, Chen CJ. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 1134-1143 [PMID: 18695135 DOI: 10.1093/jnci/djn243]
- 44 **Malaguarnera G**, Vacante M, Drago F, Bertino G, Motta M, Giordano M, Malaguarnera M. Endozepine-4 levels are increased in hepatic coma. *World J Gastroenterol* 2015; **21**: 9103-9110 [PMID: 26290636 DOI: 10.3748/wjg.v21.i30.9103]
- 45 **Saitta C**, Tripodi G, Barbera A, Bertuccio A, Smedile A, Ciancio A, Raffa G, Sangiovanni A, Navarra G, Raimondo G, Pollicino T. Hepatitis B virus (HBV) DNA integration in patients with occult HBV infection and hepatocellular carcinoma. *Liver Int* 2015; **35**: 2311-2317 [PMID: 25677098 DOI: 10.1111/liv.12807]
- 46 **Fattovich G**, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35-S50 [PMID: 15508101 DOI: 10.1053/j.gastro.2004.09.014]
- 47 **Hassan MM**, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, Beasley P, Patt YZ. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. *Hepatology* 2002; **36**: 1206-1213 [PMID: 12395331 DOI: 10.1053/jhep.2002.36780]
- 48 **Cha C**, Dematteo RP. Molecular mechanisms in hepatocellular carcinoma development. *Best Pract Res Clin Gastroenterol* 2005; **19**: 25-37 [PMID: 15757803 DOI: 10.1016/j.bpg.2004.11.005]
- 49 **Thorgeirsson SS**, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. *Nat Genet* 2002; **31**: 339-346 [PMID: 12149612 DOI: 10.1038/ng0802-339]
- 50 **Malaguarnera M**, Scuderi L, Ardiri AI, Malaguarnera G, Bertino N, Ruggeri IM, Greco C, Ozyalcin E, Bertino E, Bertino G. Type II mixed cryoglobulinemia in patients with hepatitis C Virus: treatment with pegylated-interferon and ribavirin. *Acta Medica Mediterranea* 2015; **31**: 651
- 51 **Bertino G**, Demma S, Ardiri A, Proiti M, Gruttadauria S, Toro A, Malaguarnera G, Bertino N, Malaguarnera M, Malaguarnera M, Di Carlo I. Hepatocellular carcinoma: novel molecular targets in carcinogenesis for future therapies. *Biomed Res Int* 2014; **2014**: 203693 [PMID: 25089265 DOI: 10.1155/2014/203693]
- 52 **Bertino G**, Di Carlo I, Ardiri A, Calvagno GS, Demma S, Malaguarnera G, Bertino N, Malaguarnera M, Toro A, Malaguarnera M. Systemic therapies in hepatocellular carcinoma: present and future. *Future Oncol* 2013; **9**: 1533-1548 [PMID: 24106903 DOI: 10.2217/fon.13.171]
- 53 **Biondi A**, Malaguarnera G, Vacante M, Berretta M, D'Agata V, Malaguarnera M, Basile F, Drago F, Bertino G. Elevated serum levels of Chromogranin A in hepatocellular carcinoma. *BMC Surg* 2012; **12** Suppl 1: S7 [PMID: 23173843 DOI: 10.1186/1471-2482-12-S1-S7]
- 54 **Bertino G**, Ardiri AM, Boemi PM, Ierna D, Interlandi D, Caruso L, Minona E, Trovato MA, Vicari S, Li Destri G, Puleo S. A study about mechanisms of des-gamma-carboxy prothrombin's production in hepatocellular carcinoma. *Panminerva Med* 2008; **50**: 221-226 [PMID: 18927526]
- 55 **Bertino G**, Ardiri AM, Calvagno GS, Bertino N, Boemi PM. Prognostic and diagnostic value of des-γ-carboxy prothrombin in liver cancer. *Drug News Perspect* 2010; **23**: 498-508 [PMID: 21031166 DOI: 10.1358/dnp.2010.23.8.1444236]
- 56 **Bertino G**, Ardiri A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS. Hepatocellular carcinoma serum markers. *Semin Oncol* 2012; **39**: 410-433 [PMID: 22846859 DOI: 10.1053/j.seminoncol.2012.05.001]
- 57 **Bertino G**, Neri S, Bruno CM, Ardiri AM, Calvagno GS, Malaguarnera M, Toro A, Malaguarnera M, Clementi S, Bertino N, Di Carlo I. Diagnostic and prognostic value of alpha-fetoprotein, des-γ-carboxy prothrombin and squamous cell carcinoma antigen immunoglobulin M complexes in hepatocellular carcinoma. *Minerva Med* 2011; **102**: 363-371 [PMID: 22193346]
- 58 **Sartori M**, La Terra G, Aglietta M, Manzin A, Navino C, Verzetti G. Transmission of hepatitis C via blood splash into conjunctiva. *Scand J Infect Dis* 1993; **25**: 270-271 [PMID: 8511524 DOI: 10.1093/00365549309008497]
- 59 **Bertino G**, Ardiri A, Proiti M, Rigano G, Frazzetto E, Demma S, Ruggeri MI, Scuderi L, Malaguarnera G, Bertino N, Rapisarda V, Di Carlo I, Toro A, Salomone F, Malaguarnera M, Bertino E, Malaguarnera M. Chronic hepatitis C: This and the new era of treatment. *World J Hepatol* 2016; **8**: 92-106 [PMID: 26807205 DOI: 10.4254/wjh.v8.i2.92]
- 60 **MacCannell T**, Laramie AK, Gomaa A, Perz JF. Occupational exposure of health care personnel to hepatitis B and hepatitis C: prevention and surveillance strategies. *Clin Liver Dis* 2010; **14**: 23-36, vii [PMID: 20123437 DOI: 10.1016/j.cld.2009.11.001]
- 61 **Yazdanpanah Y**, De Carli G, Miguères B, Lot F, Campins M, Colombo C, Thomas T, Deuffic-Burban S, Prevot MH, Domart M, Tarantola A, Abiteboul D, Deny P, Pol S, Desenclos JC, Puro V, Bouvet E. Risk factors for hepatitis C virus transmission to health care workers after occupational exposure: a European case-control study. *Clin Infect Dis* 2005; **41**: 1423-1430 [PMID: 16231252 DOI: 10.1086/497131]
- 62 **Malaguarnera G**, Giordano M, Nunnari G, Bertino G, Malaguarnera M. Gut microbiota in alcoholic liver disease: pathogenetic role and therapeutic perspectives. *World J Gastroenterol* 2014; **20**: 16639-16648 [PMID: 25469033 DOI: 10.3748/wjg.v20.i44.16639]
- 63 **Petrovic D**, Stamatakis Z, Dempsey E, Golden-Mason L, Freeley M, Doherty D, Prichard D, Keogh C, Conroy J, Mitchell S, Volkov Y, McKeating JA, O'Farrelly C, Kelleher D, Long A. Hepatitis C virus targets the T cell secretory machinery as a mechanism of immune evasion. *Hepatology* 2011; **53**: 1846-1853 [PMID: 21452285 DOI: 10.1002/hep.24327]
- 64 **O'Bryan JM**, Potts JA, Bonkovsky HL, Mathew A, Rothman AL. Extended interferon-alpha therapy accelerates telomere length loss in human peripheral blood T lymphocytes. *PLoS One* 2011; **6**: e20922 [PMID: 21829595 DOI: 10.1371/journal.pone.0020922]
- 65 **Pardee AD**, Butterfield LH. Immunotherapy of hepatocellular carcinoma: Unique challenges and clinical opportunities. *Oncoimmunology* 2012; **1**: 48-55 [PMID: 22720211 DOI: 10.4161/onci.1.1.18344]
- 66 **Bertino G**, Ardiri A, Boemi PM, Calvagno GS, Ruggeri IM, Speranza A, Santonocito MM, Ierna D, Bruno CM, Valenti M, Boemi R, Naimo S, Neri S. Epoetin alpha improves the response to antiviral treatment in HCV-related chronic hepatitis. *Eur J Clin Pharmacol* 2010; **66**: 1055-1063 [PMID: 20652232 DOI: 10.1007/s00228-010-0868-4]
- 67 **Malaguarnera G**, Pennisi M, Gagliano C, Vacante M, Malaguarnera M, Salomone S, Drago F, Bertino G, Caraci F, Nunnari G, Malaguarnera M. Acetyl-L-Carnitine Supplementation During HCV Therapy With Pegylated Interferon-α 2b Plus Ribavirin: Effect on Work Performance; A Randomized Clinical Trial.

- Hepat Mon* 2014; **14**: e11608 [PMID: 24910702 DOI: 10.5812/hepatmon.11608]
- 68 **Malaguarnera M**, Vacante M, Giordano M, Motta M, Bertino G, Pennisi M, Neri S, Malaguarnera M, Li Volti G, Galvano F. L-carnitine supplementation improves hematological pattern in patients affected by HCV treated with Peg interferon- $\alpha$  2b plus ribavirin. *World J Gastroenterol* 2011; **17**: 4414-4420 [PMID: 22110268 DOI: 10.3748/wjg.v17.i39.4414]
  - 69 **Malaguarnera M**, Vacante M, Bertino G, Neri S, Malaguarnera M, Gargante MP, Motta M, Lupo L, Chisari G, Bruno CM, Pennisi G, Bella R. The supplementation of acetyl-L-carnitine decreases fatigue and increases quality of life in patients with hepatitis C treated with pegylated interferon- $\alpha$  2b plus ribavirin. *J Interferon Cytokine Res* 2011; **31**: 653-659 [PMID: 21923249 DOI: 10.1089/jir.2011.0010]
  - 70 **Bruno CM**, Valenti M, Bertino G, Arditi A, Amoroso A, Consolo M, Mazzarino CM, Neri S. Relationship between circulating interleukin-10 and histological features in patients with chronic C hepatitis. *Ann Saudi Med* 2011; **31**: 360-364 [PMID: 21808111 DOI: 10.4103/0256-4947.83215]
  - 71 **Zhao F**, Korangy F, Greten TF. Cellular immune suppressor mechanisms in patients with hepatocellular carcinoma. *Dig Dis* 2012; **30**: 477-482 [PMID: 23108303 DOI: 10.1159/000341695]
  - 72 **Cai L**, Zhang Z, Zhou L, Wang H, Fu J, Zhang S, Shi M, Zhang H, Yang Y, Wu H, Tien P, Wang FS. Functional impairment in circulating and intrahepatic NK cells and relative mechanism in hepatocellular carcinoma patients. *Clin Immunol* 2008; **129**: 428-437 [PMID: 18824414 DOI: 10.1016/j.clim.2008.08.012]
  - 73 **El Ansary M**, Mogawer S, Elhamid SA, Alwakil S, Aboelkasem F, Sabaawy HE, Abdelhalim O. Immunotherapy by autologous dendritic cell vaccine in patients with advanced HCC. *J Cancer Res Clin Oncol* 2013; **139**: 39-48 [PMID: 22886490 DOI: 10.1007/s00432-012-1298-8]
  - 74 **Strosnider H**, Azziz-Baumgartner E, Banziger M, Bhat RV, Breiman R, Brune MN, DeCock K, Dilley A, Groopman J, Hell K, Henry SH, Jeffers D, Jolly C, Jolly P, Kibata GN, Lewis L, Liu X, Lubner G, McCoy L, Mensah P, Miraglia M, Misore A, Njapau H, Ong CN, Onsongo MT, Page SW, Park D, Patel M, Phillips T, Pineiro M, Pronczuk J, Rogers HS, Rubin C, Sabino M, Schaafsma A, Shephard G, Stroka J, Wild C, Williams JT, Wilson D. Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. *Environ Health Perspect* 2006; **114**: 1898-1903 [PMID: 17185282 DOI: 10.1289/ehp.9302]
  - 75 **Liu Y**, Chang CC, Marsh GM, Wu F. Population attributable risk of aflatoxin-related liver cancer: systematic review and meta-analysis. *Eur J Cancer* 2012; **48**: 2125-2136 [PMID: 22405700 DOI: 10.1016/j.ejca.2012.02.009]
  - 76 **IARC Working Group on the Evaluation of Carcinogenic Risks to Humans**. Chemical agents and related occupations. *IARC Monogr Eval Carcinog Risks Hum* 2012; **100**: 9-562 [PMID: 23189753]
  - 77 **Viegas S**, Veiga L, Figueiredo P, Almeida A, Carolino E, Viegas C. Assessment of workers' exposure to aflatoxin B1 in a Portuguese waste industry. *Ann Occup Hyg* 2015; **59**: 173-181 [PMID: 25324565 DOI: 10.1093/annhyg/meu082]
  - 78 **Autrup JL**, Schmidt J, Seremet T, Autrup H. Determination of exposure to aflatoxins among Danish workers in animal-feed production through the analysis of aflatoxin B1 adducts to serum albumin. *Scand J Work Environ Health* 1991; **17**: 436-440 [PMID: 1788537 DOI: 10.5271/sjweh.1683]
  - 79 **Diaz GJ**, Murcia HW, Cepeda SM. Cytochrome P450 enzymes involved in the metabolism of aflatoxin B1 in chickens and quail. *Poult Sci* 2010; **89**: 2461-2469 [PMID: 20952710 DOI: 10.3382/ps.2010-00864]
  - 80 **Lin ZH**, Chen JC, Wang YS, Huang TJ, Wang J, Long XD. DNA repair gene XRCC4 codon 247 polymorphism modified diffusely infiltrating astrocytoma risk and prognosis. *Int J Mol Sci* 2014; **15**: 250-260 [PMID: 24378850 DOI: 10.3390/ijms15010250]
  - 81 **Long XD**, Yao JG, Zeng Z, Ma Y, Huang XY, Wei ZH, Liu M, Zhang JJ, Xue F, Zhai B, Xia Q. Polymorphisms in the coding region of X-ray repair complementing group 4 and aflatoxin B1-related hepatocellular carcinoma. *Hepatology* 2013; **58**: 171-181 [PMID: 23390017 DOI: 10.1002/hep.26311]
  - 82 **Lai H**, Mo X, Yang Y, He K, Xiao J, Liu C, Chen J, Lin Y. Association between aflatoxin B1 occupational airway exposure and risk of hepatocellular carcinoma: a case-control study. *Tumour Biol* 2014; **35**: 9577-9584 [PMID: 24961349 DOI: 10.1007/s13277-014-2231-3]
  - 83 **Hu T**, Du Q, Ren F, Liang S, Lin D, Li J, Chen Y. Spatial analysis of the home addresses of hospital patients with hepatitis B infection or hepatoma in Shenzhen, China from 2010 to 2012. *Int J Environ Res Public Health* 2014; **11**: 3143-3155 [PMID: 24637909 DOI: 10.3390/ijerph110303143]
  - 84 **Villar S**, Ortiz-Cuaran S, Abedi-Ardekani B, Gouas D, Nogueira da Costa A, Plymoth A, Kluhnaprema T, Kalalak A, Sangrajan S, Friesen MD, Groopman JD, Hainaut P. Aflatoxin-induced TP53 R249S mutation in hepatocellular carcinoma in Thailand: association with tumors developing in the absence of liver cirrhosis. *PLoS One* 2012; **7**: e37707 [PMID: 22675488 DOI: 10.1371/journal.pone.0037707]
  - 85 **Gouas D**, Shi H, Hainaut P. The aflatoxin-induced TP53 mutation at codon 249 (R249S): biomarker of exposure, early detection and target for therapy. *Cancer Lett* 2009; **286**: 29-37 [PMID: 19376640 DOI: 10.1016/j.canlet.2009.02.057]
  - 86 **Kirk GD**, Lesi OA, Mendy M, Szymańska K, Whittle H, Goedert JJ, Hainaut P, Montesano R. 249(ser) TP53 mutation in plasma DNA, hepatitis B viral infection, and risk of hepatocellular carcinoma. *Oncogene* 2005; **24**: 5858-5867 [PMID: 16007211 DOI: 10.1038/sj.onc.1208732]
  - 87 **Jargot D**, Melin S. Characterization and validation of sampling and analytical methods for mycotoxins in workplace air. *Environ Sci Process Impacts* 2013; **15**: 633-644 [PMID: 23738362 DOI: 10.1039/c2em30566f]
  - 88 **Viegas S**, Veiga L, Malta-Vacas J, Sabino R, Figueredo P, Almeida A, Viegas C, Carolino E. Occupational exposure to aflatoxin (AFB1) in poultry production. *J Toxicol Environ Health A* 2012; **75**: 1330-1340 [PMID: 23095151 DOI: 10.1080/15287394.2012.721164]
  - 89 **Burg WA**, Shotwell OL, Saltzman BE. Measurements of airborne aflatoxins during the handling of contaminated corn. *Am Ind Hyg Assoc J* 1981; **42**: 1-11 [PMID: 6784564 DOI: 10.1080/15298668191419271]
  - 90 **Viegas S**, Faisca VM, Dias H, Clérigo A, Carolino E, Viegas C. Occupational exposure to poultry dust and effects on the respiratory system in workers. *J Toxicol Environ Health A* 2013; **76**: 230-239 [PMID: 23514065 DOI: 10.1080/15287394.2013.757199]
  - 91 **Autrup JL**, Schmidt J, Autrup H. Exposure to aflatoxin B1 in animal-feed production plant workers. *Environ Health Perspect* 1993; **99**: 195-197 [PMID: 8319623 DOI: 10.1289/ehp.9399195]
  - 92 **Ghosh SK**, Desai MR, Pandya GL, Venkaiah K. Airborne aflatoxin in the grain processing industries in India. *Am Ind Hyg Assoc J* 1997; **58**: 583-586 [PMID: 9248032 DOI: 10.1080/15428119791012513]
  - 93 **Desai MR**, Ghosh S. Occupational exposure to airborne fungi among rice mill workers with special reference to aflatoxin producing *A. flavus* strains. *Ann Agric Environ Med* 2003; **10**: 159-162 [PMID: 14677906]
  - 94 **Traverso A**, Bassoli V, Cioè A, Anselmo S, Ferro M. Assessment of aflatoxin exposure of laboratory worker during food contamination analyses. Assessment of the procedures adopted by an A.R.P.A.L. laboratory (Liguria Region Environmental Protection Agency). *Med Lav* 2010; **101**: 375-380 [PMID: 21105592]
  - 95 **Long XD**, Huang XY, Yao JG, Liao P, Tang YJ, Ma Y, Xia Q. Polymorphisms in the precursor microRNAs and aflatoxin B1-related hepatocellular carcinoma. *Mol Carcinog* 2015; Epub ahead of print [PMID: 26152337 DOI: 10.1002/mc.22350]
  - 96 **Saad-Hussein A**, Beshir S, Moubarz G, Elserougy S, Ibrahim MI. Effect of occupational exposure to aflatoxins on some liver tumor markers in textile workers. *Am J Ind Med* 2013; **56**: 818-824 [PMID: 23359393 DOI: 10.1002/ajim.22162]



- 97 **Levy BS**, Wegman DH, Baron SL, Sokas RK. Occupational and Environmental Health: Recognizing and Preventing Disease and Injury. 6th ed. New York: Oxford University Press, Inc., 2011
- 98 **IARC Working Group on the Evaluation of Carcinogenic Risks to Humans**. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 97. 1,3-butadiene, ethylene oxide and vinyl halides (vinyl fluoride, vinyl chloride and vinyl bromide). *IARC Monogr Eval Carcinog Risks Hum* 2008; **97**: 3-471 [PMID: 20232717]
- 99 **Lopez V**, Chamoux A, Tempier M, Thiel H, Ughetto S, Troussellard M, Naughton G, Duthel F. The long-term effects of occupational exposure to vinyl chloride monomer on microcirculation: a cross-sectional study 15 years after retirement. *BMJ Open* 2013; **3**: [PMID: 23794583 DOI: 10.1136/bmjopen-2013-002785]
- 100 **Uccello M**, Malaguarnera G, Corriere T, Biondi A, Basile F, Malaguarnera M. Risk of hepatocellular carcinoma in workers exposed to chemicals. *Hepat Mon* 2012; **12**: e5943 [PMID: 23162599 DOI: 10.5812/hepatmon.5943]
- 101 **Caponnetto P**, Russo C, Di Maria A, Morjaria JB, Barton S, Guarino F, Basile E, Proiti M, Bertino G, Cacciola RR, Polosa R. Circulating endothelial-coagulative activation markers after smoking cessation: a 12-month observational study. *Eur J Clin Invest* 2011; **41**: 616-626 [PMID: 21198559 DOI: 10.1111/j.1365-2362.2010.02449.x]
- 102 **Kauppinen T**, Toikkanen J, Pedersen D, Young R, Ahrens W, Boffetta P, Hansen J, Kromhout H, Maqueda Blasco J, Mirabelli D, de la Orden-Rivera V, Pannett B, Plato N, Savela A, Vincent R, Kogevinas M. Occupational exposure to carcinogens in the European Union. *Occup Environ Med* 2000; **57**: 10-18 [PMID: 10711264 DOI: 10.1136/oem.57.1.10]
- 103 **Dobecki M**, Romanowicz B. [Occupational exposure to toxic substances during the production of vinyl chloride and chlorinated organic solvents]. *Med Pr* 1993; **44**: 99-102 [PMID: 8377644]
- 104 **Fred C**, Törnqvist M, Granath F. Evaluation of cancer tests of 1,3-butadiene using internal dose, genotoxic potency, and a multiplicative risk model. *Cancer Res* 2008; **68**: 8014-8021 [PMID: 18829559 DOI: 10.1158/0008-5472.CAN-08-0334]
- 105 **Maroni M**, Mocchi F, Visentin S, Preti G, Fanetti AC. Periportal fibrosis and other liver ultrasonography findings in vinyl chloride workers. *Occup Environ Med* 2003; **60**: 60-65 [PMID: 12499459 DOI: 10.1136/oem.60.1.60]
- 106 **Dogliotti E**. Molecular mechanisms of carcinogenesis by vinyl chloride. *Ann Ist Super Sanita* 2006; **42**: 163-169 [PMID: 17033136]
- 107 **Fedeli U**, Mastroangelo G. Vinyl chloride industry in the courtroom and corporate influences on the scientific literature. *Am J Ind Med* 2011; **54**: 470-473 [PMID: 21456080 DOI: 10.1002/ajim.20941]
- 108 **Lewis R**, Rempala G, Dell LD, Mundt KA. Vinyl chloride and liver and brain cancer at a polymer production plant in Louisville, Kentucky. *J Occup Environ Med* 2003; **45**: 533-537 [PMID: 12762078 DOI: 10.1097/01.jom.0000058348.05741.1d]
- 109 **Hsieh HI**, Chen PC, Wong RH, Du CL, Chang YY, Wang JD, Cheng TJ. Mortality from liver cancer and leukaemia among polyvinyl chloride workers in Taiwan: an updated study. *Occup Environ Med* 2011; **68**: 120-125 [PMID: 20798004 DOI: 10.1136/oem.2010.056978]
- 110 **Mastrangelo G**, Martinez D, Fedeli U. Vinyl chloride and the liver: misrepresentation of epidemiological evidence. *J Hepatol* 2010; **52**: 776-777 [PMID: 20347172 DOI: 10.1016/j.jhep.2010.01.017]
- 111 **Dragani TA**, Zocchetti C. Occupational exposure to vinyl chloride and risk of hepatocellular carcinoma. *Cancer Causes Control* 2008; **19**: 1193-1200 [PMID: 18560983 DOI: 10.1007/s10552-008-9188-8]
- 112 **Mastrangelo G**, Fedeli U, Fadda E, Valentini F, Agnesi R, Magarotto G, Marchi T, Buda A, Pinzani M, Martinez D. Increased risk of hepatocellular carcinoma and liver cirrhosis in vinyl chloride workers: synergistic effect of occupational exposure with alcohol intake. *Environ Health Perspect* 2004; **112**: 1188-1192 [PMID: 15289165 DOI: 10.1289/ehp.6972]
- 113 **Malaguarnera M**, Motta M, Vacante M, Malaguarnera G, Caraci F, Nunnari G, Gagliano C, Greco C, Chisari G, Drago F, Bertino G. Silybin-vitamin E-phospholipids complex reduces liver fibrosis in patients with chronic hepatitis C treated with pegylated interferon  $\alpha$  and ribavirin. *Am J Transl Res* 2015; **7**: 2510-2518 [PMID: 26807195]
- 114 **Weihrauch M**, Lehnert G, Köckerling F, Wittekind C, Tannapfel A. p53 mutation pattern in hepatocellular carcinoma in workers exposed to vinyl chloride. *Cancer* 2000; **88**: 1030-1036 [PMID: 10699891 DOI: 10.1002/(SICI)1097-0142(20000301)88:5<1030::AID-CNCR12>3.0.CO;2-4]
- 115 **Weihrauch M**, Benicke M, Lehnert G, Wittekind C, Wrbitzky R, Tannapfel A. Frequent k-ras -2 mutations and p16(INK4A)-methylation in hepatocellular carcinomas in workers exposed to vinyl chloride. *Br J Cancer* 2001; **84**: 982-989 [PMID: 11286481 DOI: 10.1054/bjoc.2000.1675]
- 116 **Feron VJ**, Kruysse A, Til HP. One-year time sequence inhalation toxicity study of vinyl chloride in rats. I. Growth, mortality, haematology, clinical chemistry and organ weights. *Toxicology* 1979; **13**: 25-28 [PMID: 516069]
- 117 **Til HP**, Feron VJ, Immel HR. Lifetime (149-week) oral carcinogenicity study of vinyl chloride in rats. *Food Chem Toxicol* 1991; **29**: 713-718 [PMID: 1959825 DOI: 10.1016/0278-6915(91)90130-Y]
- 118 **Ward E**, Boffetta P, Andersen A, Colin D, Comba P, Daddens JA, De Santis M, Engholm G, Hagmar L, Langard S, Lundberg I, McElvenny D, Pirastu R, Sali D, Simonato L. Update of the follow-up of mortality and cancer incidence among European workers employed in the vinyl chloride industry. *Epidemiology* 2001; **12**: 710-718 [PMID: 11679801 DOI: 10.1097/00001648-200110000-00021]
- 119 **Wong RH**, Chen PC, Du CL, Wang JD, Cheng TJ. An increased standardised mortality ratio for liver cancer among polyvinyl chloride workers in Taiwan. *Occup Environ Med* 2002; **59**: 405-409 [PMID: 12040117 DOI: 10.1136/oem.59.6.405]
- 120 **Gennaro V**, Ceppi M, Crosignani P, Montanaro F. Reanalysis of updated mortality among vinyl and polyvinyl chloride workers: Confirmation of historical evidence and new findings. *BMC Public Health* 2008; **8**: 21 [PMID: 18211695 DOI: 10.1186/1471-2458-8-21]
- 121 **Bebarta V**, DeWitt C. Miscellaneous hydrocarbon solvents. *Clin Occup Environ Med* 2004; **4**: 455-479, vi [PMID: 15325316 DOI: 10.1016/j.coem.2004.03.004]
- 122 **Malaguarnera G**, Cataudella E, Giordano M, Nunnari G, Chisari G, Malaguarnera M. Toxic hepatitis in occupational exposure to solvents. *World J Gastroenterol* 2012; **18**: 2756-2766 [PMID: 22719183 DOI: 10.3748/wjg.v18.i22.2756]
- 123 **Chen R**, Seaton A. A meta-analysis of painting exposure and cancer mortality. *Cancer Detect Prev* 1998; **22**: 533-539 [PMID: 9824376 DOI: 10.1046/j.1525-1500.1998.00A47.x]
- 124 **Porru S**, Placidi D, Carta A, Gelatti U, Ribero ML, Tagger A, Boffetta P, Donato F. Primary liver cancer and occupation in men: a case-control study in a high-incidence area in Northern Italy. *Int J Cancer* 2001; **94**: 878-883 [PMID: 11745492 DOI: 10.1002/ijc.1538]
- 125 **IARC Monogr Evaluation Carcinogenesis Risks to Humans**. Dry cleaning, some chlorinated solvents and other industrial chemicals. Lyon, France, 7-14 February 1995. *IARC Monogr Eval Carcinog Risks Hum* 1995; **63**: 33-477 [PMID: 9139128]
- 126 **Alexander DD**, Kelsh MA, Mink PJ, Mandel JH, Basu R, Weingart M. A meta-analysis of occupational trichloroethylene exposure and liver cancer. *Int Arch Occup Environ Health* 2007; **81**: 127-143 [PMID: 17492303 DOI: 10.1007/s00420-007-0201-4]
- 127 **Environmental Protection Agency (EPA)**. Trichloroethylene Toxicological Review and Appendices. Office of Pesticide Programs and Toxic Substances, 2011
- 128 **Bradford BU**, Lock EF, Kosyk O, Kim S, Uehara T, Harbourt D, DeSimone M, Threadgill DW, Tryndyak V, Pogribny IP, Bleye L, Koop DR, Rusyn I. Interstrain differences in the liver effects of trichloroethylene in a multistrain panel of inbred mice. *Toxicol Sci* 2011; **120**: 206-217 [PMID: 21135412 DOI: 10.1093/toxsci/



- kfq362]
- 129 **IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.** Trichloroethylene, tetrachloroethylene, and some other chlorinated agents. *IARC Monogr Eval Carcinog Risks Hum* 2014; **106**: 1-512 [PMID: 26214861]
  - 130 **Hansen J, Sallmén M, Seldén AI, Anttila A, Pukkala E, Andersson K, Bryngelsson IL, Raaschou-Nielsen O, Olsen JH, McLaughlin JK.** Risk of cancer among workers exposed to trichloroethylene: analysis of three Nordic cohort studies. *J Natl Cancer Inst* 2013; **105**: 869-877 [PMID: 23723420 DOI: 10.1093/jnci/djt107]
  - 131 **Rusyn I, Chiu WA, Lash LH, Kromhout H, Hansen J, Guyton KZ.** Trichloroethylene: Mechanistic, epidemiologic and other supporting evidence of carcinogenic hazard. *Pharmacol Ther* 2014; **141**: 55-68 [PMID: 23973663 DOI: 10.1016/j.pharmthera.2013.08.004]
  - 132 **Pogribny IP, Rusyn I.** Role of epigenetic aberrations in the development and progression of human hepatocellular carcinoma. *Cancer Lett* 2014; **342**: 223-230 [PMID: 22306342 DOI: 10.1016/j.canlet.2012.01.038]
  - 133 **Chiu WA, Ginsberg GL.** Development and evaluation of a harmonized physiologically based pharmacokinetic (PBPK) model for perchloroethylene toxicokinetics in mice, rats, and humans. *Toxicol Appl Pharmacol* 2011; **253**: 203-234 [PMID: 21466818 DOI: 10.1016/j.taap.2011.03.020]
  - 134 **Jiang Y, Chen J, Tong J, Chen T.** Trichloroethylene-induced gene expression and DNA methylation changes in B6C3F1 mouse liver. *PLoS One* 2014; **9**: e116179 [PMID: 25549359 DOI: 10.1371/journal.pone.0116179]
  - 135 **Ramadhan DH, Kamijima M, Wang D, Ito Y, Naito H, Yanagiba Y, Hayashi Y, Tanaka N, Aoyama T, Gonzalez FJ, Nakajima T.** Differential response to trichloroethylene-induced hepatosteatosis in wild-type and PPARalpha-humanized mice. *Environ Health Perspect* 2010; **118**: 1557-1563 [PMID: 20709644 DOI: 10.1289/ehp.1001928]
  - 136 **Dunlop MH, Dray E, Zhao W, San Filippo J, Tsai MS, Leung SG, Schild D, Wiese C, Sung P.** Mechanistic insights into RAD51-associated protein 1 (RAD51AP1) action in homologous DNA repair. *J Biol Chem* 2012; **287**: 12343-12347 [PMID: 22375013 DOI: 10.1074/jbc.C112.352161]
  - 137 **Taira N, Mimoto R, Kurata M, Yamaguchi T, Kitagawa M, Miki Y, Yoshida K.** DYRK2 priming phosphorylation of c-Jun and c-Myc modulates cell cycle progression in human cancer cells. *J Clin Invest* 2012; **122**: 859-872 [PMID: 22307329 DOI: 10.1172/JCI60818]
  - 138 **Gold LS, De Roos AJ, Waters M, Stewart P.** Systematic literature review of uses and levels of occupational exposure to tetrachloroethylene. *J Occup Environ Hyg* 2008; **5**: 807-839 [PMID: 18949603 DOI: 10.1080/15459620802510866]
  - 139 **Guyton KZ, Hogan KA, Scott CS, Cooper GS, Bale AS, Kopylev L, Barone S, Makris SL, Glenn B, Subramaniam RP, Gwinn MR, Dzubow RC, Chiu WA.** Human health effects of tetrachloroethylene: key findings and scientific issues. *Environ Health Perspect* 2014; **122**: 325-334 [DOI: 10.1289/ehp.1307359]
  - 140 **Porru S, Placidi D, Carta A, Alessio L.** Prevention of injuries at work: the role of the occupational physician. *Int Arch Occup Environ Health* 2006; **79**: 177-192 [PMID: 16187126 DOI: 10.1007/s00420-005-0023-1]
  - 141 **Centers for Disease Control and Prevention (CDC).** Workplace safety and health topics. Pesticide illness and injury surveillance. 2013. Available from: URL: <http://www.cdc.gov/niosh/topics/pesticides>
  - 142 **Costa C, Rapisarda V, Catania S, Di Nola C, Ledda C, Fenga C.** Cytokine patterns in greenhouse workers occupationally exposed to  $\alpha$ -cypermethrin: an observational study. *Environ Toxicol Pharmacol* 2013; **36**: 796-800 [PMID: 23958972 DOI: 10.1016/j.etap.2013.07.004]
  - 143 **Freire C, Koifman RJ, Koifman S.** Hematological and hepatic alterations in Brazilian population heavily exposed to organochlorine pesticides. *J Toxicol Environ Health A* 2015; **78**: 534-548 [PMID: 25849770 DOI: 10.1080/15287394.2014.999396]
  - 144 **Gaikwad AS, Karunamoorthy P, Kondhalkar SJ, Ambikapathy M, Beerappa R.** Assessment of hematological, biochemical effects and genotoxicity among pesticide sprayers in grape garden. *J Occup Med Toxicol* 2015; **10**: 11 [PMID: 25759745 DOI: 10.1186/s12995-015-0049-6]
  - 145 **Anwar WA, Khaled HM, Amra HA, El-Nezami H, Loffredo CA.** Changing pattern of hepatocellular carcinoma (HCC) and its risk factors in Egypt: possibilities for prevention. *Mutat Res* 2008; **659**: 176-184 [PMID: 18346933 DOI: 10.1016/j.mrrev.2008.01.005]
  - 146 **Zhang R, Niu Y, Du H, Cao X, Shi D, Hao Q, Zhou Y.** A stable and sensitive testing system for potential carcinogens based on DNA damage-induced gene expression in human HepG2 cell. *Toxicol In Vitro* 2009; **23**: 158-165 [PMID: 19013231 DOI: 10.1016/j.tiv.2008.10.006]
  - 147 **Rojanapo W, Kupradinun P, Tepsuwan A, Tanyakaset M.** Effect of varying the onset of exposure to DDT on its modulation of AFB1-induced hepatocarcinogenesis in the rat. *Carcinogenesis* 1993; **14**: 663-667 [PMID: 8097137 DOI: 10.1093/carcin/14.4.663]
  - 148 **Occupational exposures in insecticide application, and some pesticides.** IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 16-23 October 1990. *IARC Monogr Eval Carcinog Risks Hum* 1991; **53**: 5-586 [PMID: 1688189]
  - 149 **National Toxicology Program (NTP).** Report on Carcinogens. 12th ed: Research Triangle Park North Carolina: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, 2011. Available from: URL: [http://www.academia.edu/3788147/12th\\_Report\\_on\\_Carcinogens](http://www.academia.edu/3788147/12th_Report_on_Carcinogens)
  - 150 **van den Berg H.** Global status of DDT and its alternatives for use in vector control to prevent disease. *Cien Saude Colet* 2011; **16**: 575-590 [PMID: 21340333 DOI: 10.1590/S1413-81232011000200021]
  - 151 **van den Berg H, Takken W.** A framework for decision-making in integrated vector management to prevent disease. *Trop Med Int Health* 2007; **12**: 1230-1238 [PMID: 17956506 DOI: 10.1111/j.1365-3156.2007.01905.x]
  - 152 **Persson EC, Graubard BI, Evans AA, London WT, Weber JP, LeBlanc A, Chen G, Lin W, McGlynn KA.** Dichlorodiphenyltrichloroethane and risk of hepatocellular carcinoma. *Int J Cancer* 2012; **131**: 2078-2084 [PMID: 22290210 DOI: 10.1002/ijc.27459]
  - 153 **Zhao B, Shen H, Liu F, Liu S, Niu J, Guo F, Sun X.** Exposure to organochlorine pesticides is an independent risk factor of hepatocellular carcinoma: a case-control study. *J Expo Sci Environ Epidemiol* 2012; **22**: 541-548 [PMID: 21915153 DOI: 10.1038/jes.2011.29]
  - 154 **Cocco P, Kazerouni N, Zahm SH.** Cancer mortality and environmental exposure to DDE in the United States. *Environ Health Perspect* 2000; **108**: 1-4 [PMID: 10620518 DOI: 10.2307/3454288]
  - 155 **Zhao B, Shen H, Liu F, Liu S, Niu J, Guo F, Sun X.** Exposure to organochlorine pesticides is independent risk factor of hepatocellular carcinoma: a case-control study. *J Expo Sci Environ Epidemiol* 2011; **21**: 601-608 [PMID: 21750577 DOI: 10.1038/jes.2011.24]
  - 156 **Cocco P, Fadda D, Billai B, D'Atri M, Melis M, Blair A.** Cancer mortality among men occupationally exposed to dichlorodiphenyltrichloroethane. *Cancer Res* 2005; **65**: 9588-9594 [PMID: 16230425 DOI: 10.1158/0008-5472.CAN-05-1487]
  - 157 **Chaturvedi NK, Kumar S, Negi S, Tyagi RK.** Endocrine disruptors provoke differential modulatory responses on androgen receptor and pregnane and xenobiotic receptor: potential implications in metabolic disorders. *Mol Cell Biochem* 2010; **345**: 291-308 [PMID: 20830510 DOI: 10.1007/s11010-010-0583-6]
  - 158 **Angsubhakorn S, Pradermwong A, Phanwichien K, Nguansangiam S.** Promotion of aflatoxin B1-induced hepatocarcinogenesis by dichlorodiphenyl trichloroethane (DDT). *Southeast Asian J Trop Med Public Health* 2002; **33**: 613-623 [PMID: 12693600]
  - 159 **National Toxicology Program.** N-Nitrosamines (15 listings): N-Methyl-N'-Nitro-N-Nitrosoguanidine. *Rep Carcinog* 2011; **12**: 302-303 [PMID: 21860503]
  - 160 **International Agency for Research on Cancer (IARC).** Some

- inorganic substances, chlorinated hydrocarbons, aromatic Amines, N-Nitroso Compounds and natural products. IARC Monogr Vol. 1. Lyon: France, 1972
- 161 **IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans.** Some N-nitroso compounds. *IARC Monogr Eval Carcinog Risk Chem Man* 1978; **17**: 1-349 [PMID: 150392]
  - 162 **Gentry PR**, House-Knight T, Harris A, Greene T, Campleman S. Potential occupational risk of amines in carbon capture for power generation. *Int Arch Occup Environ Health* 2014; **87**: 591-606 [PMID: 23999744 DOI: 10.1007/s00420-013-0900-y]
  - 163 **Andreotti G**, Silverman DT. Occupational risk factors and pancreatic cancer: a review of recent findings. *Mol Carcinog* 2012; **51**: 98-108 [PMID: 22162234 DOI: 10.1002/mc.20779]
  - 164 **de Vocht F**, Sobala W, Wilczynska U, Kromhout H, Szeszenia-Dabrowska N, Peplonska B. Cancer mortality and occupational exposure to aromatic amines and inhalable aerosols in rubber tire manufacturing in Poland. *Cancer Epidemiol* 2009; **33**: 94-102 [PMID: 19679054 DOI: 10.1016/j.canep.2009.06.013]
  - 165 **Bolognesi C**, Moretto A. Genotoxic risk in rubber manufacturing industry: a systematic review. *Toxicol Lett* 2014; **230**: 345-355 [PMID: 24275385 DOI: 10.1016/j.toxlet.2013.11.013]
  - 166 **Li H**, Jönsson BA, Lindh CH, Albin M, Broberg K. N-nitrosamines are associated with shorter telomere length. *Scand J Work Environ Health* 2011; **37**: 316-324 [PMID: 21321788 DOI: 10.5271/sjweh.3150]
  - 167 **Zhang X**, Lin S, Funk WE, Hou L. Environmental and occupational exposure to chemicals and telomere length in human studies. *Occup Environ Med* 2013; **70**: 743-749 [PMID: 23775864 DOI: 10.1136/oemed-2012-101350]
  - 168 **Morales L**, Dachs J, González-Gaya B, Hernán G, Abalos M, Abad E. Background concentrations of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in the global oceanic atmosphere. *Environ Sci Technol* 2014; **48**: 10198-10207 [PMID: 25083749 DOI: 10.1021/es5023619]
  - 169 **IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.** Polychlorinated Dibenzo-Para-Dioxins and Polychlorinated Dibenzofurans. Lyon, France, 4-11 February 1997. *IARC Monogr Eval Carcinog Risks Hum* 1997; **69**: 1-631 [PMID: 9379504]
  - 170 **Ovando BJ**, Ellison CA, Vezina CM, Olson JR. Toxicogenomic analysis of exposure to TCDD, PCB126 and PCB153: identification of genomic biomarkers of exposure to AhR ligands. *BMC Genomics* 2010; **11**: 583 [PMID: 20959002 DOI: 10.1186/1471-2164-11-583]
  - 171 **National Center for Environmental Assessment Research and Development U.S.** Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD and Related Compounds Part III: Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. Environmental Protection Agency Washington, DC, 2003. Available from: URL: [http://www.epa.gov/ncea/pdfs/dioxin/nas-review/pdfs/part3/dioxin\\_pt3\\_full\\_oct2004.pdf](http://www.epa.gov/ncea/pdfs/dioxin/nas-review/pdfs/part3/dioxin_pt3_full_oct2004.pdf)
  - 172 **Rivera-Austrui J**, Martinez K, Marco-Almagro L, Abalos M, Abad E. Long-term sampling of dioxin-like substances from a clinker kiln stack using alternative fuels. *Sci Total Environ* 2014; **485-486**: 528-533 [PMID: 24742561 DOI: 10.1016/j.scitotenv.2014.03.021]
  - 173 **Holma-Suutari A**, Ruokojärvi P, Laaksonen S, Kiviranta H, Nieminen M, Viluksela M, Hallikainen A. Persistent organic pollutant levels in semi-domesticated reindeer (*Rangifer tarandus tarandus* L.), feed, lichen, blood, milk, placenta, foetus and calf. *Sci Total Environ* 2014; **476-477**: 125-135 [PMID: 24463250 DOI: 10.1016/j.scitotenv.2013.12.109]
  - 174 **Sweeney MH**, Mocarelli P. Human health effects after exposure to 2,3,7,8-TCDD. *Food Addit Contam* 2000; **17**: 303-316 [PMID: 10912244 DOI: 10.1080/026520300283379]
  - 175 **Vezina CM**, Walker NJ, Olson JR. Subchronic exposure to TCDD, PeCDF, PCB126, and PCB153: effect on hepatic gene expression. *Environ Health Perspect* 2004; **112**: 1636-1644 [PMID: 15598615 DOI: 10.1289/ehp.7253]
  - 176 **Kulkarni PS**, Crespo JG, Afonso CA. Dioxins sources and current remediation technologies--a review. *Environ Int* 2008; **34**: 139-153 [PMID: 17826831 DOI: 10.1016/j.envint.2007.07.009]
  - 177 **National Institute of Occupational Safety and Health (NIOSH).** National occupational exposure survey. Cincinnati, Ohio: US Department of Health and Human Services, 2015. Available from: URL: <http://www.cdc.gov/niosh>
  - 178 **National Toxicology Program.** Toxicology and carcinogenesis studies of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (Cas No. 57117-31-4) in female Harlan Sprague-Dawley rats (gavage studies). *Natl Toxicol Program Tech Rep Ser* 2006; (**525**): 1-198 [PMID: 17160103]
  - 179 **Collins JJ**, Bodner K, Haidar S, Wilken M, Burns CJ, Lamparski LL, Budinsky RA, Martin GD, Carson ML. Chlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyl profiles of workers with trichlorophenol and pentachlorophenol exposures. *Chemosphere* 2008; **73**: S284-S289 [PMID: 18442847 DOI: 10.1016/j.chemosphere.2007.12.034]
  - 180 **Bertino G**, Ardiri A, Demma S, GiuseppeCalvagno S, Toro A, Basile E, Campagna D, Ferraro G, Frazzetto E, Proiti M, Malaguarnera G, Bertino N, Malaguarnera M, Malaguarnera M, Amaradio MD, Pricoco G, Di Carlo I. Rare benign tumors of the liver: still rare? *J Gastrointest Cancer* 2014; **45**: 202-217 [PMID: 24510731 DOI: 10.1007/s12029-014-9580-4]
  - 181 **Malaguarnera G**, Paladina I, Giordano M, Malaguarnera M, Bertino G, Berretta M. Serum markers of intrahepatic cholangiocarcinoma. *Dis Markers* 2013; **34**: 219-228 [PMID: 23396291 DOI: 10.3233/DMA-130964]
  - 182 **Collins JJ**, Bodner KM, Wilken M, Haidar S, Burns CJ, Budinsky RA, Martin GD, Carson ML, Rowlands JC. Serum concentrations of chlorinated dibenzo-p-dioxins and dibenzofurans among former Michigan trichlorophenol and pentachlorophenol workers. *J Expo Sci Environ Epidemiol* 2007; **17**: 541-548 [PMID: 17426737]
  - 183 **Aylward LL**, Bodner KM, Collins JJ, Wilken M, McBride D, Burns CJ, Hays SM, Humphry N. TCDD exposure estimation for workers at a New Zealand 2,4,5-T manufacturing facility based on serum sampling data. *J Expo Sci Environ Epidemiol* 2010; **20**: 417-426 [PMID: 19491942 DOI: 10.1038/jes.2009.31]
  - 184 **Safe S**, Lee SO, Jin UH. Role of the aryl hydrocarbon receptor in carcinogenesis and potential as a drug target. *Toxicol Sci* 2013; **135**: 1-16 [PMID: 23771949 DOI: 10.1093/toxsci/kft128]
  - 185 **Ludewig G**, Robertson LW. Polychlorinated biphenyls (PCBs) as initiating agents in hepatocellular carcinoma. *Cancer Lett* 2013; **334**: 46-55 [PMID: 23211541 DOI: 10.1016/j.canlet.2012.11.041]
  - 186 **Crinnion WJ.** Polychlorinated biphenyls: persistent pollutants with immunological, neurological, and endocrinological consequences. *Altern Med Rev* 2011; **16**: 5-13 [PMID: 21438643]
  - 187 **Holler J.** The emergency response program at the Agency for Toxic Substances and Disease Registry. *J Environ Health* 2013; **76**: 46-47 [PMID: 24288850]
  - 188 **Erickson MD**, Kaley RG. Applications of polychlorinated biphenyls. *Environ Sci Pollut Res Int* 2011; **18**: 135-151 [PMID: 20848233 DOI: 10.1007/s11356-010-0392-1]
  - 189 **Wolff MS**, Schechter A. Use of PCB blood levels to assess potential exposure following an electrical transformer explosion. *J Occup Med* 1992; **34**: 1079-1083 [PMID: 1432297 DOI: 10.1097/00043764-199211000-00009]
  - 190 **Schechter A**, Stanley J, Boggess K, Masuda Y, Mes J, Wolff M, Fürst P, Fürst C, Wilson-Yang K, Chisholm B. Polychlorinated biphenyl levels in the tissues of exposed and nonexposed humans. *Environ Health Perspect* 1994; **102** Suppl 1: 149-158 [PMID: 8187704 DOI: 10.1289/ehp.94102s1149]
  - 191 **National Toxicology Program.** NTP technical report on the toxicology and carcinogenesis studies of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) (CAS No. 35065-27-1) in female Harlan Sprague-Dawley rats (Gavage studies). *Natl Toxicol Program Tech Rep Ser* 2006; (**529**): 4-168 [PMID: 16835634]
  - 192 **Hennig B**, Reiterer G, Toborek M, Matveev SV, Daugherty A, Smart E, Robertson LW. Dietary fat interacts with PCBs to induce changes in lipid metabolism in mice deficient in low-density lipoprotein receptor. *Environ Health Perspect* 2005; **113**: 83-87

- [PMID: 15626652 DOI: 10.1289/ehp.7280]
- 193 **Zani C**, Toninelli G, Filisetti B, Donato F. Polychlorinated biphenyls and cancer: an epidemiological assessment. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 2013; **31**: 99-144 [PMID: 23672403 DOI: 10.1080/10590501.2013.782174]
  - 194 **Rayne S**, Forest K. pK(a) values of the monohydroxylated polychlorinated biphenyls (OH-PCBs), polybrominated biphenyls (OH-PBBs), polychlorinated diphenyl ethers (OH-PCDEs), and polybrominated diphenyl ethers (OH-PBDEs). *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2010; **45**: 1322-1346 [PMID: 20658412 DOI: 10.1080/10934529.2010.500885]
  - 195 **Tampal N**, Lehmler HJ, Espandiari P, Malmberg T, Robertson LW. Glucuronidation of hydroxylated polychlorinated biphenyls (PCBs). *Chem Res Toxicol* 2002; **15**: 1259-1266 [PMID: 12387623 DOI: 10.1021/tx0200212]
  - 196 **Klaunig JE**, Wang Z, Pu X, Zhou S. Oxidative stress and oxidative damage in chemical carcinogenesis. *Toxicol Appl Pharmacol* 2011; **254**: 86-99 [PMID: 21296097 DOI: 10.1016/j.taap.2009.11.028]
  - 197 **Ho PW**, Garner CE, Ho JW, Leung KC, Chu AC, Kwok KH, Kung MH, Burka LT, Ramsden DB, Ho SL. Estrogenic phenol and catechol metabolites of PCBs modulate catechol-O-methyltransferase expression via the estrogen receptor: potential contribution to cancer risk. *Curr Drug Metab* 2008; **9**: 304-309 [PMID: 18473748 DOI: 10.2174/138920008784220600]
  - 198 **Brown JF**, Mayes BA, Silkworth JB, Hamilton SB. Polychlorinated biphenyls modulated tumorigenesis in Sprague Dawley rats: correlation with mixed function oxidase activities and superoxide (O<sub>2</sub><sup>\*</sup>) formation potentials and implied mode of action. *Toxicol Sci* 2007; **98**: 375-394 [PMID: 17510085 DOI: 10.1093/toxsci/kfm122]
  - 199 **Marabini L**, Calò R, Fucile S. Genotoxic effects of polychlorinated biphenyls (PCB 153, 138, 101, 118) in a fish cell line (RTG-2). *Toxicol In Vitro* 2011; **25**: 1045-1052 [PMID: 21504788 DOI: 10.1016/j.tiv.2011.04.004]
  - 200 **Senthikumar PK**, Klingelutz AJ, Jacobus JA, Lehmler H, Robertson LW, Ludewig G. Airborne polychlorinated biphenyls (PCBs) reduce telomerase activity and shorten telomere length in immortal human skin keratinocytes (HaCat). *Toxicol Lett* 2011; **204**: 64-70 [PMID: 21530622 DOI: 10.1016/j.toxlet.2011.04.012]
  - 201 **Senthikumar PK**, Robertson LW, Ludewig G. PCB153 reduces telomerase activity and telomere length in immortalized human skin keratinocytes (HaCaT) but not in human foreskin keratinocytes (NFK). *Toxicol Appl Pharmacol* 2012; **259**: 115-123 [PMID: 22210444 DOI: 10.1016/j.taap.2011.12.015]
  - 202 **Espandiari P**, Glauert HP, Lehmler HJ, Lee EY, Srinivasan C, Robertson LW. Polychlorinated biphenyls as initiators in liver carcinogenesis: resistant hepatocyte model. *Toxicol Appl Pharmacol* 2003; **186**: 55-62 [PMID: 12583993 DOI: 10.1016/S0041-008X(02)00018-2]
  - 203 **Bencko V**, Rames J, Ondrusova M, Plesko I, Jurickova L, Trnovec T. Human exposure to polyhalogenated hydrocarbons and incidence of selected malignancies -central European experience. *Neoplasma* 2009; **56**: 353-357 [PMID: 19469657 DOI: 10.4149/neo\_2009\_04\_353]
  - 204 **Zhao G**, Wang Z, Zhou H, Zhao Q. Burdens of PBBs, PBDEs, and PCBs in tissues of the cancer patients in the e-waste disassembly sites in Zhejiang, China. *Sci Total Environ* 2009; **407**: 4831-4837 [PMID: 19539352 DOI: 10.1016/j.scitotenv.2009.05.031]
  - 205 **Mallin K**, McCann K, D'Aloisio A, Freels S, Piorkowski J, Dimos J, Persky V. Cohort mortality study of capacitor manufacturing workers, 1944-2000. *J Occup Environ Med* 2004; **46**: 565-576 [PMID: 15213519 DOI: 10.1097/01.jom.0000128156.24767.12]
  - 206 **Lauby-Secretan B**, Loomis D, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Baan R, Mattock H, Straif K. Carcinogenicity of polychlorinated biphenyls and polybrominated biphenyls. *Lancet Oncol* 2013; **14**: 287-288 [PMID: 23499544 DOI: 10.1016/S1470-2045(13)70104-9]
  - 207 **International Agency for Research on Cancer (IARC)**. Polychlorinated Biphenyls and Polybrominated Biphenyls. IARC Monogr Vol. 107. Lyon: France, 2014. Available from: URL: <http://monographs.iarc.fr/ENG/Monographs/PDFs/>
  - 208 **National Toxicology Program**. Polybrominated biphenyls. *Rep Carcinog* 2011; **12**: 347-349 [PMID: 21863083]
  - 209 **Hoque A**, Sigurdson AJ, Burau KD, Humphrey HE, Hess KR, Sweeney AM. Cancer among a Michigan cohort exposed to polybrominated biphenyls in 1973. *Epidemiology* 1998; **9**: 373-378 [PMID: 9647899 DOI: 10.1097/00001648-199807000-00005]
  - 210 Some chemicals used in plastics and elastomers. IARC Working Group. Lyon, 11-18 June 1985. *IARC Monogr Eval Carcinog Risk Chem Hum* 1986; **39**: 7-378 [PMID: 3465697]
  - 211 **Hanari N**, Kannan K, Miyake Y, Okazawa T, Kodavanti PR, Aldous KM, Yamashita N. Occurrence of polybrominated biphenyls, polybrominated dibenzo-p-dioxins, and polybrominated dibenzofurans as impurities in commercial polybrominated diphenyl ether mixtures. *Environ Sci Technol* 2006; **40**: 4400-4405 [PMID: 16903277 DOI: 10.1021/es060559k]
  - 212 **Sjödin A**, Carlsson H, Thuresson K, Sjölin S, Bergman A, Ostman C. Flame retardants in indoor air at an electronics recycling plant and at other work environments. *Environ Sci Technol* 2001; **35**: 448-454 [PMID: 11351713 DOI: 10.1021/es000077n]
  - 213 **Jira R**, Kopp E, Blaine C, McKusick BC, Röderer G. Chloroacetaldehydes. In: Ullmann's Encyclopedia of Industrial Chemistry. 2007 [DOI: 10.1002/14356007.a06\_527]
  - 214 **Boitsov AN**, Rotenberg IuS, Mulenikova VG. [Toxicologic assessment of chloral in the process of its liberation during filling and pouring of foam polyurethanes]. *Gig Tr Prof Zabol* 1970; **14**: 26-29 [PMID: 5433673]
  - 215 **Delinsky AD**, Bruckner JV, Bartlett MG. A review of analytical methods for the determination of trichloroethylene and its major metabolites chloral hydrate, trichloroacetic acid and dichloroacetic acid. *Biomed Chromatogr* 2005; **19**: 617-639 [PMID: 15828053 DOI: 10.1002/bmc.488]
  - 216 **IARC Working Group on the Evaluation of Carcinogenic Risks to Humans**. Some chemicals present in industrial and consumer products, food and drinking-water. *IARC Monogr Eval Carcinog Risks Hum* 2013; **101**: 9-549 [PMID: 24772663]
  - 217 **Leakey JE**, Seng JE, Latendresse JR, Hussain N, Allen LJ, Allaben WT. Dietary controlled carcinogenicity study of chloral hydrate in male B6C3F1 mice. *Toxicol Appl Pharmacol* 2003; **193**: 266-280 [PMID: 14644627 DOI: 10.1016/j.taap.2003.07.007]
  - 218 **US Department of Health and Human Services National Toxicology Program**. Toxicology and carcinogenesis study of chloral hydrate (ad libitum and dietary controlled) (CAS no. 302-17-0) in male B6C3F1 mice (gavage study). *Natl Toxicol Program Tech Rep Ser* 2002; **(503)**: 1-218 [PMID: 12533745]
  - 219 **Merdink JL**, Robison LM, Stevens DK, Hu M, Parker JC, Bull RJ. Kinetics of chloral hydrate and its metabolites in male human volunteers. *Toxicology* 2008; **245**: 130-140 [PMID: 18243465 DOI: 10.1016/j.tox.2007.12.018]
  - 220 **IARC Monographs Working Group on the Evaluation of Carcinogenic Risks to Humans**. Some aromatic amines, organic dyes, and related exposures. *IARC Monogr Eval Carcinog Risks Hum* 2010; **99**: 1-658 [PMID: 21528837]
  - 221 o-Toluidine and o-toluidine hydrochloride. *Rep Carcinog* 2004; **11**: III258-III259 [PMID: 21089974]
  - 222 **Kauppinen T**, Pukkala E, Saalo A, Saso AJ. Exposure to chemical carcinogens and risk of cancer among Finnish laboratory workers. *Am J Ind Med* 2003; **44**: 343-350 [PMID: 14502761 DOI: 10.1002/ajim.10278]
  - 223 **Akyüz M**, Ata S. Determination of aromatic amines in hair dye and henna samples by ion-pair extraction and gas chromatography-mass spectrometry. *J Pharm Biomed Anal* 2008; **47**: 68-80 [PMID: 18280687 DOI: 10.1016/j.jpba.2007.12.011]
  - 224 **Johansson GM**, Jönsson BA, Axmon A, Lindh CH, Lind ML, Gustavsson M, Broberg K, Boman A, Meding B, Lidén C, Albin M. Exposure of hairdressers to ortho- and meta-toluidine in hair dyes. *Occup Environ Med* 2015; **72**: 57-63 [PMID: 24912758 DOI: 10.1136/oemed-2013-101960]
  - 225 **Condorelli DF**, Kaczmarek L, Nicoletti F, Arcidiacono A, Dell'Albani P, Ingrao F, Magri G, Malaguarnera L, Avola R, Messina A. Induction of protooncogene fos by extracellular signals in primary



- glial cell cultures. *J Neurosci Res* 1989; **23**: 234-239 [PMID: 2547086 DOI: 10.1002/jnr.490230214]
- 226 **Riedel K**, Scherer G, Engl J, Hagedorn HW, Tricker AR. Determination of three carcinogenic aromatic amines in urine of smokers and nonsmokers. *J Anal Toxicol* 2006; **30**: 187-195 [PMID: 16803653 DOI: 10.1093/jat/30.3.187]
  - 227 **Sorahan T**, Hamilton L, Jackson JR. A further cohort study of workers employed at a factory manufacturing chemicals for the rubber industry, with special reference to the chemicals 2-mercaptobenzothiazole (MBT), aniline, phenyl-beta-naphthylamine and o-toluidine. *Occup Environ Med* 2000; **57**: 106-115 [PMID: 10711278 DOI: 10.1136/oem.57.2.106]
  - 228 **Sorahan T**. Bladder cancer risks in workers manufacturing chemicals for the rubber industry. *Occup Med (Lond)* 2008; **58**: 496-501 [PMID: 18725381 DOI: 10.1093/occmed/kqn104]
  - 229 **National Toxicology Program**. Bioassay of o-toluidine hydrochloride for possible carcinogenicity. *Natl Cancer Inst Carcinog Tech Rep Ser* 1979; **153**: 1-147 [PMID: 12799709]
  - 230 **National Toxicology Program**. NTP 11th Report on Carcinogens. *Rep Carcinog* 2004; **11**: 1-A32 [PMID: 19826456]
  - 231 **Venitt S**, Searle CE. Mutagenicity and possible carcinogenicity of hair colourants and constituents. *IARC Sci Publ* 1976; **(13)**: 263-271 [PMID: 793979]
  - 232 **IARC Monogr Evaluation Carcinogenesis Risks to Humans**. Occupational exposures of hairdressers and barbers and personal use of hair colourants. *IARC Monogr Eval Carcinog Risks Hum* 1993; **57**: 43-118 [PMID: 8207865]
  - 233 **Butler MA**, Guengerich FP, Kadlubar FF. Metabolic oxidation of the carcinogens 4-aminobiphenyl and 4,4'-methylene-bis(2-chloroaniline) by human hepatic microsomes and by purified rat hepatic cytochrome P-450 monooxygenases. *Cancer Res* 1989; **49**: 25-31 [PMID: 2908851]
  - 234 **Zhang YJ**. Interactions of chemical carcinogens and genetic variation in hepatocellular carcinoma. *World J Hepatol* 2010; **2**: 94-102 [PMID: 21160980 DOI: 10.4254/wjh.v2.i3.94]
  - 235 **Beyerbach A**, Rothman N, Bhatnagar VK, Kashyap R, Sabbioni G. Hemoglobin adducts in workers exposed to benzidine and azo dyes. *Carcinogenesis* 2006; **27**: 1600-1606 [PMID: 16497705 DOI: 10.1093/carcin/bgi362]
  - 236 **Collins JJ**, Strauss ME, Levinskas GJ, Conner PR. The mortality experience of workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin in a trichlorophenol process accident. *Epidemiology* 1993; **4**: 7-13 [PMID: 8420584 DOI: 10.1097/00001648-199301000-00003]
  - 237 **Collins JJ**, Strauss ME, Riordan SG. Mortalities of workers at the Nitro plant with exposure to 2-mercaptobenzothiazole. *Occup Environ Med* 1999; **56**: 667-671 [PMID: 10658544 DOI: 10.1136/oem.56.10.667]
  - 238 **Melick WF**, Naryka JJ, Kelly RE. Bladder cancer due to exposure to para-aminobiphenyl: a 17-year followup. *J Urol* 1971; **106**: 220-226 [PMID: 5099312]
  - 239 **Parsons BL**, Beland FA, Von Tungeln LS, Delongchamp RR, Fu PP, Heflich RH. Levels of 4-aminobiphenyl-induced somatic H-ras mutation in mouse liver DNA correlate with potential for liver tumor development. *Mol Carcinog* 2005; **42**: 193-201 [PMID: 15761837 DOI: 10.1002/mc.20083]
  - 240 **Lee HW**, Wang HT, Weng MW, Hu Y, Chen WS, Chou D, Liu Y, Donin N, Huang WC, Lepor H, Wu XR, Wang H, Beland FA, Tang MS. Acrolein- and 4-Aminobiphenyl-DNA adducts in human bladder mucosa and tumor tissue and their mutagenicity in human urothelial cells. *Oncotarget* 2014; **5**: 3526-3540 [PMID: 24939871 DOI: 10.18632/oncotarget.1954]
  - 241 **Huan LC**, Wu JC, Chiou BH, Chen CH, Ma N, Chang CY, Tsen YK, Chen SC. MicroRNA regulation of DNA repair gene expression in 4-aminobiphenyl-treated HepG2 cells. *Toxicology* 2014; **322**: 69-77 [PMID: 24857880 DOI: 10.1016/j.tox.2014.05.003]
  - 242 **Tao L**, Day BW, Hu B, Xiang YB, Wang R, Stern MC, Gago-Dominguez M, Cortessis VK, Conti DV, Van Den Berg D, Pike MC, Gao YT, Yu MC, Yuan JM. Elevated 4-aminobiphenyl and 2,6-dimethylaniline hemoglobin adducts and increased risk of bladder cancer among lifelong nonsmokers--The Shanghai Bladder Cancer Study. *Cancer Epidemiol Biomarkers Prev* 2013; **22**: 937-945 [PMID: 23539508 DOI: 10.1158/1055-9965.EPI-12-1447]
  - 243 **Wang S**, Sugamori KS, Brennenman D, Hsu I, Calce A, Grant DM. Influence of arylamine N-acetyltransferase, sex, and age on 4-aminobiphenyl-induced in vivo mutant frequencies and spectra in mouse liver. *Environ Mol Mutagen* 2012; **53**: 350-357 [PMID: 22508569 DOI: 10.1002/em.21695]
  - 244 **Nauwelaers G**, Bessette EE, Gu D, Tang Y, Rageul J, Fessard V, Yuan JM, Yu MC, Langouët S, Turesky RJ. DNA adduct formation of 4-aminobiphenyl and heterocyclic aromatic amines in human hepatocytes. *Chem Res Toxicol* 2011; **24**: 913-925 [PMID: 21456541 DOI: 10.1021/tx200091y]
  - 245 **NIOSH**. Special Occupational Hazard Review for Benzidine-Based Dyes. NIOSH Criteria Documents. DHHS (NIOSH) Publication No. 80-109. U.S. Department of Health, Education and Welfare, Public Health Services, Center for Disease Control, 1980: 60. Available from: URL: <http://www.ncbi.nlm.nih.gov/books/NBK304440/>
  - 246 **Ahlström LH**, Sparr Eskilsson C, Björklund E. Determination of banned azo dyes in consumer goods. *Trends in Analytical Chemistry* 2005; **24**: 49-56 [DOI: 10.1016/j.trac.2004.09.004]
  - 247 **IARC Monogr Evaluation Carcinogenesis Risks to Humans**. Some industrial chemicals and dyestuffs. *IARC Monogr Eval Carcinog Risk Chem Hum* 1982; **29**: 1-398 [PMID: 6957379]
  - 248 **Garrigós MC**, Reche F, Marín ML, Jiménez A. Determination of aromatic amines formed from azo colorants in toy products. *J Chromatogr A* 2002; **976**: 309-317 [PMID: 12462623 DOI: 10.1016/S0021-9673(02)01162-7]
  - 249 **National Toxicology Program**. Dyes metabolized to 3,3'-dimethylbenzidine (3,3'-dimethylbenzidine dye class). *Rep Carcinog* 2011; **12**: 170-171 [PMID: 21852830]
  - 250 **National Toxicology Program**. 3,3'-Dimethylbenzidine and dyes metabolized to 3,3'-dimethylbenzidine: 3,3'-dimethylbenzidine. *Rep Carcinog* 2011; **12**: 168-170 [PMID: 21852829]
  - 251 **National Toxicology Program**. Benzidine and dyes metabolized to benzidine: dyes metabolized to benzidine (benzidine dye class). *Rep Carcinog* 2011; **12**: 64-66 [PMID: 21850110]
  - 252 **National Toxicology Program**. Benzidine and dyes metabolized to benzidine: benzidine. *Rep Carcinog* 2011; **12**: 62-64 [PMID: 21850109]
  - 253 **World Health Organization (WHO)**. Preventing disease through healthy environments exposure to arsenic: a major public health concern, 2010. Available from: URL: [http://www.who.int/ipcs/features/10chemicals\\_en.pdf](http://www.who.int/ipcs/features/10chemicals_en.pdf)
  - 254 **Perlman GD**, Berman L, Leann K, Bing L. Agency for Toxic Substances and Disease Registry Brownfields/ land-reuse site tool. *J Environ Health* 2012; **75**: 30-34 [PMID: 23270111]
  - 255 **Cataudella E**, Malaguarnera G, Gagliano C, Condorelli G, Antic T, Rampello L, Erdogan Ö, Rampello L, Malaguarnera M. Pesticides exposure and the management of acute hepatic injury. *Acta Medica Mediterranea* 2012; **28**: 245
  - 256 **Amadori S**, Fenaux P, Ludwig H, O'dwyer M, Sanz M. Use of arsenic trioxide in haematological malignancies: insight into the clinical development of a novel agent. *Curr Med Res Opin* 2005; **21**: 403-411 [PMID: 15811209 DOI: 10.1185/030077904X20349]
  - 257 **Frazzetto PM**, Malaguarnera G, Gagliano C, Lucca F, Giordano M, Rampello L, Rampello L, Malaguarnera M. Biohumoral Tests in Chronic Pesticide Exposure. *Acta Medica Mediterranea* 2012; **28**: 237
  - 258 **Xi S**, Zheng Q, Zhang Q, Sun G. Metabolic profile and assessment of occupational arsenic exposure in copper- and steel-smelting workers in China. *Int Arch Occup Environ Health* 2011; **84**: 347-353 [PMID: 21132326 DOI: 10.1007/s00420-010-0574-7]
  - 259 **Liu J**, Waalkes MP. Liver is a target of arsenic carcinogenesis. *Toxicol Sci* 2008; **105**: 24-32 [PMID: 18566022 DOI: 10.1093/toxsci/kfn120]
  - 260 **Thomas DJ**. Molecular processes in cellular arsenic metabolism. *Toxicol Appl Pharmacol* 2007; **222**: 365-373 [PMID: 17397889 DOI: 10.1016/j.taap.2007.02.007]
  - 261 **Tchounwou PB**, Patlolla AK, Centeno JA. Carcinogenic and



- systemic health effects associated with arsenic exposure—a critical review. *Toxicol Pathol* 2003; **31**: 575-588 [PMID: 14585726 DOI: 10.1080/714044691]
- 262 **IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.** Some drinking-water disinfectants and contaminants, including arsenic. Monographs on chloramine, chloral and chloral hydrate, dichloroacetic acid, trichloroacetic acid and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone. *IARC Monogr Eval Carcinog Risks Hum* 2004; **84**: 269-477 [PMID: 15645578]
- 263 **IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.** Arsenic, metals, fibres, and dusts. *IARC Monogr Eval Carcinog Risks Hum* 2012; **100**: 11-465 [PMID: 23189751]
- 264 **Mazumder DN.** Effect of chronic intake of arsenic-contaminated water on liver. *Toxicol Appl Pharmacol* 2005; **206**: 169-175 [PMID: 15967205 DOI: 10.1016/j.taap.2004.08.025]
- 265 **Chen Y, Ahsan H.** Cancer burden from arsenic in drinking water in Bangladesh. *Am J Public Health* 2004; **94**: 741-744 [PMID: 15117692 DOI: 10.2105/AJPH.94.5.741]
- 266 **Abernathy CO, Thomas DJ, Calderon RL.** Health effects and risk assessment of arsenic. *J Nutr* 2003; **133**: 1536S-1538S [PMID: 12730460]
- 267 **Reichard JF, Schnekenburger M, Puga A.** Long term low-dose arsenic exposure induces loss of DNA methylation. *Biochem Biophys Res Commun* 2007; **352**: 188-192 [PMID: 17107663 DOI: 10.1016/j.bbrc.2006.11.001]
- 268 **Tokar EJ, Benbrahim-Tallaa L, Ward JM, Lunn R, Sams RL, Waalkes MP.** Cancer in experimental animals exposed to arsenic and arsenic compounds. *Crit Rev Toxicol* 2010; **40**: 912-927 [PMID: 20812815 DOI: 10.3109/10408444.2010.506641]
- 269 **Wanibuchi H, Salim EI, Kinoshita A, Shen J, Wei M, Morimura K, Yoshida K, Kuroda K, Endo G, Fukushima S.** Understanding arsenic carcinogenicity by the use of animal models. *Toxicol Appl Pharmacol* 2004; **198**: 366-376 [PMID: 15276416 DOI: 10.1016/j.taap.2003.10.032]
- 270 **Waalkes MP, Liu J, Diwan BA.** Transplacental arsenic carcinogenesis in mice. *Toxicol Appl Pharmacol* 2007; **222**: 271-280 [PMID: 17306315 DOI: 10.1016/j.taap.2006.12.034]
- 271 **Qu W, Bortner CD, Sakurai T, Hobson MJ, Waalkes MP.** Acquisition of apoptotic resistance in arsenic-induced malignant transformation: role of the JNK signal transduction pathway. *Carcinogenesis* 2002; **23**: 151-159 [PMID: 11756236 DOI: 10.1093/carcin/23.1.151]
- 272 **Waalkes MP, Keefer LK, Diwan BA.** Induction of proliferative lesions of the uterus, testes, and liver in swiss mice given repeated injections of sodium arsenate: possible estrogenic mode of action. *Toxicol Appl Pharmacol* 2000; **166**: 24-35 [PMID: 10873715 DOI: 10.1006/taap.2000.8963]
- 273 **Rossman TG.** Mechanism of arsenic carcinogenesis: an integrated approach. *Mutat Res* 2003; **533**: 37-65 [PMID: 14643412 DOI: 10.1016/j.mrfmmm.2003.07.009]
- 274 **Waalkes MP, Ward JM, Diwan BA.** Induction of tumors of the liver, lung, ovary and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers. *Carcinogenesis* 2004; **25**: 133-141 [PMID: 14514661 DOI: 10.1093/carcin/bgg181]
- 275 **National Toxicology Program.** NTP 12th Report on Carcinogens. *Rep Carcinog* 2011; **12**: iii-499 [PMID: 21822324]
- 276 **Mannino DM, Holguin F, Greves HM, Savage-Brown A, Stock AL, Jones RL.** Urinary cadmium levels predict lower lung function in current and former smokers: data from the Third National Health and Nutrition Examination Survey. *Thorax* 2004; **59**: 194-198 [PMID: 14985551 DOI: 10.1136/thorax.2003.012054]
- 277 **Huff J, Cirvello J, Haseman J, Bucher J.** Chemicals associated with site-specific neoplasia in 1394 long-term carcinogenesis experiments in laboratory rodents. *Environ Health Perspect* 1991; **93**: 247-270 [PMID: 1773796 DOI: 10.1289/ehp.9193247]
- 278 **Malaguarnera M, Drago F, Malaguarnera G, Li Volti G, Salomone S, Caraci F, Galvano F, Vacante M, Bucolo C, Malaguarnera M.** Metal fume fever. *Lancet* 2013; **381**: 2298 [PMID: 23809563 DOI: 10.1016/S0140-6736(13)60689-3]
- 279 **Satarug S, Garrett SH, Sens MA, Sens DA.** Cadmium, environmental exposure, and health outcomes. *Environ Health Perspect* 2010; **118**: 182-190 [PMID: 20123617 DOI: 10.1289/ehp.0901234]
- 280 **Satarug S.** Long-term exposure to cadmium in food and cigarette smoke, liver effects and hepatocellular carcinoma. *Curr Drug Metab* 2012; **13**: 257-271 [PMID: 22455552 DOI: 10.2174/138920012799320446]
- 281 **Rani A, Kumar A, Lal A, Pant M.** Cellular mechanisms of cadmium-induced toxicity: a review. *Int J Environ Health Res* 2014; **24**: 378-399 [PMID: 24117228 DOI: 10.1080/09603123.2013.835032]
- 282 **Alessandria I, Pennisi M, Cataudella E, Frazzetto PM, Malaguarnera M, Rampello L, Rampello L.** Neurotoxicity in cadmium-exposed workers. *Acta medica mediterranea* 2012; **28**: 253
- 283 **Liu F, Li H, Chang H, Wang J, Lu J.** Identification of hepatocellular carcinoma-associated hub genes and pathways by integrated microarray analysis. *Tumori* 2015; **101**: 206-214 [PMID: 25768320 DOI: 10.5301/tj.5000241]
- 284 **Hassan MM, Spitz MR, Thomas MB, El-Deeb AS, Glover KY, Nguyen NT, Chan W, Kaseb A, Curley SA, Vauthey JN, Ellis LM, Abdalla E, Lozano RD, Patt YZ, Brown TD, Abbruzzese JL, Li D.** Effect of different types of smoking and synergism with hepatitis C virus on risk of hepatocellular carcinoma in American men and women: case-control study. *Int J Cancer* 2008; **123**: 1883-1891 [PMID: 18688864 DOI: 10.1002/ijc.23730]
- 285 **Polosa R, Russo C, Caponnetto P, Bertino G, Sarvā M, Antic T, Mancuso S, Al-Delaimy WK.** Greater severity of new onset asthma in allergic subjects who smoke: a 10-year longitudinal study. *Respir Res* 2011; **12**: 16 [PMID: 21261960 DOI: 10.1186/1465-9921-12-16]
- 286 **Takiguchi M, Achanzar WE, Qu W, Li G, Waalkes MP.** Effects of cadmium on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation. *Exp Cell Res* 2003; **286**: 355-365 [PMID: 12749863 DOI: 10.1016/S0014-4827(03)00062-4]
- 287 **Sabolić I, Breljak D, Skarica M, Herak-Kramberger CM.** Role of metallothionein in cadmium traffic and toxicity in kidneys and other mammalian organs. *Biometals* 2010; **23**: 897-926 [PMID: 20549307 DOI: 10.1007/s10534-010-9351-z]
- 288 **Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M.** Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 2007; **317**: 121-124 [PMID: 17615358 DOI: 10.1126/science.1140485]
- 289 **Tellez-Plaza M, Navas-Acien A, Crainiceanu CM, Sharrett AR, Guallar E.** Cadmium and peripheral arterial disease: gender differences in the 1999-2004 US National Health and Nutrition Examination Survey. *Am J Epidemiol* 2010; **172**: 671-681 [PMID: 20693268 DOI: 10.1093/aje/kwq172]
- 290 **Gallagher CM, Meliker JR.** Blood and urine cadmium, blood pressure, and hypertension: a systematic review and meta-analysis. *Environ Health Perspect* 2010; **118**: 1676-1684 [PMID: 20716508 DOI: 10.1289/ehp.1002077]
- 291 **Rapisarda V, Valentino M, Bolognini S, Fenga C.** [Noise-related occupational risk aboard fishing vessels: considerations on prevention and the protection of exposed workers]. *G Ital Med Lav Ergon* 2004; **26**: 191-196 [PMID: 15551949]
- 292 **Rapisarda V, Ledda C, Castaing M, Proietti L, Ferrante M.** [Potential exposure to carcinogens in low-melting alloys processing]. *G Ital Med Lav Ergon* 2013; **35**: 73-76 [PMID: 23914599]
- 293 **Rapisarda V, Valentino M, Ravalli P, Fenga C, Duscio D.** [Occupational brucellosis in slaughtering of sheep and goats: study of five cases from a municipal abattoir in south-eastern Sicily]. *Med Lav* 2005; **96**: 134-141 [PMID: 16001513]
- 294 **Valentino M, Rapisarda V, Scalise L, Paone N, Santarelli L, Fenga C, Rossi GL.** A new method for the experimental assessment of finger haemodynamic effects induced by a hydraulic breaker in operative conditions. *J Occup Health* 2004; **46**: 253-259 [PMID: 15308823 DOI: 10.1539/joh.46.253]
- 295 **Rapisarda V, Bracci M, Nunnari G, Ferrante M, Ledda C.** Tetanus immunity in construction workers in Italy. *Occup Med (Lond)* 2014; **64**: 217-219 [PMID: 24706467 DOI: 10.1093/occmed/

kqu019]

- 296 **Valentino M**, Rapisarda V. Tetanus in a central Italian region: scope for more effective prevention among unvaccinated agricultural workers. *Occup Med (Lond)* 2001; **51**: 114-117 [PMID: 11307686 DOI: 10.1093/occmed/51.2.114]
- 297 **Malaguarnera G**, Giordano M, Cappellani A, Berretta M, Malaguarnera M, Perrotta RE. Skin cancers in elderly patients. *Anticancer Agents Med Chem* 2013; **13**: 1406-1411 [PMID: 24102278]
- 298 **Mangia A**, Cenderello G, Orlandini A, Piazzolla V, Picciotto A, Zuin M, Ciancio A, Brancaccio G, Forte P, Carretta V, Zignego AL, Minerva N, Brindicci G, Marignani M, Baroni GS, Bertino G, Cuccorese G, Mottola L, Ripoli M, Pirisi M. Individualized treatment of genotype 1 naïve patients: an Italian multicenter field practice experience. *PLoS One* 2014; **9**: e110284 [PMID: 25340799 DOI: 10.1371/journal.pone.0110284]
- 299 **Herceg Z**, Lambert MP, van Veldhoven K, Demetriou C, Vineis P, Smith MT, Straif K, Wild CP. Towards incorporating epigenetic mechanisms into carcinogen identification and evaluation. *Carcinogenesis* 2013; **34**: 1955-1967 [PMID: 23749751 DOI: 10.1093/carcin/bgt212]
- 300 **Malaguarnera G**, Gagliano C, Giordano M, Salomone S, Vacante M, Bucolo C, Caraci F, Reibaldi M, Drago F, Avitabile T, Motta M. Homocysteine serum levels in diabetic patients with non proliferative, proliferative and without retinopathy. *Biomed Res Int* 2014; **2014**: 191497 [PMID: 24877066 DOI: 10.1155/2014/191497]
- 301 **Cardin R**, Picciocchi M, Bortolami M, Kotsafti A, Barzon L, Lavezzo E, Sinigaglia A, Rodriguez-Castro KI, Rugge M, Farinati F. Oxidative damage in the progression of chronic liver disease to hepatocellular carcinoma: an intricate pathway. *World J Gastroenterol* 2014; **20**: 3078-3086 [PMID: 24696595 DOI: 10.3748/wjg.v20.i12.3078]
- 302 **Hernandez-Gea V**, Toffanin S, Friedman SL, Llovet JM. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. *Gastroenterology* 2013; **144**: 512-527 [PMID: 23313965 DOI: 10.1053/j.gastro.2013.01.002]
- 303 **Lee S**, Kim JS. Mitophagy: therapeutic potentials for liver disease and beyond. *Toxicol Res* 2014; **30**: 243-250 [PMID: 25584143 DOI: 10.5487/TR.2014.30.4.243]
- 304 **Cui J**, Gong Z, Shen HM. The role of autophagy in liver cancer: molecular mechanisms and potential therapeutic targets. *Biochim Biophys Acta* 2013; **1836**: 15-26 [PMID: 23428608 DOI: 10.1016/j.bbcan.2013.02.003]
- 305 **Czaja MJ**, Ding WX, Donohue TM, Friedman SL, Kim JS, Komatsu M, Lemasters JJ, Lemoine A, Lin JD, Ou JH, Perlmutter DH, Randall G, Ray RB, Tsung A, Yin XM. Functions of autophagy in normal and diseased liver. *Autophagy* 2013; **9**: 1131-1158 [PMID: 23774882 DOI: 10.4161/auto.25063]
- 306 **Kudchodkar SB**, Levine B. Viruses and autophagy. *Rev Med Virol* 2009; **19**: 359-378 [PMID: 19750559 DOI: 10.1002/rmv.630]
- 307 **Tang H**, Da L, Mao Y, Li Y, Li D, Xu Z, Li F, Wang Y, Tiollais P, Li T, Zhao M. Hepatitis B virus X protein sensitizes cells to starvation-induced autophagy via up-regulation of beclin 1 expression. *Hepatology* 2009; **49**: 60-71 [PMID: 19065679 DOI: 10.1002/hep.22581]
- 308 **Dreux M**, Gastaminza P, Wieland SF, Chisari FV. The autophagy machinery is required to initiate hepatitis C virus replication. *Proc Natl Acad Sci USA* 2009; **106**: 14046-14051 [PMID: 19666601 DOI: 10.1073/pnas.0907344106]
- 309 **Wang K**. Autophagy and apoptosis in liver injury. *Cell Cycle* 2015; **14**: 1631-1642 [PMID: 25927598 DOI: 10.1080/15384101.2015.1038685]

**P- Reviewer:** Tomizawa M **S- Editor:** Ji FF  
**L- Editor:** Roemmele A **E- Editor:** Liu SQ



## Innovative surgical approaches for hepatocellular carcinoma

Riccardo Memeo, Nicola de'Angelis, Vito de Blasi, Zineb Cherkaoui, Oronzo Brunetti, Vito Longo, Tullio Piardi, Daniele Sommacale, Jacques Marescaux, Didier Mutter, Patrick Pessaux

Riccardo Memeo, Vito de Blasi, Zineb Cherkaoui, Jacques Marescaux, Didier Mutter, Patrick Pessaux, Department of Digestive Surgery, University Hospital of Strasbourg, 67091 Strasbourg, France

Riccardo Memeo, Vito de Blasi, Zineb Cherkaoui, Jacques Marescaux, Didier Mutter, Patrick Pessaux, IRCAD, Research Institute Against Cancer of the Digestive Tract, 67091 Strasbourg, France

Riccardo Memeo, Vito de Blasi, Zineb Cherkaoui, Jacques Marescaux, Didier Mutter, Patrick Pessaux, IHU-Strasbourg, Institute for Image-Guided Surgery, 67091 Strasbourg, France

Riccardo Memeo, Patrick Pessaux, Inserm U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, 67091 Strasbourg, France

Nicola de'Angelis, Unit of Digestive, Hepato-Pancreato-Biliary Surgery and Liver Transplantation, Henri Mondor Hospital, AP-HP, 94010 Créteil, France

Nicola de'Angelis, Université Paris-Est, Val de Marne UPEC, 94010 Créteil, France

Oronzo Brunetti, Medical Oncology Unit, Cancer institute "Giovanni Paolo II", 70100 Bari, Italy

Vito Longo, Medical Oncology Unit, Hospital "Di Miccoli", 70124 Barletta, Italy

Tullio Piardi, Daniele Sommacale, Service de Chirurgie Générale, Digestive et Endocrinienne, Hôpital Robert Debré, Centre Hospitalier Universitaire de Reims, Université de Reims Champagne-Ardenne, 51000 Reims, France

**Author contributions:** Memeo R, de'Angelis N, de Blasi V and Cherkaoui Z performed the study conception and design; Brunetti O, Longo V and Piardi T acquired the data; Sommacale D and Marescaux J analyzed the data; Marescaux J, Mutter D and Pessaux P reviewed the paper.

**Conflict-of-interest statement:** There is no conflict of interest associated with any of the senior author or other coauthors con-

tributed their efforts in this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Riccardo Memeo, MD, PhD, Department of Digestive Surgery, University Hospital of Strasbourg, 1 Place de l'Hopital, 67091 Strasbourg, France. [riccardo.memeo@chru-strasbourg.fr](mailto:riccardo.memeo@chru-strasbourg.fr)  
**Telephone:** +33-3-69550552  
**Fax:** +33-3-69550532

**Received:** February 26, 2016  
**Peer-review started:** February 29, 2016  
**First decision:** March 23, 2016  
**Revised:** March 30, 2016  
**Accepted:** April 14, 2016  
**Article in press:** April 18, 2016  
**Published online:** May 8, 2016

### Abstract

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, with an increasing diffusion in Europe and the United States. The management of such a cancer is continuously progressing and the objective of this paper is to evaluate innovation in the surgical treatment of HCC. In this review, we will analyze the modern concept of preoperative management, the role of laparoscopic and robotic surgery, the intraoperative use of three dimensional models and augmented reality, as well as the potential application of fluorescence.

**Key words:** Hepatocellular carcinoma; Liver resection; Hepatectomy; New perspectives; Innovative surgical approaches

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, with an increasing diffusion in Europe and the United States. The management of such a cancer is continuously progressing and the objective of this paper is to evaluate innovation in the surgical treatment of HCC.

Memeo R, de'Angelis N, de Blasi V, Cherkaoui Z, Brunetti O, Longo V, Piardi T, Sommacale D, Marescaux J, Mutter D, Pessaux P. Innovative surgical approaches for hepatocellular carcinoma. *World J Hepatol* 2016; 8(13): 591-596 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i13/591.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i13.591>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, with at least 1 million new cases each year<sup>[1]</sup>. Even if liver transplantation remains the ideal treatment, hepatic resection remains the only curative treatment for HCC. Considering the early experience of liver resection for HCC in the 1980s, results were discouraging, with a mortality rate in the range of 10% and a considerable morbidity. Improvements in patient selection, early diagnosis, preoperative and postoperative management, surgical technique and development of new technologies have allowed to obtain a lower mortality and morbidity, achieving 0% in certain high-volume centers. The development of laparoscopic and robotic surgery, associated with the application of new technologies in patient care and progress in the medical treatment of HCC, represent a modern era challenge to optimize the management of HCC with the objective of improving overall and disease-free survival. The aim of this article is to describe all innovations in the surgical treatment of HCC.

## ADVANCES IN THE ASSESSMENT AND PLANNING OF SURGICAL TREATMENT

The onset of HCC in a normal liver is an extremely rare situation. It is associated with the presence of pathological liver, and cirrhosis in most cases (80%). The presence of a pathological liver requires a comprehensive study of liver function and patient condition, in order to prevent any postoperative liver failure which occurs in approximately 8% of patients after major hepatic resections<sup>[2]</sup>. The preoperative planning of a surgical procedure has improved the safety of liver resection in cirrhotic patients. The introduction of the concept of

future remnant liver (FRL)<sup>[3]</sup> as a predictor of liver failure has contributed to the development of the concept of liver volumetry. In case of pathological liver, FRL was usually set at 50% of functional liver to prevent any postoperative liver failure<sup>[3]</sup>. The necessity to calculate liver volumetry has increased the diffusion of three-dimensional (3D) surgical planning software<sup>[4]</sup>, with the double function of simulating surgery and calculating liver volumes. Even if conventional 2D images [magnetic resonance imaging (MRI) or computed tomography (CT)-scanning] reveal all the required information concerning tumors, major vessels and the biliary tract, surgeons could come across difficulties in perceiving the relationships of these structures before surgery, and during surgical planning. This platform allows to explore hepatic veins and portal triads from the hepatic pedicle up to segmental branches, allowing to evaluate spatial relationships with the tumor. This software allows to identify the vascular territory supplied by isolated vessels, allowing to simulate anatomical segmentectomy and easy planning of major and minor hepatectomies<sup>[5]</sup>. Many software tools are now available to create a 3D model, offering a visualized model of patient organs and pathologies.

## PORTAL VEIN EMBOLIZATION AND STEM CELLS APPLICATION

As previously mentioned, any hepatic resection must guarantee volume of FRL to prevent postoperative liver failure. In case of cirrhotic liver, the most common scenario in the presence of HCC, namely a portion of 40% to 50% of FRL, should be guaranteed so that liver function should not be affected. In case of insufficient FRL, portal vein embolization was suggested by Makuuchi *et al*<sup>[6]</sup> in 1990, in order to stimulate liver hypertrophy before surgery. This hypertrophy usually requires 4 to 6 wk, but in some cases, more time could be necessary, especially in case of pathological parenchyma. However, during this period, the tumor could continue its progression, and the patient could well become inoperable. One possibility to reduce this risk is to obtain a quicker hypertrophy, reducing the time between portal vein embolization and hepatectomy. Several studies have suggested that stem cells could have an important role in the process of tissue regeneration<sup>[7]</sup>. In case of acute or chronic liver suffering, stem cells can be stimulated from bone marrow. Among them, a subpopulation of cells (CD133<sup>+</sup>) have been recognized as potentially involved in liver regeneration after portal embolization, with encouraging results in some case series, demonstrating an augmented capacity of liver parenchyma regeneration<sup>[7-11]</sup>.

## 3D PRINT OF LIVER MODELS

Based on the data acquired by CT-scan which provide 2D information on geometrical measurements of tumors,



portal vein, hepatic vein and liver parenchyma, a 3D software edited model has been elaborated. 3D printing is a procedure which creates a solid 3D object based on a previous digital model. It is obtained *via* a 3D printer, which lays down thin layers of material in order to form a perfect 3D replica of the computer model. Initially developed to plan living donor liver transplantation<sup>[12]</sup>, an application is currently being found for it in liver surgery<sup>[13]</sup>. The main objective for the development of this physical liver model is to overcome the limitation of conventional 3D models and 2D images such as the absence of reliable liver surface markers, the difficult appreciation of depth, and difficulties in identifying liver segmentation as well as the relationships between vascular and biliary structures. Another advantage is the possibility to use the 3D-printed model during liver surgery, packing the prototype into a sterilized nylon bag<sup>[14]</sup>, which allows to adjust the model to the surgical situation and the surgical field in order to obtain a better understanding.

## REAL-TIME IMAGE FUSION FOR RADIOFREQUENCY ABLATION

Radiofrequency is currently considered an important support for the surgical treatment of HCC or in some cases it is considered an alternative to surgical resection<sup>[15]</sup>. This treatment is highly operator-dependent, especially for targeting, monitoring and controlling, as well as in cases of very small lesions in a pathological parenchyma. The development of a real-time image fusion system is based on the fusion of real-time sonograms with images previously obtained on CT-scan or MR<sup>[15-23]</sup>. To obtain this image fusion, a probe is equipped with a magnetic sensor, which generates a magnetic field interfaced with previously stored images. This fusion could lead to the detection of small HCCs, with an extremely high tumor-targeting success rate of 90% to 100%<sup>[21-23]</sup>. Such encouraging results could improve the performance of RFA treatment for nodules, which could not be revealed by means of sonography. The development of this tool associated with a needle tracking system could be used to assess the efficiency of RFA, hence allowing for a 3D evaluation of the treated zone.

## LAPAROSCOPIC SURGERY FOR HCC

Hepatic surgery still represents one of the most challenging and technical procedures requiring considerable experience. Despite such difficulties, some pioneers in laparoscopic surgery described the first laparoscopic liver resection in 1993<sup>[24]</sup>. Initially considered a standard procedure for patients with a single and subcapsular lesion of less than 5 cm, located in the left liver or in the anterior sectors of the right liver, it currently represents a valid alternative to open surgery for major hepatectomies, as it is considered a safe and feasible

procedure for the treatment of malignant lesions<sup>[25]</sup>. Even if the diffusion of this minimally invasive approach has rapidly gained consensus, laparoscopic resection of pathological livers was considered contraindicated due to the quality of parenchyma and condition of patients. A continuous progression of surgical devices over the last decades has improved the diffusion and safety of these complex procedures. The development of an ultrasonic scalpel (Ultracision™, Ethicon Endosurgery, Cincinnati, OH, United States) allows for a bloodless dissection of liver parenchyma. The ultrasonic dissector (Dissectron, Satelec, Mérignac, France) allows to divide and identify pedicles before being divided and clipped. Large vascular elements were divided after being secured with Hem-o-lok™ clips. Hemostasis and biliostasis of small elements were performed using saline-assisted bipolar electrocautery. Automatic vascular staplers allow for a safer and quick division and suture of large vascular structures, thereby reducing technical difficulties of manual suturing of large vessels. A crucial role during laparoscopic hepatic resection is the one played by ultrasonography, as it is used to localize hepatic veins and portal pedicles, allowing for a continuous control during parenchymal transection to check for safety margins. All these improvements, associated with the enhanced postoperative management of patients and augmented surgical skills have allowed the development of laparoscopic liver resection on cirrhosis, becoming a gold standard for treatment of HCC<sup>[26]</sup>. Despite a strong association with augmented mortality and morbidity as compared to hepatic resection on non-pathological livers, liver resection guarantees several advantages, especially in the postoperative period<sup>[27-30]</sup>, reducing blood loss, postoperative pain, abdominal wall infection, length of stay, and facilitating the surgical operation in case of future liver transplantation<sup>[31,32]</sup> due to a reduction of adhesions. As for non-pathological livers, and this is also true for HCC on cirrhosis, major hepatectomies, even associated with vascular resection<sup>[33]</sup>, are feasible with similar morbidity and mortality rates<sup>[34]</sup>.

## ROBOTIC SURGERY FOR HCC

The development and diffusion of the da Vinci™ robotic surgical system (Intuitive Surgical, Inc., Sunnyvale, CA, United States) have introduced a novel approach in general surgery with an enormous potentiality of integration. The system is made up of a patient-site with four robotic operating arms and a surgeon-site equipped with a stereoscopic 3D camera. Using the robot, this system allows to replicate human hand movements with precise downscaling. As laparoscopy had reached a standardization in hepatobiliary surgery, difficult procedures could benefit from the integrated function of robotic surgery. The aim of robotic surgery is to improve clinical outcome. The two main limitations of laparoscopic surgery (visual and ergonomic limitations) have been totally overcome by the robotic system, allowing to perform advanced procedures with safety.

Precise dissections could be achieved, due to the possibility of using articulated systems with seven degrees of freedom and advanced 3D views. Major limitations of robotic surgery include operating costs and lack of haptic feedback. Once this limitation is overcome, the diffusion will be faster, and more cases will be described in the literature. Currently, regarding HCC, few case series<sup>[35-39]</sup> are available and about 500 cases are described in the literature for liver malignant conditions. No benefits have been described as compared to laparoscopic surgery in terms of morbidity, mortality, and oncological results<sup>[35,38]</sup>. This is probably due to the shortage of series and of patients and will require further studies.

## ROBOTIC AND DEVELOPMENT OF AUGMENTED REALITY

The advent of robotic surgery has allowed the integration of the da Vinci™ robotic surgical system using virtual reality. During the surgical procedure, the 3D model reconstruction could be superimposed with real-time model mobilization, with the possibility to selectively view biliary structures, portal veins, the arterial system, hepatic veins, and lesions. This fusion is defined as augmented reality. This technique, initially described by Pessaux *et al.*<sup>[40]</sup>, use different skin landmarks associated with intra-abdominal landmarks to obtain a computer-assisted fusion of the 3D model with a real-time stereoscopic image of the operative field obtained via the 3D robotic camera. The superimposed image was used as a guide for the surgeon who, with the possibility to increase and decrease the transparency of the virtual model, could easily identify vascular structures as well as the correct localization of the lesion in order to obtain oncological resection margins. This modern era principle has found an application in other fields of surgery<sup>[40-44]</sup>, with an extremely interesting application of the lesion initially detected on CT-scan or MRI but impossible to detect preoperatively with ultrasound, called missing lesions<sup>[45]</sup>. This superimposition of 3D model reconstruction of the first bi-dimensional imaging in which the lesion was available allows the robotic image to guide resection of the liver segment in which the lesion is supposed to be located, hence allowing to achieve satisfying oncological margins.

## MINIMALLY INVASIVE APPLICATION OF FLUORESCENCE

Indocyanine green (ICG) is a non-toxic, non-radioactive and highly safe fluorophore with the capacity to appear green when excited by light in the near infrared spectrum. Historically used to predict liver failure<sup>[46]</sup>, its elimination from blood depends on hepatic blood flow. Cellular uptake and biliary excretion are measured using the ICG-plasma disappearance rate (ICG-PDR). In case of augmented values of ICG-PDR<sup>[46,47]</sup>, major

hepatectomies could be contraindicated to prevent postoperative liver failure. Considering the integration of a fluorescence camera in the robotic da Vinci™ system and laparoscopic camera, fluorescence could be integrated in operative strategies during hepatectomies. Arteries and veins are the first structures to be visualized after venous injection of ICG (5-60 s), and this could allow for an easier recognition of anatomical variations and identification of structures in the hepatic hilum. After vascular capitation, approximately 45 to 60 min after injection, ICG accumulates in the liver and is secreted in the bile. This application could be valuable to prevent complications during difficult cholecystectomies<sup>[48]</sup>, as it could allow to identify bile duct and cystic duct. In the future, it could well reduce the interest in using a perioperative cholangiogram, thereby reducing the exposure of patients to radiation.

Concerning the identification of liver neoplastic tissue, hemodynamic, metabolic and biliary excretion of ICG allow for the identification of tumoral parenchyma<sup>[49]</sup>. Poorly differentiated HCCs are characterized by a low capitation of the lesion with a fluorescent rim, due to a perilesional alteration of biliary excretion<sup>[50]</sup>. Well-differentiated HCCs have an intense fluorescent pattern<sup>[50]</sup>. This finding, as demonstrated for colorectal cancer liver metastasis, could allow to detect undetected lesions with previous conventional preoperative imaging<sup>[51]</sup>, with a potentially significant impact on disease-free survival<sup>[52]</sup>.

## CONCLUSION

HCC still represent a challenge for the surgeon of the next era. Considering the rapid evolution and quick technological progress applied to surgery, additional solutions will be put forward to achieve lower morbidity and mortality rates, guaranteeing a more precise resection, which will offer better oncological results. This progress, associated with progress in diagnosis<sup>[53]</sup>, advances in medical treatment, and an improvement of radiology and oncology will ensure a better future for our patients.

## REFERENCES

- 1 **Lau WY.** Management of hepatocellular carcinoma. *J R Coll Surg Edinb* 2002; **47**: 389-399 [PMID: 11874260]
- 2 **Vibert E, Pittau G, Gelli M, Cunha AS, Jamot L, Faivre J, Castro Benitez C, Castaing D, Adam R.** Actual incidence and long-term consequences of posthepatectomy liver failure after hepatectomy for colorectal liver metastases. *Surgery* 2014; **155**: 94-105 [PMID: 24694360 DOI: 10.1016/j.surg.2013.05.039]
- 3 **Clavien PA, Petrowsky H, DeOliveira ML, Graf R.** Strategies for safer liver surgery and partial liver transplantation. *N Engl J Med* 2007; **356**: 1545-1559 [PMID: 17429086 DOI: 10.1056/NEJMra065156]
- 4 **Bégin A, Martel G, Lapointe R, Belblidia A, Lepanto L, Soler L, Mutter D, Marescaux J, Vandenbroucke-Menu F.** Accuracy of preoperative automatic measurement of the liver volume by CT-scan combined to a 3D virtual surgical planning software (3DVSP). *Surg Endosc* 2014; **28**: 3408-3412 [PMID: 24928235 DOI: 10.1007/s00464-014-3611-x]

- 5 **Takamoto T**, Hashimoto T, Ogata S, Inoue K, Maruyama Y, Miyazaki A, Makuuchi M. Planning of anatomical liver segmentectomy and subsegmentectomy with 3-dimensional simulation software. *Am J Surg* 2013; **206**: 530-538 [PMID: 23809675 DOI: 10.1016/j.amjsurg.2013.01.041]
- 6 **Makuuchi M**, Thai BL, Takayasu K, Takayama T, Kosuge T, Gunvén P, Yamazaki S, Hasegawa H, Ozaki H. Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. *Surgery* 1990; **107**: 521-527 [PMID: 2333592]
- 7 **Theise ND**. Liver stem cells: the fall and rise of tissue biology. *Hepatology* 2003; **38**: 804-806 [PMID: 14512866 DOI: 10.1053/jhep.2003.50465]
- 8 **Franchi E**, Canepa MC, Peloso A, Barbieri L, Briani L, Panyor G, Dionigi P, Maestri M. Two-stage hepatectomy after autologous CD133+ stem cells administration: a case report. *World J Surg Oncol* 2013; **11**: 192 [PMID: 23941680 DOI: 10.1186/1477-7819-11-192]
- 9 **Fürst G**, Schulte am Esch J, Poll LW, Hosch SB, Fritz LB, Klein M, Godehardt E, Krieg A, Wecker B, Stoldt V, Stockschröder M, Eisenberger CF, Mödder U, Knoefel WT. Portal vein embolization and autologous CD133+ bone marrow stem cells for liver regeneration: initial experience. *Radiology* 2007; **243**: 171-179 [PMID: 17312278 DOI: 10.1148/radiol.2431060625]
- 10 **am Esch JS**, Knoefel WT, Klein M, Ghodsizad A, Fuerst G, Poll LW, Piechaczek C, Burchardt ER, Feifel N, Stoldt V, Stockschröder M, Stoecklein N, Tustas RY, Eisenberger CF, Peiper M, Häussinger D, Hosch SB. Portal application of autologous CD133+ bone marrow cells to the liver: a novel concept to support hepatic regeneration. *Stem Cells* 2005; **23**: 463-470 [PMID: 15790766 DOI: 10.1634/stemcells.2004-0283]
- 11 **Bellizzi A**, Sebastian S, Ceglia P, Centonze M, Divella R, Manzillo EF, Azzariti A, Silvestris N, Montemurro S, Caliendo C, De Luca R, Cicero G, Rizzo S, Russo A, Quaranta M, Simone G, Paradiso A. Co-expression of CD133(+)/CD44(+) in human colon cancer and liver metastasis. *J Cell Physiol* 2013; **228**: 408-415 [PMID: 22740326 DOI: 10.1002/jcp.24145]
- 12 **Zein NN**, Hanouneh IA, Bishop PD, Samaan M, Eghtesad B, Quintini C, Miller C, Yerian L, Klatte R. Three-dimensional print of a liver for preoperative planning in living donor liver transplantation. *Liver Transpl* 2013; **19**: 1304-1310 [PMID: 23959637 DOI: 10.1002/lt.23729]
- 13 **Takagi K**, Nanashima A, Abo T, Arai J, Matsuo N, Fukuda T, Nagayasu T. Three-dimensional printing model of liver for operative simulation in perihilar cholangiocarcinoma. *Hepatogastroenterology* 2014; **61**: 2315-2316 [PMID: 25699373]
- 14 **Igami T**, Nakamura Y, Hirose T, Ebata T, Yokoyama Y, Sugawara G, Mizuno T, Mori K, Nagino M. Application of a three-dimensional print of a liver in hepatectomy for small tumors invisible by intraoperative ultrasonography: preliminary experience. *World J Surg* 2014; **38**: 3163-3166 [PMID: 25145821 DOI: 10.1007/s00268-014-2740-7]
- 15 **Tiong L**, Maddern GJ. Systematic review and meta-analysis of survival and disease recurrence after radiofrequency ablation for hepatocellular carcinoma. *Br J Surg* 2011; **98**: 1210-1224 [PMID: 21766289 DOI: 10.1002/bjs.7669]
- 16 **Diana M**, Halvax P, Mertz D, Legner A, Brulé JM, Robinet E, Mutter D, Pessaux P, Marescaux J. Improving Echo-Guided Procedures Using an Ultrasound-CT Image Fusion System. *Surg Innov* 2015; **22**: 217-222 [PMID: 25801192 DOI: 10.1177/1553350615577483]
- 17 **Makino Y**, Imai Y, Igura T, Hori M, Fukuda K, Sawai Y, Kogita S, Fujita N, Takehara T, Murakami T. Comparative evaluation of three-dimensional Gd-EOB-DTPA-enhanced MR fusion imaging with CT fusion imaging in the assessment of treatment effect of radiofrequency ablation of hepatocellular carcinoma. *Abdom Imaging* 2015; **40**: 102-111 [PMID: 25052767 DOI: 10.1007/s00261-014-0201-2]
- 18 **Mauri G**, Cova L, De Beni S, Ierace T, Tondolo T, Cerri A, Goldberg SN, Solbiati L. Real-time US-CT/MRI image fusion for guidance of thermal ablation of liver tumors undetectable with US: results in 295 cases. *Cardiovasc Intervent Radiol* 2015; **38**: 143-151 [PMID: 24806953 DOI: 10.1007/s00270-014-0897-y]
- 19 **Park HJ**, Lee MW, Rhim H, Cha DI, Kang TW, Lim S, Song KD, Lim HK. Percutaneous ultrasonography-guided radiofrequency ablation of hepatocellular carcinomas: usefulness of image fusion with three-dimensional ultrasonography. *Clin Radiol* 2015; **70**: 387-394 [PMID: 25582889 DOI: 10.1016/j.crad.2014.12.003]
- 20 **Toshikuni N**, Tsutsumi M, Takuma Y, Arisawa T. Real-time image fusion for successful percutaneous radiofrequency ablation of hepatocellular carcinoma. *J Ultrasound Med* 2014; **33**: 2005-2010 [PMID: 25336489 DOI: 10.7863/ultra.33.11.2005]
- 21 **Kawasoe H**, Eguchi Y, Mizuta T, Yasutake T, Ozaki I, Shimonishi T, Miyazaki K, Tamai T, Kato A, Kudo S, Fujimoto K. Radiofrequency ablation with the real-time virtual sonography system for treating hepatocellular carcinoma difficult to detect by ultrasonography. *J Clin Biochem Nutr* 2007; **40**: 66-72 [PMID: 18437215 DOI: 10.3164/jcbn.40.66]
- 22 **Lee MW**, Rhim H, Cha DI, Kim YJ, Choi D, Kim YS, Lim HK. Percutaneous radiofrequency ablation of hepatocellular carcinoma: fusion imaging guidance for management of lesions with poor conspicuity at conventional sonography. *AJR Am J Roentgenol* 2012; **198**: 1438-1444 [PMID: 22623560 DOI: 10.2214/AJR.11.7568]
- 23 **Nakai M**, Sato M, Sahara S, Takasaka I, Kawai N, Minamiguchi H, Tanihata H, Kimura M, Takeuchi N. Radiofrequency ablation assisted by real-time virtual sonography and CT for hepatocellular carcinoma undetectable by conventional sonography. *Cardiovasc Intervent Radiol* 2009; **32**: 62-69 [PMID: 18987911 DOI: 10.1007/s00270-008-9462-x]
- 24 **Nguyen KT**, Marsh JW, Tsung A, Steel JJ, Gamblin TC, Geller DA. Comparative benefits of laparoscopic vs open hepatic resection: a critical appraisal. *Arch Surg* 2011; **146**: 348-356 [PMID: 21079109 DOI: 10.1001/archsurg.2010.248]
- 25 **Parks KR**, Kuo YH, Davis JM, O'Brien B, Hagopian EJ. Laparoscopic versus open liver resection: a meta-analysis of long-term outcome. *HPB (Oxford)* 2014; **16**: 109-118 [PMID: 23672270 DOI: 10.1111/hpb.12117]
- 26 **Memeo R**, de'Angelis N, Compagnon P, Salloum C, Cherqui D, Laurent A, Azoulay D. Laparoscopic vs. open liver resection for hepatocellular carcinoma of cirrhotic liver: a case-control study. *World J Surg* 2014; **38**: 2919-2926 [PMID: 24912628 DOI: 10.1007/s00268-014-2659-z]
- 27 **Vigano L**, Laurent A, Tayar C, Tomatis M, Ponti A, Cherqui D. The learning curve in laparoscopic liver resection: improved feasibility and reproducibility. *Ann Surg* 2009; **250**: 772-782 [PMID: 19801926 DOI: 10.1097/SLA.0b013e3181bd93b2]
- 28 **Lin NC**, Nitta H, Wakabayashi G. Laparoscopic major hepatectomy: a systematic literature review and comparison of 3 techniques. *Ann Surg* 2013; **257**: 205-213 [PMID: 23263192 DOI: 10.1097/SLA.0b013e31827da7fe]
- 29 **Buell JF**, Cherqui D, Geller DA, O'Rourke N, Iannitti D, Dagher I, Koffron AJ, Thomas M, Gayet B, Han HS, Wakabayashi G, Belli G, Kaneko H, Ker CG, Scatton O, Laurent A, Abdalla EK, Chaudhury P, Dutson E, Gamblin C, D'Angelica M, Nagorney D, Testa G, Labow D, Manas D, Poon RT, Nelson H, Martin R, Clary B, Pinson WC, Martinie J, Vauthey JN, Goldstein R, Roayaie S, Barlet D, Espat J, Abecassis M, Rees M, Fong Y, McMasters KM, Broelsch C, Busuttil R, Belghiti J, Strasberg S, Chari RS. The international position on laparoscopic liver surgery: The Louisville Statement, 2008. *Ann Surg* 2009; **250**: 825-830 [PMID: 19916210 DOI: 10.1097/SLA.0b013e3181b3b2d8]
- 30 **Truant S**, Bouras AF, Hebbat M, Boleslawski E, Fromont G, Dharancy S, Leteurtre E, Zerbib P, Pruvot FR. Laparoscopic resection vs. open liver resection for peripheral hepatocellular carcinoma in patients with chronic liver disease: a case-matched study. *Surg Endosc* 2011; **25**: 3668-3677 [PMID: 21688080 DOI: 10.1007/s00464-011-1775-1]
- 31 **Cherqui D**, Laurent A, Mocellin N, Tayar C, Luciani A, Van Nhieu JT, Decaens T, Hurtova M, Memeo R, Mallat A, Duvoux C. Liver resection for transplantable hepatocellular carcinoma: long-

- term survival and role of secondary liver transplantation. *Ann Surg* 2009; **250**: 738-746 [PMID: 19801927 DOI: 10.1097/SLA.0b013e3181bd582b]
- 32 **Kluger MD**, Memeo R, Laurent A, Tayar C, Cherqui D. Survey of adult liver transplantation techniques (SALT): an international study of current practices in deceased donor liver transplantation. *HPB (Oxford)* 2011; **13**: 692-698 [PMID: 21929669 DOI: 10.1111/j.1477-2574.2011.00348.x]
  - 33 **Morise Z**, Kawabe N, Tomishige H, Nagata H, Kawase J, Arakawa S, Isetani M. How Far Can We Go with Laparoscopic Liver Resection for Hepatocellular Carcinoma? Laparoscopic Sectionectomy of the Liver Combined with the Resection of the Major Hepatic Vein Main Trunk. *Biomed Res Int* 2015; **2015**: 960752 [PMID: 26448949 DOI: 10.1155/2015/960752]
  - 34 **Komatsu S**, Brustia R, Goumard C, Perdigo F, Soubrane O, Scatton O. Laparoscopic versus open major hepatectomy for hepatocellular carcinoma: a matched pair analysis. *Surg Endosc* 2015; Epub ahead of print [PMID: 26194255 DOI: 10.1007/s00464-015-4422-4]
  - 35 **Lai EC**, Yang GP, Tang CN. Robot-assisted laparoscopic liver resection for hepatocellular carcinoma: short-term outcome. *Am J Surg* 2013; **205**: 697-702 [PMID: 23561638 DOI: 10.1016/j.amjsurg.2012.08.015]
  - 36 **Smith MH**, Flanagan CL, Kemppainen JM, Sack JA, Chung H, Das S, Hollister SJ, Feinberg SE. Computed tomography-based tissue-engineered scaffolds in craniomaxillofacial surgery. *Int J Med Robot* 2007; **3**: 207-216 [PMID: 17631675 DOI: 10.1002/rcs.143]
  - 37 **Felli E**, Santoro R, Colasanti M, Vennarecci G, Lepiane P, Ettorre GM. Robotic liver surgery: preliminary experience in a tertiary hepato-biliary unit. *Updates Surg* 2015; **67**: 27-32 [PMID: 25750057 DOI: 10.1007/s13304-015-0285-4]
  - 38 **Ocuin LM**, Tsung A. Robotic liver resection for malignancy: Current status, oncologic outcomes, comparison to laparoscopy, and future applications. *J Surg Oncol* 2015; **112**: 295-301 [PMID: 26119652 DOI: 10.1002/jso.23901]
  - 39 **Salloum C**, Subar D, Memeo R, Tayar C, Laurent A, Malek A, Azoulay D. Laparoscopic robotic liver surgery: the Henri Mondor initial experience of 20 cases. *J Robot Surg* 2014; **8**: 119-124 [DOI: 10.1007/s11701-013-0437-9]
  - 40 **Pessaux P**, Diana M, Soler L, Piardi T, Mutter D, Marescaux J. Towards cybernetic surgery: robotic and augmented reality-assisted liver segmentectomy. *Langenbecks Arch Surg* 2015; **400**: 381-385 [PMID: 25392120 DOI: 10.1007/s00423-014-1256-9]
  - 41 **Hallet J**, Soler L, Diana M, Mutter D, Baumert TF, Habersetzer F, Marescaux J, Pessaux P. Trans-thoracic minimally invasive liver resection guided by augmented reality. *J Am Coll Surg* 2015; **220**: e55-e60 [PMID: 25840539 DOI: 10.1016/j.jamcollsurg.2014.12.053]
  - 42 **Marzano E**, Piardi T, Soler L, Diana M, Mutter D, Marescaux J, Pessaux P. Augmented reality-guided artery-first pancreaticoduodenectomy. *J Gastrointest Surg* 2013; **17**: 1980-1983 [PMID: 23943389 DOI: 10.1007/s11605-013-2307-1]
  - 43 **Pessaux P**, Diana M, Soler L, Piardi T, Mutter D, Marescaux J. Robotic duodenopancreatectomy assisted with augmented reality and real-time fluorescence guidance. *Surg Endosc* 2014; **28**: 2493-2498 [PMID: 24609700 DOI: 10.1007/s00464-014-3465-2]
  - 44 **Soler L**, Nicolau S, Pessaux P, Mutter D, Marescaux J. Real-time 3D image reconstruction guidance in liver resection surgery. *Hepatobiliary Surg Nutr* 2014; **3**: 73-81 [PMID: 24812598 DOI: 10.3978/j.issn.2304-3881.2014.02.03]
  - 45 **Ntourakis D**, Memeo R, Soler L, Marescaux J, Mutter D, Pessaux P. Augmented Reality Guidance for the Resection of Missing Colorectal Liver Metastases: An Initial Experience. *World J Surg* 2016; **40**: 419-426 [PMID: 26316112 DOI: 10.1007/s00268-015-3229-8]
  - 46 **Hoekstra LT**, de Graaf W, Nibourg GA, Heger M, Bennink RJ, Stieger B, van Gulik TM. Physiological and biochemical basis of clinical liver function tests: a review. *Ann Surg* 2013; **257**: 27-36 [PMID: 22836216 DOI: 10.1097/SLA.0b013e31825d5d47]
  - 47 **Lee CF**, Yu MC, Kuo LM, Chan KM, Jan YY, Chen MF, Lee WC. Using indocyanine green test to avoid post-hepatectomy liver dysfunction. *Chang Gung Med J* 2007; **30**: 333-338 [PMID: 17939263]
  - 48 **Calatayud D**, Milone L, Elli EF, Giulianotti PC. ICG-fluorescence identification of a small aberrant biliary canaliculus during robotic cholecystectomy. *Liver Int* 2012; **32**: 602 [PMID: 22292449 DOI: 10.1111/j.1478-3231.2012.02757.x]
  - 49 **Daskalaki D**, Aguilera F, Patton K, Giulianotti PC. Fluorescence in robotic surgery. *J Surg Oncol* 2015; **112**: 250-256 [PMID: 25974861 DOI: 10.1002/jso.23910]
  - 50 **Kokudo N**, Ishizawa T. Clinical application of fluorescence imaging of liver cancer using indocyanine green. *Liver Cancer* 2012; **1**: 15-21 [PMID: 24159568 DOI: 10.1159/000339017]
  - 51 **van der Vorst JR**, Schaafsma BE, Hutteman M, Verbeek FP, Liefers GJ, Hartgrink HH, Smit VT, Löwik CW, van de Velde CJ, Frangioni JV, Vahrmeijer AL. Near-infrared fluorescence-guided resection of colorectal liver metastases. *Cancer* 2013; **119**: 3411-3418 [PMID: 23794086 DOI: 10.1002/cncr.28203]
  - 52 **Murakami T**, Hiroshima Y, Zhang Y, Bouvet M, Chishima T, Tanaka K, Endo I, Hoffman RM. Improved disease-free survival and overall survival after fluorescence-guided surgery of liver metastasis in an orthotopic nude mouse model. *J Surg Oncol* 2015; **112**: 119-124 [PMID: 26266663 DOI: 10.1002/jso.23986]
  - 53 **Gnoni A**, Santini D, Scartozzi M, Russo A, Licchetta A, Palmieri V, Lupo L, Faloppi L, Palasciano G, Memeo V, Angarano G, Brunetti O, Guarini A, Pisconti S, Lorusso V, Silvestris N. Hepatocellular carcinoma treatment over sorafenib: epigenetics, microRNAs and microenvironment. Is there a light at the end of the tunnel? *Expert Opin Ther Targets* 2015; **19**: 1623-1635 [PMID: 26212068 DOI: 10.1517/14728222.2015.1071354]

**P- Reviewer:** de Santibañes E, Wang DS, Zhang ZM

**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Liu SQ





Retrospective Cohort Study

## Risk factors for deterioration of long-term liver function after radiofrequency ablation therapy

Koichi Honda, Masataka Seike, Junya Oribe, Mizuki Endo, Mie Arakawa, Hiroki Syo, Masao Iwao, Masanori Tokoro, Junko Nishimura, Tetsu Mori, Tsutomu Yamashita, Satoshi Fukuchi, Toyokichi Muro, Kazunari Murakami

Koichi Honda, Masataka Seike, Junya Oribe, Mizuki Endo, Mie Arakawa, Hiroki Syo, Masao Iwao, Masanori Tokoro, Junko Nishimura, Tetsu Mori, Kazunari Murakami, Department of Gastroenterology, Faculty of Medicine, Oita University, Yufu-City, Oita 879-5593, Japan

Tsutomu Yamashita, Satoshi Fukuchi, Toyokichi Muro, Department of Gastroenterology and Hepatology, National Hospital Organization Oita Medical Center, Oita City, Oita 870-0263, Japan

**Author contributions:** Honda K wrote the manuscript; Honda K, Seike M, Oribe J, Endo M, Arakawa M, Syo H, Iwao M, Tokoro M, Nishimura J, Mori T, Yamashita T, Fukuchi S, Muro T and Murakami K performed the clinical work; Seike M and Murakami K revised the manuscript.

**Institutional review board statement:** This research was approved by the Ethics Committee of Oita University and Oita Medical Center.

**Informed consent statement:** This is a retrospective research. The additional informed consent from the patients does not apply.

**Conflict-of-interest statement:** We have no financial relationships to disclose.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Koichi Honda, MD, PhD, Department of Gastroenterology, Faculty of Medicine, Oita University, 1-1

Idaigaoka, Hasama-machi, Yufu City, Oita 879-5593, Japan. [hondak@oita-u.ac.jp](mailto:hondak@oita-u.ac.jp)  
 Telephone: +81-97-5866193  
 Fax: +81-97-5866194

Received: January 4, 2016

Peer-review started: January 8, 2016

First decision: March 1, 2016

Revised: March 13, 2016

Accepted: March 24, 2016

Article in press: March 25, 2016

Published online: May 8, 2016

### Abstract

**AIM:** To identify factors that influence long-term liver function following radiofrequency ablation (RFA) in patients with viral hepatitis-related hepatocellular carcinoma.

**METHODS:** A total of 123 patients with hepatitis B virus- or hepatitis C virus-related hepatocellular carcinoma (HCC) ( $n = 12$  and  $n = 111$ , respectively) were enrolled. Cumulative rates of worsening Child-Pugh (CP) scores (defined as a 2-point increase) were examined.

**RESULTS:** CP score worsening was confirmed in 22 patients over a mean follow-up period of  $43.8 \pm 26.3$  mo. Multivariate analysis identified CP class, platelet count, and aspartate aminotransferase levels as significant predictors of a worsening CP score ( $P = 0.000$ ,  $P = 0.011$  and  $P = 0.024$ , respectively). In contrast, repeated RFA was not identified as a risk factor for liver function deterioration.

**CONCLUSION:** Long-term liver function following RFA was dependent on liver functional reserve, the degree

of fibrosis present, and the activity of the hepatitis condition for this cohort. Therefore, in order to maintain liver function for an extended period following RFA, suppression of viral hepatitis activity is important even after the treatment of HCC.

**Key words:** Radiofrequency ablation; Hepatocellular carcinoma; Liver function; Hepatitis B; Hepatitis C

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This study was conducted to identify risk factors for liver function deterioration following radiofrequency ablation (RFA) in patients with hepatocellular carcinoma (HCC) and viral hepatitis. A total of 123 patients with hepatitis B virus- or hepatitis C virus-related HCC were enrolled. Cumulative rates of worsening Child-Pugh (CP) scores (defined as a 2-point increase) following RFA were examined. CP class, platelet count, and aspartate aminotransferase levels were identified as significant predictors of a worsening CP score. Suppression of viral hepatitis activity with anti-viral therapy is important even after the treatment of HCC in order to maintain liver function following RFA.

Honda K, Seike M, Oribe J, Endo M, Arakawa M, Syo H, Iwao M, Tokoro M, Nishimura J, Mori T, Yamashita T, Fukuchi S, Muro T, Murakami K. Risk factors for deterioration of long-term liver function after radiofrequency ablation therapy. *World J Hepatol* 2016; 8(13): 597-604 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i13/597.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i13.597>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms worldwide<sup>[1]</sup> and most cases of HCC involve patients infected with hepatitis B virus (HBV) or hepatitis C virus (HCV)<sup>[2-4]</sup>. Radiofrequency ablation (RFA) is currently recognized as an effective local treatment for HCC<sup>[5,6]</sup> and has been shown to be a relatively low risk procedure<sup>[7-9]</sup>. However, deterioration of liver function has been observed during the long-term follow-up of these patients<sup>[10-12]</sup>. Therefore, the risk factors that contribute to deterioration of liver function need to be identified. Although a few reports have investigated changes in long-term liver function following RFA<sup>[10-12]</sup>, long-term liver function in patients with viral hepatitis-related HCC is still uncertain. The goal of this study was to identify risk factors for liver function deterioration in patients with HCC and viral hepatitis.

## MATERIALS AND METHODS

### Patients

This retrospective cohort study was based on data obtained from a prospective database maintained by

the Oita University and Oita Medical Center. Between January 2002 and December 2010, 479 patients underwent percutaneous RFA for HCC at these two institutions. This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and approved by the Ethics Committee of Oita University and Oita Medical Center.

A diagnosis of HCC was based on vascular findings obtained by dynamic computed tomography (CT) using early arterial uptake followed by washout in the porto-venous and equilibrium phase. For patients with an uncertain diagnosis, a fine-needle biopsy was performed. Prior to RFA, patients with hyper vascular tumors underwent transarterial chemoembolization. All ablations were performed with a single needle electrode (COVIDIEN, Cool-tip RF Ablation System, Ireland). Furthermore, all RFA procedures were performed percutaneously with ultrasound guidance, and diazepam and pentazocine were routinely administered prior to insertion of the electrode. If necessary, physiological saline was infused into the chest or abdominal cavity to induce artificial pleural effusion or ascites to avoid injury to adjacent organs, or to facilitate visualization of the tumor. Effects of RFA were confirmed by dynamic CT three days after treatment. If the ablated margin was insufficient, additional ablation was performed until a sufficient ablated margin was obtained.

Inclusion criteria for patient selection in the present study included: (1) HCC occurring due to HBV- or HCV-related chronic liver disease; (2) first occurrence of HCC; (3) the presence of up to four nodules per patient, with each nodule having a diameter less than 5 cm; and (4) the presence of tumors only in the liver, with complete necrosis achieved by treatment with RFA. Of the 479 patients treated for HCC, 356 patients were excluded from this study due to: Non-B or non-C HCC ( $n = 77$ ), recurrent HCC ( $n = 80$ ), complete necrosis was not obtained ( $n = 4$ ), advanced HCC ( $n = 33$ ), simultaneous other malignancies ( $n = 8$ ), nephrotic syndrome or advanced chronic kidney disease ( $n = 7$ ), portal thrombus ( $n = 3$ ), chronic debilitating disease ( $n = 1$ ), poor food intake ( $n = 2$ ), breakthrough hepatitis by resistant HBV ( $n = 2$ ), treatment with warfarin ( $n = 3$ ), received albumin around the same time as RFA treatment ( $n = 1$ ), started interferon (IFN) therapy up to 1 year after RFA treatment ( $n = 17$ ), uncontrollable progression of HCC up to 1 year after RFA treatment ( $n = 4$ ), death due to other disease within 1 year ( $n = 2$ ), documents not stored by the electronic system ( $n = 75$ ), a follow-up period less than one year ( $n = 27$ ), and treatment with a nucleoside analog within six months of RFA treatment ( $n = 10$ ). The latter was included based on reports that significant improvement in liver function had been observed within six months of lamivudine treatment for decompensated cirrhotic HBV patients<sup>[13,14]</sup>. Although it was also reported that albumin levels increased during the first two years of IFN treatment for chronic hepatitis C patients with sustained virological response (SVR)<sup>[15]</sup>, none of the patients in the current cohort met this

**Table 1 Patient characteristics (*n* = 123) at the start of the follow-up period**

Gender (male/female)	71/52
Age (yr)	69.7 ± 8.0
Hepatitis (HBV/HCV)	12/111
CP score (5/6/7/8)	79/22/15/7
CP class (A/B/C)	102/21/0
Size of tumor (mm)	20.6 ± 7.7
No. of tumor(s) (1/2/3/4)	78/30/13/2
Total bilirubin (mg/dL)	0.97 ± 0.4
Albumin (g/dL)	3.7 ± 0.6
Prothrombin time (%)	90.5 ± 15
Platelet count (10 <sup>4</sup> /μL)	11.1 ± 5.0
AST (IU/L)	58.2 ± 32.1
ALT (IU/L)	53.0 ± 39.7
Hepatitis condition RVH group/CAH group	13/110
Prior TACE with TACE/without TACE	110/13

HBV: Hepatitis B virus; CP: Child-Pugh; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; RVH: Remission status of viral hepatitis; CAH: Chronic active hepatitis; HCV: Hepatitis C virus; TACE: Transarterial chemoembolization.

criterion.

For the resulting 123 patients enrolled in this study, two groups were established in order to examine the influence of viral hepatitis activity. The first group included nine HBV patients who achieved complete remission of hepatitis (defined as a normal range of transaminase levels) by treatment with an oral nucleoside such as lamivudine, adefovir, or entecavir, two patients with non-active HBV, and four HCV patients who received IFN therapy and achieved a SVR. This group was referred to as the remission of viral hepatitis (RVH) group. The second group consisted of one HBV patient and 107 HCV patients with active hepatitis, and this group was referred to as the chronic active hepatitis (CAH) group.

#### Follow-up periods

The starting point for observation was the first day that patients underwent RFA. Follow-up periods concluded when recurrent HCC(s) were no longer able to be controlled with RFA. In addition, follow-up periods were ended when liver function was found to be deteriorating due to another disease, when treatment with IFN was initiated, when treatment with a nucleoside analog was initiated, when recurrent tumors were treated by surgery, or when a thrombus formed in the portal vein. During the follow-up period, abdominal CT or ultrasonography was performed every four months and blood assays were performed monthly.

#### Statistical analysis

All quantitative variables are presented as the mean ± SD. The endpoint used was a 2-point increase in Child-Pugh (CP) scoring. The cumulative rate of worsening CP scores (defined as a 2-point increase) was also calculated, and cumulative proportion curves were generated using the Kaplan-Meier method. Independent

factors that influenced a worsening CP score were identified by univariate and multivariate analysis using Cox's proportional hazards model. A *P*-value less than 0.05 was considered statistically significant. All statistical analyses were performed using the IBM SPSS Statistics version 20.0 for Windows.

## RESULTS

#### Patient profiles

A total of 123 patients (71 males, 52 females) with HBV infection (*n* = 12) or HCV infection (*n* = 111) were enrolled in this study. Additional characteristics of this cohort are provided in Table 1. Of the HBV patients, 9/12 were treated with nucleoside analogs [lamivudine (*n* = 1), lamivudine plus adefovir dipivoxil (*n* = 4), and entecavir (*n* = 4)] at least six months prior to RFA therapy. There were also two patients with non-active HBV carriers, and one HBV patient had an active case of hepatitis at the time of RFA. Of the HCV patients, 4/111 achieved a post-SVR state with IFN therapy. The CP class A group consisted of 102 patients which included: An active HBV carrier (*n* = 1), inactive HBV carriers that did not receive nucleoside analog treatment (*n* = 2), inactive HBV carriers that received nucleoside analog treatment (*n* = 8), patients with active hepatitis C (*n* = 87), and SVR patients with hepatitis C (*n* = 4). The CP class B group included an inactive HBV carrier who received nucleoside analog treatment (*n* = 1), and active hepatitis C patients (*n* = 20). During the follow-up period, the frequency of RFA treatment for recurrent tumors included a single treatment (*n* = 32), two treatments (*n* = 23), three treatments (*n* = 9), four treatments (*n* = 5), five treatments (*n* = 3), and six treatments (*n* = 2). There were 49 patients that did not receive any RFA treatment.

#### Liver function after RFA treatment

The follow-up period was ended for patients of this cohort due to: Loss of local control of tumor progression with RFA (*n* = 21), death or worsening of liver function due to another disease or accident (*n* = 7), induction of IFN therapy for HCV infection (*n* = 5), surgical treatment for recurrent tumors (*n* = 1), emergence of a portal thrombus (*n* = 1), and administration of a nucleoside analog for HBV infection (*n* = 1). In the latter case, a patient with HBV was enrolled in the CAH group since he initially refused treatment with entecavir. However, 12 mo later he consented to receive entecavir as a treatment, and consequently, the follow-up period for this case ended after 12 mo.

A worsening CP score was confirmed for 22 patients during a mean follow-up period of 43.8 ± 26.3 mo. Moreover, the 1-, 2-, 3-, 5- and 7-year cumulative rates for worsening CP scores calculated according to the Kaplan-Meier method were 2.4%, 6.9%, 10.0%, 19.3% and 33.2%, respectively (Figure 1). The variables listed in Table 1, as well as the frequency of RFA for

**Table 2** Univariate analysis to identify risk factors that contributed to a worsening Child-Pugh scores following radiofrequency ablation treatment ( $n = 123$ )

Variable	HR	95%CI	P-value
Gender (female <i>vs</i> male)	1.93	0.83-4.47	0.128
Age (yr) (< 70 <i>vs</i> $\geq 70$ )	1.05	0.45-2.44	0.906
CP class (B <i>vs</i> A)	5.03	2.17-11.7	0.000
Size of tumor (mm) ( $\geq 20$ <i>vs</i> < 20)	2.01	0.84-4.81	0.116
Number of tumors ( $\geq 2$ <i>vs</i> 1)	1.47	0.62-3.47	0.379
Total bilirubin (mg/dL) ( $\geq 1.0$ <i>vs</i> < 1.0)	3.48	1.35-8.99	0.010
Albumin (g/dL) (< 3.5 <i>vs</i> $\geq 3.5$ )	8.52	3.12-23.2	0.000
Prothrombin time (< 80% <i>vs</i> $\geq 80\%$ )	2.66	1.14-6.23	0.024
Platelet count ( $10^4/\mu\text{L}$ ) (< 10 <i>vs</i> $\geq 10$ )	5.04	1.86-13.7	0.001
AST (IU/L) ( $\geq 40$ <i>vs</i> < 40)	7.06	1.57-31.8	0.011
ALT (IU/L) ( $\geq 35$ <i>vs</i> < 35)	4.01	1.32-12.2	0.015
Prior TACE <i>vs</i> no TACE	1.05	0.24-4.48	0.952
Frequency of RFA treatments for recurrent HCC ( $\geq 2$ <i>vs</i> < 2)	1.51	0.64-3.53	0.344

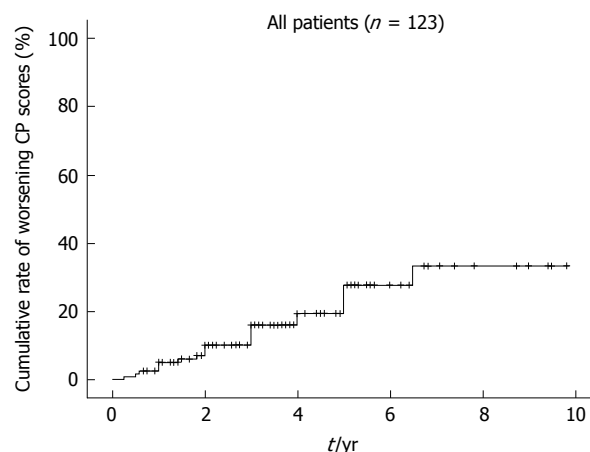
AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TACE: Transarterial chemoembolization; CP: Child-Pugh; RFA: Radiofrequency ablation; HCC: Hepatocellular carcinoma.

**Table 3** Multivariate analysis to identify risk factors that contributed to a worsening Child-Pugh scores following radiofrequency ablation ( $n = 123$ )

Variable	HR	95%CI	P-value
CP class (B <i>vs</i> A)	5.07	2.13-12.1	0.000
Platelet count ( $10^4/\mu\text{L}$ ) (< 10 <i>vs</i> $\geq 10$ )	3.83	1.36-10.8	0.011
AST (IU/L) ( $\geq 40$ <i>vs</i> < 40)	7.01	1.30-37.9	0.024
ALT (IU/L) ( $\geq 35$ <i>vs</i> < 35)	1.21	0.35-4.19	0.761

A worsening Child-Pugh (CP) scores was defined as a 2 point increase. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

recurrent HCC, were selected as factors for analysis using Cox's proportional hazards model. In contrast, the type of infection (HBV or HCV), and the presence of an active hepatitis condition (RVH or CAH), were excluded from this analysis, since none of the patients in HBV or RVH group exhibited at least a two point increase in CP scores during the follow-up period. Risk factors that were found to contribute to worsening CP scores following RFA are listed in Tables 2 and 3. In a univariate analysis performed, CP class, total bilirubin, albumin, prothrombin time, platelet count, levels of aspartate aminotransferase (AST), and levels of alanine aminotransferase were found to be associated with a worsening CP score (Table 2). Accordingly, these factors were selected for multivariate analysis. Frequency of RFA treatments for recurrent HCC was not found to be associated with deterioration of long-term liver function. Since total CP class, bilirubin, albumin, and prothrombin time are factors that indicate liver function, CP class was selected as a factor representative of these variables. In the multivariate analysis performed, CP class, platelet count, and AST were identified as significant predictors of a worsening CP score (Table 3) ( $P = 0.000$ ,  $P = 0.011$  and  $P = 0.024$ , respectively). Cumulative rates of

**Figure 1** Cumulative rate of worsening Child-Pugh scores (defined as a 2-point increase) for all patients. The 1-, 2-, 3-, 5- and 7-year cumulative rates for worsening CP scores calculated according to the Kaplan-Meier method were 2.4%, 6.9%, 10.0%, 19.3% and 33.2%, respectively. CP: Child-Pugh.

worsening CP scores were generated using the Kaplan-Meier method and are shown in Figure 2.

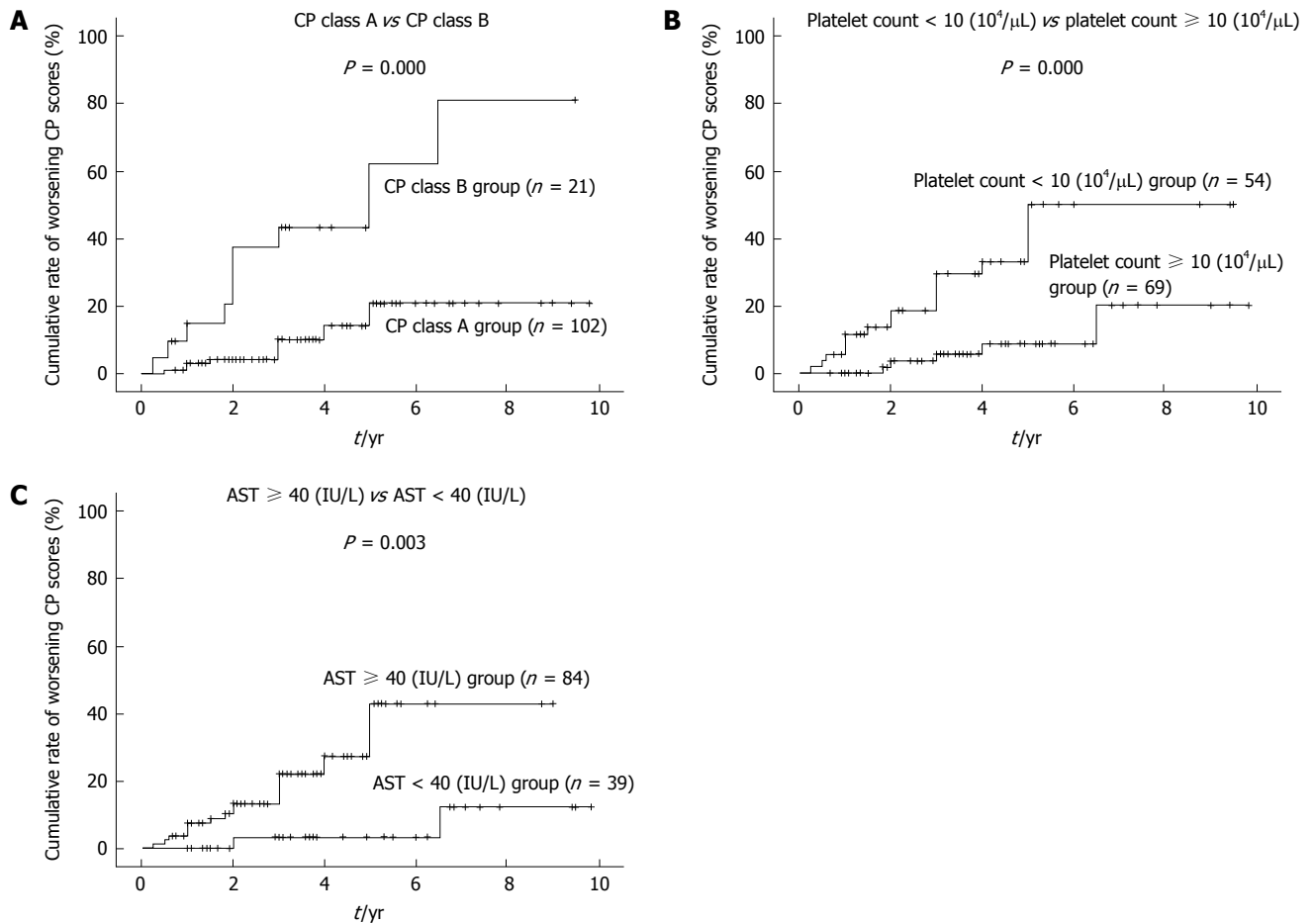
Subpopulational analyses were also performed with respect to HBV, HCV, RVH and CAH. For the HBV group ( $n = 12$ , mean follow-up period:  $64.0 \pm 28.7$  mo, CP class A ( $n = 11$ ), CP class B ( $n = 1$ ), CAH ( $n = 1$ ), RVH ( $n = 11$ ), platelet count:  $(10.4 \pm 4.3) \times 10^4/\mu\text{L}$ , AST:  $26.3 \pm 5.4$  IU/L, frequency of RFA treatment after initial treatment (0/1/2/3 times): 4/6/1/1 patients, respectively), none of the patients exhibited deterioration of long-term liver function.

For the HCV group [ $n = 111$ , mean follow-up period:  $41.6 \pm 25.2$  mo, CP class A ( $n = 91$ ), CP class B ( $n = 20$ ), CAH ( $n = 107$ ), RVH ( $n = 4$ ), platelet count:  $(11.1 \pm 5.1) \times 10^4/\mu\text{L}$ , AST:  $61.6 \pm 31.9$  IU/L, frequency of RFA treatment after initial treatment (0/1/2/3/4/5/6 times): 45/26/22/8/5/3/2 patients, respectively], CP class and platelet count were both identified as significant predictors of worsening CP scores in the multivariate analysis performed ( $P = 0.000$  and  $P = 0.009$ , respectively) (Table 4). None of the patients in the SVR group ( $n = 4$ ) exhibited worsening of CP scores.

For the RVH group [ $n = 15$ , mean follow-up period:  $65.4 \pm 28.0$  mo, HBV ( $n = 11$ ), HCV ( $n = 4$ ), CP class A ( $n = 14$ ), CP class B ( $n = 1$ ), platelet count:  $(11.7 \pm 5.1) \times 10^4/\mu\text{L}$ , AST:  $25.7 \pm 5.1$  IU/L, frequency of RFA treatment after initial treatment (0/1/2/3 times): 8/6/0/1 patients, respectively], none of the patients exhibited worsening CP scores.

For the CAH group [ $n = 108$ , mean follow-up period:  $40.8 \pm 24.7$  mo, HBV ( $n = 1$ ), HCV ( $n = 107$ ), CP class A ( $n = 88$ ), CP class B ( $n = 20$ ), platelet count:  $(11.0 \pm 5.0) \times 10^4/\mu\text{L}$ , AST:  $62.7 \pm 31.6$ , frequency of RFA treatment after initial treatment (0/1/2/3/4/5/6 times): 41/26/23/8/5/3/2 patients, respectively], CP class B and patients with a platelet count  $< 10 \times 10^4/\mu\text{L}$  were associated with CP worsening ( $P = 0.000$  and  $P = 0.010$ , respectively).





**Figure 2** Comparison of cumulative rate of worsening Child-Pugh scores (defined as a 2-point increase) according to the Kaplan-Meier method.  $P$ -values were calculated using a log-rank test. Analysis according to: A: CP class: A ( $n = 102$ ) and B ( $n = 21$ ); B: Platelet count:  $< 10 \times 10^4/\mu\text{L}$  ( $n = 54$ ) and  $\geq 10 \times 10^4/\mu\text{L}$  ( $n = 69$ ); C: AST levels:  $< 40 \text{ IU/L}$  ( $n = 39$ ) and  $\geq 40 \text{ IU/L}$  ( $n = 84$ ). CP: Child-Pugh; AST: Aspartate aminotransferase.

**Table 4** Multivariate analysis of risk factors that contributed to a worsening Child-Pugh scores following radiofrequency ablation for hepatitis C virus patients ( $n = 111$ )

Variable	HR	95%CI	P-value
CP class (B vs A)	4.90	2.05-11.7	0.000
Platelet count ( $10^4/\mu\text{L}$ ) ( $< 10$ vs $\geq 10$ )	3.96	1.40-11.2	0.009
AST (IU/L) ( $\geq 40$ vs $< 40$ )	5.25	0.98-28.0	0.052
ALT (IU/L) ( $\geq 35$ vs $< 35$ )	1.11	0.33-3.73	0.865

A worsening Child-Pugh (CP) score was defined as a 2 point increase. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

## DISCUSSION

Treatment of HCC generally involves a surgical approach and/or a non-surgical approach. In the latter case, transarterial embolization, radiation therapy, chemotherapy, and local puncture therapy are the main options available. While percutaneous ethanol injection therapy<sup>[16]</sup> is a type of local puncture therapy that has been performed since the 1980s, local ablative therapy such as microwave coagulation therapy<sup>[17]</sup> and RFA therapy were subsequently developed. Currently, RFA is the main form of local puncture therapy administered

due to its ability to provide local control of HCC. The less invasive approach of RFA also represents a key advantage of RFA over surgical resection. However, since the recurrence rate of HCC following radical treatment is generally high, repeated RFA treatments are often needed. There have been reports that the application of repeated RFA for the treatment of recurrent tumors can increase the chances of long-term survival<sup>[8,9,18]</sup>.

A few reports have referred to the influence of RFA on liver function. For example, Koda *et al.*<sup>[10]</sup> reported that liver function in patients with low pre-treatment CP scores transiently deteriorated within the first month of observation, while patients with high pre-treatment CP scores exhibited a greater extent of deterioration over a longer term of observation, approximately 6 mo. In a study by Kuroda *et al.*<sup>[11]</sup>, changes in liver function were monitored one year after RFA, and it was observed that a CP score of 9 or higher represented a major risk factor for aggravation of liver function following RFA. Furthermore, in another report by Yokoyama *et al.*<sup>[12]</sup>, the influence of RFA treatments on long-term liver function was investigated. Approximately 15% of CP class A or CP class B patients were observed to progress to CP class C five years after RFA treatment. However, the factors that influence on long-term liver function in

patients with viral hepatitis-related HCC following RFA is still uncertain. There are various factors that may contribute to changes in liver function. Since tumor progression is an obvious factor that aggravates the liver function of HCC patients, the current analyses were performed with patients where tumor progression could be excluded. Based on the analyses performed, CP class B patients, patients with a platelet count  $< 10 \times 10^4/\mu\text{L}$ , and patients with AST levels  $\geq 40$  IU/L, were found to be significantly associated with a worsening of liver function after RFA. These results suggest that worsening of long-term liver function after RFA is dependent on liver function, the degree of fibrosis present, and the activity of a patient's hepatitis condition. However, repeated RFA was not found to be a factor that aggravates long-term liver function.

None of the RVH patients exhibited CP worsening, thereby suggesting that liver function can be maintained in RVH patients if HCC is controlled. This result also suggests that short-term functional damage of the liver that is caused by RFA does not influence long-term liver function. However, since almost all of the RVH patients in the present study belonged to the CP class A group, additional studies are needed to clarify whether long-term liver function is affected following RFA for RVH patients with poor liver function.

Nucleoside analogs such as lamivudine or entecavir are used to treat active cases of hepatitis B by inhibiting DNA synthesis with termination of the nascent proviral DNA chain. As a result, levels of both serum HBV-DNA and transaminase concentrations are rapidly reduced. When viral suppression is prolonged, this can result in histological improvement, including regression of fibrosis<sup>[19-22]</sup>, and in patients with HBV-related HCC, liver function has improved<sup>[23-26]</sup>. For hepatitis C patients, IFN therapy has previously been the only treatment found to reduce levels of virus. For example, peginterferon plus ribavirin treatment has been a standard therapy for HCV infection until recently when telaprevir or simeprevir combined therapy was shown to improve the efficacy of IFN therapy<sup>[27,28]</sup>. However, since many cases of HCV-related HCC involved elderly patients, or a cirrhotic liver, there were many patients who could not receive radical treatment for HCV when HCC was detected. Other direct-acting antiviral agents have recently been investigated, and these have been found to increase SVR ratios<sup>[29,30]</sup>. Correspondingly, it is possible for HCC patients who are difficult to treat with IFN to be treated with IFN-free therapies.

While liver resection and RFA are still the standard treatments for many HCC patients, the long-term effects of surgical resection vs RFA remain controversial<sup>[31-33]</sup>. Thus, when many patients of HCV-related HCC become able to be treated with IFN-free therapies, this issue may be re-evaluated. In addition, further studies are needed to evaluate treatment modalities with respect to coexisting hepatitis conditions.

In conclusion, the results of the present study

indicate that long-term liver function following RFA is dependent on functional reserve of the liver, the degree of fibrosis present, and hepatitis activity. Since viral eradication or suppression is currently the most effective method to improve these factors, anti-viral therapy is important even after the treatment of HCC.

## COMMENTS

### Background

There are only a few reports that have examined liver function following radiofrequency ablation (RFA). In particular, long-term liver function following RFA in patients with viral hepatitis-related hepatocellular carcinoma (HCC) has not been well studied.

### Research frontiers

In the present study, long-term liver function in patients with viral hepatitis-related HCC that underwent RFA was found to be dependent on the functional reserve of the liver, the degree of fibrosis, and hepatitis activity.

### Innovations and breakthroughs

In previous studies, liver functional reserve at the time of RFA treatment was identified as a risk factor for liver function deterioration following RFA. Here, the authors demonstrate that the degree of liver fibrosis and hepatitis activity are also associated with deterioration of liver function following RFA.

### Applications

The strong and safe treatment regimen for patients with hepatitis B or hepatitis C that the authors have developed has the potential to maintain liver function following RFA treatment of patients with viral hepatitis-related HCC.

### Peer-review

This is a very well done study, it demonstrates that RFA seems to be a well tolerated therapy without relationship with deterioration of liver function.

## REFERENCES

- 1 Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]
- 2 El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]
- 3 Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; **5**: 558-567 [PMID: 16122679 DOI: 10.1016/S1473-3099(05)70216-4]
- 4 Taura N, Fukushima N, Yastuhashi H, Takami Y, Seike M, Watanabe H, Mizuta T, Sasaki Y, Nagata K, Tabara A, Komorizono Y, Taketomi A, Matsumoto S, Tamai T, Muro T, Nakao K, Fukuizumi K, Maeshiro T, Inoue O, Sata M. The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased in Kyushu area. *Med Sci Monit* 2011; **17**: PH7-P11 [PMID: 21278701]
- 5 Rossi S, Di Stasi M, Buscarini E, Quaretti P, Garbagnati F, Squassante L, Paties CT, Silverman DE, Buscarini L. Percutaneous RF interstitial thermal ablation in the treatment of hepatic cancer. *AJR Am J Roentgenol* 1996; **167**: 759-768 [PMID: 8751696 DOI: 10.2214/ajr.167.3.8751696]
- 6 Livraghi T, Goldberg SN, Lazzaroni S, Meloni F, Solbiati L, Gazelle GS. Small hepatocellular carcinoma: treatment with radiofrequency ablation versus ethanol injection. *Radiology* 1999; **210**: 655-661 [PMID: 10207464 DOI: 10.1148/radiology.210.3.r99fe40655]
- 7 Livraghi T, Solbiati L, Meloni MF, Gazelle GS, Halpern EF, Goldberg SN. Treatment of focal liver tumors with percutaneous

- radio-frequency ablation: complications encountered in a multicenter study. *Radiology* 2003; **226**: 441-451 [PMID: 12563138 DOI: 10.1148/radiol.2262012198]
- 8 **Rossi S**, Ravetta V, Rosa L, Ghittoni G, Viera FT, Garbagnati F, Silini EM, Dionigi P, Calliada F, Quaretti P, Tinelli C. Repeated radiofrequency ablation for management of patients with cirrhosis with small hepatocellular carcinomas: a long-term cohort study. *Hepatology* 2011; **53**: 136-147 [PMID: 20967759 DOI: 10.1002/hep.23965]
  - 9 **Shiina S**, Tateishi R, Arano T, Uchino K, Enooku K, Nakagawa H, Asaoka Y, Sato T, Masuzaki R, Kondo Y, Goto T, Yoshida H, Omata M, Koike K. Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. *Am J Gastroenterol* 2012; **107**: 569-577; quiz 578 [PMID: 22158026 DOI: 10.1038/ajg.2011.425]
  - 10 **Koda M**, Ueki M, Maeda Y, Mimura KI, Okamoto K, Matsunaga Y, Kawakami M, Hosho K, Murawaki Y. The influence on liver parenchymal function and complications of radiofrequency ablation or the combination with transcatheter arterial embolization for hepatocellular carcinoma. *Hepatol Res* 2004; **29**: 18-23 [PMID: 15135342 DOI: 10.1016/j.hepres.2004.02.001]
  - 11 **Kuroda H**, Kasai K, Kakisaka K, Yasumi Y, Kataoka K, Ushio A, Miyamoto Y, Sawara K, Oikawa K, Kondo K, Miura Y, Endo R, Takikawa Y, Suzuki K. Changes in liver function parameters after percutaneous radiofrequency ablation therapy in patients with hepatocellular carcinoma. *Hepatol Res* 2010; **40**: 550-554 [PMID: 20546330 DOI: 10.1111/j.1872-034X.2009.00613.x]
  - 12 **Yokoyama K**, Anan A, Iwata K, Nishizawa S, Morihara D, Ueda S, Sakurai K, Iwashita H, Hirano G, Sakamoto M, Takeyama Y, Irie M, Shakado S, Sohda T, Sakisaka S. Limitation of repeated radiofrequency ablation in hepatocellular carcinoma: proposal of a three (times)  $\times$  3 (years) index. *J Gastroenterol Hepatol* 2012; **27**: 1044-1050 [PMID: 22433056 DOI: 10.1111/j.1440-1746.2012.07134.x]
  - 13 **Tseng PL**, Lu SN, Tung HD, Wang JH, Changchien CS, Lee CM. Determinants of early mortality and benefits of lamivudine therapy in patients with hepatitis B virus-related decompensated liver cirrhosis. *J Viral Hepat* 2005; **12**: 386-392 [PMID: 15985009 DOI: 10.1111/j.1365-2893.2005.00608.x]
  - 14 **Yao FY**, Bass NM. Lamivudine treatment in patients with severely decompensated cirrhosis due to replicating hepatitis B infection. *J Hepatol* 2000; **33**: 301-307 [PMID: 10952248 DOI: 10.1016/S0168-8278(00)80371-2]
  - 15 **Maruoka D**, Imazeki F, Arai M, Kanda T, Fujiwara K, Yokosuka O. Longitudinal changes of the laboratory data of chronic hepatitis C patients with sustained virological response on long-term follow-up. *J Viral Hepat* 2012; **19**: e97-104 [PMID: 22239532 DOI: 10.1111/j.1365-2893.2011.01512.x]
  - 16 **Seki T**, Nonaka T, Kubota Y, Mizuno T, Sameshima Y. Ultrasonically guided percutaneous ethanol injection therapy for hepatocellular carcinoma. *Am J Gastroenterol* 1989; **84**: 1400-1407 [PMID: 2479262]
  - 17 **Seki T**, Wakabayashi M, Nakagawa T, Itho T, Shiro T, Kunieda K, Sato M, Uchiyama S, Inoue K. Ultrasonically guided percutaneous microwave coagulation therapy for small hepatocellular carcinoma. *Cancer* 1994; **74**: 817-825 [PMID: 8039109]
  - 18 **N'Kontchou G**, Mahamoudi A, Aout M, Ganne-Carrié N, Grando V, Coderc E, Vicaute E, Trinchet JC, Sellier N, Beaugrand M, Seror O. Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognostic factors in 235 Western patients with cirrhosis. *Hepatology* 2009; **50**: 1475-1483 [PMID: 19731239 DOI: 10.1002/hep.23181]
  - 19 **Lai CL**, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray DF. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; **339**: 61-68 [PMID: 9654535 DOI: 10.1056/NEJM199807093390201]
  - 20 **Suzuki Y**, Kumada H, Ikeda K, Chayama K, Arase Y, Saitoh S, Tsubota A, Kobayashi M, Koike M, Ogawa N, Tanikawa K. Histological changes in liver biopsies after one year of lamivudine treatment in patients with chronic hepatitis B infection. *J Hepatol* 1999; **30**: 743-748 [PMID: 10365796 DOI: 10.1016/S0168-8278(99)80123-8]
  - 21 **Dienstag JL**, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, Gardner S, Gray DF, Schiff ER. Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003; **124**: 105-117 [PMID: 12512035 DOI: 10.1053/gast.2003.50013]
  - 22 **Chang TT**, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, Safadi R, Lee SS, Halota W, Goodman Z, Chi YC, Zhang H, Hindes R, Illoeje U, Beebe S, Kreter B. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. *Hepatology* 2010; **52**: 886-893 [PMID: 20683932 DOI: 10.1002/hep.23785]
  - 23 **Kuzuya T**, Katano Y, Kumada T, Toyoda H, Nakano I, Hirooka Y, Itoh A, Ishigami M, Hayashi K, Honda T, Goto H. Efficacy of antiviral therapy with lamivudine after initial treatment for hepatitis B virus-related hepatocellular carcinoma. *J Gastroenterol Hepatol* 2007; **22**: 1929-1935 [PMID: 17914972 DOI: 10.1111/j.1440-1746.2006.04707.x]
  - 24 **Kim JH**, Park JW, Koh DW, Lee WJ, Kim CM. Efficacy of lamivudine on hepatitis B viral status and liver function in patients with hepatitis B virus-related hepatocellular carcinoma. *Liver Int* 2009; **29**: 203-207 [PMID: 18662281 DOI: 10.1111/j.1478-3231.2008.01828.x]
  - 25 **Yoshida H**, Yoshida H, Goto E, Sato T, Ohki T, Masuzaki R, Tateishi R, Goto T, Shiina S, Kawabe T, Omata M. Safety and efficacy of lamivudine after radiofrequency ablation in patients with hepatitis B virus-related hepatocellular carcinoma. *Hepatol Int* 2008; **2**: 89-94 [PMID: 19669283 DOI: 10.1007/s12072-007-9020-7]
  - 26 **Kobashi H**, Miyake Y, Ikeda F, Yasunaka T, Nishino K, Moriya A, Kubota J, Nakamura S, Takaki A, Nouse K, Yamada G, Yamamoto K. Long-term outcome and hepatocellular carcinoma development in chronic hepatitis B or cirrhosis patients after nucleoside analog treatment with entecavir or lamivudine. *Hepatol Res* 2011; **41**: 405-416 [PMID: 21435126 DOI: 10.1111/j.1872-034X.2011.00785.x]
  - 27 **McHutchison JG**, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; **360**: 1827-1838 [PMID: 19403902 DOI: 10.1056/NEJMoa0806104]
  - 28 **Fried MW**, Buti M, Dore GJ, Flisiak R, Ferenci P, Jacobson I, Marcellin P, Manns M, Nikitin I, Poordad F, Sherman M, Zeuzem S, Scott J, Gilles L, Lenz O, Peeters M, Sekar V, De Smedt G, Beumont-Mauviel M. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: the randomized PILLAR study. *Hepatology* 2013; **58**: 1918-1929 [PMID: 23907700 DOI: 10.1002/hep.26641]
  - 29 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]
  - 30 **Andreone P**, Colombo MG, Enejosa JV, Koksai I, Ferenci P, Maieron A, Müllhaupt B, Horsmans Y, Weiland O, Reesink HW, Rodrigues L, Hu YB, Podsadecki T, Bernstein B. ABT-450, ritonavir, ombitasvir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. *Gastroenterology* 2014; **147**: 359-365.e1 [PMID: 24818763 DOI: 10.1053/j.gastro.2014.04.045]
  - 31 **Chen MS**, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006; **243**: 321-328 [PMID: 16495695 DOI: 10.1097/01.sla.0000201480.65519.b8]
  - 32 **Huang J**, Hernandez-Alejandre R, Croome KP, Yan L, Wu H, Chen

- Z, Prasoon P, Zeng Y. Radiofrequency ablation versus surgical resection for hepatocellular carcinoma in Childs A cirrhotics-a retrospective study of 1,061 cases. *J Gastrointest Surg* 2011; **15**: 311-320 [PMID: 21052859 DOI: 10.1007/s11605-010-1372-y]
- 33 **Ruzzenente A**, Guglielmi A, Sandri M, Campagnaro T, Valdegamberi

A, Conci S, Bagante F, Turcato G, D'Onofrio M, Iacono C. Surgical resection versus local ablation for HCC on cirrhosis: results from a propensity case-matched study. *J Gastrointest Surg* 2012; **16**: 301-311; discussion 311 [PMID: 22095524 DOI: 10.1007/s11605-011-1745-x]

**P- Reviewer:** Tijera MFH, Xu Z, Zhong JH **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Liu SQ





## Antiviral therapy for hepatitis B virus-related hepatocellular carcinoma after surgery: A comment for moving forward

Jian-Hong Zhong, Tian Yang, Bang-De Xiang, Le-Qun Li, Liang Ma

Jian-Hong Zhong, Bang-De Xiang, Le-Qun Li, Liang Ma,  
 Department of Hepatobiliary Surgery, Affiliated Tumor Hospital  
 of Guangxi Medical University, Nanning 530021, Guangxi  
 Zhuang Autonomous Region, China

Tian Yang, Department of Hepatobiliary Surgery, Eastern Hepa-  
 tobiliary Surgery Hospital, Second Military Medical University,  
 Shanghai 201800, China

**Author contributions:** Zhong JH, Yang T and Xiang BD  
 contributed equally to this work; Zhong JH designed the study and  
 wrote the manuscript; Zhong JH, Yang T, Xiang BD, Li LQ and  
 Ma L analyzed the data; all authors reviewed the manuscript.

**Supported by** The Guangxi University of Science and Tech-  
 nology Research Projects, No. KY2015LX056; the Self-Raised  
 Scientific Research Fund of the Ministry of Health of Guangxi  
 Zhuang Autonomous Region, No. Z2015621, and No. Z2014241;  
 the Innovation Project of Guangxi Graduate Education, No.  
 YCBZ2015030; and the Guangxi Science and Technology  
 Development Projects, No. 14124003-4.

**Conflict-of-interest statement:** The authors declare no conflicts  
 of interest regarding this manuscript.

**Open-Access:** This article is an open-access article which was  
 selected by an in-house editor and fully peer-reviewed by external  
 reviewers. It is distributed in accordance with the Creative  
 Commons Attribution Non Commercial (CC BY-NC 4.0) license,  
 which permits others to distribute, remix, adapt, build upon this  
 work non-commercially, and license their derivative works on  
 different terms, provided the original work is properly cited and  
 the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Liang Ma, MD, Department of Hepato-  
 biliary Surgery, Affiliated Tumor Hospital of Guangxi Medical  
 University, He Di Rd #71, Nanning 530021, Guangxi Zhuang  
 Autonomous Region, China. [xitongpingjia@163.com](mailto:xitongpingjia@163.com)  
 Telephone: +86-771-5330855  
 Fax: +86-771-5312000

Received: February 18, 2016  
 Peer-review started: February 19, 2016  
 First decision: March 1, 2016  
 Revised: March 4, 2016

Accepted: March 22, 2016  
 Article in press: March 23, 2016  
 Published online: May 8, 2016

### Abstract

Recurrence rate of hepatocellular carcinoma remains quite high even after surgery, and no postoperative therapies have been definitively shown to prevent hepatocellular carcinoma recurrence. A previous study showed that therapy with nucleos(t)ide analogues given to such patients after surgery significantly improved survival. However, many questions still exist about the usage of nucleos(t)ide analogues for patients with hepatocellular carcinoma after surgery.

**Key words:** Antiviral therapy; Hepatocellular carcinoma; Hepatitis B virus; Unanswered question

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Some important points about the usage of nucleos(t)ide analogues for patients with hepatocellular carcinoma after surgery in clinic were pointed out.

Zhong JH, Yang T, Xiang BD, Li LQ, Ma L. Antiviral therapy for hepatitis B virus-related hepatocellular carcinoma after surgery: A comment for moving forward. *World J Hepatol* 2016; 8(13): 605-606 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i13/605.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i13.605>

### TO THE EDITOR

Recurrence rate of hepatocellular carcinoma (HCC) remains quite high even after curative resection or radiofrequency ablation (RFA), and no adjuvant thera-

pies have been definitively shown to prevent HCC recurrence<sup>[1,2]</sup>. A previous study showed that therapy with nucleos(t)ide analogues (NAs) given to HCC patients after resection significantly improved survival<sup>[3]</sup>. Whether the same holds for HCC patients after RFA was unclear until Lee *et al*<sup>[4]</sup> reported their important findings that postoperative NA therapy significantly reduced 2-year recurrence rate. The authors supported their conclusions using multivariate analysis and propensity score matching. These results provide by far the strongest evidence that postoperative NA therapy can benefit patients with hepatitis B virus (HBV)-associated HCC. At the same time, methodological limitations in that study raise several important questions that must be addressed in future work.

Several of these limitations are acknowledged by Lee *et al*<sup>[4]</sup> in their report. They did not take into account possible confounding effects due to differences in baseline viral load, HBeAg, liver function, or type of treatment after recurrence. In addition, their focus on recurrence rate as the most important outcome and the relatively short (2-year) follow-up prevented them from clarifying how NA therapy provided clinical benefit. NA therapy is not thought to directly affect tumor growth. Rather, it is thought to act in the short term by reducing the risk of HBV reactivation and improving liver function. Lee *et al*<sup>[4]</sup> did not measure these outcomes in their study, making it impossible to examine how NA therapy reduced the recurrence rate. NA therapy is also thought to act in the long term by: (1) suppressing viral replication, which might reduce the risk of *de novo* HBV-related HCC development; and (2) reducing chronic inflammation in the remnant liver, thereby improving hepatic functional reserve after surgery and improving the patient's treatment options. Lee *et al*<sup>[4]</sup> could not observe these mechanisms because they stopped follow-up at 2 years. As a result, Lee *et al*<sup>[4]</sup> were able to measure only early recurrence, not late recurrence, which occurs at least 2 years after surgery or RFA. The 2-year cut-off also prevented the authors from measuring overall survival, a key outcome for establishing the efficacy of any treatment.

The results of Lee *et al*<sup>[4]</sup> argue strongly for the therapeutic potential of postoperative NA therapy for patients with HBV-related HCC, but they fall short of definitively establishing the therapy as effective. To close this evidence gap, we recommend that future studies address the following questions<sup>[5]</sup>: (1) Do all patients with HBV-related HCC benefit from postoperative NA therapy? What are the indications for NA therapy? Should these indications include preoperative liver function and viral load? We note that most patients in the study by Lee *et al*<sup>[4]</sup> had early-stage tumors (< 3 cm) and cirrhosis. Also, all the patients in the former

randomized controlled trial were all with relatively early-stage tumors<sup>[3]</sup>. In addition, almost all patients enrolled in previous studies had Child-Pugh A liver function<sup>[3,4,6,7]</sup>. So, further studies should investigate the benefit of NA therapy for patients with Child-Pugh B or C liver function. Last but not least, is NA therapy valuable for those with serum HBV DNA less than 500 copies/mL? (2) Which NA drug(s) are the most effective and safest? Lamivudine is the first antiviral drug. Although it suppresses the virus quickly, the frequency of drug resistance is too high. Other NA drugs include adefovir dipivoxil, entecavir, and tenofovir; (3) When is the optimal time to initiate NA therapy, and how long should it last? Nowadays, doctors and patients increasingly attach importance to the phenomenon of HBV reactivation. Therefore, NA therapy should be started before surgery. One of the purposes of NA therapy is to prevent tumor recurrence. Less than two years of therapy may be not enough; and (4) Are there benefits and risks to adding a second NA drug or continuing monotherapy? Many studies reported that combined therapy with two or more NA drugs were suitable for chronic hepatitis B. However, it is unknown for patients with HCC after surgery.

Addressing these questions will be essential for defining the NA treatment regimens most likely to provide clinical benefit, as well as for identifying the most suitable patient populations.

## REFERENCES

- 1 **Zhong JH**, Ke Y, Gong WF, Xiang BD, Ma L, Ye XP, Peng T, Xie GS, Li LQ. Hepatic resection associated with good survival for selected patients with intermediate and advanced-stage hepatocellular carcinoma. *Ann Surg* 2014; **260**: 329-340 [PMID: 24096763 DOI: 10.1097/SLA.0000000000000236]
- 2 **Zhong JH**, Ma L, Li LQ. Postoperative therapy options for hepatocellular carcinoma. *Scand J Gastroenterol* 2014; **49**: 649-661 [PMID: 24716523 DOI: 10.3109/00365521.2014.905626]
- 3 **Huang G**, Lau WY, Wang ZG, Pan ZY, Yuan SX, Shen F, Zhou WP, Wu MC. Antiviral therapy improves postoperative survival in patients with hepatocellular carcinoma: a randomized controlled trial. *Ann Surg* 2015; **261**: 56-66 [PMID: 25072444 DOI: 10.1097/SLA.0000000000000858]
- 4 **Lee TY**, Lin JT, Zeng YS, Chen YJ, Wu MS, Wu CY. Association between nucleos(t)ide analogue and tumor recurrence in HBV-related hepatocellular carcinoma after radiofrequency ablation. *Hepatology* 2015; Epub ahead of print [PMID: 26426978 DOI: 10.1002/hep.28266]
- 5 **Zhong JH**. Nucleos(t)ide analogue therapy for HBV-related HCC after hepatic resection: clinical benefits and unanswered questions. *Tumour Biol* 2014; **35**: 12779-12784 [PMID: 25431264 DOI: 10.1007/s13277-014-2881-1]
- 6 **Zhou Y**, Zhang Z, Zhao Y, Wu L, Li B. Antiviral therapy decreases recurrence of hepatitis B virus-related hepatocellular carcinoma after curative resection: a meta-analysis. *World J Surg* 2014; **38**: 2395-2402 [PMID: 24791945 DOI: 10.1007/s00268-014-2586-z]
- 7 **Singal AK**, Salameh H, Kuo YF, Fontana RJ. Meta-analysis: the impact of oral anti-viral agents on the incidence of hepatocellular carcinoma in chronic hepatitis B. *Aliment Pharmacol Ther* 2013; **38**: 98-106 [PMID: 23713520 DOI: 10.1111/apt.12344]

**P- Reviewer:** He JY, Qin JM, Ramos S **S- Editor:** Qiu S  
**L- Editor:** Wang TQ **E- Editor:** Liu SQ





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 May 18; 8(14): 607-636







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vysloulzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



**MINIREVIEWS**

- 607** Liver resection for intermediate hepatocellular carcinoma  
*Yi PS, Zhang M, Zhao JT, Xu MQ*

**ORIGINAL ARTICLE****Observational Study**

- 616** Combined acoustic radiation force impulse, aminotransferase to platelet ratio index and Forns index assessment for hepatic fibrosis grading in hepatitis B  
*Dong CF, Xiao J, Shan LB, Li HY, Xiong YJ, Yang GL, Liu J, Yao SM, Li SX, Le XH, Yuan J, Zhou BP, Tipoe GL, Liu YX*

**Randomized Clinical Trial**

- 625** Co-treatment with pegylated interferon alfa-2a and entecavir for hepatitis D: A randomized trial  
*Abbas Z, Memon MS, Umer MA, Abbas M, Shazi L*

**CASE REPORT**

- 632** Direct acting antiviral therapy is curative for chronic hepatitis C/autoimmune hepatitis overlap syndrome  
*Sahebjam F, Hajdu CH, Nortey E, Sigal SH*

## ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Dr. C Ganesh Pai, MD, Professor, Department of Gastroenterology and Hepatology, Kasturba Medical College, Manipal, Karnataka 576104, India

## AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

INDEXING/  
ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

## FLYLEAF

I-IV Editorial Board

EDITORS FOR  
THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
May 18, 2016

## COPYRIGHT

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

## SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

## INSTRUCTIONS TO AUTHORS

Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjgnet.com/bpg/g_info_20160116143427.htm)

## ONLINE SUBMISSION

<http://www.wjgnet.com/esps/>

## Liver resection for intermediate hepatocellular carcinoma

Peng-Sheng Yi, Ming Zhang, Ji-Tong Zhao, Ming-Qing Xu

Peng-Sheng Yi, Ming Zhang, Ming-Qing Xu, Department of Liver Surgery, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

Ji-Tong Zhao, Department of Gynecology and Obstetrics, West China Second Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

**Author contributions:** Yi PS drafted the article; Zhang M designed the study; Zhao JT analyzed and interpreted the data; Xu MQ approved the final version to be submitted; Yi PS and Zhang M contributed equally to this work.

**Conflict-of-interest statement:** No conflict of interests is declared by the authors.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Ming-Qing Xu, MD, Department of Liver Surgery, West China Hospital, Sichuan University, Guoxue Road, Chengdu 610041, Sichuan Province, China. [xumingqing0018@163.com](mailto:xumingqing0018@163.com)  
 Telephone: +86-28-85422870  
 Fax: +86-28-85422870

Received: January 22, 2016  
 Peer-review started: January 22, 2016  
 First decision: March 1, 2016  
 Revised: March 14, 2016  
 Accepted: April 21, 2016  
 Article in press: April 22, 2016  
 Published online: May 18, 2016

### Abstract

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in China. The Barcelona

Clinic Liver Cancer (BCLC) staging system is regarded as the gold standard staging system for HCC, classifying HCC as early, intermediate, or advanced. For intermediate HCC, trans-catheter arterial chemoembolization (TACE) is recommended as the optimal strategy by the BCLC guideline. This review investigates whether liver resection is better than TACE for intermediate HCC. Based on published studies, we compare the survival benefits and complications of liver resection and TACE for intermediate HCC. We also compare the survival benefits of liver resection in early and intermediate HCC. We find that liver resection can achieve better or at least comparable survival outcomes compared with TACE for intermediate HCC; however, we do not observe a significant difference between liver resection and TACE in terms of safety and morbidity. We conclude that liver resection may improve the short- and long-term survival of carefully selected intermediate HCC patients, and the procedure may be safely performed in the management of intermediate HCC.

**Key words:** Trans-catheter arterial chemoembolization; Intermediate hepatocellular carcinoma; Liver resection; Prognostic factor

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Trans-catheter arterial chemoembolization (TACE) is recommended as the standard treatment of intermediate hepatocellular carcinoma (HCC) by the Barcelona Clinic Liver Cancer guideline, and this review investigates whether liver resection is better than TACE for intermediate HCC. Based on published studies, we compare the survival benefits and complications of liver resection and TACE for intermediate HCC. We also compare the survival benefits of liver resection in early and intermediate HCC. We find that liver resection could achieve better or at least comparable survival outcomes compared with TACE for intermediate HCC; however, we do not observe a significant difference between liver resection and TACE in terms of safety and morbidity.

Yi PS, Zhang M, Zhao JT, Xu MQ. Liver resection for intermediate hepatocellular carcinoma. *World J Hepatol* 2016; 8(14): 607-615 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i14/607.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i14.607>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer related death in the world<sup>[1]</sup>. In China, where about 120 million people are positive for hepatitis B surface antigen, HCC accounts for 300000 deaths every year<sup>[2]</sup>. It is a great challenge for clinicians to cure HCC. In order to provide standardized treatment for HCC, numerous HCC staging systems have been proposed in recent decades, including the tumor-node-metastasis (TNM) system, the Okuda system, the Barcelona Clinic Liver Cancer (BCLC) system, the Cancer of the Liver Italian Program (CLIP), the Vienna classification, the Chinese University Prognostic Index, the Japan Integrated Staging score, and the Tokyo staging system<sup>[3]</sup>. All of these staging systems rely mainly on three variables: Tumor characteristics, liver function, and general status. The TNM system is one of the oldest; however, the complexity of its variables has limited its application. The most widely adopted systems for staging HCC are the CLIP and the BCLC system (endorsed by European Association for the Study of the Liver and the American Association For the Study of the Liver)<sup>[4]</sup>. At present, the BCLC system is regarded as the optimal staging system to predict prognosis and guide treatment of HCC<sup>[5]</sup>.

The BCLC system was proposed by Llovet *et al*<sup>[6]</sup> in 1999, and validated extensively in 2002, 2005, and 2010<sup>[7,8]</sup>. Based on the BCLC grading system, the corresponding recommended treatment for each stage is stratified. Curative treatment is advocated for early HCC (defined as a single tumor less than 5 cm in diameter, or up to three tumors less than 3 cm in diameter), such as surgery, radiofrequency ablation, and liver transplantation. For intermediate HCC (a single tumor more than 5 cm in diameter; two to three tumors of which at least one is more than 3 cm in diameter; or more than 3 tumors of any diameter), trans-catheter arterial chemoembolization (TACE) is recommended as the standardized treatment<sup>[9-11]</sup>. A large proportion of patients in China are classified at diagnosis with intermediate or advanced HCC (any tumor with radiologically evident and histologically proven macro-vascular invasion, spread to lymph nodes and/or distant metastases). Therefore, only a minority of Chinese patients are eligible for radical resection or other curative treatments.

Controversy over the optimal treatment for intermediate HCC has emerged in recent years, as some evidence has suggested that due to the heterogeneity of individuals in liver function and tumor size, patients

with intermediate HCC may not all derive the same benefit from TACE. TACE cannot induce complete tumor necrosis, especially when large nodules are encountered. As the mortality and morbidity of liver resection are decreasing worldwide, surgery has been considered in some treatment models<sup>[12-14]</sup>. One study at Fudan University Hospital endorsed surgical resection for intermediate HCC<sup>[15]</sup>.

This review summarizes research on the role of liver resection in the management of intermediate HCC. Through comparison of liver resection and TACE, we seek to determine an optimal treatment for intermediate HCC.

## LIVER RESECTION VS TACE FOR INTERMEDIATE HCC

The current treatment algorithm recommends TACE as the standard treatment for intermediate HCC based on two randomized controlled trials<sup>[16,17]</sup>. However, patients with intermediate HCC vary widely in tumor size, tumor volume, overall health, and other factors, and so derive different benefit from TACE. In recent years, many studies have validated the BCLC treatment recommendation<sup>[7,18-23]</sup>. Liver resection has been widely performed in patients with intermediate HCC, and many investigators have argued that liver resection is as safe as TACE for intermediate HCC and provides better survival outcomes in selected patients<sup>[24-31]</sup>. Several centers have proposed their own criteria for judging which intermediate HCC patients are most likely to benefit from liver resection; Zhang *et al*<sup>[32]</sup> proposed that intermediate HCC cases with the following features should be considered for radical resection: Large or very large solitary tumor with swelling outward, clear border or pseudo-capsule, and less than 30% of the liver destroyed or more than 50% of hepatic hypertrophy; or multiple tumors limited to one segment or lobe. The authors also pointed out that confinement of tumors to one segment or lobe is not an absolute indication, considering that surgical outcomes could be affected by multi-center distribution and the relationship between lesions and major vessels.

Wang *et al*<sup>[24]</sup> reported that the median overall survival of patients with intermediate HCC after liver resection was significantly higher than that after TACE. Additionally, the 1-, 3- and 5-year survival rates in the liver resection group were also significantly higher than those in the TACE group. The study found that liver resection provided the best survival outcomes for patients with early and intermediate HCC. In accordance with these findings, several studies found similar survival benefits of liver resection in the management of intermediate HCC<sup>[24-31]</sup>. Another group of investigators performed a propensity score study which enrolled patients with intermediate and advanced HCC, and observed survival benefits of liver resection by total analysis and propensity-matched analysis<sup>[29]</sup>. In addition,



**Table 1** Studies related to complications of liver resection and transhepatic arterial chemotherapy and embolization for intermediate hepatocellular carcinoma

Ref.	Patient	Median OS	Survival rate	DFS	Hospital mortality	Complications
Wang <i>et al</i> <sup>[24]</sup>	LR: 243 TACE: 741	LR: 60.4 TACE: 18.2 Sig	1-, 3- and 5-yr LR: 81.5%, 64.4%, 50.5% TACE: 61.9%, 29.1%, 16.4% Sig	NR	NR	NR
Ho <i>et al</i> <sup>[25]</sup>	LR: 122 TACE: 163	LR: 41.8 TACE: 16.8 Sig	1-, 3- and 5-yr LR: 77.4%, 51.9%, 36.6% TACE: 62.6%, 25.2%, 11% Sig	1-, 3- and 5-yr LR: 60.5%, 32.3%, 24.8%	NR	NR
Lin <i>et al</i> <sup>[26]</sup>	LR: 93 TACE: 73	LR: 27.6 TACE: 18.5	1-, 2- and 3-yr LR: 83%, 62%, 49% TACE: 39%, 5%, 2% Sig	NR	LR: 3/78 (3.8%) TACE: 5/93 (5.4%) No sig	NR
Hsu <i>et al</i> <sup>[27]</sup>	LR: 268 TACE: 455	NR	1-, 3- and 5-yr LR: 81%, 68%, 63% TACE: 30%, 43%, 15% Sig	NR	90 d LR: 4/146 (2.7%) TACE: 12/146 (8.2%) Sig	LR <i>vs</i> TACE: Acute liver failure (20% <i>vs</i> 11%) Sig Biliary tract injury (6.8% <i>vs</i> 0%) Sig
Zhong <i>et al</i> <sup>[28]</sup>	LR: 660 TACE: 319	NR	1-, 3- and 5-yr LR: 91%, 67%, 44% TACE: 83%, 35%, 17% Sig	NR	NR	NR
Zhong <i>et al</i> <sup>[29]</sup>	LR: 257 TACE: 135	LR: 42.9 TACE: 21 Sig	1-, 3- and 5-yr LR: 84%, 59%, 37% TACE: 69%, 29%, 14% Sig After propensity score analysis LR: 87%, 62%, 35% TACE: 77%, 44%, 20% Sig	NR	LR <i>vs</i> TACE: 3.1% <i>vs</i> 3.7% No sig	LR <i>vs</i> TACE: 28% <i>vs</i> 18.5% Sig
Yin <i>et al</i> <sup>[31]</sup>	LR: 88 TACE: 85	LR: 41 TACE: 14 Sig	1-, 2- and 3-yr LR: 76.1%, 63.5%, 51.5% TACE: 51.8%, 34.8%, 18.1% Sig	NR	LR: 1/88 (1.1%)	NR

NR: Not reported; OS: Overall survival; DFS: Disease-free survival; Sig: Significant difference; LR: Patients with liver resection; TACE: Patients with trans-catheter arterial chemoembolization.

they conducted a subgroup analysis to detect whether patients with liver resection had better survival rates than those with TACE, and survival benefits were observed in subgroup analysis by tumor size, tumor number, macro-vascular invasion, and portal hypertension. Given that the heterogeneity of survival rates among different study cohorts, the highest and lowest 5-year survival rates were 63% and 37%, respectively. Due to the variation in regions and characteristics of enrolled patients and surveillance techniques in different centers, the survival rate might differ for these two procedures in different populations, and we cannot recommend that liver resection be the preferred treatment for intermediate HCC in all cases. However, we observed a similar linear trend of survival benefits of liver resection in the studies we examined (Table 1).

Several studies examined the complications and mortality rates of each treatment modality. Two groups of investigators observed that the incidence of complications in patients with liver resection was significantly higher than that in patients with TACE<sup>[27,29]</sup>. Hsu *et al*<sup>[27]</sup> noted that the liver resection group had a higher incidence of acute liver failure and biliary duct injury

than did the TACE group. However, the incidence of fever was lower in the resection group. Studies reached inconsistent findings about the mortality rates associated with each treatment strategy. Hsu *et al*<sup>[27]</sup> observed a higher mortality rate in the resection group than in the TACE group, which was contradicted by several other studies<sup>[26,29]</sup>. This could perhaps be explained by the fact that the proportion of patients aged < 65 years differed between the liver resection group and the TACE group, which likely biased the analysis of mortality. As we know, elements associated with the mortality of patients with HCC include liver function, surgical procedures, and age<sup>[33,34]</sup>. If the demographic characteristics of patients in different groups are not comparable, we cannot perform a reliable analysis of mortality and complications. Studies providing data related to complications of liver resection and TACE are summarized in Table 1.

## LIVER RESECTION IN PATIENTS WITH EARLY AND INTERMEDIATE HCC

The corresponding treatment recommendation for early HCC is a curative strategy such as liver resection, liver

transplantation, or radiofrequency ablation. Many multi-center studies with large sample sizes have validated liver resection for early HCC<sup>[35-37]</sup>. Generally, patients with intermediate HCC are not candidates for radical resection based on the BCLC treatment algorithm. However, in recent decades, the question of whether liver resection is indicated for intermediate HCC has been debated worldwide. Ng *et al.*<sup>[38]</sup> found the 5-year survival rate to be 39% for intermediate HCC treated by liver resection, which was fairly acceptable. They advocated to perform liver resection in patients with intermediate HCC, and they also demonstrated that liver resection in carefully selected intermediate HCC patients could be as safe as in early HCC patients. Recently, numerous studies have demonstrated that liver resection for intermediate HCC can achieve comparable survival outcomes as in early HCC<sup>[18,24,39,40]</sup>. Nevertheless, a group of investigators reported survival benefits of liver resection for early HCC<sup>[41]</sup>. This 10-center study found that disease free survival and overall survival after liver resection were significantly higher for early HCC than for intermediate HCC, but the survival outcomes of liver resection for intermediate HCC were still acceptable, with 5-year survival rate estimated at 57%. They classified the patients receiving liver resection into three groups: BCLC A, BCLC B and BCLC C. The demographic characteristics of the BCLC A and BCLC B groups were not comparable, as both tumor number and average tumor size were lower in the BCLC A group, which may have biased the analysis of survival outcomes. Furthermore, surgical procedures differed significantly between these two groups, with a higher proportion of patients with minor resection in the BCLC A group than in the BCLC B group. Despite the survival advantages in the BCLC A group, the BCLC B group also achieved favorable short- and long-term survival outcomes, in accordance with other findings<sup>[35,42,43]</sup>.

Regarding complications and mortality of liver resection for early and intermediate HCC, two groups of investigators did not observe differences in mortality and morbidity between patients with early and intermediate HCC after liver resection<sup>[38,44]</sup>. Yamashita *et al.*<sup>[42]</sup> reported that the mortality and morbidity of patients with intermediate HCC receiving liver resection were 3.8% and 24.5%, respectively, which were higher than those in other investigations. The very large tumors (> 10 cm in diameter) of patients in the Yamashita *et al.*<sup>[42]</sup> study may explain the higher mortality and morbidity of this study compared with others. Recent studies comparing liver resection in early and intermediate HCC are presented in Table 2.

A high incidence of recurrence affects the survival rate of patients with HCC after liver resection, and recurrence rate has been identified as an independent prognostic factor for long-term survival<sup>[45]</sup>. Ng *et al.*<sup>[38]</sup> reported a higher incidence of intrahepatic recurrence after liver resection in intermediate HCC, but found no difference in the extra-hepatic recurrence of patients with early and intermediate HCC after liver resection.

Torzilli *et al.*<sup>[44]</sup> conducted a prospective cohort study in 2008, which did not find significant differences in either intrahepatic or extra-hepatic recurrence between patients with early and intermediate HCC receiving liver resection. Another study reported that the estimated 1-, 2-, 3- and 5-year recurrence rates of patients with intermediate HCC after liver resection were 44.2%, 54.5%, 60.6% and 68.1%, respectively<sup>[43]</sup>. Variables that help predict the risk of HCC recurrence are serum albumin level, microscopic vascular invasion, multi-nodularity, and advanced Edmondson stage<sup>[46]</sup>. Multi-nodularity and serum albumin level were identified as independent factors of recurrence by Chang *et al.*<sup>[43]</sup>. Given that the incidence of HCC recurrence is fairly high, routine surveillance by computed tomography scan or magnetic resonance imaging is strongly recommended for patients with intermediate HCC after resection<sup>[47,48]</sup>.

## PROGNOSTIC FACTORS OF SURVIVAL

Benefits of liver resection are tightly associated with numerous variables, such as liver function, tumor size, and tumor number. Investigators have identified several important variables correlated with survival outcomes of patients with intermediate HCC after liver resection (Table 3). Overall survival is one critical endpoint for the prognosis of patients. One group of investigators found that 8 of 16 variables analyzed had a significant prognostic influence on overall survival by univariate analysis, of which, only 5 variables showed significant prognostic influence by multivariate analysis<sup>[38]</sup>, and they determined that patients without any prognostic risk factors had a higher 5-year survival rate than those with one or more prognostic risk factors. Another group of investigators identified serum albumin level, ICG-15R, tumor capsule, portal hypertension, and other measures as risk markers (variables in different studies related to overall survival are presented in Table 3). Many studies have found that tumor number is a key factor in predicting overall survival<sup>[41,49-51]</sup>, and it is a critical variable in different HCC staging systems. Incomplete radical resection and postoperative recurrence are closely associated with tumor number.

The Child-Pugh grade is another prognostic factor for overall survival that has been clarified by several studies<sup>[26,35]</sup>. To our knowledge, the Child-Pugh grading is the most widely used system for evaluating liver function. Since liver resection, particularly extensive liver resection, can lead to liver failure in patients with insufficient liver volume, preoperative assessment of liver function will undoubtedly improve the intra-operative safety and postoperative survival rate. Specifically, T4 status of HCC stage was reported to be a prognostic factor of overall survival with a hazard ratio of 5.12 by a liver cancer study group in Japan<sup>[42]</sup>. However, as this variable is based on tumor size, tumor number, and macro-vascular invasion, we do not classify it as an independent variable for overall survival.

Disease-free survival was another key endpoint in

Table 2 Studies comparing liver resection for Barcelona Clinic Liver Cancer A and B

Ref.	Group	Median OS (mo)	Median DFS (mo)	Accumulative DFS	Intrahepatic recurrence	Extra-hepatic recurrence	Survival rate	Mortality	Morbidity
Ng <i>et al</i> <sup>[38]</sup>	BCLC A: 404	A: 83.5 (67.9-99.1)	A: 77 (66, 87.9)	A: 80%, 64%, 40%	A: 139/404 (34.4%)	A: 95/404 (23.5%)	1-, 3- and 5-yr A: 88%, 76%, 58%	A: 11/404 (2.7%)	93/404 (23.0%)
	BCLC B: 380	B: 36.9 (28.9-44.8)	B: 15.6 (10.8-20.4)	B: 54%, 38%, 26%	B: 199/380 (52.4%)	B: 110/380 (29.0%)	B: 74%, 50%, 39%	No sig	104/380 (27.4%)
Cho <i>et al</i> <sup>[39]</sup>	BCLC A: 169	NR	NR	1-, 3- and 5-yr A: 71.4%, 51.8%, 44.1%	NR	NR	Sig	A: 1/169 (0.6%)	NR
	BCLC B: 61			B: 58.3%, 40.0%, 31.7%			1-, 3- and 5-yr A: 87.5%, 69.5%, 59.0%	B: 1/61 (1.6%)	
Torzilli <i>et al</i> <sup>[44]</sup>	BCLC A: 61	NR	NR	No sig	A: 19/61 (31.14%)	A: 2/61 (3.3%)	No sig	No sig	A: 13/61 (21.3%)
	BCLC B: 24			A: 77%, 30% B: 75%, 35%			1- and 3-yr A: 91.6%, 81%	A: 0	
Wang <i>et al</i> <sup>[24]</sup>	BCLC A: 202	A: Can't estimate B: 60.4	A: NR B: NR	No sig	No sig	No sig	No sig	No sig	No sig
	BCLC B: 243			A: NR B: NR			A: Cannot be estimated B: 1-, 3- and 5-yr (81.5%, 64.4%, 50.5%)		
Wei <i>et al</i> <sup>[40]</sup>	BCLC A: 52	NR	NR	1-, 2- and 3-yr A: 77.8%, 61.4%, 48.9%	NR	NR	1-, 2- and 3-yr A: 86.5%, 75.0%, 69.2%	NR	NR
	BCLC B: 51			B: 70.2%, 55.8%, 45.4%			B: 84.3%, 68.6%, 54.9%		
Chang <i>et al</i> <sup>[43]</sup>	BCLC A: NR	NR	NR	No sig	The 1-, 2-, 3- and 5-yr recurrence rates were 44.2%, 54.5%, 60.6%, and 68.1%, respectively, in BCLC stage B patients	NR	No sig	NR	NR
	BCLC B: 318			5-yr B: 28.6%			1-, 2-, 3- and 5-yr B: 81.2%, 68.1%, 59.4%, 46.5%		
Ma <i>et al</i> <sup>[49]</sup>	BCLC A: 92	A: Cannot be estimated B: 27.9 ± 3.1 (21.8-33.9)	NR	NR	NR	NR	NR	NR	NR
	BCLC B: 178			B: 16.8 ± 1.65 (13.6-20.0)			NR		
Torzilli <i>et al</i> <sup>[41]</sup>	BCLC A: 777	NR	NR	1-, 3- and 5-yr A: 77%, 41%, 21%	NR	NR	1-, 3- and 5-yr A: 95%, 80%, 61%	30 d A: 1.6% vs B: 3.1%	Not significant in major complications
	BCLC B: 633			B: 63%, 38%, 27%			B: 88%, 71%, 57%		
Cucchetti <i>et al</i> <sup>[35]</sup>	BCLC A: NR	B: 35 (26-42)	NR	Sig	NR	NR	Sig	NR	NR
	BCLC B: 247			NR			1-, 3- and 5-yr B: 77.8%, 48.7%, 33.8%		
Yamashita <i>et al</i> <sup>[42]</sup>	BCLC A: Cannot be estimated	NR	NR	5-yr	Recurrence rate	B: 32/53 (62%)	5 yr	B: 2/53 (3.8%)	B: 13/53 (24.5%)
	BCLC B: 53			B: 24%			B: 35%		

OS: Overall survival; DFS: Disease-free survival; A: Patients with HCC of BCLC A; B: Patients with HCC of BCLC B; NR: Not reported; HCC: Hepatocellular carcinoma; BCLC: Barcelona Clinic Liver Cancer; Sig: Significant difference.

prognosis analysis of patients with malignant neoplasms. Microvascular invasion and Child-Pugh class B were two independent factors for disease-free survival in patients with single large or huge HCC<sup>[39]</sup>. It is known that HCC patients with major vascular invasion have a poor survival rate and high incidence of recurrence<sup>[52]</sup>. Single large or huge HCC is normally located adjacent to biliary ducts or vessels, making vascular invasion more probable. Alpha-fetoprotein level greater than 400 ng/mL is a significant

**Table 3** Prognostic risk factors of overall survival and disease-free survival

Ref.	Prognostic factors of overall survival		Prognostic factors of disease-free survival	
	By univariate analysis	By multivariate analysis	By univariate analysis	By multivariate analysis
Ng <i>et al</i> <sup>[38]</sup>	Hepatitis B surface antigen carrier, serum AFP, symptomatic disease, presence of cirrhosis, number of tumor nodule, microvascular tumor invasion, tumor invasion of adjacent organs, histological margin involvement by tumor	Symptomatic disease, presence of cirrhosis, multi-nodular tumor, microvascular tumor invasion, positive histological margin	Serum AFP level, symptomatic disease, presence of cirrhosis, multi-nodular tumor, microvascular tumor invasion, tumor invasion of adjacent organ, positive histological margins, the presence of microsatellite nodules	Symptomatic disease, presence of cirrhosis, multi-nodular tumor, positive histological margins
Torzilli <i>et al</i> <sup>[44]</sup>	Tumor size, tumor grade	Tumor size, tumor grade	NR	NR
Chang <i>et al</i> <sup>[43]</sup>	NR	Serum albumin level, ICG-15R, serum creatinine, multi-nodularity, Edmondson stage, macro-vascular invasion	NR	NR
Ma <i>et al</i> <sup>[49]</sup>	Histopathological grade, tumor capsule, tumor number, cirrhosis, BCLC classification	Tumor capsule, BCLC classification	NR	Tumor capsule, BCLC classification
Torzilli <i>et al</i> <sup>[41]</sup>	Tumor number, tumor size, macro-vascular invasion, presence of cirrhosis, esophageal varices, major resection, BCLC classification, preoperative bilirubin values	NR	NR	NR
Cucchetti <i>et al</i> <sup>[35]</sup>	NR	Tumor number, presence of esophageal varices, Child-Pugh score	NR	NR
Cho <i>et al</i> <sup>[39]</sup>	Child-Pugh class B, AFP level > 400 ng/mL, histologically poor differentiation	Child-Pugh class B	Positivity of hepatitis B surface antigen, Child-Pugh class B, AFP level > 400 ng/mL, microvascular invasion, histologically poor differentiation	Child-Pugh class B, microvascular invasion
Yamashita <i>et al</i> <sup>[42]</sup>	NR	T4 status of HCC stage by liver cancer study group of Japan, thrombus in portal vein	NR	T4 status of HCC stage by liver cancer study group of Japan, intra-operative transfusion
Lin <i>et al</i> <sup>[26]</sup>	NR	Low albumin level, treatment modality (liver resection <i>vs</i> TACE)	NR	NR
Hsu <i>et al</i> <sup>[27]</sup>	NR	Serum AFP level, Child-Pugh class B, performance status $\geq$ 2, TACE, tumor size, vascular invasion	NR	NR
Zhong <i>et al</i> <sup>[28]</sup>	NR	Serum AFP $\geq$ 400 ng/mL, diabetes mellitus, macro-vascular invasion, portal hypertension, TACE treatment	NR	NR
Yin <i>et al</i> <sup>[31]</sup>	Treatment modality, serum AFP level, total tumor size, tumor number, gender	Tumor number, treatment modality, gender	NR	NR

TACE: Transhepatic arterial chemotherapy and embolization; NR: Not reported; HCC: Hepatocellular carcinoma; BCLC: Barcelona Clinic Liver Cancer; AFP: Alpha fetoprotein.

prognostic risk factor for disease-free survival by multivariate analysis. However, previous studies have demonstrated that minor proportions of patients with HCC do not present with up-regulation of alpha-feto-protein, which makes the surveillance of onset and recurrence of HCC challenging<sup>[53-55]</sup>. Variables in different studies related to overall survival are presented in Table 3.

## CONCLUSION

According to the current BCLC treatment guideline, TACE is recommended as the optimal treatment strategy for intermediate HCC. However, the patients with HCC in Asia distribute among BCLC A, BCLC B, and

BCLC C, despite advances in surveillance of HCC in recent years, and a large proportion of patients in Asia present as BCLC B or C when diagnosed. According to the recommendations by the BCLC guideline, these patients cannot benefit from surgical resection. Our review investigated whether liver resection is in fact a viable treatment for intermediate HCC patients.

We found that liver resection could achieve better or at least comparable survival outcomes compared with TACE for intermediate HCC. As for the safety and morbidity, controversy remains. Nevertheless, with advances in surgical equipment and perioperative management, we expect that survival benefits for intermediate HCC after liver resection will improve in the future.



In addition, we examined the outcomes of liver resection in patients with BCLC A and BCLC B. With two exceptions, most studies demonstrated that liver resection offers comparable survival benefits in intermediate HCC and early HCC<sup>[38,41]</sup>. We conclude that liver resection may improve the short- and long-term survival of intermediate HCC when patients are carefully selected and it may be safely performed in the management of intermediate HCC. However, multi-center randomized controlled trials are needed to clarify which patients are most likely to benefit from liver resection. We identified several key prognostic risk factors for overall survival and disease-free survival. We noted that patients without any prognostic risk factors achieved better short- and long-term survival than those with one or more prognostic risk factors, which indicates that careful selection of patients is critical for satisfactory outcomes in intermediate HCC patients undergoing liver resection.

Controversy remains surrounding liver resection for the management of intermediate HCC. Surgical procedures have been proposed by some treatment algorithms, and even patients beyond the Milan criteria have been selected for liver transplantation<sup>[56-58]</sup>. However, more evidence is needed about whether the indications should be expanded for liver resection for intermediate HCC.

## REFERENCES

- 1 Wörns MA, Klöckner R, Weinmann A, Galle PR. [Therapy of hepatocellular carcinoma]. *Internist (Berl)* 2014; **55**: 23-24, 26-30 [PMID: 24240604 DOI: 10.1007/s00108-013-3318-4]
- 2 Yau T, Tang VY, Yao TJ, Fan ST, Lo CM, Poon RT. Development of Hong Kong Liver Cancer staging system with treatment stratification for patients with hepatocellular carcinoma. *Gastroenterology* 2014; **146**: 1691-700.e3 [PMID: 24583061 DOI: 10.1053/j.gastro.2014.02.032]
- 3 Maida M, Orlando E, Cammà C, Cabibbo G. Staging systems of hepatocellular carcinoma: a review of literature. *World J Gastroenterol* 2014; **20**: 4141-4150 [PMID: 24764652 DOI: 10.3748/wjg.v20.i15.4141]
- 4 Gomaa AI, Hashim MS, Waked I. Comparing staging systems for predicting prognosis and survival in patients with hepatocellular carcinoma in Egypt. *PLoS One* 2014; **9**: e90929 [PMID: 24603710 DOI: 10.1371/journal.pone.0090929]
- 5 Fong ZV, Tanabe KK. The clinical management of hepatocellular carcinoma in the United States, Europe, and Asia: a comprehensive and evidence-based comparison and review. *Cancer* 2014; **120**: 2824-2838 [PMID: 24897995 DOI: 10.1002/encr.28730]
- 6 Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-338 [PMID: 10518312 DOI: 10.1055/s-2007-1007122]
- 7 Cillo U, Vitale A, Grigoletto F, Farinati F, Brolese A, Zanús G, Neri D, Boccagni P, Srsen N, D'Amico F, Ciarleglio FA, Brida A, D'Amico DF. Prospective validation of the Barcelona Clinic Liver Cancer staging system. *J Hepatol* 2006; **44**: 723-731 [PMID: 16488051 DOI: 10.1016/j.jhep.2005.12.015]
- 8 Forner A, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects. *Semin Liver Dis* 2010; **30**: 61-74 [PMID: 20175034 DOI: 10.1055/s-0030-1247133]
- 9 Ho EY, Cozen ML, Shen H, Lerrigo R, Trimble E, Ryan JC, Corvera CU, Monto A. Expanded use of aggressive therapies improves survival in early and intermediate hepatocellular carcinoma. *HPB* (Oxford) 2014; **16**: 758-767 [PMID: 24467780 DOI: 10.1111/hpb.12214]
- 10 Han KH, Kudo M, Ye SL, Choi JY, Poon RT, Seong J, Park JW, Ichida T, Chung JW, Chow P, Cheng AL. Asian consensus workshop report: expert consensus guideline for the management of intermediate and advanced hepatocellular carcinoma in Asia. *Oncology* 2011; **81** Suppl 1: 158-164 [PMID: 22212951 DOI: 10.1159/000333280]
- 11 Forner A, Gilabert M, Bruix J, Raoul JL. Treatment of intermediate-stage hepatocellular carcinoma. *Nat Rev Clin Oncol* 2014; **11**: 525-535 [PMID: 25091611 DOI: 10.1038/nrclinonc.2014.122]
- 12 Kokudo N, Makuuchi M. Evidence-based clinical practice guidelines for hepatocellular carcinoma in Japan: the J-HCC guidelines. *J Gastroenterol* 2009; **44** Suppl 19: 119-121 [PMID: 19148805 DOI: 10.1007/s00535-008-2244-z]
- 13 Choi JY. Treatment algorithm for intermediate and advanced stage hepatocellular carcinoma: Korea. *Oncology* 2011; **81** Suppl 1: 141-147 [PMID: 22212948 DOI: 10.1159/000333277]
- 14 Takayasu K, Arii S, Kudo M, Ichida T, Matsui O, Izumi N, Matsuyama Y, Sakamoto M, Nakashima O, Ku Y, Kokudo N, Makuuchi M. Superselective transarterial chemoembolization for hepatocellular carcinoma. Validation of treatment algorithm proposed by Japanese guidelines. *J Hepatol* 2012; **56**: 886-892 [PMID: 22173160 DOI: 10.1016/j.jhep.2011.10.021]
- 15 Gao Q, Wang XY, Zhou J, Fan J. Heterogeneity of intermediate-stage HCC necessitates personalized management including surgery. *Nat Rev Clin Oncol* 2015; **12**: 10 [PMID: 25421283 DOI: 10.1038/nrclinonc.2014.122-c1]
- 16 Llovet JM, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862 DOI: 10.1016/S0140-6736(02)08649-x]
- 17 Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, Fan ST, Wong J. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**: 1164-1171 [PMID: 11981766 DOI: 10.1053/jhep.2002.33156]
- 18 Vitale A, Saracino E, Boccagni P, Brolese A, D'Amico F, Gringeri E, Neri D, Srsen N, Valmasoni M, Zanús G, Carraro A, Violi P, Pauletto A, Bassi D, Polacco M, Burra P, Farinati F, Feltracco P, Romano A, D'Amico DF, Cillo U. Validation of the BCLC prognostic system in surgical hepatocellular cancer patients. *Transplant Proc* 2009; **41**: 1260-1263 [PMID: 19460533 DOI: 10.1016/j.transproceed.2009.03.054]
- 19 Huitzil-Melendez FD, Capanu M, O'Reilly EM, Duffy A, Gansukh B, Saltz LL, Abou-Alfa GK. Advanced hepatocellular carcinoma: which staging systems best predict prognosis? *J Clin Oncol* 2010; **28**: 2889-2895 [PMID: 20458042 DOI: 10.1200/jco.2009.25.9895]
- 20 Santambrogio R, Salceda J, Costa M, Kluger MD, Barabino M, Laurent A, Opocher E, Azoulay D, Cherqui D. External validation of a simplified BCLC staging system for early hepatocellular carcinoma. *Eur J Surg Oncol* 2013; **39**: 850-857 [PMID: 23726257 DOI: 10.1016/j.ejso.2013.05.001]
- 21 Kitai S, Kudo M, Izumi N, Kaneko S, Ku Y, Kokudo N, Sakamoto M, Takayama T, Nakashima O, Kadoya M, Matsuyama Y, Matsunaga T. Validation of three staging systems for hepatocellular carcinoma (JIS score, biomarker-combined JIS score and BCLC system) in 4,649 cases from a Japanese nationwide survey. *Dig Dis* 2014; **32**: 717-724 [PMID: 25376289 DOI: 10.1159/000368008]
- 22 Radu P, Groza I, Iancu C, Al Hajjar N, Andreica V, Sparchez Z. Treatment of hepatocellular carcinoma in a tertiary Romanian center. Deviations from BCLC recommendations and influence on survival rate. *J Gastrointest Liver Dis* 2013; **22**: 291-297 [PMID: 24078986]
- 23 Vitale A, Burra P, Frigo AC, Trevisani F, Farinati F, Spolverato G, Volk M, Giannini EG, Ciccarese F, Piscaglia F, Rapaccini GL, Di Marco M, Caturelli E, Zoli M, Borzio F, Cabibbo G, Felder M,

- Gasbarrini A, Sacco R, Foschi FG, Missale G, Morisco F, Svegliati Baroni G, Virdone R, Cillo U. Survival benefit of liver resection for patients with hepatocellular carcinoma across different Barcelona Clinic Liver Cancer stages: a multicentre study. *J Hepatol* 2015; **62**: 617-624 [PMID: 25450706 DOI: 10.1016/j.jhep.2014.10.037]
- 24 **Wang JH**, Changchien CS, Hu TH, Lee CM, Kee KM, Lin CY, Chen CL, Chen TY, Huang YJ, Lu SN. The efficacy of treatment schedules according to Barcelona Clinic Liver Cancer staging for hepatocellular carcinoma - Survival analysis of 3892 patients. *Eur J Cancer* 2008; **44**: 1000-1006 [PMID: 18337087 DOI: 10.1016/j.ejca.2008.02.018]
  - 25 **Ho MC**, Huang GT, Tsang YM, Lee PH, Chen DS, Sheu JC, Chen CH. Liver resection improves the survival of patients with multiple hepatocellular carcinomas. *Ann Surg Oncol* 2009; **16**: 848-855 [PMID: 19159983 DOI: 10.1245/s10434-008-0282-7]
  - 26 **Lin CT**, Hsu KF, Chen TW, Yu JC, Chan DC, Yu CY, Hsieh TY, Fan HL, Kuo SM, Chung KP, Hsieh CB. Comparing hepatic resection and transarterial chemoembolization for Barcelona Clinic Liver Cancer (BCLC) stage B hepatocellular carcinoma: change for treatment of choice? *World J Surg* 2010; **34**: 2155-2161 [PMID: 20407768 DOI: 10.1007/s00268-010-0598-x]
  - 27 **Hsu CY**, Hsia CY, Huang YH, Su CW, Lin HC, Pai JT, Loong CC, Chiou YY, Lee RC, Lee FY, Huo TI, Lee SD. Comparison of surgical resection and transarterial chemoembolization for hepatocellular carcinoma beyond the Milan criteria: a propensity score analysis. *Ann Surg Oncol* 2012; **19**: 842-849 [PMID: 21913008 DOI: 10.1245/s10434-011-2060-1]
  - 28 **Zhong JH**, Ke Y, Gong WF, Xiang BD, Ma L, Ye XP, Peng T, Xie GS, Li LQ. Hepatic resection associated with good survival for selected patients with intermediate and advanced-stage hepatocellular carcinoma. *Ann Surg* 2014; **260**: 329-340 [PMID: 24096763 DOI: 10.1097/sla.0000000000000235]
  - 29 **Zhong JH**, Xiang BD, Gong WF, Ke Y, Mo QG, Ma L, Liu X, Li LQ. Comparison of long-term survival of patients with BCLC stage B hepatocellular carcinoma after liver resection or transarterial chemoembolization. *PLoS One* 2013; **8**: e68193 [PMID: 23874536 DOI: 10.1371/journal.pone.0068193]
  - 30 **Ke Y**, Zhong J, Guo Z, Liang Y, Li L, Xiang B. [Comparison liver resection with transarterial chemoembolization for Barcelona Clinic Liver Cancer stage B hepatocellular carcinoma patients on long-term survival after SPSS propensity score matching]. *Zhonghua Yixue Zazhi* 2014; **94**: 747-750 [PMID: 24844957]
  - 31 **Yin L**, Li H, Li AJ, Lau WY, Pan ZY, Lai EC, Wu MC, Zhou WP. Partial hepatectomy vs. transcatheter arterial chemoembolization for resectable multiple hepatocellular carcinoma beyond Milan Criteria: a RCT. *J Hepatol* 2014; **61**: 82-88 [PMID: 24650695 DOI: 10.1016/j.jhep.2014.03.012]
  - 32 **Zhang ZM**, Guo JX, Zhang ZC, Jiang N, Zhang ZY, Pan LJ. Therapeutic options for intermediate-advanced hepatocellular carcinoma. *World J Gastroenterol* 2011; **17**: 1685-1689 [PMID: 21483627 DOI: 10.3748/wjg.v17.i13.1685]
  - 33 **Thiele M**, Glud LL, Fialla AD, Dahl EK, Krag A. Large variations in risk of hepatocellular carcinoma and mortality in treatment naïve hepatitis B patients: systematic review with meta-analyses. *PLoS One* 2014; **9**: e107177 [PMID: 25225801 DOI: 10.1371/journal.pone.0107177]
  - 34 **Kansagara D**, Papak J, Pasha AS, O'Neil M, Freeman M, Relevo R, Quiñones A, Motu'apuaka M, Jou JH. Screening for hepatocellular carcinoma in chronic liver disease: a systematic review. *Ann Intern Med* 2014; **161**: 261-269 [PMID: 24934699 DOI: 10.7326/m14-0558]
  - 35 **Cucchetti A**, Djulbegovic B, Tsalatsanis A, Vitale A, Hozo I, Piscaglia F, Cescon M, Ercolani G, Tuci F, Cillo U, Pinna AD. When to perform hepatic resection for intermediate-stage hepatocellular carcinoma. *Hepatology* 2015; **61**: 905-914 [PMID: 25048515 DOI: 10.1002/hep.27321]
  - 36 **Vitale A**, Morales RR, Zanús G, Farinati F, Burra P, Angeli P, Frigo AC, Del Poggio P, Rapaccini G, Di Nolfo MA, Benvegnù L, Zoli M, Borzio F, Giannini EG, Caturelli E, Chiaramonte M, Trevisani F, Cillo U. Barcelona Clinic Liver Cancer staging and transplant survival benefit for patients with hepatocellular carcinoma: a multicentre, cohort study. *Lancet Oncol* 2011; **12**: 654-662 [PMID: 21684210 DOI: 10.1016/s1470-2045(11)70144-9]
  - 37 **Gómez Rodríguez R**, Romero Gutiérrez M, González de Frutos C, de Artaza Varasa T, de la Cruz Perez G, Ciampi Dopazo JJ, Lanciego Pérez C, Gómez Moreno AZ. [Clinical characteristics, staging and treatment of patients with hepatocellular carcinoma in clinical practice. Prospective study of 136 patients]. *Gastroenterol Hepatol* 2011; **34**: 524-531 [PMID: 21940068 DOI: 10.1016/j.gastrohep.2011.06.009]
  - 38 **Ng KK**, Vauthey JN, Pawlik TM, Lauwers GY, Regimbeau JM, Belghiti J, Ikai I, Yamaoka Y, Curley SA, Nagorney DM, Ng IO, Fan ST, Poon RT. Is hepatic resection for large or multinodular hepatocellular carcinoma justified? Results from a multi-institutional database. *Ann Surg Oncol* 2005; **12**: 364-373 [PMID: 15915370 DOI: 10.1245/aso.2005.06.004]
  - 39 **Cho YB**, Lee KU, Lee HW, Cho EH, Yang SH, Cho JY, Yi NJ, Suh KS. Outcomes of hepatic resection for a single large hepatocellular carcinoma. *World J Surg* 2007; **31**: 795-801 [PMID: 17345125 DOI: 10.1007/s00268-006-0359-z]
  - 40 **Wei S**, Hao X, Zhan D, Xiong M, Li K, Chen X, Huang Z. Are surgical indications of Barcelona Clinic Liver Cancer staging classification justified? *J Huazhong Univ Sci Technolog Med Sci* 2011; **31**: 637-641 [PMID: 22038353 DOI: 10.1007/s11596-011-0574-1]
  - 41 **Torzilli G**, Belghiti J, Kokudo N, Takayama T, Capussotti L, Nuzzo G, Vauthey JN, Choti MA, De Santibanes E, Donadon M, Morenghi E, Makuuchi M. A snapshot of the effective indications and results of surgery for hepatocellular carcinoma in tertiary referral centers: is it adherent to the EASL/AASLD recommendations?: an observational study of the HCC East-West study group. *Ann Surg* 2013; **257**: 929-937 [PMID: 23426336 DOI: 10.1097/SLA.0b013e31828329b8]
  - 42 **Yamashita Y**, Taketomi A, Shirabe K, Aishima S, Tsujita E, Morita K, Kayashima H, Maehara Y. Outcomes of hepatic resection for huge hepatocellular carcinoma ( $\geq 10$  cm in diameter). *J Surg Oncol* 2011; **104**: 292-298 [PMID: 21465490 DOI: 10.1002/jso.21931]
  - 43 **Chang WT**, Kao WY, Chau GY, Su CW, Lei HJ, Wu JC, Hsia CY, Lui WY, King KL, Lee SD. Hepatic resection can provide long-term survival of patients with non-early-stage hepatocellular carcinoma: extending the indication for resection? *Surgery* 2012; **152**: 809-820 [PMID: 22766361 DOI: 10.1016/j.surg.2012.03.024]
  - 44 **Torzilli G**, Donadon M, Marconi M, Palmisano A, Del Fabbro D, Spinelli A, Botea F, Montorsi M. Hepatectomy for stage B and stage C hepatocellular carcinoma in the Barcelona Clinic Liver Cancer classification: results of a prospective analysis. *Arch Surg* 2008; **143**: 1082-1090 [PMID: 19015467 DOI: 10.1001/archsurg.143.11.1082]
  - 45 **Lee JH**, Kim HY, Kim YJ, Yoon JH, Chung JW, Lee HS. Barcelona Clinic Liver Cancer staging system and survival of untreated hepatocellular carcinoma in a hepatitis B virus endemic area. *J Gastroenterol Hepatol* 2015; **30**: 696-705 [PMID: 25250761 DOI: 10.1111/jgh.12788]
  - 46 **Liccioni A**, Reig M, Bruix J. Treatment of hepatocellular carcinoma. *Dig Dis* 2014; **32**: 554-563 [PMID: 25034288 DOI: 10.1159/000360501]
  - 47 **You MW**, Kim SY, Kim KW, Lee SJ, Shin YM, Kim JH, Lee MG. Recent advances in the imaging of hepatocellular carcinoma. *Clin Mol Hepatol* 2015; **21**: 95-103 [PMID: 25834808 DOI: 10.3350/cmh.2015.21.1.95]
  - 48 **Kim MN**, Han KH, Ahn SH. Prevention of hepatocellular carcinoma: beyond hepatitis B vaccination. *Semin Oncol* 2015; **42**: 316-328 [PMID: 25843736 DOI: 10.1053/j.seminoncol.2014.12.018]
  - 49 **Ma C**, Chi M, Su H, Cheng X, Chen L, Kan Y, Wei W, Huang X, Li Y, Li L, Lin K, Huang Y, Wu Y, Huang X, Huang A, Liu J. Evaluation of the clinical features of HCC following hepatectomy for different stages of HCC. *Hepatogastroenterology* 2012; **59**: 2104-2111 [PMID: 23435129 DOI: 10.5754/hge12109]

- 50 **Cai ZQ**, Si SB, Chen C, Zhao Y, Ma YY, Wang L, Geng ZM. Analysis of prognostic factors for survival after hepatectomy for hepatocellular carcinoma based on a bayesian network. *PLoS One* 2015; **10**: e0120805 [PMID: 25826337 DOI: 10.1371/journal.pone.0120805]
- 51 **Hsu CY**, Liu PH, Lee YH, Hsia CY, Huang YH, Lin HC, Chiou YY, Lee FY, Huo TI. Using serum  $\alpha$ -fetoprotein for prognostic prediction in patients with hepatocellular carcinoma: what is the most optimal cutoff? *PLoS One* 2015; **10**: e0118825 [PMID: 25738614 DOI: 10.1371/journal.pone.0118825]
- 52 **Okuyama H**, Ikeda M, Kuwahara A, Takahashi H, Ohno I, Shimizu S, Mitsunaga S, Senda S, Okusaka T. Prognostic factors in patients with hepatocellular carcinoma refractory or intolerant to sorafenib. *Oncology* 2015; **88**: 241-246 [PMID: 25503567 DOI: 10.1159/000369351]
- 53 **Zhao YJ**, Ju Q, Li GC. Tumor markers for hepatocellular carcinoma. *Mol Clin Oncol* 2013; **1**: 593-598 [PMID: 24649215 DOI: 10.3892/mco.2013.119]
- 54 **Jia X**, Liu J, Gao Y, Huang Y, Du Z. Diagnosis accuracy of serum glypican-3 in patients with hepatocellular carcinoma: a systematic review with meta-analysis. *Arch Med Res* 2014; **45**: 580-588 [PMID: 25446613 DOI: 10.1016/j.arcmed.2014.11.002]
- 55 **Rich N**, Singal AG. Hepatocellular carcinoma tumour markers: current role and expectations. *Best Pract Res Clin Gastroenterol* 2014; **28**: 843-853 [PMID: 25260312 DOI: 10.1016/j.bpg.2014.07.018]
- 56 **Andreou A**, Gül S, Pascher A, Schöning W, Al-Abadi H, Bahra M, Klein F, Denecke T, Strücker B, Puhl G, Pratschke J, Seehofer D. Patient and tumour biology predict survival beyond the Milan criteria in liver transplantation for hepatocellular carcinoma. *HPB (Oxford)* 2015; **17**: 168-175 [PMID: 25263399 DOI: 10.1111/hpb.12345]
- 57 **Shirabe K**, Yoshiya S, Yoshizumi T, Uchiyama H, Soejima Y, Kawanaka H, Ikegami T, Yamashita Y, Ikeda T, Maehara Y. [Liver transplantation in the patients with hepatocellular carcinoma beyond Milan criteria -with special reference to extended criteria]. *Nihon Shokakibyo Gakkai Zasshi* 2014; **111**: 885-891 [PMID: 24806231]
- 58 **Tuci F**, Vitale A, D'Amico F, Gringeri E, Neri D, Zanusi G, Bassi D, Polacco M, Boetto R, Lodo E, Germani G, Burra P, Angeli P, Cillo U. Survival benefit of transplantation for recurrence of hepatocellular carcinoma after liver resection. *Transplant Proc* 2014; **46**: 2287-2289 [PMID: 25242770 DOI: 10.1016/j.transproceed.2014.07.031]

**P- Reviewer:** He JY, Qin JM, Romero MR, Wong GLH  
**S- Editor:** Qiu S **L- Editor:** Wang TQ **E- Editor:** Liu SQ



Observational Study

# Combined acoustic radiation force impulse, aminotransferase to platelet ratio index and Forns index assessment for hepatic fibrosis grading in hepatitis B

Chang-Feng Dong, Jia Xiao, Ling-Bo Shan, Han-Ying Li, Yong-Jia Xiong, Gui-Lin Yang, Jing Liu, Si-Min Yao, Sha-Xi Li, Xiao-Hua Le, Jing Yuan, Bo-Ping Zhou, George L Tipoe, Ying-Xia Liu

Chang-Feng Dong, Han-Ying Li, Division of Ultrasound, Shenzhen Third People's Hospital, Shenzhen 518112, China

Jia Xiao, Ling-Bo Shan, Gui-Lin Yang, Jing Liu, Si-Min Yao, Sha-Xi Li, Jing Yuan, Bo-Ping Zhou, Ying-Xia Liu, National Key Disciplines for Infectious Diseases, Shenzhen Third People's Hospital, Shenzhen 518112, China

Jia Xiao, Yong-Jia Xiong, Department of Immunobiology, Institute of Tissue Transplantation and Immunology, Jinan University, Guangzhou 510632, Guangdong Province, China

Xiao-Hua Le, Department of Pathology, Shenzhen Third People's Hospital, Shenzhen 518112, China

George L Tipoe, School of Biomedical Sciences, LKS Faculty of Medicine, the University of Hong Kong, Hong Kong, China

**Author contributions:** Xiao J, Li HY and Liu YX are the guarantors of integrity of entire study; all authors contributed to study concepts/study design or data acquisition or data analysis/interpretation; all authors contributed to manuscript drafting or manuscript revision for important intellectual content; all authors gave approval of final version of submitted manuscript; Dong CF, Shan LB, Yang GL, Liu J, Yao SM, Le XH, Yuan J and Liu YX contributed to clinical studies; Dong CF, Li HY, Xiong YJ, Li SX and Liu YX contributed to statistical analysis; Dong CF, Xiao J, Xiong YJ, Zhou BP, Tipoe GL and Liu YX contributed to manuscript editing; Dong CF, Xiao J and Shan LB contributed equally to this work.

**Supported by** Shenzhen Municipal Science and Technology Innovation Fund, Nos. CXZZ20130322170220544 and JCYJ20140411112047885.

**Institutional review board statement:** The study was reviewed and approved by the Ethics Committee Office of Shenzhen Third People's Hospital.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** There are no conflicts of interest to report.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** George L Tipoe, MD, PhD, School of Biomedical Sciences, LKS Faculty of Medicine, the University of Hong Kong, Pokfulam, Hong Kong, China. [tgeorge@hku.hk](mailto:tgeorge@hku.hk)  
Telephone: +852-39179185

**Received:** January 8, 2016

**Peer-review started:** January 8, 2016

**First decision:** February 26, 2016

**Revised:** March 8, 2016

**Accepted:** April 20, 2016

**Article in press:** April 22, 2016

**Published online:** May 18, 2016

## Abstract

**AIM:** To investigate the combined diagnostic accuracy of acoustic radiation force impulse (ARFI), aspartate aminotransferase to platelet ratio index (APRI) and Forns index for a non-invasive assessment of liver fibrosis in patients with chronic hepatitis B (CHB).

**METHODS:** In this prospective study, 206 patients had CHB with liver fibrosis stages F0-F4 classified by METAVIR and 40 were healthy volunteers were



measured by ARFI, APRI and Forns index separately or combined as indicated.

**RESULTS:** ARFI, APRI or Forns index demonstrated a significant correlation with the histological stage (all  $P < 0.001$ ). According to the AUROC of ARFI and APRI for evaluating fibrotic stages more than F2, ARFI showed an enhanced diagnostic accuracy than APRI ( $P < 0.05$ ). The combined measurement of ARFI and APRI exhibited better accuracy than ARFI alone when evaluating  $\geq$  F2 fibrotic stage ( $Z = 2.77$ ,  $P = 0.006$ ). Combination of ARFI, APRI and Forns index did not obviously improve the diagnostic accuracy compared to the combination of ARFI and APRI ( $Z = 0.958$ ,  $P = 0.338$ ).

**CONCLUSION:** ARFI + APRI showed enhanced diagnostic accuracy than ARFI or APRI alone for significant liver fibrosis and ARFI + APRI + Forns index shows the same effect with ARFI + APRI.

**Key words:** Acoustic radiation force impulse; Aspartate aminotransferase to platelet ratio index; Forns index; Hepatitis B virus; Non-invasive diagnosis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Chronic hepatitis B (CHB) is a major health problem in a lot of countries all over the world, particularly in China. An accurate staging of liver fibrosis is critical for prognosticating this disease. However, although it is still the golden standard, liver biopsy is hindered by its inherent drawbacks in clinical applications. In this study, we demonstrated that non-invasive methods, including acoustic radiation force impulse (ARFI), aspartate aminotransferase to platelet ratio index (APRI) and Forns index showed significant correlations with the histological staging results from liver biopsy. The combined measurement of ARFI and APRI had the best diagnostic accuracy, which provided an ideal and convenient non-invasive diagnostic method for the detection of hepatic fibrosis of CHB patients in clinical practice.

Dong CF, Xiao J, Shan LB, Li HY, Xiong YJ, Yang GL, Liu J, Yao SM, Li SX, Le XH, Yuan J, Zhou BP, Tipoe GL, Liu YX. Combined acoustic radiation force impulse, aminotransferase to platelet ratio index and Forns index assessment for hepatic fibrosis grading in hepatitis B. *World J Hepatol* 2016; 8(14): 616-624 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i14/616.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i14.616>

of progressing to liver cirrhosis<sup>[1]</sup>. Unlike cirrhosis, hepatic fibrosis is reversible at its early stage when proper clinical therapeutic interventions are applied<sup>[2]</sup>. Therefore, an accurate staging of liver fibrosis is critical for prognosticating this disease. To date, the gold standard for staging hepatic fibrosis is still the liver biopsy, which cannot be routinely performed because of its inherent limitations, such as pain, bleeding, inaccurate staging from sampling error, and variability of biopsy interpretation<sup>[3]</sup>. During the past decades, considerable efforts have been invested in developing non-invasive methods of assessments, which may provide accurate evaluation of liver fibrosis comparable to liver biopsy. Indeed, these non-invasive methods have several advantages such as high safety margin, simple, convenient, reproducible, and inexpensive.

Acoustic radiation force impulse (ARFI) is a new quantitative assessment method of estimating tissue stiffness through measurement of shear wave velocity (SWV, measured in m/s). Its quantitative representation is named as virtual touch tissue quantification, which gives an objective numerical evaluation of the tissue stiffness<sup>[4-6]</sup>. ARFI imaging offers a quantitative assessment of the hepatic parenchyma elasticity to non-invasively grade and stage hepatic fibrosis. It has been used to diagnose hepatic fibrosis of patients with CHB<sup>[7]</sup>, hepatitis C<sup>[8]</sup>, cirrhosis<sup>[9]</sup>, and non-alcoholic fatty liver disease (NAFLD)<sup>[10]</sup>. In addition, ARFI is often performed with serum liver functions tests [e.g., alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins, and albumin] to generate better prediction and evaluation of liver fibrosis<sup>[11]</sup>. Among these, AST platelet ratio (APRI) is a serum hepatic function test which has been proposed as a non-invasive tool for the assessment of liver fibrosis in CHB<sup>[12]</sup> or chronic hepatitis C<sup>[13]</sup>. Another important serum test is Forns index method, which uses simple obtained parameters including age, gamma-glutamyltransferase (GGT), cholesterol, and platelet count (PLT), but it requires a relatively complicated calculation<sup>[14]</sup>. One of the advantages of APRI and Forns index over the other non-invasive tests is that they are based on readily available blood tests and simple to use. Although these strategies have been widely applied in the past decade for hepatitis C evaluation<sup>[15,16]</sup>, their accuracy for CHB grading are still not comparable with liver biopsy. Therefore, a combined use of these non-invasive methods may be another promising and practical diagnostic application in CHB patients. In the current study, we aimed to compare the accuracy among ARFI, APRI, Forns index and their combinations for non-invasive diagnosis grading and prognosis of human CHB-induced hepatic fibrosis.

## INTRODUCTION

Chronic liver injury, such as chronic hepatitis B (CHB), may cause inflammation and necrosis of hepatocytes, leading to hepatic fibrosis. It is a long-term pathological change with certain possibility (about 20%)

## MATERIALS AND METHODS

### Subjects of study

This prospective study was approved by the ethical committee of Shenzhen Third People's Hospital. All study procedures and methods were in accordance with

the approved guidelines. All patients in this study were fully informed about the research protocol including the data handling and the privacy of personal data. After this procedure, patients signed the written consent. A total of 246 subjects were consecutively enrolled in this study, including 206 CHB subjects and 40 healthy subjects. These 206 CHB cases were selected from 245 CHB patients diagnosed by liver biopsy in Shenzhen Third People's Hospital, from May 2011 to December 2014. Of the 206 CHB patients, there were 39 female cases (18.9%) and 167 male cases (81.1%). Inclusion criteria are: (1) patients must be 18-65 years old; (2) with hepatitis B surface antigen positive for more than 6 mo; (3) without receiving antiviral treatment before this study; (4) ALT and AST were  $< 2 \times$  upper limit of normal (ULN) in the past 6 mo; (5)  $18.5 <$  body mass index (BMI)  $< 31.0$ ; (6) length of liver biopsy tissue  $\geq 15$  mm and contains at least 10 periportal areas; (7) hemoglobin  $> 90$  g/L, prothrombin time 11-15.1 s; (8) activated partial thromboplastin time and thrombin time were at a normal range; and (9) cardiac and renal functions were normal. Negative for the following: Human immunodeficiency virus, hepatitis A virus, hepatitis C virus (HCV), hepatitis D virus, hepatitis E virus super-infection or co-infection, auto-immune liver diseases, alcoholic steatosis, NAFLD, hepatocellular carcinoma (HCC), pregnancy, ascites, as well as jaundice. Of the 245 eligible CHB patients, 39 were excluded because of the following: NAFLD ( $n = 10$ ), received antiviral treatment before this study ( $n = 8$ ), jaundice ( $n = 5$ ), alcoholic steatosis ( $n = 6$ ), HCV infection ( $n = 2$ ), auto-immune liver disease ( $n = 1$ ), with age  $< 18$  ( $n = 4$ ), with age  $> 65$  ( $n = 1$ ), and declined to participate ( $n = 2$ ). Healthy group consisted of 40 volunteers, with 30 males and 10 females, aged range from 20-53 years old, with mean age of  $39.8 \pm 11.45$  years and no hepatitis B virus (HBV) or HCV infection, no hypertension, diabetes, fatty liver and other apparent diseases. The BMI of healthy subjects were between 18.5 and 31.0. Other parameters were similar to the CHB patients. All CHB patients were examined by ARFI one day before or on the day of liver biopsy. All the subjects had blood or sera drawn for the detection of platelet and fibrotic serological markers.

#### Liver biopsy and pathological staging

Liver biopsy tissue specimens were collected by needle puncture (MN1613, Bard Biopsy Systems, Tempe, AZ) under the Color Doppler Ultrasound guidance in a separate clinic setting for diagnostic purposes. The liver specimen was 15-20 mm in length, including at least 10 portal vein areas. Then it was embedded by paraffin and stained by Sirius Red (Sigma-Aldrich, St. Louis, MO). Liver fibrosis stage was assessed by the METAVIR scoring system (F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis and a few septa; F3 = numerous fibrosis without cirrhosis; and F4 = cirrhosis)<sup>[17]</sup>. The METAVIR scoring system was previously used in other reports on CHB<sup>[18,19]</sup>. Two independent pathologists were responsible for the

staging of all samples without additional information about the specimens they checked.

#### ARFI

The detection of ARFI in the liver was performed under fasting conditions using Siemens Acuson S2000 with probe detector 4C1, frequency 2.0-4.0 MHz (Siemens Healthcare, Erlangen, Germany) according to routine instructions. ARFI was mainly conducted by a radiologist (Dong CF) with assistant from another physician and a nurse. Dong CF has 11-year experience in clinical radiology and 4-year experience in ARFI diagnosis. Form of the liver capsule and the echogenicity of hepatic parenchyma were recorded. Detection of SWV (m/s) of hepatic segments s5, s6, s7 and s8 was repeated for 3 times and the mean values were calculated. Thus, 12 measurements of hepatic segments s5, s6, s7, s8 were recorded. Our pilot study in healthy volunteers showed that when compared with conventional ARFI protocol (mean value from 10 measurements), the current protocol exhibited similar results with smaller standard deviation ( $1.08 \pm 0.21$  m/s vs  $1.11 \pm 0.12$  m/s;  $t = 0.6794$ ,  $P > 0.05$ ). This is consistent with a report that showed the reproducibility of measurements in the right lobe was higher<sup>[20]</sup>. Images and data of ARFI were saved for analysis.

#### Blood markers for APRI and Forns index evaluations

AST was determined in the same laboratory prior to the liver biopsy using Siemens ADVIA 2400 Chemistry system (Siemens Healthcare). Enzymatic activity was measured at 37 °C, according to International Federation of Clinical Chemistry standards. Platelet count was assessed by an automatic blood cell analyzer (XE-5000 Automated Hematology System, Sysmex, Lincolnshire, IL). The ULN range of AST was considered as 40 U/L.

$APRI = AST(ULN)/PLT(10^9/L) \times 100$ .

$Forns\ index = 7.811 - 3.131 \times \ln(PLT) + 0.781 \times \ln(GGT) + 3.467 \times \ln(age) - 0.014 \times (cholesterol)$

#### Combined assessments of ARFI + APRI/ARFI + Forns index

A logistic regression analysis model for hepatic fibrosis  $\geq F2$  has been established by using the ENTER method.

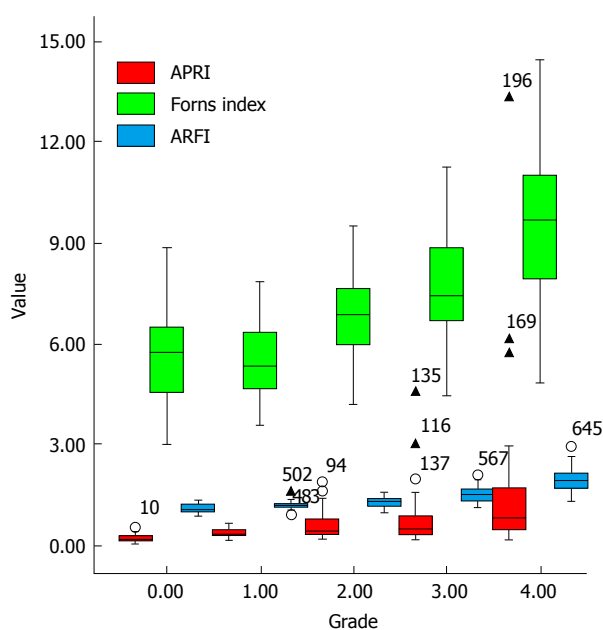
#### Statistical analysis

Continuous normal distribution data were represented with means  $\pm$  SD. Categorical normal distribution data were represented with median  $\pm$  quartile ( $M \pm Q$ ). Kruskal-Wallis test was used to analyze the differences among these different groups. When there was a statistical significance ( $P < 0.05$ ), a post-hoc Bonferroni test was applied to analyze data between two groups.  $P < 0.05$  was considered to be statistically significant using a SPSS 13.0, IBM, Armonk, NY. The box plot was used to record the mean and degree of variation. MedCalc software (Ostend, Belgium) was used to draw receiver operating characteristic curve (ROC) and calculate cut-off value, sensitivity, specificity, positive predictive

**Table 1** Results of basic information and acoustic radiation force impulse/aspartate aminotransferase to platelet ratio index/Forns index of all examinees

Group	Age (yr)	Gender (male/female)	BMI	ARFI	APRI	Forns index
F0 ( <i>n</i> = 40)	39.8 ± 11.45	30/10	22.91 ± 2.31	1.09 (1.01, 1.21)	0.19 (0.14, 0.28)	5.58 ± 1.33
F1 ( <i>n</i> = 41)	33.07 ± 7.97 <sup>1</sup>	33/8	22.37 ± 2.24	1.19 (1.15, 1.28) <sup>1</sup>	0.34 (0.28, 0.44) <sup>1</sup>	5.60 ± 1.19
F2 ( <i>n</i> = 52)	38.27 ± 7.66 <sup>2</sup>	43/9	22.26 ± 2.41	1.31 (1.21, 1.43) <sup>1,2</sup>	0.42 (0.32, 0.64) <sup>1</sup>	6.73 ± 1.09 <sup>1,2</sup>
F3 ( <i>n</i> = 59)	39.83 ± 8.73 <sup>2</sup>	47/12	22.44 ± 2.57	1.52 (1.35, 1.64) <sup>1,2,3</sup>	0.45 (0.32, 0.86) <sup>1,2</sup>	7.58 ± 1.55 <sup>1,2,3</sup>
F4 ( <i>n</i> = 54)	43.85 ± 10.81 <sup>1,2,3,4</sup>	44/10	22.35 ± 2.47	1.92 (1.74, 2.14) <sup>1,2,3,4</sup>	0.80 (0.51, 1.68) <sup>1,2,3,4</sup>	9.43 ± 2.30 <sup>1,2,3,4</sup>
$\chi^2/F$	7.907	0.947	0.477	176.043	107.992	49.501
<i>P</i> value	< 0.001	0.918	0.753	< 0.001	< 0.001	< 0.001

For age and Forns index, data were represented in mean ± SD. For ARFI and APRI data, results were exhibited in median ± quartile. <sup>1</sup>Means significant change against the F0 group; <sup>2</sup>Means significant change against the F1 group; <sup>3</sup>Means significant change against the F2 group; <sup>4</sup>Means significant change against the F3 group. For gender, ARFI and APRI comparisons, size of test  $\alpha' = \alpha/n = 0.005$ ; for age and Forns index comparison, size of test  $\alpha = 0.05$ . ARFI: Acoustic radiation force impulse; APRI: Aspartate aminotransferase to platelet ratio index; BMI: Body mass index.



**Figure 1** Box plots show correlation between noninvasive tests and histological stages from liver biopsy. Top and bottom of boxes represent first and third quartiles, respectively. Length of box represents interquartile range within which 50% of values are located. Line through each box represents median. Error bars mark the minimum and maximum values (range). Small circles represent the outliers. Triangles represent the extreme value, which is  $> 3 \times$  interquartile range. ARFI: Acoustic radiation force impulse; APRI: Aspartate transaminase to platelet ratio index.

values, negative predictive values, AUROC of ARFI and APRI for every liver fibrotic stage. The ROC curve of two variables combination (ARFI + APRI and ARFI + Forns index) and three variables combination (ARFI + APRI + Forns index) for significant hepatic fibrosis ( $\geq F2$ ) was also analyzed. When AUROC  $> 0.5$ , the closer of AUROC to 1, the better diagnostic outcome it provided. Comparison of AUROC among these parameters and their combination was analyzed by the Delong test<sup>[21]</sup>.

## RESULTS

### Results of basic information, ARFI, APRI, and Forns index

Basic information (e.g., age and gender) and assess-

ment results of ARFI, APRI, and Forns index of all subjects were shown in Table 1. The average ages of subjects with significant or serious fibrosis (F2, F3 and F4) were significantly higher than subjects with mild fibrosis (F1) ( $P = 0.009$  for F2 vs F3,  $P < 0.001$  for F2 vs F4, and  $P < 0.001$  for F3 vs F4). Also, male patients showed higher incidence of hepatic fibrosis (from F1 to F4) than female patients. The differences of ARFI results among F0, F1, F2, F3 and F4 groups were significant ( $P < 0.05$ ). For Forns index, except for F0 and F1 group, the differences among other groups were significant ( $P < 0.05$ ). Results of APRI indicated that only F4 showed significant change from other groups (F0, F1, F2 and F3) (all  $P < 0.001$ ), while the F1, F2, and F3 groups showed significantly higher values than the F0 group (all  $P < 0.001$ ) (Table 1).

### Correlations between ARFI, APRI, Forns index and hepatic pathology

The median, quartile, minimum value, maximum value and outlier image (Figure 1). There was a high correlation between the staging of ARFI/APRI/Forns index and the hepatic histology, with correlation coefficient 0.845 ( $P < 0.001$ ), 0.641 ( $P < 0.001$ ) and 0.644 ( $P < 0.001$ ), respectively (Table 2). In ENTER model, Y axis was the result from liver biopsy and the X axis was the results from ARFI + APRI or ARFI + Forns Index combined assessments. The equation for ARFI + APRI was  $y = -13.27 + 9.11 \text{ ARFI} + 5.03 \text{ APRI}$ , while the equation for ARFI + Forns index was  $y = -15.08 + 8.67 \text{ ARFI} + 0.70 \text{ Forns index}$ .

### Determination of the cut-off values of hepatic fibrosis staging

There were significantly different interval ranges between different liver fibrotic stages and the corresponding ARFI and APRI results. In order to determine the cut-off value of each fibrotic stage, we applied ROC to analyze the data from both ARFI and APRI (Figure 2). From the result, it showed that the diagnostic performance of ARFI for predicting stages more than F2, F3 and F4 was 91% (95%CI: AUROC = 0.87-0.95,  $P < 0.05$ ), 94% (95%CI:

**Table 2** Correlations of non-invasive tests with histological fibrosis stage by rank correlation analysis

Histological staging	Noninvasive test	Correlation (Spearman coefficient)	95%CI	P value
METAVIR classification	ARFI	0.845	0.805-0.877	< 0.001
	APRI	0.641	0.561-0.709	< 0.001
	Forns index	0.644	0.564-0.711	< 0.001

ARFI: Acoustic radiation force impulse; APRI: Aspartate aminotransferase to platelet ratio index.

**Table 3** Cut-off values of acoustic radiation force impulse and aspartate aminotransferase to platelet ratio index for the diagnosis of liver fibrosis (95%CI)

	≥ F1	≥ F2	≥ F3	F4
ARFI				
Cut-off (m/s)	1.26	1.29	1.43	1.62
Sensitivity	76.2% (69.80-81.90)	83.6% (77.10-88.90)	82.3% (74.00-88.80)	90.7% (79.70-96.90)
Specificity	95.0% (83.10-99.40)	90.1% (89.50-97.60)	89.5% (83.00-94.10)	92.2% (87.40-95.60)
PPV	99.1% (96.20-99.90)	94.5% (91.90-99.10)	86.9% (79.10-92.70)	76.0% (64.40-86.30)
NPV	35.9% (22.50-47.40)	73.0% (63.10-81.40)	85.6% (78.60-91.00)	97.2% (93.70-99.10)
AUROC	0.90 (0.86-0.94) <sup>a</sup>	0.91 (0.87-0.95) <sup>a</sup>	0.94 (0.90-0.96) <sup>a</sup>	0.96 (0.93-0.98) <sup>a</sup>
APRI				
Cut-off (m/s)	0.30	0.41	0.49	0.44
Sensitivity	84.0% (78.20-88.70)	68.5% (60.80-75.50)	63.7% (54.10-72.60)	83.3% (70.70-92.10)
Specificity	85.0% (70.20-94.30)	82.7% (72.70-90.20)	79.7% (71.90-86.20)	67.2% (70.10-73.80)
PPV	97.6% (94.20-99.30)	89.0% (82.20-93.80)	72.8% (62.90-81.20)	41.7% (32.30-51.60)
NPV	42.7% (30.00-56.10)	56.3% (46.80-65.40)	72.1% (64.00-79.20)	93.5% (87.90-97.00)
AUROC	0.92 (0.88-0.95) <sup>a</sup>	0.84 (0.79-0.89) <sup>a</sup>	0.79 (0.73-0.84) <sup>a</sup>	0.82 (0.76-0.86) <sup>a</sup>

<sup>a</sup>P < 0.05 for all values. ARFI: Acoustic radiation force impulse; APRI: Aspartate aminotransferase to platelet ratio index; AUROC: Area under the receiver operating characteristic curve; NPV: Negative predictive value; PPV: Positive predictive value.

**Table 4** Binary logistic regression of two variables in hepatic fibrosis ≥ F2

Combination	Variable	RC	SD of RC	Wald	P value	OR	95%CI of OR
ARFI + APRI	ARFI	9.11	1.48	37.68	< 0.001	9085.54	494.92-166789.07
	APRI	5.03	1.30	15.07	< 0.001	153.01	12.07-1939.04
	Constant	-13.27	1.95	46.09	< 0.001	-	-
ARFI + Forns index	ARFI	8.67	1.44	36.16	< 0.001	5824.00	345.12-98280.97
	Forns index	0.70	0.17	16.27	< 0.001	2.01	1.43-2.82
	Constant	-15.08	2.08	52.68	< 0.001	-	-

ARFI: Acoustic radiation force impulse; APRI: Aspartate aminotransferase to platelet ratio index; OR: Odds ratio; RC: Regression coefficient.

AUROC = 0.90-0.96,  $P < 0.05$ ), 96% (95%CI: AUROC = 0.93-0.98,  $P < 0.05$ ), and the best cut-off value of F2, F3 and F4 was 1.29, 1.43 and 1.62 m/s. However, APRI measurement showed decreased accuracy of diagnosing significant fibrosis when compared with ARFI (Table 3).

#### Combined assessment of ARFI + APRI/ARFI + Forns index/ARFI + APRI + Forns index for hepatic fibrosis ≥ F2

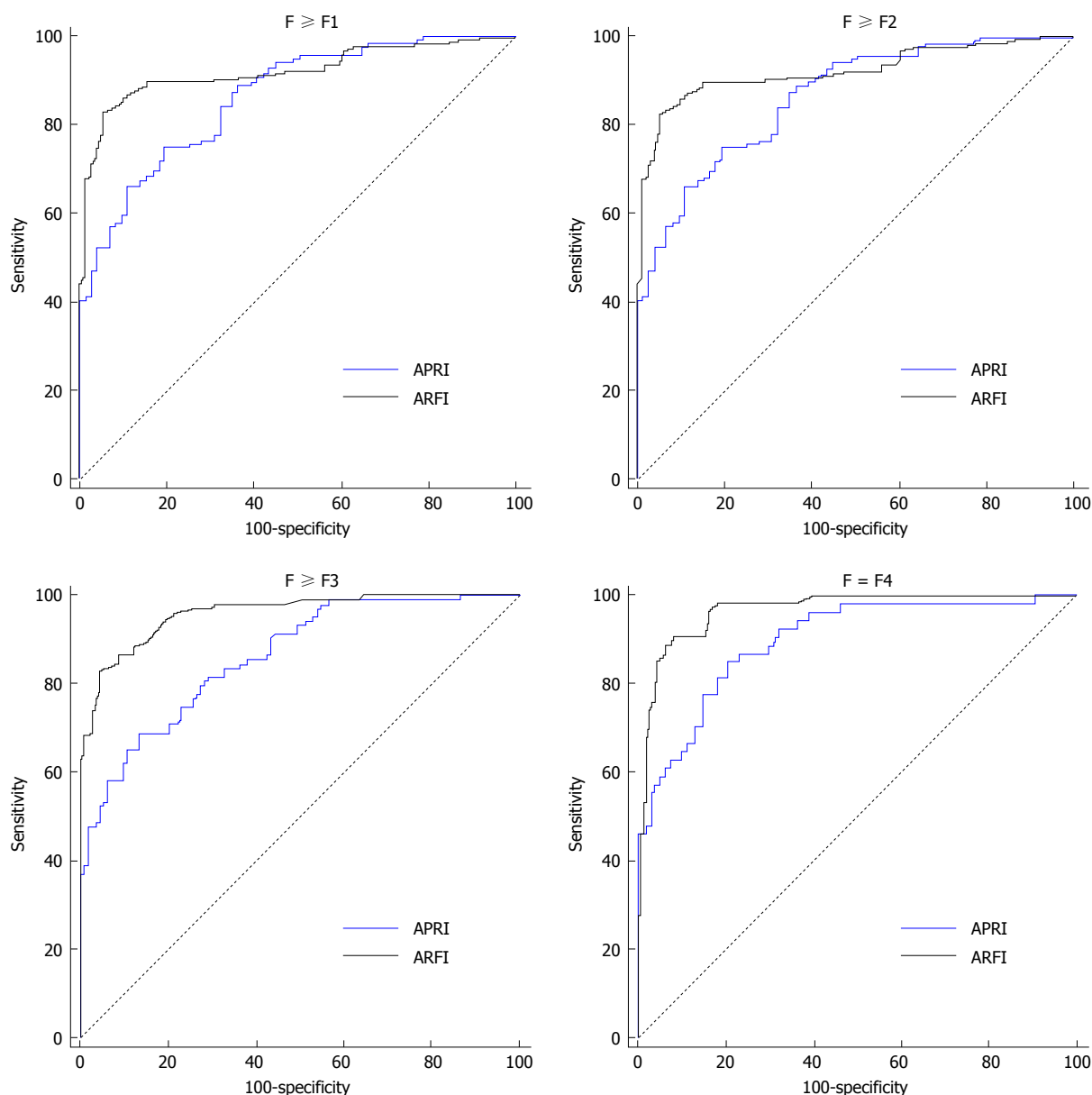
Firstly we established a logistic regression analysis model for hepatic fibrosis ≥ F2 in which the Y axis was the result from liver biopsy and the X axis was the results from combined ARFI + APRI/ARFI + Forns index assessment (Table 4). From the AUROC results of Table 5, when evaluating patients with hepatic fibrosis ≥ F2, there was a significant change between the AUROCs of ARFI + APRI and ARFI alone (0.940 and 0.913, respectively;  $Z = 2.77$ ,  $P = 0.006$ ), also

between ARFI + Forns index and ARFI alone (0.933 and 0.913, respectively;  $Z = 2.091$ ,  $P = 0.037$ ), ARFI + APRI + Forns index and ARFI alone (0.944 and 0.913, respectively;  $Z = 2.893$ ,  $P = 0.004$ ), indicating an enhanced screening ability of the combined assessment than ARFI alone. However, the change between ARFI + APRI and ARFI + APRI + Forns index was not significant (0.940 and 0.944, respectively;  $Z = 0.958$ ,  $P = 0.338$ ), suggesting that Forns index cannot further improve the diagnostic accuracy for staging hepatic fibrosis ≥ F2 when using a combined method of ARFI + APRI (Figure 3).

## DISCUSSION

To date, the gold standard for the diagnosis of liver fibrosis remains to be liver biopsy. In most circumstances, patients find it difficult to accept liver biopsy due





**Figure 2** Receiver operating characteristic curves for acoustic radiation force impulse and aspartate transaminase to platelet ratio index for diagnosis of hepatic fibrosis (F1-F4). ARFI: Acoustic radiation force impulse; APRI: Aspartate transaminase to platelet ratio index.

to its complications. From 2009, with the introduction of ARFI, the clinical research on non-invasive assessment of fibrosis rapidly progressed. As an advanced imaging technology, ARFI is capable of providing biomechanical information on the tissue stiffness and elasticity using conventional ultrasound scanning of anatomical location and structure<sup>[22,23]</sup>. However, its utility, particularly in combination with other non-invasive methods in hepatitis B, has not been adequately evaluated.

In the current study, CHB patients with different stages of liver fibrosis were diagnosed by ARFI, APRI, Forns index and their combined assessments. Our results demonstrated that the mean SWV value from ARFI was highly correlated with the staging of liver fibrosis classified by liver biopsy (METAVIR classification). This result indicated that biomechanical properties (e.g., hepatic elasticity and stiffness) had progressed

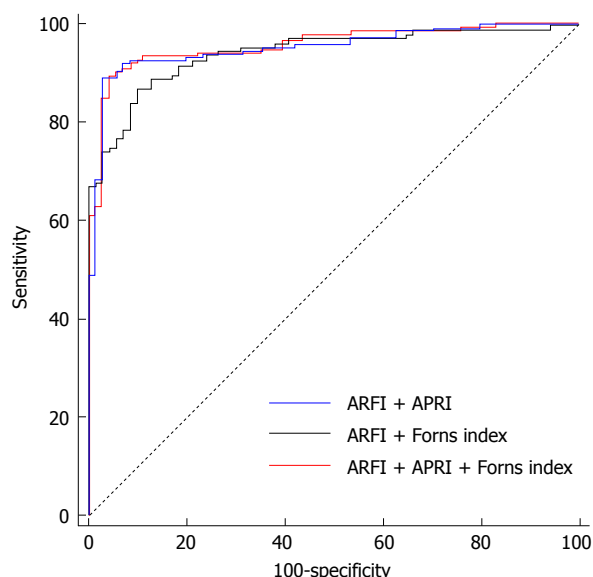
from liver fibrosis to cirrhosis during the development of CHB, which was consistent with the pathological progression of hepatocyte degeneration, necrosis, inflammation reaction, hepatocyte regeneration, formation of connective tissue fiber intervals, and liver lobule structural failure during the course of liver fibrosis of HBV infection<sup>[24]</sup>.

With the progression of liver fibrosis from F2 to F4, the effectiveness of ARFI on the diagnosis of liver fibrosis also increased. That is, when the value of SWV was lower than 1.29 m/s (clinically F0 and F1 patients), hepatic fibrosis could be unlikely significant. SWV higher than 1.43 m/s could be likely considered as an indication for serious liver fibrosis (F3, sensitivity 82.3% and specificity 89.5%), and SWV > 1.62 m/s could be diagnosed as early cirrhosis (F4, sensitivity 90.7% and specificity 92.2%). In addition, when they were used

**Table 5** Comparing area under the receiver operating characteristic curve of acoustic radiation force impulse/acoustic radiation force impulse + aspartate aminotransferase to platelet ratio index/acoustic radiation force impulse + Forns index/acoustic radiation force impulse + aspartate aminotransferase to platelet ratio index + Forns index in patients with fibrosis stage  $\geq$  F2

Comparison	AUROC	Difference	95%CI		Z	P value
			Lower limit	Upper limit		
ARFI	0.913	0.027	0.008	0.046	2.770	0.006
ARFI + APRI	0.940					
ARFI	0.913	0.020	0.001	0.040	2.091	0.037
ARFI + Forns index	0.933					
ARFI	0.913	0.031	0.010	0.053	2.893	0.004
ARFI + APRI + Forns index	0.944					
ARFI + APRI	0.940	0.007	-0.011	0.025	0.728	0.466
ARFI + Forns index	0.933					
ARFI + APRI	0.940	0.005	-0.005	0.014	0.958	0.338
ARFI + APRI + Forns index	0.944					
ARFI + Forns index	0.933	0.011	-0.001	0.023	1.789	0.074
ARFI + APRI + Forns index	0.944					

AUROC: Area under the receiver operating characteristic curve; ARFI: Acoustic radiation force impulse; APRI: Aspartate aminotransferase to platelet ratio index.



**Figure 3** Receiver operating characteristic curves of acoustic radiation force impulse + aspartate transaminase to platelet ratio index/acoustic radiation force impulse + Forns index/ acoustic radiation force impulse + aspartate transaminase to platelet ratio index + Forns index assessment for the diagnosis of liver fibrosis  $\geq$  F2 in patient with chronic hepatitis B. ARFI: Acoustic radiation force impulse; APRI: Aspartate transaminase to platelet ratio index.

independently, ARFI was the best way for the diagnosis of fibrosis  $\geq$  F2; ARFI provides a dynamic technical support for non-invasive diagnosis of liver fibrosis. This result is in line with a report found that ARFI correlated well with liver biopsy and thus was a reliable ultrasound-based method for the assessment of advanced fibrosis induced by CHB<sup>[25]</sup>.

Currently it is difficult for non-invasive diagnostic methods to differentiate F0 and F1 fibrotic stages. However, in this study, we found that there was a significant change of ARFI readings between the F0 and F1 groups (Table 1). It is known that stage F2 posse-

sses significant diagnostic value in determining the progression of liver disease and anti-viral therapy choice. At this stage, patients have more risk in developing complications such as portal hypertension, cirrhosis, and HCC than patients without significant liver fibrosis<sup>[26]</sup>. If patients receive anti-viral therapy promptly during this period, it is possible to retard or even reverse the pathological progression of fibrosis<sup>[27]</sup>. Thus, early accurate diagnosis and appropriate therapy to patients at F2 fibrosis evidently decreases the morbidity and mortality of patients with CHB<sup>[28,29]</sup>.

Similar to the FibroScan method which is partially affected by obesity<sup>[30]</sup>, ARFI also has some disadvantages. For example, certain hepatic disorders (e.g., ascites and acute icteric hepatitis) may affect the ARFI results. However, in our study, all the enrolled subjects including obese patients with BMI of 30.81 successfully got SWV values. Thus, ARFI may have a wider application range than FibroScan. In general, ARFI overcome a spectrum of disadvantages of conventional ultrasound technologies, such as no manual operation of pressing, improved depth limitation (5 cm of the earlier machines and 8 cm of the newer machines) and location of imaging. Compared to other methods, ARFI has no pain, with good reproducibility of data and simple operation. Indeed, ARFI is potentially limited by patients with a BMI > 40 or after contrast-enhanced ultrasonography. Thus, its combination with other non-invasive methods is necessary to enhance the diagnostic accuracy<sup>[31]</sup>.

Currently, serological diagnostic assays for non-invasive assessment of liver fibrosis are available including direct and indirect methods. The main purpose of these methods is to identify the existence of fibrosis but not the grading or staging. In this study, APRI and Forns index were also used to stage liver fibrotic stage. Although the sensitivity and specificity of these methods for the diagnosis of liver fibrosis was lower than ARFI,

they partially reflected the pro-inflammatory response and hepatic compensation. The most important finding of this study was that combined measurement of ARFI and APRI exhibited better accuracy than ARFI or APRI alone when evaluating  $\geq$  F2 fibrosis stage. Combination of ARFI, APRI and Forns index did not further improve the diagnostic effect than the combination of ARFI and APRI.

In conclusion, ARFI, APRI and Forns index correlated well with the histological liver fibrosis stages in CHB patients. ARFI showed better accuracy than APRI when evaluating F2, F3 and F4 stages. Combined check with ARFI and APRI showed a significant enhancement of diagnostic accuracy than ARFI or APRI alone. ARFI + APRI exhibited similar enhancement of diagnostic accuracy of hepatic fibrosis with ARFI + APRI + Forns index when evaluating fibrotic stages more than F2 in CHB patients. This study provides an ideal and convenient non-invasive diagnostic method for the detection of hepatic fibrosis of CHB patients in clinical practice.

## COMMENTS

### Background

Hepatitis B virus (HBV) infection-mediated chronic injury of hepatocytes induces fibrosis, which may progress to end-stage liver diseases like cirrhosis and hepatocellular carcinoma. Thus, accurate grading of hepatic fibrosis is important for the application of appropriate intervening strategy to retard the progression. To date, the "golden standard" of fibrotic grading is still liver biopsy, which wide clinical application is hindered by its inherent drawbacks. In recent years, biomechanical-based ultrasonic elastography received mass attention. However, several clinical studies found that the sole application of ultrasonic elastography may bring evident errors in diagnosing hepatic fibrosis. It is suggested that a combination of ultrasonic elastography and serum liver functions tests holds the potential to overcome those disadvantages.

### Research frontiers

There are an increasing number of hospitals using non-invasive ultrasonic elastography techniques, such as acoustic radiation force impulse (ARFI) and Fibroscan to grade hepatic fibrosis of chronic hepatitis B (CHB) patients in China and chronic hepatitis C patients in Western countries. Combination of different ultrasonic elastography techniques has been reported by a number of reports. However, few studies investigate the accuracy of the combination of ultrasonic elastography and serum liver functions tests.

### Innovations and breakthroughs

This study evaluated the accuracy of one ultrasound elastography method (ARFI) and two serum biochemical tests [aspartate aminotransferase to platelet ratio index (APRI) and Forns index], as well as their combination in the assessment of liver fibrosis in CHB. The authors found that ARFI + APRI exhibited similar enhancement of diagnostic accuracy of hepatic fibrosis with ARFI + APRI + Forns index when evaluating fibrotic stages more than F2 in CHB patients.

### Applications

The data in this study suggest that doctor can yield favorable outcomes through the accumulation of technical experience. Furthermore, this study also provides readers with important information regarding an ideal and convenient non-invasive diagnostic method for the grading of hepatic fibrosis of CHB patients.

### Terminology

ARFI imaging involves mechanically exciting a localized region of interest in the tissue with acoustic radiation force to induce a shear wave in the tissue. The displacement of the shear wave is tracked using a pulse-echo mode ultrasound

at several lateral locations along the propagation path of the shear wave. By measuring the time to peak displacement at each location, the shear wave velocity was calculated, which is directly related to the elasticity of the tissue.  $APRI = AST(ULN)/PLT(109/L) \times 100$ . Forns index =  $7.811 - 3.131 \times \ln(PLT) + 0.781 \times \ln(GGT) + 3.467 \times \ln(\text{age}) - 0.014 \times (\text{cholesterol})$ .

### Peer-review

This is a good attempt by Dong *et al* to compare ARFI, APRI and Forns to determine fibrosis stage in chronic HBV patients. As these are not new techniques for fibrosis evaluation and they wanted to establish that combination of ARFI/APRI and ARFI/Forns as better non-invasive technique.

## REFERENCES

- 1 **Pinzani M**, Vizzutti F. Fibrosis and cirrhosis reversibility: clinical features and implications. *Clin Liver Dis* 2008; **12**: 901-913, x [PMID: 18984473 DOI: 10.1016/j.cld.2008.07.006]
- 2 **Popov Y**, Schuppan D. Targeting liver fibrosis: strategies for development and validation of antifibrotic therapies. *Hepatology* 2009; **50**: 1294-1306 [PMID: 19711424 DOI: 10.1002/hep.23123]
- 3 **Nguyen D**, Talwalkar JA. Noninvasive assessment of liver fibrosis. *Hepatology* 2011; **53**: 2107-2110 [PMID: 21547935 DOI: 10.1002/hep.24401]
- 4 **Kaminuma C**, Tsushima Y, Matsumoto N, Kurabayashi T, Taketomi-Takahashi A, Endo K. Reliable measurement procedure of virtual touch tissue quantification with acoustic radiation force impulse imaging. *J Ultrasound Med* 2011; **30**: 745-751 [PMID: 21632988]
- 5 **Palmeri ML**, Wang MH, Dahl JJ, Frinkley KD, Nightingale KR. Quantifying hepatic shear modulus in vivo using acoustic radiation force. *Ultrasound Med Biol* 2008; **34**: 546-558 [PMID: 18222031 DOI: 10.1016/j.ultrasmedbio.2007.10.009]
- 6 **Gallotti A**, D'Onofrio M, Pozzi Mucelli R. Acoustic Radiation Force Impulse (ARFI) technique in ultrasound with Virtual Touch tissue quantification of the upper abdomen. *Radiol Med* 2010; **115**: 889-897 [PMID: 20082227 DOI: 10.1007/s11547-010-0504-5]
- 7 **Sporea I**, Sirli R, Bota S, Popescu A, Sendroiu M, Jurchis A. Comparative study concerning the value of acoustic radiation force impulse elastography (ARFI) in comparison with transient elastography (TE) for the assessment of liver fibrosis in patients with chronic hepatitis B and C. *Ultrasound Med Biol* 2012; **38**: 1310-1316 [PMID: 22698510 DOI: 10.1016/j.ultrasmedbio.2012.03.011]
- 8 **Haque M**, Robinson C, Owen D, Yoshida EM, Harris A. Comparison of acoustic radiation force impulse imaging (ARFI) to liver biopsy histologic scores in the evaluation of chronic liver disease: A pilot study. *Ann Hepatol* 2010; **9**: 289-293 [PMID: 2072070]
- 9 **Piscaglia F**, Salvatore V, Di Donato R, D'Onofrio M, Gualandi S, Gallotti A, Peri E, Borghi A, Conti F, Fattovich G, Sagrini E, Cucchetti A, Andreone P, Bolondi L. Accuracy of VirtualTouch Acoustic Radiation Force Impulse (ARFI) imaging for the diagnosis of cirrhosis during liver ultrasonography. *Ultraschall Med* 2011; **32**: 167-175 [PMID: 21321842 DOI: 10.1055/s-0029-1245948]
- 10 **Yoneda M**, Suzuki K, Kato S, Fujita K, Nozaki Y, Hosono K, Saito S, Nakajima A. Nonalcoholic fatty liver disease: US-based acoustic radiation force impulse elastography. *Radiology* 2010; **256**: 640-647 [PMID: 20529989 DOI: 10.1148/radiol.10091662]
- 11 **D'Onofrio M**, Crosara S, De Robertis R, Canestrini S, Demozzi E, Gallotti A, Pozzi Mucelli R. Acoustic radiation force impulse of the liver. *World J Gastroenterol* 2013; **19**: 4841-4849 [PMID: 23946588 DOI: 10.3748/wjg.v19.i30.4841]
- 12 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]
- 13 **Shin WG**, Park SH, Jang MK, Hahn TH, Kim JB, Lee MS, Kim DJ, Jun SY, Park CK. Aspartate aminotransferase to platelet ratio index (APRI) can predict liver fibrosis in chronic hepatitis B. *Dig Liver Dis* 2008; **40**: 267-274 [PMID: 18055281 DOI: 10.1016/

j.dld.2007.10.011]

- 14 **Forns X**, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM, Rodés J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986-992 [PMID: 12297848 DOI: 10.1053/jhep.2002.36128]
- 15 **Jeong JY**, Kim TY, Sohn JH, Kim Y, Jeong WK, Oh YH, Yoo KS. Real time shear wave elastography in chronic liver diseases: accuracy for predicting liver fibrosis, in comparison with serum markers. *World J Gastroenterol* 2014; **20**: 13920-13929 [PMID: 25320528 DOI: 10.3748/wjg.v20.i38.13920]
- 16 **Ferraioli G**, Tinelli C, Dal Bello B, Zicchetti M, Filice G, Filice C. Accuracy of real-time shear wave elastography for assessing liver fibrosis in chronic hepatitis C: a pilot study. *Hepatology* 2012; **56**: 2125-2133 [PMID: 22767302 DOI: 10.1002/hep.25936]
- 17 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832 [PMID: 9121257 DOI: 10.1016/S0140-6736(96)07642-8]
- 18 **Myers RP**, Tainturier MH, Ratziu V, Piton A, Thibault V, Imbert-Bismut F, Messous D, Charlotte F, Di Martino V, Benhamou Y, Poynard T. Prediction of liver histological lesions with biochemical markers in patients with chronic hepatitis B. *J Hepatol* 2003; **39**: 222-230 [PMID: 12873819 DOI: 10.1016/S0168-8278(03)00171-5]
- 19 **Poynard T**, Zoulim F, Ratziu V, Degos F, Imbert-Bismut F, Deny P, Landais P, El Hasnaoui A, Slama A, Blin P, Thibault V, Parvaz P, Munteanu M, Trepo C. Longitudinal assessment of histology surrogate markers (FibroTest-ActiTest) during lamivudine therapy in patients with chronic hepatitis B infection. *Am J Gastroenterol* 2005; **100**: 1970-1980 [PMID: 16128941 DOI: 10.1111/j.1572-0241.2005.41957.x]
- 20 **Boursier J**, Isselin G, Fouchard-Hubert I, Oberti F, Dib N, Lebigot J, Bertrais S, Gallois Y, Calès P, Aubé C. Acoustic radiation force impulse: a new ultrasonographic technology for the widespread noninvasive diagnosis of liver fibrosis. *Eur J Gastroenterol Hepatol* 2010; **22**: 1074-1084 [PMID: 20440210 DOI: 10.1097/MEG.0b013e328339e0a1]
- 21 **DeLong ER**, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; **44**: 837-845 [PMID: 3203132 DOI: 10.2307/2531595]
- 22 **Behler RH**, Nichols TC, Zhu H, Merricks EP, Gallippi CM. ARFI imaging for noninvasive material characterization of atherosclerosis. Part II: toward in vivo characterization. *Ultrasound Med Biol* 2009; **35**: 278-295 [PMID: 19026483 DOI: 10.1016/j.ultrasmedbio.2008.08.015]
- 23 **Goertz RS**, Zopf Y, Jugl V, Heide R, Janson C, Strobel D, Bernatik T, Haendl T. Measurement of liver elasticity with acoustic radiation force impulse (ARFI) technology: an alternative noninvasive method for staging liver fibrosis in viral hepatitis. *Ultraschall Med* 2010; **31**: 151-155 [PMID: 20306380 DOI: 10.1055/s-0029-1245244]
- 24 **Arzumanyan A**, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer* 2013; **13**: 123-135 [PMID: 23344543 DOI: 10.1038/nrc3449]
- 25 **Friedrich-Rust M**, Buggisch P, de Knegt RJ, Dries V, Shi Y, Matschenz K, Schneider MD, Herrmann E, Petersen J, Schulze F, Zeuzem S, Sarrazin C. Acoustic radiation force impulse imaging for non-invasive assessment of liver fibrosis in chronic hepatitis B. *J Viral Hepat* 2013; **20**: 240-247 [PMID: 23490368 DOI: 10.1111/j.1365-2893.2012.01646.x]
- 26 **Poynard T**, Halfon P, Castera L, Munteanu M, Imbert-Bismut F, Ratziu V, Benhamou Y, Bourlière M, de Ledinghen V. Standardization of ROC curve areas for diagnostic evaluation of liver fibrosis markers based on prevalences of fibrosis stages. *Clin Chem* 2007; **53**: 1615-1622 [PMID: 17634213 DOI: 10.1373/clinchem.2007.085795]
- 27 **Huwart L**, Sempoux C, Vicaux E, Salameh N, Annet L, Danse E, Peeters F, ter Beek LC, Rahier J, Sinkus R, Horsmans Y, Van Beers BE. Magnetic resonance elastography for the noninvasive staging of liver fibrosis. *Gastroenterology* 2008; **135**: 32-40 [PMID: 18471441 DOI: 10.1053/j.gastro.2008.03.076]
- 28 **Poynard T**, Munteanu M, Imbert-Bismut F, Charlotte F, Thabut D, Le Calvez S, Messous D, Thibault V, Benhamou Y, Moussalli J, Ratziu V. Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. *Clin Chem* 2004; **50**: 1344-1355 [PMID: 15192028 DOI: 10.1373/clinchem.2004.032227]
- 29 **Poynard T**, Halfon P, Castera L, Charlotte F, Le Bail B, Munteanu M, Messous D, Ratziu V, Benhamou Y, Bourlière M, De Ledinghen V. Variability of the area under the receiver operating characteristic curves in the diagnostic evaluation of liver fibrosis markers: impact of biopsy length and fragmentation. *Aliment Pharmacol Ther* 2007; **25**: 733-739 [PMID: 17311607 DOI: 10.1111/j.1365-2036.2007.03252.x]
- 30 **Sasso M**, Miette V, Sandrin L, Beaugrand M. The controlled attenuation parameter (CAP): a novel tool for the non-invasive evaluation of steatosis using Fibroscan. *Clin Res Hepatol Gastroenterol* 2012; **36**: 13-20 [PMID: 21920839 DOI: 10.1016/j.clinre.2011.08.001]
- 31 **Palmeri ML**, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B, Diehl AM, Nightingale KR. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. *J Hepatol* 2011; **55**: 666-672 [PMID: 21256907 DOI: 10.1016/j.jhep.2010.12.019]

**P-Reviewer:** Banerjee S, Malnick SDH, Pai CG **S-Editor:** Qi Y  
**L-Editor:** A **E-Editor:** Liu SQ





Randomized Clinical Trial

# Co-treatment with pegylated interferon alfa-2a and entecavir for hepatitis D: A randomized trial

Zaigham Abbas, Mohammad Sadik Memon, Muhammad Amir Umer, Minaam Abbas, Lubna Shazi

Zaigham Abbas, Department of Gastroenterology, Ziauddin University Hospital, Karachi 74400, Pakistan

Zaigham Abbas, Muhammad Amir Umer, Department of Medicine, Orthopedic and Medical Institute, Karachi 74400, Pakistan

Zaigham Abbas, Minaam Abbas, Lubna Shazi, Liver Stomach Clinic Zamzama, Karachi 75500, Pakistan

Mohammad Sadik Memon, Asian Institute of Medical Sciences, Hyderabad 71000, Pakistan

**Author contributions:** Abbas Z was responsible for the study conception and design, data analysis and interpretation, and manuscript drafting; Abbas Z and Memon MS recruited patients for the study; Umer MA, Abbas M and Shazi L coordinated study related activities and helped in manuscript drafting; all authors reviewed and approved the final version to be submitted for publication.

**Institutional review board statement:** The study was approved by Independent Ethics Review Board and Institutional Review Board.

**Clinical trial registration statement:** This investigator initiated trial was registered with the Roche (ML25746).

**Informed consent statement:** All study participants provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** Authors do not have any conflict of interest to declare.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Zaigham Abbas, FCPS, FRCP, Department of Gastroenterology, Ziauddin University Hospital, Shahrah-e-Ghalib, Karachi 74400, Pakistan. [drzabbas@gmail.com](mailto:drzabbas@gmail.com)  
 Telephone: +92-21-35862939  
 Fax: +92-21-35862940

Received: January 25, 2016  
 Peer-review started: January 27, 2016  
 First decision: March 9, 2016  
 Revised: March 30, 2016  
 Accepted: April 14, 2016  
 Article in press: April 18, 2016  
 Published online: May 18, 2016

## Abstract

**AIM:** To investigate the efficacy of pegylated interferon alfa (PEG-IFN $\alpha$ ) therapy with and without entecavir in patients with chronic hepatitis D.

**METHODS:** Forty hepatitis D virus (HDV) RNA positive patients were randomized to receive either PEG-IFN $\alpha$ -2a 180  $\mu$ g weekly in combination with entecavir 0.5 mg daily ( $n = 21$ ) or PEG-IFN $\alpha$  alone ( $n = 19$ ). Patients who failed to show 2 log reduction in HDV RNA level at 24 wk of treatment, or had detectable HDV RNA at 48 wk of therapy were considered as treatment failure. Treatment was continued for 72 wk in the rest of the patients. All the patients were followed for 24 wk post treatment. Intention to treat analysis was performed.

**RESULTS:** The mean age of the patients was  $26.7 \pm 6.8$  years, 31 were male. Two log reduction in HDV RNA levels at 24 wk of therapy was achieved in 9 (43%) patients receiving combination therapy and 12 (63%) patients receiving PEG-IFN $\alpha$  alone ( $P = 0.199$ ). Decline in hepatitis B surface antigen (HBsAg) levels was insignificant. At the end of treatment, HDV RNA was negative in 8 patients (38%) receiving combination

therapy and 10 patients (53%) receiving PEG-IFN $\alpha$ -2a alone. Virological response persisted in 7 (33%) and 8 (42%) patients, respectively at the end of the 24 wk follow-up period. One responder patient in the combination arm lost HBsAg and became hepatitis B surface antibody positive. Six out of 14 baseline hepatitis B e antigen reactive patients seroconverted and four of these seroconverted patients had persistent HDV RNA clearance.

**CONCLUSION:** Administration of PEG-IFN $\alpha$ -2a with or without entecavir, resulted in persistent HDV RNA clearance in 37% of patients. The addition of entecavir did not improve the overall response.

**Key words:** Hepatitis D; Entecavir; Hepatitis B surface antigen; Pegylated interferon; Hepatitis D virus RNA; Treatment

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Chronic hepatitis D is a difficult to treat infection. Six months post treatment response is seen only in one quarter of the patients treated with pegylated interferon alfa (PEG-IFN $\alpha$ ). In an attempt to improve the response of PEG-IFN, we combined entecavir. This is the first study to evaluate the efficacy of PEG-IFN with entecavir compared to PEG-IFN alone for the treatment of hepatitis D infection. Our study showed that the combination treatment did not have any additional benefit in terms of hepatitis D virus RNA suppression and hepatitis B surface antigen reduction as compared to PEG-IFN alone.

Abbas Z, Memon MS, Umer MA, Abbas M, Shazi L. Co-treatment with pegylated interferon alfa-2a and entecavir for hepatitis D: A randomized trial. *World J Hepatol* 2016; 8(14): 625-631 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i14/625.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i14.625>

## INTRODUCTION

The prevalence of hepatitis B surface antigen (HBsAg) positive individuals in Pakistan is 2.5%<sup>[1]</sup> and it is estimated that 5 million persons are HBsAg positive. In a large series, hepatitis D virus (HDV) antibodies in HBsAg positive individuals were found to be present in 16.6% cases<sup>[2]</sup>. So there is a large pool of patients exposed to hepatitis D in this country.

Chronic hepatitis D is a difficult to treat infection. Standard interferon-alfa is not an ideal treatment<sup>[3]</sup>. Recent few trials with pegylated interferon alfa (PEG-IFN $\alpha$ ) have shown a better response of 25%-30% six months post treatment<sup>[4,5]</sup>. In an attempt to improve the response of PEG-IFN $\alpha$ , the International Hepatitis Delta Network evaluated adefovir and later tenofovir in combination with PEG-IFN $\alpha$  in HIDIT-1 and HIDIT-2

studies<sup>[6,7]</sup>. It was expected that HIDIT-2 will yield better results due to use of potent nucleotide analogue in combination with PEG-IFN $\alpha$  for a longer duration of therapy.

Recently presented results of HITID-2 trial<sup>[7]</sup> showed that 96 wk of PEG-IFN $\alpha$ -2a and tenofovir therapy was associated with a high frequency of serious adverse events. Combination treatment had similar effects on HBsAg reduction as compared to PEG-IFN $\alpha$  alone. More than one third of the on-treatment responders experienced a posttreatment HDV RNA relapse despite prolonged therapy. The results of the long term post treatment follow-up are awaited. Combination therapy with tenofovir did not provide obvious benefits in hepatitis D patients with low baseline hepatitis B virus (HBV)-DNA levels and prolongation of treatment to 96 wk did not provide higher off-treatment HDV RNA responses (compared to 48 wk in the HIDIT-1 study).

The aim of this study is to evaluate the efficacy of PEG-IFN $\alpha$ -2a with entecavir for the treatment of chronic hepatitis D. The reason for choosing entecavir was that HIDIT-2 study using tenofovir was in progress with high hopes and no data was available for entecavir in combination with PEG-IFN $\alpha$ -2a in the chronic hepatitis D setting. This drug, particularly in combination with PEG-IFN $\alpha$ -2a, have shown good results in HBsAg decline<sup>[8]</sup>.

## MATERIALS AND METHODS

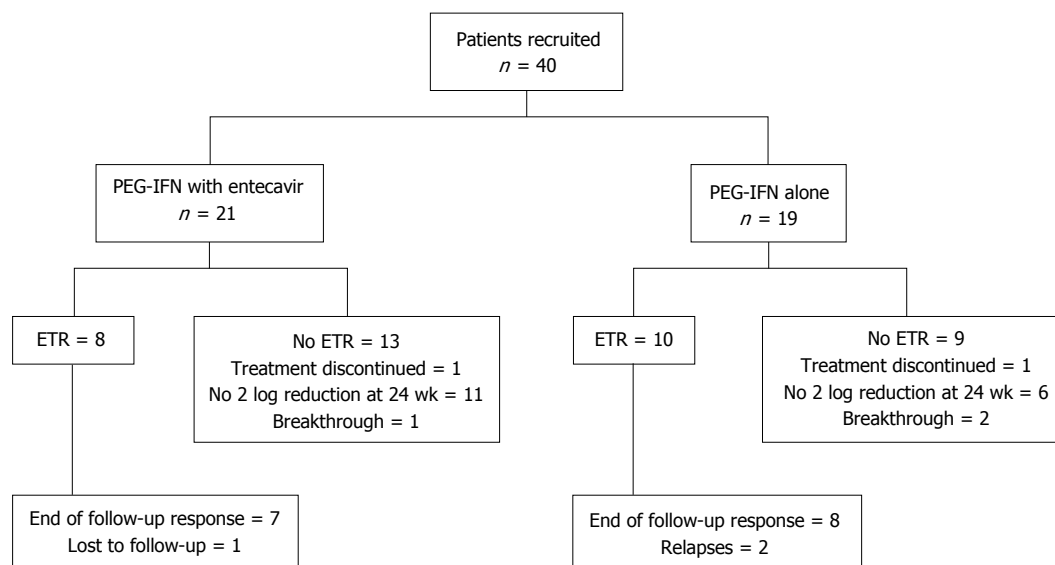
### Trial design

This randomized study compared the efficacy of PEG-IFN $\alpha$ -2a plus entecavir vs PEG-IFN $\alpha$ -2a alone for the treatment of chronic hepatitis D. Patients were randomized 1:1 into two groups. Duration of treatment was 72 wk with a post-treatment follow-up of 24 wk.

### Participants

Inclusion criteria were age 15-60 years, anti-HDV anti body positive and detectable serum HDV RNA at enrolment by real time polymerase chain reaction (PCR), elevated alanine aminotransferase (ALT) on two occasions in last 3 mo during screening phase, patients with compensated liver disease, *i.e.*, Child Pugh class A, hemoglobin > 12.0 g/dL for males and > 11.0 g/dL for females at screening, total leucocyte count > 3.000/mm<sup>3</sup>, and neutrophils > 1500/mm<sup>3</sup>, platelets > 80.000/mm<sup>3</sup>, serum creatinine level < 1.5 mg/dL and liver biopsy within 6 mo prior to randomization.

Exclusion criteria were patients who had received therapy for chronic hepatitis D, co-infection with hepatitis C or human immunodeficiency virus, serum total bilirubin greater than twice the upper limit of normal at screening, evidence of decompensated liver disease (Childs B-C), history or other evidence of a medical condition associated with chronic liver disease (*e.g.*, Wilson's disease, hemochromatosis, autoimmune hepatitis, alcoholic liver disease, alpha1 anti-trypsin deficiency, toxin exposures, thalassemia), women with ongoing pregnancy or who are breast feeding, evidence of drug



**Figure 1** Flow diagram of study patients. PEG-IFN: Pegylated interferon; ETR: End of treatment response.

and/or alcohol abuse, history of severe cardiac or pulmonary disease, inability or unwillingness to provide informed consent or abide by the requirements of the study.

### Settings

The study was conducted at the Orthopaedic and Medical Institute, Karachi, Liver Stomach Clinic, Karachi and Asian Institute of Medical Sciences, Hyderabad, Pakistan. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and principles of Good Clinical Practice. Patients gave the informed consent and the ethics committee approved the study.

### Interventions

The dose of PEG-IFN $\alpha$ -2a in each arm was 180  $\mu$ g weekly (Pegasys<sup>®</sup>, F. Hoffmann-La Roche Ltd, Basel). Entecavir was given in a dose of 0.5 mg per oral daily in the combination arm.

### Outcomes

Virological response was defined as HDV RNA clearance at the end of treatment and at follow-up six months post treatment. Biochemical response was normalization of ALT at the end of treatment and at follow-up.

Treatment failure was defined as failure to show 2 log reduction in HDV RNA level at 24 wk of treatment, or presence of detectable HDV RNA at 48 wk of therapy or relapse at 24 wk post treatment follow-up. Development of decompensation (ascites or hepatic encephalopathy) during the treatment, drop outs, and lost to follow-up were also considered as treatment failure in an intention to treat analysis (Figure 1).

### Randomization

Randomization (1:1 allocation) was computer-generated. The investigators were not involved in sequence

generation or allocation concealment steps and were provided with sealed envelopes containing the treatment code to administer, in increasing numbers, according to chronological inclusion in the study.

### Viral nucleic acids and serologic testing

Viral nucleic acids were isolated from patients' serum samples by High Pure Viral Nucleic Acid Kit, according to the manufacturer's instructions (Roche Diagnostics, United States). Serum HBV DNA levels were measured using the Cobas TaqMan (Roche Diagnostics Systems, Basel, Switzerland) with a lower limit of quantification 20 IU/mL. For HDV RNA, qualitative test was based on the reverse transcription PCR of the target gene. Quantification of HDV RNA was done by real time PCR having a lower limit of detection of 500 IU/mL. Hepatitis B e antigen (HBeAg) and antibody against HBeAg (anti-HBe) status was determined using enzyme immunoassays. Serum HBsAg was quantified using the Architect HBsAg assay (Abbott Laboratories, Abbott Park, IL, United States). Grading of inflammation and the staging of fibrosis was performed according to the Batt's and Ludwig's classification<sup>[9]</sup>.

### On-treatment evaluation

The Patients were educated regarding administration of subcutaneous pegylated interferon and oral entecavir, expected adverse events, schedule for laboratory monitoring, and clinic appointment. Patients were evaluated as outpatients for safety, tolerance and efficacy every 4 wk during treatment until week 72 and then at 92 wk, *i.e.*, 24 wk post treatment during the follow-up period. At each visit complete blood count and biochemistry was assessed. HDV RNA levels were measured at baseline, 24, 48, 72 and 96 wk. HBsAg levels were measured at baseline and 24 wk. HBeAg and anti-HBe antibodies were checked at baseline. In case of HBeAg reactive, tests for HBeAg and anti-HBeAg

**Table 1** Baseline characteristics of study patients *n* (%)

	PEG-IFN $\alpha$ with entecavir ( <i>n</i> = 21)	PEG-IFN $\alpha$ alone ( <i>n</i> = 19)	<i>P</i> value
Age (mean, yr)	26.4 $\pm$ 6.4	27 $\pm$ 7.4	0.946
Gender (male:female)	16:5	15:4	1.00
Body mass index (kg/m <sup>2</sup> )	21.8 $\pm$ 3.6	23.6 $\pm$ 4.3	0.151
Hemoglobin (g/dL)	13.7 $\pm$ 1.59	13.9 $\pm$ 1.3	0.473
Total leucocyte count ( $\times 10^6$ /L)	7.1 $\pm$ 2.0	6.7 $\pm$ 1.9	0.626
Platelets count ( $\times 10^9$ /L)	237 $\pm$ 83	185 $\pm$ 59	0.023 <sup>1</sup>
Total bilirubin (mg/dL)	0.67 $\pm$ 0.34	0.70 $\pm$ 0.38	0.828
ALT (IU/L)	87 $\pm$ 55	89 $\pm$ 41	0.379
GGT (IU/L)	49 $\pm$ 41	69 $\pm$ 72	0.255
Inflammatory grade on biopsy			0.186
0-1	5 (31)	1 (5)	
2-3	16 (69)	18 (95)	
Fibrosis stage on biopsy			0.105
0-2	12 (57)	6 (32)	
3-4	9 (43)	13 (68)	
Cirrhosis	2 (10)	6 (32)	0.120
HBeAg reactive	7 (33)	7 (37)	0.816
HDV RNA (mean log10 IU/mL)	7.5 $\pm$ 1.1	6.9 $\pm$ 1.2	0.119
HBV DNA detected	5 (24)	5 (26)	1.00
HBV DNA (mean log10 IU/mL)	1.08 $\pm$ 2.10	1.48 $\pm$ 2.70	0.656
HBsAg (mean log10 IU/mL)	4.50 $\pm$ 0.42	4.20 $\pm$ 0.64	0.068

<sup>1</sup>Significant *P* value. ALT: Alanine aminotransferase; GGT: Gamma glutamyl transferase; HBeAg: Hepatitis B e antigen; HDV: Hepatitis D virus; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; PEG-IFN $\alpha$ : Pegylated interferon alfa.

done were checked at 24, 48 and 72 wk to document seroconversion.

### Adverse events

Adverse events and clinical laboratory parameters were recorded. Serious adverse events were defined as those that were fatal, life-threatening, required inpatient hospitalization or discontinuation of treatment. These included decompensation of liver disease and mortality. Non-serious adverse events and laboratory abnormalities leading to dose modifications and premature withdrawal from therapy were noted.

### Statistical analysis

Data were expressed as the number of subjects with percentages for nominal variables. These variables were compared by  $\chi^2$  or Fisher exact test. Continuous variables were presented as mean (standard deviation), and compared using Mann-Whitney *U* test. The degree of the relationship between linear related variables was measured by the Pearson *r* correlation test. Statistical analyses were performed using SPSS 20.0 software (IBM SPSS Statistics, New York, NY, United States). All tests were 2-tailed and a two-tailed *P* value < 0.05 was required for statistical significance. Intention-to-treat analysis was done to include all randomized patients.

## RESULTS

A total of 40 patients with chronic hepatitis D was included during the study period of 2012-2014. Twenty-one patients were treated with PEG-IFN $\alpha$  plus entecavir (combination arm) and 19 with PEG-IFN $\alpha$  alone (mono-

therapy arm). The mean age of the patients was 26.7  $\pm$  6.8 years, 31 were male and 14 were HBeAg reactive; 7 in each arm.

Demographic and baseline clinical characteristics of the patients are shown in Table 1. Age, gender, body mass index, degree of fibrosis and inflammation on liver biopsy, ALT, HBeAg status, and HDV RNA levels were comparable to the combination and monotherapy arm patients. However, platelet count in monotherapy arm was lower than in the combination arm. Baseline HBsAg and HBV DNA levels were correlated (Pearson correlation = 0.625, *P* < 0.001). Liver biopsy was available in all cases as one of the inclusion criteria. Mild inflammation was seen in 6 and moderate to severe in 34 patients. Stage of the disease was 0-2 in 18 and 3-4 in 22 patients. Cirrhosis of the liver as evident from histology, ultrasound or clinical examination was present in 8 (20%) patients.

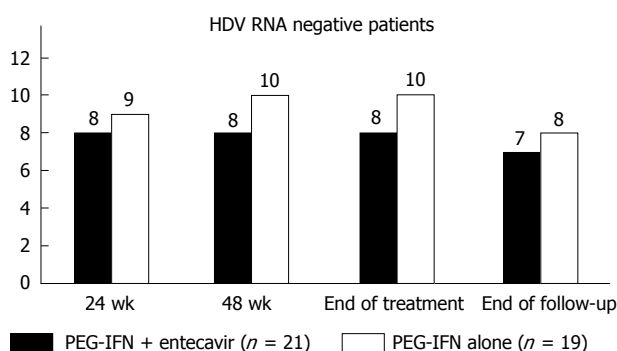
Two log reduction in HDV RNA levels at 24 wk of therapy was achieved in 9 (43%) patients receiving combination therapy and in 12 (63%) patients receiving PEG-IFN $\alpha$  alone (*P* = 0.199) (Figure 1). There was no significant difference in the HBsAg log10 levels after six months of therapy; 4.13  $\pm$  0.91 in the combination arm vs 4.01  $\pm$  0.51 in the monotherapy arm (*P* = 0.608), and a mean decline in HBsAg levels (*P* = 0.579). At the end of treatment, HDV RNA was negative in 8 patients (38%) receiving combination therapy and in 10 patients (53%) receiving PEG-IFN $\alpha$ -2a alone by intention to treat analysis (*P* = 0.356). ALT normalization was seen in 4 (19%) patients of the combination arm and 7 (37%) patients of the monotherapy arm (*P* = 0.293). One 29-year-old male patient in the combination arm



**Table 2** Factors associated with hepatitis D virus RNA negativity at 24 wk post-treatment *n* (%)

Variable	Responders ( <i>n</i> = 15)	Non-responders ( <i>n</i> = 25)	<i>P</i> value
Age (mean, yr)	27.9 ± 8.4	25.9 ± 5.7	0.654
Gender (male:female)	12:3	19:6	1.00
Body mass index (kg/m <sup>2</sup> )	23.8 ± 4.2	21.9 ± 3.8	0.158
ALT (mean IU/L)	103 ± 44	80 ± 50	0.033 <sup>1</sup>
GGT (mean IU/L)	45 ± 21	66 ± 72	0.700
Inflammatory activity on biopsy			0.493
0-1	3 (20)	3 (12)	
2-3	12 (80)	22 (88)	
Fibrosis on biopsy			0.412
0-2	8 (53)	10 (40)	
3-4	7 (47)	15 (60)	
Cirrhosis	2 (13)	6 (24)	0.686
HBeAg reactive	4 (27)	10 (40)	0.502
Baseline HDV RNA	7.01 ± 1.25	4.61 ± 1.91	0.072
Baseline HBsAg	4.38 ± 0.63	4.32 ± 0.53	0.727
Treatment arm			0.567
PEG-IFN $\alpha$ + entecavir ( <i>n</i> = 21)	7 (33)	14 (67)	
PEG-IFN $\alpha$ alone ( <i>n</i> = 19)	8 (42)	11 (58)	
Two log of HDV RNA reduction at week 24	15 (100)	6 (24)	< 0.001 <sup>1</sup>
Baseline HBeAg reactive	4 (27)	10 (40)	0.502
24 wk HBsAg level	3.97 ± 1.01	4.31 ± 0.45	0.476
One log reduction of HBsAg level at 24 wk	5 (33)	4 (16)	0.255
Patients with decrease in HBsAg level at 24 wk from baseline	12 (80)	12 (48)	0.056
HBeAg reactive patients ( <i>n</i> = 14) who seroconverted	4/4 (100)	2/10 (20)	0.015 <sup>1</sup>

<sup>1</sup>Significant *P* value. ALT: Alanine aminotransferase; GGT: Gamma glutamyl transferase; HBeAg: Hepatitis B e antigen; HDV: Hepatitis D virus; PEG-IFN $\alpha$ : Pegylated interferon alfa; HBsAg: Hepatitis B surface antigen.



**Figure 2** Number of patients who became hepatitis D virus RNA negative. HDV: Hepatitis D virus; PEG-IFN: Pegylated interferon.

lost HBsAg and became anti-HBs antibody positive. His baseline parameters were HBsAg 2903 IU/mL (log<sub>10</sub> = 3.46), HBeAg negative with undetectable HBV DNA, and HDV RNA 158000 IU/mL (log<sub>10</sub> = 5.20). At 24 wk of treatment, his HBsAg level was 11.6 IU/mL, and HDV RNA negative. HBsAg became undetectable at 48 wk and he developed anti-HBs antibodies.

Two patients in the monotherapy arm relapsed during the 24 wk post-treatment period and one patient in the combination arm was lost to follow-up, decreasing the persistent virological clearance 24 wk post treatment to 7 (33%) in the combination arm and 8 (42%) in the monotherapy arm (*P* = 0.567) (Table 2 and Figure 2) in an intention to treat analysis. End of follow-up biochemical response was seen in only patients with the virologic response; 5/21 (24%) and 7/19 (37%)

patients of combination arm and monotherapy arm respectively (*P* = 0.369).

Though there were no statistical differences in log reduction of HBsAg levels at 24 wk of treatment between responders and non responders, there was a trend of decrease in HBsAg levels (*P* = 0.056). Out of 14 HBeAg reactive patients, HBeAg seroconversion was seen in 2 patients on combination arm and 4 patients of monotherapy arm. Both patients of the combination arm and 2 patients of the monotherapy arm achieved persistence HDV RNA clearance during the follow-up period while one patient relapsed and another had virological breakthrough during treatment. In contrast, 7 out of 8 patients, who did not seroconvert, were null responders and one patient could not complete treatment due to complications of treatment (combination arm).

One patient from the combination arm could achieve only one log reduction in the HDV RNA levels at 24 wk of treatment, and was considered as a non-responder according to the protocol. He was taken off the study and was considered a treatment failure in an intention to treat analysis. However, he continued to receive PEG-IFN $\alpha$  monotherapy for another 24 wk and became HDV RNA negative at the end of treatment and the response persisted 24 wk post treatment.

The side effects reported by these patients were usually of PEG-IFN and included nausea, weakness, fever, decreased appetite, bloating, body aches, headaches, weight loss. These side effects did not require a dose reduction. One patient developed transient

neutropenia and responded to subcutaneous filgrastim. Two patients discontinued treatment; one in each arm during the treatment before 24 wk of therapy. One patient in the monotherapy arm developed ascites while treatment was stopped in another patient in the combination arm due to severe depression.

## DISCUSSION

We used PEG-IFN $\alpha$  in combination with entecavir to treat HDV patients for the first time. The results of our study are in congruence with the previous studies that the combination treatment of nucleos(t)ides with PEG-IFN $\alpha$  does not have any edge over PEG-IFN monotherapy in terms of sustained clearance of HDV RNA<sup>[10]</sup>.

Some of the previous studies, including one of ours, have shown that response to the treatment can be predicted by the HDV RNA assessment at six months, and may give a clue whether to stop treatment<sup>[11,12]</sup>. Patients with negative HDV RNA at six months are more likely to have sustained virologic response<sup>[5]</sup> while non-responders could be identified by a less than 3 log decrease of HDV RNA at 6 mo of treatment<sup>[11]</sup>. We used two log reduction at 24 wk as a criterion to continue treatment in our protocol. One of our patients from the combination arm had one log reduction at 24 wk, was taken off the study as non-responder but he continued treatment and showed persistent virological clearance. As there are not many treatment options for chronic hepatitis D, we may suggest that the patients, even with one log reduction in the viral load at 24 wk of therapy, who continue to show steady decline in HDV RNA levels may remain on treatment.

We had a low relapse rate in this study compared to our previous experience<sup>[5]</sup> as according to the protocol only better responders continued treatment, *i.e.*, patients who had a 2 log reduction in HDV RNA levels at 24 wk of treatment. Moreover, treatment was extended to 72 wk instead of stopping at 48 wk. It may be beneficial to extend treatment duration beyond 48 wk in good responders to decrease the relapse rate, *i.e.*, patients who show a reduction in HDV RNA and HBsAg levels, and HBeAg reactive patients who seroconvert during the treatment. However, proper way to judge this dictum could be a randomized trial comparing 48 wk vs 72-96 wk of therapy. Extending duration of therapy may also be useful in patients with slow but steady decline of HDV RNA and HBsAg levels.

Heller *et al.*<sup>[13]</sup> studied prolonging therapy of chronic hepatitis D with PEG-IFN $\alpha$  for up to 5 years. Only three of 12 patients treated achieved a complete virologic response, endpoint defined as the combination of undetectable HDV RNA with loss of HBsAg and anti-HBsAg seroconversion in serum. Thus, given the poor response rates, and long-term risks of interferon-based therapies, we have to be selective in choosing our patients for a prolonged therapy. The long term results of HIDIT-1 study<sup>[6]</sup> where patients were treated for 48 wk

vs HIDIT-2 study<sup>[7]</sup> when the treatment was extended for 92 wk were not much different. Thus, given the poor response rates, and long-term risks of interferon-based therapies, we have to be selective in choosing our patients for a prolonged therapy and we cannot make it a rule. Optimized HBsAg titer monitoring and checking HDV RNA levels may improve the outcome<sup>[14,15]</sup>.

In our study, HBeAg reactive patients who seroconverted during the treatment had a less chance of relapse. One of our responder patients in the combination arm, who had a lower HBsAg level as compared to the average of the cohort, lost HBsAg during the treatment. Interferon based therapy is known to induce HBsAg seroconversion and it is usually associated with low pretreatment HBsAg levels<sup>[16]</sup>.

We did not check for genotypes of HBV and HDV for this study as it is already known that the genotype of hepatitis D is 1<sup>[17]</sup> and of hepatitis B is D in our region<sup>[18]</sup>. We followed our patients for six months post treatment. Late HDV RNA relapses may occur after PEG-IFN $\alpha$  therapy of hepatitis delta and thus the term "sustained virological response" should be used with caution in HDV infection<sup>[19]</sup>. There was a possibility of a higher relapse rate in our patients if we had followed up our patients for a longer period.

In conclusion, combination treatment did not show any additional benefit in terms of HDV RNA suppression and HBsAg reduction as compared to PEG-IFN $\alpha$  alone. Liver fibrosis and HBsAg levels did not predict HDV RNA response. HDV RNA response at 24 wk, HBeAg seroconversion and any reduction in HBsAg levels during treatment may predict the patients who are going to have a better outcome.

## ACKNOWLEDGMENTS

We are thankful to Dr. Syed Salman Ali and Dr. Muhammad Mustafa for their help as coordinator in the initial phases of study.

## COMMENTS

### Background

Chronic hepatitis D is a difficult to treat infection. Six months post treatment response is seen only in one quarter of the patients treated with pegylated interferon alfa (PEG-IFN $\alpha$ ).

### Research frontiers

In an attempt to improve the response of PEG-IFN, the authors combined entecavir which is a reverse transcriptase inhibitor.

### Innovations and breakthroughs

This is the first study to evaluate the efficacy of subcutaneous PEG-IFN with oral entecavir compared to PEG-IFN alone for the treatment of hepatitis D infection.

### Applications

This study showed that the combination treatment did not have any additional benefit in terms of hepatitis D virus (HDV) RNA suppression and hepatitis B surface antigen reduction as compared to PEG-IFN alone.

## Terminology

HDV is a small spherical enveloped RNA virus. It is considered to be a subviral satellite because it can propagate only in the presence of the hepatitis B virus. There is no satisfactory treatment available to treat this infection. However, PEG-IFN is often used.

## Peer-review

The paper indicates in a randomized trial that the addition of entecavir to PEG-IFN does not increase efficacy vs PEG-IFN monotherapy in the treatment of chronic hepatitis D. The data are valuable as they extend and confirm previous anecdotal reports.

## REFERENCES

- 1 **Qureshi H**, Bile KM, Jooma R, Alam SE, Afridi HU. Prevalence of hepatitis B and C viral infections in Pakistan: findings of a national survey appealing for effective prevention and control measures. *East Mediterr Health J* 2010; **16** Suppl: S15-S23 [PMID: 21495584]
- 2 **Mumtaz K**, Hamid SS, Adil S, Afaq A, Islam M, Abid S, Shah HA, Jafri W. Epidemiology and clinical pattern of hepatitis delta virus infection in Pakistan. *J Gastroenterol Hepatol* 2005; **20**: 1503-1507 [PMID: 16174065 DOI: 10.1111/j.1440-1746.2005.03857.x]
- 3 **Abbas Z**, Khan MA, Salih M, Jafri W. Interferon alpha for chronic hepatitis D. *Cochrane Database Syst Rev* 2011; **(12)**: CD006002 [PMID: 22161394 DOI: 10.1002/14651858.CD006002.pub2]
- 4 **Wedemeyer H**. Hepatitis D revival. *Liver Int* 2011; **31** Suppl 1: 140-144 [PMID: 21205152 DOI: 10.1111/j.1478-3231.2010.02408.x]
- 5 **Abbas Z**, Memon MS, Mithani H, Jafri W, Hamid S. Treatment of chronic hepatitis D patients with pegylated interferon: a real-world experience. *Antivir Ther* 2014; **19**: 463-468 [PMID: 24423484 DOI: 10.3851/IMP2728]
- 6 **Wedemeyer H**, Yurdaydin C, Dalekos GN, Erhardt A, Çakaloğlu Y, Değertekin H, Gürel S, Zeuzem S, Zachou K, Bozkaya H, Koch A, Bock T, Dienes HP, Manns MP. Peginterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med* 2011; **364**: 322-331 [PMID: 21268724 DOI: 10.1056/NEJMoa0912696]
- 7 **Wedemeyer H**, Yurdaydin C, Ernst S, Caruntu FA, Curescu MG, Yalcin K, Akarca US, Gurel SG, Zeuzem S, Erhardt A, Luth S, Papatheodoridis GV, Keskin O, Port K, Radu M, Celen MK, Ildeman R, Stift J, Heidrich B, Mederacke I, Hardtke S, Koch A, H.P. Dienes HP, Manns MP, HIDIT-2 Study Group. Prolonged therapy of hepatitis delta for 96 weeks with pegylated-interferon-a-2a plus tenofovir or Placebo does not prevent HDV RNA relapse after Treatment: the HIDIT-2 study. *J Hepatol* 2014; **60** (Suppl 1): S2-S3
- 8 **Brouwer WP**, Xie Q, Sonneveld MJ, Zhang N, Zhang Q, Tabak F, Streinu-Cercel A, Wang JY, Idilman R, Reesink HW, Diculescu M, Simon K, Voiculescu M, Akdogan M, Mazur W, Reijnders JG, Verhey E, Hansen BE, Janssen HL. Adding pegylated interferon to entecavir for hepatitis B e antigen-positive chronic hepatitis B: A multicenter randomized trial (ARES study). *Hepatology* 2015; **61**: 1512-1522 [PMID: 25348661 DOI: 10.1002/hep.27586]
- 9 **Batts KP**, Ludwig J. Chronic hepatitis. An update on terminology and reporting. *Am J Surg Pathol* 1995; **19**: 1409-1417 [PMID: 7503362]
- 10 **Yurdaydin C**. Treatment of chronic delta hepatitis. *Semin Liver Dis* 2012; **32**: 237-244 [PMID: 22932972 DOI: 10.1055/s-0032-1323629]
- 11 **Erhardt A**, Gerlich W, Starke C, Wend U, Donner A, Sagir A, Heintges T, Häussinger D. Treatment of chronic hepatitis delta with pegylated interferon-alpha2b. *Liver Int* 2006; **26**: 805-810 [PMID: 16911462 DOI: 10.1111/j.1478-3231.2006.01279.x]
- 12 **Castelnau C**, Le Gal F, Ripault MP, Gordien E, Martinot-Peignoux M, Boyer N, Pham BN, Maylin S, Bedossa P, Dény P, Marcellin P, Gault E. Efficacy of peginterferon alpha-2b in chronic hepatitis delta: relevance of quantitative RT-PCR for follow-up. *Hepatology* 2006; **44**: 728-735 [PMID: 16941695 DOI: 10.1002/hep.21325]
- 13 **Heller T**, Rotman Y, Koh C, Clark S, Haynes-Williams V, Chang R, McBurney R, Schmid P, Albrecht J, Kleiner DE, Ghany MG, Liang TJ, Hoofnagle JH. Long-term therapy of chronic delta hepatitis with peginterferon alfa. *Aliment Pharmacol Ther* 2014; **40**: 93-104 [PMID: 24815494 DOI: 10.1111/apt.12788]
- 14 **Manesis EK**, Schina M, Le Gal F, Agelopoulos O, Papaioannou C, Kalligeros C, Arseniou V, Manolakopoulos S, Hadziyannis ES, Gault E, Koskinas J, Papatheodoridis G, Archimandritis AJ. Quantitative analysis of hepatitis D virus RNA and hepatitis B surface antigen serum levels in chronic delta hepatitis improves treatment monitoring. *Antivir Ther* 2007; **12**: 381-388 [PMID: 17591028]
- 15 **Ouzan D**, Pénaranda G, Joly H, Halfon P. Optimized HBsAg titer monitoring improves interferon therapy in patients with chronic hepatitis delta. *J Hepatol* 2013; **58**: 1258-1259 [PMID: 23318602 DOI: 10.1016/j.jhep.2012.12.019]
- 16 **Manesis EK**, Hadziyannis ES, Angelopoulou OP, Hadziyannis SJ. Prediction of treatment-related HBsAg loss in HBeAg-negative chronic hepatitis B: a clue from serum HBsAg levels. *Antivir Ther* 2007; **12**: 73-82 [PMID: 17503750]
- 17 **Moatter T**, Abbas Z, Shabir S, Jafri W. Clinical presentation and genotype of hepatitis delta in Karachi. *World J Gastroenterol* 2007; **13**: 2604-2607 [PMID: 17552010 DOI: 10.3748/wjg.v13.i18.2604]
- 18 **Abbas Z**, Muzaffar R, Siddiqui A, Naqvi SA, Rizvi SA. Genetic variability in the precore and core promoter regions of hepatitis B virus strains in Karachi. *BMC Gastroenterol* 2006; **6**: 20 [PMID: 16863587 DOI: 10.1186/1471-230X-6-20]
- 19 **Heidrich B**, Yurdaydin C, Kabaçam G, Ratsch BA, Zachou K, Bremer B, Dalekos GN, Erhardt A, Tabak F, Yalcin K, Gürel S, Zeuzem S, Cornberg M, Bock CT, Manns MP, Wedemeyer H. Late HDV RNA relapse after peginterferon alpha-based therapy of chronic hepatitis delta. *Hepatology* 2014; **60**: 87-97 [PMID: 24585488 DOI: 10.1002/hep.27102]

**P- Reviewer:** Cunha C, Gatselis NK, Rizzetto M

**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Liu SQ



## Direct acting antiviral therapy is curative for chronic hepatitis C/autoimmune hepatitis overlap syndrome

Farhad Sahebjam, Cristina H Hajdu, Esther Nortey, Samuel H Sigal

Farhad Sahebjam, Esther Nortey, Samuel H Sigal, Division of Gastroenterology, New York University School of Medicine, New York, NY 10016, United States

Cristina H Hajdu, Department of Pathology, New York University School of Medicine, New York, NY 10016, United States

Samuel H Sigal, Division of Gastroenterology and Liver Diseases, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY 10467, United States

**Author contributions:** Sahebjam F and Sigal SH designed the report; Hajdu CH performed pathologic analysis; Nortey E acquired clinical data.

**Institutional review board statement:** This case report was exempt from the Institutional Review Board standards at the New York University School of Medicine.

**Informed consent statement:** The patients involved in this case report gave verbal informed consent to be included. Written informed consent was exempt from the Institutional Review Board standards at the New York University School of Medicine.

**Conflict-of-interest statement:** Samuel H Sigal received research support from AbbVie and Gilead, consulting fees from AbbVie and Gilead, and was a member of the Gilead Speakers' Bureau. The other authors have no conflicts of interest to declare.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Samuel H Sigal, MD, Division of Gastroenterology and Liver Diseases, Montefiore Medical Center and Albert Einstein College of Medicine, 111 210<sup>th</sup> Street, Rosenthal 2, Bronx, NY 10467, United States. [ssigal@montefiore.org](mailto:ssigal@montefiore.org)  
 Telephone: +1-718-9206240

Fax: +1-917-3988466

Received: December 29, 2015  
 Peer-review started: January 1, 2016  
 First decision: February 2, 2016  
 Revised: February 19, 2016  
 Accepted: March 9, 2016  
 Article in press: March 14, 2016  
 Published online: May 18, 2016

### Abstract

Autoimmune phenomena are common in patients with chronic hepatitis C. Management of chronic hepatitis C/autoimmune hepatitis syndrome has until recently been problematic due to the adverse effects of interferon on autoimmune processes and immunosuppression on viral replication. In this report we describe 3 patients with chronic hepatitis C/autoimmune hepatitis overlap syndrome who responded rapidly to direct acting antiviral therapy. The resolution of the autoimmune process supports a direct viral role in its pathophysiology.

**Key words:** Hepatitis C; Autoimmune hepatitis; Overlap syndrome; Direct acting antiviral therapy

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Autoimmune phenomena are common in patients with chronic hepatitis C, and occasionally patients with chronic hepatitis C have concomitant features of autoimmune hepatitis (AIH). Management of these patients has until recently been problematic due to the adverse effects of interferon on autoimmune processes and immunosuppression on viral replication. In this report we describe 3 patients with chronic hepatitis C/AIH overlap syndrome who responded rapidly to direct acting anti-viral therapy with prompt normalization of liver tests and progressive decrease in the serologic



markers of AIH. The resolution of the autoimmune process supports a direct viral role in its pathophysiology.

Sahebjam F, Hajdu CH, Nortey E, Sigal SH. Direct acting antiviral therapy is curative for chronic hepatitis C/autoimmune hepatitis overlap syndrome. *World J Hepatol* 2016; 8(14): 632-636 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i14/632.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i14.632>

## INTRODUCTION

Chronic hepatitis C virus (HCV) infection has a worldwide prevalence of 2%-3% and is a leading cause of cirrhosis and hepatocellular carcinoma in Western countries<sup>[1]</sup>. In 40%-74% of patients, HCV is associated with autoimmune phenomena ranging from positive serologic markers to wide spread autoimmune diseases, including rheumatoid arthritis, mixed cryoglobulinemia, B-cell lymphoma, systemic lupus erythematosus, sicca syndrome, autoimmune thyroiditis, and autoimmune hepatitis (AIH).

AIH is a condition of unknown etiology characterized by a progressive inflammatory process with histopathologic changes that include interface hepatitis with a predominant lymphoplasmacytic infiltrate, elevated transaminases and immunoglobulin levels, and the presence of autoantibodies. To standardize diagnostic criteria, the International Autoimmune Hepatitis Group (IAHG) devised a scoring system to categorize patients as definite AIH, probable AIH and not AIH<sup>[2]</sup> in which points are distributed based on the presence of anti-nuclear antibody (ANA), anti smooth antibody (ASMA or F-Actin Antibody), anti-soluble liver/liver pancreas antigen, immunoglobulin G (IgG) level, liver histology and the absence of viral hepatitis.

In patients with chronic hepatitis C, markers of AIH are frequently present. Up to 40% of HCV patients may have positive ANA, SMA, and LKM-1 autoantibodies<sup>[3]</sup>. In most cases, titers are usually low, and cases with positive serologies are in general histologically indistinguishable from those without detectable antibodies. However, patients with an autoimmune overlap syndrome in whom liver biopsies reveal features of both chronic hepatitis C and inflammatory features characteristic of AIH are occasionally encountered<sup>[4]</sup>.

The treatment of patients with HCV/AIH overlap syndrome has until recently been challenging. Because interferon (IFN) therapy for chronic HCV can trigger latent AIH and lead to severe hepatic failure, there are significant concerns about its use in patients with preexisting autoimmune processes<sup>[5]</sup>. Immunosuppression, on the hand, has an adverse effect on viral replication<sup>[6]</sup>. In this report, we present three patients with AIH/hepatitis C overlap syndrome in whom both processes rapidly responded to interferon-free antiviral therapy.

## CASE REPORT

### Case report 1

A 22-year-old Caucasian man with chronic hepatitis C presented with mild generalized fatigue and anhedonia. Risk factors for infection included intravenous drug use and possible vertical transmission. Physical examination did not reveal stigmata of advanced liver disease. Initial laboratory evaluation was remarkable for markedly elevated aspartate aminotransferase (AST) 314 U/L (normal, 15-46) and alanine aminotransferase (ALT) 608 U/L (normal, 15-65) levels, total bilirubin 0.6 mg/dL (normal, 0-1), albumin 4.5 g/dL (normal, 3.5-5), international normalized ratio 1.1, platelet count of 156 K/uL (normal 150-400), HCV RNA viral load of 1410000 IU/mL (6.15 logs), HCV genotype 1. Serological markers of AIH were remarkable for elevated IgG at 2100 mg/dL (normal, 700-1600), positive ANA (1:80), and positive F-actin antibody of 37 units (normal, 0-19).

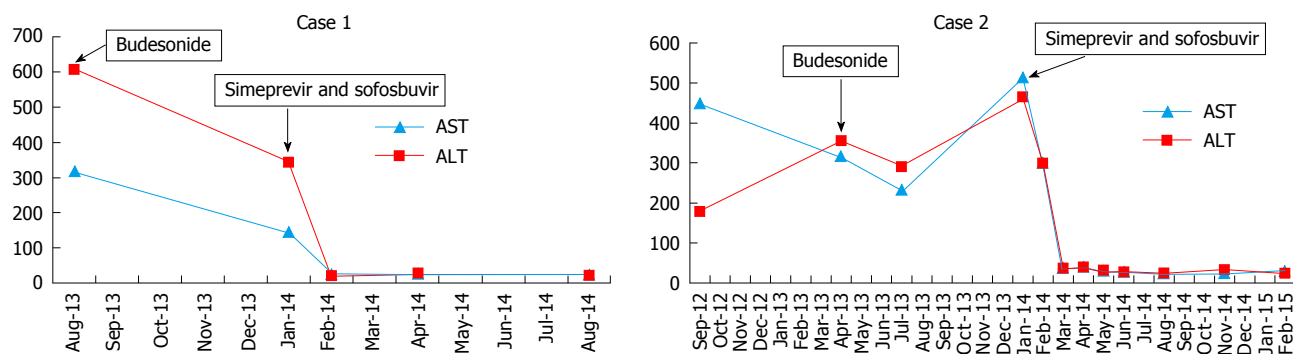
Liver biopsy showed established cirrhosis with mild to moderate activity. Inflammatory infiltrates composed of lymphocytes with lymphoid aggregate formation, polymorphonuclear cells and scattered plasma cells were present in the portal tracts, interface and fibrous septae. A brisk lobular lymphoplasmacytic infiltrate with rare acidophil bodies was also present. Due to the severity of the inflammatory activity, the overall histologic appearance was suggestive of an autoimmune process with a simplified IAHG, diagnostic score of 6 (probable AIH).

The patient was treated with budesonide 3 mg three times daily for two months with limited biochemical response (ALT, 343 U/L) and response in either the total protein level (8.2 g/dL, pre-; 7.6 g/dL, post-) or HCV viral load [1523252 IU/ML (6.18 logs)]. Budesonide was then discontinued, and a 12 wk interferon-free regimen of simeprevir and sofosbuvir started with prompt normalization of aminotransferase levels, normalization of the IgG level (1350 mg/dL), and achievement of a sustained response. F-Actin antibody titer did not change following treatment (37 U before, 36 U after) (Figure 1).

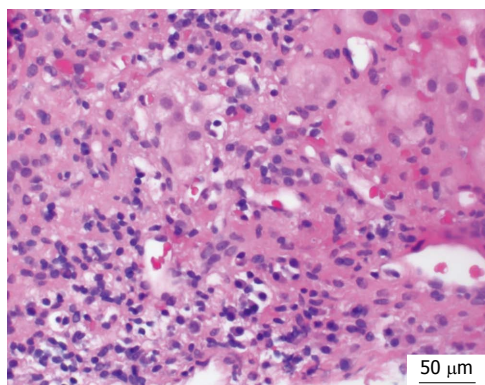
### Case report 2

A 62-year-old man with chronic hepatitis C presented with generalized fatigue. Risk factors for hepatitis C infection included a blood transfusion at birth. Past medical history was significant for epilepsy and depression. Physical examination did not reveal stigmata of chronic liver disease. Laboratory evaluation was remarkable for AST 447 U/L, ALT 480 U/L, total bilirubin of 0.8 mg/dL, albumin 3.9 g/dL, international normalized ratio 1.4, HCV genotype 1, platelet count of 115 K/uL, HCV RNA viral load 1660000 IU/mL. Serologic markers of AIH were remarkable for elevated IgG at 3030 mg/dL, ANA titer 1:80, and F-actin antibody titer of 24 U.

Liver biopsy revealed mild to moderate portal infiltration consisting of lymphocytes with lymphoid aggregate formation, plasma cells and eosinophils. Interface



**Figure 1** Aspartate aminotransferase and alanine aminotransferase levels in case 1 and case 2 prior and after immunosuppression and hepatitis C infection treatment. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.



**Figure 2** Liver biopsy of case 3 demonstrating interface activity with abundant plasma cell infiltration (600 × H and E staining).

activity was moderate with plasma cells easily identified and lobular inflammation was mild to moderate with acidophil bodies readily found. Macrovesicular steatosis in 30% to 40% of the specimen with focal ballooning degeneration and focal bridging fibrosis (stage 3) was also present. The moderate interface activity with numerous plasma cells was consistent with an autoimmune process with a simplified IAHG diagnostic score of 6 (probable AIH)<sup>[7]</sup>.

The patient declined interferon therapy and was started on budesonide 3 mg twice daily without significant effect on ALT or IgG levels (Figure 1) and HCV viral load remain unchanged (1177912 IU/mL). After 6 mo, budesonide was tapered to 3 mg daily, and a repeat liver biopsy was performed which revealed persistent portal and lobular inflammation, worsening ballooning degeneration and progression to cirrhosis. Budesonide was discontinued, and interferon-free therapy with 12 wk of simeprevir and sofosbuvir initiated. Aminotransferase levels promptly normalized. HCV RNA was undetectable by treatment week 8, and a sustained virologic response was achieved. During and after completion of therapy, IgG and F-actin levels progressively decreased (2070 mg/dL, 15 units respectively), and ANA titer was negative one year after completion of antiviral therapy.

### Case report 3

A 62-year-old African American woman with a history

of alcoholism was referred for treatment of chronic hepatitis C. Risk factors included a history of intravenous drug abuse. Liver biopsy 3 years previously revealed stage IV fibrosis and moderate necroinflammatory activity with plasma cell component (Figure 2). Physical examination was significant for an enlarged left lobe of liver. Initial laboratory evaluation was remarkable for mildly elevated AST 68 U/L, ALT of 84 U/L levels, total bilirubin 0.8 mg/dL, albumin 3.6 g/dL, total protein 8.89 g/dL, international normalized ratio 1.2, platelet count of 85 K/uL, HCV RNA viral load of 285000 IU/mL (5.46 logs), HCV genotype 1a. Serological markers of AIH were remarkable for elevated IgG 3250 mg/dL, positive ANA, F-actin antibody titer of 37.

The patient was started on ledipasvir/sofosbuvir. Viral load became undetectable within 4 wk, and she achieved SVR with 12 wk of treatment. At the end of therapy, aminotransferase levels were normal (AST, 36; ALT, 27). ANA became negative, serum total protein decreased to normal level of 7.6 g/dL (normal, 6.3-8.2), and serum IgG decreased to 2300 mg/dL (normal, 700-1600). F-actin antibody titer also decreased to the normal range (17 U).

## DISCUSSION

In this report, we present the response of HCV/AIH overlap syndrome to direct acting antiviral (DAA) therapy. The diagnosis of overlap syndrome was established by the presence of active viremia and characteristic biochemical, serologic, and histopathologic features of AIH. Although only a simplified AIH score of 6 was present, it is important to note that only a maximum score of 6 is possible if viral hepatitis component is not included. There were no biochemical or immunologic responses, but rather worsening pathologic changes in the one case in which a repeat liver biopsy was performed. In contrast, there was a prompt normalization of liver biochemistries and resolution of serologic features of AIH in response to DAA therapy in all three cases.

Although the pathogenesis of AIH is incompletely understood, a frequently cited mechanism is a reaction to viral infections in genetically susceptible persons. Cross-reaction between viral particles and liver auto-

antigens has been proposed as a trigger mechanism of virus induced AIH. Activation of resting T cells by inducing the release of a variety of cytokines, and polyclonal activation of lymphocytes has also been proposed to play a role. An association with measles virus was first proposed in 1987 after identification of persistent measles virus genome in lymphocytes and high antibody titers in 12 of 18 patients with AIH<sup>[8]</sup>. Vento *et al*<sup>[9]</sup> reported the development of AIH in healthy relatives of patients with AIH that was associated with cases of infectious mononucleosis due to Epstein-Barr virus (EBV) infection. In these cases, the development and persistence of autoantibodies to the asialoglycoprotein receptor were documented, and it was proposed that cross reactivity between asialoglycoprotein and EBV antibodies caused an autoimmune reaction. Recently, high prevalence of hepatitis E antibody positivity was found in patients with AIH, compared to healthy, and individuals with HCV or HBV infection<sup>[10]</sup>. Other viral infections that have been associated with AIH include hepatitis B, varicella-zoster and rubella<sup>[11]</sup>.

There are several proposed mechanisms in the pathogenesis of autoimmunity in HCV. HCV facilitates lymphotropism in which clonal B-lymphocyte expansion leads to widespread autoantibody production. The HCV envelope protein E2 is able to bind to the CD81 molecule expressed on hepatocytes and B-lymphocytes, resulting in a dysregulation of cytokines with an enhanced Th1 immune response. This may cause self-reactive lymphocytes to induce autoimmunity in the chronic HCV patient. Recently, cross-reactivity between CYP2E1 and specific sequences in HCV-NS5b protein has been shown responsible for the production of auto-antibodies targeting self-proteins<sup>[12]</sup>.

There are no established treatment strategies for HCV/AIH overlap syndrome. IFN alone previously was avoided due to the concern about its potential to induce an autoimmune flare. Early reports of steroid therapy prior to the era in which HCV RNA testing was available frequently included RNA negative patients, making determination of efficacy difficult to assess. Several small series and case reports have advocated pre-treatment with corticosteroids with or without azathioprine followed by IFN-based therapy to prevent an IFN-induced flare<sup>[13-15]</sup>.

The development of interferon-free direct acting antiviral regimens has revolutionized the treatment of HCV. These new treatments are potent, safe, and achieve rapid normalization of aminotransferase levels and viral suppression within the first few weeks of therapy. This is the first description of DAA therapy for HCV/AIH overlap syndrome. The rapid normalization of aminotransferase level and suppression of viral RNA followed by a gradual disappearance of autoimmune markers without immunosuppression supports the hypothesis that the viral infection triggers the autoimmune response. Based on our cases, we propose DAA agents as an initial treatment for patients with HCV/AIH overlap syndrome and early reassessment of response.

Corticosteroids and immunosuppression should be reserved for those who are refractory to this approach.

## COMMENTS

### Case characteristics

Three patients presented for treatment of chronic hepatitis C.

### Clinical diagnosis

Severe hepatitis with markedly elevated aminotransferase levels.

### Differential diagnosis

Chronic hepatitis C with severe activity or superimposed second process such as autoimmune hepatitis (AIH).

### Laboratory diagnosis

Positive anti-nuclear antibody and anti-smooth muscle antibody, elevated immunoglobulin G (IgG) level.

### Pathologic diagnosis

Liver biopsy reveal in all three cases prominent numbers of plasma cells compatible with AIH.

### Treatment

Treatment with steroids in the form of budesonide was not effective. However, there was prompt resolution of both the chronic hepatitis C and AIH with direct acting anti-viral therapy.

### Related reports

Reports have suggested an infectious precipitant for AIH. Previous therapeutic approach for the treatment of chronic hepatitis C/AIH have usually involved steroid therapy followed by interferon-based therapy with variable success.

### Term explanation

In chronic hepatitis C/AIH overlap syndrome, hepatitis C viremia is present in patients with AIH as defined by the presence of anti-nuclear and anti-smooth muscle antibodies, elevated IgG levels, and lymphoplasmacytic infiltrates on liver biopsy.

### Experiences and lessons

Resolution of both the viral and AIH in response to direct acting antiviral therapy supports the hypothesis that the autoimmune process is caused by the viral infection.

### Peer-review

In the present manuscript, the authors described 3 case patients with chronic HCV/AIH syndrome who were treated with direct acting antiviral (DAA). DAA treatment promptly induced the normalization of liver biochemistries and the resolution of serological features of AIH. The present report is potentially easily understandable and very interesting.

## REFERENCES

- 1 Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41-52 [PMID: 11439948 DOI: 10.1056/NEJM200107053450107]
- 2 Yeoman AD, Westbrook RH, Al-Chalabi T, Carey I, Heaton ND, Portmann BC, Heneghan MA. Diagnostic value and utility of the simplified International Autoimmune Hepatitis Group (IAIHG) criteria in acute and chronic liver disease. *Hepatology* 2009; **50**: 538-545 [PMID: 19575457 DOI: 10.1002/hep.23042]
- 3 Chrétien P, Chousterman M, Abd Alsamad I, Ozenne V, Rosa I, Barrault C, Lons T, Hagège H. Non-organ-specific autoantibodies in chronic hepatitis C patients: association with histological activity

- and fibrosis. *J Autoimmun* 2009; **32**: 201-205 [PMID: 19324518 DOI: 10.1016/j.jaut.2009.02.005]
- 4 **Czaja AJ**, Carpenter HA. Histological findings in chronic hepatitis C with autoimmune features. *Hepatology* 1997; **26**: 459-466 [PMID: 9252159 DOI: 10.1002/hep.510260229]
- 5 **Kogure T**, Ueno Y, Fukushima K, Nagasaki F, Inoue J, Kakazu E, Matsuda Y, Kido O, Nakagome Y, Kimura O, Obara N, Wakui Y, Iwasaki T, Shimosegawa T. Fulminant hepatic failure in a case of autoimmune hepatitis in hepatitis C during peg-interferon-alpha 2b plus ribavirin treatment. *World J Gastroenterol* 2007; **13**: 4394-4397 [PMID: 17708618 DOI: 10.3748/wjg.v13.i32.4394]
- 6 **Calleja JL**, Albillos A, Cacho G, Iborra J, Abreu L, Escartín P. Interferon and prednisone therapy in chronic hepatitis C with non-organ-specific antibodies. *J Hepatol* 1996; **24**: 308-312 [PMID: 8778197 DOI: 10.1016/S0168-8278(96)80009-2]
- 7 **Hennes EM**, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL, Bittencourt PL, Porta G, Boberg KM, Hofer H, Bianchi FB, Shibata M, Schramm C, Eisenmann de Torres B, Galle PR, McFarlane I, Dienes HP, Lohse AW. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008; **48**: 169-176 [PMID: 18537184 DOI: 10.1002/hep.22322]
- 8 **Robertson DA**, Zhang SL, Guy EC, Wright R. Persistent measles virus genome in autoimmune chronic active hepatitis. *Lancet* 1987; **2**: 9-11 [PMID: 2885546 DOI: 10.1016/S0140-6736(87)93051-0]
- 9 **Vento S**, Guella L, Mirandola F, Cainelli F, Di Perri G, Solbiati M, Ferraro T, Concia E. Epstein-Barr virus as a trigger for autoimmune hepatitis in susceptible individuals. *Lancet* 1995; **346**: 608-609 [PMID: 7651006 DOI: 10.1016/S0140-6736(95)91438-2]
- 10 **Pischke S**, Gisa A, Suneetha PV, Wiegand SB, Taubert R, Schlue J, Wursthorn K, Bantel H, Raupach R, Bremer B, Zacher BJ, Schmidt RE, Manns MP, Rifai K, Witte T, Wedemeyer H. Increased HEV seroprevalence in patients with autoimmune hepatitis. *PLoS One* 2014; **9**: e85330 [PMID: 24465537 DOI: 10.1371/journal.pone.0085330]
- 11 **Kalvenes MB**, Haukenes G, Nysaeter G, Kalland KH, Myrmet H. Raised levels of antibodies to human viruses at the clinical onset of autoimmune chronic active hepatitis. *J Viral Hepat* 1995; **2**: 159-164 [PMID: 7493312 DOI: 10.1111/jvh.12492]
- 12 **Sutti S**, Vidali M, Mombello C, Sartori M, Ingelman-Sundberg M, Albano E. Breaking self-tolerance toward cytochrome P4502E1 (CYP2E1) in chronic hepatitis C: possible role for molecular mimicry. *J Hepatol* 2010; **53**: 431-438 [PMID: 20576306 DOI: 10.1016/j.jhep.2010.03.030]
- 13 **Azhar A**, Niazi MA, Tufail K, Malek AH, Balasubramanian M, Araya V. A new approach for treatment of hepatitis C in hepatitis C-autoimmune hepatitis overlap syndrome. *Gastroenterol Hepatol (N Y)* 2010; **6**: 233-236 [PMID: 20567575]
- 14 **Oeda S**, Mizuta T, Isoda H, Kuwashiro T, Oza N, Iwane S, Takahashi H, Kawaguchi Y, Eguchi Y, Toda S, Ozaki I, Anzai K, Fujimoto K. Efficacy of pegylated interferon plus ribavirin in combination with corticosteroid for two cases of combined hepatitis C and autoimmune hepatitis. *Clin J Gastroenterol* 2012; **5**: 141-145 [PMID: 22593772 DOI: 10.1007/s12328-012-0295-4]
- 15 **Schiano TD**, Te HS, Thomas RM, Hussain H, Bond K, Black M. Results of steroid-based therapy for the hepatitis C-autoimmune hepatitis overlap syndrome. *Am J Gastroenterol* 2001; **96**: 2984-2991 [PMID: 11693337 DOI: 10.1111/j.1572-0241.2001.04672.x]

**P- Reviewer:** Ito H, Lau WY, Morales-Gonzalez J  
**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

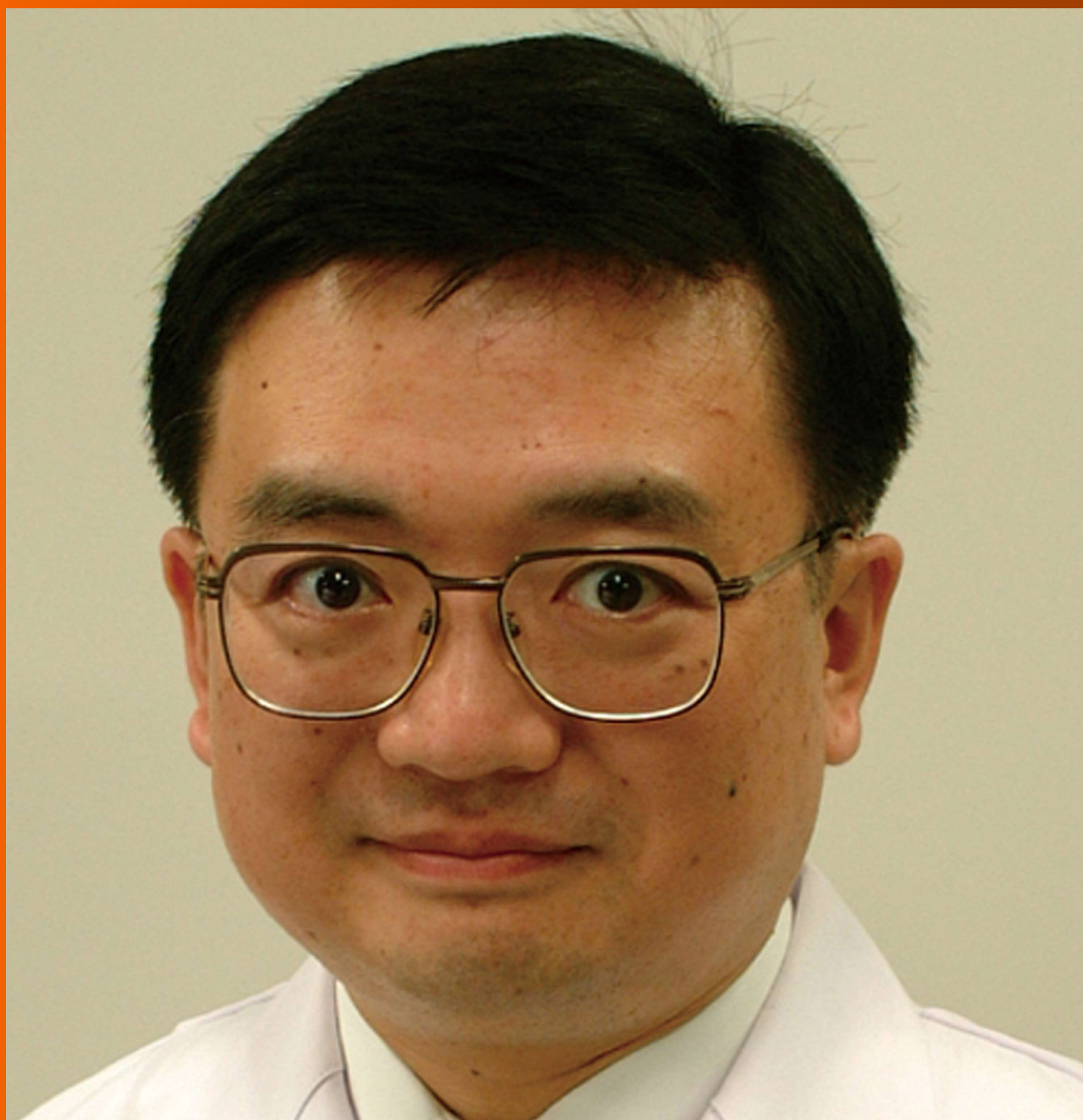
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 May 28; 8(15): 637-664





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**Stephen DH Malnick, *Rehovot***Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*

**Jordan**Kamal E Bani-Hani, *Zarqa***Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**Angela Peltec, *Chishinev***Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**CA Asabamaka Onyekwere, *Lagos***Pakistan**Bikha Ram Devrajani, *Jamshoro***Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**Jacek Zielinski, *Gdansk***Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**Reem Al Olaby, *Doha***Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**Ser Yee Lee, *Singapore***South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**ORIGINAL ARTICLE****Retrospective Cohort Study**

- 637 Hepatitis B surface antigen clearance in inactive hepatitis B surface antigen carriers treated with peginterferon alfa-2a

*Li MH, Xie Y, Zhang L, Lu Y, Shen G, Wu SL, Chang M, Mu CQ, Hu LP, Hua WH, Song SJ, Zhang SF, Cheng J, Xu DZ*

**Retrospective Study**

- 644 Outcome analysis of management of liver trauma: A 10-year experience at a trauma center

*She WH, Cheung TT, Dai WC, Tsang SHY, Chan ACY, Tong DKH, Leung GKK, Lo CM*

**Observational Study**

- 649 Hepatitis C virus infection in Argentina: Burden of chronic disease

*Ridruejo E, Bessone F, Daruich JR, Estes C, Gadano AC, Razavi H, Villamil FG, Silva MO*

**CASE REPORT**

- 659 Host factors are dominant in the development of post-liver transplant non-alcoholic steatohepatitis

*Boga S, Munoz-Abraham AS, Rodriguez-Davalos MI, Emre SH, Jain D, Schilsky ML*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Kazuaki Inoue, MD, PhD, Associate Professor, Department of Internal Medicine, Division of Gastroenterology, Showa University Fujigaoka Hospital, Yokohama 227-8501, Japan

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Jin-Lei Wang*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: editorialoffice@wjnet.com  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjnet.com](mailto:bpgoffice@wjnet.com)  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

**PUBLICATION DATE**  
May 28, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

Full instructions are available online at [http://www.wjnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjnet.com/bpg/g_info_20160116143427.htm)

**ONLINE SUBMISSION**

<http://www.wjnet.com/esps/>



Retrospective Cohort Study

# Hepatitis B surface antigen clearance in inactive hepatitis B surface antigen carriers treated with peginterferon alfa-2a

Ming-Hui Li, Yao Xie, Lu Zhang, Yao Lu, Ge Shen, Shu-Ling Wu, Min Chang, Cai-Qin Mu, Lei-Ping Hu, Wen-Hao Hua, Shu-Jing Song, Shu-Feng Zhang, Jun Cheng, Dao-Zhen Xu

Ming-Hui Li, Yao Xie, Lu Zhang, Yao Lu, Ge Shen, Shu-Ling Wu, Min Chang, Cai-Qin Mu, Lei-Ping Hu, Jun Cheng, Dao-Zhen Xu, Liver Disease Center, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China

Wen-Hao Hua, Shu-Jing Song, Shu-Feng Zhang, Clinical Test Center, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China

**Author contributions:** Li MH and Xie Y conceived the study, participated in the study design, and drafted the manuscript; Zhang L, Lu Y, Shen G, Wu SL, Chang M, Mu CQ and Hu LP supervised all aspects of the study implementation; the remaining authors coordinated testing and helped to draft the manuscript.

**Institutional review board statement:** The study was reviewed and approved by the Beijing Ditan Hospital Institutional Review Board.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** There was no conflict of interest and this study was carried out as a part of our routine work.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Yao Xie, PhD, Liver Disease Center, Beijing Ditan Hospital, Capital Medical University, 8 Jingshun East Street, Beijing 100015, China. [xieyao00120184@sina.com](mailto:xieyao00120184@sina.com)

Telephone: +86-10-84322146  
Fax: +86-10-84322146

Received: February 11, 2016  
Peer-review started: February 12, 2016  
First decision: March 9, 2016  
Revised: April 6, 2016  
Accepted: May 7, 2016  
Article in press: May 9, 2016  
Published online: May 28, 2016

## Abstract

**AIM:** To examine the association between interferon (IFN) therapy and loss of hepatitis B surface antigen (HBsAg) in inactive HBsAg carriers.

**METHODS:** This was a retrospective cohort study in inactive HBsAg carriers, who were treatment-naïve, with a serum HBsAg level < 100 IU/mL and an undetectable hepatitis B virus (HBV) DNA level (< 100 IU/mL). All the 20 treated patients received subcutaneous PEG-IFN alfa-2a 180 µg/wk for 72 wk and were then followed for 24 wk. There were 40 untreated controls matched with 96 wk of observation. Serum HBsAg, HBV DNA, and alanine aminotransferases were monitored every 3 mo in the treatment group and every 3-6 mo in the control group.

**RESULTS:** Thirteen (65.0%) of 20 treated patients achieved HBsAg loss, 12 of whom achieved HBsAg seroconversion. Mean HBsAg level in treated patients decreased to  $6.69 \pm 13.04$  IU/mL after 24 wk of treatment from a baseline level of  $26.22 \pm 33.00$  IU/mL. Serum HBV DNA level remained undetectable (< 100 IU/mL) in all treated patients during the study. HBsAg level of the control group decreased from  $25.72 \pm 25.58$  IU/mL at baseline to  $17.11 \pm 21.62$  IU/mL at

week 96 ( $P = 0.108$ ). In the control group, no patient experienced HBsAg loss/seroconversion, and two (5.0%) developed HBV reactivation.

**CONCLUSION:** IFN treatment results in HBsAg loss and seroconversion in a considerable proportion of inactive HBsAg carriers with low HBsAg concentrations.

**Key words:** Chronic hepatitis B surface antigen carriers; Inactive hepatitis B surface antigen carriers; Interferon; Peginterferon alfa-2a; Hepatitis B surface antigen loss/seroconversion

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This study examined the association between interferon (IFN) therapy and loss of hepatitis B surface antigen (HBsAg) in inactive HBsAg carriers. This was a retrospective cohort study in inactive HBsAg carriers with a serum HBsAg level  $< 100$  IU/mL and a persistently undetectable hepatitis B virus (HBV) DNA level ( $< 100$  IU/mL). All the 20 treated patients received subcutaneous PEG-IFN alfa-2a 180  $\mu$ g/wk for 72 wk and were then followed for 24 wk. IFN treatment resulted in HBsAg loss (65.0%) and seroconversion in a considerable proportion of inactive HBsAg carriers with low HBsAg concentrations. In the control group, no patient experienced HBsAg loss/seroconversion, and 2 (5.0%) developed HBV reactivation.

Li MH, Xie Y, Zhang L, Lu Y, Shen G, Wu SL, Chang M, Mu CQ, Hu LP, Hua WH, Song SJ, Zhang SF, Cheng J, Xu DZ. Hepatitis B surface antigen clearance in inactive hepatitis B surface antigen carriers treated with peginterferon alfa-2a. *World J Hepatol* 2016; 8(15): 637-643 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i15/637.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i15.637>

## INTRODUCTION

Chronic hepatitis B virus (HBV) infection is the leading cause of end-stage liver disease or hepatocellular carcinoma (HCC) throughout the world. In the nature history of chronic HBV infection, inactive hepatitis B surface antigen (HBsAg) carriers, defined as HBsAg positive, hepatitis B envelope antigen (HBeAg)-negative/antiHBe-positive, undetectable HBV DNA level and normal alanine aminotransferases (ALT) levels, frequently have good long-term clinical outcomes and thus are not recommended for antiviral treatment<sup>[1-3]</sup>. However, this inactive carrier status was not always sustained. Fourteen percent to 24% of inactive carriers have reactivation after years of quiescent disease, and 4.2% to 20% of them reverse back to HBeAg positivity<sup>[4-7]</sup>, with increased cumulative probabilities of reactivation of hepatitis B after years of follow-up<sup>[8]</sup>. Compared to a control subcohort (negative for HBsAg), inactive HBsAg

carriers have higher risks of hepatocellular carcinoma and liver-related death<sup>[9]</sup>, especially in countries with a high prevalence of HBV infection<sup>[8,9]</sup>. In contrast, 100% and 90% of patients had improvement and stable liver inflammation and liver fibrosis<sup>[10]</sup>, respectively, and no HCC occurred in patients with HBsAg clearance after interferon (IFN) treatment<sup>[11]</sup>. Nevertheless, spontaneous HBsAg loss occurred in inactive carriers only at rates from 1% to 1.5% per year observed in Caucasians<sup>[12]</sup> and Asians<sup>[13]</sup>. HBsAg clearance usually indicates recovery from HBV infection, and has been an aim of antiviral therapy<sup>[2]</sup>. In the real life, HBsAg positive people were restricted in many aspects such as work, diet, and cosmetic surgery, in China, and many of them hope to obtain HBsAg loss through effective methods.

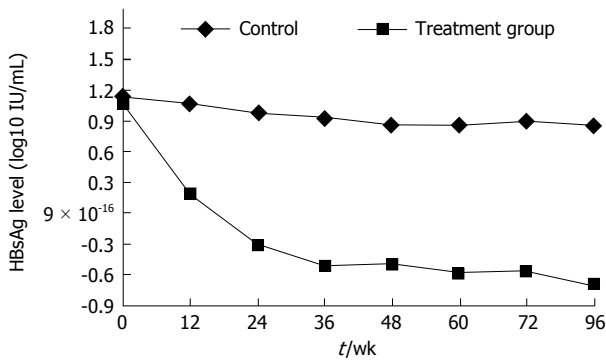
IFN treatment exerts direct antiviral as well as immunoregulatory effects<sup>[14]</sup>, and can induce specific and nonhepatotoxic degradation of nuclear HBV covalently closed circular DNA (cccDNA)<sup>[15]</sup>, and increased HBV-specific T-cell responses in chronic HBV infected patients with undetectable levels of serum HBV DNA<sup>[16]</sup>. This retrospective cohort study was conducted to evaluate the efficacy of PEG-IFN alfa-2a treatment in chronic inactive carriers with a low HBsAg level.

## MATERIALS AND METHODS

### Selection of patients

A retrospective cohort study including inactive HBsAg carriers attending the department of hepatology, Beijing Ditan Hospital, Capital Medical University between May 2008 and August 2012 was conducted. We diagnosed inactive HBsAg carriers based on their history of HBV infection, HBV DNA level, serological markers, and liver function. Patients with cirrhosis, which was diagnosed as liver stiffness  $> 9$  kPa or presence of portal hypertension (spleen enlargement with a reduction in platelet count) by FibroScan and ultrasonic examinations, were excluded. Patients who were treatment-naïve, HBsAg positive, anti-HBs-negative and HBeAg negative for more than 6 mo, had a persistently undetectable HBV DNA level ( $< 100$  IU/mL) and normal ALT levels ( $< 19$  IU/mL for females and  $< 30$  IU/mL for males, measured every 3-6 mo) during the preceding 2 years, and serum HBsAg  $< 100$  IU/mL on two occasions during the month prior to enrollment were included in the study. Patients with other liver diseases or co-infection of hepatitis C virus, hepatitis D virus, and human immunodeficiency virus, as well as those who had a history of immunosuppressive or antiviral drug usage were excluded. HBV genotyping cannot be performed due to an undetectable HBV DNA level in the subjects; however, epidemiological studies showed the HBV genotypes in China were mainly genotypes B and C<sup>[17,18]</sup>.

Participants in the treatment group contained all the patients who were willing to receive IFN treatment for achieve HBsAg clearance and had completed 72 wk of treatment with PEG-IFN alfa-2a and 24 wk of follow-up after completing the treatment. There were



**Figure 1** Mean hepatitis B surface antigen level decreased in a time-dependent manner in treated patients and was significantly lower at week 24 than at baseline. HBsAg: Hepatitis B surface antigen.

40 controls matched for age, sex, and HBsAg level, and undetectable HBV DNA with persistently normal ALT levels, and they were selected from 284 untreated patients who attended the clinic and completed 96 wk of observation during the same period as treated patients.

#### Ethics approval

The study adhered to the Declaration of Helsinki and ethics approval was obtained from the Beijing Ditan Hospital of Capital Medical University Institutional Review Board. Written informed consent was obtained from all subjects before enrolment.

#### Treatment and follow-up

The treated cohort comprised 20 patients who had received subcutaneous PEG-IFN alfa-2a at a dose of 180 µg/wk for 72 wk and had been followed for 24 wk after completing the treatment, while the control cohort comprised 40 matched patients who had finished 96 wk of observation.

None of the participants received immunosuppressive or oral antiviral drugs during the study period. In the treated patients, serum HBsAg, anti-HBs and HBV DNA levels were measured once every 3 mo, and peripheral blood neutrophil and platelet counts, and liver and kidney function tests were performed once every 1-3 mo. These biomarkers were measured once every 3-6 mo in controls.

#### Safety and efficacy assessments

Kidney and liver function biomarkers, including serum creatinine, blood urea nitrogen, ALT, aspartate aminotransferase, albumin and total bilirubin (Tbil), were measured with an automated biochemical analyzer. Peripheral blood neutrophil and platelet counts were measured with an automatic blood cell analyzer.

HBV DNA was measured with a commercially available real-time fluorescence PCR kit with a detection limit of 100 IU/mL (Piji Company, Shenzhen City, China). HBsAg concentrations were quantified by an automated chemoluminescent microparticle immunoassay (Architect i2000 HBsAg quantitative assay, Abbott Laboratories,

Abbott Park, IL, United States, sensitivity < 0.05 IU/mL; dynamic range 0.05-250 IU/mL). HBsAg loss was defined as HBsAg concentration < 0.05 IU/mL. Anti-HBs was measured with an Architect i2000 kit (Abbott Laboratories, dynamic range of 0.00-1000 mIU/mL), with concentrations ≥ 10 mIU/L being considered positive. The primary efficacy endpoints were HBsAg loss and seroconversion.

#### Statistical analysis

Unless otherwise stated, clinical and biological outcomes before and after treatment are expressed as mean ± SD or median (range), and were compared using paired Student's *t*-tests, with a *P*-value less than 0.05 being considered statistically significant. Qualitative variables are presented as counts and percentages and were compared using Fisher's exact tests. All statistical analyses were performed using SPSS statistical software version 13.0 (Chicago, IL, United States).

## RESULTS

#### Patients and clinical characteristics

A total of 60 inactive chronic HBsAg carriers were included in the study, 20 of whom were in the treated group and 40 in the control group. There were no significant differences in the baseline characteristics between the treated and control groups (Table 1). However, in the treatment group, the patients who achieved HBsAg loss had a lower baseline HBsAg level of 8.09 (3.81-22.50) IU/mL and were younger (age of 31.46 ± 12.16 years) than patients without HBsAg loss after treatment [baseline HBsAg level of 18.95 (2.85-83.00) IU/mL and age of 38.24 ± 9.25 years], but there was no significant difference.

#### HBsAg kinetics and clinical outcomes

HBsAg levels decreased with increasing treatment period in the treated group (Figure 1). Among patients treated with PEG-IFN alfa-2a, the mean HBsAg level decreased by 55.98% from baseline to week 12 (from 26.22 ± 33.00 to 11.59 ± 20.83 IU/mL, *P* = 0.108), by 74.59% from baseline to week 24 (to 6.69 ± 13.04 IU/mL, *P* = 0.024 vs baseline), and was 0.045 IU/mL (range, 0.02-2.44 IU/mL) at the end of follow-up (week 96). Of the 20 treated patients, 13 achieved HBsAg loss, of whom 12 occurred during treatment and 1 at follow-up time, with a mean of 40.62 ± 22.74 mo after the initiation of treatment, in which 12 achieved HBsAg seroconversion (Table 1). Eighty percent (8/10) of patients with an HBsAg level < 10 IU/mL achieved HBsAg loss after treatment. In the remaining seven treated patients, the mean HBsAg level decreased by 66.93% from baseline to the end of follow-up (from 37.43 ± 38.69 to 8.20 ± 15.69 IU/mL, *P* = 0.049). Serum HBV DNA remained undetectable (< 100 IU/mL) in all treated patients during the treatment and follow-up periods, and no return to HBsAg positivity occurred in all patients during the study course. In contrast,

**Table 1** Baseline characteristics and outcomes at the end of treatment and follow-up

Characteristic	Treatment group	Control group	P-value
No.	20	40	
Mean age at entry in year $\pm$ SD	33.80 $\pm$ 11.45	33.85 $\pm$ 8.37	0.985
Age > 40 yr, <i>n</i> (%)	4 (20.0)	11 (27.5)	0.527
Men:women, <i>n</i>	15:5	30:10	1.000
Mean baseline ALT (U/L) $\pm$ SD	23.46 $\pm$ 8.78	21.24 $\pm$ 10.26	0.874
HBsAg level (IU/mL)			
Mean $\pm$ SD	26.22 $\pm$ 33.00	25.72 $\pm$ 5.58	0.949
Median (Q1, Q3)	11.36 (3.52-37.40)	15.81 (4.59-40.15)	0.714
95%CI of patients with 10-100 IU/mL, <i>n</i> (%)	(10.77, 41.75), 10 (50.0)	(17.54, 33.90), 22 (55.0)	
Patients with < 10 IU/mL, <i>n</i> (%)	10 (50.0)	18 (45.0)	
Mean decline in HBsAg level at EOT (IU/mL) $\pm$ SD	22.33 $\pm$ 29.45	5.76 $\pm$ 17.67	0.009
Median HBsAg level at EOT (IU/mL)	0.04 (0.02, 0.55)	13.21 (2.97, 30.31)	0.003
(Q1, Q3)	95%CI: (-0.68, 8.53)	95%CI: (12.8, 27.12)	
Mean decline in HBsAg level at EOF (IU/mL) $\pm$ SD	23.36 $\pm$ 29.47	8.61 $\pm$ 19.32	0.023
Median HBsAg level at EOF (IU/mL)	0.045 (0.02, 2.44)	5.69 (1.50, 20.88)	0.007
(Q1, Q3)	95%CI: (-1.63, 7.43)	95%CI: (10.20, 24.03)	
HBsAg loss, <i>n</i> (%)	13 (65.0)	0 (0)	0.000
HBsAg seroconversion, <i>n</i> (%)	12 (60.0)	0 (0)	0.000
HBV DNA reactivation, <i>n</i> (%)	0 (0)	2 (5.0)	0.309

ALT: Alanine aminotransferase; EOF: End of follow-up; EOT: End of treatment; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

the mean HBsAg level of the control group remained relatively stable over 96 wk and was 25.72  $\pm$  25.58 IU/mL at baseline and 17.11  $\pm$  21.62 IU/mL at week 96 ( $P$  = 0.108; Figure 1). No patients in the control group experienced HBsAg loss/seroconversion, and two (5.0%) experienced HBV reactivation, defined as return of serum HBV DNA to positivity from undetectable level (< 100 IU/mL) (Table 1).

### Safety

Among all patients in the treated group, peripheral blood neutrophil count decreased, which was lower than  $0.85 \times 10^9/L$  in 13 (65.0%) individuals. The platelet count also decreased, which was lower than  $6.0 \times 10^{12}/L$  in eight (40.0%) patients, and dose reductions were not required. Serum creatinine and blood urea nitrogen remained stable during treatment with PEG-IFN alfa-2a. Five patients had a loss of body weight and six patients had mild hair loss during treatment. There were no thyroid dysfunction and neuropsychiatric adverse effects, including depression, delirium, irritability and agitation. All adverse reactions disappeared 3-6 mo after the therapy was discontinued.

ALT levels increased during treatment in 18 of 20 (90.0%) treated patients, and 9 (45.0%) individuals experienced an ALT level > 80 IU/L. However, bilirubin levels remained within normal limits throughout the treatment and follow-up periods in all treated patients. Normalization of ALT levels coincided with HBsAg loss and/or the end of treatment, and was maintained during follow-up.

## DISCUSSION

HBsAg level reflects the transcriptional activity of the cccDNA and is used as a proxy measure of HBV

infection and for treatment guidance<sup>[19-21]</sup>. HBsAg declines during treatment and its level at the end of treatment can predict HBeAg seroconversion in HBeAg-positive patients<sup>[22-24]</sup> and sustained viral response in HBeAg-negative patients<sup>[25-27]</sup>. Thus, inactive HBsAg carriers were not recommended for antiviral therapy<sup>[1-3]</sup>. However, this inactive state was not always sustained. A long-term follow-up study showed cumulative probabilities of hepatitis relapse in inactive HBsAg carriers of 10.2%, 17.4%, 19.3%, 20.2% and 20.2% after 5, 10, 15, 20 and 25 years of follow-up, respectively, with an annual rate of 1.55%<sup>[28]</sup>. Another long-term longitudinal study (up to 23 years) showed that 1%-17% of inactive carriers reverted back to HBeAg-positive chronic hepatitis<sup>[4]</sup>. Cirrhosis and HCC may still develop in some inactive HBsAg carriers<sup>[28-30]</sup>. In contrast, no cirrhosis or HCC occurred in patients with HBsAg loss after IFN treatment, indicating that HBsAg clearance is currently the only parameter associated with an excellent long-term prognosis<sup>[10]</sup>, and the strongest factor predicting excellent long-term outcome in HBV infected individuals is HBsAg loss, spontaneously or after treatment<sup>[10]</sup>. Therefore, it could be speculated that inactive HBsAg carriers can get further improvement in outcomes if HBsAg loss could be achieved after IFN treatment.

This study contained all participants who were inactive carriers with HBsAg < 100 IU/mL and wished to achieve HBsAg clearance by PEG-IFN alfa-2a treatment during the study period. Despite the lack of liver pathology for diagnosis, the patients could be considered as inactive for having undetectable HBV DNA and persistent normal ALT for 2 years, serum HBV DNA < 100 IU/mL and HBsAg < 100 IU/mL at enrollment. It has been reported that HBsAg < 1000 IU/mL with HBV DNA < 2000 IU/mL can distinguish inactive from active carriers with a diagnostic accuracy of 94.3%, sensitivity



of 91.1%, specificity of 95.4%, positive predictive value of 87.9%, and negative predictive value of 96.7%<sup>[31]</sup>. Although the present study was not a randomized controlled study, all treated inactive carriers with HBsAg < 100 IU/mL and matched controls according to age, sex, and HBsAg and ALT levels were included for eliminating the bias.

Effects, including the probability of HBsAg clearance, can be enhanced by extended therapy with PEG-IFN alfa-2a<sup>[32]</sup>. In our study the patients were given 72 wk of treatment. After 12 wk of treatment with PEG-IFN alfa-2a, HBsAg levels decreased significantly compared with baseline levels. Furthermore, at the end of study, HBsAg loss occurred in most of treated patients, and HBsAg levels in the remaining seven treated carriers who did not achieve HBsAg loss decreased significantly. In contrast, mean HBsAg level of the control group remained constant during 96 wk of observation and no patients experienced HBsAg loss. These results suggest that inactive HBsAg carriers could benefit from PEG-IFN alfa-2a treatment.

In the present study, all participants had HBsAg < 100 IU/mL and they may have a good long-term clinical outcome, even HBsAg loss, after long-term follow-up. However, it was reported that spontaneous HBsAg loss in patients with HBsAg < 100 IU/mL occurred in a mean period of  $86.6 \pm 29$  mo (range, 26-115) after the baseline visit with an annual rate of 1.6%<sup>[33]</sup>, and in the present study after 72 wk treatment of PEG-IFN alfa-2a, HBsAg clearance occurred in 65% of treated objects. In a study by Tseng *et al.*<sup>[34]</sup>, HBsAg level < 10 IU/mL at baseline was the strongest predictor of HBsAg loss. However, the rate of HBsAg loss was only 7.4 per 100 persons per year and it occurred in a mean period of  $5.8 \pm 4.2$  years. Although half of the subjects included in this study had HBsAg < 10 IU/mL and undetectable HBV DNA, 80% (8/10) of them achieved HBsAg loss after 72 wk of IFN treatment, suggesting that PEG-IFN alfa-2a treatment can make inactive carriers achieve HBsAg clearance in a short-term period compared with spontaneous HBsAg loss occurring in the nature history. Although Chen *et al.*<sup>[35]</sup> reported in a case-control study that the positive predictive value of HBsAg level of 200 IU/mL in predicting HBsAg loss occurring within 1 year was 36%, their study design was different from ours. The aim of their study was to observe the difference in HBsAg decrease between 46 patients who underwent spontaneous HBsAg loss and 46 patients who had no HBsAg loss during the same observation course. The aim of our study was to compare the rate of HBsAg clearance in patients treated with PEG-IFN alfa-2a compared with untreated patients, and the result showed that the rate of HBsAg clearance was significantly higher in patients treated with PEG-IFN alfa-2a than in untreated patients. The results of our study suggested that inactive carriers can receive PEG-IFN alfa-2a therapy to increase the probability of HBsAg clearance and shorten the time compared with that occurring spontaneously.

In conclusion, our study demonstrated that treat-

ment with PEG-IFN alfa-2a produced a high rate of HBsAg loss/seroconversion in inactive carriers with low HBsAg levels. However, whether inactive carriers with HBsAg levels more than 100 IU/mL could benefit from PEG-IFN alfa-2a treatment needs further study.

## ACKNOWLEDGMENTS

We are grateful to staff for effectively facilitating the present project.

## COMMENTS

### Background

Although inactive hepatitis B surface antigen (HBsAg) carriers often have no liver inflammation and are not recommended to undergo treatment, they may develop hepatitis relapse or revert back to HBeAg-positive chronic hepatitis, and cirrhosis and hepatocellular carcinoma (HCC) may still develop in some inactive HBsAg carriers in a long-term follow-up period. In contrast, no cirrhosis or HCC occurred in patients with HBsAg loss after interferon (IFN) treatment. So, HBsAg loss is generally considered to be the ultimate goal of therapy, indicating a complete response to treatment and the resolution of the disease. It was suggested that inactive HBsAg carriers could get benefits from IFN treatment if HBsAg loss was achieved after treatment.

### Research frontiers

HBsAg loss is the goal and ideal end-point of treatment in chronic hepatitis B. The spontaneous rate of HBsAg loss in inactive carriers was only 0.5%-2.5% per year, and HBsAg clearance occurred in a mean period of  $86.6 \pm 29$  mo (range, 26-115) after the initial visit. Even in patients with an HBsAg level < 10 IU/mL, a mean period of  $5.8 \pm 4.2$  years is required to achieve HBsAg clearance.

### Innovations and breakthroughs

In contrast to chronic hepatitis B, in which a low rate of HBsAg loss is achieved after IFN treatment, inactive HBsAg carriers with HBsAg < 100 IU/mL could obtain a high rate of HBsAg loss after PEG-IFN treatment in a shorter period than that occurring spontaneously.

### Applications

Inactive HBsAg carriers will benefit from PEG-IFN treatment, if HBsAg loss can be achieved after a short period of PEG-IFN therapy.

### Terminology

Inactive HBsAg carriers are patients who were HBsAg-positive, with low hepatitis B virus (HBV) replication and no liver inflammation. HBsAg loss was defined as an HBsAg concentration < 0.05 IU/mL, and seroconversion defined as an HBsAg concentration < 0.05 IU/mL and an anti-HBs level  $\geq 10$  mIU/L. HBsAg loss often indicates recovery from HBV infection.

### Peer-review

The manuscript entitled "Hepatitis B surface antigen clearance in inactive hepatitis B surface antigen carriers treated with peginterferon alfa-2a" discusses a possible application of an IFN therapy in inactive HBsAg carriers with a very low HBsAg level. The authors report that in their study the HBsAg disappeared in 65% of treated patients. This result seems to be very good, taking into account that usually HBsAg clearance is rarely observed.

## REFERENCES

- 1 Hou JL, Lai W. [The guideline of prevention and treatment for chronic hepatitis B: a 2015 update]. *Zhonghua Ganzangbing Zazhi* 2015; **23**: 888-905 [PMID: 26739464 DOI: 10.3760/cma.j.issn.1007-3418.2015.12.002]
- 2 European Association For The Study Of The Liver. EASL

- clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]
- 3 **Sarin SK**, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, Chen DS, Chen HL, Chen PJ, Chien RN, Dokmeci AK, Gane E, Hou JL, Jafri W, Jia J, Kim JH, Lai CL, Lee HC, Lim SG, Liu CJ, Locarnini S, Al Mahtab M, Mohamed R, Omata M, Park J, Piratvisuth T, Sharma BC, Sollano J, Wang FS, Wei L, Yuen MF, Zheng SS, Kao JH. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int* 2016; **10**: 1-98 [PMID: 26563120 DOI: 10.1007/s12072-015-9675-4]
  - 4 **Fattovich G**, Olivari N, Pasino M, D'Onofrio M, Martone E, Donato F. Long-term outcome of chronic hepatitis B in Caucasian patients: mortality after 25 years. *Gut* 2008; **57**: 84-90 [PMID: 17715267 DOI: 10.1136/gut.2007.128496]
  - 5 **Chu CM**, Hung SJ, Lin J, Tai DI, Liaw YF. Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. *Am J Med* 2004; **116**: 829-834 [PMID: 15178498 DOI: 10.1016/j.amjmed.2003.12.040]
  - 6 **McMahon BJ**, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. *Ann Intern Med* 2001; **135**: 759-768 [PMID: 11694101 DOI: 10.7326/0003-4819-135-9-200111060-00006]
  - 7 **Hsu YS**, Chien RN, Yeh CT, Sheen IS, Chiou HY, Chu CM, Liaw YF. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. *Hepatology* 2002; **35**: 1522-1527 [PMID: 12029639 DOI: 10.1053/jhep.2002.33638]
  - 8 **Chu CM**, Liaw YF. Incidence and risk factors of progression to cirrhosis in inactive carriers of hepatitis B virus. *Am J Gastroenterol* 2009; **104**: 1693-1699 [PMID: 19455130 DOI: 10.1038/ajg.2009.187]
  - 9 **Chen JD**, Yang HI, Iloeje UH, You SL, Lu SN, Wang LY, Su J, Sun CA, Liaw YF, Chen CJ. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. *Gastroenterology* 2010; **138**: 1747-1754 [PMID: 20114048 DOI: 10.1053/j.gastro.2010.01.042]
  - 10 **Moucari R**, Korevaar A, Lada O, Martinot-Peignoux M, Boyer N, Mackiewicz V, Dauvergne A, Cardoso AC, Asselah T, Nicolas-Chanoine MH, Vidaud M, Valla D, Bedossa P, Marcellin P. High rates of HBsAg seroconversion in HBeAg-positive chronic hepatitis B patients responding to interferon: a long-term follow-up study. *J Hepatol* 2009; **50**: 1084-1092 [PMID: 19376603 DOI: 10.1016/j.jhep.2009.01.016]
  - 11 **Chen YC**, Sheen IS, Chu CM, Liaw YF. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. *Gastroenterology* 2002; **123**: 1084-1089 [PMID: 12360470 DOI: 10.1053/gast.2002.36026]
  - 12 **Manno M**, Cammà C, Schepis F, Bassi F, Gelmini R, Giannini F, Miselli F, Grotola A, Ferretti I, Vecchi C, De Palma M, Villa E. Natural history of chronic HBV carriers in northern Italy: morbidity and mortality after 30 years. *Gastroenterology* 2004; **127**: 756-763 [PMID: 15362032 DOI: 10.1053/j.gastro.2004.06.021]
  - 13 **Chu CM**, Liaw YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. *Hepatology* 2007; **45**: 1187-1192 [PMID: 17465003 DOI: 10.1002/hep.21612]
  - 14 **Randall RE**, Goodbourn S. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. *J Gen Virol* 2008; **89**: 1-47 [PMID: 18089727 DOI: 10.1099/vir.0.83391-0]
  - 15 **Lucifora J**, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, Sprinzl MF, Koppensteiner H, Makowska Z, Volz T, Remouchamps C, Chou WM, Thasler WE, Hüser N, Durantel D, Liang TJ, Münk C, Heim MH, Browning JL, Dejardin E, Dandri M, Schindler M, Heikenwalder M, Protzer U. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science* 2014; **343**: 1221-1228 [PMID: 24557838 DOI: 10.1126/science.1243462]
  - 16 **Sprinzl MF**, Russo C, Kittner J, Allgayer S, Grambihler A, Bartsch B, Weinmann A, Galle PR, Schuchmann M, Protzer U, Bauer T. Hepatitis B virus-specific T-cell responses during IFN administration in a small cohort of chronic hepatitis B patients under nucleos(t)ide analogue treatment. *J Viral Hepat* 2014; **21**: 633-641 [PMID: 24251783 DOI: 10.1111/jvh.12189]
  - 17 **Li HM**, Wang JQ, Wang R, Zhao Q, Li L, Zhang JP, Shen T. Hepatitis B virus genotypes and genome characteristics in China. *World J Gastroenterol* 2015; **21**: 6684-6697 [PMID: 26074707 DOI: 10.3748/wjg.v21.i21.6684]
  - 18 **Wei DH**, Liu HZ, Huang AM, Liu XL, Liu JF. A new trend of genotype distribution of hepatitis B virus infection in southeast China (Fujian), 2006-2013. *Epidemiol Infect* 2015; **143**: 2822-2826 [PMID: 25648505 DOI: 10.1017/S0950268815000059]
  - 19 **Chan HL**, Wong VW, Tse AM, Tse CH, Chim AM, Chan HY, Wong GL, Sung JJ. Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. *Clin Gastroenterol Hepatol* 2007; **5**: 1462-1468 [PMID: 18054753 DOI: 10.1016/j.cgh.2007.09.005]
  - 20 **Wurstthorn K**, Lutgehetmann M, Dandri M, Volz T, Buggisch P, Zollner B, Longerich T, Schirmacher P, Metzler F, Zankel M, Fischer C, Currie G, Brosgart C, Petersen J. Peginterferon alpha-2b plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. *Hepatology* 2006; **44**: 675-684 [PMID: 16941693]
  - 21 **Chan HL**, Thompson A, Martinot-Peignoux M, Piratvisuth T, Cornberg M, Brunetto MR, Tillmann HL, Kao JH, Jia JD, Wedemeyer H, Locarnini S, Janssen HL, Marcellin P. Hepatitis B surface antigen quantification: why and how to use it in 2011 - a core group report. *J Hepatol* 2011; **55**: 1121-1131 [PMID: 21718667 DOI: 10.1016/j.jhep.2011.06.006]
  - 22 **Sonneveld MJ**, Hansen BE, Piratvisuth T, Jia JD, Zeuzem S, Gane E, Liaw YF, Xie Q, Heathcote EJ, Chan HL, Janssen HL. Response-guided peginterferon therapy in hepatitis B e antigen-positive chronic hepatitis B using serum hepatitis B surface antigen levels. *Hepatology* 2013; **58**: 872-880 [PMID: 23553752 DOI: 10.1002/hep.26436]
  - 23 **Piratvisuth T**, Marcellin P, Popescu M, Kapprell HP, Rothe V, Lu ZM. Hepatitis B surface antigen: association with sustained response to peginterferon alfa-2a in hepatitis B e antigen-positive patients. *Hepatol Int* 2013; **7**: 429-436 [PMID: 21701902 DOI: 10.1007/s12072-011-9280-0]
  - 24 **Liaw YF**, Jia JD, Chan HL, Han KH, Tanwandee T, Chuang WL, Tan DM, Chen XY, Gane E, Piratvisuth T, Chen L, Xie Q, Sung JJ, Wat C, Bernaards C, Cui Y, Marcellin P. Shorter durations and lower doses of peginterferon alfa-2a are associated with inferior hepatitis B e antigen seroconversion rates in hepatitis B virus genotypes B or C. *Hepatology* 2011; **54**: 1591-1599 [PMID: 22045673 DOI: 10.1002/hep.24555]
  - 25 **Marcellin P**, Bonino F, Yurdaydin C, Hadziyannis S, Moucari R, Kapprell HP, Rothe V, Popescu M, Brunetto MR. Hepatitis B surface antigen levels: association with 5-year response to peginterferon alfa-2a in hepatitis B e-antigen-negative patients. *Hepatol Int* 2013; **7**: 88-97 [PMID: 23518903 DOI: 10.1007/s12072-012-9343-x]
  - 26 **Moucari R**, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, Dauvergne A, Asselah T, Boyer N, Bedossa P, Valla D, Vidaud M, Nicolas-Chanoine MH, Marcellin P. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. *Hepatology* 2009; **49**: 1151-1157 [PMID: 19115222 DOI: 10.1002/hep.22744]
  - 27 **Brunetto MR**, Moriconi F, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, Luo K, Wang Y, Hadziyannis S, Wolf E, McCloud P, Batrla R, Marcellin P. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. *Hepatology* 2009; **49**: 1141-1150 [PMID: 19338056 DOI: 10.1002/hep.22760]
  - 28 **Chu CM**, Liaw YF. Spontaneous relapse of hepatitis in inactive HBsAg carriers. *Hepatol Int* 2007; **1**: 311-315 [PMID: 19669355]
  - 29 **Yang HI**, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; **347**: 168-174 [PMID: 12124405]

- 30 **Huo TI**, Wu JC, Lee PC, Chau GY, Lui WY, Tsay SH, Ting LT, Chang FY, Lee SD. Sero-clearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. *Hepatology* 1998; **28**: 231-236 [PMID: 9657117]
- 31 **Brunetto MR**, Oliveri F, Colombatto P, Moriconi F, Ciccorossi P, Coco B, Romagnoli V, Cherubini B, Moscato G, Maina AM, Cavallone D, Bonino F. Hepatitis B surface antigen serum levels help to distinguish active from inactive hepatitis B virus genotype D carriers. *Gastroenterology* 2010; **139**: 483-490 [PMID: 20451520 DOI: 10.1053/j.gastro.2010.04.052]
- 32 **Lampertico P**, Viganò M, Di Costanzo GG, Sagnelli E, Fasano M, Di Marco V, Boninsegna S, Farci P, Fargion S, Giuberti T, Iannaccone C, Regep L, Massetto B, Facchetti F, Colombo M. Randomised study comparing 48 and 96 weeks peginterferon  $\alpha$ -2a therapy in genotype D HBsAg-negative chronic hepatitis B. *Gut* 2013; **62**: 290-298 [PMID: 22859496 DOI: 10.1136/gutjnl-2011-301430]
- 33 **Chan HL**, Wong GL, Tse CH, Chan HY, Wong VW. Viral determinants of hepatitis B surface antigen seroclearance in hepatitis B e antigen-negative chronic hepatitis B patients. *J Infect Dis* 2011; **204**: 408-414 [PMID: 21742839 DOI: 10.1093/infdis/jir283]
- 34 **Tseng TC**, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, Kuo SF, Liu CH, Chen PJ, Chen DS, Kao JH. Determinants of spontaneous surface antigen loss in hepatitis B e antigen-negative patients with a low viral load. *Hepatology* 2012; **55**: 68-76 [PMID: 21858846 DOI: 10.1002/hep.24615]
- 35 **Chen YC**, Jeng WJ, Chu CM, Liaw YF. Decreasing levels of HBsAg predict HBsAg seroclearance in patients with inactive chronic hepatitis B virus infection. *Clin Gastroenterol Hepatol* 2012; **10**: 297-302 [PMID: 21893131 DOI: 10.1016/j.cgh.2011.08.029]

**P- Reviewer:** Belopolskaya M, Charuorn P, Lee HC

**S- Editor:** Qi Y **L- Editor:** Wang TQ **E- Editor:** Liu SQ



Retrospective Study

## Outcome analysis of management of liver trauma: A 10-year experience at a trauma center

Wong Hoi She, Tan To Cheung, Wing Chiu Dai, Simon HY Tsang, Albert CY Chan, Daniel KH Tong, Gilberto KK Leung, Chung Mau Lo

Wong Hoi She, Tan To Cheung, Wing Chiu Dai, Simon HY Tsang, Albert CY Chan, Daniel KH Tong, Gilberto KK Leung, Chung Mau Lo, Department of Surgery, Queen Mary Hospital, the University of Hong Kong, Hong Kong, China

Hong Kong, No. 102 Pok Fu Lam Road, Hong Kong, China. [tantocheung@hotmail.com](mailto:tantocheung@hotmail.com)  
Telephone: +852-22553025  
Fax: +852-28165284

**Author contributions:** She WH was responsible for study design, literature search, collection, analysis and interpretation of data, and manuscript drafting; Cheung TT was responsible for study design, analysis and interpretation of data, and writing and critical revision of manuscript; Dai WC, Tsang SHY and Chan ACY were responsible for study design and critical revision of manuscript; Tong DKH, Leung GKK and Lo CM were responsible for study design; Cheung TT is the guarantor.

Received: January 21, 2016  
Peer-review started: January 22, 2016  
First decision: February 22, 2016  
Revised: March 11, 2016  
Accepted: May 7, 2016  
Article in press: May 9, 2016  
Published online: May 28, 2016

**Institutional review board statement:** Review and approval of this particular study by the Institutional Review Board of The University of Hong Kong are not necessary since the Board permits all use of patient data in retrospective studies provided that no individual patients can be identified.

**Informed consent statement:** Consent by patients was not required for this particular study since this is an overall review of patient data with no individual patients being identified.

**Conflict-of-interest statement:** None of the authors has any conflict of interest to report.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Tan To Cheung, Associate Professor, Department of Surgery, Queen Mary Hospital, the University of

### Abstract

**AIM:** To review the outcomes of liver trauma in patients with hepatic injuries only and in patients with associated injuries outside the liver.

**METHODS:** Data of liver trauma patients presented to our center from January 2003 to October 2013 were reviewed. The patients were divided into two groups. Group 1 consisted of patients who had hepatic injuries only. Group 2 consisted of patients who also had associated injuries outside the liver.

**RESULTS:** Seven (30.4%) patients in group 1 and 10 (28.6%) patients in group 2 received non-operative management; the rest underwent operation. Blunt trauma occurred in 82.8% (48/58) of the patients and penetrative trauma in 17.2% (10/58). A higher injury severity score (ISS) was observed in group 2 (median 45 vs 25,  $P < 0.0001$ ). More patients in group 1 were hemodynamically stable (65.2% vs 37.1%,  $P = 0.036$ ). Other parameters were comparable between groups. Group 1 had better 30-d survival (91.3% vs 71.4%,  $P = 0.045$ ). On multivariate analysis using the logistic regression model, ISS was found to be associated with mortality ( $P = 0.004$ , hazard ratio = 1.035, 95%CI:



1.011-1.060).

**CONCLUSION:** Liver trauma patients with multiple injuries are relatively unstable on presentation. Despite a higher ISS in group 2, non-operative management was possible for selected patients. Associated injuries outside the liver usually account for morbidity and mortality.

**Key words:** Non-operative management; Liver trauma; Multiple injuries; Penetrative trauma; Liver laceration; Blunt trauma

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Liver trauma patients who have not only liver injury but also associated injury outside the liver usually have a high injury severity score (ISS) and a bigger chance of morbidity and death. Management of liver trauma features surgical and nonsurgical approaches. Choice of approach should depend on individual patients' overall clinical condition rather than just ISS or imaging findings. The applicability of nonsurgical approach has extended to penetrative injuries with success.

She WH, Cheung TT, Dai WC, Tsang SHY, Chan ACY, Tong DKH, Leung GKK, Lo CM. Outcome analysis of management of liver trauma: A 10-year experience at a trauma center. *World J Hepatol* 2016; 8(15): 644-648 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i15/644.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i15.644>

## INTRODUCTION

The liver is well known to be the most frequently injured internal organ in abdominal injury despite its relatively hidden location behind the subcostal region<sup>[1]</sup>. In liver trauma management, the widespread use of ultrasonography and computed tomography (CT) has facilitated decision-making, and non-operative management (NOM) has been shown to reduce mortality<sup>[2]</sup>. NOM is now the standard of care for blunt liver injury in hemodynamically stable patients<sup>[3-7]</sup>. A contrast CT scan of the abdomen can accurately identify the pathology, presence of complication and proper severity grade of injury in hemodynamically stable patients. For hemodynamically unstable patients, operative management (OM) may be necessary. Other considerations should also be taken into account as patients may suffer multiple injuries. Some injuries call for OM. In such cases, the liver injury can be dealt with in the laparotomy required by associated injuries. Treatment outcomes depend on the severity of injuries to organs. This study reviewed the management of liver trauma with or without associated injuries over 10 years at a level-1 trauma center in Hong Kong.

## MATERIALS AND METHODS

This is a retrospective study. The period for review is from January 2003 to October 2013. Patients at Department of Surgery, Queen Mary Hospital, the University of Hong Kong, who had liver trauma from blunt or penetrative injuries in the period were reviewed. Data of interest included demographic data, presentation, associated injury, mechanism of injury, grade of liver injury, injury severity score (ISS), and management outcome. The data were retrieved by a dedicated trauma nurse coordinator and then screened and reviewed by the authors.

The patients were divided into two groups. Group 1 consisted of patients who had hepatic injuries only. Group 2 was comprised of patients who also had associated injuries outside the liver. The presence of associated injuries was checked for either during the primary and the secondary surveys according to the Advanced Trauma Life Support principle and then by imaging (X-ray or CT scan) of various regions, or during operation. Patients (with or without initial fluid resuscitation) were regarded as hemodynamically stable if they had a patent airway, satisfactory oxygen saturation of > 95%, good volume pulse, heart rate of < 100 beats/min, and systolic blood pressure of > 90 mmHg.

The patients' grade of liver injury was determined according to the Organ Injury Scaling developed by the Organ Injury Scaling Committee of the American Association for the Surgery of Trauma<sup>[8]</sup>, with grade 1 being the least severe and Grade 6 being unsurvivable. For patients who received NOM, grade of liver injury was determined with a CT scan; for those who received OM, it was determined during operation.

The ISS is an anatomical scoring system that provides an overall score (0-75) for patients with multiple injuries. Calculation of each patient's ISS was based on signs shown upon physical examination, results of investigation, and findings in operation. Each injury in the six body regions (head, face, chest, abdomen, extremities and external) was assigned an Abbreviated Injury Score (AIS) according to the Abbreviated Injury Scale, and only the highest AIS in each body region were used. Each patient's three most severely injured body regions had their AIS squared and added together to produce an ISS for the patient. An AIS of 6 (unsurvivable injury) always entailed an ISS of 75 (fatality)<sup>[9]</sup>.

NOM was adopted for hemodynamically stable patients whose abdominal examination showed no peritoneal signs and whose imaging scans (X-ray, CT or ultrasonography) showed no intraperitoneal, retroperitoneal or extra-abdominal injuries requiring operative intervention. OM was indicated otherwise and when NOM failed.

All patients were closely monitored in the intensive care unit. Reassessment measures included physical examination, daily blood tests, and reassessment CT scan. Reassessment CT scan of the abdomen was performed 3 to 5 d after initial insult. CT scan for other

**Table 1** Comparison of perioperative data of the two groups *n* (%)

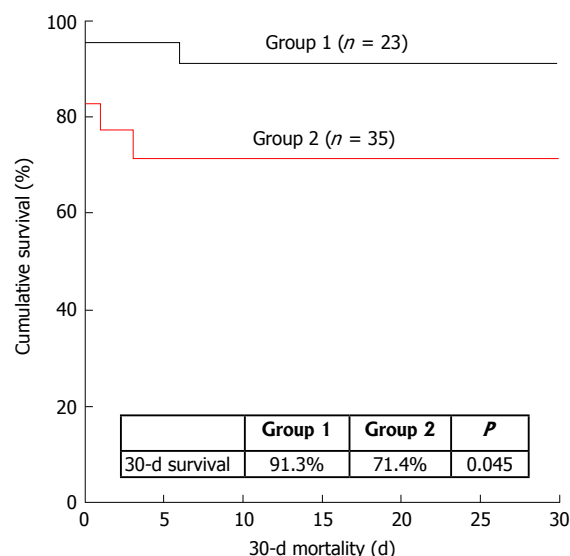
	Group 1 ( <i>n</i> = 23)	Group 2 ( <i>n</i> = 35)	<i>P</i> value
Age (yr)	36 (4-79)	36 (5-75)	0.762
Male:female	16:7	23:12	0.760
Health background			0.208
Good past health	15 (65.2)	28 (80.0)	
With comorbidity	8 (34.8)	7 (20.0)	
Type of trauma			1
Blunt	19 (82.6)	29 (82.9)	
Penetrative	4 (17.4)	6 (17.1)	
Mechanism of injury			0.077
Blunt injury	5 (21.7)	2 (5.7)	
Fall from a height	2 (8.7)	6 (17.1)	
Penetrative injury	4 (17.4)	3 (8.6)	
Road traffic accident	9 (39.1)	23 (65.7)	
Slip and fall	3 (13.0)	1 (2.9)	
With initial CT done	17 (73.9)	25 (71.4)	1
Reassessment CT			0.367
Not done	8 (34.8)	15 (42.9)	
Problem resolved	15 (65.2)	18 (51.4)	
Complication seen	0 (0)	2 (5.7)	
Hemodynamics			0.036
Stable	15 (65.2)	13 (37.1)	
Unstable	8 (34.8)	22 (62.9)	
Management			0.879
NOM	7 (30.4)	10 (28.6)	
OM	16 (69.6)	25 (71.4)	
Blood loss in OM (mL)	300 (0-20000)	1250 (0-24000)	0.133
Blood transfusion			0.018
No	14 (60.9)	10 (29.4)	
Yes	9 (39.1)	24 (70.6)	
Packed cells transfused (mL)	0 (0-2390)	1050 (0-10240)	0.001
Radiological intervention			1
No	21 (91.3)	30 (90.9)	
Yes	2 (8.7)	3 (9.1)	
ISS	25 (16-75)	45 (17-75)	< 0.0001
Grade of liver injury <sup>1</sup>			0.354
1	4 (17.4)	3 (8.8)	
2	5 (21.8)	5 (14.7)	
3	11 (47.8)	12 (35.3)	
4	2 (8.7)	8 (23.5)	
5	1 (4.3)	5 (14.7)	
6	0 (0)	1 (2.9)	
With complication	4 (18.2)	4 (11.4)	0.747
Follow-up duration (mo)	6 (0-60)	3 (0-128)	0.339

<sup>1</sup>There is one missing datum in group 2. Data are presented as median with range or number with percentage. ISS: Injury severity score; NOM: Non-operative management; OM: Operative management; CT: Computed tomography.

regions was performed if necessary.

### Statistical analysis

At the Department of Surgery, The University of Hong Kong, we have our own statistical staff. The biostatistics in this study was performed by our own statistical staff. The computer software SPSS, version 21.0, from IBM SPSS Statistics was used for statistical analyses. Continuous variables were compared by the Mann-Whitney *U* test and expressed as median with interquartile range. Student's *t*-test and Pearson's  $\chi^2$  test were employed. Thirty-day survival was measured. The Kaplan-Meier method was used for survival estimation



**Figure 1** Thirty-day survival in the two groups.

and the log-rank test was used for survival comparison. Multivariate analysis was performed to identify the risks for mortality. *P* values < 0.05 were considered statistically significant.

## RESULTS

Fifty-eight patients were included in the study, with 23 patients in group 1 and 35 patients in group 2. Seven (30.4%) patients in group 1 and 10 (28.6%) patients in group 2 received NOM. No change in management plan occurred. The median age was 32 years in patients receiving NOM and 39 years in patients receiving OM (*P* = 0.140). Comparison of group 1 and group 2 is shown in Table 1. The amounts of blood loss in patients who received OM were similar in the two groups (300 mL vs 1250 mL, *P* = 0.133); the amounts of blood transfused were also similar (2700 mL vs 2880 mL, *P* = 0.799). However, significantly more patients in Group 2 required transfusion (70.6% vs 39.1%, *P* = 0.018). In the 58 patients, 48 (82.8%) suffered blunt trauma and 10 (17.2%) suffered penetrative trauma. Both group 1 and group 2 had road traffic accident as the commonest cause of injury. ISS (*P* < 0.0001) and hemodynamic stability (*P* = 0.036) were significantly different between the two groups. Group 1 had significantly better 30-d survival (91.3% vs 71.4%, *P* = 0.045), as shown in Figure 1. Figure 2 shows 30-d survival stratified by grade of liver injury (*P* = 0.104). On multivariate analysis using the logistic regression model, ISS was found to be associated with mortality (*P* = 0.004, hazard ratio = 1.035, 95%CI: 1.011-1.060) (Table 2).

## DISCUSSION

The liver is the most commonly injured abdominal organ despite its well-protected position<sup>[1]</sup>. Management of liver injury depends on the patient's condition, diagnosis,

**Table 2 Multivariate analysis of risk factors for mortality**

Dependent factor			
Mortality			
Variables put into the system for model selection			
ISS			
Location of injury (0: Liver only; 1: Liver and outside the liver)			
Hemodynamics (0: Stable; 1: Unstable)			
Variable remaining in the final logistic regression model			
Factor	<i>P</i>	Hazard ratio	95% CI
ISS	0.004	1.035	1.011-1.060

ISS: Injury severity score.

transfusion requirement and complications, as well as facilities for monitoring. NOM of liver injuries has gained wide support; it was adopted for approximately 60% of cases of liver injuries from low grades to high grades<sup>[8,10]</sup>. Its application has been extended to penetrative injuries<sup>[11]</sup>.

At our center, liver trauma patients (with blunt or penetrative injuries) are subjected to CT for diagnostic purpose if they are hemodynamically stable; otherwise they are resuscitated and stabilized in the Accident and Emergency department, with a brief examination by a Focused Assessment with Sonography for Trauma scan, and then sent to the operation theater. Severity of injuries and presence of associated injuries are checked with CT or during laparotomy.

CT scan of the abdomen is widely used to evaluate intra-abdominal injuries in patients with stable hemodynamics; it should not be used if a patient has unstable hemodynamics since the patient's condition may deteriorate rapidly during scanning. CT scan can present the precise grade of liver injury, thereby allowing formulation of a proper management plan. A high grade (Grade 3-5) represents relatively severe injury. Patients with a high grade of liver injury tend to be more unstable and require OM<sup>[12]</sup>. But NOM is becoming more applicable to these patients because of improvement in intensive clinical care and increased use of interventional radiology. If NOM is adopted, reassessment CT scan should be performed within 7 to 10 d after the initial CT scan to check if there are any delayed complications<sup>[5]</sup>.

Grade of liver injury can reflect the degree of hepatic parenchymal damage, but it is not indicative of complication development or need for OM<sup>[13]</sup>. Grade-6 injuries are by definition not salvageable. In our present study, morbidity and mortality tended to worsen with a higher grade of liver injury. However, the presence of associated injuries also mattered; patients in group 2 with a high ISS fared the worst. Our 30-d survival curves by grade of liver injury reflected worsening survival with rising grades in both groups. However, further subgroup analysis showed that grade of liver injury did not make difference in survival ( $P = 0.104$ ). In fact, if there are associated injuries outside the liver, grade of liver injury cannot reflect the overall severity of injuries.

Multiple injuries, which can be caused by more than one mechanism of injury, often lead to major trauma (or

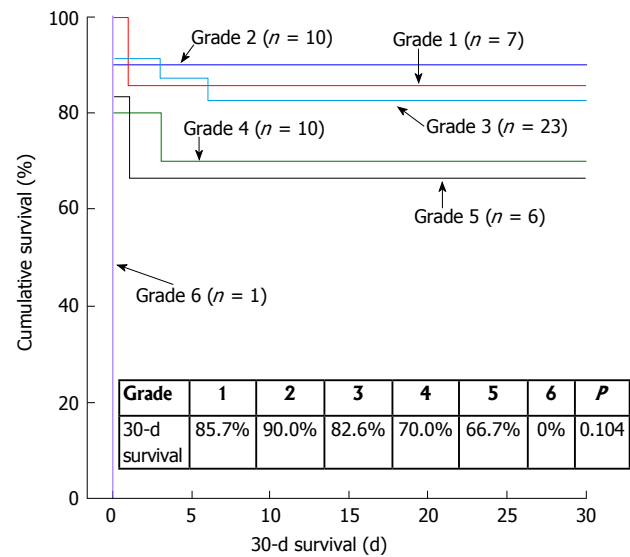


Figure 2 Thirty-day survival by grade of liver injury (with 1 missing datum).

multi-trauma) and result in serious physical complications and physiological decompensation. Major trauma is defined as ISS > 15<sup>[14]</sup>. It is usually caused by a high impact of energy, and the commonest cause is road traffic accident<sup>[15]</sup>. The higher median ISS in group 2 was due to significant associated injuries outside the liver. And more patients in this group had transfusion need, a reflection of the severity of injury. It is not surprising that group 2 had a lower survival rate<sup>[16]</sup> as it has been reported that ISS could predict length of intensive care unit stay as well as mortality and survival<sup>[17]</sup>. Our multivariate analysis also found that patients with a higher ISS were more likely to have shorter survival.

Most of the patients who suffered blunt injuries in group 2 were unstable. At our center, the decision on management approach is based on individual patients' clinical condition rather than ISS. Although ISS is used as an index for quality assurance at most trauma centers, it is not an accurate indicator and it does not reflect multiple injuries in the same body region. Hence, a high ISS should not be an indicator for OM. OM is required if a patient's hemodynamics is unstable; it is also required in the presence of another operative indication (e.g., the need for thoracotomy, neurosurgery, orthopedic operation, repair of viscera, management of pelvic bleeding, etc). Decision on initial and subsequent management approaches should be based on clinical condition as well as mechanism and site of injury. Understanding the mechanism of the injury helps to identify potential life-threatening and limb-threatening conditions, which can maximize the chance of salvage and prevent functional deficit.

This study is not rid of the inherent limitations of a single-center retrospective study, and the patients were heterogeneous in terms of premorbid status, mechanism of injury and severity of injury. The use of ISS was to quantify severity of injuries for a more standardized representation.

In conclusion, liver trauma patients with multiple injuries are relatively unstable on presentation. Despite a significantly higher ISS in group 2, NOM was possible for selected patients. Associated injuries outside the liver usually account for morbidity and mortality.

## COMMENTS

### Background

In liver trauma management, the widespread use of ultrasonography and computed tomography (CT) has facilitated decision-making, and non-operative management (NOM) has been shown to reduce mortality. NOM is now the standard of care for blunt liver injury in hemodynamically stable patients.

### Research frontiers

This study reviewed the management of liver trauma with or without associated injuries over 10 years at a level-1 trauma center in Hong Kong.

### Innovations and breakthroughs

Liver trauma patients with multiple injuries are relatively unstable on presentation. Despite a significantly higher injury severity score in group 2, NOM was possible for selected patients. Associated injuries outside the liver usually account for morbidity and mortality.

### Peer-review

It is well written and documented and discussed paper, it has valuable points to stress the nonoperative management of liver trauma in addition to that on behalf of the scope of your journal this type of articles may increase the impact effect of it since the paper is discussed a huge number of cases even they collected them in 10 years but it is meaningful and it likes a review of liver trauma.

## REFERENCES

- 1 **Feliciano DV**. Surgery for liver trauma. *Surg Clin North Am* 1989; **69**: 273-284 [PMID: 2648616]
- 2 **David Richardson J**, Franklin GA, Lukan JK, Carrillo EH, Spain DA, Miller FB, Wilson MA, Polk HC, Flint LM. Evolution in the management of hepatic trauma: a 25-year perspective. *Ann Surg* 2000; **232**: 324-330 [PMID: 10973382 DOI: 10.1097/0000658-20009000-00004]
- 3 **Croce MA**, Fabian TC, Menke PG, Waddle-Smith L, Minard G, Kudsk KA, Patton JH, Schurr MJ, Pritchard FE. Nonoperative management of blunt hepatic trauma is the treatment of choice for hemodynamically stable patients. Results of a prospective trial. *Ann Surg* 1995; **221**: 744-753; discussion 753-755 [PMID: 7794078 DOI: 10.1097/0000658-199506000-00013]
- 4 **Meredith JW**, Young JS, Bowling J, Roboussin D. Nonoperative management of blunt hepatic trauma: the exception or the rule? *J*

- Trauma* 1994; **36**: 529-534; discussion 534-535 [PMID: 8158715 DOI: 10.1097/00005373-199404000-00012]
- 5 **Pachter HL**, Hofstetter SR. The current status of nonoperative management of adult blunt hepatic injuries. *Am J Surg* 1995; **169**: 442-454 [PMID: 7694987 DOI: 10.1016/S0002-9610(99)80194-9]
- 6 **Malhotra AK**, Fabian TC, Croce MA, Gavin TJ, Kudsk KA, Minard G, Pritchard FE. Blunt hepatic injury: a paradigm shift from operative to nonoperative management in the 1990s. *Ann Surg* 2000; **231**: 804-813 [PMID: 10816623 DOI: 10.1097/0000658-200006000-00004]
- 7 **Coimbra R**, Hoyt DB, Engelhart S, Fortlage D. Nonoperative management reduces the overall mortality of grades 3 and 4 blunt liver injuries. *Int Surg* 2006; **91**: 251-257 [PMID: 17061668]
- 8 **Moore EE**, Cogbill TH, Jurkovich GJ, Shackford SR, Malangoni MA, Champion HR. Organ injury scaling: spleen and liver (1994 revision). *J Trauma* 1995; **38**: 323-324 [PMID: 7897707 DOI: 10.1097/00005373-199503000-00001]
- 9 **Baker SP**, O'Neill B, Haddon W, Long WB. The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. *J Trauma* 1974; **14**: 187-196 [PMID: 4814394 DOI: 10.1097/00005373-197403000-00001]
- 10 **Cachecho R**, Clas D, Gersin K, Grindlinger GA. Evolution in the management of the complex liver injury at a Level I trauma center. *J Trauma* 1998; **45**: 79-82 [PMID: 9680016 DOI: 10.1097/00005373-199807000-00016]
- 11 **Petrovsky H**, Raeder S, Zuercher L, Platz A, Simmen HP, Puhon MA, Keel MJ, Clavien PA. A quarter century experience in liver trauma: a plea for early computed tomography and conservative management for all hemodynamically stable patients. *World J Surg* 2012; **36**: 247-254 [PMID: 22170476 DOI: 10.1007/s00268-011-1384-0]
- 12 **Tinkoff G**, Esposito TJ, Reed J, Kilgo P, Fildes J, Pasquale M, Meredith JW. American Association for the Surgery of Trauma Organ Injury Scale I: spleen, liver, and kidney, validation based on the National Trauma Data Bank. *J Am Coll Surg* 2008; **207**: 646-655 [PMID: 18954775 DOI: 10.1016/j.jamcollsurg.2008.06.342]
- 13 **Becker CD**, Gal I, Baer HU, Vock P. Blunt hepatic trauma in adults: correlation of CT injury grading with outcome. *Radiology* 1996; **201**: 215-220 [PMID: 8816546 DOI: 10.1148/radiology.201.1.8816546]
- 14 **Keel M**, Trentz O. Pathophysiology of polytrauma. *Injury* 2005; **36**: 691-709 [PMID: 15910820 DOI: 10.1016/j.injury.2004.12.037]
- 15 **von Rüden C**, Woltmann A, Röse M, Wurm S, Rüger M, Hierholzer C, Bühren V. Outcome after severe multiple trauma: a retrospective analysis. *J Trauma Manag Outcomes* 2013; **7**: 4 [PMID: 23675931 DOI: 10.1186/1752-2897-7-4]
- 16 **Pracht E**. Inpatient hospital outcomes following injury in Suriname: lessons for prevention. *Glob Health Promot* 2014; **21**: 29-39 [PMID: 24449798 DOI: 10.1177/1757975913509655]
- 17 **Akhavan Akbari G**, Mohammadian A. Comparison of the RTS and ISS scores on prediction of survival chances in multiple trauma patients. *Acta Chir Orthop Traumatol Cech* 2012; **79**: 535-539 [PMID: 23286687]

**P- Reviewer:** Guneren E, Ximenes RO **S- Editor:** Qi Y  
**L- Editor:** A **E- Editor:** Liu SQ





Observational Study

## Hepatitis C virus infection in Argentina: Burden of chronic disease

Ezequiel Ridruejo, Fernando Bessone, Jorge R Daruich, Chris Estes, Adrián C Gadano, Homie Razavi, Federico G Villamil, Marcelo O Silva

Ezequiel Ridruejo, Hepatology Section, Department of Medicine, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno, Ciudad Autónoma de Buenos Aires C1425ASG, Argentina

Ezequiel Ridruejo, Marcelo O Silva, Hepatology and Liver Transplant Unit, Hospital Universitario Austral, Pilar 1629, Prov. de Buenos Aires, Argentina

Fernando Bessone, Hepatology Section, Escuela de Medicina, Universidad de Rosario, Rosario 2000, Prov. de Santa Fe, Argentina

Jorge R Daruich, Hepatology Section, Hospital de Clínicas San Martín, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires C1120AAF, Argentina

Chris Estes, Homie Razavi, Center for Disease Analysis, Louisville, CO 80026, United States

Adrián C Gadano, Hepatology and Liver Transplant Unit, Hospital Italiano de Buenos Aires, Ciudad Autónoma de Buenos Aires C1181ACH, Argentina

Federico G Villamil, Liver Transplant Unit, Hospital Británico, Ciudad Autónoma de Buenos Aires C1280AEB, Argentina

**Author contributions:** Ridruejo E drafted the manuscript; Estes C and Razavi H participated in study design and performed statistical analysis; all authors were involved with data collection, assisted with data analysis, read and approved the final manuscript.

**Institutional review board statement:** None.

**Informed consent statement:** None.

**Conflict-of-interest statement:** Ridruejo E, Bessone F, Daruich JR, Gadano AC, Villamil FG and Silva MO have no conflicts of interest to declare; Estes C and Razavi H and are employees of the Center for Disease Analysis.

**Data sharing statement:** There is no additional data available.

**Open-Access:** This article is an open-access article which was

selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Ezequiel Ridruejo, MD, Hepatology Section, Department of Medicine, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno, Avda. Las Heras 2939, Ciudad Autónoma de Buenos Aires C1425ASG, Argentina. [eridruejo@gmail.com](mailto:eridruejo@gmail.com)  
**Telephone:** +54-11-52991221  
**Fax:** +54-11-52990600

**Received:** January 28, 2016

**Peer-review started:** January 28, 2016

**First decision:** February 29, 2016

**Revised:** April 4, 2016

**Accepted:** May 10, 2016

**Article in press:** May 11, 2016

**Published online:** May 28, 2016

### Abstract

**AIM:** To estimate the progression of the hepatitis C virus (HCV) epidemic and measure the burden of HCV-related morbidity and mortality.

**METHODS:** Age- and gender-defined cohorts were used to follow the viremic population in Argentina and estimate HCV incidence, prevalence, hepatic complications, and mortality. The relative impact of two scenarios on HCV-related outcomes was assessed: (1) increased sustained virologic response (SVR); and (2) increased SVR and treatment.

**RESULTS:** Under scenario 1, SVR raised to 85%-95% in 2016. Compared to the base case scenario, there was a 0.3% reduction in prevalent cases and liver-related deaths by 2030. Given low treatment rates, cases of hepatocellular carcinoma and decompensated cirrhosis decreased < 1%, in contrast to the base case in 2030. Under scenario 2, the same increases in SVR were modeled, with gradual increases in the annual diagnosed and treated populations. This scenario decreased prevalent infections 45%, liver-related deaths 55%, liver cancer cases 60%, and decompensated cirrhosis 55%, as compared to the base case by 2030.

**CONCLUSION:** In Argentina, cases of end stage liver disease and liver-related deaths due to HCV are still growing, while its prevalence is decreasing. Increasing in SVR rates is not enough, and increasing in the number of patients diagnosed and candidates for treatment is needed to reduce the HCV disease burden. Based on this scenario, strategies to increase diagnosis and treatment uptake must be developed to reduce HCV burden in Argentina.

**Key words:** Diagnosis; Disease burden; Epidemiology; Incidence; Mortality; Prevalence; Treatment; Argentina; Hepatitis C

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This is a study evaluating potential policies to diminish hepatitis C virus (HCV) disease burden. Increasing diagnoses and treated individuals with the high current sustained virologic response rates, will diminish HCV disease burden.

Ridruejo E, Bessone F, Daruich JR, Estes C, Gadano AC, Razavi H, Villamil FG, Silva MO. Hepatitis C virus infection in Argentina: Burden of chronic disease. *World J Hepatol* 2016; 8(15): 649-658 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i15/649.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i15.649>

## INTRODUCTION

Chronic hepatitis C virus (HCV) liver disease is a global public health issue, with an estimated prevalence of 170 million infected people. Every year, 3000000 to 4000000 new HCV infections are diagnosed, and a mean global seroprevalence of nearly 3%<sup>[1]</sup>.

In many countries, while HCV prevalence is decreasing, its morbidity and mortality is increasing<sup>[2]</sup>. Population aging results in a rise in all-cause mortality. This leads to a reduction in the total of infected patients. Progression to advanced HCV related liver disease combined with populace aging, is associated with a rising in mortality due to advanced liver disease<sup>[2,3]</sup>.

In Argentina, the exact HCV prevalence is unknown. According to different studies it varies between 0.17%

to 5.6%; in some areas of high endemicity it may vary between 2.2% to 7.3%<sup>[4]</sup>. Nosocomial transmission appears to be the main route of infection, and genotype 1 is most prevalent in the infected population<sup>[5,6]</sup>. Precise data for incidence and prevalence estimates are lacking in Argentina. Also, there are no data about the burden of the disease and its impact on public health. Data on the percentage of HCV patients treated and their outcomes are also scarce. It has been estimated that only 0.15% of HCV patients have been treated in the last 15 years in Argentina<sup>[7]</sup>. These results are comparable to other countries in the region. Our aim was, using a modeling method, to describe HCV-related disease progression at the national level.

A model was also used to evaluate the influence of distinct actions aimed at diminishing the burden of HCV disease (*e.g.*, multiply the percentage of treated patients, improved cure rates and improved case identification). This model has been already validated and used in similar studies in different countries<sup>[8-11]</sup>.

## MATERIALS AND METHODS

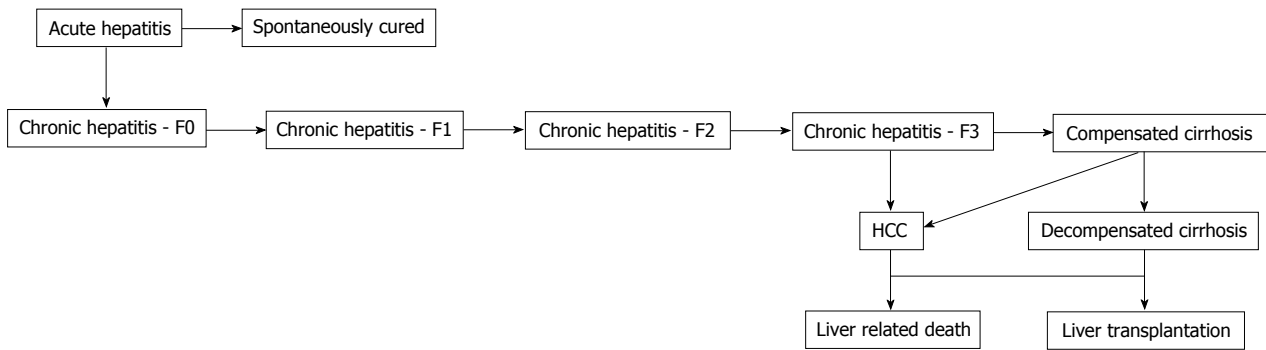
A systematic review of the literature was done to find studies addressing the proportion of HCV patients who had been diagnosed, received treatment and achieve sustained virologic response (SVR) in Argentina. The review included all studies published between January 1990 and July 2014.

PubMed and EMBASE databases were consulted looking for indexed articles. Non-indexed sources were identified by searching in the National Ministry of Health Website, proceedings of local medical meetings, unpublished data and data from large liver centers.

Also, an expert panel including epidemiologists, hepatologists, infectious disease specialists, public health professionals and virologists, gathered in a person to person meeting to analyze all the retrieved information.

Data from countries with similar healthcare practices and/or risk factors, or expert consensus were used there was no input data available. Some of these data were included in a previous global report<sup>[2,3]</sup>. To populate a disease progression model and to assess the magnitude of the HCV-infected populace according to liver fibrosis stages (METAVIR score F0-F4), country-specific inputs from 2013-2030 were loaded in Microsoft Excel® database (Microsoft Corp., Redmond, WA) (Figure 1). Crystal Ball, an Excel add-in by Oracle, was utilized for uncertainty and sensitivity analyses. For the uncertainty model, beta-PERT distributions were utilized associated with all inputs. To analyze the incertitudes that had the biggest repercussion on in 2030 HCV prevalence, a sensitivity analysis was utilized.

Populace information were arranged by sex, five-year age groups, and year (1950-2100) and obtained from the United Nations population database<sup>[12]</sup>. Based on expert inputs, in adults (persons aged ≥ 20 years) HCV viremia prevalence in Argentina in 2013, was estimated at 1.5%. The HCV viremic rate in Argentina is



**Figure 1** The flow of the hepatitis C virus disease progression model. HCC: Hepatocellular carcinoma.

80%, as previously reported<sup>[13]</sup>.

Using a 0.83% viremic prevalence, it was calculated that 342000 persons had HCV RNA detectable in 2013.

A hybrid distribution was constructed to calculate age and gender specific HCV diagnosis rates by five-year age group using notification inputs for HCV infection for persons aged 0 to 59 years<sup>[14]</sup>, and transplant inputs classified by age and gender for persons aged  $\geq 60$  years<sup>[15]</sup>. The notified and transplanted people were weighted to the national estimate for total prevalence and aged to the year 2013, accounting for mortality and cured patients.

To estimate HCV genotype distribution, data from over 200 treated patients was used<sup>[16]</sup>. Genotype 1 (G1) subtypes distribution was calculated using data from another study<sup>[5]</sup>. The genotype distribution applied in the model was G1/other = 63%, G2 = 25%, G3 = 11%, G4 = 1%.

As outlined in a previous work, annual patients progress through each disease state were include in the model using age and gender specific transition probabilities<sup>[2,3]</sup>.

Changes in historical HCV incidence were estimated according to expert opinion. Changes in historical HCV incidence were estimated according to expert opinion. After an estimated peak incidence in 1989, it has markedly decrease with the introduction of antiHCV screening in blood donors. In Argentina, it was estimated that 1850 new infections were diagnosed in 2013.

It was estimated that 350 and 200 patients receive treatment in 2014 and 2015, respectively, based on expert consensus and IMS data for pegylated-interferon (IFN) units sold in Argentina<sup>[17]</sup>. A multiplier was used to account for under-reporting in IMS data. The Argentinean genotype distribution was used to estimate the average number of weeks of treatment per patient with 85% compliance/persistence.

In 2013, 74 of 329 (22.4%) patients receiving a liver transplant were related to HCV end stage liver disease. Data from the national organ registry for the years 1999 to 2013 showed that the percentage of liver transplant in HCV patients was 22.0% before adoption of model for end stage liver disease (MELD) based allocation and 22.4% after MELD implementation<sup>[15,16]</sup>.

Database from the Pan American Health Organization

allow us to estimate the diagnosed population based upon data for HCV positive blood donors<sup>[7]</sup>. The annual number of confirmed cases was balanced to account for diagnosis in other settings. It was assumed that 118800 persons were previously diagnosed and 6560 new cases were confirmed in 2010. The Berkeley Human Mortality database was used to estimate mortality rate by year, age group and gender<sup>[18]</sup> (Table 1).

Using estimates of 65000 active injection drug users (IDU) and a 54.6% HCV prevalence in Argentina, it was calculated that in 2001, 9.3% of the HCV population were IDU<sup>[19,20]</sup>.

Using a standard mortality ratio (SMR) of 10.0 for persons between 15 and 44 years old, a raised mortality was estimated among active IDU<sup>[21-26]</sup>.

It was estimated that 20.8% of the HCV patients were related blood transfusions in 2005, according to data from a national study<sup>[6]</sup>. In this subgroup of patients, a SMR of 1.5 was applied for all age groups<sup>[27]</sup>.

## Scenarios

**Base scenario:** Patients aged 15-69 years were considered for treatment and 60% of potential patients in Argentina were considered candidates for antiHCV therapy. It was considered that median SVR rates were 60% (G1), 75% (G2/4), and 65% (G3). Treated populations of 350 patients in 2014 and 200 patients annually during 2015-2030 were modeled, was and were restricted to patients with fibrosis stages  $\geq F3$  (G1) and  $\geq F2$  (G2/3/4).

It was considered that until 2016 patients with severe liver disease such as decompensated cirrhosis or eligible for transplantation, or those with hepatocellular carcinoma (HCC), were not candidates for treatment.

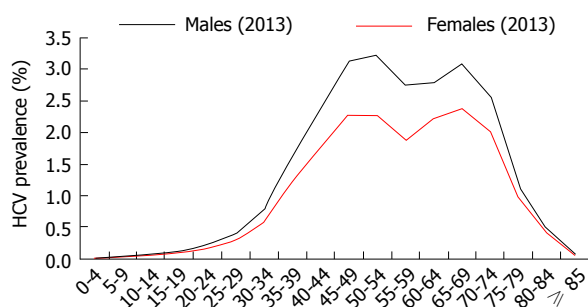
**Scenario 1:** Increased efficacy: It was assumed that by 2016, treatment eligibility raised to 95% for all genotypes and SVR rates steadily raised to 90% (G1/4), 95% (G2), and 85% (G3). The number of patients treated and newly diagnosed every year remained constant, while treatment was extended to fibrosis stages  $\geq F2$  in all genotypes (Figure 2).

**Scenario 2:** Increased efficacy and treatment: SVR, treatment eligibility, and fibrosis restriction increases

**Table 1 Model inputs and 2013 estimates**

	Historical	Year	2013 (Est.)
HCV infected cases	427890 (132720-829480)	2013	428260
AntiHCV prevalence	1.0% (0.3%-2.0%)		1.0%
Total viremic cases	342310 (106170-663580)	2013	342310
Viremic prevalence	0.8% (0.3%-1.6%)		0.8%
Viremic rate	80.0%		80.0%
HCV diagnosed (viremic)	112270	2010	117250
Viremic diagnosis rate	32.8%		34.2%
Annual newly diagnosed	4920	2010	4920
New infections			1950
New infection rate (per 100K)			4.7
Treated			
Number treated			650
Annual treatment rate			0.2%
Risk factors			
Number of active IDU with HCV			31950
Percent active IDU			9.3%
Previous blood transfusion			48420
Percent previous blood transfusion			14.1%

HCV: Hepatitis C virus; IDU: Injection drug users.



**Figure 2 Age and gender distribution of anti-hepatitis C virus prevalence, Argentina, 2013.** HCV: Hepatitis C virus.

were the same as in scenario 1. The number of patients newly diagnosed every year progressively escalated to 14770 in 2016, while the number of patients treated every year progressively escalated to 12000 by 2020 (Figure 3).

## RESULTS

### **Prevalence of chronic hepatitis C and complications**

According to the model, the HCV prevalence in Argentina peaked in 2002 at 376000 viremic individuals. In 2013, there were an estimated 342000 (95%CI: 146000-517000) infected individuals, a 10% decline from 2002. In the base scenario, viremic cases are estimated at 241000 in 2030, a decline of 30% from 2014 (Figure 4). The incidence of HCV in Argentina peaked in 1989 with an estimated 21340 new infections, and declined by 90% in 2013 with an estimated 1850 cases new infections.

There were 42910 compensated cirrhotic patients in 2013 and it was calculated that there will be 69600 by 2030. Also by 2030 there will be 2500 new cases of HCC 7830 patients will develop decompensated

cirrhosis. By 2030, 2890 patients will die from HCV related liver disease in contrast to 1520 patients who died in 2013. The proportion of viremic patients who have compensated cirrhosis or decompensated cirrhosis or HCC will increase to 34% in 2030, as compared with 14% in 2013 (Figures 5 and 6).

New HCV treatment strategies imply an increase in SVR rates. Based on recent results SVR rates will increase to at least 90% (G1/4), 95% (G2), and 85% (G3) by 2016. In the same period, treatment eligibility will increase to 95% for all genotypes. According to the model, increasing treatment efficacy but keeping the same low number of treated patients (scenario 1) will result in 660 fewer viremic patients in 2030, a 0.3% reduction as compared to the base case.

Compared with the base case, by 2030 it was estimated a 0.3% decrease in the number of HCC cases (2490 cases), a 0.3% decrease in liver related deaths (2880 cases), a 0.2% decrease in decompensated and 0.3% in compensated cirrhosis new cases (7800 and 69380 cases, respectively) (Figure 7).

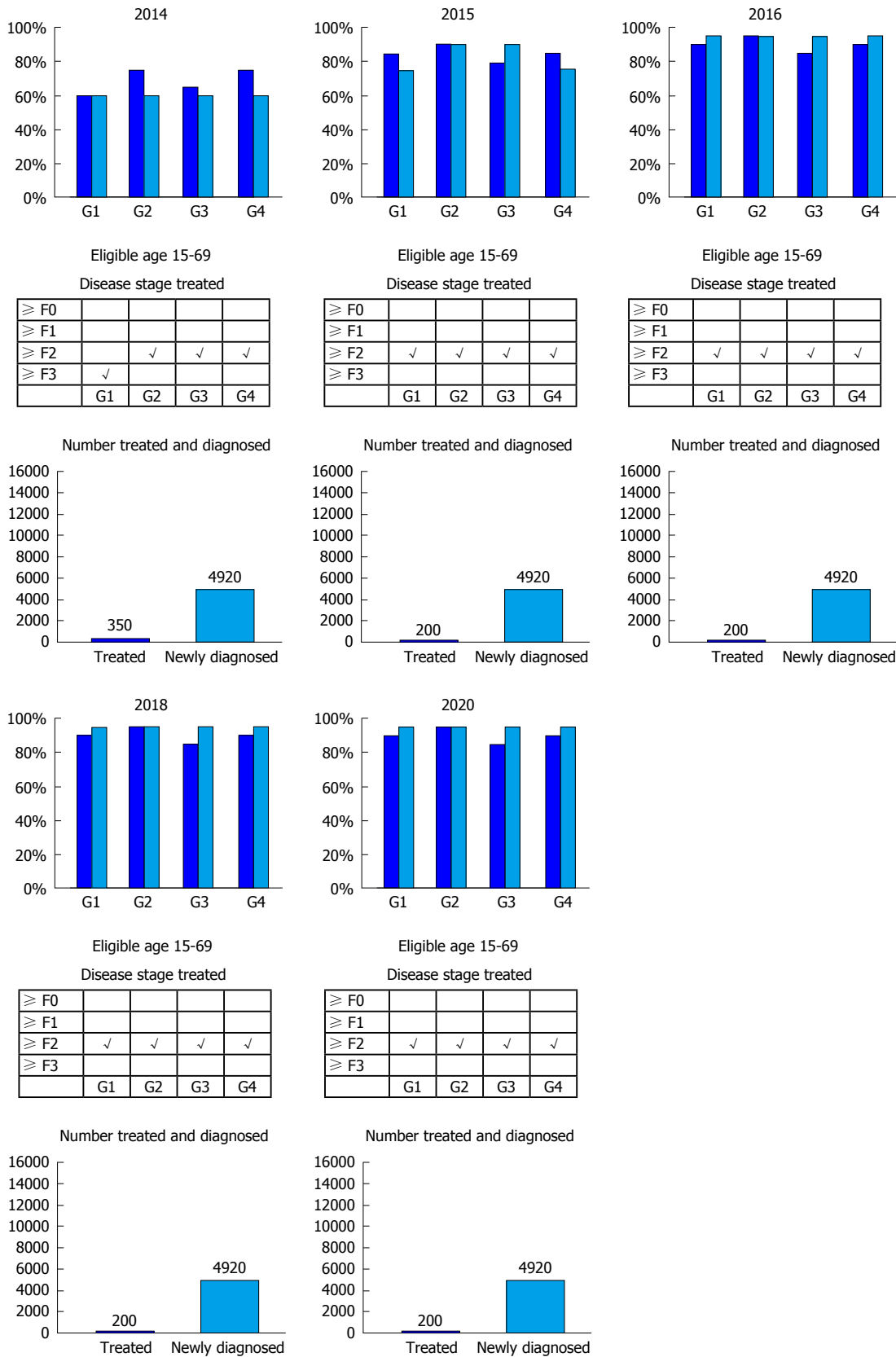
Increased treatment efficacy alone seems to have little impact in decreasing HCV burden, so another scenario was developed with the same SVR rates but increasing numbers of patients diagnosed and treated (scenario 2).

If the number of diagnosed and treated patients is markedly increased, a 45% reduction in the number of viremic patients can be obtained by 2030, meaning 107000 fewer infected patients. A 60% reduction in HCC cases is expected, with 1000 new HCC cases diagnosed by 2030. It is expected that the number of liver related deaths will also decrease with 1260 by 2030, meaning a 55% reduction when compared to the base case. New cirrhosis cases will decrease by 55% in decompensated and by 60% in compensated cases by 2030 (3390 and 29210, respectively) (Figure 7).



■ SVR ■ Eligibility

Scenario 1



Scenario 2

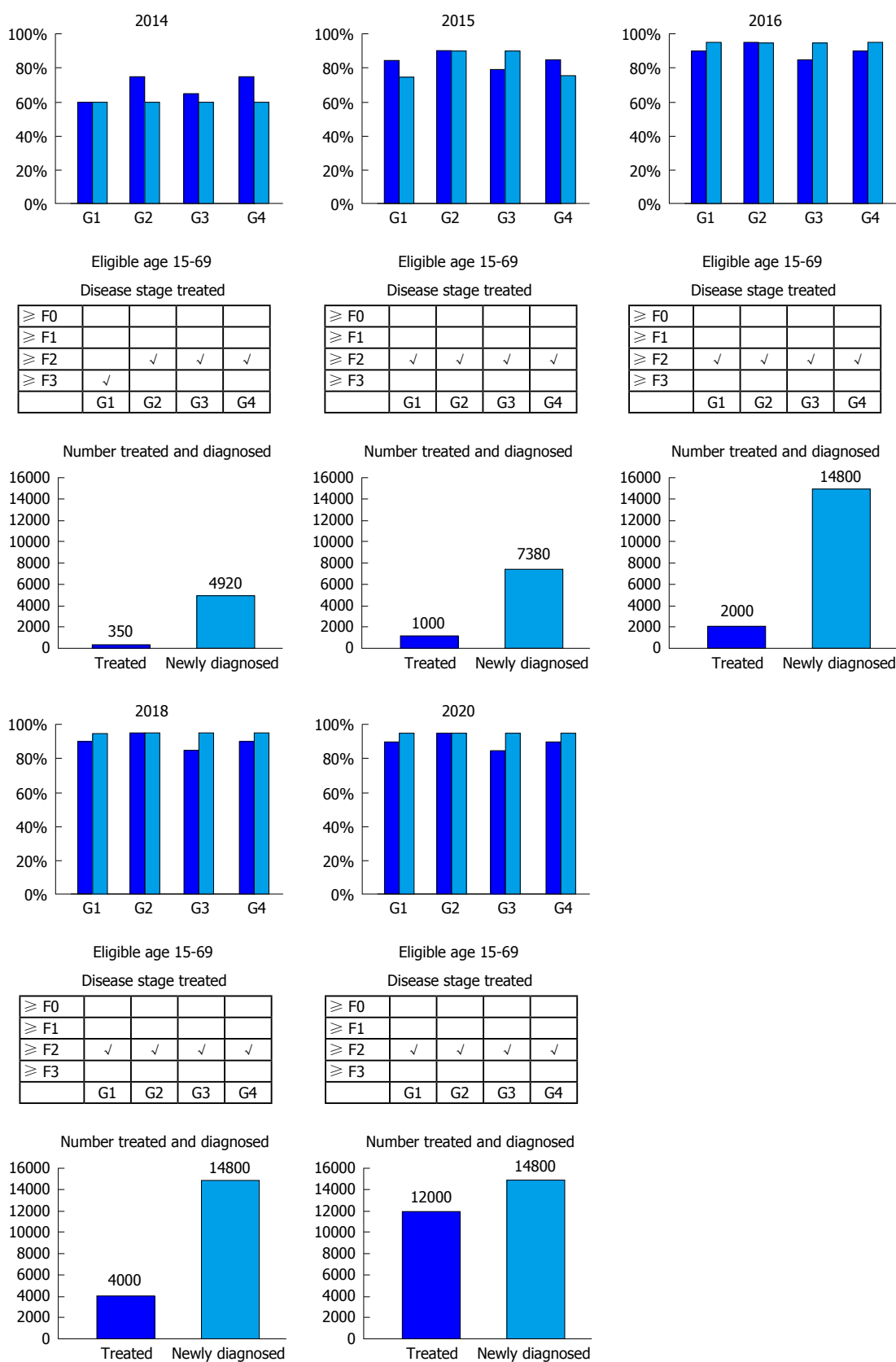
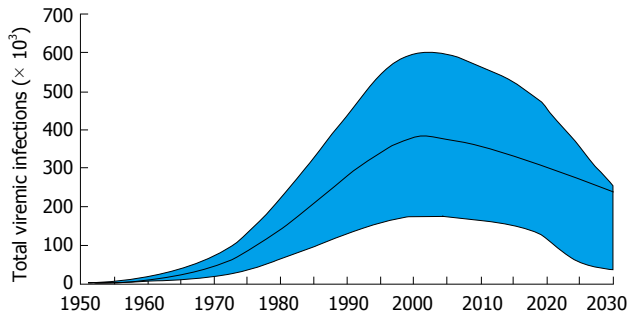
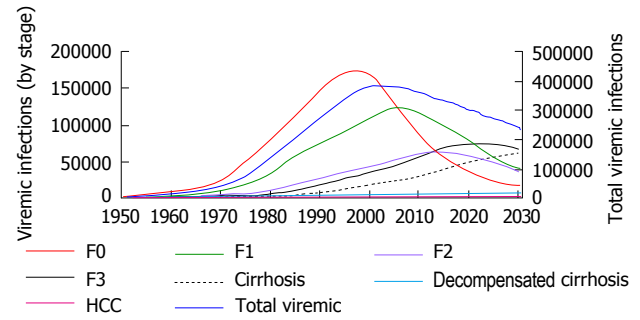


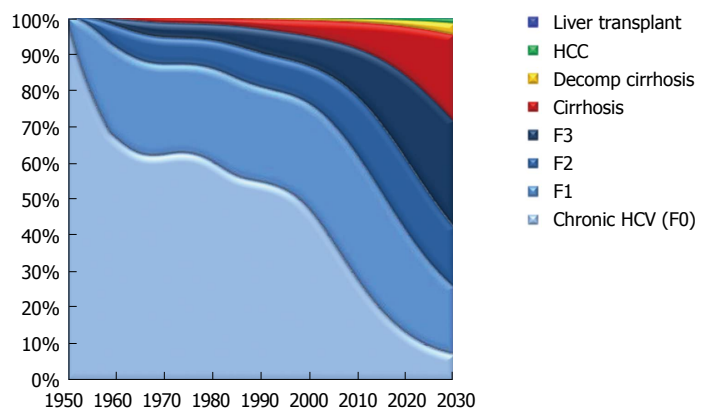
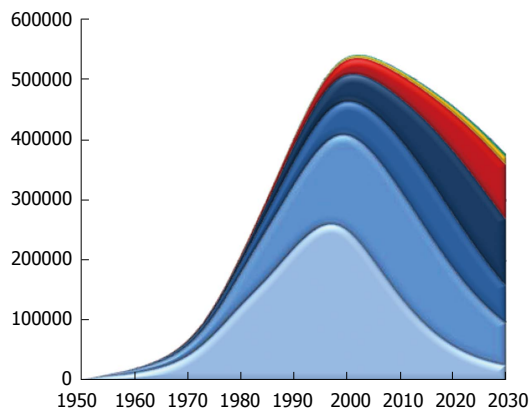
Figure 3 Model inputs for scenarios 1 and 2. SVR: Sustained virologic response; G1: Genotype 1.



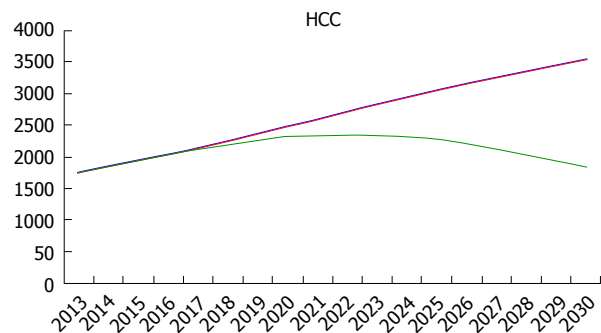
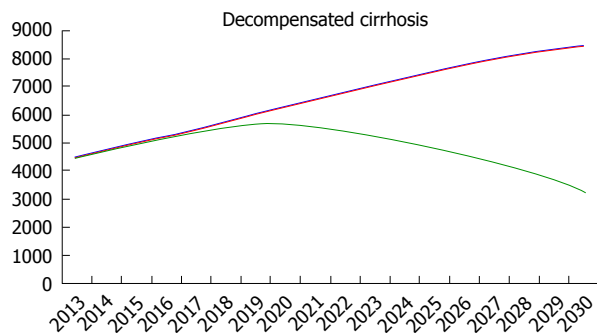
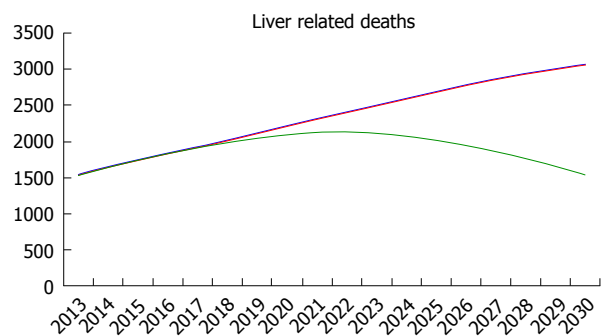
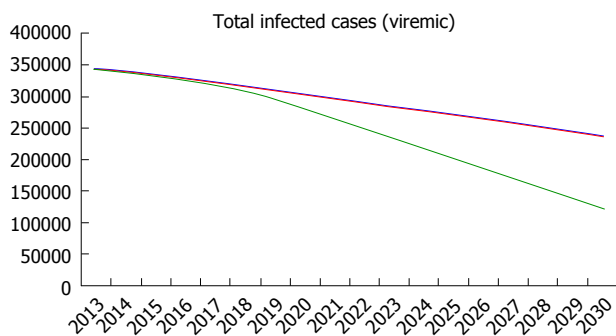
**Figure 4** Total number of viremic hepatitis C virus cases (with uncertainty intervals) according to year, 1950 to 2030.



**Figure 5** Number of viremic hepatitis C virus cases, in total and according to disease stage. F: Fibrosis stage; HCC: Hepatocellular carcinoma.



**Figure 6** Proportion of all viremic hepatitis C virus cases according to disease stage, 1950 to 2030. Decomp: Decompensated; F: Fibrosis stage; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus.



— Baseline — Scenario 1 — Scenario 2

**Figure 7** Selected hepatitis C virus-related outcomes by scenario - Argentina, 2013-2030. Scenario 1: Increased treatment efficacy; Scenario 2: Increased treatment efficacy and increased annual diagnosed/treated populations; HCC: Hepatocellular carcinoma.

## DISCUSSION

Increasing access to HCV diagnosis and treatment are pending actions in Argentina and in Latin-America. It is estimated that less than 20%-30% of patients are diagnosed and only 1%-2% of those diagnosed have been treated<sup>[7]</sup>. Approval of new HCV treatments in the region is delayed compared with Europe or the United States. In the last months of 2015, three novel regimens were approved in Argentina. Upcoming IFN and ribavirin free regimens are safe and effective, offering SVR rates over 90%-95% for most genotypes. To impact the burden of disease, patients must be diagnosed and treatment availability must increase.

Our study shows important results for our country. The greatest burden of HCV-related advanced liver disease will come in the next 5 to 15 years. HCV burden will increase if no action is taken. Our model showed that the only way to significantly reduce HCV burden is to increase diagnosed and treated patients 10 times the current number of treated persons. Similar results have been reported in many countries around the world, including some in Latin-America, including Brazil and Mexico<sup>[2,3]</sup>.

The main challenge in the region is to develop strategies to increase diagnosis. Strategies must be country specific since epidemiology and risk factors for HCV infection vary between countries. For example, the United States Centers for Disease Control and Prevention has recommended a birth-year based screening strategy: Persons born during 1945-1965 in the United States have an increased rate of HCV infection and focused screening of this cohort is an efficient use of resources<sup>[28]</sup>. But this strategy might not be effective in Argentina, since in 2013 the majority of HCV patients are estimated to be 40 to 75 years old (Figure 2), meaning that they were born between 1938 and 1973. The same was shown in Brazil where most patients were born between 1950 and 1980<sup>[29]</sup>. Country specific screening campaigns must be developed to achieve this goal.

Another pending issue is adequate access to care and treatment. This means that all people involved in HCV management must make an effort to achieve this goal. Patients need greater access to new therapies, but the main restriction is treatment cost. In resource constrained countries, treating all patients with current drug costs is unaffordable. There must be strategies to reduce HCV treatment costs and at the beginning, prioritization of treatment may be necessary. For example, the sickest patients will be treated first with the safest and more effective drugs. Then earlier stage patients will be treated later to reduce the impact of the disease.

This is the first study evaluating HCV burden in Argentina. These results might help public health authorities take action to reduce its impact. But it has to be mentioned that our results have some limitations.

First, each input may have its limitations, but to our knowledge the best data from published and unpu-

blished studies available in Argentina were applied in our model. Second, some patients may have progressive liver disease despite achieving SVR; progression of cured patients was not evaluated in this model<sup>[30]</sup>. And finally, we did not include extrahepatic manifestations of HCV infection in the model, which may have contributed to all-cause mortality and may lead to underestimation in mortality among viremic patients<sup>[9]</sup>.

In conclusion, the present analysis, with the available data, showed that HCV prevalence is decreasing in Argentina, but advanced liver disease prevalence is expected to raise as HCV infected patients get older. There is an urgent need to enhance diagnosis and treatment rates to reduce the future disease burden and its impact on Argentina's public health.

## COMMENTS

### Background

Chronic hepatitis C virus (HCV) infection is one of the main causes of end stage liver disease, liver transplantation, hepatocellular carcinoma (HCC) and liver-related mortality in Argentina. Burden of HCV disease is unknown, and strategies to reduce it are not yet developed.

### Research frontiers

An epidemiological model has been developed to estimate HCV disease burden and to evaluate different diagnostic and therapeutic strategies that may impact in HCV natural history.

### Innovations and breakthroughs

This model allows them for the first time to evaluate HCV burden in Argentina. This estimated data can help health authorities to develop a national plan to manage HCV disease. Also, it permits the authors to estimate the number of persons needing treatment to reduce HCV burden in the next 15 years.

### Applications

This study shows that HCV treatment impacts in its disease burden and that a major work has to be done in improving its diagnosis and access to treatment.

### Terminology

HCV disease burden implies the development of liver related disease: Cirrhosis, HCC, liver failure, liver transplantation and death.

### Peer-review

In this study the authors have used a modeling approach to describe HCV-related disease progression in Argentina. The methods are well designed and are exposed very clearly for the reader. In general, it is a good manuscript.

## REFERENCES

- 1 **Mohd Hanafiah K**, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]
- 2 **Hatzakis A**, Chulanov V, Gadano AC, Bergin C, Ben-Ari Z, Mossong J, Schr ter I, Baatarkhuu O, Acharya S, Aho I, Anand AC, Andersson MI, Arendt V, Arkkila P, Barclay K, Bessone F, Blach S, Blokhina N, Brunton CR, Choudhuri G, Cisneros L, Croes EA, Dahgwaahdorj YA, Dalgard O, Daruich JR, Dashdorj NR, Davaadorj D, de Knecht RJ, de Vree M, Estes C, Flisiak R, Gane E, Gower E, Halota W, Henderson C, Hoffmann P, Hornell J, Houlihan D, Hrusovsky S, Jar  uska P, Kershenobich D, Kostrzewska K, Kristian P, Leshno M, Lurie Y, Mahomed A, Mamonova N, Mendez-Sanchez N, Norris S, Nurmukhametova E, Nymadawa P, Oltman



- M, Oyunbileg J, Oyunsuren Ts, Papatheodoridis G, Pimenov N, Prabdhial-Sing N, Prins M, Radke S, Rakhmanova A, Razavi-Shearer K, Reesink HW, Ridruejo E, Safadi R, Sagalova O, Sanchez Avila JF, Sanduivav R, Saraswat V, Seguin-Devaux C, Shah SR, Shestakova I, Shevaldin A, Shibolet O, Silva MO, Sokolov S, Sonderup M, Souliotis K, Spearman CW, Staub T, Stedman C, Strebkova EA, Struck D, Sypsa V, Tomasiewicz K, Undram L, van der Meer AJ, van Santen D, Veldhuijzen I, Villamil FG, Willemse S, Zuckerman E, Zuure FR, Puri P, Razavi H. The present and future disease burden of hepatitis C virus (HCV) infections with today's treatment paradigm - volume 2. *J Viral Hepat* 2015; **22** Suppl 1: 26-45 [PMID: 25560840 DOI: 10.1111/jvh.12351]
- 3 **Saraswat V**, Norris S, de Knecht RJ, Sanchez Avila JF, Sonderup M, Zuckerman E, Arkkila P, Stedman C, Acharya S, Aho I, Anand AC, Andersson MI, Arendt V, Baatarkhuu O, Barclay K, Ben-Ari Z, Bergin C, Bessone F, Blach S, Blokhina N, Brunton CR, Choudhuri G, Chulanov V, Cisneros L, Croes EA, Dahgwahdorj YA, Dalgard O, Daruich JR, Dashdorj NR, Davaadorj D, de Vree M, Estes C, Flisiak R, Gadano AC, Gane E, Halota W, Hatzakis A, Henderson C, Hoffmann P, Hornell J, Houlihan D, Hrusovsky S, Jarčuška P, Kershenobich D, Kostrzewska K, Kristian P, Leshno M, Lurie Y, Mahomed A, Mamonova N, Mendez-Sanchez N, Mossong J, Nurmukhametova E, Nymadawa P, Oltman M, Oyunbileg J, Oyunsuren Ts, Papatheodoridis G, Pimenov N, Prabdhial-Sing N, Prins M, Puri P, Radke S, Rakhmanova A, Razavi H, Razavi-Shearer K, Reesink HW, Ridruejo E, Safadi R, Sagalova O, Sanduivav R, Schröter I, Seguin-Devaux C, Shah SR, Shestakova I, Shevaldin A, Shibolet O, Sokolov S, Souliotis K, Spearman CW, Staub T, Strebkova EA, Struck D, Tomasiewicz K, Undram L, van der Meer AJ, van Santen D, Veldhuijzen I, Villamil FG, Willemse S, Zuure FR, Silva MO, Sypsa V, Gower E. Historical epidemiology of hepatitis C virus (HCV) in select countries - volume 2. *J Viral Hepat* 2015; **22** Suppl 1: 6-25 [PMID: 25560839 DOI: 10.1111/jvh.12350]
  - 4 **Reggiardo MV**, Tanno F, Mendizabal M, Galdame O. [Argentine consensus on hepatitis C 2013]. *Acta Gastroenterol Latinoam* 2014; **44**: 154-173 [PMID: 25199310]
  - 5 **Vladimirsky S**, Silvina MM, Otegui L, Altabert N, Soto S, Brajerterman L, Echenique H, González J; Unidades Centinela para Hepatitis Virales. [Surveillance of viral hepatitis in Argentina: analysis of information from sentinel units 2007-2010]. *Acta Gastroenterol Latinoam* 2013; **43**: 22-30 [PMID: 23650830]
  - 6 **Ridruejo E**, Adrover R, Cocozzella D, Fernández N, Reggiardo MV. Efficacy, tolerability and safety in the treatment of chronic hepatitis C with combination of PEG-Interferon - Ribavirin in daily practice. *Ann Hepatol* 2010; **9**: 46-51 [PMID: 20308722]
  - 7 **Kershenobich D**, Razavi HA, Sánchez-Avila JF, Bessone F, Coelho HS, Dagher L, Gonçalves FL, Quiroz JF, Rodriguez-Perez F, Rosado B, Wallace C, Negro F, Silva M. Trends and projections of hepatitis C virus epidemiology in Latin America. *Liver Int* 2011; **31** Suppl 2: 18-29 [PMID: 21651701 DOI: 10.1111/j.1478-3231.2011.02538.x]
  - 8 **Razavi H**, Elkhoury AC, Elbasha E, Estes C, Pasini K, Poynard T, Kumar R. Chronic hepatitis C virus (HCV) disease burden and cost in the United States. *Hepatology* 2013; **57**: 2164-2170 [PMID: 23280550 DOI: 10.1002/hep.26218]
  - 9 **Myers RP**, Krajden M, Bilodeau M, Kaita K, Marotta P, Peltekian K, Ramji A, Estes C, Razavi H, Sherman M. Burden of disease and cost of chronic hepatitis C infection in Canada. *Can J Gastroenterol Hepatol* 2014; **28**: 243-250 [PMID: 24839620 DOI: 10.1155/2014/317623]
  - 10 **Flisiak R**, Halota W, Tomasiewicz K, Kostrzewska K, Razavi HA, Gower EE. Forecasting the disease burden of chronic hepatitis C virus in Poland. *Eur J Gastroenterol Hepatol* 2015; **27**: 70-76 [PMID: 25426979 DOI: 10.1097/MEG.0000000000000237]
  - 11 **Willemse SB**, Razavi-Shearer D, Zuure FR, Veldhuijzen IK, Croes EA, van der Meer AJ, van Santen DK, de Vree JM, de Knecht RJ, Zaaijer HL, Reesink HW, Prins M, Razavi H. The estimated future disease burden of hepatitis C virus in the Netherlands with different treatment paradigms. *Neth J Med* 2015; **73**: 417-431 [PMID: 26582807]
  - 12 **United Nations, Department of Economic and Social Affairs.** Population division (2011). World population prospects: The 2010 revision. Volume I: comprehensive tables. New York New York United Nations, 2010
  - 13 **del Pino N**, Oubiña JR, Rodríguez-Frías F, Esteban JI, Buti M, Otero T, Gregori J, García-Cehic D, Camos S, Cubero M, Casillas R, Guàrdia J, Esteban R, Quer J. Molecular epidemiology and putative origin of hepatitis C virus in random volunteers from Argentina. *World J Gastroenterol* 2013; **19**: 5813-5827 [PMID: 24124326 DOI: 10.3748/wjg.v19.i35.5813]
  - 14 **Personal Communication.** Situación epidemiológica en Argentina. 2014
  - 15 **Instituto Nacional Central Único Coordinador de Ablación e Implante.** El Sistema Nacional de Información de Procuración y Trasplante de la República Argentina. 2014
  - 16 **Cejas NG**, Villamil FG, Lendoire JC, Tagliafichi V, Lopez A, Krogh DH, Soratti CA, Bisigniano L. Improved waiting-list outcomes in Argentina after the adoption of a model for end-stage liver disease-based liver allocation policy. *Liver Transpl* 2013; **19**: 711-720 [PMID: 23775946 DOI: 10.1002/lt.23665]
  - 17 **IMS Health.** IMS Health MIDAS. Data. IMS Health, 2013
  - 18 **Wilmoth JR**, Shkolnikov V. Human Mortality Database. Berkeley, United States: University of California. Rostock, Germany: Mack Planck Institute for Demographic Research, 2013
  - 19 **Aceijas C**, Rhodes T. Global estimates of prevalence of HCV infection among injecting drug users. *Int J Drug Policy* 2007; **18**: 352-358 [PMID: 17854722 DOI: 10.1016/j.drugpo.2007.04.004]
  - 20 **Nelson PK**, Mathers BM, Cowie B, Hagan H, Des Jarlais D, Horyniak D, Degenhardt L. Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. *Lancet* 2011; **378**: 571-583 [PMID: 21802134 DOI: 10.1016/S0140-6736(11)61097-0]
  - 21 **Engström A**, Adamsson C, Allebeck P, Rydberg U. Mortality in patients with substance abuse: a follow-up in Stockholm County, 1973-1984. *Int J Addict* 1991; **26**: 91-106 [PMID: 2066174 DOI: 10.3109/10826089109056241]
  - 22 **Frischer M**, Goldberg D, Rahman M, Berney L. Mortality and survival among a cohort of drug injectors in Glasgow, 1982-1994. *Addiction* 1997; **92**: 419-427 [PMID: 9177063 DOI: 10.1111/j.1360-0443.1997.tb03373.x]
  - 23 **Hickman M**, Carnwath Z, Madden P, Farrell M, Rooney C, Ashcroft R, Judd A, Stimson G. Drug-related mortality and fatal overdose risk: pilot cohort study of heroin users recruited from specialist drug treatment sites in London. *J Urban Health* 2003; **80**: 274-287 [PMID: 12791803 DOI: 10.1093/jurban/jtg030]
  - 24 **Oppenheimer E**, Tobutt C, Taylor C, Andrew T. Death and survival in a cohort of heroin addicts from London clinics: a 22-year follow-up study. *Addiction* 1994; **89**: 1299-1308 [PMID: 7804091 DOI: 10.1111/j.1360-0443.1994.tb03309.x]
  - 25 **Perucci CA**, Davoli M, Rapiti E, Abeni DD, Forastiere F. Mortality of intravenous drug users in Rome: a cohort study. *Am J Public Health* 1991; **81**: 1307-1310 [PMID: 1656799]
  - 26 **Bjornaas MA**, Bekken AS, Ojlert A, Haldorsen T, Jacobsen D, Rostrup M, Ekeberg O. A 20-year prospective study of mortality and causes of death among hospitalized opioid addicts in Oslo. *BMC Psychiatry* 2008; **8**: 8 [PMID: 18271956 DOI: 10.1186/1471-244X-8-8]
  - 27 **Kamper-Jørgensen M**, Ahlgren M, Rostgaard K, Melbye M, Edgren G, Nyrén O, Reilly M, Norda R, Titlestad K, Tynell E, Hjalgrim H. Survival after blood transfusion. *Transfusion* 2008; **48**: 2577-2584 [PMID: 18673342 DOI: 10.1111/j.1537-2995.2008.01881.x]
  - 28 **Smith BD**, Morgan RL, Beckett GA, Falck-Ytter Y, Holtzman D, Teo CG, Jewett A, Baack B, Rein DB, Patel N, Alter M, Yartel A, Ward JW. Recommendations for the identification of chronic hepatitis C virus infection among persons born during 1945-1965. *MMWR Recomm Rep* 2012; **61**: 1-32 [PMID: 22895429]
  - 29 **Razavi H**, Waked I, Sarrazin C, Myers RP, Idilman R, Calinas F, Vogel W, Mendes Correa MC, Hézode C, Lázaro P, Akarca U, Aleman S, Balık I, Berg T, Bihl F, Bilodeau M, Blasco AJ,

Brandão Mello CE, Bruggmann P, Buti M, Calleja JL, Cheinquer H, Christensen PB, Clausen M, Coelho HS, Cramp ME, Dore GJ, Doss W, Duberg AS, El-Sayed MH, Ergör G, Esmat G, Falconer K, Félix J, Ferraz ML, Ferreira PR, Frankova S, García-Samaniego J, Gerstoft J, Giria JA, Gonçalves FL, Gower E, Gschwandler M, Guimarães Pessoa M, Hindman SJ, Hofer H, Husa P, Kåberg M, Kaita KD, Kautz A, Kaymakoglu S, Krajden M, Krarup H, Laleman W, Lavanchy D, Marinho RT, Marotta P, Mauss S, Moreno C, Murphy K, Negro F, Nemecek V, Örmeci N, Øvrehus AL, Parkes J, Pasini K, Peltekian KM, Ramji A, Reis N, Roberts SK, Rosenberg WM, Roudot-Thoraval F, Ryder SD, Sarmiento-Castro R, Semela D, Sherman M, Shiha GE, Sievert W, Sperl J,

Stärkel P, Stauber RE, Thompson AJ, Urbanek P, Van Damme P, van Thiel I, Van Vlierberghe H, Vandijck D, Wedemeyer H, Weis N, Wiegand J, Yosry A, Zekry A, Cornberg M, Müllhaupt B, Estes C. The present and future disease burden of hepatitis C virus (HCV) infection with today's treatment paradigm. *J Viral Hepat* 2014; **21** Suppl 1: 34-59 [PMID: 24713005 DOI: 10.1111/jvh.12248]

- 30 **Aleman S**, Rahbin N, Weiland O, Davidsdottir L, Hedenstierna M, Rose N, Verbaan H, Stål P, Carlsson T, Norrgren H, Ekbom A, Granath F, Hultcrantz R. A risk for hepatocellular carcinoma persists long-term after sustained virologic response in patients with hepatitis C-associated liver cirrhosis. *Clin Infect Dis* 2013; **57**: 230-236 [PMID: 23616492 DOI: 10.1093/cid/cit234]

**P- Reviewer:** Ciftci S, Medina P **S- Editor:** Gong XM  
**L- Editor:** A **E- Editor:** Liu SQ



## Host factors are dominant in the development of post-liver transplant non-alcoholic steatohepatitis

Salih Boga, Armando Salim Munoz-Abraham, Manuel I Rodriguez-Davalos, Sukru H Emre, Dhanpat Jain, Michael L Schilsky

Salih Boga, Michael L Schilsky, Division of Digestive Diseases, Section of Transplantation and Immunology, Department of Medicine and Surgery, Yale University School of Medicine, New Haven, CT 06520, United States

Armando Salim Munoz-Abraham, Manuel I Rodriguez-Davalos, Sukru H Emre, Department of Surgery, Section of Transplantation and Immunology, Yale-New Haven Transplantation Center, Yale University School of Medicine, New Haven, CT 06520, United States

Dhanpat Jain, Department of Pathology, Yale University School of Medicine, New Haven, CT 06520, United States

**Author contributions:** Boga S and Munoz-Abraham AS collected the data from patients' file, reviewed literature and drafted manuscript; Jain D evaluated the biopsy specimens from pathologic points of view, prepared demonstrative pathology pictures; Rodriguez-Davalos MI, Emre SH and Schilsky ML supervised in designing and drafting the manuscript, revised the manuscript critically for important intellectual content; Emre SH and Schilsky ML presented patients' clinical data, constituted the final form of manuscript; all authors read and approved the final manuscript.

**Institutional review board statement:** This case report was exempt from the Institutional Review Board standards at Yale University.

**Informed consent statement:** The patients involved in this report gave their written informed consents authorizing use and disclosure of their protected health information.

**Conflict-of-interest statement:** All the authors have no conflicts of interests to declare.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

[licenses/by-nc/4.0/](http://creativecommons.org/licenses/by-nc/4.0/)

**Manuscript source:** Invited manuscript

**Correspondence to:** Salih Boga, MD, Postdoctoral Fellow, Division of Digestive Diseases, Section of Transplantation and Immunology, Department of Medicine and Surgery, Yale University School of Medicine, 333 Cedar Street, LMP 1080, New Haven, CT 06520, United States. [salihboga@yahoo.com](mailto:salihboga@yahoo.com)  
**Telephone:** +1-203-7371592  
**Fax:** +1-203-7856645

**Received:** January 25, 2016

**Peer-review started:** January 25, 2016

**First decision:** February 29, 2016

**Revised:** March 30, 2016

**Accepted:** May 7, 2016

**Article in press:** May 9, 2016

**Published online:** May 28, 2016

### Abstract

Non-alcoholic fatty liver disease (NAFLD) is a recognized problem in patients after orthotopic liver transplantation and may lead to recurrent graft injury. As the increased demand for liver allografts fail to match the available supply of donor organs, split liver transplantation (SLT) has emerged as an important technique to increase the supply of liver grafts. SLT allows two transplants to occur from one donor organ, and provides a unique model for observing the pathogenesis of NAFLD with respect to the role of recipient environmental and genetic factors. Here we report on two recipients of a SLT from the same deceased donor where only one developed non-alcoholic steatohepatitis (NASH), suggesting that host factors are critical for the development of NASH.

**Key words:** Liver; Split graft; Steatohepatitis; Host factors; Transplant

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Split liver transplantation provides a unique model of the pathogenesis of non-alcoholic fatty liver disease with respect to the role of recipient environmental risk factors and genetic background because the same donor graft is shared by two distinct recipients. Here we present two recipients of a split liver transplantation from same deceased donor, with one developing nonalcoholic steatohepatitis and the other without any evidence of hepatic steatosis three years after they were transplanted. These cases provide a unique natural experiment to explore host factors that contributed to the development of nonalcoholic steatohepatitis after liver transplantation.

Boga S, Munoz-Abraham AS, Rodriguez-Davalos MI, Emre SH, Jain D, Schilsky ML. Host factors are dominant in the development of post-liver transplant non-alcoholic steatohepatitis. *World J Hepatol* 2016; 8(15): 659-664 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i15/659.htm> DOI: <http://dx.doi.org/10.4254/wjgh.v8.i15.659>

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) affects around one third of the western population with an incidence that continues to grow in other parts of the world<sup>[1]</sup>. Histopathological findings of NAFLD in the liver range from simple steatosis to non-alcoholic steatohepatitis (NASH), and can eventually progress to cirrhosis and liver cancer<sup>[2]</sup>. NAFLD is recognized as a potential complication following LT and studies are being conducted to determine the prevalence and risk factors for development of NAFLD in LT recipients<sup>[3,4]</sup>.

Split liver transplantation (SLT) has emerged as an important strategy to increase the supply of liver grafts by allowing two transplants to occur from one donor organ, and provide a unique opportunity to observe the role of host factors in the development of NAFLD. The technique of SLT is continuously evolving with reduced ischemia times and reduced vascular and biliary complications, and when performed *in situ*, SLT has yielded excellent outcomes<sup>[5]</sup>. However, SLT still involves significant complexity and short and long term complications of the split grafts need to be continually analyzed. Here we present data on the clinical course and outcomes of two recipients of a SLT from the same deceased donor where only one developed NASH, suggesting that extrahepatic host factors are critical for the development of NASH.

## CASE REPORT

### Case 1

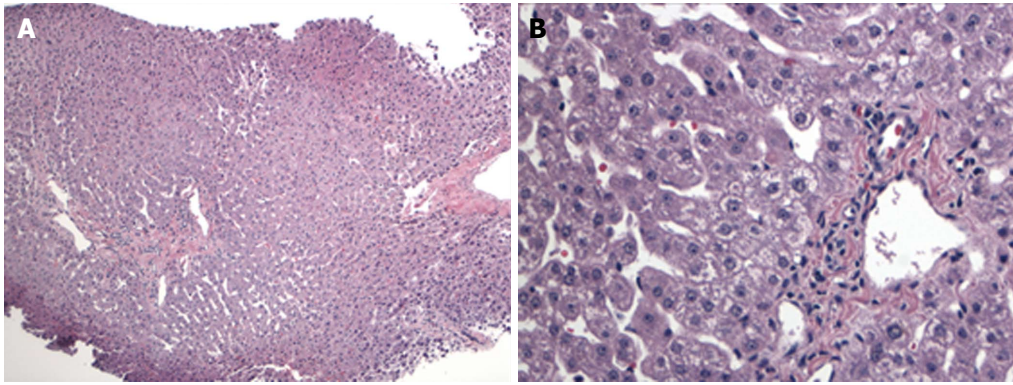
A female infant with a diagnosis of Crigler Najjar syn-

drome underwent a SLT at age 15 mo old, receiving a left lateral liver segment from a deceased donor who was exitus because of head trauma at age 16 years without any history of obesity, diabetes, hyperlipidemia or hypertension. The explanted liver did not reveal any significant histopathological abnormality and the donor pre-reperfusion biopsy was also negative for any significant pathologic findings, including inflammation, fibrosis, necrosis and steatosis (Figure 1). During the first year following transplantation, several episodes of liver test elevations were noted. Histology of the liver biopsy performed 19 mo post-SLT was not consistent with acute cellular rejection but showed minimal lobular inflammation, mild periportal edema and mild fibrosis (stage 1-2/4) without significant ductular reaction. No steatosis was present. A second biopsy performed 25 mo post-transplant due to continued liver test abnormalities revealed no histologic evidence of steatosis or progression of fibrosis (Figure 2). At this time the liver biopsy showed minimal portal fibrosis and no evidence of rejection, duct injury or duct loss. Subsequently magnetic resonance cholangiopancreatography was performed and an anatomic biliary stricture and dilated intrahepatic biliary ducts were identified. The patient underwent biliary reconstruction and a Roux-en-Y hepatojejunostomy and biliary stenting with internal-external drain placement at age 4 years. Three years following transplantation and two months after the biliary repair, liver tests improved [alanine aminotransferase (ALT): 29 U/L, aspartate aminotransferase (AST): 39 U/L, T/D Bil: 0.34/0.10 mg/dL, international normalized ratio (INR): 0.93]. Growth was in the normal range with a body mass index (BMI): 17.58 kg/m<sup>2</sup>. She was maintained on tacrolimus, mycophenolate mofetil and ursodeoxycholic acid treatment with routine biliary drain checks and close follow-up.

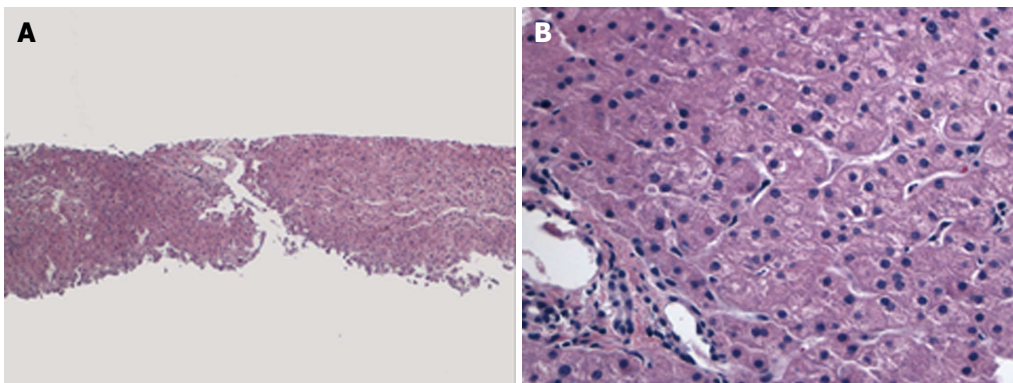
### Case 2

A 69-year-old male patient with a history of heterozygosity for genetic hemochromatosis (single copy of C282Y for the *HFE* gene) and alcoholic cirrhosis complicated by development of hepatocellular carcinoma (HCC) within Milan criteria (a 2.6 cm in segment III, and 1.5 and 1.1 cm lesions in segment VI). He was treated with chemoembolization and 8 mo later underwent an extended right lobe LT (segments I-IV-VIII) from the same deceased donor. The explanted liver revealed cirrhosis with residual viable nodules of moderately differentiated hepatocellular carcinoma without any vascular invasion, mild steatosis with steatohepatitis and increased hepatocellular siderosis. His past medical history was remarkable for hypertension, hyperlipidemia and diabetes mellitus. Initial immunosuppression was with steroid and tacrolimus, and he was changed to sirolimus and a lower dosage of tacrolimus at eight weeks post-transplant to try to reduce the risk of recurrent HCC. Although his postoperative course was uncomplicated but he remained on insulin for glycemic control. Twenty-four months after SLT, he had HCC

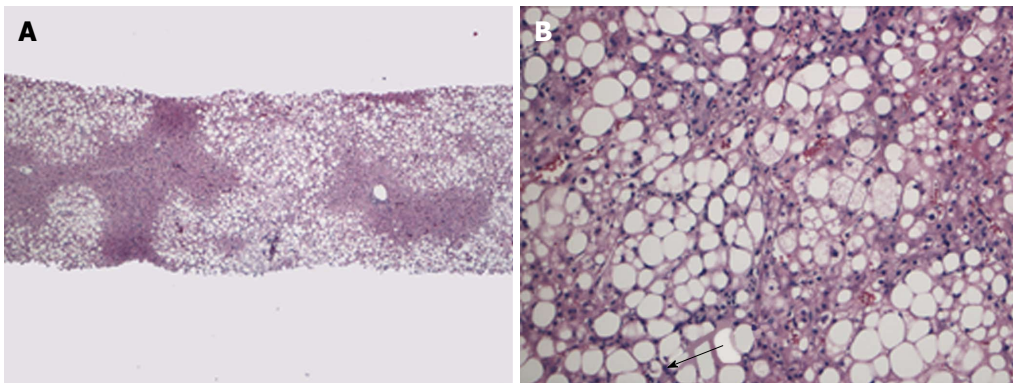




**Figure 1** Biopsy of the donor liver showing a lack of obvious pathologic changes. Specifically there is no steatosis, fibrosis or inflammation (H and E stain). A: Low magnification ( $\times 100$ ); B: Higher magnification ( $\times 200$ ) of the biopsy.



**Figure 2** Biopsy of liver graft from case 1 performed 25 mo later showing a lack of significant pathologic changes. Specifically, no steatosis is noted (H and E stain). A: Low magnification ( $\times 100$ ); B: Higher magnification ( $\times 200$ ) of the biopsy.



**Figure 3** Biopsy of the liver graft from case 2 about 3 years post split liver transplantation showing marked macrovesicular steatosis (H and E stain). A: Low magnification ( $\times 100$ ); B: Higher magnification showing rare Mallory-Denk bodies (arrow), ballooned hepatocytes and mild lobular inflammation ( $\times 200$ ). Trichrome stain revealed mild sinusoidal fibrosis (not shown here).

recurrence with a solitary 1.5 cm nodule in segment VIII found on surveillance imaging. The tumor was treated by selective chemoembolization and he is without recurrence on follow up magnetic resonance images; the most recent being 34 mo post-transplant. Three years after transplantation, his liver function tests were found to be elevated on routine testing, and increased cholesterol and triglyceride levels were noted (ALT: 154 U/L, AST: 125 U/L, T/D Bil: 0.85/0.20 mg/dL, INR:

1.1, total cholesterol: 218 mg/dL, HDL cholesterol: 34 mg/dL, triglyceride: 501 mg/dL). At the same time, his weight had increased by 12% (had BMI increased from 30.5 kg/m<sup>2</sup> to 34 kg/m<sup>2</sup>). He denied drinking alcohol. A liver biopsy was performed and showed no evidence of acute or chronic cellular rejection but was notable for marked macrovesicular steatosis involving about 70% of liver parenchyma, steatohepatitis and perisinusoidal fibrosis (grade 1 of 3, stage 1 of 4, Brunt system) (Figure

3).

## DISCUSSION

LT is the accepted treatment of end-stage liver disease. The establishment of standard transplantation techniques, development of better immunosuppressive medications and accumulated experience in their safe use, improvement of intensive care and anesthesia all have played a major role in improving current 1-year survival after LT to 90%. Long-term outcomes, however, are still compromised by recurrent liver disease, increased risk of cancer, adverse effects of immunosuppressive drugs and possible metabolic complications<sup>[6,7]</sup>. One of the possible metabolic complications is the development of NASH/NAFLD.

SLT has developed as an alternative to increase the donor pool of organ for LT. The concept of splitting a liver allograft between two recipients was reported almost simultaneously by Pichlmayr *et al.*<sup>[8]</sup> and Bismuth *et al.*<sup>[9]</sup>. Recipients of SLT in the mid 1990s<sup>[10,11]</sup> were primarily one child who received the left-lateral segments and one adult who received the extended right lobe. SLT provides a unique model of the pathogenesis of NAFLD with respect to the role of recipient environmental risk factors and genetic background because the same donor graft is shared by two distinct recipients. Here we present two recipients of a SLT from same deceased donor, with one developing NASH and the other without any evidence of hepatic steatosis three years after they were transplanted. These cases provide a unique natural experiment to explore host factors that contributed to the development of NASH after LT.

LT recipients have several risk factors that put them at risk for NAFLD. Age and rapid weight gain causing obesity and long-term exposure to immunosuppressive medications can in part be responsible for NAFLD. Hyperlipidemia occurs frequently following solid-organ transplantation. Between 16% and 43% of adult LT recipients can have increased plasma cholesterol levels<sup>[7,12,13]</sup>. Furthermore corticosteroids and calcineurin inhibitors promote hypertension and hypercholesterolemia, prednisone, tacrolimus, and cyclosporine A are diabetogenic, and sirolimus induces hyperlipidemia. Although both of our recipients were placed on tacrolimus, the older patient was also treated with sirolimus. In our adult recipient, use of tacrolimus and the aberrant gain in weight likely increased his already present insulin resistance and contributed to further deterioration of glucose regulation, causing an increase in hepatic fatty infiltration and inflammation that ended with steatohepatitis.

In the first few months after liver LT, weight gain may be regarded as one of the positive effects of transplantation, especially in patients with advanced liver disease and pre-transplant cachexia. However, within two years of transplantation, an excess body weight is recorded in up to 60% to 70% of patients and 20% of previously non-obese transplant recipients become

obese<sup>[14,15]</sup>. The recipient with steatohepatitis showed an increase in BMI from 30.5 kg/m<sup>2</sup> at time of transplant to 34 kg/m<sup>2</sup>, corresponding to a 12% increase in weight over 3 years time. NAFLD is strongly linked to obesity, (BMI > 30 kg/m<sup>2</sup>) with a reported prevalence as high as 80% in obese patients and only 16% in individuals with a normal BMI<sup>[16]</sup>. Although the exact mechanisms leading to excessive weight gain in post-LT patients are uncertain, a major role is attributed to the development of post-LT insulin resistance, diabetes mellitus, arterial hypertension, hyperlipidemia and the metabolic effects of immunosuppressive medications (corticosteroids, mTOR inhibitors and calcineurin inhibitors).

There are other factors in our steatohepatic recipient that may have increased hepatic fatty infiltration. The patient had a history of alcoholic cirrhosis, and some patients transplanted for alcoholic disease have a significantly higher risk of post-LT NAFLD even in the absence of recurrent alcoholic intoxication. Kim *et al.*<sup>[4]</sup>, reported that even though in 156 patients who had stopped drinking or had a limited amount of alcohol after LT, pre-LT alcoholic liver cirrhosis was a significant factor for their development of post-LT NAFLD. Similarly Dumortier *et al.*<sup>[17]</sup> suggested that many patients with post-LT NAFLD have an unrecognized combination of alcoholic and non-alcoholic steatohepatitis that put them at risk of secondary liver failure because of persistent metabolic abnormalities. Recently Hejlova *et al.*<sup>[18]</sup> examined 2360 post-transplant biopsies of 548 LT recipients to identify risk factors for the development of significant steatosis and found alcohol induced cirrhosis as a pre-transplant factor that is associated with significant post-transplant steatosis. It is likely patients with this combination of alcoholic and non-alcoholic steatohepatitis pre-transplant have an increased risk of persistence of metabolic abnormalities post-LT due to newly *de-novo* or aggravated insulin resistance.

Though the adult recipient had a pre-transplant diagnosis of iron overload disorder, genetic hemochromatosis is cured by liver transplantation<sup>[19]</sup>. There are instances where recipients received organs from patients with hemochromatosis and iron accumulation has occurred<sup>[20]</sup>. In the adult recipient, we did not find iron accumulation in his liver biopsy, suggesting iron did not play any additional role in the genesis of his steatohepatitis. Of the factors mentioned above; age, genetic background and even pretransplant history of alcoholic cirrhosis may be considered as unchangeable host factors where as post-transplant life style changes, diet, glycemic control by anti-diabetic medications, control of weight gain, hyperlipidemia therapy and immunosuppressive medications are changeable host factors that can affect the presence and progression of post-LT NASH.

In conclusion, this SLT provided a unique opportunity to observe the pathogenesis of NAFLD in the post-transplant setting. Although we can not exclude an interaction of donor and host factors, our data suggest host factors may be dominant for the development of

post-LT NASH.

Because we can not change the genetic background of the donor organ, careful attention to potentially alterable host factors or treatments like diet, life style changes, hyperlipidemia therapy and immunosuppressive medications can result in improved long term outcomes for recipients.

## COMMENTS

### Case characteristics

A female infant with a diagnosis of Crigler Najjar syndrome and a 69-year-old male patient with cirrhosis complicated by development of hepatocellular carcinoma (HCC) underwent split liver transplantation (SLT).

### Clinical diagnosis

Infant had jaundice and the elderly patient had signs of cirrhosis such as jaundice, ascites and spider angioma.

### Differential diagnosis

Inherited disorders of bilirubin metabolism for the first patient and primary and metastatic malignities of the liver for the second patient.

### Laboratory diagnosis

The first patient had elevated bilirubin levels (total bilirubin: 21 mg/dL, direct bilirubin: 0.25 mg/dL) and the second patient had an alpha fetoprotein of 109 ng/mL.

### Imaging diagnosis

The adult patient had a 2.6 cm HCC lesion in segment III, and 1.5 and 1.1 cm HCC lesions in segment VI on magnetic resonance scan.

### Pathological diagnosis

While the explanted liver did not reveal any significant histopathological abnormality in the first patient and revealed cirrhosis with residual viable nodules of HCC in the second patient; post-transplant liver biopsies showed minimal portal fibrosis and no histologic evidence of steatosis in the first patient and showed macrovesicular steatosis, steatohepatitis and perisinusoidal fibrosis in the second patient.

### Treatment

First patient underwent biliary reconstruction, a Roux-en-Y hepatojejunostomy and biliary stenting with internal-external drain placement and was maintained on tacrolimus, mycophenolate mofetil and ursodeoxycholic acid treatment and the second patient had an initial immunosuppression with steroid and tacrolimus, and then was changed to sirolimus and a lower dosage of tacrolimus at eight weeks post-transplant and had selective chemoembolization for recurrent HCC.

### Related reports

Even though emerging literature puts non-alcoholic fatty liver disease (NAFLD) forward as a potential complication following liver transplantation, SLT presented in this report provided a unique model of the pathogenesis of NAFLD with respect to the role of recipient environmental risk factors and genetic background because the same donor graft was shared by two distinct recipients.

### Term explanation

Crigler-Najjar syndrome is a rare hereditary disorder of bilirubin metabolism characterized by unconjugated hyperbilirubinemia due to deficiency of the enzymatic activity of glucuronosyltransferase.

### Experiences and lessons

This SLT provided a unique opportunity to observe the pathogenesis of

NAFLD in the post-transplant setting. The data suggest host factors may be dominant for the development of post-LT non-alcoholic steatohepatitis (NASH). The authors recommend paying careful attention to potentially alterable host factors or treatments like diet, life style changes, hyperlipidemia therapy and immunosuppressive medications to improve the long term outcomes for recipients.

### Peer-review

This is an interesting case report about post transplant NASH comparing two different scenarios (two different hosts) for a unique donor from a split liver transplant.

## REFERENCES

- Milić S, Stimac D. Nonalcoholic fatty liver disease/steatohepatitis: epidemiology, pathogenesis, clinical presentation and treatment. *Dig Dis* 2012; **30**: 158-162 [PMID: 22722431 DOI: 10.1159/000336669]
- Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140 [PMID: 12105842]
- Seo S, Maganti K, Khehra M, Ramsamooj R, Tsodikov A, Bowlus C, McVicar J, Zern M, Torok N. De novo nonalcoholic fatty liver disease after liver transplantation. *Liver Transpl* 2007; **13**: 844-847 [PMID: 17029282 DOI: 10.1002/lt.20932]
- Kim H, Lee K, Lee KW, Yi NJ, Lee HW, Hong G, Choi Y, You T, Suh SW, Jang JJ, Suh KS. Histologically proven non-alcoholic fatty liver disease and clinically related factors in recipients after liver transplantation. *Clin Transplant* 2014; **28**: 521-529 [PMID: 24579874 DOI: 10.1111/ctr.12343]
- Emre S, Umman V. Split liver transplantation: an overview. *Transplant Proc* 2011; **43**: 884-887 [PMID: 21486620 DOI: 10.1016/j.transproceed.2013.02.063]
- Reuben A. Long-term management of the liver transplant patient: diabetes, hyperlipidemia, and obesity. *Liver Transpl* 2001; **7**: S13-S21 [PMID: 11689772 DOI: 10.1053/jlts.2001.29167]
- Sheiner PA, Magliocca JF, Bodian CA, Kim-Schluger L, Altaca G, Guarrera JV, Emre S, Fishbein TM, Guy SR, Schwartz ME, Miller CM. Long-term medical complications in patients surviving > or = 5 years after liver transplant. *Transplantation* 2000; **69**: 781-789 [PMID: 10755526 DOI: 10.1097/00007890-200003150-00018]
- Pichlmayr R, Ringe B, Gubernatis G, Hauss J, Bunzendahl H. [Transplantation of a donor liver to 2 recipients (splitting transplantation)--a new method in the further development of segmental liver transplantation]. *Langenbecks Arch Chir* 1988; **373**: 127-130 [PMID: 3287073]
- Bismuth H, Morino M, Castaing D, Gillon MC, Descorps Declere A, Saliba F, Samuel D. Emergency orthotopic liver transplantation in two patients using one donor liver. *Br J Surg* 1989; **76**: 722-724 [PMID: 2670054 DOI: 10.1002/bjs.1800760723]
- Azoulay D, Astarcioglu I, Bismuth H, Castaing D, Majno P, Adam R, Johann M. Split-liver transplantation. The Paul Brousse policy. *Ann Surg* 1996; **224**: 737-746; discussion 746-748 [PMID: 8968228]
- Rogiers X, Malagó M, Gawad K, Jauch KW, Olausson M, Knoefel WT, Gundlach M, Bassas A, Fischer L, Sterneck M, Burdelski M, Broelsch CE. In situ splitting of cadaveric livers. The ultimate expansion of a limited donor pool. *Ann Surg* 1996; **224**: 331-339; discussion 339-341 [PMID: 8813261]
- Imagawa DK, Dawson S, Holt CD, Kirk PS, Kaldas FM, Shackleton CR, Seu P, Rudich SM, Kinkhabwala MM, Martin P, Goldstein LI, Murray NG, Terasaki PI, Busuttil RW. Hyperlipidemia after liver transplantation: natural history and treatment with the hydroxy-methylglutaryl-coenzyme A reductase inhibitor pravastatin. *Transplantation* 1996; **62**: 934-942 [PMID: 8878387 DOI: 10.1097/00007890-199610150-00011]
- Gisbert C, Prieto M, Berenguer M, Bretó M, Carrasco D, de



- Juan M, Mir J, Berenguer J. Hyperlipidemia in liver transplant recipients: prevalence and risk factors. *Liver Transpl Surg* 1997; **3**: 416-422 [PMID: 9346772 DOI: 10.1002/lt.500030409]
- 14 **Everhart JE**, Lombardero M, Lake JR, Wiesner RH, Zetterman RK, Hoofnagle JH. Weight change and obesity after liver transplantation: incidence and risk factors. *Liver Transpl Surg* 1998; **4**: 285-296 [PMID: 9649642 DOI: 10.1002/lt.500040402]
- 15 **Palmer M**, Schaffner F, Thung SN. Excessive weight gain after liver transplantation. *Transplantation* 1991; **51**: 797-800 [PMID: 2014532 DOI: 10.1097/00007890-199104000-00012]
- 16 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]
- 17 **Dumortier J**, Giostra E, Belbouab S, Morard I, Guillaud O, Spahr L, Boillot O, Rubbia-Brandt L, Scoazec JY, Hadengue A. Non-alcoholic fatty liver disease in liver transplant recipients: another story of “seed and soil”. *Am J Gastroenterol* 2010; **105**: 613-620 [PMID: 20040915 DOI: 10.1038/ajg.2009.717]
- 18 **Hejlova I**, Honsova E, Sticova E, Lanska V, Hucl T, Spicak J, Jirsa M, Trunecka P. Prevalence and risk factors of steatosis after liver transplantation and patient outcomes. *Liver Transpl* 2016; **22**: 644-655 [PMID: 26707008 DOI: 10.1002/lt.24393]
- 19 **Moini M**, Mistry P, Schilsky ML. Liver transplantation for inherited metabolic disorders of the liver. *Curr Opin Organ Transplant* 2010; **15**: 269-276 [PMID: 20489626 DOI: 10.1097/MOT.0b013e3283399dbd]
- 20 **Dwyer JP**, Sarwar S, Egan B, Nolan N, Hegarty J. Hepatic iron overload following liver transplantation of a C282y homozygous allograft: a case report and literature review. *Liver Int* 2011; **31**: 1589-1592 [PMID: 22093334 DOI: 10.1111/j.1478-3231.2011.02606.x]

**P- Reviewer:** Inoue K, Ikura Y, Komatsu H, Villela-Nogueira CA

**S- Editor:** Gong XM **L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 June 8; 8(16): 665-702





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**MINIREVIEWS**

- 665 Mechanisms of adaptation of the hepatic vasculature to the deteriorating conditions of blood circulation in liver cirrhosis

*Garbuzenko DV, Arefyev NO, Belov DV*

**ORIGINAL ARTICLE****Basic Study**

- 673 Obese diet-induced mouse models of nonalcoholic steatohepatitis-tracking disease by liver biopsy

*Kristiansen MNB, Veidal SS, Rigbolt KTG, Tølbøl KS, Roth JD, Jelsing J, Vrang N, Feigh M*

**Retrospective Study**

- 685 Hepatocellular carcinoma after locoregional therapy: Magnetic resonance imaging findings in falsely negative exams

*Becker-Weidman D, Civan JM, Deshmukh SP, Roth CG, Herrine SK, Parker L, Mitchell DG*

**SYSTEMATIC REVIEWS**

- 691 Redefining Budd-Chiari syndrome: A systematic review

*Shin N, Kim YH, Xu H, Shi HB, Zhang QQ, Colon Pons JP, Kim D, Xu Y, Wu FY, Han S, Lee BB, Li LS*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Bum-Joon Kim, PhD, Professor, Department of Microbiology and Immunology, and Liver Research Institute, Seoul National University, College of Medicine, Seoul 151-742, South Korea

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Su-Qing Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjnet.com](mailto:editorialoffice@wjnet.com)  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjnet.com](mailto:bpgoffice@wjnet.com)  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

PUBLICATION DATE  
June 8, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

Full instructions are available online at [http://www.wjnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjnet.com/bpg/g_info_20160116143427.htm)

**ONLINE SUBMISSION**

<http://www.wjnet.com/esps/>



## Mechanisms of adaptation of the hepatic vasculature to the deteriorating conditions of blood circulation in liver cirrhosis

Dmitry Victorovich Garbuzenko, Nikolay Olegovich Arefyev, Dmitry Vladimirovich Belov

Dmitry Victorovich Garbuzenko, Nikolay Olegovich Arefyev, Department of Faculty Surgery, South Ural State Medical University, 454092 Chelyabinsk, Russia

Dmitry Vladimirovich Belov, Department of Hospital Surgery, South Ural State Medical University, 454092 Chelyabinsk, Russia

**Author contributions:** Garbuzenko DV contributed to the conception and design; acquisition, analysis and interpretation of data; drafting the article; final approval of the version; all authors wrote this manuscript.

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dmitry Victorovich Garbuzenko, MD, PhD, Professor, Department of Faculty Surgery, South Ural State Medical University, Box 12317, 454092 Chelyabinsk, Russia. [garb@inbox.ru](mailto:garb@inbox.ru)  
 Telephone: +8-909-7459826  
 Fax: +8-351-2687772

Received: March 12, 2016  
 Peer-review started: March 13, 2016  
 First decision: April 18, 2016  
 Revised: April 25, 2016  
 Accepted: May 17, 2016  
 Article in press: May 27, 2016  
 Published online: June 8, 2016

### Abstract

PubMed, EMBASE, Orphanet, MIDLINE, Google Scholar and Cochrane Library were searched for articles published between 1983 and 2015. Relevant articles were selected by using the following terms: "Liver cirrhosis", "Endothelial dysfunction", "Sinusoidal remodeling", "Intrahepatic angiogenesis" and "Pathogenesis of portal hypertension". Then the reference lists of identified articles were searched for other relevant publications as well. Besides gross hepatic structural disorders related to diffuse fibrosis and formation of regenerative nodules, the complex morphofunctional rearrangement of the hepatic microvascular bed and intrahepatic angiogenesis also play important roles in hemodynamic disturbances in liver cirrhosis. It is characterized by endothelial dysfunction and impaired paracrine interaction between activated stellate hepatocytes and sinusoidal endotheliocytes, sinusoidal remodeling and capillarization, as well as development of the collateral microcirculation. In spite of the fact that complex morphofunctional rearrangement of the hepatic microvascular bed and intrahepatic angiogenesis in liver cirrhosis are the compensatory-adaptive reaction to the deteriorating conditions of blood circulation, they contribute to progression of disease and development of serious complications, in particular, related to portal hypertension.

**Key words:** Liver cirrhosis; Endothelial dysfunction; Sinusoidal remodeling; Intrahepatic angiogenesis; Pathogenesis of portal hypertension

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Besides gross hepatic structural disorders related to diffuse fibrosis and formation of regenerative nodules, the complex morphofunctional rearrangement

of the hepatic microvascular bed and intrahepatic angiogenesis play important roles in hemodynamic disturbances in liver cirrhosis. In spite of the fact that these changes of the hepatic vasculature are the compensatory-adaptive reaction to the deteriorating conditions of blood circulation, they contribute to the progression of disease and development of serious complications, in particular, related to portal hypertension.

Garbuzenko DV, Arefyev NO, Belov DV. Mechanisms of adaptation of the hepatic vasculature to the deteriorating conditions of blood circulation in liver cirrhosis. *World J Hepatol* 2016; 8(16): 665-672 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i16/665.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i16.665>

## MORPHOFUNCTIONAL REARRANGEMENT OF THE HEPATIC MICROVASCULAR BED IN LIVER CIRRHOSIS

Besides gross hepatic structural disorders related to diffuse fibrosis and formation of regenerative nodules, the complex morphofunctional rearrangement of the hepatic microvascular bed in liver cirrhosis also contributes to the development of severe complications, in particular, associated with portal hypertension<sup>[1]</sup>. In this situation, the main place of resistance to portal blood flow is in pathologically modified sinusoids. Sinusoidal endothelial cells (SEC) become dysfunctional and among other features acquire a vasoconstrictor phenotype. It leads to increasing of SEC sensitivity to endogenous vasoconstrictors, such as endothelin, norepinephrine, angiotensin II, vasopressin, leukotrienes, thromboxane A2. In contrast, the production of nitric oxide (the most studied vasodilator involved in the regulation of hepatic vascular tone) is reduced. The reason for this may be insufficient activity of endothelial nitric oxide synthase (eNOS) due to its increased interaction with caveolin-1. Furthermore, endothelin-1 activates G-protein-coupled receptor kinase-2 which directly interacts with and inhibits protein kinase B (Akt) phosphorylation and decreases the production of nitric oxide (NO)<sup>[2]</sup>.

One of the main factors of sinusoidal endothelial dysfunction in cirrhosis is intrahepatic oxidative stress, which is associated with a decrease of eNOS expression and NO bioavailability. For example, cyclooxygenase attenuates Akt-eNOS signalization by stimulating thromboxane A2, which inhibits Akt phosphorylation in endothelial cells, as well as excessive activation of Rho-kinase. Asymmetric dimethylarginine, an endogenous inhibitor of NOS, causes uncoupling of NOS leading to generation of reactive nitrogen species such as peroxynitrite, and down-regulated tetrahydrobiopterin

expression promotes that the eNOS cannot generate NO but instead produces O<sub>2</sub><sup>-</sup>, thereby leading to further decreases in NO production. In addition, it was reported that a possible reason for the insufficient bioavailability of nitric oxide might be a reduction of superoxide dismutase ("an enzyme that saves NO") and increase of homocysteine level in the serum due to reduced expression of cystathionine-γ-lyase and cystathionine-β-synthase<sup>[3]</sup>.

Activated hepatic stellate cells (HSCs) and its paracrine interaction with SEC play very important roles in the sinusoidal microcirculation in liver cirrhosis. In pathological conditions violation of the structure and function of HSCs accompanied by a loss of retinoids reserve and HSCs transformation into myofibroblasts. Activated HSCs start to perform the functions of pericytes. This is confirmed by the expression of its phenotypic markers such as α-smooth muscle actin, desmin, NG2, glial fibrillary acidic protein, as well as emergence or increase of receptors for growth factors, cytokines and endothelin, and a number of cell adhesion molecules on its surface<sup>[4]</sup>.

HSCs, located in the subendothelial Disse spaces between the SEC and hepatocytes, are contacted because of the long branching cytoplasmic processes with nerve endings, which contains various neurotransmitters such as substance P, vasoactive intestinal peptide, somatostatin, cholecystokinin, neurotensin, NO, calcitonin gene-related peptide, and neuropeptide Y. Some vasoactive substances are able to regulate the tone of HSCs. Substances for instance endothelin-1, substance P, angiotensin II, norepinephrine, prostaglandin F2, thromboxane A2, platelet activating factor (PAF) and thrombin can trigger HSC contractility. In contrast, vasoactive substances such as acetylcholine, vasoactive intestinal peptide, NO, carbon monoxide, hydrogen sulfide, prostaglandin E2, and adrenomedullin are known for the ability to relax HSCs<sup>[5]</sup>.

Myosin II is involved in the HSCs contraction, and Ca<sup>2+</sup>-dependent and Ca<sup>2+</sup>-independent pathways mediate this process. In a Ca<sup>2+</sup>-dependent pathway, myosin light chains are phosphorylated by activated myosin light chain kinase, whose activation is induced in response to an increase in intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) and subsequent formation of the Ca<sup>2+</sup>/calmodulin complex. In a Ca<sup>2+</sup>-independent pathway, Rho kinase and protein kinase C inhibit the activity of myosin light chain phosphatase, an enzyme that dephosphorylates phosphorylated myosin light chains and induces relaxation<sup>[6]</sup>.

Endothelin (ET) as a powerful endogenous vasoconstrictor modulates the tone of the HSCs. ET has three kinds of isoform, ET-1, 2, and 3, which are synthesized from ET by endothelin-converting enzymes. They interact with conjugated protein G receptors A and B types, which are well expressed in the HSCs. ET-1 is the most studied. The main site of its synthesis in liver

cirrhosis is activated HSCs. Stimulation of endothelin A receptors leads to its proliferation<sup>[7]</sup>. Angiotensin II has a similar effect. In liver cirrhosis, HSCs increase its synthesis because of increased expression of angiotensin-converting enzyme. HSCs constriction may also be caused by decreased NO production and/or bioavailability in cirrhotic liver<sup>[8]</sup>. In contrast, carbon monoxide overproduction by Kupffer cells causes a dilation of the sinusoids and a decrease of hepatic vascular resistance (HVR) because of paracrine impact on HSCs and SEC<sup>[9]</sup>.

Increased HSCs mobility and migration in liver cirrhosis are required to promote enhanced coverage of HSCs around an EC-lined sinusoid, contributing to the process of sinusoidal remodeling<sup>[10]</sup>. Changes in the structure of the HSCs membrane plays an important role in this process. Cellular locomotion requires dynamic but regulated actin remodeling to form membrane structures that facilitate cell extension. These include lamellipodia, which are membrane protrusions that form the leading edge toward directed cell migration, and filopodia, which are thin, actin filament-structured spikes emanating from the plasma membrane. Small guanosine triphosphatases from the Rho family including RhoA (Rho), Rac1 (Rac), and Cdc42 in turn, closely regulate formation of actin-based structures. Proved that if Rac contributes to HSC migration due to formation of filopodia, the Rho causes a resistant to the inhibitory action of NO and restores the chemotactic response to platelet-derived growth factor (PDGF) in the absence of a functional Rac<sup>[11]</sup>.

A key molecule responsible for proliferation, migration, mobility and recruitment of HSCs is PDGF, which is secreted by endothelial cells and binds to its cognate PDGF receptor (PDGFR- $\beta$ ) on pericytes, in particular due to an ephrin-B2/EphB4 signaling pathway<sup>[12]</sup>. Moreover, activation of the PDGFR- $\beta$  causes to stimulation of Raf-1 kinase, MEK kinase and extracellular-signal regulated kinase (ERK), which leads to the proliferation of the HSCs. Phosphatidylinositol 3-kinase activation is also necessary for both mitogenesis and chemotaxis induced by PDGF<sup>[13]</sup>. In addition, it is shown that the axonal guidance molecule neuropilin-1 contributes to the chemotactic response to PDGF too<sup>[14]</sup>.

Activated HSCs are a rich source of polypeptides, eicosanoids and various other molecules with paracrine, juxtacrine, and autocrine signalization or chemoattractant activity, which include: (1) polypeptides which enhance cells proliferation in an autocrine and paracrine manner: Hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), endothelin-1, insulin-like growth factor, transforming growth factor (TGF)- $\alpha$ , epidermal growth factor (EGF) and acidic fibroblast growth factor (aFGF); (2) members of the TGF- $\beta$  family; (3) neurotrophins; and (4) hematopoietic growth factors such as erythropoietin<sup>[15]</sup>.

When the liver is damaged, activated HSC proliferate and migrate to areas of inflammation and

necrosis of hepatocytes, producing excessive amounts of extracellular matrix components. TGF- $\beta$ 1, PDGF, connective tissue growth factor and FGF regulate this process<sup>[16]</sup>.

Overall there are three general sources of fibrogenic cells in the liver: (1) endogenous (resident) fibroblast or myofibroblast-like cells, mainly represented by HSCs, but also by portal fibroblast, vascular smooth muscle cells and pericytes; (2) the epithelial-mesenchymal transition that may occur in the liver as well as in other organs and lead to transdifferentiation of parenchymal cells; and (3) recruitment of fibrocytes from the bone marrow<sup>[17]</sup>.

## INTRAHEPATIC ANGIOGENESIS IN LIVER CIRRHOSIS

In 1983, Rappaport *et al.*<sup>[18]</sup> were among the first who had described the collateral microcirculation in cirrhotic liver. Nowadays, pathological angiogenesis well characterized in experimental liver fibrosis, as well as in patients with chronic viral and autoimmune liver diseases and nonalcoholic steatohepatitis<sup>[19]</sup>.

Angiogenesis is the complicated physiological process through which new blood vessels form from pre-existing vessels. It is accomplished by the activation of endothelial cells, expression in it proteases, destruction of the extracellular matrix, proliferation, migration of the endothelial cells and formation of high permeability primary vascular structures<sup>[20]</sup>.

### Molecular insights into the angiogenic process

The primary inducer of angiogenesis in physiological and pathological conditions is hypoxia. Cells respond to hypoxic stress through multiple mechanisms, including the stabilization of hypoxia-inducible factors (HIFs), which directly regulate the expression of angiogenic growth factors. The family of HIFs includes three  $\alpha$ -subunits, which are associated with a common  $\beta$ -subunit (HIF-1 $\beta$ ). HIF-1 $\alpha$  appears to be ubiquitously expressed, whereas HIF-2 $\alpha$  is detected in a more restricted set of cell types, including vascular endothelial cells, hepatocytes, type II pneumocytes, and macrophages. A third mammalian HIF- $\alpha$  subunit, HIF-3 $\alpha$ , has also been described, although its role in hypoxic responses is less well understood<sup>[21]</sup>.

NADPH oxidase is an important mediator of angiogenic signaling pathways. It was noted that the increased NADPH oxidase expression because of NADPH oxidase subunit p 47phox phosphorylation leads to an increase in the reactive oxygen species (ROS) levels, contributing to HIF-1 $\alpha$  induction, VEGF-receptors (VEGFR) activation and EGF-receptors transactivation<sup>[22]</sup>.

The important role of miRNA has been shown recently in the regulation of cellular response to hypoxia. In particular, Let-7 and miR-103/107 favor the VEGF induction by targeting argonaute 1 protein<sup>[23]</sup>.

The most studied angiogenic growth factors include

VEGF family consisting of five homologs: VEGF-A, B, C, D and placental growth factor (PlGF). VEGF stimulates both physiological and pathological angiogenesis. All members of this family are connected to different homologous receptors: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), VEGFR-3 (Flt-4), of which only the first and second responsible for angiogenic signals transmitting. Besides that, the binding of VEGF-A to VEGFR-2 and increasing vascular permeability through the nitric oxide are the mechanisms triggering angiogenesis and vasculogenesis.

PlGF, a homolog of VEGF binding VEGFR-1, enhances angiogenesis, but only in pathological conditions affecting, directly and indirectly, multiple cell types, including endothelial cells. In addition, it is assumed that PlGF, breaking the binding of VEGF with VEGFR-1, makes the binding of VEGF with VEGFR-2 more probable. Mass spectrometry studies showed that PlGF and VEGF each induce the phosphorylation of distinct tyrosine residues in VEGFR-1, further indicating that PlGF and VEGF transmit distinct angiogenic signals through VEGFR-1.

Different mechanisms are the basis of synergism between PlGF and VEGF. By activating VEGFR-1, PlGF induces an intermolecular cross talk between VEGFR-1 and VEGFR-2, which thereby is more response to VEGF. PlGF, as a subunit of PlGF/VEGF heterodimer, induces the formation of VEGFR-1/2 heterodimers, which transphosphorylate each other in an intramolecular reaction. By producing PlGF, endothelial cells are thus capable of enhancing their own responsiveness to VEGF but adjacent stromal or inflammatory cells may also release PlGF.

PlGF directly affects smooth muscle cells and fibroblasts, which express VEGFR-1, but may also indirectly influence its proliferation and migration through cytokine release from activated endothelial cells. Through these effects, PlGF recruits smooth muscle cells around nascent vessels, thereby stabilizing them into mature, durable and non-leaky vessels.

PlGF also mobilizes VEGFR-1 positive hematopoietic progenitor cells from the bone marrow and recruits, indirectly *via* upregulation of VEGF expression, VEGFR-2-positive endothelial progenitor cells to the ischemic tissue. PlGF is also chemoattractive for monocytes and macrophages, which express VEGFR-1<sup>[24]</sup>.

FGF family members are also able to stimulate angiogenesis. Cellular response to FGFs occurs through specific binding FGF-receptor (FGFR), which has internal tyrosine kinase activity. FGFR dimerization is a prerequisite for phosphorylation and activation of signaling molecules with the participation of heparin-binding proteins. This causes migration, proliferation, cell differentiation and destruction of extracellular matrix. It should be noted that while VEGF family members are involved mainly in the formation of the capillaries, FGFs primarily involved in arteriogenesis<sup>[25]</sup>.

Although the angiogenic effect of PDGF is not so expressed as in VEGF, PlGF and FGF, studies *in vivo*

have shown that it may induce the formation of blood vessels and regulate their tone<sup>[26]</sup>.

Tie-2 (Tek), an endothelial-specific receptor tyrosine kinase, and its ligands, the angiopoietins, have been identified as critical mediators of vascular development. Angiopoietin-1 induces endothelial cells migration, inhibits endothelial cells apoptosis and stimulates its formation, promoting stabilization of vessels. At the same time, NADPH oxidase is involved in the ang-1-mediated activation of Akt and mitogen-activated protein kinase (p42/p44 MAPK, or ERK2 and ERK1) and subsequent modulation of endothelial cell migration and angiogenesis<sup>[27]</sup>. In contrast, angiopoietin-2 causes vascular destabilization by shifting the endothelial cells from the stable state to the proliferative phenotype. However, it may also stimulate angiogenesis in the presence of VEGF<sup>[28]</sup>.

Integrin  $\alpha V\beta 3$  and  $\alpha V\beta 5$  are adhesion receptors promoting angiogenesis by mediating migration and proliferation of endothelial cells and the formation of new blood vessels<sup>[29]</sup>.

Endothelial-specific adhesion molecule vascular endothelial cadherin contributes to cell-cell junctions during neovascularization and controls the passage of molecules through the endothelial lining<sup>[30]</sup>.

Thrombospondin-1 - one of the five known thrombospondins - is an adhesive protein that regulates the interaction of cells with each other and with the extracellular matrix. Its expression increases with the progression of liver cirrhosis and strongly correlated with the severity of fibrosis and angiogenesis. However, the precise role of thrombospondin-1 is not defined in this process. It may function as a promoter or inhibitor of angiogenesis that may depend on its concentration, the type of domain being activated and the type of receptors present on endothelial cells<sup>[31]</sup>.

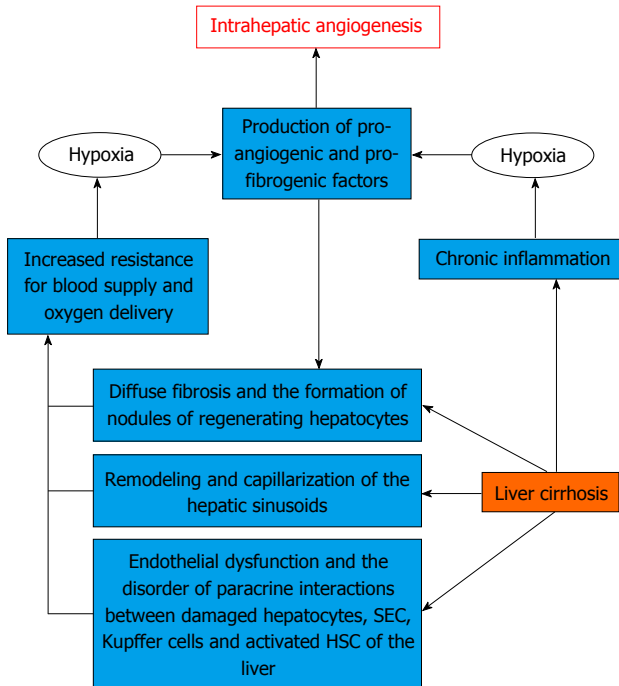
Angiostatin - a fragment of plasminogen, and endostatin - a fragment of the C-terminal part of the collagen XVIII  $\alpha 1$ -chain, inhibits the migration of human endothelial cells stimulated with FGF and VEGF and do not affect intracellular signaling pathways stimulated by FGF and VEGF<sup>[32]</sup>.

Toll-like receptor 4, which recognizes bacterial lipopolysaccharide, is expressed by SECs involved in fibrosis-associated angiogenesis in cirrhotic liver. These properties are related through the cytosolic adapter protein MyD88, which is involved in the production of extracellular protease regulating the invasive ability of SECs<sup>[33]</sup>.

Hepatic apelin system (apelin/APJ-receptor) - the connecting link between chronic inflammation and subsequent fibrogenic and angiogenic processes in liver cirrhosis. On the one hand, hypoxia and inflammation initiate expression of APJ, on the other profibrogenic activation of APJ mediates the induction of profibrogenic genes, HSCs proliferation and secretion of pro-angiogenic factors<sup>[34]</sup>.

Aquaporin-1 is an integral membrane channel pro-





**Figure 1** Two main ways of intrahepatic angiogenesis in liver cirrhosis. SEC: Sinusoidal endothelial cells; HSC: Hepatic stellate cell.

tein, overexpressed in cirrhosis, that promotes angiogenesis by enhancing endothelial invasion<sup>[35]</sup>.

It is known that chemokines from CXC family are involved in angiogenesis. ELR-positive chemokines stimulate this process, and ELR-negative suppress it<sup>[36]</sup>.

Neuropilin-1 and neuropilin-2 are transmembrane glycoproteins with large extracellular domains that interact with both class 3 semaphorins, VEGF and the classical receptors for VEGF, VEGFR-1 and -2, mediating signal transduction. Neuropilin-1 expressed mainly by arterial endothelium, whereas neuropilin-2 is only expressed by venous and lymphatic endothelium. Both neuropilins are commonly over-expressed in regions of physiological and pathological angiogenesis, but the definitive role of neuropilins in angiogenic processes are not fully characterized<sup>[37]</sup>.

### Mechanisms of intrahepatic angiogenesis in liver cirrhosis

Hepatic angiogenesis may substantially differ from homologous processes in other organs or tissue on the basis of: (1) the rather unique phenotypic profile and functional role of activated HSCs and of other liver myofibroblasts; (2) the presence of two different microvascular structures described (*i.e.*, sinusoids lined by fenestrated endothelium vs large vessels lined by a continuous one); and (3) the existence of ANGPTL3, a liver-specific angiogenic factor.

There are two main ways of forming new blood vessels in liver cirrhosis<sup>[38]</sup> (Figure 1). One of them is associated with increased expression of pro-angiogenic growth factors, cytokines and matrix metalloproteinases

on the background of chronic inflammation<sup>[39]</sup>. Proinflammatory mediators produced by Kupffer cells, mast cells and leukocytes may produce angiogenic response due to induction and increased transcriptional activity of HIF-1 $\alpha$ <sup>[40]</sup>.

It is believed that macrophages in the normal state are not directly involved in angiogenesis. In contrast, activated Kupffer cells contribute to the formation of new blood vessels through the production of cytokines, ROS and PAF in liver cirrhosis<sup>[41]</sup>.

Kupffer cells also produce tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), which induces the migration of cells and regulates apoptosis and angiogenesis<sup>[42]</sup>. The increase of ROS in the liver stimulates angiogenesis due to enhanced expression of TNF- $\alpha$ , NO, HIF-1 and VEGF<sup>[43]</sup>. PAF promotes the development of VEGF by the activation of nuclear transcription factor nuclear factor  $\kappa$ B (NF- $\kappa$ B)<sup>[44]</sup>. Mast cells involved in the formation of new blood vessels through the production of heparin, histamine, tryptase, cytokines (TGF- $\beta$ 1, TNF- $\alpha$ , interleukins) and VEGF. They can also increase the number of SECs *in vitro*<sup>[45]</sup>. Soluble mediators, in particular, pro-inflammatory cytokines, growth factors, proteases, and products of oxidative stress regulate increased expression of chemokines in chronic inflammation of the liver. Leukocytes can thereby penetrate into the liver tissue where they produce angiogenic factors such as VEGF, PlGF, PDGF, FGF, TGF- $\beta$ 1, EGF, angiopoietin-2 and different interleukins<sup>[46]</sup>.

On the one hand, hypoxia, caused by HIF-1 $\alpha$  stimulation, activates the HSCs and leads to the production of various angiogenic and fibrogenic factors (PlGF, VEGF, NO, HGF, PDGF)<sup>[47]</sup>, promoting angiogenesis and progression of hepatic fibrosis<sup>[48]</sup>. On the other hand, diffuse fibrosis, regenerative liver nodules and forming of sinusoidal capillarization cause an increase of HVR and impair the oxygen supply to the liver cells<sup>[49]</sup>. Accumulation of HIFs, in particular, HIF-1 $\alpha$ , increases the VEGF, angiopoietin-1 and their receptors expression on activated HSCs. This leads to involvement and stimulation of SECs, stabilizing the newly formed vessels and providing them with strength<sup>[50]</sup>. In turn, SECs generate PDGF and TGF- $\beta$ , helping to attract and migration of HSCs. This process includes ROS-mediated activation of ERK and c-Jun-NH2-terminal kinase (JNK) followed by a delayed- and HIF-1 $\alpha$ -dependent up-regulation and release of VEGF<sup>[51]</sup>.

Respectively there are two different phases of an angiogenic process occurring in the liver cirrhosis. Initially, the formation of blood vessels occurs in developing incomplete septa in which concomitant expression of VEGF, Flk-1, and Tie-2 is restricted by HSCs. In a later phase, angiogenesis occurs in large bridging septa and the expression of this proangiogenic panel is limited to endothelial cells and aims to stabilize the newly formed blood vessels<sup>[52]</sup>. Some of them occur mainly along areas of active inflammation and fibrous septa, probably favors inflammation, tissue repair, and gives rise to intrahepatic shunts. Some of them probably needed

for compensation of insufficient intrahepatic blood flow. Other forms intrahepatic shunts bypassing sinusoids and draining blood from the portal to the central venule. Although they perform the decompression role, they can lead to liver dysfunction due to declining oxygen delivery and nutrients to the liver tissues and limiting the free exchange between hepatocytes and sinusoids<sup>[53]</sup>.

During last years it has been found that endothelial progenitor cells produced by stem cells of the bone marrow are capable of causing *in situ* neovascularization in both physiological and pathological conditions (post-natal vasculogenesis). In particular, they may play an important paracrine role in liver angiogenesis by stimulating resident SECs through as factors as PDGF and VEGF in liver cirrhosis<sup>[54]</sup>. However, their angiogenic ability is significantly reduced in patients in this category, especially with severe hepatic dysfunction. Perhaps this is because chronic inflammation stimulates the release of angiogenic factors by resident HSCs and SECs, and inhibits the endothelial progenitor cells mobilization into the bloodstream<sup>[55]</sup>.

## CONCLUSION

Endothelial dysfunction and impaired paracrine interaction between activated HSCs and SECs, as well as sinusoidal remodeling and capillarization play an important role in improving the HVR to portal blood flow, adding structural changes associated with diffuse fibrosis and regenerative nodules in liver cirrhosis. The development of intrahepatic angiogenesis can be regarded as a compensatory mechanism that is aimed at decompression of the portal system. However, the newly formed vessels carrying blood to bypass the sinusoids are unable to provide oxygen and nutrients to the liver tissue which leads to progression of the disease. A comprehensive assessment of morphological and functional changes of hepatic vessels in the liver cirrhosis might allow to develop some new correction methods of its specific hemodynamic disorders. Moreover, it could help to enhance the effectiveness of therapeutic interventions aimed at the prevention of portal hypertension complications.

## REFERENCES

- 1 **Fernandez M.** Molecular pathophysiology of portal hypertension. *Hepatology* 2015; **61**: 1406-1415 [PMID: 25092403 DOI: 10.1002/hep.27343]
- 2 **García-Pagán JC,** Gracia-Sancho J, Bosch J. Functional aspects on the pathophysiology of portal hypertension in cirrhosis. *J Hepatol* 2012; **57**: 458-461 [PMID: 22504334 DOI: 10.1016/j.jhep.2012.03.007]
- 3 **Hu LS,** George J, Wang JH. Current concepts on the role of nitric oxide in portal hypertension. *World J Gastroenterol* 2013; **19**: 1707-1717 [PMID: 23555159 DOI: 10.3748/wjg.v19.i11.1707]
- 4 **Hellerbrand C.** Hepatic stellate cells--the pericytes in the liver. *Pflugers Arch* 2013; **465**: 775-778 [PMID: 23292551 DOI: 10.1007/s00424-012-1209-5]
- 5 **Ueno T,** Bioulac-Sage P, Balabaud C, Rosenbaum J. Innervation of the sinusoidal wall: regulation of the sinusoidal diameter. *Anat Rec A Discov Mol Cell Evol Biol* 2004; **280**: 868-873 [PMID: 15382014]
- 6 **Iizuka M,** Murata T, Hori M, Ozaki H. Increased contractility of hepatic stellate cells in cirrhosis is mediated by enhanced Ca<sup>2+</sup>-dependent and Ca<sup>2+</sup>-sensitization pathways. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G1010-G1021 [PMID: 21393429 DOI: 10.1152/ajpgi.00350.2010]
- 7 **Takashimizu S,** Kojima S, Nishizaki Y, Kagawa T, Shiraishi K, Mine T, Watanabe N. Effect of endothelin A receptor antagonist on hepatic hemodynamics in cirrhotic rats. Implications for endothelin-1 in portal hypertension. *Tokai J Exp Clin Med* 2011; **36**: 37-43 [PMID: 21769771]
- 8 **Lugo-Baruqui A,** Muñoz-Valle JF, Arévalo-Gallegos S, Armendariz-Borunda J. Role of angiotensin II in liver fibrosis-induced portal hypertension and therapeutic implications. *Hepatol Res* 2010; **40**: 95-104 [PMID: 19737316 DOI: 10.1111/j.1872-034X.2009.00581.x]
- 9 **Reynaert H,** Urbain D, Geerts A. Regulation of sinusoidal perfusion in portal hypertension. *Anat Rec (Hoboken)* 2008; **291**: 693-698 [PMID: 18484616 DOI: 10.1002/ar.20669]
- 10 **Lee JS,** Semela D, Iredale J, Shah VH. Sinusoidal remodeling and angiogenesis: a new function for the liver-specific pericyte? *Hepatology* 2007; **45**: 817-825 [PMID: 17326208]
- 11 **Lee JS,** Kang Decker N, Chatterjee S, Yao J, Friedman S, Shah V. Mechanisms of nitric oxide interplay with Rho GTPase family members in modulation of actin membrane dynamics in pericytes and fibroblasts. *Am J Pathol* 2005; **166**: 1861-1870 [PMID: 15920170]
- 12 **Semela D,** Das A, Langer D, Kang N, Leof E, Shah V. Platelet-derived growth factor signaling through ephrin-b2 regulates hepatic vascular structure and function. *Gastroenterology* 2008; **135**: 671-679 [PMID: 18570897 DOI: 10.1053/j.gastro.2008.04.010]
- 13 **Pinzani M.** PDGF and signal transduction in hepatic stellate cells. *Front Biosci* 2002; **7**: d1720-d1726 [PMID: 12133817]
- 14 **Cao S,** Yaqoob U, Das A, Shergill U, Jagavelu K, Huebert RC, Routray C, Abdelmoneim S, Vasdev M, Leof E, Charlton M, Watts RJ, Mukhopadhyay D, Shah VH. Neuropilin-1 promotes cirrhosis of the rodent and human liver by enhancing PDGF/TGF-beta signaling in hepatic stellate cells. *J Clin Invest* 2010; **120**: 2379-2394 [PMID: 20577048 DOI: 10.1172/JCI41203]
- 15 **Friedman SL.** Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008; **88**: 125-172 [PMID: 18195085 DOI: 10.1152/physrev.00013.2007]
- 16 **Seki E,** Brenner DA. Recent advancement of molecular mechanisms of liver fibrosis. *J Hepatobiliary Pancreat Sci* 2015; **22**: 512-518 [PMID: 25869468 DOI: 10.1002/jhbp.245]
- 17 **Svegliati-Baroni G,** De Minicis S, Marziani M. Hepatic fibrogenesis in response to chronic liver injury: novel insights on the role of cell-to-cell interaction and transition. *Liver Int* 2008; **28**: 1052-1064 [PMID: 18783548 DOI: 10.1111/j.1478-3231.2008.01825.x]
- 18 **Rappaport AM,** MacPhee PJ, Fisher MM, Phillips MJ. The scarring of the liver acini (Cirrhosis). Tridimensional and micro-circulatory considerations. *Virchows Arch A Pathol Anat Histopathol* 1983; **402**: 107-137 [PMID: 6420982]
- 19 **Elpek GÖ.** Angiogenesis and liver fibrosis. *World J Hepatol* 2015; **7**: 377-391 [PMID: 25848465 DOI: 10.4254/wjh.v7.i3.377]
- 20 **Folkman J.** Angiogenesis: an organizing principle for drug discovery? *Nat Rev Drug Discov* 2007; **6**: 273-286 [PMID: 17396134]
- 21 **Skuli N,** Majmundar AJ, Krock BL, Mesquita RC, Mathew LK, Quinn ZL, Runge A, Liu L, Kim MN, Liang J, Schenkel S, Yodh AG, Keith B, Simon MC. Endothelial HIF-2 $\alpha$  regulates murine pathological angiogenesis and revascularization processes. *J Clin Invest* 2012; **122**: 1427-1443 [PMID: 22426208 DOI: 10.1172/JCI57322]
- 22 **Brandes RP,** Miller FJ, Beer S, Haendeler J, Hoffmann J, Ha T, Holland SM, Görlach A, Busse R. The vascular NADPH oxidase subunit p47phox is involved in redox-mediated gene expression.

- Free Radic Biol Med* 2002; **32**: 1116-1122 [PMID: 12031896]
- 23 **Chen Z**, Lai TC, Jan YH, Lin FM, Wang WC, Xiao H, Wang YT, Sun W, Cui X, Li YS, Fang T, Zhao H, Padmanabhan C, Sun R, Wang DL, Jin H, Chau GY, Huang HD, Hsiao M, Shyy JY. Hypoxia-responsive miRNAs target argonaute 1 to promote angiogenesis. *J Clin Invest* 2013; **123**: 1057-1067 [PMID: 23426184 DOI: 10.1172/JCI65344]
  - 24 **Carmeliet P**. Manipulating angiogenesis in medicine. *J Intern Med* 2004; **255**: 538-561 [PMID: 15078497]
  - 25 **Klein S**, Roghani M, Rifkin DB. Fibroblast growth factors as angiogenesis factors: new insights into their mechanism of action. *EXS* 1997; **79**: 159-192 [PMID: 9002232]
  - 26 **Hellberg C**, Ostman A, Heldin CH. PDGF and vessel maturation. *Recent Results Cancer Res* 2010; **180**: 103-114 [PMID: 20033380 DOI: 10.1007/978-3-540-78281-0\_7]
  - 27 **Chen JX**, Zeng H, Lawrence ML, Blackwell TS, Meyrick B. Angiopoietin-1-induced angiogenesis is modulated by endothelial NADPH oxidase. *Am J Physiol Heart Circ Physiol* 2006; **291**: H1563-H1572 [PMID: 16679392]
  - 28 **Pauta M**, Ribera J, Melgar-Lesmes P, Casals G, Rodríguez-Vita J, Reichenbach V, Fernandez-Varo G, Morales-Romero B, Bataller R, Michelena J, Altamirano J, Jiménez W, Morales-Ruiz M. Overexpression of angiopoietin-2 in rats and patients with liver fibrosis. Therapeutic consequences of its inhibition. *Liver Int* 2015; **35**: 1383-1392 [PMID: 24612347 DOI: 10.1111/liv.12505]
  - 29 **Patsenker E**, Popov Y, Stickel F, Schneider V, Ledermann M, Sägeser H, Niedobitek G, Goodman SL, Schuppan D. Pharmacological inhibition of integrin  $\alpha$ 5 $\beta$ 1 aggravates experimental liver fibrosis and suppresses hepatic angiogenesis. *Hepatology* 2009; **50**: 1501-1511 [PMID: 19725105 DOI: 10.1002/hep.23144]
  - 30 **Kevil CG**, Payne DK, Mire E, Alexander JS. Vascular permeability factor/vascular endothelial cell growth factor-mediated permeability occurs through disorganization of endothelial junctional proteins. *J Biol Chem* 1998; **273**: 15099-15103 [PMID: 9614120]
  - 31 **Elpek GO**, Gokhan GA, Bozova S. Thrombospondin-1 expression correlates with angiogenesis in experimental cirrhosis. *World J Gastroenterol* 2008; **14**: 2213-2217 [PMID: 18407596]
  - 32 **Eriksson K**, Magnusson P, Dixelius J, Claesson-Welsh L, Cross MJ. Angiostatin and endostatin inhibit endothelial cell migration in response to FGF and VEGF without interfering with specific intracellular signal transduction pathways. *FEBS Lett* 2003; **536**: 19-24 [PMID: 12586331]
  - 33 **Jagavelu K**, Routray C, Shergill U, O'Hara SP, Faubion W, Shah VH. Endothelial cell toll-like receptor 4 regulates fibrosis-associated angiogenesis in the liver. *Hepatology* 2010; **52**: 590-601 [PMID: 20564354 DOI: 10.1002/hep.23739]
  - 34 **Melgar-Lesmes P**, Pauta M, Reichenbach V, Casals G, Ros J, Bataller R, Morales-Ruiz M, Jiménez W. Hypoxia and proinflammatory factors upregulate apelin receptor expression in human stellate cells and hepatocytes. *Gut* 2011; **60**: 1404-1411 [PMID: 21450694 DOI: 10.1136/gut.2010.234690]
  - 35 **Huebert RC**, Jagavelu K, Hendrickson HI, Vasdev MM, Arab JP, Splinter PL, Trussoni CE, Larusso NF, Shah VH. Aquaporin-1 promotes angiogenesis, fibrosis, and portal hypertension through mechanisms dependent on osmotically sensitive microRNAs. *Am J Pathol* 2011; **179**: 1851-1860 [PMID: 21854740 DOI: 10.1016/j.ajpath.2011.06.045]
  - 36 **Sahin H**, Borkham-Kamphorst E, Kuppe C, Zaldivar MM, Grouls C, Al-samman M, Nellen A, Schmitz P, Heinrichs D, Berres ML, Doleschel D, Scholten D, Weiskirchen R, Moeller MJ, Kiessling F, Trautwein C, Wasmuth HE. Chemokine Cxcl9 attenuates liver fibrosis-associated angiogenesis in mice. *Hepatology* 2012; **55**: 1610-1619 [PMID: 22237831 DOI: 10.1002/hep.25545]
  - 37 **Staton CA**, Kumar I, Reed MW, Brown NJ. Neuropilins in physiological and pathological angiogenesis. *J Pathol* 2007; **212**: 237-248 [PMID: 17503412]
  - 38 **Fernández M**, Semela D, Bruix J, Colle I, Pinzani M, Bosch J. Angiogenesis in liver disease. *J Hepatol* 2009; **50**: 604-620 [PMID: 19157625 DOI: 10.1016/j.jhep.2008.12.011]
  - 39 **Ehling J**, Bartneck M, Wei X, Gremse F, Fecht V, Möckel D, Baeck C, Hittatiya K, Eulberg D, Luedde T, Kiessling F, Trautwein C, Lammers T, Tacke F. CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. *Gut* 2014; **63**: 1960-1971 [PMID: 24561613 DOI: 10.1136/gutjnl-2013-306294]
  - 40 **Chaparro M**, Sanz-Cameno P, Trapero-Marugan M, Garcia-Buey L, Moreno-Otero R. Mechanisms of angiogenesis in chronic inflammatory liver disease. *Ann Hepatol* 2007; **6**: 208-213 [PMID: 18007549]
  - 41 **Steib CJ**. Kupffer cell activation and portal hypertension. *Gut* 2011; **60**: 1307-1308 [PMID: 21708827 DOI: 10.1136/gut.2011.242560]
  - 42 **Lochhead PA**, Gilley R, Cook SJ. ERK5 and its role in tumour development. *Biochem Soc Trans* 2012; **40**: 251-256 [PMID: 22260700 DOI: 10.1042/BST20110663]
  - 43 **Dewhirst MW**, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. *Nat Rev Cancer* 2008; **8**: 425-437 [PMID: 18500244 DOI: 10.1038/nrc2397]
  - 44 **Ko HM**, Seo KH, Han SJ, Ahn KY, Choi IH, Koh GY, Lee HK, Ra MS, Im SY. Nuclear factor kappaB dependency of platelet-activating factor-induced angiogenesis. *Cancer Res* 2002; **62**: 1809-1814 [PMID: 11912159]
  - 45 **Franceschini B**, Ceva-Grimaldi G, Russo C, Dioguardi N, Grizzi F. The complex functions of mast cells in chronic human liver diseases. *Dig Dis Sci* 2006; **51**: 2248-2256 [PMID: 17103041]
  - 46 **Marra F**. Chemokines in liver inflammation and fibrosis. *Front Biosci* 2002; **7**: d1899-d1914 [PMID: 12161342]
  - 47 **Copple BL**, Bai S, Burgoon LD, Moon JO. Hypoxia-inducible factor-1 $\alpha$  regulates the expression of genes in hypoxic hepatic stellate cells important for collagen deposition and angiogenesis. *Liver Int* 2011; **31**: 230-244 [PMID: 20880076 DOI: 10.1111/j.1478-3231.2010.02347.x]
  - 48 **Lemoine S**, Cadoret A, El Mourabit H, Thabut D, Housset C. Origins and functions of liver myofibroblasts. *Biochim Biophys Acta* 2013; **1832**: 948-954 [PMID: 23470555 DOI: 10.1016/j.bbdis.2013.02.019]
  - 49 **Yokomori H**, Oda M, Yoshimura K, Hibi T. Enhanced expressions of apelin on proliferative hepatic arterial capillaries in human cirrhotic liver. *Hepatol Res* 2012; **42**: 508-514 [PMID: 22502744 DOI: 10.1111/j.1872-034X.2011.00945.x]
  - 50 **Coulon S**, Heindryckx F, Geerts A, Van Steenkiste C, Colle I, Van Vlierberghe H. Angiogenesis in chronic liver disease and its complications. *Liver Int* 2011; **31**: 146-162 [PMID: 21073649 DOI: 10.1111/j.1478-3231.2010.02369.x]
  - 51 **Novo E**, Povero D, Busletta C, Paternostro C, di Bonzo LV, Cannito S, Compagnone A, Bandino A, Marra F, Colombatto S, David E, Pinzani M, Parola M. The biphasic nature of hypoxia-induced directional migration of activated human hepatic stellate cells. *J Pathol* 2012; **226**: 588-597 [PMID: 21959987 DOI: 10.1002/path.3005]
  - 52 **Novo E**, Cannito S, Zamara E, Valfrè di Bonzo L, Caligiuri A, Cravanzola C, Compagnone A, Colombatto S, Marra F, Pinzani M, Parola M. Proangiogenic cytokines as hypoxia-dependent factors stimulating migration of human hepatic stellate cells. *Am J Pathol* 2007; **170**: 1942-1953 [PMID: 17525262]
  - 53 **Vanheule E**, Geerts AM, Van Huysse J, Schelfhout D, Praet M, Van Vlierberghe H, De Vos M, Colle I. An intravital microscopic study of the hepatic microcirculation in cirrhotic mice models: relationship between fibrosis and angiogenesis. *Int J Exp Pathol* 2008; **89**: 419-432 [PMID: 19134051 DOI: 10.1111/j.1365-2613.2008.00608.x]
  - 54 **Kaur S**, Tripathi D, Dongre K, Garg V, Rooge S, Mukopadhyay A, Sakhuja P, Sarin SK. Increased number and function of endothelial progenitor cells stimulate angiogenesis by resident liver sinusoidal endothelial cells (SECs) in cirrhosis through paracrine factors. *J Hepatol* 2012; **57**: 1193-1198 [PMID: 22824816 DOI: 10.1016/j.jhep.2012.07.016]
  - 55 **Chen CH**, Chang LT, Tung WC, Chen YL, Chang CL, Leu S, Sun CK, Tsai TH, Tsai IT, Chang HW, Yip HK. Levels and values

of circulating endothelial progenitor cells, soluble angiogenic factors, and mononuclear cell apoptosis in liver cirrhosis patients.

*J Biomed Sci* 2012; **19**: 66 [PMID: 22809449 DOI: 10.1186/1-423-0127-19-66]

**P- Reviewer:** Abdel-Razik A, Sharma M **S- Editor:** Qi Y  
**L- Editor:** A **E- Editor:** Liu SQ





## Basic Study

# Obese diet-induced mouse models of nonalcoholic steatohepatitis-tracking disease by liver biopsy

Maria Nicoline Baandrup Kristiansen, Sanne Skovgård Veidal, Kristoffer Tobias Gustav Rigbolt, Kirstine Sloth Tølbøl, Jonathan David Roth, Jacob Jelsing, Niels Vrang, Michael Feigh

Maria Nicoline Baandrup Kristiansen, Sanne Skovgård Veidal, Kristoffer Tobias Gustav Rigbolt, Kirstine Sloth Tølbøl, Jacob Jelsing, Niels Vrang, Michael Feigh, Gubra Aps, 2970 Hørsholm, Denmark

Jonathan David Roth, Intercept Pharmaceuticals, Inc., San Diego, CA 9212, United States

**Author contributions:** Kristiansen MNB, Veidal SS, Rigbolt KTG, Tølbøl KS and Feigh M performed the experiments and analyzed the data; Rigbolt KTG performed the molecular investigations; Kristiansen MNB and Veidal SS performed the histological analysis; Veidal SS, Rigbolt KTG, Roth JD, Jelsing J, Vrang N and Feigh M designed and coordinated the research; Kristiansen MNB, Veidal SS, Rigbolt KTG, Tølbøl KS, Roth JD, Jelsing J, Vrang N and Feigh M wrote the paper.

**Institutional review board statement:** This study includes no data or material from patients. We confirm that all of the required permissions for this study were obtained from our local authorities as mentioned in the Institutional animal care and use committee statement.

**Institutional animal care and use committee statement:** All procedures involving animals were reviewed and approved by the Danish Committee for animal research and covered by a personal license for Jacob Jelsing (2013-15-2934-00784). All of the institutional and national guidelines for the care and use of laboratory animals were followed.

**Conflict-of-interest statement:** There are no patents, products in development or marked products to declare.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

[licenses/by-nc/4.0/](http://creativecommons.org/licenses/by-nc/4.0/)

**Correspondence to:** Michael Feigh, PhD, Gubra Aps, Hørsholm Kongevej 11B, 2970 Hørsholm, Denmark. [mfe@gubra.dk](mailto:mfe@gubra.dk)  
Telephone: +45-31522651

Received: February 11, 2016

Peer-review started: February 12, 2016

First decision: March 9, 2016

Revised: April 1, 2016

Accepted: April 20, 2016

Article in press: April 22, 2016

Published online: June 8, 2016

## Abstract

**AIM:** To characterize development of diet-induced nonalcoholic steatohepatitis (NASH) by performing liver biopsy in wild-type and genetically obese mice.

**METHODS:** Male wild-type C57BL/6J (C57) mice (DIO-NASH) and male *Lep<sup>ob</sup>/Lep<sup>ob</sup>* (*ob/ob*) mice (*ob/ob*-NASH) were maintained on a diet high in trans-fat (40%), fructose (22%) and cholesterol (2%) for 26 and 12 wk, respectively. A normal chow diet served as control in C57 mice (lean chow) and *ob/ob* mice (*ob/ob* chow). After the diet-induction period, mice were liver biopsied and a blinded histological assessment of steatosis and fibrosis was conducted. Mice were then stratified into groups counterbalanced for steatosis score and fibrosis stage and continued on diet and to receive daily PO dosing of vehicle for 8 wk. Global gene expression in liver tissue was assessed by RNA sequencing and bioinformatics. Metabolic parameters, plasma liver enzymes and lipids (total cholesterol, triglycerides) as well as hepatic lipids and collagen content were measured by biochemical analysis. Non-alcoholic fatty liver disease activity score (NAS) (steatosis/inflammation/ballooning

degeneration) and fibrosis were scored. Steatosis and fibrosis were also quantified using percent fractional area.

**RESULTS:** Diet-induction for 26 and 12 wk in DIO-NASH and *ob/ob*-NASH mice, respectively, elicited progressive metabolic perturbations characterized by increased adiposity, total cholesterol and elevated plasma liver enzymes. The diet also induced clear histological features of NASH including hepatosteatosis and fibrosis. Overall, the metabolic NASH phenotype was more pronounced in *ob/ob*-NASH *vs* DIO-NASH mice. During the eight week repeated vehicle dosing period, the metabolic phenotype was sustained in DIO-NASH and *ob/ob*-NASH mice in conjunction with hepatomegaly and increased hepatic lipids and collagen accumulation. Histopathological scoring demonstrated significantly increased NAS of DIO-NASH mice (0 *vs*  $4.7 \pm 0.4$ ,  $P < 0.001$  compared to lean chow) and *ob/ob*-NASH mice ( $2.4 \pm 0.3$  *vs*  $6.3 \pm 0.2$ ,  $P < 0.001$  compared to *ob/ob* chow), respectively. Furthermore, fibrosis stage was significantly elevated for DIO-NASH mice (0 *vs*  $1.2 \pm 0.2$ ,  $P < 0.05$  compared to lean chow) and *ob/ob* NASH ( $0.1 \pm 0.1$  *vs*  $3.0 \pm 0.2$ ,  $P < 0.001$  compared to *ob/ob* chow). Notably, fibrosis stage was significantly ( $P < 0.001$ ) increased in *ob/ob*-NASH mice, when compared to DIO-NASH mice.

**CONCLUSION:** These data introduce the obese diet-induced DIO-NASH and *ob/ob*-NASH mouse models with biopsy-confirmed individual disease staging as a preclinical platform for evaluation of novel NASH therapeutics.

**Key words:** Nonalcoholic steatohepatitis; Liver biopsy; Diet-induced obesity; Nonalcoholic fatty liver disease; Fibrosis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We characterize the development and progression of diet-induced nonalcoholic steatohepatitis (NASH) in a wild-type and a genetically obese mouse model. We confirm that a diet high in trans-fat, fructose and cholesterol, develops key histological hallmarks of NASH (steatosis, inflammation, ballooning degeneration) in conjunction with fibrosis. Concomitantly, marked alterations in NASH associated gene expression pathways can be evaluated by RNAseq analysis. In addition, we describe that performing a baseline liver biopsy enables individual disease staging for subsequent stratified randomization of animals into study groups. Finally, we show these models' utility for a chronic repeated dosing study to evaluate pharmacological intervention.

Kristiansen MNB, Veidal SS, Rigbolt KTG, Tølbøl KS, Roth JD, Jelsing J, Vrang N, Feigh M. Obese diet-induced mouse models of nonalcoholic steatohepatitis-tracking disease by liver biopsy. *World J Hepatol* 2016; 8(16): 673-684 Available from: URL:

<http://www.wjgnet.com/1948-5182/full/v8/i16/673.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i16.673>

## INTRODUCTION

It is generally accepted that along with increasing rates of obesity, type 2 diabetes and metabolic syndrome, the incidence and prevalence of patients with nonalcoholic fatty liver disease (NAFLD) continues to rise<sup>[1-4]</sup>. NAFLD is considered the hepatic manifestation of the metabolic syndrome and covers a variety of pathologies ranging from simple hepatic steatosis (accumulation of triglycerides in hepatocytes) to nonalcoholic steatohepatitis (NASH), characterized by inflammation, cellular ballooning and fibrosis in varying degrees<sup>[1-3]</sup>. The pathogenesis of NASH is described by the "two-hit" hypothesis, the first hit being fat accumulation in hepatocytes, while the "second hit", *e.g.*, oxidative stress, apoptosis or mitochondrial dysfunction, causes development of inflammation and fibrosis<sup>[5]</sup>.

There are currently no pharmacological agents specifically approved for the treatment of NASH and disease management is consequently focused on the correction of underlying risk factors (*e.g.*, obesity, insulin resistance and dyslipidemia)<sup>[1,6]</sup>. A likely contributor to the absence of therapeutics is the paucity of preclinical models resembling human NAFLD/NASH<sup>[6]</sup>. Historically, several animal models have been developed to represent the pathophysiology, morphological findings, biochemical changes, and clinical features of human NAFLD/NASH. These models are usually divided into two main categories: The diet-induced models and the genetically modified models (transgenic or knockout models)<sup>[1]</sup>. Some diet-induced models are based on *ad libitum* feeding of diets enriched with various combinations of fat, cholesterol and sugars (*e.g.*, fructose) thereby developing a metabolic phenotype reflected by adiposity and hepatosteatosis, albeit only presenting mild characteristics of NASH and typically lack of liver fibrosis<sup>[7,8]</sup>. Other dietary models involve feeding nutrient-deficient diets such as the methionine- and choline-deficient diet (MCD). Methionine and choline deficiency impairs liver  $\beta$ -oxidation and the production of very-low density lipoproteins (VLDL) hereby generating a "second hit"<sup>[1]</sup>, eliciting a more severe fibrotic NASH phenotype within hepatic tissue<sup>[8,9]</sup>. However, these models fail to recapitulate a clinically relevant overall metabolic phenotype as MCD animals demonstrate pronounced weight loss and perturbed energy- and glucose homeostasis<sup>[10]</sup>. Recently, a novel wild-type diet-induced obese fibrotic NASH mouse model was introduced by Trevaskis *et al*<sup>[11]</sup>, based on the ALIOS diet model<sup>[12]</sup>. This model, generated by feeding an *ad libitum* diet high in trans-fat, fructose and cholesterol to wild-type C57Bl/6J mice [the Amylin liver NASH model (AMLN)], displayed key hallmarks of clinical NASH<sup>[11]</sup>. The AMLN mouse model was further optimized by demonstrating a liver biopsy technique for

assessing individual steatosis, inflammation, ballooning degeneration and fibrosis staging, prior to a putative study intervention<sup>[6]</sup>. Not only does the baseline liver biopsy reduce biological variability by excluding mice that fail to develop NASH prior to initiating therapy, but it also allows for within-subject comparisons over time, thereby increasing statistical power<sup>[6]</sup>.

For the genetically modified NASH models, several studies have implicated a role of individual genes involved in the development of NASH using deletion or overexpression models<sup>[7,9]</sup>. For example, mice that overexpress the transcription factor sterol regulatory element-binding proteins (SREBPs), a feedback regulatory system controlling intracellular levels of cholesterol and free fatty acids develop a hepatic phenotype resembling NASH. However, like MCD-fed mice, SREBP overexpression does not induce a metabolic profile consistent with obesity and insulin resistance<sup>[13]</sup>. In contrast, impairment of leptin signaling (e.g., *db/db* mice) results in obesity, insulin resistance and diabetes<sup>[14]</sup>. Leptin-deficient mice (*Lep<sup>ob</sup>/Lep<sup>ob</sup>*) are predisposed to develop steatohepatitis, however, when maintained on regular rodent chow they do not develop fibrosis<sup>[11]</sup>. In fact, it was previously postulated that *Lep<sup>ob</sup>/Lep<sup>ob</sup>* mice are incapable of developing hepatic fibrosis<sup>[9]</sup>. This notion was dispelled by the observation that *Lep<sup>ob</sup>/Lep<sup>ob</sup>* mice maintained on the AMLN diet for at least 12 wk do in fact develop the key hallmarks of NASH, including fibrosis<sup>[11]</sup>.

The present study assessed key NASH diagnostic characteristics (e.g., steatosis score, inflammation, ballooning degeneration and fibrosis stage), metabolic endpoints and gene expression signatures in wild-type C57Bl/6J and *Lep<sup>ob</sup>/Lep<sup>ob</sup>* mice, fed the AMLN diet for a total of 34 and 20 wk, respectively, including an eight-week repeated vehicle dosing period. In addition, we demonstrate how a baseline liver biopsy allows for individual disease staging and for stratified randomization into experimental groups with reduced biological variability and for a clear cut analysis of individual response to pharmacological intervention.

## MATERIALS AND METHODS

### Animals and experimental set-up

All animal experiments were conformed to international accepted principles for the care and use of laboratory animals and were covered by a personal license for Jacob Jelsing (2013-15-2934-00784) issued by the Danish Committee for animal research.

Male C57Bl/6J (C57) and *Lep<sup>ob</sup>/Lep<sup>ob</sup>* (*ob/ob*) mice at 5 wk of age were obtained from JanVier (JanVier labs, France), and group housed 5 animals pr. cage under a 12/12 h dark-light cycle. Room temperature was controlled to 22 °C ± 1 °C, with 50% ± 10% humidity. Animals had *ad libitum* access to diet high in fat (40%, of these 18% trans-fat), 40% carbohydrates (20% fructose) and 2% cholesterol (D09100301, Research Diet, United States) previously described as the AMLN diet<sup>[6]</sup>, or regular rodent chow (Altromin 1324, Brogaar-

den, Denmark), and tap water. Both strains had *ad libitum* access to either the AMLN diet (DIO-NASH, *n* = 110; *ob/ob* NASH, *n* = 40) or chow (lean chow, *n* = 10; *ob/ob* chow, *n* = 10). After 26 (DIO-NASH) or 12 wk (*ob/ob*-NASH) a liver biopsy was performed for histological assessment of individual fibrosis and steatosis staging at baseline. Following biopsy procedure animals were single housed. An 8-wk vehicle intervention period was conducted in a representative subset of DIO-NASH and *ob/ob*-NASH mice, and their respective chow controls. Vehicle dosing consisted of once daily per oral dose of carboxymethyl cellulose (C57 and *ob/ob*) and subcutaneous injection with PBS (C57). The rationale was to mimic repeated dosing administration and animal handling in combination with AMLN diet-maintenance. After a total of 34 and 20 wk on AMLN diet for DIO-NASH and *ob/ob*-NASH mice, respectively, animals were euthanized and liver tissue collected for histological and biochemical analysis. Total animal numbers for each experiment is indicated in the figures and table.

### Baseline liver biopsy after diet-induction

Mice were pretreated with enrofloxacin (Bayer, Germany) (5 mg/mL-1 mL/kg) one day before being biopsied. Prior to biopsy, mice were anesthetized with isoflurane (2%-3%) in 100% oxygen. A small abdominal incision in the midline was made and the left lateral lobe of the liver was exposed. A cone shaped wedge of liver tissue (50-100 mg) was excised from the distal portion of the lobe fixed in 4% paraformaldehyde for histology. The biopsy procedure previously described by Clapper *et al*<sup>[6]</sup> 2013 was refined using electrocoagulation of the cut surface of the liver by means of bipolar coagulation using ERBE VIO 100C electrosurgical unit (ERBE, United States). The liver was returned to the abdominal cavity, abdominal wall was sutured and skin stapled. Carprofen (Pfizer, United States) (5 mg/mL-0.01 mL/10 g) and enrofloxacin (5 mg/mL-1 mL/kg) were administered intraperitoneal at the time of surgery and at post-operation day one and two, to control postoperative pain relief and infection, respectively.

### Hepatic gene expression changes

Gene expression changes were measured in a representative subset of DIO-NASH mice and *ob/ob*-NASH. Liver tissue was harvested from the left lateral lobe and snap frozen in liquid nitrogen. Tissue sections (about 50 mg) were homogenized in lysis buffer containing protease inhibitors and used for RNA extraction using NucleoSpin Plus RNA columns (Macherey-Nagel). The quantity of the RNA was analyzed using a Nano Drop 2000 spectrophotometer (Thermo Scientific, United States). RNAseq libraries were prepared with the KAPA poly-A kit (Kapa Biosystems, United States) and sequenced on the NextSeq 500 (Illumina, United States) (single-end, 75 bp reads). Reads were aligned to the GRCh38 Ensembl Mus musculus genome using STAR v.2.4.0<sup>[15]</sup> and feature counts were obtained using HTseq v.0.6.1<sup>[16]</sup>, both with default parameters. Differential

expression analysis was performed with edgeR<sup>[17]</sup> and genes with a  $P \leq 0.05$  after correction for multiple testing using the Benjamini and Hochberg method was regarded as significantly regulated. Pathway analysis of WikiPathways<sup>[18]</sup> was performed using the statistics module in PathVisio<sup>[19]</sup>.

### **Body weight and body composition analysis**

Body weight was intermittently monitored during the diet-induction period and once daily during the intervention period. Whole-body fat mass was analyzed at baseline (week -1) and week 8 of the intervention period by non-invasive EchoMRI scanning using EchoMRI-900 (EchoMRI, United States). During the scanning procedure the mice were placed in a restrainer for 90–120 s.

### **Plasma biochemistry analysis**

After diet-induction, a baseline blood sample was collected from the submandibular vein in non-fasted conscious animals and blood sampling was repeated following the intervention period. Plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG) and total cholesterol (TC) were measured using the auto analyzer Cobas C-111 (Roche Diagnostics, Germany). Plasma levels of insulin were measured in duplicates using an AlphaLisa kit (Perkin Elmer), according to the manufacturer's instructions.

### **Oral glucose tolerance test**

An oral glucose tolerance test (OGTT) was performed in week 4 of the intervention period. Animals were fasted for 4 h prior to OGTT. At  $t = 0$  an oral glucose load [2 g/kg glucose 200 mg/mL, (Fresenius Kabi, Sweden)] was administered *via* a gastrically placed tube. Blood samples for measuring blood glucose (BG) were collected from the tail vein at  $t = 0, 15, 30, 60$  and 120 min. Glucose area under the curve (AUC) calculations were determined as total AUC from the sampling period of 0 to 120 min.

### **Whole blood glucose analysis**

Blood samples for BG analysis were collected into 10  $\mu$ L heparinized glass capillary tubes and immediately suspended in buffer [0.5 mL of glucose/lactate system solution (EKF-diagnostics, Germany)] and analyzed for glucose using a BIOSEN c-Line glucose meter (EKF-diagnostics, Germany) according to the manufacturer's instructions.

### **Terminal hepatic hydroxyproline content**

Formalin fixed (50 mg) liver tissue was homogenized in 500  $\mu$ L water. Five hundred microliter concentrated HCl was added to the samples and hydrolyzed at 120 °C for three hours. Supernatants were transferred to a 96 well plate and wells were allowed to evaporate dry overnight. Total collagen content in the liver was measured by colorimetric determination of hydroxyproline residues

by acid hydrolysis of collagen (Cat no. MAK008, Sigma Aldrich).

### **Terminal hepatic triglyceride and total cholesterol content**

A liver piece (about 100 mg) was collected in FastPrep tubes and snap-frozen in liquid nitrogen. One milliliter 5% NH<sub>4</sub>OH/ddH<sub>2</sub>O solution (ab142227, Abcam) was added to the FastPrep tube. The tubes were homogenized in a FastPrep homogenizer and shaken for 2  $\times$  60 s. After homogenization the samples were slowly heated to 80 °C–100 °C in a heating block for three minutes. Samples were allowed to return to room temperature prior to a second round of heating. Next, samples were centrifuged for two minutes at top speed using a microcentrifuge to remove any insoluble material. TG and TC content in liver homogenates are measured in single determinations using auto analyzer Cobas C-111 with commercial kit (Roche Diagnostics, Germany) according to manufacturer's instructions.

### **Histology assessment and digital image analysis**

Baseline liver biopsy and terminal samples were collected from the left lateral lobe (about 100 mg) and fixed overnight in 4% paraformaldehyde. Liver tissue was paraffin embedded and sectioned (3  $\mu$ m thickness). To assess hepatic morphology and fibrosis, sections were stained with Hematoxylin and Eosin and Sirius Red, respectively, followed by analysis with Visiormorph software (Visiopharm, Denmark). Histological assessment and scoring was performed by a pathologist blinded to the study. NAFLD activity score (NAS) (steatosis/inflammation/ballooning degeneration) and fibrosis stage were performed using the clinical criteria outlined by Kleiner *et al.*<sup>[20]</sup>.

### **Statistical analysis**

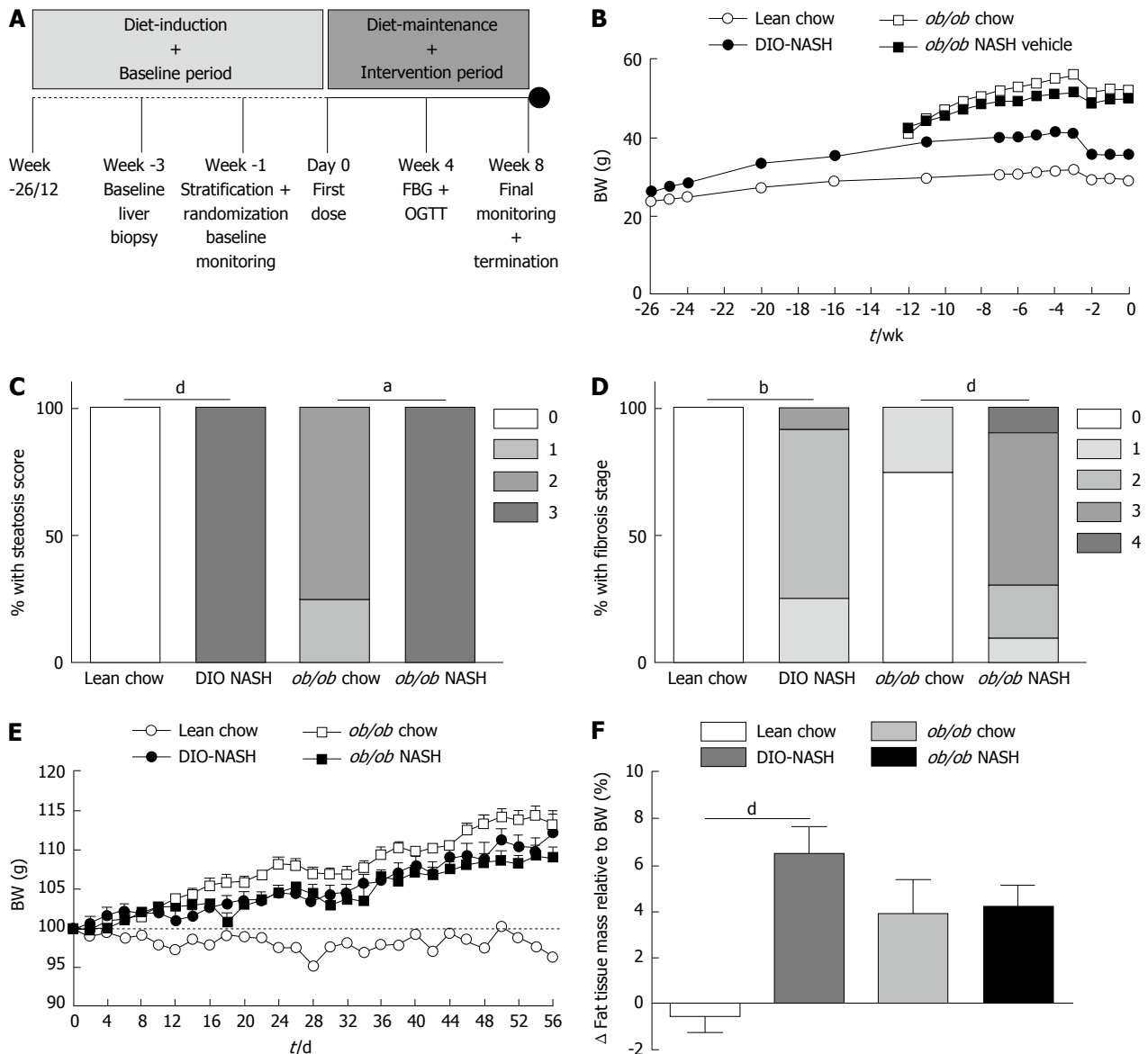
All data were analyzed using GraphPad Prism 5.0. The results are presented as mean  $\pm$  standard error of the mean. Statistical significance was evaluated using One-way analysis of variance with Turkey's multiple comparison test, and for histological analysis using Kruskal-Wallis test with Dunn's multiple comparison test.  $P < 0.05$  was considered statistical significant.

## **RESULTS**

### **Male C57 and ob/ob mice developed adiposity and elevated plasma metabolic parameters after AMLN diet-induction**

The overall study design is outlined in Figure 1A. Following a diet-induction period of 26 wk, C57 (DIO-NASH) mice demonstrated increased body weight (adiposity), when compared to lean chow animals. In the already obese *ob/ob* strain there was no additional effect on body weight noted in *ob/ob*-NASH mice relative to chow controls. Whereas all mice experienced slight weight loss following the biopsy, they returned





**Figure 1** Study design, body weight regulation and liver biopsy-confirmed disease development. A: Study design of the DIO-NASH and *ob/ob* NASH mouse models; B: BW during diet-induction and baseline period (monitoring and biopsy-recovery); C and D: Liver biopsy-derived histopathological assessment of (C) steatosis score (0-3), and (D) fibrosis stage (0-4); E and F: Change in BW (E), and change in adiposity (F), during diet-maintenance and intervention period (repeated vehicle dosing). <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$ . The results are presented as mean  $\pm$  SEM. Lean chow ( $n = 9$ ), DIO-NASH ( $n = 12$ ), *ob/ob* chow ( $n = 8$ ), *ob/ob* NASH ( $n = 10$ ). NASH: Nonalcoholic steatohepatitis; BW: Body weight; OGTT: Oral glucose tolerance test; FBG: Fed blood glucose; SEM: Standard error of the mean.

to a weight stable state within one week (Figure 1B). After diet-induction, DIO-NASH and *ob/ob*-NASH mice demonstrated a metabolic NAFLD phenotype, as reflected by elevated levels of plasma total cholesterol (TC) and liver enzymes ALT and AST, when compared to respective chow fed animals. Overall, the *ob/ob*-NASH mice demonstrated an accelerated and more pronounced metabolic phenotype, when compared to DIO-NASH mice (Table 1).

**Male C57 and *ob/ob* mice demonstrated biopsy-proven hepatic steatosis and fibrosis after AMLN diet-induction**  
Histological assessments of biopsied liver tissue revealed that lean chow animals did not develop hepatic steatosis or fibrosis over the 26-wk diet-induction (Figure

1C and D). In contrast, DIO-NASH mice presented with high levels of steatosis (score 3) (Figure 1C) and fibrosis stage ranging from 1-3 (Figure 1D). The *ob/ob* chow phenotype displayed mild steatosis (score 1-2) and lack of or only mild fibrosis (stage 1) whereas all *ob/ob*-NASH mice displayed a steatosis score of 3 (Figure 1C) and a fibrosis stage ranging from 1-4 (Figure 1D).

#### Altered hepatic gene expression in male C57 and *ob/ob* mice after AMLN diet-induction

To characterize the effect of 26 wk diet-induction on global liver gene expression, the transcriptome of lean chow vs DIO-NASH mice were analyzed by RNAseq<sup>[21]</sup>. Principal component analysis identified a clear separation between the two groups along the first component,

**Table 1** Effect of Amylin liver nonalcoholic steatohepatitis model diet on metabolic parameters, non-alcoholic fatty liver disease activity score/fibrosis stage, body weight/composition and liver weight

	Lean chow <i>n</i> = 9	DIO-NASH <i>n</i> = 12	<i>ob/ob</i> chow <i>n</i> = 8	<i>ob/ob</i> NASH <i>n</i> = 10
Baseline plasma ALT (U/L)	30.7 ± 0.8	133.6 ± 16.3	207.0 ± 58.7	577.4 ± 43.4 <sup>d,f</sup>
Terminal plasma ALT (U/L)	31.5 ± 2.9	126.1 ± 19.8	249.7 ± 47.4	670.0 ± 59.0 <sup>d,f</sup>
Baseline plasma AST (U/L)	46.5 ± 2.2	134.8 ± 11.6 <sup>b</sup>	174.2 ± 41.1	436.7 ± 36.8 <sup>d,f</sup>
Terminal plasma AST (U/L)	139.0 ± 28.2	213.8 ± 31.6	338.9 ± 87.7	552.6 ± 49.5 <sup>c,f</sup>
Baseline plasma TC (mmol/L)	2.1 ± 0.1	6.8 ± 0.3 <sup>b</sup>	3.5 ± 0.2	10.4 ± 0.9 <sup>d,f</sup>
Terminal plasma TC (mmol/L)	2.3 ± 0.1	6.7 ± 0.4 <sup>b</sup>	4.4 ± 0.2	10.8 ± 0.6 <sup>d,f</sup>
Baseline plasma TG (mmol/L)	0.7 ± 0.1	0.9 ± 0.1	1.1 ± 0.2	0.8 ± 0.1
Terminal plasma TG (mmol/L)	0.8 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	0.7 ± 0.1 <sup>d</sup>
OGTT-AUC	1104 ± 49.7	1217 ± 39.9	1612 ± 173.4	1319 ± 61.4
Fasting blood glucose (mmol/L)	7.3 ± 0.3	7.6 ± 0.2	8.1 ± 0.3	7.6 ± 0.3
Plasma insulin (pmol/L)	30.6 ± 6.7	97.4 ± 18.3	1189 ± 94	567.3 ± 123 <sup>d,e</sup>
Baseline steatosis score (0-3)	0	2.7 ± 0.3 <sup>b</sup>	1.8 ± 0.2	2.7 ± 0.3
Baseline fibrosis stage (0-4)	0	1.8 ± 0.2	0.25 ± 0.2	2.7 ± 0.3 <sup>d,f</sup>
Terminal NAFLD activity score (0-8)	0	4.7 ± 0.4 <sup>b</sup>	2.4 ± 0.3	6.3 ± 0.2 <sup>d</sup>
Terminal steatosis score (0-3)	0	2.8 ± 0.1 <sup>b</sup>	2.1 ± 0.2	2.1 ± 0.2
Terminal inflammation score (0-3)	0	1.4 ± 0.2 <sup>b</sup>	0.3 ± 0.2	2.4 ± 0.2 <sup>d</sup>
Terminal ballooning degeneration score (0-2)	0	0.4 ± 0.1	0	0.9 ± 0.1 <sup>d</sup>
Terminal fibrosis stage (0-4)	0	1.2 ± 0.2 <sup>a</sup>	0.1 ± 0.1	3.0 ± 0.2 <sup>d,e</sup>
Terminal steatosis (% area)	5.4 ± 0.5	33.9 ± 2.6 <sup>b</sup>	29.5 ± 2.3	41.2 ± 1.0 <sup>c</sup>
Terminal fibrosis (% area)	0.3 ± 0.1	1.1 ± 0.2	1.2 ± 0.2	4.9 ± 0.7 <sup>d,e</sup>
Terminal BW (g)	28.0 ± 0.3	39.1 ± 1.1	59 ± 1.1	54.8 ± 0.8 <sup>e</sup>
Terminal lean tissue mass (g)	14.6 ± 0.8	18.8 ± 0.5 <sup>b</sup>	19.9 ± 0.9	17.3 ± 0.4 <sup>c</sup>
Terminal lean tissue mass (% of BW)	49.6 ± 2.6	47.7 ± 1.5	33.6 ± 1.1	31.9 ± 0.6 <sup>f</sup>
Terminal fat tissue mass (g)	1.5 ± 0.1	8.1 ± 0.7 <sup>b</sup>	25.1 ± 0.7	22.5 ± 0.5 <sup>c,f</sup>
Terminal fat tissue mass (% of BW)	5.1 ± 0.5	20.0 ± 1.3 <sup>b</sup>	42.3 ± 0.8	41.5 ± 0.5 <sup>f</sup>
Liver weight (g)	1.1 ± 0.1	2.5 ± 0.3 <sup>b</sup>	2.9 ± 0.2	5.4 ± 0.2 <sup>d,f</sup>
Liver weight (% of BW)	3.9 ± 0.3	6.3 ± 0.6 <sup>b</sup>	4.9 ± 0.3	9.9 ± 0.3 <sup>d,f</sup>

<sup>a</sup>*P* < 0.05 vs lean chow, <sup>b</sup>*P* < 0.01 vs lean chow, <sup>c</sup>*P* < 0.05 vs *ob/ob* chow, <sup>d</sup>*P* < 0.01 vs *ob/ob* chow, <sup>e</sup>*P* < 0.05 vs DIO-NASH and <sup>f</sup>*P* < 0.01 vs DIO-NASH. NASH: Nonalcoholic steatohepatitis; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BW: Body weight; OGTT: Oral glucose tolerance test; AUC: Area under the curve.

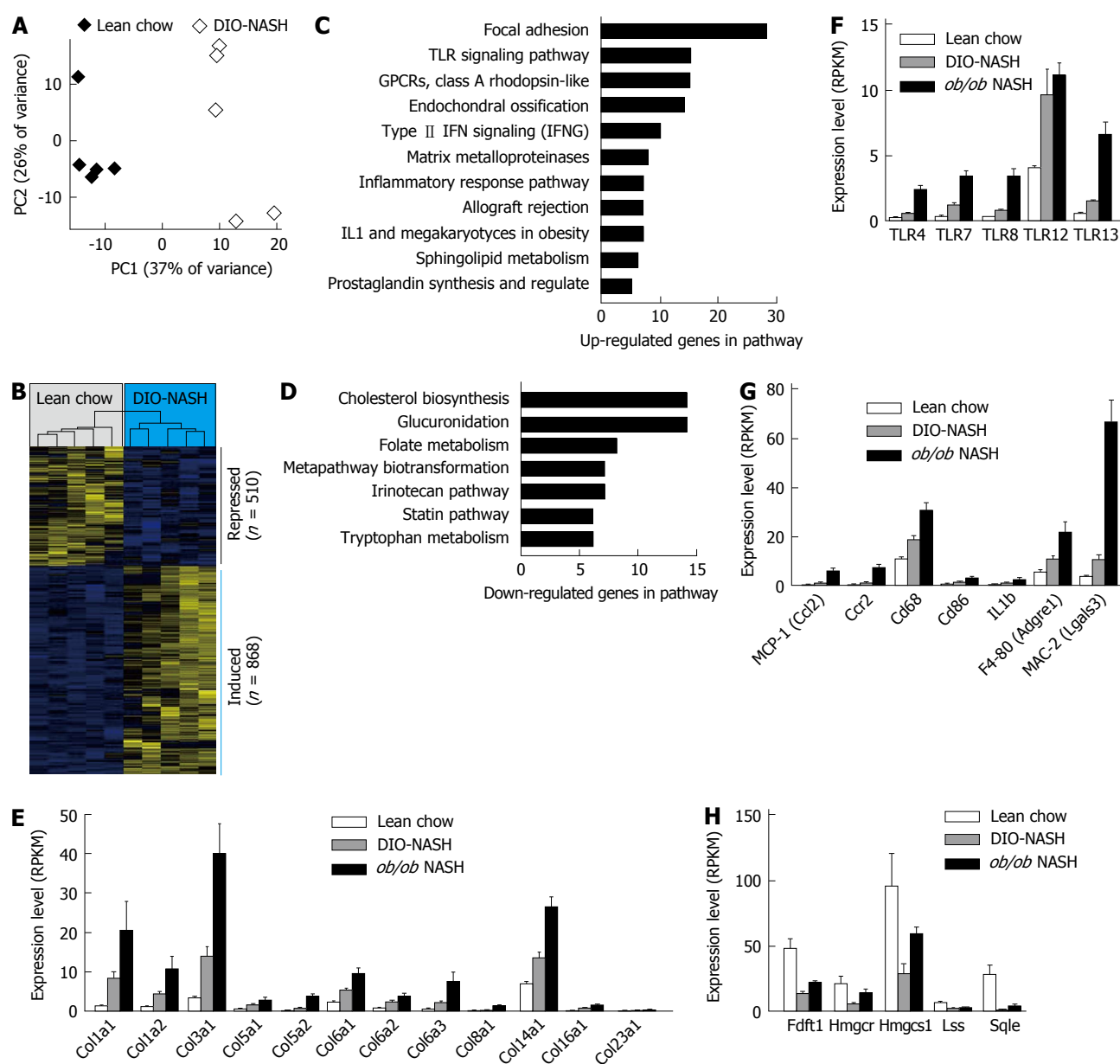
indicating that the NASH diet markedly alters the overall gene expression profile (Figure 2A). We identified a total of 1378 differentially expressed genes, composed of 510 repressed and 868 induced genes (Figure 2B). To explore biological processes affected, sets of significantly altered signaling pathways were extracted (Figure 2C and D). Many of these pathways are consistent with the observed NASH phenotype including focal adhesion, toll-like receptor (TLR) signaling pathway, matrix metalloproteinases and inflammatory response pathway. Consistent with the identification of focal adhesion as the top affected pathways multiple collagen subtypes showed increased expression (Figure 2E).

Similar analyses were conducted on a subset of samples from *ob/ob*-NASH animals which confirmed the exaggerated expression levels of collagen types (Figure 2E). The pathway analysis also highlighted TLR signaling as one of the primary affected signaling processes, an observation supported by the increased expression of TLR4, which was recently demonstrated as an important pro-inflammatory mediator in the pathogenesis of NASH<sup>[22,23]</sup>. Notably, mRNA expression levels of a number of other TLR subtypes (TLR7, TLR8, TLR12 and TLR13) were upregulated to greater extent than TLR4 (Figure 2F). Furthermore, a large collection of pro-inflammatory factors ranging from chemokines, such as monocyte chemoattractant protein-1 (MCP-1), to chemokine receptors, such as C-C motif chemokine

receptor-2 (Ccr2) and macrophage markers (*i.e.*, CD68, CD86, F4-80 and MAC-2) (Figure 2G) were significantly induced. Finally, in line with observed hepatosteatosis, expression levels of genes involved in triglyceride biosynthesis were significantly increased in C57 and *ob/ob* animals exposed to the AMLN diet. Conversely, cholesterol biosynthesis expression was significantly decreased in the DIO-NASH and *ob/ob*-NASH mice (Figure 2H).

#### **Male C57 and *ob/ob* mice sustained adiposity and elevated plasma metabolic parameters after AMLN diet-maintenance and repeated dosing intervention period**

During the intervention period with diet-maintenance and repeated vehicle dosing for a total of 8 wk, DIO-NASH mice progressively gained body weight (adiposity), when compared to lean chow animals. Leptin-deficient mice also gained fat mass during the 8-wk intervention period (Figure 1E and F). Fat gain was somewhat less in the *ob/ob*-NASH mice, however, these mice began the study with a higher % adiposity (37%, *n* = 10) relative to DIO-NASH mice (14%, *n* = 12). At study end (termination), DIO-NASH and *ob/ob*-NASH animals sustained the elevated levels of plasma liver enzymes and hypercholesterolemia, when compared to respective chow-fed mice (Table 1). In contrast, terminal plasma TG levels were unchanged in DIO-NASH mice, and were significantly decreased for *ob/ob*-NASH animals,



**Figure 2 Gene expression analysis by RNAsequencing and bioinformatics.** A: Principal component analysis of the 500 most variable genes, [principal component (PC)]; B: Hierarchical clustering of the differentially expressed genes, bars on the right indicate the induced and repressed genes; C: Pathways enriched for the induced (up-regulated) genes, filtered for  $P < 0.01$  and  $n > 4$ ; D: Same as (C) for the down-regulated genes; E-H: Expression levels of selected differentially expressed genes shown as mean  $\pm$  SEM. Lean chow ( $n = 5$ ), DIO-NASH ( $n = 5$ ), *ob/ob* NASH ( $n = 5$ ). NASH: Nonalcoholic steatohepatitis; BW: Body weight; TLR: Toll-like receptor; IFN: Interferon; IL1: Interleukine 1; MCP-1: Monocyte chemoattractant protein-1; GPCRs: G protein-coupled receptors; IFNG: Interferon gamma; RPKM: Reads per kilobase of transcript per million mapped reads; SEM: Standard error of the mean.

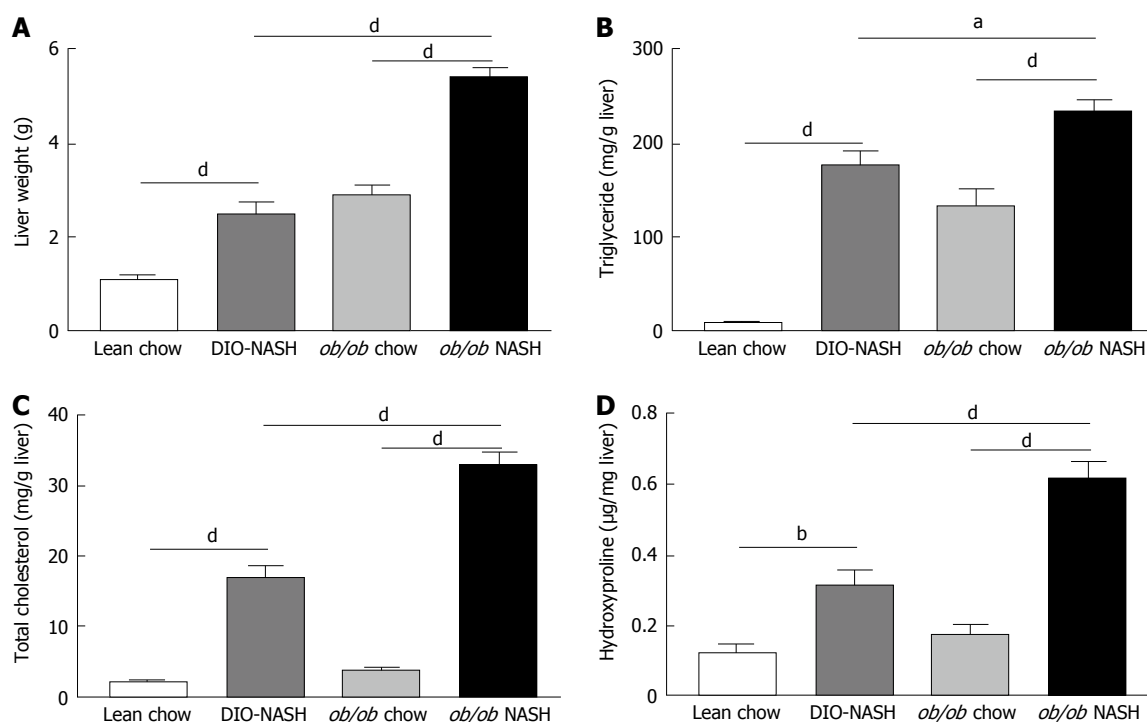
when compared to chow (Table 1). Collectively, terminal plasma levels of ALT, AST and TC were markedly elevated in *ob/ob*-NASH, when compared to DIO-NASH mice (Table 1).

An OGTT was performed four weeks into the intervention period. Fasting blood glucose and OGTT AUC for blood glucose were unchanged in DIO-NASH and *ob/ob*-NASH mice, as compared to respective chow fed animals (Table 1). Diet effects on glycemic status are supported by the elevation in plasma insulin levels of about 3-fold (NS) in DIO-NASH when compared to lean chow, whereas *ob/ob*-NASH showed a surprisingly decrease in plasma insulin levels at study end when

compared to *ob/ob* chow animals (Table 1).

#### Male C57 and *ob/ob* mice demonstrated hepatomegaly with increased hepatic lipids and collagen content after AMLN diet-maintenance and repeated dosing intervention period

At study end, terminal liver weight was significantly increased in DIO-NASH and *ob/ob*-NASH mice, when compared to respective chow animals (Figure 3A). Additionally, liver weight of *ob/ob*-NASH was significantly higher than liver weight of DIO-NASH animals (Figure 3A). Both strains demonstrated increased deposition of liver TG and TC when compared to respective chow mice



**Figure 3 Liver weight and hepatic lipid and collagen content at study end.** Liver weight at termination (A), hepatic triglyceride content (B), hepatic total cholesterol content (C), and hepatic hydroxyproline (collagen) content (D) at study end. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>d</sup> $P < 0.001$ . The results are presented as mean  $\pm$  SEM. Lean chow ( $n = 9$ ), DIO-NASH ( $n = 12$ ), *ob/ob* chow ( $n = 8$ ), *ob/ob* NASH ( $n = 10$ ). NASH: Nonalcoholic steatohepatitis; BW: Body weight; SEM: Standard error of the mean.

(Figure 3B and C). Furthermore, liver TG and TC content were significantly increased in *ob/ob*-NASH mice, when compared to DIO-NASH animals (Figure 3B and C). Notably, DIO-NASH and *ob/ob*-NASH mice showed elevated levels of liver hydroxyproline (collagen) content, when compared to chow controls (Figure 3D). Overall, levels of liver hydroxyproline content were higher in *ob/ob*-NASH animals relative to DIO-NASH mice (Figure 3D).

#### **Histopathological scoring of liver steatosis, inflammation and ballooning degeneration after AMLN diet-maintenance and repeated dosing intervention period in male C57 and *ob/ob* mice**

Blinded histological assessment of NAS was performed on hematoxylin and eosin stained terminal hepatic tissue (Table 1). No evidence of steatosis, inflammation and ballooning degeneration was observed in lean chow controls (Figure 4A). In *ob/ob* chow mice steatosis was categorized as pronounced microvesicular with mild microvesicular steatosis (Figure 4B). Despite increased steatosis when maintained on chow diet, neither ballooning degeneration nor inflammation was observed in *ob/ob* chow animals (Figure 4B). In contrast, DIO-NASH mice developed micro- and macro-vesicular steatosis, with inflammation and ballooning degeneration (Figure 4C). Similarly, *ob/ob*-NASH mice developed micro- and macro-vesicular steatosis, and more pronounced inflammation and ballooning degeneration (Figure 4D). Thus, the NASH phenotypes of both strains of mice were clearly reflected in significantly increased NAS,

when compared to respective chow animals (Figure 4E). Finally, image analysis confirmed hepatic steatosis in DIO-NASH and *ob/ob*-NASH mice (Figure 4F).

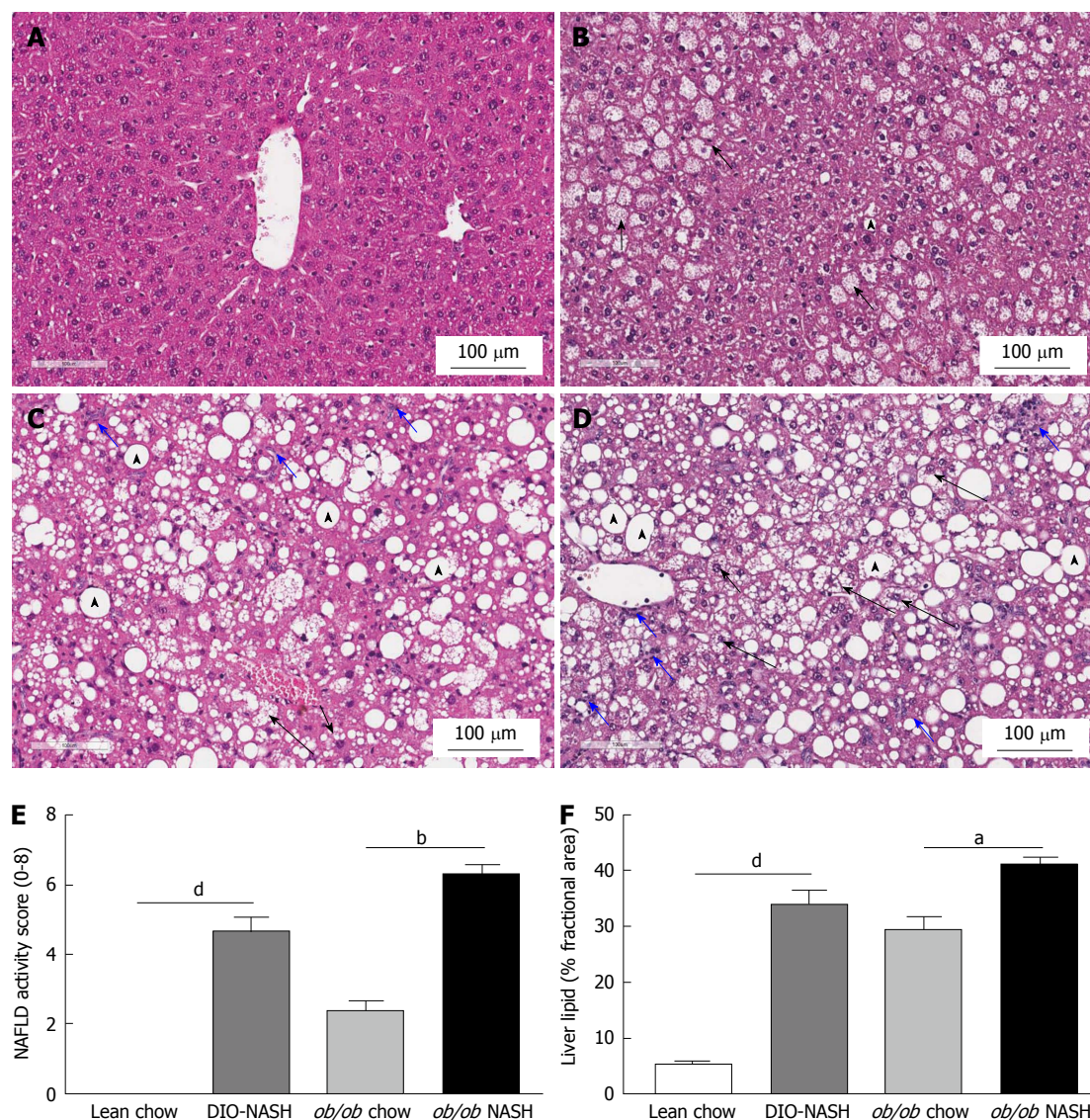
#### **Histopathological scoring of liver fibrosis after AMLN diet-maintenance and repeated dosing intervention period in male C57 and *ob/ob* mice**

Fibrosis stage was assessed by blinded histological evaluation using Sirius red staining of terminal liver tissue (Table 1). Hepatic fibrosis was not observed in lean chow or *ob/ob* chow mice (Figure 5A and B). In contrast, fibrosis was observed in DIO-NASH mice (Figure 5C) and to a greater extent in *ob/ob*-NASH animals who progressed to bridging fibrosis (Figure 5D). Fibrosis was most evident at tissue margins, but also penetrated into the tissue (Figure 5A-D). The fibrotic phenotypes of the DIO-NASH and *ob/ob*-NASH mice were mirrored by an increase in fibrosis stage compared to respective chow animals (Figure 5E). Increases in fibrosis stage were reflected by our image analyses showing an increase in % fractional area of Sirius Red (Figure 5F). Notably, the *ob/ob*-NASH animals were more fibrotic than DIO-NASH mice (Figure 5E and F).

## **DISCUSSION**

In the present study two obese mouse models of diet-induced NASH were evaluated; the C57 DIO-NASH and the *ob/ob*-NASH. We confirm that a diet high in trans-fat, fructose and cholesterol produces a metabolic NASH phenotype with elevated plasma liver enzymes,



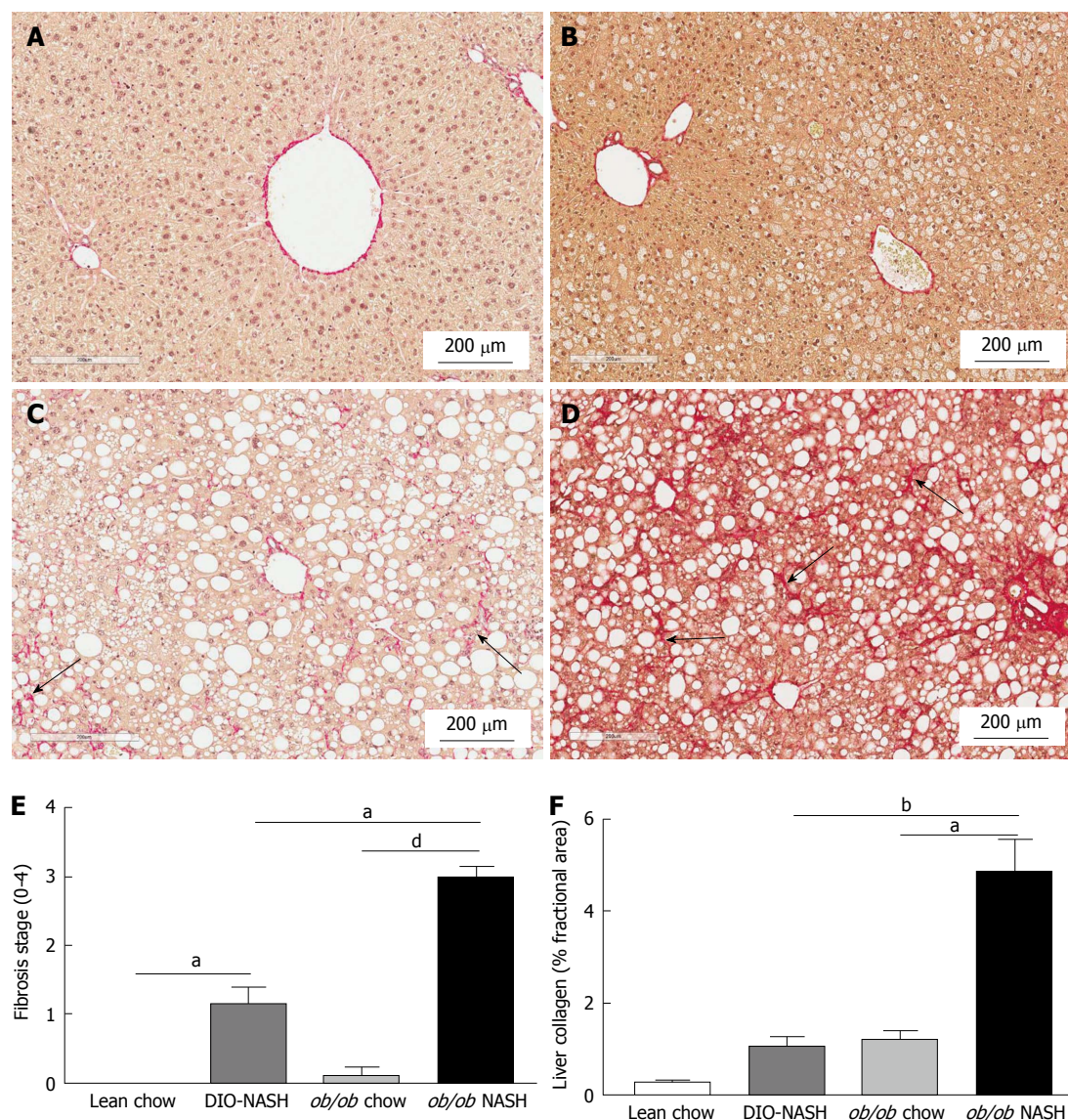


**Figure 4** Histological assessment of non-alcoholic fatty liver disease activity score and liver lipid at study end. Representative H and E stained sections from; lean chow (A), *ob/ob* chow (B), DIO-NASH (C) and *ob/ob* NASH (D) mouse models. NAFLD activity score (steatosis, inflammation and ballooning degeneration) performed by a blinded pathologist at study end (E), and quantitatively image analysis of steatosis (% area) from H and E staining using visiomorph software (F). Macrovesicular steatosis indicated by arrowheads, microvesicular steatosis indicated by short black arrows, inflammation indicated by short blue arrows, ballooning degeneration indicated by long black arrows. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>d</sup> $P < 0.001$ . The results are presented as mean  $\pm$  SEM. Lean chow ( $n = 9$ ), DIO-NASH ( $n = 12$ ), *ob/ob* chow ( $n = 8$ ), *ob/ob* NASH ( $n = 10$ ). NASH: Nonalcoholic steatohepatitis; NAFLD: Nonalcoholic fatty liver disease; SEM: Standard error of the mean.

hepatomegaly and recapitulates multiple clinical features including key hallmarks of NASH (steatosis, inflammation, ballooning degeneration and fibrosis). These changes were associated with marked alterations in associated gene expression pathways implicated in NASH and development of fibrosis. The mouse models are also suitable for pharmacological intervention studies, with a paired baseline liver biopsy procedure enabling individual disease stage before a repeated dosing period as is customary in NASH preclinical studies. Whereas all mice experienced slight weight loss following the biopsy, they returned to a weight stable state within one week moreover DIO-NASH and *ob/ob*-NASH sustained hepatomegaly, hepatic steatosis, inflammation, ballooning degeneration and fibrosis following repeated dosing intervention for a total of 8 wk.

DIO-NASH and *ob/ob*-NASH mice developed key hallmarks of fibrotic NASH including marked hepatosteatosis with evident inflammation and ballooning degeneration, as assessed by a clinical-derived histological NAS and fibrosis stage classification system developed by Kleiner *et al.*<sup>[20]</sup>. This is in line with recent findings by Clapper *et al.*<sup>[6]</sup> and Honda *et al.*<sup>[24]</sup> in C57 AMLN mice, thus supporting the wild-type C57 DIO-NASH mouse as a suitable preclinical model for diet-induced obesity and NASH. In addition, the genetically obese *ob/ob* mouse model was also recently demonstrated to exhibit fibrotic NASH when fed AMLN diet<sup>[11,25]</sup>. In the leptin-deficient model superimposing the NASH diet high in trans-fat, fructose and cholesterol represents a “second hit” in development of preclinical NASH. The present study demonstrates that the NAS





**Figure 5** Histological assessment of fibrosis stage and liver collagen at study end. Representative sirius red stained sections from; lean chow (A), *ob/ob* chow (B), DIO-NASH (C) and *ob/ob* NASH (D) mouse model. Liver fibrosis stage performed by a blinded pathologist at study end (E), and quantitatively image analysis of collagen (% area) from sirius red staining using visiomorph software (F). Fibrous band formation indicated by black arrows. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>d</sup> $P < 0.001$ . The results are presented as mean  $\pm$  SEM. Lean chow ( $n = 9$ ), DIO-NASH ( $n = 12$ ), *ob/ob* chow ( $n = 8$ ), *ob/ob* NASH ( $n = 10$ ). NASH: Nonalcoholic steatohepatitis; SEM: Standard error of the mean.

and fibrosis stage can also be integrated in analyses of the *ob/ob*-NASH mouse - and in conjunction with the excessive accumulation of hepatic lipids and collagen content - introduces an accelerated and aggressive diet-induced NASH phenotype relative to the C57 DIO-NASH model.

In accordance with histological observations of hepatic inflammation, mRNA analyses revealed within inflammatory pathways that toll-like receptors and downstream pro-inflammatory effectors (e.g., IL1b, MCP-1) were markedly upregulated after the diet-induction period in DIO-NASH and *ob/ob*-NASH. In line with previous findings in diet-induced NASH mouse models<sup>[26]</sup>, we observed increased expression of TLR4, a key receptor in fibrogenic development as demonstrated in high-fat diet-induced TLR4 knockout<sup>[22]</sup>, and bile duct

ligation models<sup>[23]</sup>. In addition, TLR4 KO in *ob/ob* mice was protective against NASH development as evinced by reduced NAS compared to regular *ob/ob* mice<sup>[27]</sup>. Interestingly, we observed higher expression levels of four additional TLRs: TLR7, TLR8, TLR12 and TLR13, which could be of relevance in future elucidation of the pathogenesis of NASH and for pharmacological intervention.

CCR2 mRNA levels were also increased in *ob/ob*-NASH mice. CCR2 has been implicated in the development of liver fibrosis, with *Ccr2*<sup>-/-</sup> mice showing reduced fibrosis following bile duct ligation or CCl<sub>4</sub> exposure<sup>[28]</sup>. CCR2 is a functional receptor for MCP-1, and is involved in the migration of macrophages during obesity<sup>[29]</sup>. Together, the impact on TLR signaling and macrophage abundance indicates an accelerated inflammatory NASH

phenotype in the *ob/ob*-genotype. Furthermore, markers of macrophage infiltration CD68 and F4-80, were up-regulated in DIO-NASH and to a larger extent in *ob/ob*-NASH mice, hereby corroborating the histological finding of increased inflammation in the two models.

In accordance with histological observations of hepatic fibrosis, our mRNA analyses also revealed increased expression of fibrillary collagens. Of particular interest is the increased diet- and strain-induced regulation of type I, III and type IV collagen, as these are known to be abundantly increased in liver fibrosis<sup>[30,31]</sup>. Interestingly, we also report altered expression of type I collagen  $\alpha 1$  and  $\alpha 2$  chain, type III collagen  $\alpha 1$ , as well as type XIV collagen  $\alpha 1$ , which could be of relevance in development/progression from NASH to fibrosis and future design of anti-fibrotic agents.

Pathway analyses also shed light on the transcriptional regulation of the main enzymes involved in triglyceride and cholesterol biosynthesis induced by the AMLN diet in DIO-NASH and *ob/ob*-NASH mice. Expression of these enzymes are all regulated by SREBP transcription factors, with SREBP1 regulating triglyceride synthesis and SREBP2 regulating cholesterol synthesis<sup>[32]</sup>. We report significantly increased levels of total cholesterol in plasma and livers of DIO-NASH and *ob/ob*-NASH mice compared to respective chow groups. Interestingly, the gene markers for biosynthesis of cholesterol in the liver appear to be dramatically reduced for DIO-NASH and *ob/ob*-NASH, presumably to compensate for the intake of high level of cholesterol in the diet. However, to our surprise the same was not observed for the triglyceride synthesis as the main lipid enzymes showed increased expression (data not shown), albeit plasma levels of triglycerides were significantly decreased for *ob/ob*-NASH mice compared to *ob/ob* chow. This could be caused by impairment in VLDL secretion from the liver<sup>[6]</sup>, as relative triglyceride content in the liver was significantly increased in livers of *ob/ob*-NASH compared to levels in livers from *ob/ob* chow. Dysfunctional VLDL synthesis and secretion has been suggested to be a key factor in the progression of simple steatosis to NASH<sup>[33]</sup>. The mechanism(s) of action involved in the perturbed lipid metabolism awaits further investigations.

In conclusion, the diet-induced DIO-NASH and *ob/ob*-NASH mouse models demonstrate metabolic and histological key hallmarks of NASH. A clinically-derived histopathological scoring system can be applied in the DIO-NASH and *ob/ob*-NASH mouse models, thereby introducing a preclinical platform for evaluation of novel NASH therapeutics. Finally, a liver biopsy procedure at baseline allows for evaluation of individual disease staging prior to pharmacological intervention hereby reducing biological variability.

## COMMENTS

### Background

Nonalcoholic steatohepatitis (NASH) is an emerging liver disease with

increasing prevalence. There are currently no pharmacological agents specifically approved for the treatment of NASH and disease management is consequently focused on the correction of underlying risk factors such as obesity, insulin resistance and dyslipidemia.

### Research frontiers

The lack of approved therapeutics has to some degree been attributed to the failure of animal models to faithfully represent the clinical condition (e.g., disease progression and metabolic background) and the way NASH is assessed clinically (paired biopsies and validated histological methods). Hence, novel diet-induced NASH models that develop the appropriate metabolic phenotype with improved liver sampling methods are highly desirable as a preclinical platform for exploring novel NASH treatments.

### Innovations and breakthroughs

The authors describe and characterize a wild-type C57 and a genetically (*ob/ob*) obese diet-induced mouse model of NASH and confirm previous findings demonstrating key hallmarks of metabolic deregulation and fibrotic NASH using biochemical, histological and gene expression endpoints. Notably, a liver biopsy-confirmed and clinically-derived histological NASH scoring and fibrosis staging are being performed in *ob/ob* mice, which the authors are the first to report. Finally, the utility of the diet-induced NASH mouse models for pharmacological investigations is being demonstrated by performing a chronic intervention period with repeated dosing following a baseline liver biopsy procedure.

### Applications

A baseline liver biopsy performed after diet-induction allows for individual disease staging for stratification and randomization into study groups and for evaluation of novel NASH therapeutics.

### Peer-review

The results of this study demonstrated a useful animal model for evaluation the disease progression and treatment of NASH. The data were appropriately presented and interpreted. The manuscript was well prepared.

## REFERENCES

- 1 Ariz U, Mato JM, Lu SC, Martínez Chantar ML. Nonalcoholic steatohepatitis, animal models, and biomarkers: what is new? *Methods Mol Biol* 2010; **593**: 109-136 [PMID: 19957147 DOI: 10.1007/978-1-60327-194-3]
- 2 Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138 [PMID: 15629518 DOI: 10.1016/j.jhep.2004.09.012]
- 3 Zeevaert JG, Wang L, Thakur VV, Leung CS, Tirado-Rives J, Bailey CM, Domaoal RA, Anderson KS, Jorgensen WL. Optimization of azoles as anti-human immunodeficiency virus agents guided by free-energy calculations. *J Am Chem Soc* 2008; **130**: 9492-9499 [PMID: 18588301 DOI: 10.1021/ja8019214]
- 4 Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**: S99-S112 [PMID: 16447287 DOI: 10.1002/hep.20973]
- 5 Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/S0016-5085(98)70599-2]
- 6 Clapper JR, Hendricks MD, Gu G, Wittmer C, Dolman CS, Herich J, Athanacio J, Villescaz C, Ghosh SS, Heilig JS, Lowe C, Roth JD. Diet-induced mouse model of fatty liver disease and nonalcoholic steatohepatitis reflecting clinical disease progression and methods of assessment. *Am J Physiol Gastrointest Liver Physiol* 2013; **305**: G483-G495 [PMID: 23886860 DOI: 10.1152/ajpgi.00079.2013]
- 7 Sanches SC, Ramalho LN, Augusto MJ, da Silva DM, Ramalho FS. Nonalcoholic Steatohepatitis: A Search for Factual Animal Models. *Biomed Res Int* 2015; **2015**: 574832 [PMID: 26064924 DOI: 10.1155/2015/574832]
- 8 Machado MV, Michelotti GA, Xie G, Almeida Pereira T, Boursier



- J, Bohnic B, Guy CD, Diehl AM. Mouse models of diet-induced nonalcoholic steatohepatitis reproduce the heterogeneity of the human disease. *PLoS One* 2015; **10**: e0127991 [PMID: 26017539 DOI: 10.1371/journal.pone.0127991]
- 9 **Takahashi Y**, Soejima Y, Fukusato T. Animal models of non-alcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2012; **18**: 2300-2308 [PMID: 22654421 DOI: 10.3748/wjg.v18.i19.2300]
  - 10 **Starkel P**, Leclercq IA. Animal models for the study of hepatic fibrosis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 319-333 [PMID: 21497748 DOI: 10.1016/j.bpg.2011.02.004]
  - 11 **Trevaskis JL**, Griffin PS, Wittmer C, Neuschwander-Tetri BA, Brunt EM, Dolman CS, Erickson MR, Napora J, Parkes DG, Roth JD. Glucagon-like peptide-1 receptor agonism improves metabolic, biochemical, and histopathological indices of nonalcoholic steatohepatitis in mice. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G762-G772 [PMID: 22268099 DOI: 10.1152/ajpgi.00476.2011]
  - 12 **Tetri LH**, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G987-G995 [PMID: 18772365 DOI: 10.1152/ajpgi.90272.2008]
  - 13 **Nakayama H**, Otabe S, Ueno T, Hirota N, Yuan X, Fukutani T, Hashinaga T, Wada N, Yamada K. Transgenic mice expressing nuclear sterol regulatory element-binding protein 1c in adipose tissue exhibit liver histology similar to nonalcoholic steatohepatitis. *Metabolism* 2007; **56**: 470-475 [PMID: 17379003 DOI: 10.1016/j.metabol.2006.11.004]
  - 14 **Chen H**, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, Morgenstern JP. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* 1996; **84**: 491-495 [PMID: 8608603 DOI: 10.1016/S0092-8674(00)81294-5]
  - 15 **Dobin A**, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 2013; **29**: 15-21 [PMID: 23104886 DOI: 10.1093/bioinformatics/bts635]
  - 16 **Anders S**, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* 2015; **31**: 166-169 [PMID: 25260700 DOI: 10.1101/002824]
  - 17 **Robinson MD**, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 2010; **26**: 139-140 [PMID: 19910308 DOI: 10.1093/bioinformatics/btp616]
  - 18 **Kutmon M**, Riutta A, Nunes N, Hanspers K, Willighagen EL, Bohler A, Mélius J, Waagmeester A, Sinha SR, Miller R, Coort SL, Cirillo E, Smeets B, Evelo CT, Pico AR. WikiPathways: capturing the full diversity of pathway knowledge. *Nucleic Acids Res* 2016; **44**: D488-D494 [PMID: 26481357 DOI: 10.1093/nar/gkv1024]
  - 19 **Kutmon M**, van Iersel MP, Bohler A, Kelder T, Nunes N, Pico AR, Evelo CT. PathVisio 3: an extendable pathway analysis toolbox. *PLoS Comput Biol* 2015; **11**: e1004085 [PMID: 25706687 DOI: 10.1371/journal.pcbi.1004085]
  - 20 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]
  - 21 **Wang Z**, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 2009; **10**: 57-63 [PMID: 19015660 DOI: 10.1038/nrg2484]
  - 22 **Sutter AG**, Palanisamy AP, Lench JH, Eskilsen S, Geng T, Lewin DN, Cowart LA, Chavin KD. Dietary Saturated Fat Promotes Development of Hepatic Inflammation Through Toll-Like Receptor 4 in Mice. *J Cell Biochem* 2016; **117**: 1613-1621 [PMID: 26600310 DOI: 10.1002/jcb.25453]
  - 23 **Seki E**, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007; **13**: 1324-1332 [PMID: 17952090 DOI: 10.1038/nm1663]
  - 24 **Honda Y**, Imajo K, Kato T, Kessoku T, Ogawa Y, Tomeno W, Kato S, Mawatari H, Fujita K, Yoneda M, Saito S, Nakajima A. The Selective SGLT2 Inhibitor Ipragliflozin Has a Therapeutic Effect on Nonalcoholic Steatohepatitis in Mice. *PLoS One* 2016; **11**: e0146337 [PMID: 26731267 DOI: 10.1371/journal.pone.0146337]
  - 25 **Griffett K**, Welch RD, Flaveny CA, Kolar GR, Neuschwander-Tetri BA, Burris TP. The LXR inverse agonist SR9238 suppresses fibrosis in a model of non-alcoholic steatohepatitis. *Mol Metab* 2015; **4**: 353-357 [PMID: 25830098 DOI: 10.1016/j.molmet.2015.01.009]
  - 26 **Rivera CA**, Adegboyega P, van Rooijen N, Tagalicud A, Allman M, Wallace M. Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. *J Hepatol* 2007; **47**: 571-579 [PMID: 17644211 DOI: 10.1016/j.jhep.2007.04.019]
  - 27 **Sutter AG**, Palanisamy AP, Lench JH, Jessmore AP, Chavin KD. Development of steatohepatitis in Ob/Ob mice is dependent on Toll-like receptor 4. *Ann Hepatol* 2015; **14**: 735-743 [PMID: 26256903]
  - 28 **Seki E**, de Minicis S, Inokuchi S, Taura K, Miyai K, van Rooijen N, Schwabe RF, Brenner DA. CCR2 promotes hepatic fibrosis in mice. *Hepatology* 2009; **50**: 185-197 [PMID: 19441102 DOI: 10.1002/hep.22952.CCR2]
  - 29 **Weisberg SP**, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, Charo I, Leibel RL, Ferrante AW. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J Clin Invest* 2006; **116**: 115-124 [PMID: 16341265 DOI: 10.1172/JCI24335]
  - 30 **Battaller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI200524282]
  - 31 **Friedman SL**. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; **134**: 1655-1669 [PMID: 18471545 DOI: 10.1053/j.gastro.2008.03.003]
  - 32 **Horton JD**, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; **109**: 1125-1131 [PMID: 11994399 DOI: 10.1172/JCI200215593]
  - 33 **Fujita K**, Nozaki Y, Wada K, Yoneda M, Fujimoto Y, Fujitake M, Endo H, Takahashi H, Inamori M, Kobayashi N, Kirikoshi H, Kubota K, Saito S, Nakajima A. Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. *Hepatology* 2009; **50**: 772-780 [PMID: 19650159 DOI: 10.1002/hep.23094]

**P- Reviewer:** Chuang WL, Kim JS, Miura K **S- Editor:** Qi Y  
**L- Editor:** A **E- Editor:** Liu SQ





Retrospective Study

# Hepatocellular carcinoma after locoregional therapy: Magnetic resonance imaging findings in falsely negative exams

David Becker-Weidman, Jesse M Civan, Sandeep P Deshmukh, Christopher G Roth, Steven K Herrine, Laurence Parker, Donald G Mitchell

David Becker-Weidman, Sandeep P Deshmukh, Christopher G Roth, Laurence Parker, Donald G Mitchell, Department of Radiology, Thomas Jefferson University, Philadelphia, PA 19107, United States

Jesse M Civan, Steven K Herrine, Division of Gastroenterology and Hepatology, Department of Medicine, Thomas Jefferson University, Philadelphia, PA 19107, United States

**Author contributions:** Becker-Weidman D designed the study, collected the data, and drafted the manuscript; Civan JM developed the concept, collected the data and drafted the manuscript; Deshmukh SP and Roth CG interpreted MRI images and edited the manuscript; Herrine SK developed the concept and edited the manuscript; Parker L performed statistical analysis and edited the manuscript; Mitchell DG developed the concept, interpreted MRI images, and edited the manuscript.

**Institutional review board statement:** This study was reviewed and approved by the Ethics Committee of the Thomas Jefferson University.

**Informed consent statement:** Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

**Conflict-of-interest statement:** We have no financial relationships to disclose.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

[licenses/by-nc/4.0/](http://creativecommons.org/licenses/by-nc/4.0/)

**Manuscript source:** Invited manuscript

**Correspondence to:** Donald G Mitchell, MD, Department of Radiology, Thomas Jefferson University, 132 S 10<sup>th</sup> St, Main Building, Room 1094, Philadelphia, PA 19107, United States. [donald.mitchell@jefferson.edu](mailto:donald.mitchell@jefferson.edu)  
 Telephone: +1-215-9554809  
 Fax: +1-215-9558270

**Received:** March 2, 2016

**Peer-review started:** March 2, 2016

**First decision:** March 22, 2016

**Revised:** April 7, 2016

**Accepted:** May 10, 2016

**Article in press:** May 11, 2016

**Published online:** June 8, 2016

## Abstract

**AIM:** To elucidate causes for false negative magnetic resonance imaging (MRI) exams by identifying imaging characteristics that predict viable hepatocellular carcinoma (HCC) in lesions previously treated with locoregional therapy when obvious findings of recurrence are absent.

**METHODS:** This retrospective institutional review board-approved and Health Insurance Portability and Accountability Act-compliant study included patients who underwent liver transplantation at our center between 1/1/2000 and 12/31/2012 after being treated for HCC with locoregional therapy. All selected patients had a contrast-enhanced MRI after locoregional therapy within 90 d of transplant that was prospectively interpreted as without evidence of residual or recurrent

tumor. Retrospectively, 2 radiologists, blinded to clinical and pathological data, independently reviewed the pre-transplant MRIs for 7 imaging features. Liver explant histopathology provided the reference standard, with clinically significant tumor defined as viable tumor  $\geq 1.0$  cm in maximum dimension. Fisher's exact test was first performed to identify significant imaging features.

**RESULTS:** Inclusion criteria selected for 42 patients with 65 treated lesions. Fourteen of 42 patients (33%) and 16 of 65 treated lesions (25%) had clinically significant viable tumor on explant histology. None of the 7 imaging findings examined could reliably and reproducibly determine which treated lesion had viable tumor when the exam had been prospectively read as without evidence of viable HCC.

**CONCLUSION:** After locoregional therapy some treated lesions that do not demonstrate any MRI evidence of HCC will contain viable tumor. As such even patients with a negative MRI following treatment should receive regular short-term imaging surveillance because some have occult viable tumor. The possibility of occult tumor should be a consideration when contemplating any action which might delay liver transplant.

**Key words:** Hepatocellular carcinoma; Transarterial chemoembolization; Tumor recurrence; Locoregional therapy; Imaging surveillance

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatocellular carcinoma (HCC) is often treated with locoregional therapy such as transarterial chemoembolization as a bridge to transplantation. Detecting residual or recurrent tumor within these treated lesions is challenging and some treated lesions that do not demonstrate any magnetic resonance imaging (MRI) evidence of HCC will contain foci of viable tumor. Regular, short-term imaging surveillance is clinically important for patients being considered for liver transplantation even when prior MRIs have been negative and the possibility of a false negative MRI exam needs to be considered when managing these patients.

Becker-Weidman D, Civan JM, Deshmukh SP, Roth CG, Herrine SK, Parker L, Mitchell DG. Hepatocellular carcinoma after locoregional therapy: Magnetic resonance imaging findings in falsely negative exams. *World J Hepatol* 2016; 8(16): 685-690 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i16/685.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i16.685>

## INTRODUCTION

The American College of Radiology developed the liver imaging reporting and data system (LI-RADS) to standardize how hepatocellular carcinoma (HCC) is

diagnosed<sup>[1]</sup>. These criteria were validated in untreated lesions and therefore do not apply to lesions after treatment with locoregional therapy. Although certain imaging findings are associated with the presence of viable HCC in a treated lesion there is currently no formal system to assess the probability of viable tumor.

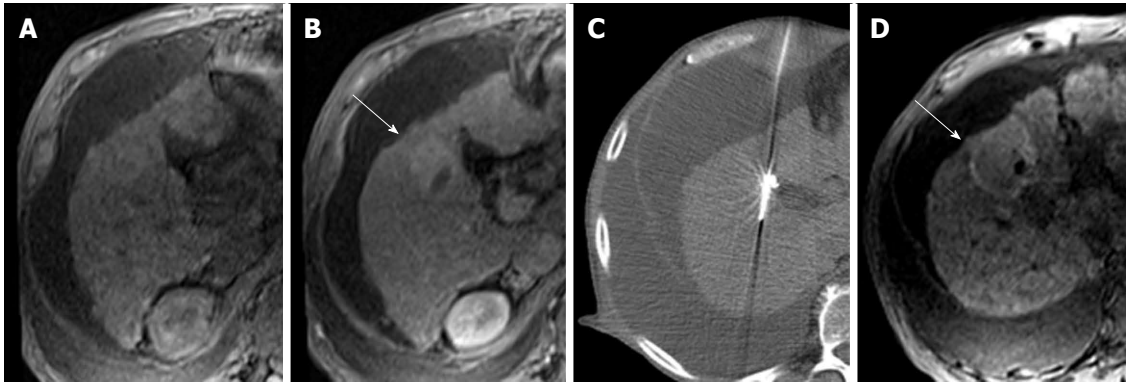
Magnetic resonance imaging (MRI) is commonly used status post locoregional therapy to evaluate for recurrent or residual viable tumor. Because the hallmark of HCC is avid arterial-phase enhancement, dynamic imaging following gadolinium-based contrast administration should be a core component of the examination. Arterial-phase enhancement following locoregional therapy has a reported sensitivity and specificity of 82% to 100% and 79% to > 90% respectively<sup>[2,3]</sup>. Subtle arterial-phase enhancement can be obscured in treated lesions as they often demonstrate heterogeneous high signal on T1-weighted images due to the presence of blood products (Figure 1). HCC is a very cellular tumor<sup>[4]</sup> and will typically restrict the diffusion of water molecules giving it high signal on diffusion-weighted imaging (DWI) and corresponding low signal on the computer generated apparent diffusion coefficient map. Diffusion restriction following locoregional therapy has a reported sensitivity and specificity of 61% to 75% and 88% to > 90% respectively<sup>[2,3]</sup>. Identifying restricted diffusion in treated areas is complicated by the fact that these areas typically demonstrate high signal on T2-weighted images due to fibrosis (Figure 2), appearing as T2 shine through on DWI. Signal intensity on T2-weighted images is not typically helpful as it is affected by treatment and can be variable, although it is typically mildly to moderately hyperintense. Signal intensity on precontrast T1-weighted images is quite variable and generally not helpful. HCC is usually hypointense or isointense but can be hyperintense with intratumoral fat.

Unresectable HCC is often treated with locoregional therapy to decrease disease burden and as a bridge to transplant. In these patients accurate assessment of tumor response is integral to directing patient care. False negative MRI exams are due to a number of factors including technical limitations and inherent MR signal alteration of the treated areas. In addition there is no formal system for evaluating treated lesions. The goal of this study was to retrospectively determine which MRI features were most predictive of histological findings of residual or recurrent HCC in a population that does not demonstrate obvious recurrence.

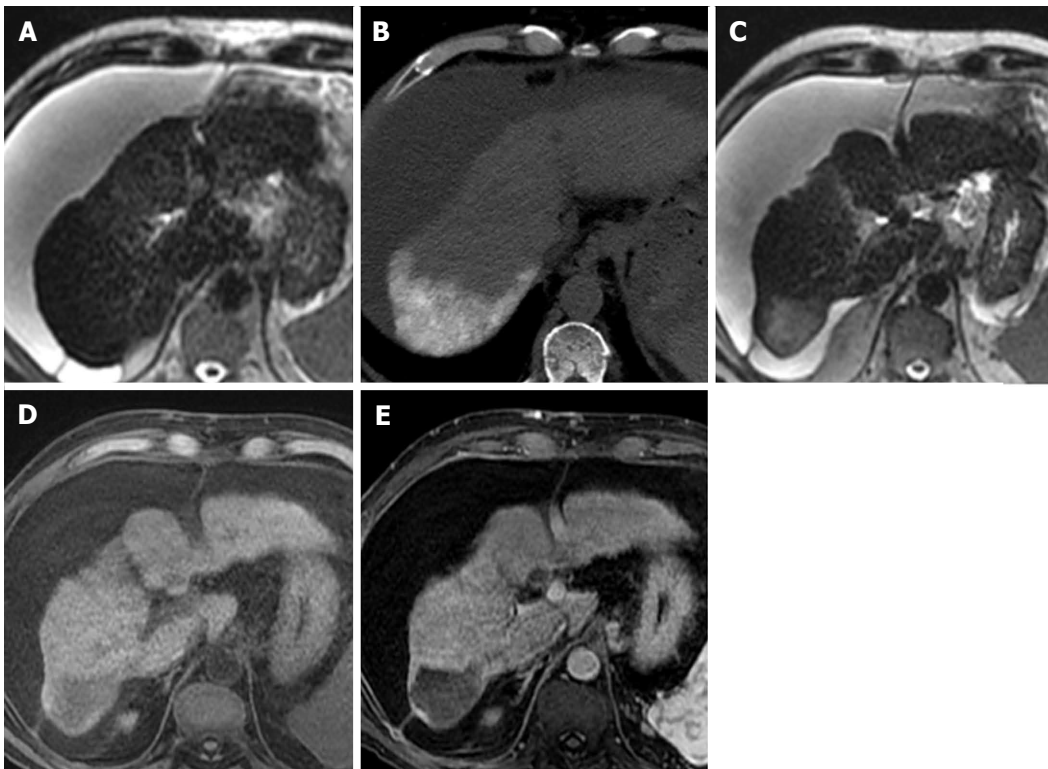
## MATERIALS AND METHODS

### Patient and lesion inclusion criteria

This retrospective study was approved by our institutional review board and was compliant with the Health Insurance Portability and Accountability Act. Our study included patients with HCC who underwent liver transplantation at our center between 1/1/2000 and 12/31/2012 after being treated with locoregional therapy. Inclusion criteria selected patients that had a



**Figure 1 Treatment-related signal alterations on T1-weighted images.** A 59-year-old man with HCV cirrhosis and a segment V LI-RADS 5B lesion measuring up to 3.6 cm. Fat-saturated T1-weighted precontrast (A) and arterial-phase (B) images demonstrate a 3.6 cm enhancing focus of viable tumor (B, arrow) within an area previously treated with TACE. Mass could not be visualized upon selective angiography so repeat TACE was not performed. Non-contrast CT from a radiofrequency ablation procedure (C) demonstrates an electrode positioned within the tumor. One month later a fat-saturated T1-weighted precontrast image (D) demonstrates peripheral high signal related to hemorrhage from coagulative necrosis (D, arrow). LI-RADS: Liver imaging reporting and data system; TACE: Transarterial chemoembolization; HCV: Hepatitis C virus.



**Figure 2 Treatment-related signal alterations on T2-weighted images.** A 63-year-old man with HCV/EtOH cirrhosis and a segment VII LI-RADS 5B lesion measuring 2.4 cm × 1.8 cm. T2-weighted image (A) just superior to the segment VI lesion before treatment demonstrates a cirrhotic liver with homogeneous low signal intensity. Non-contrast CT (B) performed immediately after a TACE procedure shows high attenuation Lipiodol® in segment VII confirming that the appropriate segment was treated. T2-weighted image (C) from an MRI performed one month later shows high signal intensity in the area that was treated due to fibrotic change. Precontrast (D) and arterial-phase images (E) from that exam demonstrate that the treated area is completely necrotic. LI-RADS: Liver imaging reporting and data system; TACE: Transarterial chemoembolization; HCV: Hepatitis C virus; CT: Computed tomography; MRI: Magnetic resonance imaging.

contrast-enhanced MRI after locoregional therapy within 90 d of transplant that was prospectively interpreted as without evidence of residual or recurrent tumor. Patients were identified through our electronic medical record.

While HCC is a radiologic diagnosis, subcentimeter lesions cannot be designated as “definite” HCC by either the American Association for the Study of Liver Diseases or the LI-RADS criteria in recognition of the fact that

early tumors may not demonstrate hypervascularity and technical limitations preclude adequate assessment of lesions below this threshold<sup>[1,5]</sup>. Therefore, we considered foci of HCC identified on explant significant for the purpose of our study only if it measured  $\geq 1.0$  cm in maximal diameter.

Foci of viable tumor detected histologically on explant, distinct from a previously treated lesion were



not included in our analysis. These foci were treated as incidental findings as our current analysis regards the MRI findings in lesions previously identified as HCC subsequently undergoing treatment.

### MR image analysis

All MRI data sets were reviewed on a workstation equipped with image review software (iSite, version 3.6; Philips, Andover, MA). Retrospective image interpretations were performed independently by two body MRI specialists, each with more than 10 years of experience. The study coordinator, a radiology resident, prepared the exams for review by correlating the lesions described in the explant pathology report with the treated lesions on the MRI, marking the lesions to be evaluated with an arrow. Exams were reviewed in random order by the interpreting radiologists, who were blinded to all other patient history, including pathology and other imaging results.

Each liver lesion was assessed by the interpreting radiologist for the presence or absence of: Arterial-phase nodular enhancement, arterial-phase non-nodular enhancement, gradual enhancement, partial or complete T1 signal hypointensity, partial or complete T2 signal hyperintensity, lipid as determined by comparison of in-phase and opposed-phase images, and restricted diffusion if DWI was performed. Findings were recorded in prepared data sheets.

### Gross pathology and histopathologic analysis

All explanted livers were received as surgical resection specimens. Each explant was serially sectioned in contiguous slices at 5 mm intervals, and processed for routine Hematoxylin and Eosin stains. These slides were prospectively reviewed for the presence of viable HCC and the pathology report produced was used to retrospectively correlate the histologic findings with the pretransplant MRI.

### Statistical analysis

Statistical review of the study was performed by a biomedical statistician. Statistical software (SAS version 9.4; SAS Institute, Cary, NC) was used for all data analysis. Fisher's exact test was first performed to identify significant imaging features in a bivariate analysis followed by a step-wise logistic regression if more than one variable was significant. The significance threshold was set at a *P*-value of 0.05 and any variable with *P* > 0.05 was removed from the model and determined to be insignificant. The agreement level between readers was measured by using *k* coefficient. We defined *k* values for level of agreement as follows: 0.81-0.99, almost perfect agreement; 0.61-0.80, substantial agreement; 0.41-0.60, moderate agreement; 0.21-0.40, fair agreement; and 0.01-0.20, slight agreement<sup>[6]</sup>.

## RESULTS

### Patients

A search of our electronic medical record showed

that 488 patients had a liver transplant at our center between 1/1/2000 and 12/31/2012, of which 167 (34.2%) had HCC, all of whom were treated with locoregional therapy prior to transplant. Of these patients, 84 (50.3%) had findings suspicious for recurrent or residual HCC on the pre-transplant MRI, 24 (14.4%) underwent locoregional treatment between the pre-transplant MRI and transplant, 16 (9.6%) had the pre-transplant MRI over 90 d before transplant, and 1 (0.6%) did not receive intravenous contrast and were excluded from our study. Patient accrual details are presented in Figure 3. The final cohort of 42 patients (mean age, 59 years; age range, 46-73 years) included 34 men (mean age, 59 years; age range, 46-73 years) and 8 women (mean age, 59 years; age range, 53-70 years). Patients had cirrhosis secondary to hepatitis C (*n* = 29), hepatitis C and alcohol abuse (*n* = 5), alcohol abuse (*n* = 4), nonalcoholic steatohepatitis (*n* = 1), or an unknown cause (*n* = 3). MRI was performed an average of 40 d before transplant (range, 1-89 d).

Prior to transplant 33 (79%) patients were treated with transarterial chemoembolization (TACE) only, 3 (7%) were treated with radiofrequency ablation only, 2 (5%) were treated with radioactive embolization only, 1 (2%) was treated with bland transarterial embolization only, and 3 (7%) were treated with TACE and radiofrequency ablation.

### Reference histopathologic analysis

The 42 patients who met our inclusion criteria included 18 (43%) who had no viable tumor, 10 (24%) who had viable tumor that was considered clinically insignificant, and 14 (33%) who had at least one focus of clinically-significant viable tumor on explant pathology. Two patients had two foci of clinically-significant viable tumor. The explant Pathology report mentioned a single lesion in 27 patients (64%), 2 lesions in 9 patients (21%), 3 lesions in 3 patients (7%), 4 lesions in 2 patients (5%), and 5 lesions in 1 patient (3%) for a total of 65 treated lesions. Sixteen treated lesions (25%) had clinically significant viable tumor (mean size, 1.5 cm; range, 1.0-3.5 cm), 13 treated lesions (20%) had a focus of tumor < 1.0 cm (mean size, 0.5 cm; range, 0.1-0.9 cm), and 36 (55%) treated lesions had no viable tumor.

### MR image and statistical analysis

Of the 42 patients, 15 received gadoxetate disodium (Eovist) (36%), 13 received gadopentate dimeglumine (Magnevist) (31%), 8 received gadobutrol (Gadavist) (19%), and 6 received gadobenate dimeglumine (MultiHance) (14%). DWI was only performed in 19 patients (45%) as DWI was not included as a part of our routine MRI exam of the abdomen until 2011.

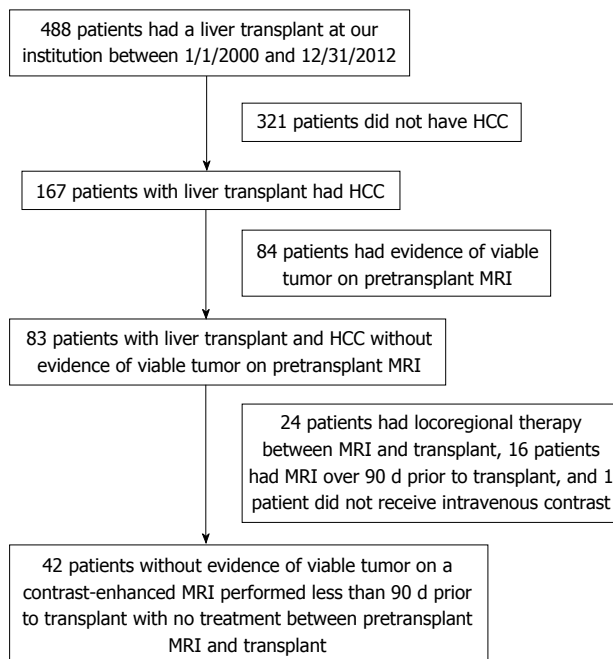
For reader #1 "arterial-phase non-nodular enhancement" and "partial or complete T1 signal hypointensity" were significant predictors of viable tumor. For reader #2 "partial or complete T2 signal hyperintensity" was the sole significant predictor of viable tumor.



**Table 1** *P*-values and Kappa values associated with the 7 different imaging features

	<i>P</i> -values		Kappa values
	Reader #1	Reader #2	
Arterial-phase nodular enhancement	0.44	0.14	0.37 (fair agreement)
Arterial-phase non-nodular enhancement	0.25	0.008 <sup>b</sup>	0.23 (fair agreement)
Gradual enhancement	0.47	0.15	0.10 (slight agreement)
Partial or complete T1 signal hypointensity	0.21	0.001 <sup>b</sup>	0.15 (slight agreement)
Partial or complete T2 signal hyperintensity	0.047 <sup>a</sup>	0.47	0.07 (slight agreement)
Lipid	0.56	0.44	-0.03 (no agreement)
Restricted diffusion	N/A <sup>1</sup>	N/A <sup>1</sup>	N/A <sup>1</sup>

<sup>a,b</sup>*P*-values reached significance; <sup>1</sup>Could not be assessed due to collinearity. N/A: Not applicable.



**Figure 3 Patient accrual flowchart.** HCC: Hepatocellular carcinoma; MRI: Magnetic resonance imaging.

There was fair agreement for arterial-phase nodular enhancement and non-nodular enhancement ( $k = 0.37, 0.23$  respectively); slight agreement for gradual enhancement, partial or complete T1 signal hypointensity, and partial or complete T2 signal hyperintensity ( $k = 0.07, 0.10, 0.15$  respectively); and no agreement for the presence of lipid ( $k = -0.03$ ). The *P*-values and kappa values are presented in Table 1.

### Patient outcomes

There was a single post-transplant recurrence in the 18 patients without viable tumor (mean length of followup, 4.9 years; range, 1.1-9.0 years). There was a single recurrence in the 10 patients with clinically insignificant cancer (mean length of followup, 5.2 years; range, 2.6-13.4 years). There was a single recurrence in the 14 patients with clinically significant viable tumor (mean length of followup, 3.4 years; range, 0.2-7.1 years). One patient with only 0.2 years of followup died from a stroke.

## DISCUSSION

Retrospective review of true negative and false negative MR exams did not identify any subtle findings that can reliably and reproducibly indicate the presence of viable HCC in studies that were prospectively interpreted as negative. T1 and T2 signal intensity are highly variable after locoregional therapy and are not reliable indicators of viable tumor. Delayed enhancement is often seen after treatment and indicates fibrosis. Arterial-phase enhancement is associated with viable tumor but may be subtle or absent and has a reported sensitivity of as low as 82%<sup>[2]</sup>. In other words, some patients who do not have evidence of HCC on MRI will have viable tumor on explant pathology.

A limitation of this study was the low level of agreement between readers. This can be partially explained due to the low number of "positive" imaging features. When there is a low base rate a small number of discordant findings will have a disproportionately large effect on Cohen's kappa coefficient<sup>[7]</sup>. Agreement regarding enhancement characteristics is only slight to fair because any case that demonstrated obvious enhancement was prospectively read as suspicious for viable HCC and excluded from our study. The only cases that remained were those that demonstrated subtle enhancement. Agreement for signal intensity on T1- and T2-weighted images is only slight due to the inherent difficulty in classifying a highly heterogeneous area. That being said the low kappa value limits the value and reliability of any imaging finding that was positively associated with viable HCC. Therefore, we do not propose that signal intensity on unenhanced T1-weighted or T2-weighted images is predictive of viable tumor. It is possible that non-nodular arterial-phase enhancement is predictive of viable tumor but this finding was not reliable enough in our study for clinical use.

Another limitation was the inconsistency of the explant pathology reports. Some pathology reports measured the size of the viable component or state the percentage of necrosis within the measured treated lesion, whereas, other reports used subjective terminology such as "partially necrotic" or "largely necrotic" which made exact measurement of the viable component difficult. Another problem was that some of

the treated lesions demonstrated partial diffuse necrosis and contained only microscopic islands of tumor. These lesions are considered viable by histology but impossible to identify by imaging.

Noting the limitations above it is clear that occasionally treated lesions without evidence of viable HCC by MRI can contain foci of viable tumor. This supports the utilization of regular short-term imaging surveillance even when prior MRIs have been negative and is clinically important for patients being considered for liver transplantation. For example, a decision to delay transplant to allow treatment of underlying chronic viral hepatitis C should be made with caution, without over-reliance on a sense of security suggested by surveillance imaging with no definite evidence of viable HCC. The possibility of a false negative MRI exam needs to be considered when managing patients after locoregional therapy.

## COMMENTS

### Background

Unresectable hepatocellular carcinoma (HCC) is often treated with locoregional therapy to decrease disease burden and as a bridge to transplant. After locoregional therapy magnetic resonance imaging (MRI) interpretation can be more difficult due to a number of factors. Several imaging findings have been shown to correlate with the presence of viable HCC in this setting including diffusion restriction and arterial-phase enhancement.

### Research frontiers

Liver imaging reporting and data system (LI-RADS) was not developed to be applied to treated lesions and as such these lesions are designated "LR-treated". Further investigation into the imaging characteristics of treated lesions could lead to the development of a version of LI-RADS that can be applied to these lesions.

### Innovations and breakthroughs

In this study, the authors demonstrate that treated lesions can harbor foci of viable HCC but demonstrate no MRI findings.

### Applications

The research supports the utilization of regular short-term imaging surveillance

in patients with HCC treated with locoregional therapy even when prior MRIs have been negative due to the possibility of a false negative exam.

### Terminology

Diffusion-weighted imaging: MRI sequence that measures random Brownian motion of water molecules within a voxel of tissue; LI-RADS: Set of terminology developed by the American College of Radiology to standardize the reporting of imaging findings of liver lesions; Locoregional therapy: Transarterial and/or local ablative therapy.

### Peer-review

This is an interesting manuscript providing information for an easily neglected field. It is thus of value to be considered for publication.

## REFERENCES

- 1 **Mitchell DG**, Bruix J, Sherman M, Sirlin CB. LI-RADS (Liver Imaging Reporting and Data System): summary, discussion, and consensus of the LI-RADS Management Working Group and future directions. *Hepatology* 2015; **61**: 1056-1065 [PMID: 25041904 DOI: 10.1002/hep.27304]
- 2 **Goshima S**, Kanematsu M, Kondo H, Yokoyama R, Tsuge Y, Shiratori Y, Onozuka M, Moriyama N. Evaluating local hepatocellular carcinoma recurrence post-transcatheter arterial chemoembolization: is diffusion-weighted MRI reliable as an indicator? *J Magn Reson Imaging* 2008; **27**: 834-839 [PMID: 18383261 DOI: 10.1002/jmri.21316]
- 3 **Mannelli L**, Kim S, Hajdu CH, Babb JS, Clark TW, Taouli B. Assessment of tumor necrosis of hepatocellular carcinoma after chemoembolization: diffusion-weighted and contrast-enhanced MRI with histopathologic correlation of the explanted liver. *AJR Am J Roentgenol* 2009; **193**: 1044-1052 [PMID: 19770328 DOI: 10.2214/AJR.08.1461]
- 4 **Taouli B**, Vilgrain V, Dumont E, Daire JL, Fan B, Menu Y. Evaluation of liver diffusion isotropy and characterization of focal hepatic lesions with two single-shot echo-planar MR imaging sequences: prospective study in 66 patients. *Radiology* 2003; **226**: 71-78 [PMID: 12511671 DOI: 10.1148/radiol.2261011904]
- 5 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
- 6 **Viera AJ**, Garrett JM. Understanding interobserver agreement: the kappa statistic. *Fam Med* 2005; **37**: 360-363 [PMID: 15883903]
- 7 **Sim J**, Wright CC. The kappa statistic in reliability studies: use, interpretation, and sample size requirements. *Phys Ther* 2005; **85**: 257-268 [PMID: 15733050]

**P- Reviewer:** Kayaalp C, Zhang Q **S- Editor:** Ji FF

**L- Editor:** A **E- Editor:** Liu SQ



## Redefining Budd-Chiari syndrome: A systematic review

Naomi Shin, Young H Kim, Hao Xu, Hai-Bin Shi, Qing-Qiao Zhang, Jean Paul Colon Pons, Ducksoo Kim, Yi Xu, Fei-Yun Wu, Samuel Han, Byung-Boong Lee, Lin-Sun Li

Naomi Shin, Young H Kim, Department of Radiology, University of Massachusetts Medical School, Worcester, MA 01655, United States

Hao Xu, Qing-Qiao Zhang, Department of Interventional Radiology, the Affiliated Hospital of Xuzhou Medical College, Xuzhou 221006, Jiangsu Province, China

Hai-Bin Shi, Yi Xu, Fei-Yun Wu, Lin-Sun Li, Department of Radiology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Jean Paul Colon Pons, Ducksoo Kim, Department of Radiology, Boston University School of Medicine, Boston, MA 02118, United States

Samuel Han, Department of Gastroenterology, University of Massachusetts Medical School, Worcester, MA 01655, United States

Byung-Boong Lee, Department of Surgery, George Washington University School of Medicine, Washington, DC 20037, United States

**Author contributions:** Shin N, Kim YH, Xu H, Shi HB, Zhang QQ and Xu Y designed the research; Shin N, Colon Pons JP, Kim D, Xu Y, Wu FY, Lee BB and Li LS conducted the research; Shin N, Kim YH, Xu H and Han S wrote the paper.

**Conflict-of-interest statement:** The authors declare no conflicts of interest.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at [young.kim@umassmemorial.org](mailto:young.kim@umassmemorial.org).

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

**Correspondence to:** Young H Kim, MD, PhD, Associate Professor, Department of Radiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, United States. [young.kim@umassmemorial.org](mailto:young.kim@umassmemorial.org)  
Telephone: +1-508-3342087  
Fax: +1-508-8564910

Received: January 13, 2016

Peer-review started: January 15, 2016

First decision: March 23, 2016

Revised: April 8, 2016

Accepted: May 17, 2016

Article in press: May 27, 2016

Published online: June 8, 2016

### Abstract

**AIM:** To re-examine whether hepatic vein thrombosis (HVT) (classical Budd-Chiari syndrome) and hepatic vena cava-Budd Chiari syndrome (HVC-BCS) are the same disorder.

**METHODS:** A systematic review of observational studies conducted in adult subjects with primary BCS, hepatic vein outflow tract obstruction, membranous obstruction of the inferior vena cava (IVC), obliterative hepatocavopathy, or HVT during the period of January 2000 until February 2015 was conducted using the following databases: Cochrane Library, CINAHL, MEDLINE, PubMed and Scopus.

**RESULTS:** Of 1299 articles identified, 26 were included in this study. Classical BCS is more common in women with a pure hepatic vein obstruction (49%-74%). HVC-BCS is more common in men with the obstruction often located in both the inferior vena cava and hepatic veins (14%-84%). Classical BCS presents with acute abdominal pain, ascites, and hepatomegaly. HVC-BCS presents with chronic abdominal pain and abdominal

wall varices. Myeloproliferative neoplasms (MPN) are the most common etiology of classical BCS (16%-62%) with the JAK2V617-F mutation found in 26%-52%. In HVC-BCS, MPN are found in 4%-5%, and the JAK2V617-F mutation in 2%-5%. Classical BCS responds well to medical management alone and 1<sup>st</sup> line management of HVC-BCS involves percutaneous recanalization, with few managed with medical management alone.

**CONCLUSION:** Systematic review of recent data suggests that classical BCS and HVC-BCS may be two clinically different disorders that involve the disruption of hepatic venous outflow.

**Key words:** Budd-Chiari; Hepatic vein outflow tract obstruction; Membranous obstruction of the inferior vena cava; Obliterative hepatocavopathy; Hepatic vein thrombosis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** With improved diagnostic methods, the terminology for Budd-Chiari syndrome (BCS) has expanded discordantly. This systematic review discusses recent population studies of BCS and proposes the delineation of two clinically unique syndromes.

Shin N, Kim YH, Xu H, Shi HB, Zhang QQ, Colon Pons JP, Kim D, Xu Y, Wu FY, Han S, Lee BB, Li LS. Redefining Budd-Chiari syndrome: A systematic review. *World J Hepatol* 2016; 8(16): 691-702 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i16/691.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i16.691>

## INTRODUCTION

Budd-Chiari syndrome (BCS) was originally described as a rare vascular disorder that encompasses an array of symptoms due to obstruction of hepatic blood outflow at the level of the hepatic veins or hepatic portion of the inferior vena cava (IVC)<sup>[1]</sup>. The symptoms resulting from this type of occlusion of the hepatic outflow, "classical BCS", were first described by Budd<sup>[2,3]</sup> in 1845 and later by Hans Chiari in 1899. With the advancement of diagnostic and therapeutic techniques, providers have expanded upon these initial characterizations<sup>[4]</sup>. Historically, identifying the precise location of the obstruction was challenging, leading to the propagation of simplified descriptions. The precise location of the obstruction(s) is however clinically and prognostically significant. As Valla<sup>[5]</sup> proposed, the clinical manifestations of BCS (the selective group of symptoms that characterize the syndrome) can be explained by the location of the obstruction: Within the hepatic veins vs within the IVC at the level of the hepatic ostia. Over time, in order to incorporate novel and more detailed findings associated with BCS, the lexicon has evolved discordantly. The

lexicon now includes a myriad of ambiguous terms or eponyms: Budd's disease, Chiari's disease, Chiari's syndrome, Rokitansky's disease, von Rokitansky disease, Hepatic vein outflow tract obstruction, membranous obstruction of the IVC, obliterative hepatocavopathy, Hepatic vena cava disease, Budd-Chiari syndrome with occlusion of hepatic vein, or hepatic vein thrombosis<sup>[6-8]</sup>. These eponyms have been used at some point during the course of further discovery; this disarray of terms, some of which are unclear and nonspecific, reflects not only the heterogeneous presentation of BCS, but also the possibility of distinct entities within this syndrome.

The currently accepted definition of primary BCS is hepatic outflow obstruction regardless of the cause or level of obstruction<sup>[6,9]</sup>. The obstruction can range from the small hepatic veins to the orifice of the IVC into the right atrium. Sinusoidal obstruction syndrome is excluded from this definition<sup>[6,9]</sup>. Secondary BCS is defined as a hepatic venous outflow obstruction due to compression or invasion by extravascular lesions, including benign or malignant diseases such as abscesses, hepatocellular carcinomas, and renal cell carcinomas, or secondary to cardiac or pericardial diseases<sup>[6,9]</sup>.

In 1998, Okuda *et al*<sup>[4]</sup> proposed that primary hepatic venous thrombosis (classical BCS) and thrombosis of the IVC at the level of the IVC were two separate syndromes. Recent studies continue to suggest a clear division within the definition of "primary BCS" based on the location of the obstructive lesion<sup>[4,10]</sup>. Obstruction of the hepatic veins or "classical BCS" appears to be more common in Western patient populations and usually has a known etiology<sup>[11,12]</sup>, acute onset of symptoms, and a greater severity of symptoms requiring a different therapeutic approach than obstruction of the IVC at the level of the hepatic veins<sup>[4,13,14]</sup>. In comparison with "classical BCS", hepatic vena cava (HVC)-BCS appears to be more common in East Asian patient populations, and is more often idiopathic or due to membranous obstruction. HVC-BCS more commonly presents with a chronic onset of less severe symptoms, thus requiring a different therapeutic approach than "classical BCS"<sup>[15]</sup>. The location, size, and chronicity is clinically important as it dictates the patient's symptoms and directs the therapeutic approach for patient management<sup>[10]</sup>.

## Precedence

Historically, hepatic sinusoidal obstruction syndrome (SOS) or veno-occlusive disease was included under the general term BCS<sup>[1,16-18]</sup>. SOS is specifically defined as obstruction of the sinusoids or hepatic veins resulting from sinusoidal wall injury. Several distinct clinical characteristics differentiate SOS from BCS and the two conditions are now considered separate entities as the distinct etiology and pathophysiology of SOS necessitates different management strategies. SOS is caused by pyrrolizidine alkaloid toxicity, whereas BCS is caused by multifactorial prothrombotic condition(s) or membranous obstruction of the IVC and/or HV<sup>[18]</sup>. Pyrrolizidine alkaloids include over 150 compounds that



occur naturally in several plant families<sup>[18]</sup>. Historically, they were ingested in indigenous herbal teas or inadvertently *via* crop contamination in developing countries. Currently, pyrrolizidine alkaloids are used as myeloablative regimens for patients preparing for hematopoietic stem cell transplantation. Thus, SOS almost exclusively affects hematopoietic stem cell transplant patients, while BCS can affect a wide range of patient populations<sup>[9]</sup>. Clinically, both SOS and BCS can present with abdominal pain, portal hypertension, jaundice, and non-cirrhotic ascites. Management of SOS is challenging and involves preventive measures (avoiding pyrrolizidine alkaloids in susceptible patients) and a few interventional therapeutic options (defibrotide, heparin, shunt procedures, *etc.*). In contrast, management of BCS ranges from medical management (*e.g.*, anticoagulation) to interventional procedures (angioplasty, stents, shunt procedures, *etc.*)<sup>[19]</sup>.

Due to the low incidence of “BCS” in many countries, published data tended to include only small case series. Recently, there have been an increasing number of larger observational studies (both retrospective and prospective), particularly from Asia (China) and Europe. Advancing imaging technologies, such as computed tomography (CT) angiography, magnetic resonance (MR) angiography, Doppler ultrasound (US), and angiography have allowed for better identification and delineation of this disease. This may signal the start of prospective, randomized, controlled therapeutic trials which can differentiate classical BCS from HVC-BCS and their management strategies. Other investigators have suggested various novel classification systems, including those that forego the eponym “Budd-Chiari” altogether<sup>[8-16]</sup>. However, given that both classical and HVC-BCS reflect an obstruction in hepatic venous outflow, we propose a clarification of the general BCS term into classical BCS and HVC-BCS.

## MATERIALS AND METHODS

A systematic literature search yielded 818 results in the PubMed database; 428 in the Scopus database; 18 in the CINAHL database; and 17 in the Cochrane database. All duplicates were removed. After 18 additional studies (from the references within included studies) were added, 1178 study abstracts were screened. Of these, 591 were excluded because of the publication type and/or subject (reviews, case reports including less than 20 patients, non-human studies, or studies not on BCS (*e.g.*, Chiari malformations, acute liver failure, *etc.*)). The full text articles of the remaining 587 studies were acquired to determine eligibility.

### Inclusion criteria

Clinical trials and observational studies (prospective or retrospective) conducted in predominantly adult subjects with primary BCS were included in this study. All of the included studies needed to explicitly delineate diagnostic methods for BCS (namely standard imaging

studies such as US, CT, MR imaging, or venography) and to explicitly describe inclusion and exclusion criteria to ensure the focus on primary BCS (vs secondary BCS). For multiple studies published from the same institution(s) within a close time frame, we reviewed years of subject recruitment, methodology specifics, and results. In addition, we also investigated if there were possible overlapping subjects and/or results. Only the most recent eligible studies were included in this review, unless distinctly specific and separate findings were previously reported<sup>[20,21]</sup>.

Of the 587 studies, the following were excluded: 390 were missing key clinical information (*e.g.*, clear inclusion and exclusion criteria) or focused on a subpopulation within the BCS population (*e.g.*, only BCS patients requiring liver transplantation, *etc.*); 71 studies were not limited to primary BCS; 86 studies were not mainly focused on BCS, but rather broader topics associated with BCS (*e.g.*, causes of liver transplantation, *etc.*); 17 studies were older versions of recently published subject populations with similar study aims. Twenty-six studies were included for analysis in this review (Figure 1).

## RESULTS

### Epidemiology

Many observational studies have recently been published on “BCS” (Table 1). For clarity and compromise, only the terms classical BCS and HVC-BCS will be used to differentiate between the two types of BCS in this review. After considering the location of the obstruction and clinical manifestations of the subjects, studies were grouped as majority-classical BCS or majority-HVC-BCS studies in Table 1. It has previously been suspected that classical BCS is more likely to present in women with a pure hepatic vein obstruction<sup>[9,13]</sup>. This review continues to support this observation as 13 of the 14 included studies reported a higher incidence of classical BCS in women; 55%-76% of the reported population is female. In addition, recent studies continue to report pure obstruction in the majority of cases 49%-85%. Most studies reported pure hepatic vein obstruction in > 71% of patients (Table 1). Compared with classical BCS, HVC-BCS is more common in men (51%-66%) and more likely to present with an IVC obstruction with or without involvement of the HVs (69%-100%) (Table 1).

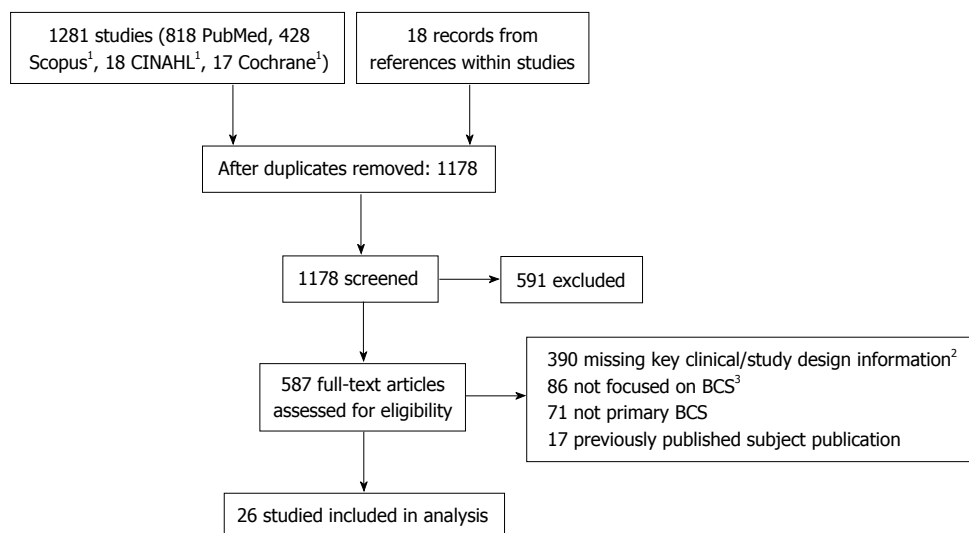
### Clinical manifestations in classical BCS vs HVC-BCS

Classical BCS typically presents with an acute onset of symptoms with most studies reporting the duration of symptoms < 6 mo (Table 2) with 60%-85% of patients having an acute presentation of symptoms; however, one study from Egypt designated 80% of their 94 patients as chronic, but the definitions of chronic vs acute were not explicitly delineated<sup>[22]</sup>. Classical BCS typically presents with abdominal pain (45%-86% of patients), ascites (76%-100%), and hepatomegaly (43%-83%) (Table 2). In comparison, HVC-BCS typically presents

**Table 1** Epidemiology of classical Budd-Chiari syndrome and hepatic vena cava-Budd Chiari syndrome

Ref.	Country	Publication date	Recruitment years	n	Age (median)	Gender		Location of obstruction (%)		
						M (%)	F (%)	HV	IVC	Both
Janssen <i>et al</i> <sup>[22]</sup>	The Netherlands	2000	1984-1997	43	40	16 (37)	27 (63)			
Perelló <i>et al</i> <sup>[40]</sup>	Spain	2002	1990-2000	21	36 <sup>1</sup>	5 (24)	16 (76)	17 (81)	0 (0)	4 (19)
Colaizzo <i>et al</i> <sup>[30]</sup>	Italy	2008	1997-2006	32	35	9 (28)	23 (72)			
Darwish Murad <i>et al</i> <sup>[24]</sup>	Europe	2009	2003-2005	163	38	70 (43)	93 (57)	80 (49)	4 (2)	79 (48)
Xavier <i>et al</i> <sup>[31]</sup>	Brazil	2010	2000-2008	31	33	11 (35)	20 (65)			
Sakr <i>et al</i> <sup>[22]</sup>	Egypt	2011	2009-2011	94	28.8 <sup>1</sup>	36 (38)	58 (62)	70 (74)	3 (3)	16 (17)
Deepak <i>et al</i> <sup>[29]</sup>	India	2011	2006-2009	20	36.6	14 (70)	6 (30)	17 (85)	1 (5)	2 (10)
Rautou <i>et al</i> <sup>[37]</sup>	France	2011	1995-2005	94	38 <sup>1</sup>	34 (36)	60 (64)	73 (78)		13 (14)
Raszeja-Wyszomirska <i>et al</i> <sup>[45]</sup>	Poland	2012	2004-2011	20	38	9 (45)	11 (55)			
Westbrook <i>et al</i> <sup>[32]</sup>	United Kingdom	2012	1985-2008	66	36	27 (41)	39 (59)			
D'Amico <i>et al</i> <sup>[34]</sup>	Italy	2013	2005-2011	31	46	14 (45)	17 (55)			
Harmanci <i>et al</i> <sup>[42]</sup>	Turkey	2013	1989-2011	62	42.8 <sup>1</sup>	26 (42)	36 (58)	35 (56)	8 (14)	19 (30)
Nozari <i>et al</i> <sup>[47]</sup>	Iran	2013	1989-2012	55	29 <sup>1</sup>	22 (40)	33 (60)			
Pavri <i>et al</i> <sup>[38]</sup>	United States	2014	2008-2013	47	42.4	16 (34)	31 (66)			
Faraoun <i>et al</i> <sup>[25]</sup>	Algeria	2015	2008-2012	176	33 <sup>1</sup>	75 (43)	101 (57)	125 (71)	0 (0)	51 (29)
De <i>et al</i> <sup>[23]</sup>	India	2001	1992-1998	40	35.2 <sup>1</sup>	26 (65)	14 (35)	N/A	23 (72)	9 (28)
Xu <i>et al</i> <sup>[41]</sup>	China	2004	1983-2003	1360	33.2 <sup>1</sup>	833 (61)	527 (39)	2 (0)	1358 (100) <sup>2</sup>	
Ebrahimi <i>et al</i> <sup>[46]</sup>	Iran	2011	2002-2008	21	42 <sup>1</sup>	11 (52)	10 (48)	6 (29)	12 (57)	3 (14)
Park <i>et al</i> <sup>[51]</sup>	South Korea	2012	1988-2008	67	47	34 (51)	33 (49)	5 (7)	56 (84)	6 (9)
Qi <i>et al</i> <sup>[35]</sup>	China	2013	1999-2011	169	38.3 <sup>1</sup>	66 (52)	61 (48)	53 (31)	20 (12)	96 (57)
Cheng <i>et al</i> <sup>[13]</sup>	China	2013	2010-2011	145	46	90 (6)	55 (38)	45 (31)	8 (6)	92 (63)
Qi <i>et al</i> <sup>[36]</sup>	China	2014	2012-2012	25	35.7 <sup>1</sup>	14 (56)	11 (44)	4 (16)	0 (0)	21 (84)
Zhou <i>et al</i> <sup>[26]</sup>	China	2014	2006-2010	338	41.7 <sup>1</sup>	209 (62)	129 (38)	45 (13)	8 (2)	285 (84)
Gao <i>et al</i> <sup>[49]</sup>	China	2015	2008-2012							
R				98	36 <sup>3</sup>	62 (63)	36 (37)	31 (32)	26 (27)	41 (42)
NR				373	45 <sup>3</sup>	193 (52)	180 (48)	82 (22)	169 (45)	122 (33)

<sup>1</sup>Mean values; <sup>2</sup>No differentiation between IVC alone *vs* both IVC and hepatic vein; <sup>3</sup>Provided median ages for the two groups separately. R: Recurrence of disease, NR: Non-recurrence of the disease; HV: Hepatic vein; IVC: Inferior vena cava; M: Male; F: Female; N/A: Not available.



**Figure 1** Flow diagram of studies selection. <sup>1</sup>Searches conducted with MEDLINE results removed; <sup>2</sup>Studies missing key clinical information including clear inclusion and exclusion criteria, clear diagnostic parameters, *etc.*, and studies that investigated subpopulations (*e.g.*, BCS patients requiring liver transplantation, BCS patients without MPN, *etc.*); <sup>3</sup>Studies focused on other categories (*e.g.*, causes of liver failure). BCS: Budd-Chiari syndrome; MPN: Myeloproliferative neoplasms.

with chronic onset of symptoms (75%-86% of patients), with an average duration of symptoms prior to diagnosis ranging from 44-96 mo. Nine to seventy percent of patients (most studies reporting < 29%) with HVC-BCS present with abdominal pain, 32%-90% with ascites, and 28%-95% with hepatomegaly. Splenomegaly, abdominal wall varices, lower extremity varices, and discoloration are more commonly associated with HVC-

BCS (Table 2)<sup>[13,23]</sup>. The severity of disease depends upon both the extent of disease (the number of occluded vessels, complete or incomplete occlusion), the presence of associated symptoms (refractory ascites, portal vein thrombosis, *etc.*), and the chronicity of symptoms. Patients with the chronic variation of disease generally have several milder episodes of vague symptoms (abdominal pain or leg swelling), providing sufficient time for

Table 2 Signs and symptoms in classical Budd-Chiari syndrome and hepatic vena cava-Budd Chiari syndrome

	Classical BCS						HVC-BCS								
	Perelló <i>et al</i> <sup>[40]</sup>	Darwish Murad <i>et al</i> <sup>[24]</sup>	Sakr <i>et al</i> <sup>[22]</sup>	Rautou <i>et al</i> <sup>[46]</sup>	Raszeja-Wyzomirska <i>et al</i> <sup>[45]</sup>	Westbrook <i>et al</i> <sup>[32]</sup>	D'Amico <i>et al</i> <sup>[34]</sup>	Harmanci <i>et al</i> <sup>[42]</sup>	Nozari <i>et al</i> <sup>[47]</sup>	De <i>et al</i> <sup>[23]</sup>	Xu <i>et al</i> <sup>[41]</sup>	Ebrahimi <i>et al</i> <sup>[46]</sup>	Qi <i>et al</i> <sup>[35]</sup>	Cheng <i>et al</i> <sup>[31]</sup>	Gao <i>et al</i> <sup>[49]</sup> R vs NR
Country	Spain	Europe	Egypt	France	Poland	United Kingdom	Italy	Sweden	Iran	India	China	Iran	China	China	China
<i>n</i> (%)	21	163	94	94	20	66	31	62	55	40	1360	21	169	145	98
Abdominal pain	18 (86)	99 (61)	78 (83)	73 (78)		36 (55)		28 (45)	33 (60)	28 (70)	122 (9)	5 (29)		30 (21)	
Ascites	18 (86)	135 (83)	80 (85)		20 (100)	57 (87)			42 (76)	30 (75)	914 (67)	19 (90)	95 (56)	77 (53)	76 (78)
Hepatomegaly	9 (43)	109 (67)	78 (83)						33 (60)	38 (95)	1124 (83)	8 (38)		40 (28)	61 (62)
Splenomegaly		85 (52)	48 (51)						19 (34)	26 (65)	683 (50)			113 (78)	165 (44)
Abdominal wall varices			39 (41)							38 (95)	821 (60)		50 (30)	73 (50)	
Esophageal varices		45 (58) <sup>1</sup>		53 (56)			18 (58)								
Lower extremity edema			46 (49)					28 (45)		28 (70)		14 (67)	86 (51)	76 (52)	
Jaundice	10 (48)		36 (38)						10 (18)	15 (38)	116 (9)				
Encephalopathy	1 (5)	15 (9)	29 (31)	7 (7)		32 (48)						12 (57)	1 (1)	31 (21)	
Bleeding episodes	1 (5)	8 (5)	15 (16)		5 (25)		7 (23)			6 (15)	162 (12)		25 (15)	96	
Duration of symptoms	1.4 <sup>2</sup>	< 1						1-6	6				44		
Chronic, > 6 mo		23 (14)	75 (80) <sup>3</sup>					25 (40)	21 (38)	30 (75)				125 (86)	
Acute, < 6 mo		138 (85)	18 (19)					37 (60)	34 (62)					20 (14)	

<sup>1</sup>77 patients underwent EGD; <sup>2</sup>Mean, not median. Most studies reported median duration of symptoms in months; when the median was not available, the mean is reported; <sup>3</sup>No definition of "chronic" was provided. BCS: Budd-Chiari syndrome; HVC: Hepatic vena cava; R: Recurrence of BCS; NR: Non-recurrence of BCS.

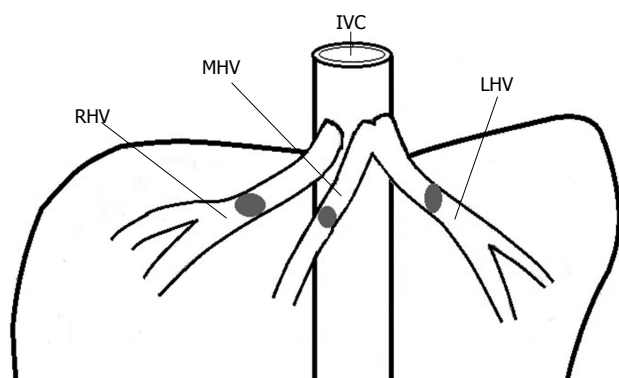
the development of collateral vessels<sup>[13,24]</sup>. In contrast, acute onset and/or significant obstruction (e.g., complete occlusion of several hepatic veins) increases the risk of acute hepatic failure.

### Obstruction characteristics

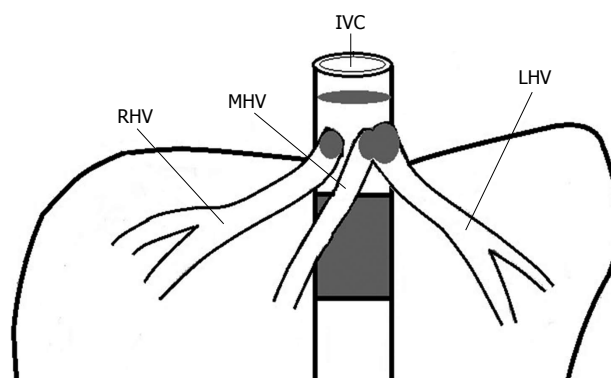
Patients with classical BCS typically have an obstructing thrombus within the hepatic veins (Figure 2)<sup>[5,24]</sup>. In contrast, patients with HVC-BCS typically have a membranous or segmental obstruction involving the IVC<sup>[24]</sup> (proximal to the ostia of the hepatic veins), but the obstruction can extend into, or secondarily involve the hepatic veins themselves (Figure 3). Observational studies continue to reflect this difference between classical BCS and HVC-BCS patients; several studies from Europe to northern Africa consistently describe a thrombotic obstruction (87%-95%) limited to the hepatic veins (49%-85%) and rarely describe a membranous obstruction (1%-5%) located at only the IVC (0%-14%). However, in HVC-BCS patients, many studies report a membranous obstruction (30%-61%) only located at the IVC (57%-72%) or both the IVC and HV (63%-84%). Obstructions in HVC-BCS patients are not commonly isolated in the hepatic veins (0%-31%)<sup>[24-26]</sup>. The development of collateral circulation takes time; given that the chronic form of BCS is more commonly associated with HVC-BCS, it is not surprising that the development of collateral circulation is more typical with HVC-BCS patients (63%-65%) than with classical BCS patients (Table 3).

### Etiology

Uncovering the etiology of BCS can be challenging. In classical BCS, however, thrombotic risk factors are consistently identified in the majority of patients<sup>[1]</sup>. Findings reported in recent studies continue to report myeloproliferative neoplasms [MPN, previously called myeloproliferative disorders (MPD)] as the most common etiology of classical BCS; 9 out of 14 studies found that it is the most common cause of classical BCS affecting 16%-62% of patients, with many reporting between 41%-62% (Table 4). The most commonly observed MPNs include polycythemia vera (PV) and essential thrombocythemia (ET) found in 18%-43% and 6%-14% of classical BCS patients, respectively. The JAK2V617-F mutation is a sensitive marker for MPN and has been observed in 26%-52% of patients with classical BCS<sup>[27-34]</sup>. In contrast, in several large Chinese studies,



**Figure 2** Classical Budd-Chiari syndrome - Occlusions are within the hepatic veins themselves and usually thrombi. RHV: Right hepatic vein; MHV: Middle hepatic vein; LHV: Left hepatic vein; IVC: Inferior vena cava.



**Figure 3** Hepatic vena cava-Budd Chiari syndrome - Occlusions are thin or thick (membranous or segmental) and within the inferior vena cava and occlusion can extend into the hepatic veins and generally involve the ostia to the inferior vena cava. RHV: Right hepatic vein; MHV: Middle hepatic vein; LHV: Left hepatic vein; IVC: Inferior vena cava.

MPN were only found in 4%-5% of patients (PV in 2% and ET in 1%-2%) and the JAK2V617-F mutation in only 0%-5% of patients diagnosed with primary HVC-BCS (Table 4)<sup>[13,35,36]</sup>.

Hereditary prothrombotic conditions such as factor V Leiden mutation (FVL), prothrombin (PT) 20210A mutation, protein C deficiency (PCD), protein S deficiency (PSD), antithrombin deficiency (ATD), plasminogen activator inhibitor [PAI-1 (4G-4G)], and the 5,10-methylenetetrahydrofolate reductase enzyme mutation (MTHFR C677T) often also play a significant role in the development of classical BCS. Following MPNs, the FVL mutation is the second most common cause of classical BCS and was found in 2%-53% of patients. Thrombophilic conditions also may contribute to the development of classical BCS. Mutations in PT were found in 2%-8% of patients with classical BCS vs 0% of patients with HVC-BCS. PCD, PSD, and ATD were found in 3%-26%, 1%-9% and 3%-15% of patients with classical BCS, respectively, vs 0% of patients with HVC-BCS (Table 4). Interestingly, this pattern is not apparent with MTHFR C677T mutations; these mutations were found in 26%-52% of patients with classical BCS and 71%-72% of patients with HVC-BCS. Less common but established prothrombotic or associated conditions further include antiphospholipid antibodies (classical BCS: 3%-29% vs HVC-BCS: 0%-17%), hyperhomocysteinemia (10%-18% vs 21%-50%), and paroxysmal nocturnal hemoglobinuria (0%-19% vs 0%-4%). Several systemic conditions (classical BCS: 5%-24% vs HVC-BCS: 1%-19%) including connective tissue disorders such as systemic lupus erythematosus (5%-12% vs 1%) are generally associated more frequently with classical BCS. Hormonal factors such as oral contraceptives, pregnancy, or puerperium can also increase the risk of thrombosis as can local insults such as recent surgery. Of these numerous differences between classical BCS and HVC-BCS, one consistent difference is the greater influence of hormonal changes (be it oral contraceptive use or pregnancy) in classical BCS patients (4%-52% of the female population) (Table 4)<sup>[29,37-39]</sup>.

Membranous obstruction of the IVC (and/or HV) is consistently listed as the etiology of a significant number of HVC-BCS patients (52%-61%). In classical BCS patients, membranous obstruction is rare (1%) or rarely explicitly delineated, except in one study of 23 consecutive patients diagnosed with BCS in Germany where 5 patients (22%) were found to have a membranous obstruction of the IVC<sup>[24]</sup>. Furthermore, despite comprehensive work-up, an etiologic factor is often not identified in HVC-BCS patients (19%-29% vs classical BCS: 5%-30%) (Table 4).

Data from recent studies continues to support the possibility of two different types of BCS with separate etiologies: Classical BCS, where thrombophilic risk factors and often multiple concomitant factors are common vs HVC-BCS, where thrombophilic risk factors are uncommon, but membranous obstruction and idiopathic hepatic venous outflow obstruction are more common.

### Management and outcomes

Treatment and prognosis of BCS depends on a few key factors: Acuity of symptoms, location and extent of the obstruction, and etiology<sup>[24]</sup>. In 2013, Seijo *et al.*<sup>[11]</sup> outlined a step-wise management approach for BCS patients from the analysis of the extended follow-up data of 157 patients from 9 European countries. This management approach starts with medical management alone (*e.g.*, salt-restriction, anticoagulation, diuretics), including concomitant management of any underlying etiological processes. Diagnostic work-up for classical BCS patients generally includes hematologic work-up for MPN, JAK2V617F mutation screening for MPN<sup>[29-31,33]</sup>, testing for FVL mutation<sup>[28]</sup>, and the aforementioned thrombophilic risk factors. In addition, some studies recommend continued monitoring of JAK2-mutation-positive-patients for occult MPNs<sup>[5,9,33]</sup>. In general, the medical management of classical BCS patients involves anticoagulation and ascites management with diuretics. Patients with MPN require additional aspirin and cyto-



Table 3 Obstruction characteristics: Location, type, and associated findings

	Classical BCS						HVC-BCS				
	Perelló <i>et al.</i> <sup>[40]</sup>	Darwish <i>Murad et al.</i> <sup>[24]</sup>	Sakr <i>et al.</i> <sup>[22]</sup>	Deepak <i>et al.</i> <sup>[29]</sup>	Harmanci <i>et al.</i> <sup>[42]</sup>	Faraoun <i>et al.</i> <sup>[25]</sup>	De <i>et al.</i> <sup>[23]</sup>	Xu <i>et al.</i> <sup>[41]</sup>	Ebrahimi <i>et al.</i> <sup>[45]</sup>	Cheng <i>et al.</i> <sup>[13]</sup>	Zhou <i>et al.</i> <sup>[26]</sup>
Country	Spain	Europe	Egypt	India	Turkey	Algeria	India	China	Iran	China	China
<i>n</i> (%)	21	163	94	20	62	176	40	1360	21	145	338
Obstruction location											
HV only	17 (81)	80 (49)	70 (74)	17 (85)	35 (56)	125 (71)	N/A	2 (0)	6 (29)	45 (31)	45 (13)
IVC only	0 (0)	4 (2)	3 (3)	1 (5)	8 (14)	0 (0)	23 (72)	1358 (100)	12 (57)	8 (6)	8 (2)
Both HV and IVC	4 (19)	79 (48)	16 (17)	2 (10)	19 (30)	51 (29)	9 (28)		3 (14)	92 (63)	285 (84)
HV thrombosis	20 (95)				54 (87)	170 (97)		DNS		15 (10)	
IVC thrombosis	3 (14)	DNS	DNS	DNS	27 (44)	DNS	12 (30)	123 (9)	11 (52)	89 (61)	
IVC web/membrane	1 (5)	DNS	2 (1)	DNS	DNS	DNS		717 (53)		92 (63)	220 (65)
Collateral circulation										36 (25) <sup>1</sup>	79 (23)
Benign regenerative nodules											

<sup>1</sup>Described as "benign nodules", not benign regenerative nodules. DNS: Study mentions generally, but, does not provide specific counts; BCS: Budd-Chiari syndrome; HV: Hepatic vein; HVC: Hepatic vena cava; IVC: Inferior vena cava.

reductive medications (e.g., hydroxyurea). Patients with autoimmune diseases (e.g., antiphospholipid syndrome, Behçet's disease, etc.) require additional corticosteroids and/or immunosuppressive drugs. If patients fail medical management, therapy is then escalated to minimally invasive procedures including percutaneous transluminal angioplasty (PTA) and/or thrombolysis. If patients fail to respond to these measures, developing refractory ascites, variceal bleeding, or liver failure, they are then treated with transjugular intrahepatic portosystemic shunt (TIPS) or other shunt operations<sup>[11,40,41]</sup>, with liver transplantation as a final option<sup>[32]</sup>. Such an approach appears to result in good long-term survival (Table 5)<sup>[11]</sup>.

Recent studies continue to support that medical management alone can be appropriate for classical BCS patients; 33%-54% of the classical BCS patients treated with medical management alone have good outcomes. In contrast, only 0%-7% of HVC-BCS patients are treated with medical management alone. While both classical and HVC-BCS patients benefit from interventional therapy, the specific interventions are different. Classical BCS patients commonly undergo TIPS (classical BCS: 4%-62% vs HVC-BCS: 1%-4.5%) and liver transplantation (9%-55% vs 0%-1%). In a study of 62 predominantly classical BCS patients from Turkey, none of the patients underwent liver transplantations, but that was due to a lack of donor availability<sup>[42]</sup>. In contrast, first line management of HVC-BCS with percutaneous re-canalization (with or without stent deployment) has good outcomes<sup>[43]</sup>. In one large study from China, Han *et al.*<sup>[44]</sup> found that all 187 consecutively diagnosed primary BCS patients at one institution were eligible for percutaneous recanalization, regardless of the location of the obstruction. Recent studies report that HVC-BCS patients undergo PTA more frequently compared to classical BCS patients (HVC-BCS: 43%-92% vs classical BCS: 3%-18%). After percutaneous recanalization, patients are anticoagulated with an international normalized ratio goal of 2-3 for a minimum of 6-8 mo per standard post-endovascular intervention management guidelines<sup>[44]</sup>.

Median follow-up for both classical BCS and HVC-BCS patients were similar (classical BCS: 17-58 mo and HVC-BCS: 12-103 mo). Both groups of patients fared well with their respective management strategies. One-year and five-year survival was 79%-96% and 56%-79% among classical BCS patients, respectively. One-year and five-year survival for HVC-BCS patients was 67%-99% and 75%-86%, respectively (Table 5). Poor prognostic factors for classical BCS patients include: Severe BCS (e.g., ascites requiring diuretics or paracentesis, pleural effusion, higher Clichey Prognostic index score), older age, cirrhosis at diagnosis of BCS, and chronic kidney disease<sup>[37,38]</sup>. Development of cirrhosis or hepatocellular carcinoma (HCC) are poor prognostic factors for HVC-BCS patients<sup>[23]</sup>.

## DISCUSSION

This systematic literature review highlights the numerous differences between classical BCS and HVC-BCS. Despite the growing cognizance of this difference<sup>[4,5]</sup> and despite

Table 4 Risk factors and/or etiologies of classical Budd-Chiari syndrome and hepatic vena cava-Budd Chiari syndrome

	Classical BCS										HVC-BCS							
	Perelló <i>et al.</i> <sup>140</sup>	Smalberg <i>et al.</i> <sup>139</sup>	Colaizzo <i>et al.</i> <sup>101</sup>	Xavier <i>et al.</i> <sup>131</sup>	Sakr <i>et al.</i> <sup>122</sup>	Deepak <i>et al.</i> <sup>129</sup>	Rautou <i>et al.</i> <sup>137</sup>	Raszeja-Wyszomirska <i>et al.</i> <sup>145</sup>	Westbrook <i>et al.</i> <sup>132</sup>	D'Amico <i>et al.</i> <sup>134</sup>	Seijo <i>et al.</i> <sup>111</sup>	Harmanci <i>et al.</i> <sup>142</sup>	Nozari <i>et al.</i> <sup>147</sup>	Pavri <i>et al.</i> <sup>138</sup>	Ebrahimi <i>et al.</i> <sup>146</sup>	Qi <i>et al.</i> <sup>135</sup>	Cheng <i>et al.</i> <sup>133</sup>	Qi <i>et al.</i> <sup>136</sup>
Country	Spain	Netherlands	Italy	Brazil	Egypt	India	France	Poland	United Kingdom	Italy	Europe	Turkey	Iran	United States	Iran	China	China	China
<i>n</i> (%)	21	40	32	31	94	20	94	20	66	31	157	62	55	47	21	169	145	25
MPN (%)	13 (62)	13 (33)	13 (41)	5 (16)	8 (40)	8 (40)	51 (59)	8 (40)	37 (56)	17 (55)	52 (33)	19 (31)	9 (16)			7 (4) <sup>9</sup>	5 (5) <sup>10</sup>	
JAK2V617-F	N/A	7 (41)		8 (26)	18 (29) <sup>4</sup>	8 (40)			34 (52)							4 (2)	5 (5) <sup>10</sup>	0 (0)
PV	9 (43)										28 (18)			14 (30)		3 (2)	2 (2) <sup>10</sup>	
ET	3 (14)										12 (8)			3 (6)		1 (1)	2 (2) <sup>10</sup>	
FVL	2 (10)	5 (15)	6 (19)	3 (10)	34 (53) <sup>5</sup>	5 (25)	15 (19)	1 (5)	1 (2)	9 (29)	19 (12)	15 (30)	10 (18)	4 (9)		0 (0)	0 (0) <sup>11</sup>	0 (0)
PT 20210A		2 (8)	1 (3)	1 (3)	3 (5) <sup>6</sup>		6 (8)		1 (2)	1 (3)	5 (3)	1 (2)				0 (0)	0 (0) <sup>11</sup>	0 (0)
Protein C deficiency		2 (7) <sup>2</sup>			4 (4)	2 (10)	6 (12)	3 (15)	2 (3)		5 (3)	16 (31)	12 (20)	2 (4)		0 (0)	0 (0) <sup>12</sup>	
Protein S deficiency		2 (7) <sup>2</sup>			1 (1)	1 (5)	5 (9)				3 (2)	5 (10)	3 (6)	1 (2)		0 (0)	0 (0) <sup>12</sup>	
AT deficiency					4 (4)	3 (15)	3 (4)			17 (55)	4 (3)	6 (15)	3 (6)			0 (0)	0 (0) <sup>12</sup>	
PAI-1 (4G-4G)										8 (26)		19 (39)				96 (71)		18 (72)
MTHFR C677T					31 (52) <sup>6</sup>						29 (18)					64 (50)	30 (21)	
HH					2 (10)						15 (10)	1 (2)		3 (6)		1 (1)	0 (0) <sup>13</sup>	1 (4)
PNH	4 (19)	2 (9)	1 (3)		2 (2)	1 (5)	8 (12)	1 (5)	0 (0)		35 (39)	4 (11)	3 (9)	2 (6)		2 (4)		
OCP, pregnancy, or puerperium <sup>1</sup>		13 (52) (OC) <sup>3</sup>	1 (4) (OC) <sup>3</sup>	7 (35)	19 (33)		21 (35) (OC) <sup>3</sup>			4 (24)				(OC) <sup>3</sup>	4 (19) <sup>8</sup>	2 (1)	1 (1)	
Systemic diseases		2 (5)			12 (13) <sup>7</sup>				3 (5) <sup>7</sup>		37 (24)	8 (13) <sup>7</sup>	5 (9)					
or local factors												autoimmune						
NAD/idiopathic	1 (5)				8 (9)			6 (30)		2 (6)		6 (10)	10 (18)		6 (29)		28 (19)	
Web/membrane															11 (52)		89 (61)	
MOV C																	6 (4)	
MOV C + HV	1 (5)																60 (41)	
MOHV																	23 (16)	

<sup>1</sup>Percentage of total female patients; <sup>2</sup>Patients on anticoagulation were not tested; <sup>3</sup>Number of women on OC only, no specific information on number of pregnant women; <sup>4</sup>Out of 62 tested; <sup>5</sup>Out of 64 tested; <sup>6</sup>Out of 60 tested; <sup>7</sup>Mainly Behcet's disease; <sup>8</sup>Behcet's disease (2), Hepatitis C (1), Leukemia (1); <sup>9</sup>2 of these 7 patients were diagnosed with latent MPN; <sup>10</sup>In this study, most (105 patients), but, not all were tested; percentages are out of 105; <sup>11</sup>Out of 96 patients were tested; <sup>12</sup>Out of 80 patients tested; <sup>13</sup>Out of 83 patients tested. BCS: Budd-Chiari syndrome; HV: Hepatic vein; HVC: Hepatic vena cava; ET: Essential thrombocythemia; PNH: Paroxysmal nocturnal hemoglobinuria; OCP: Oral contraception; MOV C: Membranous obstruction of IVC; NAD: No associated disease/etiology found; MPN: Myeloproliferative neoplasms; FVL: Factor V Leiden mutation; HH: Hyperhomocysteinemia.

the abundance of recent publications on BCS, there is still a paucity of large, randomized clinical trials with regard to classical and/or HVC-BCS. The vast heterogeneity of these recent publications with regards to recruitment of solely primary vs secondary BCS, of solely classical or HVC-BCS, and of patients who have not previously been recruited and described, may lead to disparate and skewed patient populations that preclude certain data analyses and conclusions. Thus, this review specifically sought out studies that recruited BCS subjects that were representative of the indigenous BCS patient population. We attempted to minimize selection bias by excluding studies that focused on a particular subgroup of patients (e.g., BCS patients requiring liver transplantation, exclusion of patients with specific previously diagnosed etiologies, etc.). We also attempted to minimize multiple representations of the same patient population by comparing recruitment periods from studies that were similar in geographic location (institution, city, and country) or similar in authorship. The selected studies thereby provide an unadulterated presentation of the differences between classical vs HVC-BCS according to geography, patient demographics, location of obstruction, and treatment strategies and outcomes. The examination of these differences is important as they impact the diagnostic work-up and the therapeutic management strategies for individual patients and healthcare communities alike.

Table 5 Management and outcomes in classical Budd-Chiari syndrome and hepatic vena cava-Budd Chiari syndrome

	Classical BCS						HVC-BCS						
	Perelló <i>et al</i> <sup>[40]</sup> <i>et al</i> <sup>[37]</sup>	Raszeja-Wyszomirska <i>et al</i> <sup>[45]</sup>	Westbrook <i>et al</i> <sup>[32]</sup>	Harmandi <i>et al</i> <sup>[42]</sup>	Seijo <i>et al</i> <sup>[11]</sup>	Nozari <i>et al</i> <sup>[47]</sup>	Pavri <i>et al</i> <sup>[38]</sup>	De <i>et al</i> <sup>[23]</sup>	Xu <i>et al</i> <sup>[41]</sup>	Ebrahimi <i>et al</i> <sup>[46]</sup>	Park <i>et al</i> <sup>[51]</sup>	Cheng <i>et al</i> <sup>[13]</sup>	Gao <i>et al</i> <sup>[49]</sup>
Country	Spain	Poland	United Kingdom	Turkey	Europe	Iran	United States	India	China	Iran	South Korea	China	China
<i>n</i> (%)	21	20	66	62	157	55	47	40	1360	21	67	145	471
Medical management	21 (100)	20 (100)	61 (92)	61 (98)	139 (89)	55 (100)	≥ 40 (85)			12 (57)	32 (48)		
Medical management only (%) <sup>1</sup>	7 (33)				69 (54)				0 (0)			4 (3)	31 (7)
Interventional therapy			34 (52) <sup>5</sup>		49 (71) alive	10 (18)		23 (58)	1360 (100)	9 (43)		141 (97)	440 (93)
PTA	1 (5)			2 (3) in IVC	72 (82) alive			23 (58)	1318 alive				
Shunt operation			32 (48.5) <sup>6</sup>		22 (14)	2 (4)				9 (43)	27 (40)	134 (92)	
TIPS <sup>2</sup>	2 (10)			4 (6)	62 (39) <sup>3</sup>	2 (4)	21 (45)		330 (24)	3 (14)	4 (5.9)	1 <sup>2</sup>	
Liver transplantation	13 (62)	2 (10)	36 (55)	0 (0) <sup>8</sup>	20 <sup>4</sup> (13)	5 (9)	8 (17)		0 (0)			0 (0) <sup>12</sup>	2 (1) <sup>12</sup>
	15 (16)	10 (50)										2 (1) <sup>12</sup>	
Median follow-up (in months)	58 <sup>13</sup>	17	40-73 <sup>9</sup>	25.2 <sup>12</sup>	50	65 mo <sup>10</sup>	32	56	81.6		103	12	19
Survival	7 (100)	15 (75)		96%	95 (73)		37 (79)			67% <sup>7</sup>		99%	401 (94)
At 1 yr		80%	88%				37 (79)	75 %			86%		
At 5 yr			56%		95 (73)			10 <sup>11</sup>				2 (1)	
Mortality													

<sup>1</sup>Medical management includes anticoagulation, diuretics, and medical treatment of any underlying causes; <sup>2</sup>After PTA failed; <sup>3</sup>Of the 22 patients who initially were treated with PTA/thrombolitics, 12 subsequently underwent TIPS and 2 underwent OLT; <sup>4</sup>Among the 62 patients who underwent TIPS, 4 subsequently underwent OLT; <sup>5</sup>Patients who failed medical management (namely anticoagulation with heparin and warfarin and diuretics) as defined by persistent transaminitis, resistant ascites or worsening hepatic function) moved on to receive stenting, shunting, or TIPS; <sup>6</sup>50% success rate (16/32); <sup>7</sup>Per study, 7 out of 21 patients died before hospital discharge; <sup>8</sup>No patients underwent liver transplantations due to a lack of donor availability; <sup>9</sup>Median follow-up post liver transplantation was 40 mo and median follow-up of patients with MPN was 73 mo; <sup>10</sup>Mean survival time was 65 mo; <sup>11</sup>10 patients died by the 5-yr follow-up period; <sup>12</sup>At the end of the follow-up period, 2 patients were waiting to receive OLT from another hospital; <sup>13</sup>Mean, not median values provided. Interventional therapy includes both endovascular and surgical procedures. OLT: Orthotopic liver transplant; TIPS: Transjugular intrahepatic portosystemic shunt; PTA: Percutaneous transluminal angioplasty; BCS: Budd-Chiari syndrome; HVC: Hepatic vena cava; IVC: Inferior vena cava.

Differentiation between classical and HVC-BCS is important because it dictates what constitutes comprehensive and appropriate diagnostic strategies. Given the likelihood of multiple pro-thrombotic risk factors contributing to the development of classical BCS, recommendations for extensive routine work-up for multiple possible etiologies include testing for MPN; JAK2V617F mutation screening for MPN<sup>[42,45]</sup>; continued monitoring of JAK2-mutation-positive-patients for occult MPN<sup>[5,9,24,31]</sup>; further testing for TET2 mutation when the JAK2 screening is negative<sup>[32]</sup>; FVL mutation<sup>[28]</sup>; PT 20210A mutation; protein C and S deficiencies; AT deficiencies; PAI-1 and MTHFR C677T mutations<sup>[24]</sup>. Since more than one thrombophilic condition often manifests in classical BCS patients, such an extensive work-up is appropriate, but recent data continues to suggest that those same recommendations may not be appropriate for HVC-BCS patients<sup>[24]</sup>. For instance, screening for the JAK2V617F mutation is important for classical BCS patients because it has been reported to be a better diagnostic test for MPN when compared to traditional hematologic tests<sup>[42]</sup>. The JAK2V617F mutation has consistently been found in a significant number of "idiopathic" cases of classical BCS (although this is not observed in "idiopathic" HVC-BCS). In a study of 41 classical BCS patients from England, the JAK2V617F mutation was detected in 58.5% of idiopathic BCS cases<sup>[32]</sup>. Furthermore, 93% of the patients who later developed latent MPN were positive for the mutation suggesting that the JAK2V617F mutation is a highly sensitive marker to detect overt or covert MPN<sup>[33]</sup>. Given the possible geographic distribution of classical and HVC-BCS in regions with limited healthcare resources, a clear delineation of standard of care would benefit patients and providers alike. Historically, it has been speculated that there is association between lower standards of living and HVC-BCS. However, a recent prospective study including 53 consecutive BCS patients from Western India found no association between socioeconomic status and location of hepatic venous outflow obstruction although, a correlation between living in mud houses and IVC membranous obstruction was observed<sup>[28]</sup>. Therefore, according to this review, balancing the costs of diagnostic work-up for numerous potential genetic or acquired pro-thrombotic factors

with the actual benefit the patient may gain should be BCS-type specific.

Treatment and prognosis of BCS depends on a few key factors: Acuity and severity of the symptoms, location and the extent of the obstruction, and etiology of the obstruction<sup>[24]</sup>. While anticoagulation (initially with heparin and chronically with warfarin) is the initial treatment of choice for both classical BCS and HVC-BCS patients<sup>[45]</sup>, the expected response and course of therapy differs dramatically. Classical BCS patients often present with acute thrombosis of the hepatic veins. This rapid blockage of hepatic venous outflow precludes the ability to adapt *via* the development of collateral circulation. It is not surprising then that acute fulminant liver failure (with its sequelae) is more common among classical BCS patients, thus requiring shunt operations and liver transplantations more frequently than in HVC-BCS patients<sup>[13]</sup>. In contrast, HVC-BCS patients generally present with chronic symptoms that may lead to the transformation of an old thrombus into a fibrous, membranous obstruction<sup>[7,25]</sup>. Depending on the thickness and the extent of the obstruction, early interventional therapy (most commonly PTA with or without stent deployment), is very effective and thus more commonly utilized among HVC-BCS patients<sup>[13,46]</sup>. The thrombotic nature of obstruction observed in classical BCS may explain why these obstructions are more susceptible and responsive to medical management (namely anticoagulation) alone. As noted in two long-term follow up studies by Perelló *et al.*<sup>[40]</sup> and Darwish Murad *et al.*<sup>[24]</sup> (with median follow-ups of 58 and 17 mo respectively) of predominantly classical BCS patients, 33%-44% of patients that were maintained on medical management alone had good outcomes: 100% and 44% (at 12 mo), respectively. In both studies, very few classical BCS patients (5%-9%) required percutaneous recanalization. In HVC-BCS patients, the role of anticoagulation is often adjunctive and temporary; the use of warfarin before angioplasty can improve outcomes in patients with IVC obstruction<sup>[47]</sup>. Few HVC-BCS patients are managed with medical management alone because of previously reported poor outcomes<sup>[38]</sup>. In terms of the pathophysiology, Simonetto *et al.*<sup>[48]</sup> recently used a murine model to demonstrate that hepatic venous outflow obstruction as seen in congestive heart failure or veno-occlusive disease led to liver fibrosis not *via* an inflammatory pathway, but *via* sinusoidal thrombosis and mechanical strain, while also showing that anticoagulation may have a beneficial effect in decreasing fibrosis. This aids our understanding of the mechanism by which BCS and HVC-BCS can result in fibrosis, and emphasizes the need for relief of obstruction for proper management. Given the different presentations and treatment courses of the two entities, it would be relevant to further study the pathophysiology of these conditions to better optimize management.

Factors that contribute poor prognosis in classical BCS include: Increasing age, cirrhosis at the time of diagnosis, chronic kidney disease, and portal vein

thrombosis<sup>[25,38]</sup>. The Child-Pugh and MELD scores also play an unclear role in terms of practical management, while asymptomatic patients generally have better prognoses<sup>[45,49]</sup>. For HVC-BCS patients, factors that contribute to poor prognosis include the development of cirrhosis and HCC. Recent studies have suggested that the risk of developing HCC in HVC-BCS patients (unlike classical BCS patients) is directly attributable to the disease vs Hepatitis B or C infections<sup>[7]</sup>. Furthermore, the incidence of developing HCC in HVC-BCS patients is similar to those of cirrhotic patients<sup>[50,51]</sup>. These findings suggest that HVC-BCS patients, unlike classic BCS patients, should be routinely monitored for the development of HCC. Specific interventions to address and reduce the high pressure gradient in BCS patients may reduce the risk of HCC development.

In conclusion, clarification in the terminology describing hepatic venous outflow obstruction would enable both clinicians and investigators to identify patients with comparable signs and symptoms, thus enabling the execution of sound (randomized and controlled) and separate research studies on pathogenesis, therapy, and prognosis of what seems to be two different etiologies of Budd-Chiari syndrome. As summarized in this review, recent studies continue to support that classical and HVC-BCS have distinct demographics, characteristics, etiologies, therapeutic strategies, and prognoses. To address gaps in knowledge within classical BCS and HVC-BCS patients, these differences should be acknowledged and future research should be performed on these two conditions separately.

## COMMENTS

### Background

Budd-Chiari syndrome (BCS) encompasses a wide array of symptoms that are caused by hepatic venous outflow tract obstruction and has been known by many different names. While reviewing the recent literature, this paper delineates the difference between primary hepatic venous thrombosis and thrombosis of the inferior vena cava (IVC), which have both previously been referred to as BCS.

### Research frontiers

With the influx of new studies examining the wide spectrum of BCS, there has been a growing argument for the separation of primary hepatic venous thrombosis (classical BCS) and thrombosis of the IVC at the level of the IVC (hepatic vena cava-BCS) given the difference in their etiology and management.

### Innovations and breakthroughs

This paper supports the clarification of terminology used to describe hepatic venous outflow obstruction, which will help guide future research and allow for more specific treatment modalities for this condition.

### Applications

The evidence presented helps clinicians to understand the difference in etiologies of this syndrome and their influence in the management of the separate entities of this condition.

### Terminology

Classical BCS refers to primary hepatic venous thrombosis. HVC-BCS refers to thrombosis of the IVC at the level of the IVC.



# Peer-review

This review is well organized and comprehensive, has a good clinical message about BCS, and should be of great interest to the readers.

## REFERENCES

- Valla DC. Vascular disorders of the liver. *Acta Gastroenterol Belg* 2003; **66**: 294-297 [PMID: 14989053]
- Budd G. On diseases of the liver. Philadelphia: Lea and Blanchard, 1846
- Budd G. On diseases of the liver. Philadelphia: Blanchard and Lea, 1857
- Okuda K, Kage M, Shrestha SM. Proposal of a new nomenclature for Budd-Chiari syndrome: hepatic vein thrombosis versus thrombosis of the inferior vena cava at its hepatic portion. *Hepatology* 1998; **28**: 1191-1198 [PMID: 9794901 DOI: 10.1002/hep.510280505]
- Valla DC. The diagnosis and management of the Budd-Chiari syndrome: consensus and controversies. *Hepatology* 2003; **38**: 793-803 [PMID: 14512865 DOI: 10.1002/hep.1840380404]
- Janssen HL, Garcia-Pagan JC, Elias E, Mentha G, Hadengue A, Valla DC. Budd-Chiari syndrome: a review by an expert panel. *J Hepatol* 2003; **38**: 364-371 [PMID: 12586305 DOI: 10.1016/S0168-8278(02)00434-8]
- Shrestha SM. Liver cirrhosis and hepatocellular carcinoma in hepatic vena cava disease, a liver disease caused by obstruction of inferior vena cava. *Hepatol Int* 2009; **3**: 392-402 [PMID: 19669366 DOI: 10.1007/s12072-009-9122-5]
- Okuda K. Membranous obstruction of the inferior vena cava (obliterative hepatocavopathy, Okuda). *J Gastroenterol Hepatol* 2001; **16**: 1179-1183 [PMID: 11903732 DOI: 10.1046/j.1440-1746.2001.02577.x]
- DeLeve LD, Valla DC, Garcia-Tsao G. Vascular disorders of the liver. *Hepatology* 2009; **49**: 1729-1764 [PMID: 19399912 DOI: 10.1002/hep.22772]
- Valla DC. Hepatic venous outflow tract obstruction etiopathogenesis: Asia versus the West. *J Gastroenterol Hepatol* 2004; **19**: S204-S211 [DOI: 10.1111/j.1440-1746.2004.03642.x]
- Seijo S, Plessier A, Hoekstra J, Dell'era A, Mandair D, Rifai K, Trebicka J, Morard I, Lasser L, Abiralde JG, Darwish Murad S, Heller J, Hadengue A, Primignani M, Elias E, Janssen HL, Valla DC, Garcia-Pagan JC. Good long-term outcome of Budd-Chiari syndrome with a step-wise management. *Hepatology* 2013; **57**: 1962-1968 [PMID: 23389867 DOI: 10.1002/hep.26306]
- Cazals-Hatem D, Vilgrain V, Genin P, Denninger MH, Durand F, Belghiti J, Valla D, Degott C. Arterial and portal circulation and parenchymal changes in Budd-Chiari syndrome: a study in 17 explanted livers. *Hepatology* 2003; **37**: 510-519 [PMID: 12601347 DOI: 10.1053/jhep.2003.50076]
- Cheng D, Xu H, Lu ZJ, Hua R, Qiu H, Du H, Xu X, Zhang J. Clinical features and etiology of Budd-Chiari syndrome in Chinese patients: a single-center study. *J Gastroenterol Hepatol* 2013; **28**: 1061-1067 [PMID: 23425079 DOI: 10.1111/jgh.12140]
- Denninger MH, Chaït Y, Casadevall N, Hillaire S, Guillin MC, Bezeaud A, Erlinger S, Briere J, Valla D. Cause of portal or hepatic venous thrombosis in adults: the role of multiple concurrent factors. *Hepatology* 2000; **31**: 587-591 [PMID: 10706547 DOI: 10.1002/hep.510310307]
- Qi X, Zhang C, Han G, Zhang W, He C, Yin Z, Liu Z, Bai W, Li R, Bai M, Yang Z, Wu K, Fan D. Prevalence of the JAK2V617F mutation in Chinese patients with Budd-Chiari syndrome and portal vein thrombosis: a prospective study. *J Gastroenterol Hepatol* 2012; **27**: 1036-1043 [PMID: 22142461 DOI: 10.1111/j.1440-1746.2011.07040.x]
- Dilawari JB, Bamberg P, Chawla Y, Kaur U, Bhusnurmath SR, Malhotra HS, Sood GK, Mitra SK, Khanna SK, Walia BS. Hepatic outflow obstruction (Budd-Chiari syndrome). Experience with 177 patients and a review of the literature. *Medicine* (Baltimore) 1994; **73**: 21-36 [PMID: 8309360 DOI: 10.1097/00005792-199401000-00003]
- Ludwig J, Hashimoto E, McGill DB, van Heerden JA. Classification of hepatic venous outflow obstruction: ambiguous terminology of the Budd-Chiari syndrome. *Mayo Clin Proc* 1990; **65**: 51-55 [PMID: 2296212 DOI: 10.1016/S0025-6196(12)62109-0]
- McDermott WV, Ridker PM. The Budd-Chiari syndrome and hepatic veno-occlusive disease. Recognition and treatment. *Arch Surg* 1990; **125**: 525-527 [PMID: 2322120 DOI: 10.1001/archsurg.1990.01410160111022]
- Plessier A, Rautou PE, Valla DC. Management of hepatic vascular diseases. *J Hepatol* 2012; **56** Suppl 1: S25-S38 [PMID: 22300463 DOI: 10.1016/S0168-8278(12)60004-X]
- Schein M, Paladugu R. Redundant surgical publications: tip of the iceberg? *Surgery* 2001; **129**: 655-661 [PMID: 11391360 DOI: 10.1067/msy.2001.114549]
- Qi X, Ren W, Liu L, Yang Z, Yang M, Fan D, Han G. Prevalence of covert duplicate publications in Budd-Chiari syndrome articles in China: a systematic analysis. *Am J Med* 2013; **126**: 633-9.e2 [PMID: 23787196 DOI: 10.1016/j.amjmed.2012.12.021]
- Sakr M, Barakat E, Abdelhakam S, Dabbous H, Youssef S, Shaker M, Eldorri A. Epidemiological aspects of Budd-Chiari in Egyptian patients: a single-center study. *World J Gastroenterol* 2011; **17**: 4704-4710 [PMID: 22180713 DOI: 10.3748/wjg.v17.i42.4704]
- De BK, Biswas PK, Sen S, Das D, De KK, Das U, Mandal SK, Majumdar D. Management of the Budd-Chiari syndrome by balloon cavoplasty. *Indian J Gastroenterol* 2001; **20**: 151-154 [PMID: 11497174]
- Darwish Murad S, Plessier A, Hernandez-Guerra M, Fabris F, Eapen CE, Bahr MJ, Trebicka J, Morard I, Lasser L, Heller J, Hadengue A, Langlet P, Miranda H, Primignani M, Elias E, Leebeek FW, Rosendaal FR, Garcia-Pagan JC, Valla DC, Janssen HL. Etiology, management, and outcome of the Budd-Chiari syndrome. *Ann Intern Med* 2009; **151**: 167-175 [PMID: 19652186 DOI: 10.7326/0003-4819-151-3-200908040-00004]
- Faraoun SA, Boudjella Mel A, Debzi N, Afredj N, Guerrache Y, Benidir N, Bouzid C, Bentabak K, Soyer P, Bendib SE. Budd-Chiari syndrome: a prospective analysis of hepatic vein obstruction on ultrasonography, multidetector-row computed tomography and MR imaging. *Abdom Imaging* 2015; **40**: 1500-1509 [PMID: 25687630 DOI: 10.1007/s00261-015-0380-5]
- Zhou P, Ren J, Han X, Wu G, Zhang W, Ding P, Bi Y. Initial imaging analysis of Budd-Chiari syndrome in Henan province of China: most cases have combined inferior vena cava and hepatic veins involvement. *PLoS One* 2014; **9**: e85135 [PMID: 24416352 DOI: 10.1371/journal.pone.0085135]
- Janssen HL, Meinardi JR, Vleggaar FP, van Uum SH, Haagsma EB, van Der Meer FJ, van Hattum J, Chamuleau RA, Adang RP, Vandenbroucke JP, van Hoek B, Rosendaal FR. Factor V Leiden mutation, prothrombin gene mutation, and deficiencies in coagulation inhibitors associated with Budd-Chiari syndrome and portal vein thrombosis: results of a case-control study. *Blood* 2000; **96**: 2364-2368 [PMID: 11001884]
- Mohanty D, Shetty S, Ghosh K, Pawar A, Abraham P. Hereditary thrombophilia as a cause of Budd-Chiari syndrome: a study from Western India. *Hepatology* 2001; **34**: 666-670 [PMID: 11584361 DOI: 10.1053/jhep.2001.27948]
- Deepak A, Punamiya S, Patel N, Parekh S, Mehta S, Shah N. Prevalence of JAK2V617F mutation in intra-abdominal venous thrombosis. *Trop Gastroenterol* 2011; **32**: 279-284 [PMID: 22696908]
- Colaizzo D, Amitrano L, Tiscia GL, Iannaccone L, Gallone A, Grandone E, Guardascione MA, Margaglione M. Occurrence of the JAK2 V617F mutation in the Budd-Chiari syndrome. *Blood Coagul Fibrinolysis* 2008; **19**: 459-462 [PMID: 18600100 DOI: 10.1097/MBC.0b013e3283049662]
- Xavier SG, Gadelha T, Pimenta G, Eugenio AM, Ribeiro DD, Gomes FM, Bonamino M, Zalcberg IR, Spector N. JAK2V617F mutation in patients with splanchnic vein thrombosis. *Dig Dis Sci* 2010; **55**: 1770-1777 [PMID: 19690956 DOI: 10.1007/s10620-009-0933-y]
- Westbrook RH, Lea NC, Mohamedali AM, Smith AE, Orr DW, Roberts LN, Heaton ND, Wendon JA, O'Grady JG, Heneghan MA, Mufti GJ. Prevalence and clinical outcomes of the 46/1

- haplotype, Janus kinase 2 mutations, and ten-eleven translocation 2 mutations in Budd-Chiari syndrome and their impact on thrombotic complications post liver transplantation. *Liver Transpl* 2012; **18**: 819-827 [PMID: 22467227 DOI: 10.1002/lt.23443]
- 33 **Goulding C**, Uttenthal B, Foroni L, Duke V, Traore A, Kottaridis P, Hoffbrand AV, Patch D, McNamara C. The JAK2(V617F) tyrosine kinase mutation identifies clinically latent myeloproliferative disorders in patients presenting with hepatic or portal vein thrombosis. *Int J Lab Hematol* 2008; **30**: 415-419 [PMID: 19046316 DOI: 10.1111/j.1751-553X.2007.00973.x]
  - 34 **D'Amico M**, Sammarco P, Pasta L. Thrombophilic Genetic Factors PAI-1, MTHFR C677T, V Leiden 506Q, and Prothrombin 20210A in Noncirrhotic Portal Vein Thrombosis and Budd-Chiari Syndrome in a Caucasian Population. *Int J Vasc Med* 2013; **2013**: 717480 [PMID: 24455271 DOI: 10.1155/2013/717480]
  - 35 **Qi X**, Wu F, Ren W, He C, Yin Z, Niu J, Bai M, Yang Z, Wu K, Fan D, Han G. Thrombotic risk factors in Chinese Budd-Chiari syndrome patients. An observational study with a systematic review of the literature. *Thromb Haemost* 2013; **109**: 878-884 [PMID: 23447059 DOI: 10.1160/TH12-10-0784]
  - 36 **Qi X**, Wu F, Fan D, Han G. Prevalence of thrombotic risk factors in Chinese Budd-Chiari syndrome patients: results of a prospective validation study. *Eur J Gastroenterol Hepatol* 2014; **26**: 576-577 [PMID: 24694738 DOI: 10.1097/MEG.000000000000056]
  - 37 **Rautou PE**, Douarin L, Denninger MH, Escolano S, Lebre C, Moreau R, Vidaud M, Itzykson R, Moucari R, Bezeaud A, Valla D, Plessier A. Bleeding in patients with Budd-Chiari syndrome. *J Hepatol* 2011; **54**: 56-63 [PMID: 20889223 DOI: 10.1016/j.jhep.2010.06.019]
  - 38 **Pavri TM**, Herbst A, Reddy R, Forde KA. Budd-Chiari syndrome: a single-center experience. *World J Gastroenterol* 2014; **20**: 16236-16244 [PMID: 25473178 DOI: 10.3748/wjg.v20.i43.16236]
  - 39 **Smalberg JH**, Darwish Murad S, Braakman E, Valk PJ, Janssen HL, Leebeek FW. Myeloproliferative disease in the pathogenesis and survival of Budd-Chiari syndrome. *Haematologica* 2006; **91**: 1712-1713 [PMID: 17145613]
  - 40 **Perelló A**, García-Pagán JC, Gilabert R, Suárez Y, Moitinho E, Cervantes F, Reverter JC, Escorsell A, Bosch J, Rodés J. TIPS is a useful long-term derivative therapy for patients with Budd-Chiari syndrome uncontrolled by medical therapy. *Hepatology* 2002; **35**: 132-139 [PMID: 11786969 DOI: 10.1053/jhep.2002.30274]
  - 41 **Xu PQ**, Ma XX, Ye XX, Feng LS, Dang XW, Zhao YF, Zhang SJ, Zhao LS, Tang Z, Lu XB. Surgical treatment of 1360 cases of Budd-Chiari syndrome: 20-year experience. *Hepatobiliary Pancreat Dis Int* 2004; **3**: 391-394 [PMID: 15313675]
  - 42 **Harmanci O**, Kav T, Peynircioglu B, Buyukasik Y, Sokmensuer C, Bayraktar Y. Long-term follow-up study in Budd-Chiari syndrome: single-center experience in 22 years. *J Clin Gastroenterol* 2013; **47**: 706-712 [PMID: 22495815 DOI: 10.1097/MCG.0b013e31824fd63]
  - 43 **Wang R**, Meng Q, Qu L, Wu X, Sun N, Jin X. Treatment of Budd-Chiari syndrome with inferior vena cava thrombosis. *Exp Ther Med* 2013; **5**: 1254-1258 [PMID: 23596497]
  - 44 **Han G**, Qi X, Zhang W, He C, Yin Z, Wang J, Xia J, Xu K, Guo W, Niu J, Wu K, Fan D. Percutaneous recanalization for Budd-Chiari syndrome: an 11-year retrospective study on patency and survival in 177 Chinese patients from a single center. *Radiology* 2013; **266**: 657-667 [PMID: 23143028 DOI: 10.1148/radiol.12120856]
  - 45 **Raszeja-Wyszomirska J**, Mieżyńska-Kurtycz J, Marlicz W, Lawniczak M, Wójcicki M. Primary Budd-Chiari syndrome - a single center experience. *Hepatogastroenterology* 2012; **59**: 1879-1882 [PMID: 22819909]
  - 46 **Ebrahimi M**, Modaghegh MH, Esmaeilzadeh A. Presentation of hospital outcomes and different treatment methods of patients with budd-Chiari syndrome: a report from two tertiary hospitals in iran. *Med Princ Pract* 2011; **20**: 287-290 [PMID: 21455002 DOI: 10.1159/000323755]
  - 47 **Nozari N**, Vossoghnia H, Malekzadeh F, Kafami L, Mirheidari M, Malekzadeh R. Long-term Outcome of Budd-Chiari Syndrome: A Single Center Experience. *Middle East J Dig Dis* 2013; **5**: 146-150 [PMID: 24829685]
  - 48 **Simonetto DA**, Yang HY, Yin M, de Assuncao TM, Kwon JH, Hilscher M, Pan S, Yang L, Bi Y, Beyder A, Cao S, Simari RD, Ehman R, Kamath PS, Shah VH. Chronic passive venous congestion drives hepatic fibrogenesis via sinusoidal thrombosis and mechanical forces. *Hepatology* 2015; **61**: 648-659 [PMID: 25142214 DOI: 10.1002/hep.27387]
  - 49 **Gao X**, Gui E, Lu Z, Ning X, Zu M, Zhang P, Sun G. Risk factors of recurrence among 471 Chinese patients with Budd-Chiari syndrome. *Clin Res Hepatol Gastroenterol* 2015; **39**: 620-626 [PMID: 25656980 DOI: 10.1016/j.clinre.2014.12.010]
  - 50 **Gwon D**, Ko GY, Yoon HK, Sung KB, Kim JH, Lee SS, Lee JM, Ohm JY, Shin JH, Song HY. Hepatocellular carcinoma associated with membranous obstruction of the inferior vena cava: incidence, characteristics, and risk factors and clinical efficacy of TACE. *Radiology* 2010; **254**: 617-626 [PMID: 20093533 DOI: 10.1148/radiol.09090738]
  - 51 **Park H**, Yoon JY, Park KH, Kim do Y, Ahn SH, Han KH, Chon CY, Park JY. Hepatocellular carcinoma in Budd-Chiari syndrome: a single center experience with long-term follow-up in South Korea. *World J Gastroenterol* 2012; **18**: 1946-1952 [PMID: 22563176 DOI: 10.3748/wjg.v18.i16.1946]

**P- Reviewer:** Balaceanu LA, Bayraktar Y, Cetinkunar S, Mancuso A

**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Liu SQ





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 June 18; 8(17): 703-748







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verducci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



**TOPIC HIGHLIGHT**

- 703** Usefulness of staging systems and prognostic scores for hepatocellular carcinoma treatments  
*Adhoue X, Penaranda G, Raoul JL, Le Treut P, Bollon E, Hardwigsen J, Castellani P, Perrier H, Bourlière M*
- 716** Innate immune targets of hepatitis B virus infection  
*Zou ZQ, Wang L, Wang K, Yu JG*

**ORIGINAL ARTICLE****Basic Study**

- 726** Anti-CD163-dexamethasone conjugate inhibits the acute phase response to lipopolysaccharide in rats  
*Thomsen KL, Møller HJ, Graversen JH, Magnusson NE, Moestrup SK, Vilstrup H, Grønbaek H*

**Clinical Trials Study**

- 731** Therapeutic usability of two different fiducial gold markers for robotic stereotactic radiosurgery of liver malignancies: Pilot study  
*Marsico M, Gabbani T, Livi L, Biagini MR, Galli A*

**Prospective Study**

- 739** Circulating insulin-like growth factor-binding protein 3 as prognostic biomarker in liver cirrhosis  
*Correa CG, Colombo BS, Ronsoni MF, Soares e Silva PE, Fayad L, Silva TE, Wildner LM, Bazzo ML, Dantas-Correa EB, Narciso-Schiavon JL, Schiavon LL*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Leonardo L Schiavon, MD, PhD, Associate Professor, Department of Internal Medicine, Federal University of Santa Catarina, Florianópolis, Santa Catarina 88051150, Brazil

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

**I-IV** Editorial Board

**EDITORS FOR  
THIS ISSUE**

**Responsible Assistant Editor:** *Xiang Li*  
**Responsible Electronic Editor:** *Su-Qing Liu*  
**Proofing Editor-in-Chief:** *Lian-Sheng Ma*

**Responsible Science Editor:** *Yuan Qi*  
**Proofing Editorial Office Director:** *Xin-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
June 18, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjgnet.com/bpg/g_info_20160116143427.htm)

**ONLINE SUBMISSION**

<http://www.wjgnet.com/esps/>

## 2016 Hepatocellular Carcinoma: Global view

# Usefulness of staging systems and prognostic scores for hepatocellular carcinoma treatments

Xavier Adhoute, Guillaume Penaranda, Jean Luc Raoul, Patrice Le Treut, Emilie Bollon, Jean Hardwigsen, Paul Castellani, Hervé Perrier, Marc Bourlière

Xavier Adhoute, Paul Castellani, Hervé Perrier, Marc Bourlière, Department of Hepato-Gastroenterology, Hôpital Saint-Joseph, 13008 Marseille, France

Guillaume Penaranda, AlphaBio Laboratory, 13008 Marseille, France

Jean Luc Raoul, Department of Hepato-Gastroenterology and Digestive Oncology, Institut Paoli-Calmette, 13008 Marseille, France

Patrice Le Treut, Emilie Bollon, Jean Hardwigsen, Department of Hepatobiliary Surgery, Centre Hospitalo-Universitaire Timone, 13008 Marseille, France

**Author contributions:** Adhoute X, Le Treut P, Bollon E, Hardwigsen J, Castellani P, Perrier H and Bourlière M collected the data; Penaranda G proceeded to statistical analysis; Adhoute X, Penaranda G, Raoul JL and Bourlière M wrote the manuscript.

**Conflict-of-interest statement:** Raoul JL, Board member (Bayer, BMS, Daichi). Bourlière M, Board member (Merck-Schering Plough, Gilead, Janssen, Vertex, Boehringer-Ingelheim, BMS, Roche, Abbvie, GSK); Speaker (Merck-Schering Plough - Gilead, Janssen, Vertex, Boehringer-Ingelheim, BMS, Roche, Abbvie, Novartis, GSK). Adhoute X, Penaranda G, Bollon E, Castellani P, Perrier H, Hardwigsen J and Le Treut P declare that they have no conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Dr. Xavier Adhoute, Department of Hepato-Gastroenterology, Hôpital Saint-Joseph, 26 Bd de

Louvain, 13008 Marseille, France. [adhoute.xavier@neuf.fr](mailto:adhoute.xavier@neuf.fr)  
Telephone: +33-49-1807065  
Fax: +33-49-1806912

Received: February 17, 2016  
Peer-review started: February 19, 2016  
First decision: April 5, 2016  
Revised: April 24, 2016  
Accepted: May 17, 2016  
Article in press: May 27, 2016  
Published online: June 18, 2016

## Abstract

Therapeutic management of hepatocellular carcinoma (HCC) is quite complex owing to the underlying cirrhosis and portal vein hypertension. Different scores or classification systems based on liver function and tumoral stages have been published in the recent years. If none of them is currently "universally" recognized, the Barcelona Clinic Liver Cancer (BCLC) staging system has become the reference classification system in Western countries. Based on a robust treatment algorithm associated with stage stratification, it relies on a high level of evidence. However, BCLC stage B and C HCC include a broad spectrum of tumors but are only matched with a single therapeutic option. Some experts have thus suggested to extend the indications for surgery or for transarterial chemoembolization. In clinical practice, many patients are already treated beyond the scope of recommendations. Additional alternative prognostic scores that could be applied to any therapeutic modality have been recently proposed. They could represent complementary tools to the BCLC staging system and improve the stratification of HCC patients enrolled in clinical trials, as illustrated by the NIACE score. Prospective studies are needed to compare these scores and refine their role in the decision making process.

**Key words:** Scoring system; Hepatocellular carcinoma; Barcelona Clinic Liver Cancer staging system; NIACE; Transarterial chemoembolization

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Different scores or classification systems have been proposed to refine hepatocellular carcinoma prognosis and better guide medical treatment. The Barcelona Clinic Liver Cancer (BCLC) system has become the reference classification in Western countries. Its treatment algorithm is based on randomized studies, but only offers one recommendation for BCLC stages B and C, whereas they include a broad spectrum of tumors. In clinical practice, many patients are treated out of the scope of these recommendations. In this context, alternative scores or classifications, which have been opposed for a long time, could be complementary tools for the benefit of the treatment.

Adhoute X, Penaranda G, Raoul JL, Le Treut P, Bollon E, Hardwigen J, Castellani P, Perrier H, Bourlière M. Usefulness of staging systems and prognostic scores for hepatocellular carcinoma treatments. *World J Hepatol* 2016; 8(17): 703-715 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i17/703.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i17.703>

## INTRODUCTION

Most hepatocellular carcinomas (HCC) develop upon chronic diseases of the liver, mainly B or C viral hepatitis. HCC is a frequent and serious cancer, often diagnosed at an inoperable stage<sup>[1]</sup>. It is singular as its prognosis not only relies on the tumor characteristics but also on the underlying liver disease, frequently at a cirrhotic stage. The tumor-node-metastasis (TNM) classification of solid tumors failed to impose itself as the reference system for such a dual pathology, despite its recognized prognostic value even for non-operated tumors<sup>[2]</sup>. In order to refine the prognosis and provide better medical care, different scores or classifications originating from Asian or Western countries have been published recently. Most of them use regression models based on the prognostic variables of the studied populations. If they all share common parameters including liver function, tumor characteristics, age-related clinical consequences, comorbidities or cirrhosis (Figure 1), there is no universally recognized score or classification to date.

In the first part, we will focus on the main scores and classification systems published in the recent years, following a chronological order and revealing the differences between Western and Asian countries, the corresponding affected populations, treatment modalities and recommendations being distinct. The second part highlights the complementarity between the two systems in the decision making process (excluding graft), as successively exemplified by the sorafenib,

the transarterial chemoembolization (TACE), the radio-frequency ablation (RFA) and the surgical resection treatments.

## HCC PROGNOSIS: SCORES OR CLASSIFICATIONS?

The OKUDA score, published in the eighties, was the first to combine tumor-associated parameters (more vs less than 50% of invaded parenchyma) and liver function (ascites, albumin, bilirubin) (Tables 1 and 2). It classifies patients into three stages [lowly ( I ), moderately ( II ) or highly advanced ( III )] with different outcomes, depending on their number of positive variables (0 vs 1-2 vs 3-4, respectively). This score was initially validated on a population of 850 patients, either non-treated or treated according to the modalities applicable at that time (surgery, intra-arterial or systemic chemotherapy, arterial embolization)<sup>[3]</sup>. Although approximative and hardly differentiating the less advanced patients (e.g., the median survival of stage I patients was 11.5 mo independently of the treatment vs 25.6 mo when operated), this score has been widely used.

Published in the late nineties, the Italian Cancer of the Liver Italian Program (CLIP) score was calculated from the prognostic values of 435 patients originating from 16 centers (Tables 1 and 2)<sup>[4]</sup>. It includes other tumor-linked parameters such as portal vein thrombosis or alpha-fetoprotein (AFP) serum levels and better estimates the liver function using the Child-Pugh score. Easy to calculate (4 variables to add), it is well correlated with survival (CLIP 0, 1, 2, 3, 4, 5-6: 42.5 vs 32 vs 16.5 vs 4.5 vs 2.5 vs 1.0 mo). The CLIP score was first assessed on a prospective cohort<sup>[5,6]</sup> and subsequently validated on Asian cohorts<sup>[7]</sup>. Still recently ranked first for its ability to predict survival<sup>[8]</sup>, it was criticized for its lack of treatment offer, approximation in tumor morphology and extension, for the absence of clinical status consideration and its inability to classify intermediate stages. Another issue is that studies evaluating the CLIP system mainly included patients with scores only ranging from 0 to 2<sup>[7-9]</sup>.

French speaking teams have created the GRETCH score in 1999. Quite similar to the CLIP, it further includes the patients' overall condition but lacks tumor morphology information<sup>[10]</sup>. Also determined from a multivariate analysis including 761 patients (mainly non-treated) from 24 centers, it identifies 3 different groups (A: 0, B: 1 to 5 and C: 6 to 11 points) with distinct prognosis [overall survival after a year: A (72%), B (34%), C (7%), respectively]. Less evaluated than the CLIP, it faces the same limitations.

The BCLC classification was published at the same time<sup>[11]</sup>. Differently built as it is not based on a regression model but results from the combination of different studies, it distinguishes 4 different stages [A: (very) early, B: intermediate, C: advanced, D: terminal] with different prognosis, according to the liver function, the



**Table 1 Hepatocellular carcinoma scores and staging systems published in the recent years**

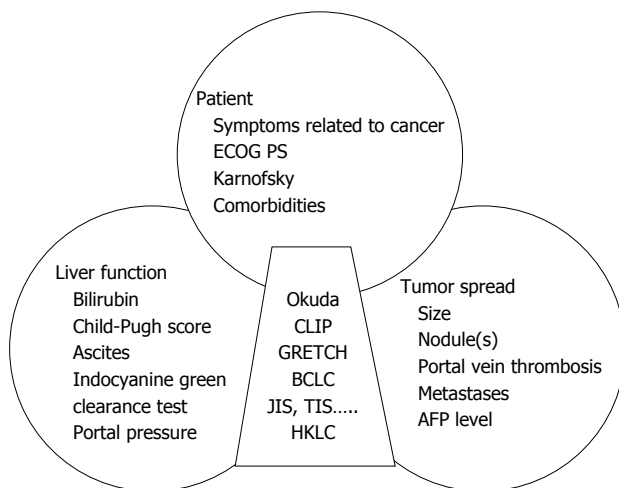
Scores and classifications	Liver function	AFP	PS	Tumor spread
Okuda 1985	Ascites, albumin, bilirubin	No	No	Hepatic spread 50% < vs > 50%
CLIP 1998	Child-Pugh score	< 400 ng/mL vs ≥ 400 ng/mL	No	Nodule(s), hepatic spread 50% ≤ vs > 50%
GRETCH 1999	Bilirubine, phosphatases alcalines	< 35 ng/mL vs ≥ 35 ng/mL	Yes	Portal vein thrombosis
BCLC 1999	Child-Pugh score	No	Yes	Portal vein thrombosis
c-JIS 2003	Child-Pugh score	No	No	Nodule(s), size
bm-JIS 2008	Child-Pugh score	Yes (+ AFP-L3 + DCP)	No	TNM LSCGJ
TIS 2010	Child-Pugh score	< 400 g/mL vs ≥ 400 ng/mL	No	TNM LSCGJ
HKLC 2014	Child-Pugh score	No	Yes	Total tumor volume
				Nodule(s), size
				Portal vein thrombosis

BCLC: Barcelona Clinic Liver Cancer; CLIP: Cancer of the Liver Italian Program; JIS: Japan Integrated Staging; HKLC: Hong Kong Liver Cancer; TIS: Taipei Integrated Scoring System; AFP: Alpha-fetoprotein; PS: Performance Status; bm-JIS: Biomarker combined JIS; DCP: Des-gamma-carboxy prothrombin; LSCGJ: Liver Cancer Study Group of Japan; TNM: Tumor-node-metastasis.

**Table 2 Definitions of the Okuda score and the Cancer of the Liver Italian Program score**

Okuda score			CLIP score		
Parameters	(+) 1 point	(-) 0 point	0 point	1 point	2 points
Tumor spread	> 50%	< 50%			
Albumin, g/dL	< 3	> 3			
Bilirubin, mg/dL	> 3	< 3			
Ascites	Yes	No			
Child-Pugh score			A	B	C
Tumor spread			Unipolar and hepatic spread ≤ 50%	Multinodular and hepatic spread ≤ 50%	Massive or hepatic spread > 50%
Portal vein thrombosis			No	Yes	
AFP, ng/dL			< 400	≥ 400	

AFP: Alpha-fetoprotein; CLIP: Cancer of the Liver Italian Program.



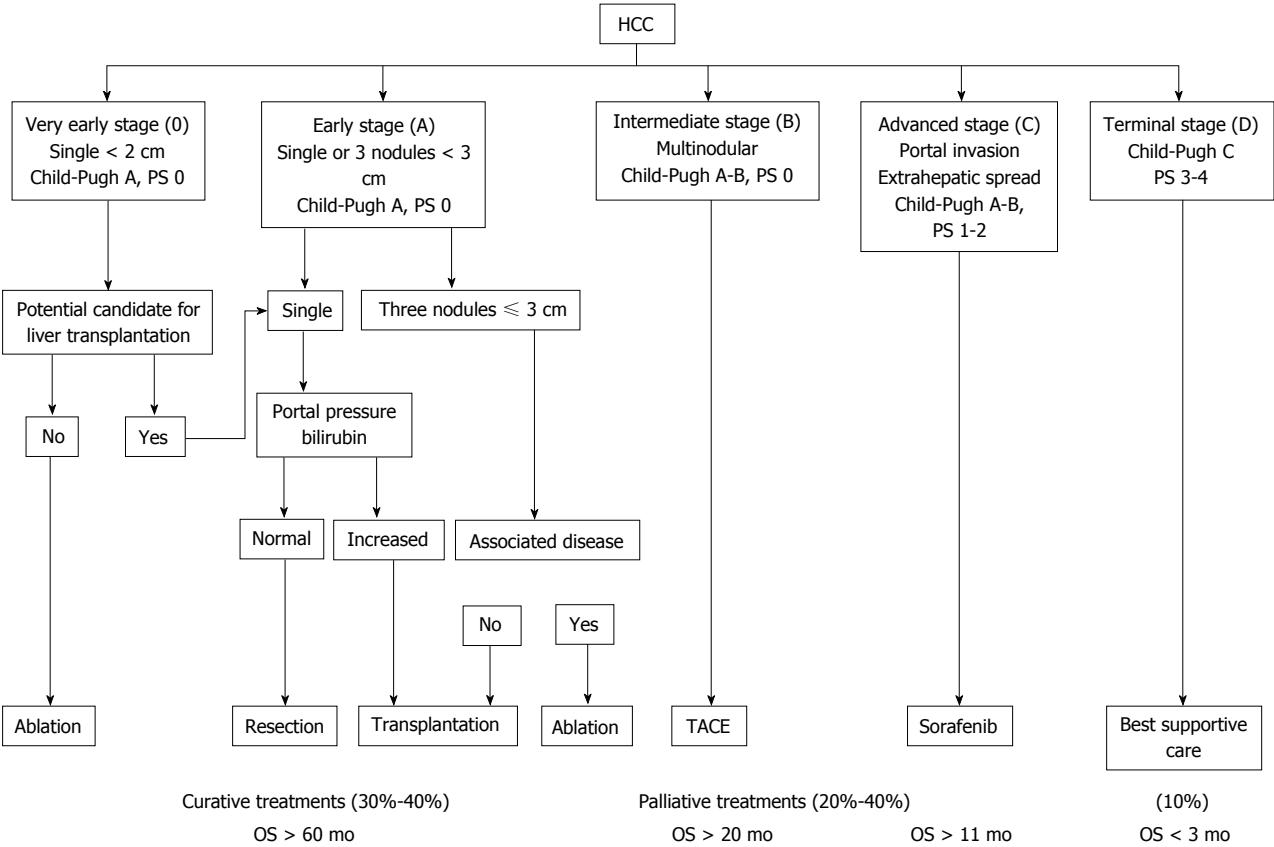
**Figure 1 Common parameters between hepatocellular carcinoma classifications and scores.** AFP: Alpha-fetoprotein; CLIP: Cancer of the Liver Italian Program; BCLC: Barcelona Clinic Liver Cancer; JIS: Japan Integrated Staging; HKLC: Hong Kong Liver Cancer; TIS: Taipei Integrated Scoring System; ECOG (PS): Eastern Cooperative Oncology Group (performance status).

extent of the tumor and its consequences (Figure 2). As opposed to the previous scores, the early stages are well defined according to the number and size of nodules, the

associated comorbidities and the portal vein pressure. The BCLC staging system was assessed on Western and Asian cohorts<sup>[12,13]</sup> and demonstrated a better ability to predict survival than most other scores<sup>[9,14]</sup>. This classification has imposed itself from its practical aspect and for being the only one linked to a treatment algorithm relying on a high level of evidence for each modality. Endorsed by both the European Associations for the Study of the Liver (EASL)<sup>[15]</sup> and the American Associations for the Study of the Liver (AASLD)<sup>[16]</sup>, it has become the reference classification in Western countries and is being used in day-to-day practice and clinical trials.

However, BCLC is not the reference classification in Asia, notably as HCC treatment modalities differ according to the countries (*e.g.*, external radiotherapy, intra-arterial and systemic chemotherapy or TACE being indicated for advanced HCC despite a low level of evidence<sup>[17]</sup>). Such recommendations are based on studies but, as opposed to the BCLC, also rely on personal experience, experts advice and consensus conferences. Alternative scores or classifications have thus been proposed.

The Japan Integrated Staging (JIS) score was published in 2003 (Table 3)<sup>[18]</sup>. Also easy to calculate, it associates the Child-Pugh score and the Japanese TNM,



**Figure 2** Barcelona Clinic Liver Cancer system. HCC: Hepatocellular carcinoma; PS: Performance status; TACE: Transarterial chemoembolization.

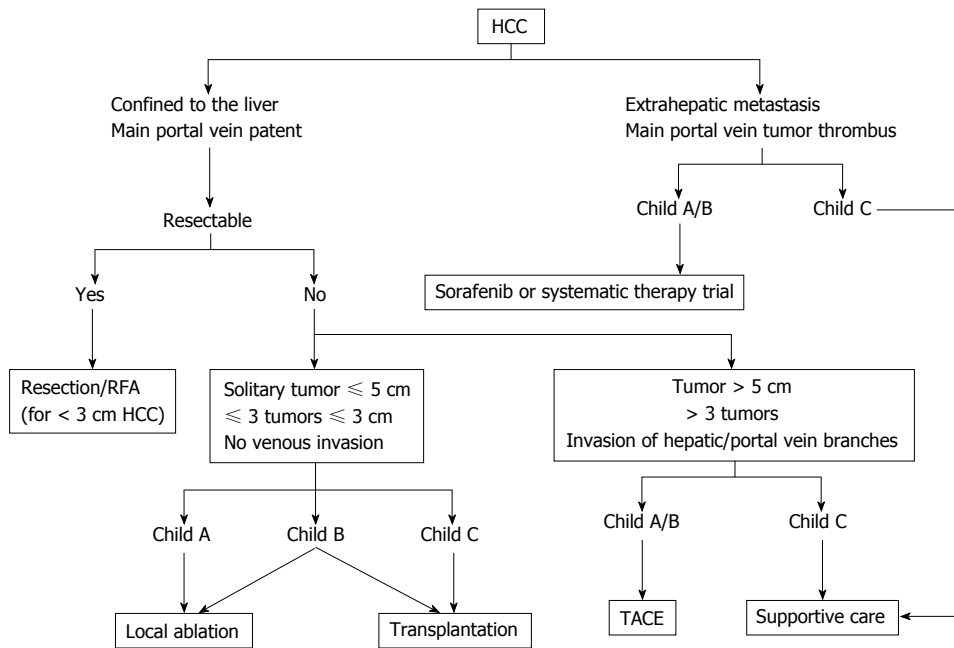
Table 3 Definitions of the c-Japan Integrated Staging score and the biomarker combined Japan Integrated Staging score				
	0 point	1 point	2 points	3 points
Score c-JIS				
Child-Pugh stage	A	B	C	
TNM stage by LSCGJ <sup>1</sup>	I	II	III	IV
Score bm-JIS				
Child-Pugh stage	A	B	C	
TNM stage by LSCGJ <sup>1</sup>	I	II	III	IV
Elevated tumor markers, n (AFP, AFP-L3, DCP)	0	1	2 or 3	

<sup>1</sup>Definitions of the TNM stage by the LSCGJ; Stage I: T1 (fulfilling 3 T factors) N0 M0; Stage II: T2 (fulfilling 2 T factors) N0 M0; Stage III: T3 (fulfilling 1 T factor) N0 M0; Stage IV: T4 (fulfilling 0 T factor) N0 M0 or any T N0 - 1 M1; T factor: (1) single; (2) < 2 cm; and (3) no vascular involvement. LSCGJ: Liver Cancer Study Group of Japan; bm-JIS: Biomarker combined-Japan Integrated Staging.

which is based on three parameters (vascular invasion, unique vs multiple nodules, diameter  $\leq$  vs  $>$  20 mm) determined from a population of 13772 operated patients. It defines six groups with different prognosis (excluding JIS 4-5). This score has demonstrated a better ability to predict survival than the CLIP and was further improved a few years later with the modified-JIS<sup>[19]</sup>, in which the encephalopathy item is replaced by the indocyanine green clearance, due to an early HCC screening in Japan and a preferred surgical orientation. In 2008, the JIS score became the biomarker combined

JIS with the inclusion of three HCC serum markers [AFP, AFP-L3 (AFP-Lens culinaris agglutinin-reactive) and des-gamma-carboxy prothrombin], which allowed better survival predictions (Table 3)<sup>[20]</sup>. However, two of those markers are not frequently used in Western countries where HCC is also often being diagnosed at more advanced stages. Thus, this score, without treatment guidelines, has not been evaluated on patients from Western countries.

The Taipei Integrated Scoring system (TIS) was published in 2010<sup>[21]</sup> arising from the lack of a reference classification and the opposite results from studies regarding the performance of classification systems. TIS is a point scoring system combining AFP levels (< 400 vs > 400 ng/mL: 0 vs 1 point), Child-Pugh score (A, B and C : 0, 1 and 2 points, respectively) and the sum of the volume of each tumor (total tumor volume), calculated from the following formulae:  $[(4/3) \times 3.14 \times (\text{radius of tumor in cm})^3]$ , and which defines 4 different groups (< 50 cm<sup>3</sup>, 50-250 cm<sup>3</sup>, 250-500 cm<sup>3</sup>, > 500 cm<sup>3</sup>: 0, 1, 2 or 3 points, respectively). From a cohort of 2030 patients, mainly with viral hepatitis (hepatitis B virus 51%, hepatitis C virus 27%), the score identified six distinct prognostic groups, with a score evolution inversely correlated to survival. The predictive ability of the TIS score was better than the JIS and the BCLC for the whole cohort, independently of the treatment modality (curative or palliative), but not as good as the CLIP for the 936 patients treated with curative intent.



**Figure 3** Asian Pacific Association for the Study of the Liver guidelines on the treatment algorithm for hepatocellular carcinoma. HCC: Hepatocellular carcinoma; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation.

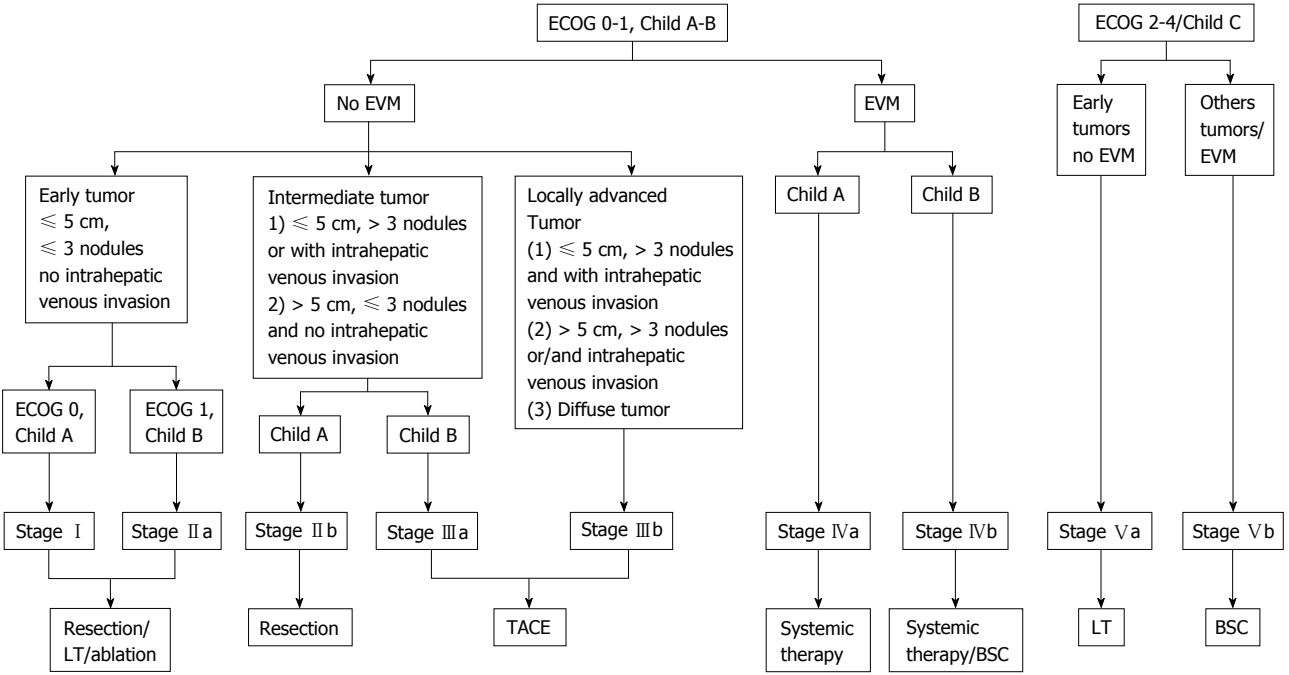
Vascular invasion that was observed in 36.7% of the patients is taken into account in the CLIP but not in the TIS, which probably participates in this discrepancy. Again, this score appears promising, but lacks a linkage to any treatment decision choice and has not been validated on any Western country patient.

In 2011, an Asian experts meeting has suggested to adopt a common classification and common recommendations. TACE was then proposed for HCC with limited vascular invasion, despite a low level of evidence (Figure 3)<sup>[17]</sup>. The competing Hong Kong Liver Cancer (HKLC) classification, which is close to the BCLC system, was published in 2014<sup>[22]</sup>. Built from a population of 3856 patients (median age: 58 years old), mainly affected by viral hepatitis B, with Child-Pugh A scores (73%), it identifies five groups and nine sub-groups to further refine the prognosis (Figure 4). The associated treatment algorithm recommended surgery at more advanced stages and subsequently increased survival according to the authors. However, its prognostic value was comparable to the BCLC system for a European cohort of HCC linked to viral hepatitis C or alcohol, the II a/II b, IIIb/IVa, IVb/Vb subgroups presenting similar survival<sup>[23]</sup>, which limits the impact of such a stratification within this population. A prospective study is currently on-going to further evaluate this score.

Overall, the BCLC classification has become the reference in Western countries and has replaced the other prognostic scores. Limitations have however been highlighted since several years. The intermediate BCLC B stage, which gathers multifocal tumors lacking vascular invasion and excludes unique and large HCC, now part of the BCLC A group in newer version of the BCLC classification<sup>[24]</sup>, remains heterogeneous<sup>[25]</sup>. Thus, a diffuse multinodular HCC or four nodules of one

centimeter in size within the same lobe are categorized within the same BCLC B group, and only a single therapeutic option is offered (*i.e.*, chemoembolization). Advanced (BCLC C) stages encompass a broad spectrum of tumors, including cancers with or without symptoms, metastatic or locally advanced diseases, eventually associated with portal thrombosis, nodular or infiltrating tumors, uni- or multi-nodular tumors, associated with Child-Pugh A or B grade, which are, again, only associated with a single treatment (sorafenib)<sup>[24]</sup>. It has thus been suggested to extend the indication for surgery<sup>[26-28]</sup> or chemoembolization to some advanced stages<sup>[29,30]</sup>. Stage C HCC were defined using a population limited to 102 patients<sup>[31]</sup>. Furthermore, comparative studies have shown lower prognostic ability for the BCLC than the CLIP score regarding advanced HCC<sup>[32-34]</sup>, and several studies have suggested a possible stratification for the BCLC C HCC<sup>[35-37]</sup>. For example, Yau *et al.*<sup>[36]</sup> have proposed a new score called Advanced Liver Cancer Prognostic System (ALCPS), separating 3 groups according to their survival after 3 mo, and aiming at improving patients selection before their enrollment into clinical trials (Table 4). However, the ALCPS score is too complex for daily clinical practice as it includes eleven variables with different coefficients, as is the Chinese University Prognostic Index score<sup>[37]</sup>.

Conversely, the recently published NIACE score<sup>[38]</sup> (Table 5) was determined from a population of advanced HCC and validated using an external Asian cohort, independently of the BCLC stage<sup>[39]</sup>. Easy to calculate and well correlated to survival, it distinguishes 2 subgroups with different prognosis within BCLC stage C patients. Advanced HCC are classified according to their morphology as infiltrating or diffuse (hardly delimited lesion, with a heterogeneous enhancement, more easily



**Figure 4 Hong Kong Liver Cancer classification.** EVM: Extrahepatic vascular invasion/metastasis; BSC: Best supportive care; TACE: Transarterial chemoembolization; ECOG: Eastern Cooperative Oncology Group.

Table 4 Barcelona Clinic Liver Cancer C hepatocellular carcinoma, a broad spectrum of tumors; example of the Advanced Liver Cancer Prognostic System score <sup>[36]</sup>	
Parameters	Points
Ascites	2
Abdominal pain	2
Weight loss	2
Child-Pugh grade A/B/C	0/2/5
alkaline phosphatase, UI/L > 200	3
Bilirubin, mcml/L ≤ 33/> 33-≤ 50/> 50	0/1/3
Urea, mmol/L > 8.9	2
Portal vein thrombosis	3
Tumor size: Diffuse/> 5 cm/≤ 5 cm	4/3/0
Lung metastases	3
AFP, ng/mL > 400	4
Probability of patients surviving at least 3 mo estimated by the ALCPS score <sup>[36]</sup>	
Score ≤ 8 points: 82.0% (95%CI: 76.5%-87.5%)	
Score 9-15 points: 53.4% (95%CI: 48.3%-57.7%)	
Score ≥ 16 points: 18.9% (95%CI: 14.7%-23.3%)	

Probability of patients surviving at least 3 mo estimated by the ALCPS score<sup>[36]</sup>. AFP: Alpha-fetoprotein; ALCPS: Advanced Liver Cancer Prognostic System.

characterized using magnetic resonance imaging<sup>[40]</sup> and frequently associated with portal vein thrombosis<sup>[41]</sup> or bile duct invasion), as opposed to the nodular HCC meeting the EASL/AASLD diagnosis criteria<sup>[42]</sup>. It also considers the AFP level ( $\pm$  200 ng/mL), whose prognostic value has been demonstrated independently of the stage of the disease<sup>[4,10,43]</sup>; those two last criteria missing from the BCLC system.

The predictive value of the NIACE score has been compared to those of the CLIP score and both the BCLC

Table 5 NIACE score <sup>[38]</sup>	
Score NIACE	Points
Nodules < 3	0
Nodules ≥ 3	1
Infiltrative HCC: No	0
Infiltrative HCC: Yes	1.5
AFP < 200 ng/mL (at baseline)	0
AFP ≥ 200 ng/mL (at baseline)	1.5
Child-Pugh grade A	0
Child-Pugh grade B	1.5
ECOG PS 0	0
ECOG PS ≥ 1	1.5

AFP: Alpha-fetoprotein; HCC: Hepatocellular carcinoma; ECOG (PS): Eastern Cooperative Oncology Group (Performance Status).

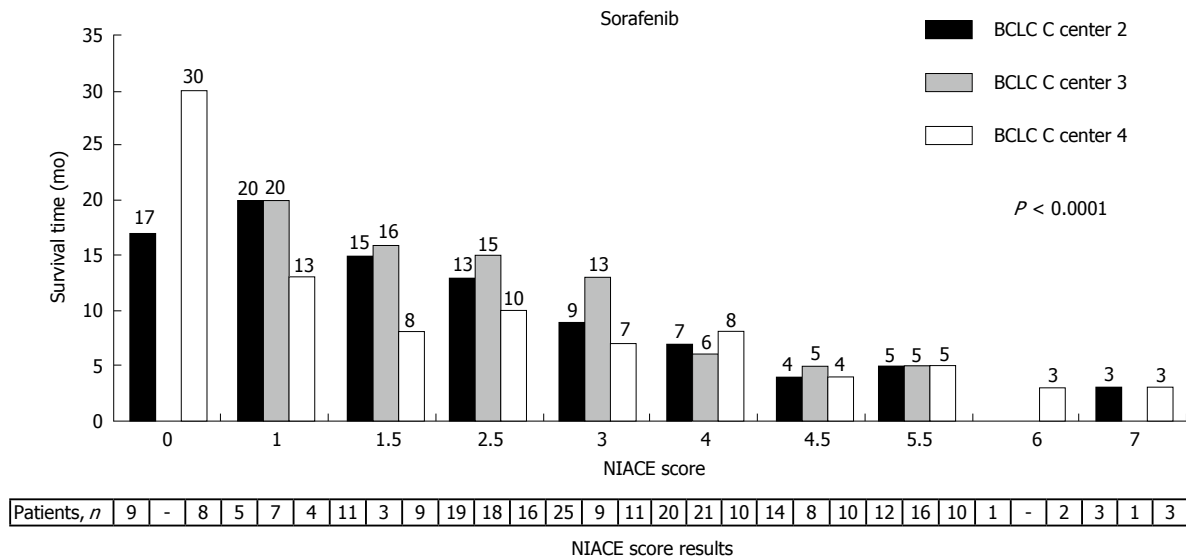
and HKLC classifications using a French multicenter HCC cohort of 1102 patients, of 68 (60-74) years of age, mostly with cirrhosis (81%), often linked to alcohol (41%) or hepatitis C (28%) or B (6%) viruses; most of the patients with Child-Pugh A and BCLC C scores, and treated according to the following modalities: Curative treatment in 22% of the cases (surgical resection or RFA), palliative treatment in 66% of the cases (TACE, sorafenib) and supportive care in 12% of the cases<sup>[44]</sup>. Each scoring system identified different prognosis subgroups ( $P < 0.0001$ ), with scores and classifications correlated with survival. The NIACE score showed the best homogeneity ( $LR \chi^2 = 532.0369$ ,  $P < 0.0001$ ), the best discriminative ability ( $LT \chi^2 = 91.6906$ ,  $P < 0.0001$ ), the lowest Akaike information criterion (AIC 10648.198) and the highest C-index [C-index 0.718 (0.688-0.748)] (Table 6). Using a threshold value of 1 or 2.5, the NIACE score identified 2 distinct prognosis groups within the



**Table 6** Comparison of prognostic performance of the NIACE, Barcelona Clinic Liver Cancer, Hong Kong Liver Cancer, and Cancer of the Liver Italian Program systems<sup>[44]</sup>

Score	Discriminatory ability linear trend test		Homogeneity likelihood ratio test		Akaike information criterion	C-index (95%CI)
	LT ( $\chi^2$ )	P value	LR ( $\chi^2$ )	P value		
NIACE	91.6906	< 0.0001	532.0369	< 0.0001	10648.198	0.718 (0.688-0.748)
BCLC	79.0342	< 0.0001	380.4100	< 0.0001	10805.825	0.674 (0.645-0.704)
HKLC	71.8861	< 0.0001	455.3169	< 0.0001	10740.918	0.698 (0.673-0.731)
CLIP	87.2785	< 0.0001	430.3872	< 0.0001	10749.848	0.716 (0.687-0.746)

BCLC: Barcelona Clinic Liver Cancer; CLIP: Cancer of the Liver Italian Program; HKLC: Hong Kong Liver Cancer; LR: Likelihood ratio; LT:  $\chi^2$  linear trend test.



**Figure 5** Evolution of the median overall survival according to the NIACE score in Barcelona Clinic Liver Cancer stage C patients from a French multicenter study, treated by sorafenib (black bars center 2, grey bars center 3, white bars center 4)<sup>[38]</sup>. BCLC: Barcelona Clinic Liver Cancer.

CLIP 0, 1, 2 and 3 groups ( $P < 0.0001$ ). As opposed to the HKLC, when applied to the various HKLC groups with similar survival (*i.e.*, II a/II b, IIIb/IVa, IVb/Vb), the NIACE score highlighted 2 different prognosis sub-groups using a threshold value of 3 ( $P < 0.0001$ ). The same results were obtained when investigating the HKLC I group using a threshold value of 1 ( $P < 0.0001$ )<sup>[45]</sup>.

In conclusion, the use of additional prognostic scores improves the stratification of HCC selected according to the BCLC system.

## HCC CLASSIFICATION AND PROGNOSTIC SCORES: A USEFUL COMPLEMENTARITY FOR TREATMENT CHOICE

### Prognostic scores benefit in HCC treatment: Before sorafenib

Sorafenib is recommended for BCLC stage C HCC<sup>[46,47]</sup> and is also a possible alternative for some BCLC stage B HCC being either progressive or confronted with chemoembolization contraindication<sup>[48]</sup>. The NIACE score allows to further stratify the BCLC stage C patients

treated with sorafenib (Figure 5), by separating two distinct groups with different survival using a threshold value of 3<sup>[38]</sup>. The survival of patients with a NIACE score  $> 3$  is limited to around 5.0 mo, despite a median treatment duration of 2 mo. Thus, this population does not seem to really benefit from the treatment and the NIACE score could be helpful in the treatment choice process or even earlier, to better classify patients before their enrollment into clinical trials.

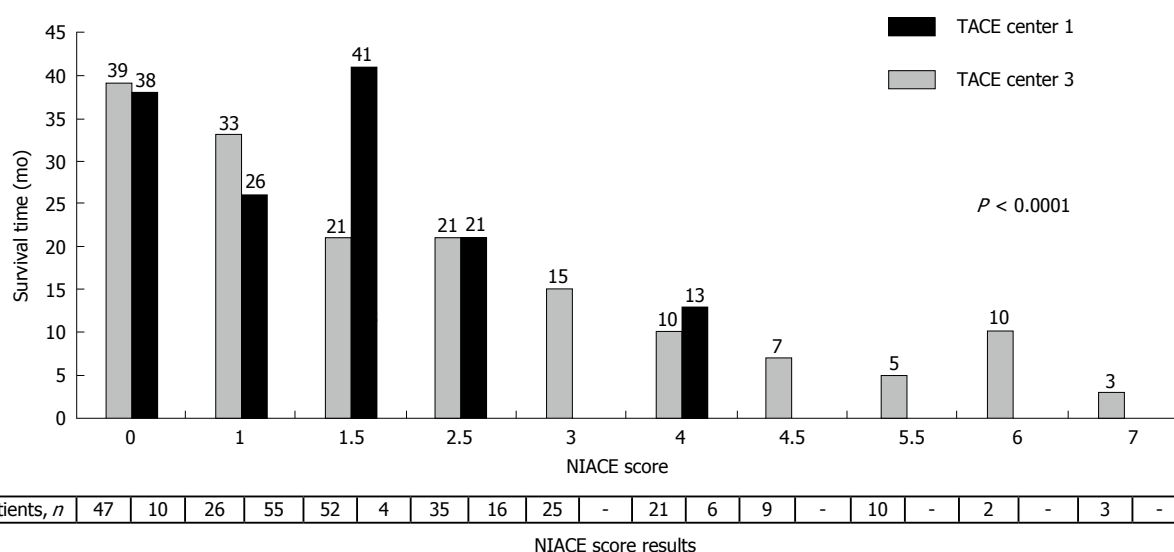
### Prognostic scores benefit in HCC treatment: Before chemoembolization

As chemoembolization is mainly recommended for intermediate BCLC stage B HCC<sup>[24]</sup>, the usefulness of any additional prognostic score for such cases appears limited to some experts. However, if TACE remains the main treatment modality in most countries confronted with this disease<sup>[1]</sup>, it is controversial. Its validation relies on two randomized studies with limited patients groups, mainly including intermediate and advanced HCC, and each offering a different treatment option<sup>[49,50]</sup>. Metadata analyses show contradictory results<sup>[51,52]</sup> and, despite the improvement of the selection criteria, the radiological response (according to the EASL or the mRECIST criteria)<sup>[53,54]</sup>, the existing contraindications<sup>[55]</sup>

**Table 7** Prognostic scores before the first transarterial chemoembolization

HAP (0 to 4 points)		NIACE (0 to 7 points)		STATE
Before the first TACE				
Albumin < 36 g/dL	1 point	≥ 3 nodules	1 point	Albumin (g/L)
Bilirubin > 17 mcmol/L	1 point	infiltrative HCC <i>vs</i> nodular HCC	1.5 points	-12 (tumour load exceeding the up-to-7 criteria)
			0	
AFP > 400 ng/mL	1 point	AFP ≥ 200 ng/mL	1.5 points	
		Child-Pugh A <i>vs</i> Child-Pugh B	0	
			1.5 points	
Size of dominant tumour > 70 mm	1 point	ECOG PS ≥ 1	1.5 points	-12 (if CRP ≥ 1 mg/dL)
No chemoembolization				
≥ 2 points		> 3 points		< 18 points

HAP: Hepatoma arterial-embolisation prognostic; AFP: Alpha-fetoprotein; TACE: Transarterial chemoembolization; HCC: Hepatocellular carcinoma; ECOG: Eastern Cooperative Oncology Group; PS: Performance status; CRP: C reactive protein; STATE: Selection for transarterial chemoembolisation treatment.



**Figure 6** Evolution of the median overall survival according to the NIACE score in hepatocellular carcinoma patients from a French multicenter study treated by transarterial chemoembolization (grey bars center 1, black bars center 2)<sup>[60]</sup>. TACE: Transarterial chemoembolization.

or treatment termination criteria<sup>[56]</sup>, there is still no consensus regarding the treatment strategy (on-demand or sequential), the number of treatments before reassessment<sup>[57]</sup>, the overall aim (stability or response)<sup>[55,56]</sup> or concerning the TACE mode (using conventional techniques or calibrated drug-eluting beads). An additional score could thus facilitate the treatment strategy choice.

### Before the first treatment

Several scores have been proposed recently to improve candidate patient selection (Table 7), as TACE is a potentially toxic treatment, with limited survival benefit. Among these pre-therapeutic scores, the Hepatoma Arterial-embolisation Prognostic (HAP) and the selection for transarterial chemoembolisation treatment (STATE) scores were determined from the prognostic variables of around a hundred of BCLC stage A, B (HAP, STATE) or even C (HAP) patients treated by TACE<sup>[58,59]</sup>. The NIACE score was also evaluated on two cohorts adding up 321 BCLC A, B or sometimes C (with distal portal vein thrombosis) patients treated by TACE. Using a threshold value of 3, the NIACE score identified two

groups presenting a significantly different survival (NIACE ≤ 3: 27 mo (24-31) *vs* NIACE > 3: 7 mo (6-10),  $P < 0.0001$ ), even without any stage C patients (Figure 6)<sup>[60]</sup>. It also separated two subgroups with distinct prognosis from an Asian cohort of patients treated by TACE<sup>[39]</sup>, as opposed to the HAP score which failed to prove its ability to select all the “good” candidates for TACE from a multicenter European cohort (with similar survival between the subgroups)<sup>[61]</sup>. Such a result could be anticipated as the same rating (1 point) is attributed to each variable and only HCC > 70 mm are taken into account, whereas the efficiency of the TACE treatment relies on the size (generally < 50 mm) and the number of nodules. The more recent STATE score, which mainly focuses on multinodular (BCLC B) HCC, still needs to be evaluated. The list presented here is not exhaustive and some relatively new scores now include indocyanine green clearance to better evaluate the liver function before TACE<sup>[29]</sup>, but often at the expense of simplicity, which should remain a priority.

The continuation of a TACE treatment is determined by the radiological response (which is correlated to

**Table 8** Pronostic scores before retreatment with transarterial chemoembolization

ART (0 to 8 points)		ABCR (-3 to 6 points)	
Before the second, the third TACE.....			
No radiological response	1 point	AFP < 200 ng/mL	0
		AFP ≥ 200 ng/mL	1 point
AST increased > 25%	4 points	BCLC A/B/C	0/2/3 points
Child-Pugh increased: 1 point	1.5 points	Child-Pugh increased ≥ 2 points	2 points
Increased: ≥ 2 points	3 points	Radiological response	-3 points
No chemoembolization			
ART ≥ 2.5 points		ABCR > 2 points	

TACE: Transarterial chemoembolization; ART: Assessment for retreatment with TACE; AST: Aspartate aminotransferase; AFP: Alpha-fetoprotein; BCLC: Barcelona Clinic Liver Cancer.

survival after TACE<sup>[53]</sup>, a decrease in AFP levels and the impact of the treatment on the liver function.

**After the first TACE:** Two scores easy to calculate were proposed to improve the selection of patients before repeating the treatment: The Assessment for Retreatment with TACE (ART) and the ABCR scores, both defined using regression models<sup>[62,63]</sup>. The ART score associates its higher coefficient with a possible increase in ASAT levels (4 points), the lower being associated with the radiological response (1 point). It is recommended not to repeat the treatment in case of a score worsening ≥ 2.5 points (Table 8). Conversely, the ABCR system assigns a higher coefficient to the radiological response (-3 points), which is correlated to survival after TACE and to the initial stage of the disease (BCLC A/B/C: 0/2/3 points). The associated threshold value is a score worsening > 2 points. Both scores are usable after the second treatment. From a European multicenter cohort, the ART score calculated before the second or the third TACE failed to orientate the treatment option for all the patients<sup>[61,63]</sup>. If, unlike the ABCR, it did discriminate two different prognosis subgroups, the evolution of the ART score was not correlated with survival. As expected, patients with an ART score of 1 (*i.e.*, no radiological response) presented a lower survival than the ART 4 (ASAT levels increase > 25%) patients. Among the ABCR score limitations stands the possible absence of radiological response after the first TACE, which affects almost 25% of the "late responders", depending on the series<sup>[64]</sup>. The score being contributory after the second TACE, it is recommended to repeat the treatment in the absence of obvious progression and in case of worsening hepatic function.

The prognostic ability of the ABCR score was higher than the HAP and ART systems on both Western<sup>[65]</sup> and Asian cohorts<sup>[66]</sup>.

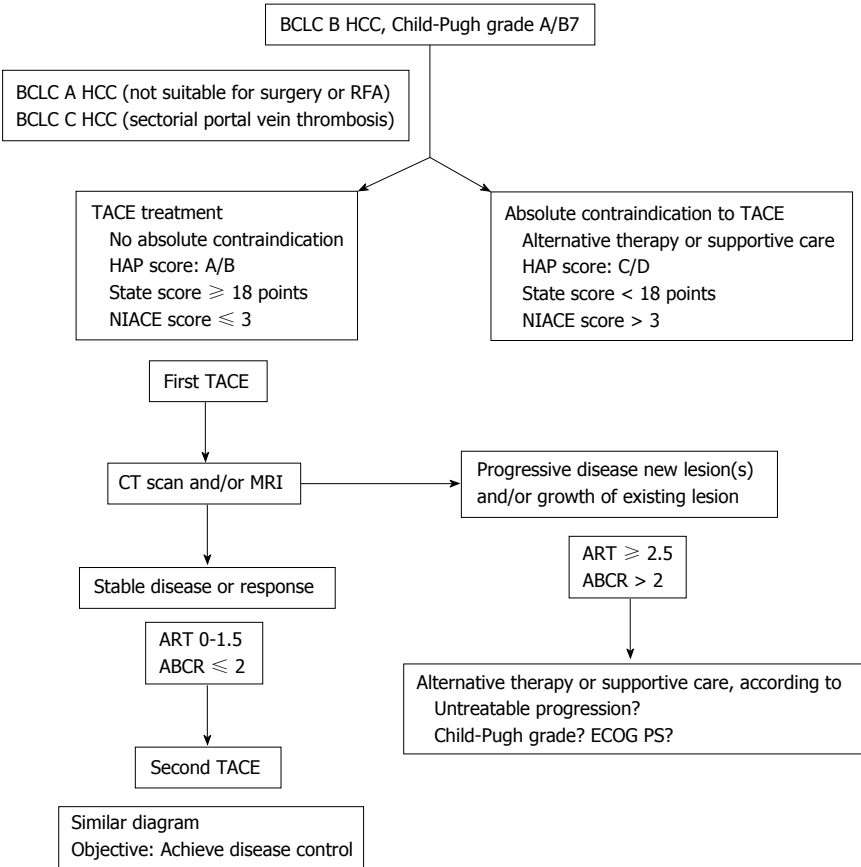
Overall, these pre-chemoembolization scores are not able to embrace all the patients or situations and cannot replace a multidisciplinary meeting. However, owing to the high number of patients treated following this modality, the heterogeneity of HCC and day-to-day practices, such scores could help in the therapy decision making process (Figure 7).

### Prognostic scores benefit in HCC treatment: Before surgical resection or radiofrequency

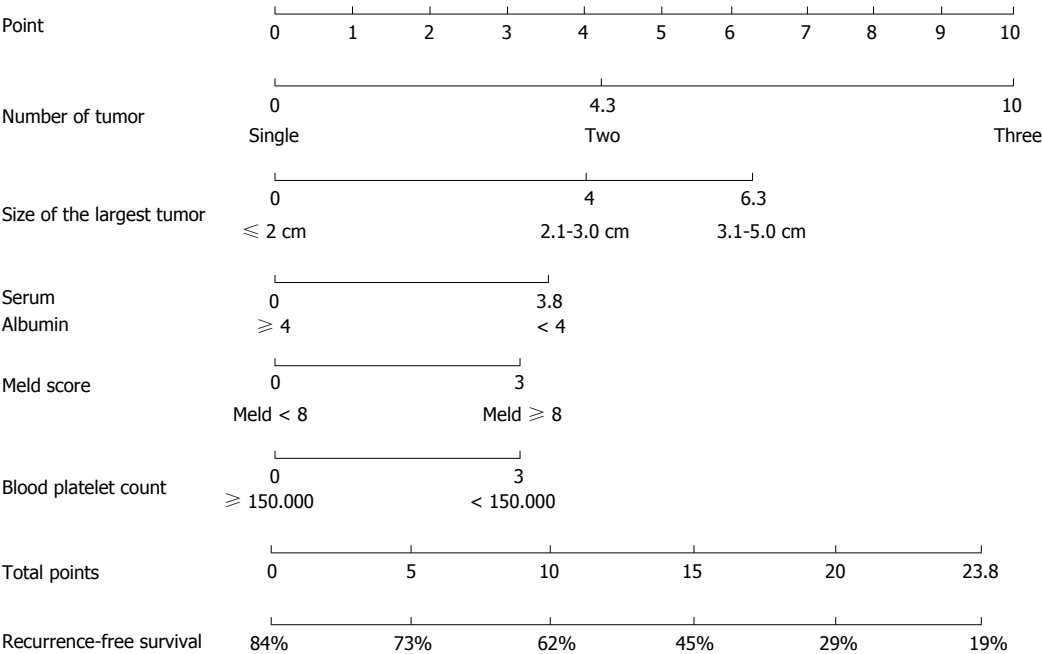
Surgical resection and radiofrequency ablation are curative treatments for HCC. In such cases, a score is not meant to exclude patients from the treatment when they meet the Barcelona criteria, early (BCLC A) stages being more homogeneous (single nodule or 3 nodules ≤ 3 cm), but to further evaluate their prognosis (overall survival and recurrence), in the prospect of a possible complementary treatment. This is illustrated by the nomogram recently proposed by Liu *et al.*<sup>[67]</sup> which orientates stage A HCC towards surgery or RFA according to the risk of recurrence (Figure 8). However, some experts have proposed to extend the indication for surgery beyond the Barcelona criteria to some intermediate or advanced HCC, which are more heterogeneous<sup>[27]</sup>. Despite some interesting results, only a proper randomized comparative study could address this question using a prognostic score to improve patient classification.

The NIACE score was tested on two French cohorts, both including around one hundred BCLC A/B and even C (single nodule with segmental portal vein thrombosis or above) HCC patients treated by surgery, thus beyond the scope of the BCLC recommendation, but in agreement with day-to-day practice. Using the more stringent threshold value of 1, it identified two different prognosis groups regarding the median overall survival (NIACE ≤ 1: 61 mo (36-81) vs NIACE > 1: 18 mo (9-73),  $P = 0.0005$ ) and the mean time to progression (NIACE ≤ 1, 26.9 ± 16.3 mo vs NIACE > 1, 9.2 ± 9.7 mo,  $P < 0.0001$ )<sup>[68]</sup>. The score evolution was inversely correlated to survival (Figure 9). Similar results were observed using an Asian cohort comprising around one hundred BCLC A/B/C HCC patients treated by surgery<sup>[39]</sup>.

When tested on a group of BCLC A HCC patients treated by surgery, selected from a French multicenter cohort, the NIACE score also highlighted two subgroups with distinct prognosis (median OS NIACE ≤ 1: 80 (58-81) mo vs NIACE > 1: 39 (28-58) mo,  $P = 0.0011$ ), notably among patients with a single tumor exceeding 50 mm in the longest axis (median OS NIACE ≤ 1: 80 (58-80) mo vs NIACE > 1: 35 (18-58) mo,  $P = 0.0024$ )<sup>[44]</sup>.



**Figure 7** Prognostic scores designed to transarterial chemoembolization, an aid to the decision making process: In practice. BCLC: Barcelona Clinic Liver Cancer; HCC: Hepatocellular carcinoma; TACE: Transarterial chemoembolization; CT: Computed tomography; ART: Assessment for Retreatment with TACE; ECOG (PS), Eastern Cooperative Oncology Group (Performance Status); RFA: Radiofrequency ablation; HAP: Hepatoma Arterial-embolisation Prognostic; MRI: Magnetic resonance imaging.



**Figure 8** Nomogram for hepatocellular carcinoma recurrence after radiofrequency ablation<sup>[67]</sup>.

These results should be further confirmed by a prospective study but, again, an additional prognostic score could provide complementary information to the BCLC system.





- for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607]
- 16 **Bruix J**, Sherman M, Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
- 17 **Han KH**, Kudo M, Ye SL, Choi JY, Poon RT, Seong J, Park JW, Ichida T, Chung JW, Chow P, Cheng AL. Asian consensus workshop report: expert consensus guideline for the management of intermediate and advanced hepatocellular carcinoma in Asia. *Oncology* 2011; **81** Suppl 1: 158-164 [PMID: 22212951 DOI: 10.1159/000333280]
- 18 **Kudo M**, Chung H, Osaki Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). *J Gastroenterol* 2003; **38**: 207-215 [PMID: 12673442 DOI: 10.1007/s005350300038]
- 19 **Ikai I**, Takayasu K, Omata M, Okita K, Nakanuma Y, Matsuyama Y, Makuuchi M, Kojiro M, Ichida T, Arii S, Yamaoka Y. A modified Japan Integrated Stage score for prognostic assessment in patients with hepatocellular carcinoma. *J Gastroenterol* 2006; **41**: 884-892 [PMID: 17048053 DOI: 10.1007/s00535-006-1878-y]
- 20 **Kitai S**, Kudo M, Minami Y, Ueshima K, Chung H, Hagiwara S, Inoue T, Ishikawa E, Takahashi S, Asakuma Y, Haji S, Osaki Y, Oka H, Seki T, Kasugai H, Sasaki Y, Matsunaga T. A new prognostic staging system for hepatocellular carcinoma: value of the biomarker combined Japan integrated staging score. *Intervirology* 2008; **51** Suppl 1: 86-94 [PMID: 18544953 DOI: 10.1159/000122599]
- 21 **Hsu CY**, Huang YH, Hsia CY, Su CW, Lin HC, Loong CC, Chiou YY, Chiang JH, Lee PC, Huo TI, Lee SD. A new prognostic model for hepatocellular carcinoma based on total tumor volume: the Taipei Integrated Scoring System. *J Hepatol* 2010; **53**: 108-117 [PMID: 20451283 DOI: 10.1016/j.jhep.2010.01.038]
- 22 **Yau T**, Tang VY, Yao TJ, Fan ST, Lo CM, Poon RT. Development of Hong Kong Liver Cancer staging system with treatment stratification for patients with hepatocellular carcinoma. *Gastroenterology* 2014; **146**: 1691-1700.e3 [PMID: 24583061 DOI: 10.1053/j.gastro.2014.02.032]
- 23 **Adhoute X**, Penaranda G, Bronowicki JP, Raoul JL. Usefulness of the HKLC vs. the BCLC staging system in a European HCC cohort. *J Hepatol* 2015; **62**: 492-493 [PMID: 25194894 DOI: 10.1016/j.jhep.2014.08.035]
- 24 **European Association For The Study Of The Liver**; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]
- 25 **Bolondi L**, Burroughs A, Dufour JF, Galle PR, Mazzaferro V, Piscaglia F, Raoul JL, Sangro B. Heterogeneity of patients with intermediate (BCLC B) Hepatocellular Carcinoma: proposal for a subclassification to facilitate treatment decisions. *Semin Liver Dis* 2012; **32**: 348-359 [PMID: 23397536 DOI: 10.1055/s-0032-1329906]
- 26 **Chang WT**, Kao WY, Chau GY, Su CW, Lei HJ, Wu JC, Hsia CY, Lui WY, King KL, Lee SD. Hepatic resection can provide long-term survival of patients with non-early-stage hepatocellular carcinoma: extending the indication for resection? *Surgery* 2012; **152**: 809-820 [PMID: 22766361 DOI: 10.1016/j.surg.2012.03.024]
- 27 **Torzilli G**, Belghiti J, Kokudo N, Takayama T, Capussotti L, Nuzzo G, Vauthey JN, Choti MA, De Santibanes E, Donadon M, Morengi E, Makuuchi M. A snapshot of the effective indications and results of surgery for hepatocellular carcinoma in tertiary referral centers: is it adherent to the EASL/AASLD recommendations?: an observational study of the HCC East-West study group. *Ann Surg* 2013; **257**: 929-937 [PMID: 23426336 DOI: 10.1097/SLA.0b013e31828329b8]
- 28 **Vitale A**, Burra P, Frigo AC, Trevisani F, Farinati F, Spolverato G, Volk M, Giannini EG, Ciccarese F, Piscaglia F, Rapaccini GL, Di Marco M, Caturelli E, Zoli M, Borzio F, Cabibbo G, Felder M, Gasbarrini A, Sacco R, Foschi FG, Missale G, Morisco F, Svegliati Baroni G, Virdone R, Cillo U. Survival benefit of liver resection for patients with hepatocellular carcinoma across different Barcelona Clinic Liver Cancer stages: a multicentre study. *J Hepatol* 2015; **62**: 617-624 [PMID: 25450706 DOI: 10.1016/j.jhep.2014.10.037]
- 29 **Xu L**, Peng ZW, Chen MS, Shi M, Zhang YJ, Guo RP, Lin XJ, Lau WY. Prognostic nomogram for patients with unresectable hepatocellular carcinoma after transcatheter arterial chemoembolization. *J Hepatol* 2015; **63**: 122-130 [PMID: 25725438 DOI: 10.1016/j.jhep.2015.02.034]
- 30 **Xue TC**, Xie XY, Zhang L, Yin X, Zhang BH, Ren ZG. Transarterial chemoembolization for hepatocellular carcinoma with portal vein tumor thrombus: a meta-analysis. *BMC Gastroenterol* 2013; **13**: 60 [PMID: 23566041 DOI: 10.1186/1471-230X-13-60]
- 31 **Llovet JM**, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, Brú C, Rodés J, Bruix J. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. *Hepatology* 1999; **29**: 62-67 [PMID: 9862851 DOI: 10.1002/hep.510290145]
- 32 **Huitzil-Melendez FD**, Capanu M, O'Reilly EM, Duffy A, Gansukh B, Saltz LL, Abou-Alfa GK. Advanced hepatocellular carcinoma: which staging systems best predict prognosis? *J Clin Oncol* 2010; **28**: 2889-2895 [PMID: 20458042 DOI: 10.1200/JCO.2009.25.9895]
- 33 **Collette S**, Bonnetain F, Paoletti X, Doffoel M, Bouché O, Raoul JL, Rougier P, Masskouri F, Bedenne L, Barbare JC. Prognosis of advanced hepatocellular carcinoma: comparison of three staging systems in two French clinical trials. *Ann Oncol* 2008; **19**: 1117-1126 [PMID: 18303031 DOI: 10.1093/annonc/mdn030]
- 34 **Zhang JF**, Shu ZJ, Xie CY, Li Q, Jin XH, Gu W, Jiang FJ, Ling CQ. Prognosis of unresectable hepatocellular carcinoma: comparison of seven staging systems (TNM, Okuda, BCLC, CLIP, CUPI, JIS, CIS) in a Chinese cohort. *PLoS One* 2014; **9**: e88182 [PMID: 24609114 DOI: 10.1371/journal.pone.0088182]
- 35 **Zhao Y**, Wang WJ, Guan S, Li HL, Xu RC, Wu JB, Liu JS, Li HP, Bai W, Yin ZX, Fan DM, Zhang ZL, Han GH. Sorafenib combined with transarterial chemoembolization for the treatment of advanced hepatocellular carcinoma: a large-scale multicenter study of 222 patients. *Ann Oncol* 2013; **24**: 1786-1792 [PMID: 23508822 DOI: 10.1093/annonc/mdt072]
- 36 **Yau T**, Yao TJ, Chan P, Ng K, Fan ST, Poon RT. A new prognostic score system in patients with advanced hepatocellular carcinoma not amenable to locoregional therapy: implication for patient selection in systemic therapy trials. *Cancer* 2008; **113**: 2742-2751 [PMID: 18853421 DOI: 10.1002/cncr.23878]
- 37 **Leung TW**, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, Lau JT, Yu SC, Johnson PJ. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system: a study based on 926 patients. *Cancer* 2002; **94**: 1760-1769 [PMID: 11920539]
- 38 **Adhoute X**, Penaranda G, Raoul JL, Blanc JF, Edeline J, Conroy G, Perrier H, Pol B, Bayle O, Monnet O, Beaurain P, Muller C, Castellani P, Bronowicki JP, Bourlière M. Prognosis of advanced hepatocellular carcinoma: a new stratification of Barcelona Clinic Liver Cancer stage C: results from a French multicenter study. *Eur J Gastroenterol Hepatol* 2016; **28**: 433-440 [PMID: 26695429 DOI: 10.1097/MEG.0000000000000558]
- 39 **Su TH**, Liu CJ, Yang HC, Liu CH, Chen PJ, Chen DS. The NIACE score helps predict the survival of Asian hepatocellular carcinoma patients. *J Gastroenterol Hepatol* 2015; **30**: 23
- 40 **Rosenkrantz AB**, Lee L, Matza BW, Kim S. Infiltrative hepatocellular carcinoma: comparison of MRI sequences for lesion conspicuity. *Clin Radiol* 2012; **67**: e105-e111 [PMID: 23026725 DOI: 10.1016/j.crad.2012.08.019]
- 41 **Benvegnù L**, Noventa F, Bernardinello E, Pontisso P, Gatta A, Alberti A. Evidence for an association between the aetiology of cirrhosis and pattern of hepatocellular carcinoma development. *Gut* 2001; **48**: 110-115 [PMID: 11115831]
- 42 **Bruix J**, Sherman M, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

- 43 **Ochiai T**, Sonoyama T, Ichikawa D, Fujiwara H, Okamoto K, Sakakura C, Ueda Y, Otsuji E, Itoi H, Hagiwara A, Yamagishi H. Poor prognostic factors of hepatectomy in patients with resectable small hepatocellular carcinoma and cirrhosis. *J Cancer Res Clin Oncol* 2004; **130**: 197-202 [PMID: 14770307 DOI: 10.1007/s00432-003-0533-8]
- 44 **Adhoute X**, Penaranda G, Raoul JL, Bourlière M. Hepatocellular carcinoma scoring and staging systems. Do we need new tools? *J Hepatol* 2016; **64**: 1449-1450 [PMID: 26912407 DOI: 10.1016/j.jhep.2016.01.038]
- 45 **Adhoute X**, Penaranda G, Raoul JL, Bourlière M. "Staging of Hepatocellular Carcinoma: BCLC system, what else?" *Liver Int* 2016; Epub ahead of print [PMID: 26778275 DOI: 10.1111/liv.13066]
- 46 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]
- 47 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]
- 48 **Bruix J**, Raoul JL, Sherman M, Mazzaferro V, Bolondi L, Craxi A, Galle PR, Santoro A, Beaugrand M, Sangiovanni A, Porta C, Gerken G, Marrero JA, Nadel A, Shan M, Moscovici M, Voliotis D, Llovet JM. Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma: subanalyses of a phase III trial. *J Hepatol* 2012; **57**: 821-829 [PMID: 22727733 DOI: 10.1016/j.jhep.2012.06.014]
- 49 **Lo CM**, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, Fan ST, Wong J. Randomised controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**: 1164-1171 [PMID: 11981766 DOI: 10.1053/jhep.2002.33156]
- 50 **Llovet JM**, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Murchat J, Solà R, Rodés J, Bruix J. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862 DOI: 10.1016/S0140-6736(02)08649-X]
- 51 **Oliveri RS**, Wetterslev J, Gluud C. Transarterial (chemo)embolisation for unresectable hepatocellular carcinoma. *Cochrane Database Syst Rev* 2011; **(3)**: CD004787 [PMID: 21412886 DOI: 10.1002/14651858.CD004787.pub2]
- 52 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794 DOI: 10.1053/jhep.2003.50047]
- 53 **Gillmore R**, Stuart S, Kirkwood A, Hameeduddin A, Woodward N, Burroughs AK, Meyer T. EASL and mRECIST responses are independent prognostic factors for survival in hepatocellular cancer patients treated with transarterial embolization. *J Hepatol* 2011; **55**: 1309-1316 [PMID: 21703196 DOI: 10.1016/j.jhep.2011.03.007]
- 54 **Kim BK**, Kim KA, Park JY, Ahn SH, Chon CY, Han KH, Kim SU, Kim MJ. Prospective comparison of prognostic values of modified Response Evaluation Criteria in Solid Tumours with European Association for the Study of the Liver criteria in hepatocellular carcinoma following chemoembolisation. *Eur J Cancer* 2013; **49**: 826-834 [PMID: 22995582 DOI: 10.1016/j.ejca.2012.08.022]
- 55 **Raoul JL**, Sangro B, Forner A, Mazzaferro V, Piscaglia F, Bolondi L, Lencioni R. Evolving strategies for the management of intermediate-stage hepatocellular carcinoma: available evidence and expert opinion on the use of transarterial chemoembolization. *Cancer Treat Rev* 2011; **37**: 212-220 [PMID: 20724077 DOI: 10.1016/j.ctrv.2010.07.006]
- 56 **Bruix J**, Reig M, Rimola J, Forner A, Burrel M, Vilana R, Ayuso C. Clinical decision making and research in hepatocellular carcinoma: pivotal role of imaging techniques. *Hepatology* 2011; **54**: 2238-2244 [PMID: 21932394 DOI: 10.1002/hep.24670]
- 57 **Wang W**, Zhao Y, Bai W, Han G. Response assessment for HCC patients treated with repeated TACE: The optimal time-point is still an open issue. *J Hepatol* 2015; **63**: 1530-1531 [PMID: 26256436 DOI: 10.1016/j.jhep.2015.07.031]
- 58 **Kadalayil L**, Benini R, Pallan L, O'Beirne J, Marelli L, Yu D, Hackshaw A, Fox R, Johnson P, Burroughs AK, Palmer DH, Meyer T. A simple prognostic scoring system for patients receiving transarterial embolisation for hepatocellular cancer. *Ann Oncol* 2013; **24**: 2565-2570 [PMID: 23857958 DOI: 10.1093/annonc/ndt247]
- 59 **Hucke F**, Pinter M, Graziadei I, Bota S, Vogel W, Müller C, Heinzl H, Waneck F, Trauner M, Peck-Radosavljevic M, Sieghart W. How to STATE suitability and START transarterial chemoembolization in patients with intermediate stage hepatocellular carcinoma. *J Hepatol* 2014; **61**: 1287-1296 [PMID: 25016222 DOI: 10.1016/j.jhep.2014.07.002]
- 60 **Adhoute X**, Penaranda G, Raoul JL, Perrier H, Castellani P, Conroy G. Hepatocellular carcinoma, NIACE score: an aid to the decision-making process before the first transarterial chemoembolization. *J Gastroenterol Hepatol* 2015; **30**: 339-340
- 61 **Adhoute X**, Penaranda G, Castellani P, Perrier H, Bourlière M. Recommendations for the use of chemoembolization in patients with hepatocellular carcinoma: Usefulness of scoring system? *World J Hepatol* 2015; **7**: 521-531 [PMID: 25848475 DOI: 10.4254/wjh.v7.i3.521]
- 62 **Sieghart W**, Hucke F, Pinter M, Graziadei I, Vogel W, Müller C, Heinzl H, Trauner M, Peck-Radosavljevic M. The ART of decision making: retreatment with transarterial chemoembolization in patients with hepatocellular carcinoma. *Hepatology* 2013; **57**: 2261-2273 [PMID: 23316013 DOI: 10.1002/hep.26256]
- 63 **Adhoute X**, Penaranda G, Naude S, Raoul JL, Perrier H, Bayle O, Monnet O, Beaurain P, Bazin C, Pol B, Folgoc GL, Castellani P, Bronowicki JP, Bourlière M. Retreatment with TACE: the ABCR SCORE, an aid to the decision-making process. *J Hepatol* 2015; **62**: 855-862 [PMID: 25463541 DOI: 10.1016/j.jhep.2014.11.014]
- 64 **Georgiades C**, Geschwind JF, Harrison N, Hines-Peralta A, Liapi E, Hong K, Wu Z, Kamel I, Frangakis C. Lack of response after initial chemoembolization for hepatocellular carcinoma: does it predict failure of subsequent treatment? *Radiology* 2012; **265**: 115-123 [PMID: 22891361 DOI: 10.1148/radiol.12112264]
- 65 **Adhoute X**, Penaranda G, Castellani P, Perrier H, Naude S, Monnet M. Unresectable hepatocellular carcinoma (HCC) treated by chemoembolization. What prognostic score use: ART, HAP, ABCR? A comparative French multicenter study. *J Gastroenterol Hepatol* 2014; **29**: 178-179
- 66 **Yang H**, Bae SH, Lee S, Lee HL, Jang JW, Choi JY. Korean validation and comparison of prognostic scores for transarterial chemoembolization: ART, ABCR, HAP and modified HAP. *Hepatology* 2015; **62**: 437A
- 67 **Liu PH**, Hsu CY, Lee YH, Hsia CY, Huang YH, Su CW, Chiou YY, Lin HC, Huo TI. When to Perform Surgical Resection or Radiofrequency Ablation for Early Hepatocellular Carcinoma?: A Nomogram-guided Treatment Strategy. *Medicine (Baltimore)* 2015; **94**: e1808 [PMID: 26512576 DOI: 10.1097/MD.0000000000001808]
- 68 **Adhoute X**, Penaranda G, Raoul JL, Pol B, Bollon E, Perrier H. Hepatocellular carcinoma, NIACE score: A simple tool to better distinguish patients at risk of relapse after surgery. *J Gastroenterol Hepatol* 2015; **30**: 341

**P- Reviewer:** Cerwenka HR, Frenette C, Johnson PJ

**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Liu SQ



2016 Hepatitis B Virus: Global view

## Innate immune targets of hepatitis B virus infection

Zhi-Qiang Zou, Li Wang, Kai Wang, Ji-Guang Yu

Zhi-Qiang Zou, Li Wang, Ji-Guang Yu, Infectious Disease Hospital of Yantai, Yantai 264001, Shandong Province, China

Kai Wang, Hepatology Department, Qilu Hospital of Shandong University, Jinan 250012, Shandong Province, China

**Author contributions:** Zou ZQ, Wang L and Yu JG performed literature search; Wang L and Wang K designed and wrote manuscript.

**Conflict-of-interest statement:** All the authors of the manuscript declare that they have no conflict of interest in connection with this paper.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Li Wang, PhD, Infectious Disease Hospital of Yantai, 62 Huanshan Road, Zhifu District, Yantai 264001, Shandong Province, China. [liliwang2200@163.com](mailto:liliwang2200@163.com)  
 Telephone: +86-535-6232253  
 Fax: +86-535-6628542

Received: March 25, 2016

Peer-review started: March 25, 2016

First decision: April 19, 2016

Revised: May 19, 2016

Accepted: June 1, 2016

Article in press: June 3, 2016

Published online: June 18, 2016

### Abstract

Approximately 400 million people are chronically infected with hepatitis B virus (HBV) globally despite

the widespread immunization of HBV vaccine and the development of antiviral therapies. The immunopathogenesis of HBV infection is initiated and driven by complexed interactions between the host immune system and the virus. Host immune responses to viral particles and proteins are regarded as the main determinants of viral clearance or persistent infection and hepatocyte injury. Innate immune system is the first defending line of host preventing from virus invasion. It is acknowledged that HBV has developed active tactics to escape innate immune recognition or actively interfere with innate immune signaling pathways and induce immunosuppression, which favor their replication. HBV reduces the expression of pattern-recognition receptors in the innate immune cells in humans. Also, HBV may interrupt different parts of antiviral signaling pathways, leading to the reduced production of antiviral cytokines such as interferons that contribute to HBV immunopathogenesis. A full comprehension of the mechanisms as to how HBV inactivates various elements of the innate immune response to initiate and maintain a persistent infection can be helpful in designing new immunotherapeutic methods for preventing and eradicating the virus. In this review, we aimed to summarize different branches the innate immune targeted by HBV infection. The review paper provides evidence that multiple components of immune responses should be activated in combination with antiviral therapy to disrupt the tolerance to HBV for eliminating HBV infection.

**Key words:** Hepatitis B virus; Infection; Targets; Innate immune response; Signaling pathway

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The pathogenesis of hepatitis B virus (HBV) infection is initiated and driven by complicated interplays between the virus and the host immune system. HBV DNA and different HBV proteins have various effects on different arms of innate immune system. The extent of HBV replication as well as the amounts of circulating



HBV antigens and different source of HBV proteins have heterogeneous effects on innate immune responses and antiviral signaling pathways. Other factors, such as liver inflammation may also have impact on innate immune response. Multiple components of immune responses should be activated in combination with antiviral therapy to disrupt the tolerance to HBV for eliminating HBV infection.

Zou ZQ, Wang L, Wang K, Yu JG. Innate immune targets of hepatitis B virus infection. *World J Hepatol* 2016; 8(17): 716-725 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i17/716.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i17.716>

## INTRODUCTION

Though hepatitis B virus (HBV) vaccine has been available for several decades and much progress has been made on anti-HBV therapeutics, there are still more than 350 million people chronically infected with HBV worldwide. The immunopathogenesis of HBV infection is initiated and propelled by complicated interactions between the host immune system and the virus<sup>[1]</sup>.

It is recognized that host immune responses to HBV antigens are the major determinants of HBV pathogenesis and hepatocytes damage in the liver. On the contrary, viruses also exert immune regulatory effects to favor their replication. HBV has evolved active tactics to escape innate immune recognition and induce immunosuppression<sup>[2]</sup>. This has been displayed through the fact that HBV particles and proteins can be detectable around 5 wk postinfection, after which viral loads reach a logarithmic amplification stage<sup>[3]</sup>. The reason of the lag of viral replication is that the virus manages to evade being sensed by the innate immune system in the early phase of infection when the adaptive immune system has not been fully activated. HBV genome is 3.2 kbp in length and contains four overlapping genes that encode for the nucleocapsid (precore and core), polymerase, envelope (pre S and S), and hepatitis X proteins. The abundant HBV particles and viral proteins in the circulation in chronic HBV-infected patients allow multiple interactions among the virus, its viral proteins, and the immune system. HBV DNA and different HBV proteins have various effects on different parts of host immune systems, including immune cells and signaling pathways. The extent of HBV replication as well as the amounts of circulating HBV antigens, especially surface antigen (HBsAg), leads to heterogeneous profiles of the immune response, particularly in the context of chronic infection manifested as patients' different clinical profiles<sup>[4-6]</sup>. The immune tolerance phase has the highest level of serum HBsAg and hepatitis B e antigen (HBeAg) quantitation<sup>[7]</sup>. High levels of viremia, particularly high amounts of HBsAg, not only suppress innate immune cells, including monocytes, dendritic cells (DCs), natural

killer (NK) cells, and NKT cells, through direct interaction, but also lead to exhaustion of cytotoxic T lymphocytes (CTLs) and helper T (Th) cells<sup>[8]</sup>. HBsAg mutations which enhanced the capability to avoid immune response were associated with HBV reactivation in a quite different clinical profiles<sup>[9]</sup>. Also, reduced viremia through antiviral therapy partially restores the impaired immune response<sup>[10]</sup> and the restored immune response status correlated with the levels of HBV infection parameters<sup>[11]</sup>, which indirectly demonstrated the immune suppressive effect of HBV and its proteins.

A full understanding of the mechanisms as to how the virus inactivates various components of the immune system to maintain a persistent infection can help establish a new theory for designing novel immunotherapeutic methods and aid the eradication of the virus in chronic HBV infection.

Innate immune pathways are the targets of HBV to evade host antiviral responses contributing to chronicity of infection. The components of the innate immune system targeted by HBV include pattern-recognition receptors (PRRs), DCs, NKs, NKT cells, and antiviral signaling pathways<sup>[2]</sup>. In addition to directly regulating the innate immune response, HBV also modulates the innate immunity through alteration of the expression of microRNAs (miRNAs)<sup>[12]</sup>.

### PRRs

PRRs, including toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I) - like receptors, and NOD-like receptors (NLRs), are crucial for sensing invading pathogens, initiating innate immune responses, restricting the spread of infection, and facilitating effective adaptive immune responses<sup>[13]</sup>. The early inhibition of the innate immune response by HBV is mainly through TLR-3 and RIG-I /melanoma differentiation-associated gene 5 (MDA5) signaling pathways, which leads to decreased expression of several proinflammatory and antiviral cytokine genes<sup>[14]</sup>. In the setting of chronic HBV infection, reduced TLR expression and interference of PRRs signaling pathways lead to the impairment of host innate immune response.

### TLRs

TLRs sense pathogen-associated molecule patterns (PAMPs), including nucleic acid sequences in degraded viral particles, and activate antiviral mechanisms, including intracellular antiviral pathways, production of antiviral effector interferons (IFNs) and proinflammatory cytokines, and initiation of adaptive immunity<sup>[15]</sup>. TLR signaling pathways are important parts of the innate immune response in HBV infection. It has been demonstrated that TLR ligands could suppress HBV replication<sup>[16]</sup>. Also, the activation of TLRs plays an important part in preventing intrauterine HBV transmission<sup>[17]</sup>. Accumulating evidence has consistently shown that the expression and function of TLRs in immune cells reduced during chronic HBV infection<sup>[18]</sup>. Expressions of TLR2 mRNA and protein were remarkably reduced

in peripheral blood monocytes (PBMCs) derived from chronic hepatitis B (CHB) patients<sup>[19]</sup>.

HBV virions or proteins such as HBsAg and HBeAg may reduce TLR expression and abrogate TLR-induced antiviral activity. The inhibitory mechanisms include suppressing IFN- $\beta$  production and induction of IFN-stimulated genes (ISG) and transcription factors, such as IFN regulatory factor 3 (IRF3) and nuclear factor- $\kappa$ B (NF- $\kappa$ B)<sup>[20]</sup>. HBsAg, HBeAg, and HBV particles could inhibit the activation of nonparenchymal liver cells by TLR3 ligands<sup>[20]</sup>. Jiang *et al.*<sup>[21]</sup> demonstrated that TLR-induced the expression of IFN- $\gamma$ , ISGs, and proinflammatory cytokines in murine Kupffer cells (KCs) and liver sinusoidal endothelial cells (LSECs), and the activation of NF- $\kappa$ B, IRF3, and mitogen-activated protein kinases (MAPKs) in hepatocytes were strongly suppressed by HBsAg. TLR3-stimulated KCs and LSECs mediated T-cell activation was also suppressed by HBsAg. Visvanathan *et al.*<sup>[22]</sup> first showed that TLR2 expression on liver cells, KCs, and PBMCs significantly reduced in HBeAg-positive CHB patients compared with HBeAg-negative CHB and controls. TLR2 detects several microbial PAMPs and subsequently activates NF- $\kappa$ B in a myeloid differentiation primary response gene 88 (MyD88)-dependent manner. Therefore, decreased TLR2 expression may lead to impairment of immune responses to HBV infection<sup>[23]</sup>. In addition to directly inhibits the TLR2-mediated c-Jun N-terminal kinase/MAPK pathway, HBsAg may also induce interleukin (IL)-10 production in monocytes indirectly<sup>[24,25]</sup>. Thus, TLR2 is an important immune target of HBV infection.

Toll/IL-1 receptor (TIR) domain-containing adapter protein inducing IFN- $\beta$  (TRIF) is an important component in innate immune signaling pathways. It is one of the main intracellular adapter proteins required for TLR3 and TLR4 signaling. Ayoobi *et al.*<sup>[26]</sup> suggested that the expression of TRIF significantly decreased in PBMCs isolated from CHB patients compared with those isolated from healthy subjects. TRIF protein was also downregulated in human hepatoma cell lines and liver tissue specimens infected with HBV<sup>[27]</sup>. HBeAg interacted with TRIF-related adaptor molecule (TRAM), Mal, and TLR2 at the subcellular level, and mutated HBeAg not only may disrupt the interaction between Mal and MyD88 but also ablate homotypic TIR:TIR interaction, which is crucial for TLR-mediated signaling<sup>[28]</sup>. Furthermore, HBeAg can suppress TIR and IL-1 $\beta$ -mediated activation of the inflammatory transcription factors, such as NF- $\kappa$ B and inhibit NF- $\kappa$ B and IFN- $\beta$  promoter activity<sup>[29]</sup>. These results suggest the presence of intracellular precore protein in addition to secreted extracellular HBeAg.

Hepatitis B virus X (HBx) and polymerase (Pol) are the proteins that interfere with the PRRs pathways most frequently<sup>[2,30]</sup>. For instance, HBx reduced TRIF protein expression *via* the proteasomal pathway in a dose-dependent manner<sup>[31]</sup>. However, no direct convincing evidence indicating that HBV RNAs, DNAs and proteins are authentically recognized by TLRs is available up to date. The interplay between HBV proteins and TLRs

should be verified directly *in vivo* through further investigation<sup>[15]</sup>.

### RIG- I - MDA5 pathway

MDA5 and RIG-1 as the PRRs play important roles in viral mRNA recognition. HBx and HBV Pol are involved most frequently in the inactivation of the RIG- I pathways and ultimately impaired IFN production. HBx is a pivotally protein involved in HBV-associated liver diseases. Studies<sup>[32]</sup> indicate that HBx can interact with the mitochondrial membrane protein virus-induced signaling adapter (VISA), which is a key adapter protein downstream RIG- I and MDA5, and interrupts the association of VISA with its upstream and downstream parts. This inhibits the induction of type I IFNs through the activation of transcription factors, including NF- $\kappa$ B and IRF3. Human cell line studies<sup>[33]</sup> have also suggested that adapter protein mitochondrial antiviral signaling (MAVS) is another target for HBx. The RIG- I /MDA5 pathway and IFN- $\beta$  induction is inhibited due to degradation of MAVS promoted by HBx. A recent study<sup>[34]</sup> showed that mRNA levels of MDA5 and RIG- I dramatically decreased in CHB patients in comparison with healthy controls. However, these mRNA levels have little alteration among CHB patients with different states of HBeAg and HBV DNA viral loads. Moreover, RIG- I could also offset the interaction of HBV Pol with the 5'- $\epsilon$  region, which suggest that RIG- I dually actions as an HBV sensor activating innate signaling and counteracting viral Pol in human liver cells<sup>[35]</sup>. Therefore, the mechanism underlying the downregulation of MDA5 may attribute to several reasons in patients with CHB<sup>[34]</sup>. DDX3, an HBV Pol binding protein, belonging to the DEAD-box RNA helicase family, is associated with mRNA metabolism. HBV Pol blocks PRRs signaling *via* interaction with DDX3<sup>[36]</sup>. This may explain the mechanism of how HBV evading the innate immune response.

In contrast, Luangsay *et al.*<sup>[14]</sup> found that the early inhibition of dsRNA-mediated response resulted from the HBV inoculum, but not HBsAg or HBeAg itself. Whereas, the significance of these results in the human needs to be confirmed.

### DCs

DCs are key cells in the initiation of adaptive immune responses because of their ability of processing foreign antigens and presenting them to effector cells. Also, mature DCs can efficiently induce T-cell polarization to Th1 and generate HBcAg-specific CTLs<sup>[37]</sup>. A long-lasting debate exists on the functionality and phenotypes of DCs in chronic HBV infection. Several studies demonstrated that DCs functions were impairment in CHB patients, which included decreased expression of co-stimulatory molecules, defective cytokine production, and reduced allostimulatory capacity compared with healthy people<sup>[38,39]</sup>.

However, Gehring *et al.*<sup>[40]</sup> suggested that the fre-

quency and function of myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) were largely intact *ex vivo* in HBV infected patients except for the reduced IFN- $\alpha$  production compared with those of healthy donor DCs. They found that reduced IFN- $\alpha$  production did not correlate with viral titer, which suggested that viral antigens had slight impact on DCs function. The major confusion about the function of DCs resulted from studies which indicated that function of healthy donor DCs was impaired exposing to various sources of HBV *in vitro*, which are in contrast to the results obtained from CHB patients. Tavakoli *et al.*<sup>[41]</sup> also demonstrated that phenotypes and functionality of circulating total DCs, mDCs, or pDCs are unaffected in chronically HBV infected patients whether experimented *ex vivo* or after *in vitro* activation and maturation. They demonstrated that isolated mDCs and pDCs from chronic HBV carriers showed the similar expression of co-stimulatory molecules and alloreactive T cell stimulation as that of the control DCs.

However, other studies showed that pDCs were the targets of HBV. Both HBV virions and purified HBsAg have immune modulatory functions and may directly contribute to the impairment of mDCs functions in chronic HBV infection<sup>[42]</sup>. HBV particles and HBsAg were capable of abrogating TLR9-induced IFN- $\alpha$  gene transcription *via* combining to TLR9-triggered pDC directly<sup>[43]</sup>. HBV not only directly interfered with pDC function, but also indirectly disturbed monocyte-pDC interaction. In addition, the ability of inducing the cytolytic activity of NK cells by TLR9-activated pDCs from CHB patients were also compromised<sup>[44]</sup>. Virus-like particles (VLPs) comprising small HBV envelope protein (HBsAgS) impaired IFN- $\alpha$  production of pDCs in response to CpG *in vitro*<sup>[45]</sup>. Op den Brouw *et al.*<sup>[42]</sup> suggested that in the presence of HBV or HBsAg, cytokine-induced maturation resulted in a more tolerogenic mDC phenotype, as demonstrated by a significantly reduced upregulation of co-stimulatory molecules and a decreased T-cell stimulatory capacity, as demonstrated by T-cell proliferation and production of IFN- $\gamma$  and IL-12. It has been shown that DCs from immune tolerant patients showed a prominently lower expression of CD80, CD86, and HLA-DR and demonstrated an injured stimulatory capacity in mixed lymphocyte reactions and decreased production of IL-12, compared with those in the inactive HBsAg carrier state. Also, no remarkable difference was observed between the indexes from inactive carrier and healthy controls<sup>[46]</sup>. Several studies<sup>[47,48]</sup> revealed that mDC frequency could return to the level of healthy donors, IL12p70 production increased, and lower expression of phenotypic molecules was restored with antiviral therapy of adefovir and lamivudine. These results indirectly demonstrated the suppressive effect of high loads of HBV particles and proteins on DCs.

Though with suppressive effect on DCs functions, HBsAg is also a component of HBV vaccine. HBsAg-pulsed DCs might promote HBV-specific immune response in CHB patients<sup>[49]</sup>. HBsAgS VLPs can deliver an

antigen to both major histocompatibility complex (MHC)-I and MHC-II in primary DCs and facilitate cytotoxic and helper T-cell priming<sup>[45]</sup>. Also, Ag-Ab immune complexes could be easily captured and taken up by DCs<sup>[50]</sup>, and could efficiently induce HBs-specific T cells. A clinical study showed that the immunity was enhanced by autologous HBsAg-activated DC-cytokine-induced killer cells as adoptive immunotherapy<sup>[51]</sup>. Martinet *et al.*<sup>[52]</sup> showed that the vaccination of Hepato-HuPBL mice with the HBc/HBs peptide-loaded pDCs induced HBV-specific T cells with specific ability of lysing the transfected hepatocytes. In addition, HBeAg might have a negative effect on the generation of DCs from bone marrow precursors<sup>[53]</sup>.

The mechanism underlying the suppressive influence of HBV and HBV proteins on the function of DCs has not been fully elucidated. Some reports indicated that HBV and HBsAg can enter the DCs and cause damage, leading to a decline in the number of DCs and functional impairment<sup>[45,54]</sup>. However, Tavakoli *et al.*<sup>[41]</sup> found that viral mRNA was not detectable by reverse transcription-polymerase chain reaction in both DC populations, which argues against viral replication in DCs.

The arguments regarding the functions and phenotypes of DCs result from the heterogeneous source of HBV antigens, variability of patients, and assay methods of DC maturation and cytokine production *in vitro* across studies<sup>[40]</sup>. In addition, liver pathology also likely affects the function of pDCs. Studies show that IFN- $\alpha$  production by pDCs is negatively correlated with the serum alanine aminotransferase (ALT) level in patients with CHB<sup>[39,43]</sup>. Furthermore, the expression of inhibitory molecule programmed death-ligand 1 (PD-1) in mDCs tended to more closely relate to ALT level than to viral load<sup>[55]</sup>.

## NK AND NKT CELLS

NK cells, the main innate immune cells, play indispensable roles in the clearance of HBV from hepatocytes. Although the numbers, subset distribution, and cytotoxic capacity of NK cells were retained, their activation and IFN- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$  production, particularly of the CD56(dim) subset, were strongly hampered in patients with CHB compared with healthy controls<sup>[56]</sup>. NK cells express several kinds of stimulatory and inhibitory receptors, which interact with their respective ligands results in functional activation and suppression<sup>[57]</sup>. Activation status and surface receptor expression patterns of NK cells may be altered in HBV infection<sup>[2]</sup>. Natural killer group 2D (NKG2D) is a well-characterized activating receptor expressed on NK cells, NKT cells, and CD8(+) cytotoxic T cells, which binds to a diverse group of ligands that resemble the MHC-class I molecules. Accumulating evidence has shown that NKG2D-ligand interactions play a crucial role in the persistence of HBV infection and the development of liver injury and hepatocellular carcinoma. The expression of NKG2D ligands may be modulated post-trans-

criptionally by HBV<sup>[1]</sup>. Also, high serum HBV DNA loads upregulate the expression of inhibitory receptors such as NKG2A, but downregulate activating receptors, CD16, NKp30, NKG2D, and NKp46<sup>[56,58,59]</sup>. One study showed that the expression of NKp46 negatively correlated with the HBV DNA level and was much higher in inactive HBsAg carriers compared with active infection patients. NKp46 activation may restore NK cell cytolytic activity to HepG2 and HepG2.215 cell lines *in vitro*<sup>[59]</sup>. Furthermore, NK cell phenotype and functionality may partially be restored by viral load reduction through antiviral therapy, as shown by downregulated expression of NKG2A and improved IFN- $\gamma$  production as a result of an increased ability of CD56(dim) NK cells<sup>[56]</sup>. And the recovered function of NK cells was strongly associated with HBsAg clearance<sup>[60]</sup>. Under the combination treatment of pegylated IFN- $\alpha$ -2a and adefovir, compared with nonresponders, responders had a remarkably lower expression of NKG2A on CD56(dim) NK cells and higher CD56 (bright) TNF-related apoptosis-inducing ligand expression and IFN- $\gamma$  production at the end of the treatment. These results were not observed in HBeAg-positive patients who developed HBeAg seroconversion without HBsAg clearance<sup>[60]</sup>. The spontaneous reduction of HBV loads had similar results<sup>[61]</sup>.

In addition, HBeAg may inhibit IFN- $\gamma$  production by NK cells mediated by IL-18 in a dose-dependent manner<sup>[62]</sup>. HBV may specifically suppress pDC-induced IFN- $\gamma$  production by NK cells without affecting their cytolytic ability through pDC-NK cell cross-talk<sup>[63]</sup>. Although NK cell IFN- $\gamma$  production was impaired in response to TLR9 stimulation in CHB patients compared with controls, the upregulation of CD69 expression in response to TLR9 was maintained<sup>[64]</sup>.

NKT cells are a unique subgroup of T-cells expressing both NK cell surface marker-CD56 and a T-cell receptor CD3 - which are stimulated by lipid antigens. NK and NKT are the two cell types that are promptly activated in the early phase of HBV infection, which probably contribute to controlling the HBV invasion and allowing timely induction of adaptive immune responses<sup>[65]</sup>. While, it has demonstrated that the frequency of hepatic NKT cells from HBV transgenic mice was low and the capability of producing IFN- $\gamma$  was impaired<sup>[66]</sup>. Reports by Jiang *et al*<sup>[67]</sup> and Zhu *et al*<sup>[68]</sup> indicated that the frequency of peripheral invariant NKT (iNKT) cells is lower in patients with chronic HBV infection than in healthy subjects, and returns to normal levels during viral control with telbivudine. In patients treated with PEG-IFN- $\alpha$ , the ratio of peripheral blood NKT cells in T lymphocytes before, during, and after treatment significantly elevated in the significant-effect group compared with the effect and no-effect groups<sup>[69]</sup>. This implies that NKT cells modulate the innate immune response against HBV infection and play a major role in effective antiviral treatment.

The mechanism underlying the decrease in the number and function of circulating iNKT cells in patients with CHB remains unclear. It is believed that this

reduction is at least partially due to trafficking to the liver<sup>[70]</sup> because iNKT cells express a high level of CC chemokine receptor 5 (CCR5) and CCR6<sup>[67]</sup> which enable iNKT cells migrate toward the liver. Other mechanism may involved in the high expression of inhibitory molecules PD-1 and Tim-3, and lower the expression of CD28<sup>[66,71]</sup>. In addition, HBV-induced lipid alterations also contributed to a change in NKT cell function<sup>[72]</sup>.

## IMMUNE TARGET OF SIGNALING PATHWAYS OF THE ANTIVIRAL RESPONSE AND CYTOKINES

Recognition of viral infections by PRRs, such as TLRs and RIG- I /MDA5, activates signaling pathways and leads to the induction of inflammatory and antiviral cytokines, such as type I IFN, that limit viral replication and initiation of adaptive immunity. The expression of TLR signaling molecules, such as MyD88, IL-1 receptor-associated kinase 1 (IRAK1), and IRAK4, significantly decreased in PBMCs from CHB patients compared with healthy controls<sup>[73,74]</sup>.

HBV proteins, such as HBV Pol and HBx, could interfere with multiple sites of intracellular signaling pathways triggered by HBV infection, preventing IFN production and antiviral responses in hepatocytes<sup>[30,75]</sup>. HBV Pol can inhibit TANK-binding kinase 1 (TBK1)/IkappaB kinase-epsilon (IKKi), the effector kinases of IRF signaling. It can block IRF signaling activation mediated by TLR-3 or RIG- I recognizing dsRNA in the endosomes or in the cytosol through interaction with DDX3, a transcriptional factor of the IFN- $\beta$  promoter in human hepatoma cell lines<sup>[30,32,36]</sup>. HBV Pol mediates blockage of IFN- $\alpha$  signaling through suppressing IFN- $\alpha$ -induced signal transducers and activators of transcription 1 (STAT1) serine 727 phosphorylation and STAT1/2 nuclear accumulation<sup>[76]</sup>. Pol also affects STAT methylation through increasing protein phosphatase 2A (PP2A) expression, which inhibits protein arginine methyltransferase 1, the enzyme that catalyzes the methylation of STAT1<sup>[77]</sup>. This may be responsible for HBV resistance to PEG-IFN- $\alpha$  therapy<sup>[78]</sup>. However, HBV Pol does not interfere with STAT1 degradation and phosphorylation<sup>[79]</sup>. The cytosolic DNA sensor and key adaptor stimulator of IFN genes (STING) has been suggested to be critical in multiple foreign DNA-elicited innate immune signaling. Screening analysis demonstrated that the reverse transcriptase and the RNase H (RH) domains of HBV Pol were responsible for the inhibition of STING-stimulated IRF3 activation and IFN- $\beta$  induction<sup>[80]</sup>. One study has demonstrated that HBV Pol preferentially suppresses TNF- $\alpha$ -, TLR3- or TLR4-induced NF- $\kappa$ B signaling by inhibiting the activity of IKK complex through disrupting the association of IKK/NF- $\kappa$ B essential modulator (NEMO) with Cdc37/Hsp90 $\beta$  in hepatoma cells<sup>[81]</sup>. Therefore, in addition to its inherent catalytic function, HBV Pol has multifunctional immunomodulatory effects. It may counteract the innate



**Table 1** Innate immune cells, molecules and signaling pathways targeted by hepatitis B virus and hepatitis B virus proteins

HBV and HBV proteins	Innate immune cells, molecules and signaling pathways	Ref.
HBs	TLR, ISG, IRF3, IFN- $\beta$ and NF- $\kappa$ B	[20]
	KCs, LSECs, IFN- $\gamma$ , ISGs, MAPKs, TLR3	[21]
	JNK/MAPK, $\kappa$ B $\alpha$	[24,25,87]
	mDCs, pDCs, TLR-9	[42,43]
HBe	Hepatocytes, KCs, PBMCs, and TLR2, ISG, IRF3, IFN- $\beta$ and NF- $\kappa$ B	[20,22]
	TRAM, Mal, TLR2, and TIR:TIR	[28]
	NF- $\kappa$ B and IFN- $\beta$ promoter, IFN- $\gamma$	[29,62]
	RIPK2	[83]
HBx	TRIF, RIG-I/MDA5, VISA, MAVS, NF- $\kappa$ B	[31-33,82]
	NEMO, TBK1, IKKi, and IRF3	[75]
HBV Pol	RIG-I, DDX3, NEMO-Cdc37/Hsp90 $\beta$	[35,36,81]
	TBK1/IKKi, STAT1, PP2A, STING	[32,33,36,76,77,80]
HBV	NK, NKG2D, NKG2A, CD16, NKp30, and NKp46	[56,58,59]
	pDC-NK, NKT	[63,72]
	CTHRC1	[84]

HBs: Hepatitis B surface antigen; HBe: Hepatitis B e antigen; HBx: Hepatitis B x protein; HBV Pol: Hepatitis B polymerase; TLRs: Toll-like receptors; ISG: Interferon-stimulated genes; IFN: Interferon; NF- $\kappa$ B: Nuclear factor  $\kappa$ B; KCs: Kupffer cells; LSECs: Liver sinusoidal endothelial cells; MAPKs: Mitogen-activated protein kinases; JNK: c-Jun N-terminal kinase; mDCs: Myeloid dendritic cells; pDCs: Plasmacytoid DCs; PBMCs: Peripheral blood mononuclear cells; TRAM: TRIF-related adaptor molecule; TIR: Toll/interleukin-1 receptor; RIPK2: Receptor-interacting serine/threonine protein kinase 2; TRIF: TIR domain-containing adapter protein inducing IFN- $\beta$ ; RIG-I: Retinoic acid inducible gene I; IRF: Interferon-regulatory factors; MDA5: Melanoma differentiation associated gene 5; VISA: Virus-induced signaling adapter; MAVS: Mitochondrial antiviral signaling; TBK1: TANK-binding kinase 1; IKKi: KappaB kinase-epsilon; STAT1: Signal transducers and activators of transcription 1; PP2A: Protein phosphatase 2A; STING: Stimulator of IFN genes; NK: Natural killer; NKG2D: NK group 2D; NKG2A: NK group 2A; NKT: NK T cell; CTHRC1: Collagen triple helix repeat containing 1; HBV: Hepatitis B virus; MAPK: Mitogen-activated protein kinase; NEMO: NF- $\kappa$ B essential modulator.

responses at different steps.

Similar to HBV Pol, HBx can target multiple points of signaling pathways negatively regulating type I IFN production. In addition to RIG-I, TNF receptor-associated factor 3, and TRIF, HBx also interacts with NEMO, TBK1, kinase-epsilon (IKKi), and IRF3<sup>[75]</sup>. HBx can also transactivate multiple transcription factors including NF- $\kappa$ B that regulates inflammatory-related genes. A recent report has suggested that HBx-evolutionarily conserved signaling intermediate in toll pathways interaction plays an important role in IL-1 $\beta$  induction of NF- $\kappa$ B activation<sup>[82]</sup>.

In addition to HBV Pol and HBx proteins, HBeAg may also modulate the intracellular signaling pathways. HBeAg may target receptor-interacting serine/threonine protein kinase 2 through inhibiting its expression and interacting with it<sup>[83]</sup> which may results in inactivation of NF- $\kappa$ B. Experiments indicate that collagen triple helix repeat containing 1 (CTHRC1) expressed in HBV-transfected cells facilitates HBV replication in cultured cells and BALB/c mice. On the other hand, HBV increases CTHRC1 expression, which downregulates the activity of type I IFN, the transcription of ISGs, and the phosphorylation of STAT1/2<sup>[84]</sup>.

However, some of the signaling pathways are important in restraining HBV replication. Tzeng *et al.*<sup>[85]</sup> demonstrated that not IFN- $\alpha/\beta$  receptor, RIG-I, MDA5, MyD88, NLR pyrin containing 3, caspase recruitment domain, and IL-1R but TNF- $\alpha$  is essential for HBV eradication. In the absence of TNF- $\alpha$ , or early treatment

with the soluble blocker of TNF receptor in mice leads to HBV persistence<sup>[86]</sup>. This may explain the mechanism of HBV reactivation during TNF blockage agents therapy.

In contrast to HBeAg, research has reported that the treatment of human monocyte-derived DCs with HBsAg resulted in enhanced cell surface expression of CD80, CD83, CD86, and MHC-II, and increased IL-12 p40, IL-12p70, and IL-10 production through decreasing inhibition of  $\kappa$ B $\alpha$  concentrations and MAPK phosphorylation<sup>[87]</sup>.

## CONCLUSION

The suppression of various innate immune components targeted by HBV and HBV proteins may result in virus spread and subsequent inefficient adaptive immune responses, leading to HBV persistence. However, still controversies exist regarding the effects of HBV on the functionalities and phenotypes of innate immune cells, especially DCs. The conflicting results may be due to patient diversity, divergence of antigen sources, and inconsistent assay methods. Some of the findings derived from cell line and animal models remain to be defined for the human HBV infection. Furthermore, the knowledge of the exact mechanism of action of HBV and HBV proteins on some of the sites of the complicated innate signaling pathways is lacking. The updated findings of innate immune cells, molecules and signaling pathways targeted by HBV and HBV proteins are summarized in Table 1. The present study

provides evidence that multiple components of immune responses should be activated in combination with antiviral therapy to disrupt the tolerance to HBV for eliminating HBV infection.

## REFERENCES

- Pollicino T, Koumbi L. Role natural killer group 2D-ligand interactions in hepatitis B infection. *World J Hepatol* 2015; **7**: 819-824 [PMID: 25937859 DOI: 10.4254/wjh.v7.i6.819]
- Busca A, Kumar A. Innate immune responses in hepatitis B virus (HBV) infection. *Viral J* 2014; **11**: 22 [PMID: 24507433 DOI: 10.1186/1743-422X-11-22]
- Fong TL, Di Bisceglie AM, Biswas R, Waggoner JG, Wilson L, Claggett J, Hoofnagle JH. High levels of viral replication during acute hepatitis B infection predict progression to chronicity. *J Med Virol* 1994; **43**: 155-158 [PMID: 8083663 DOI: 10.1002/jmv.1890430210]
- Webster GJ, Reingart S, Brown D, Ogg GS, Jones L, Seneviratne SL, Williams R, Dusheiko G, Bertoletti A. Longitudinal analysis of CD8+ T cells specific for structural and nonstructural hepatitis B virus proteins in patients with chronic hepatitis B: implications for immunotherapy. *J Virol* 2004; **78**: 5707-5719 [PMID: 15140968 DOI: 10.1128/JVI.78.11.5707-5719.2004]
- Boni C, Fiscaro P, Valdatta C, Amadei B, Di Vincenzo P, Giuberti T, Laccabue D, Zerbini A, Cavalli A, Missale G, Bertoletti A, Ferrari C. Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. *J Virol* 2007; **81**: 4215-4225 [PMID: 17287266 DOI: 10.1128/JVI.02844-06]
- Loggi E, Bihl FK, Cursaro C, Granieri C, Galli S, Brodosi L, Furlini G, Bernardi M, Brander C, Andreone P. Virus-specific immune response in HBeAg-negative chronic hepatitis B: relationship with clinical profile and HBsAg serum levels. *PLoS One* 2013; **8**: e65327 [PMID: 23750252 DOI: 10.1371/journal.pone.0065327]
- Wang L, Zou ZQ, Wang K, Yu JG, Liu XZ. Role of serum hepatitis B virus marker quantitation to differentiate natural history phases of HBV infection. *Hepatol Int* 2016; **10**: 133-138 [PMID: 26427997 DOI: 10.1007/s12072-015-9657-6]
- Kondo Y, Ninomiya M, Kakazu E, Kimura O, Shimosegawa T. Hepatitis B surface antigen could contribute to the immunopathogenesis of hepatitis B virus infection. *ISRN Gastroenterol* 2013; **2013**: 935295 [PMID: 23401786 DOI: 10.1155/2013/935295]
- Salpini R, Colagrossi L, Bellocchi MC, Surdo M, Becker C, Alteri C, Aragri M, Ricciardi A, Armenia D, Pollicita M, Di Santo F, Carioti L, Louzoun Y, Mastroianni CM, Lichtner M, Paoloni M, Esposito M, D'Amore C, Marrone A, Marignani M, Sarrecchia C, Sarmati L, Andreoni M, Angelico M, Verheyen J, Perno CF, Svicher V. Hepatitis B surface antigen genetic elements critical for immune escape correlate with hepatitis B virus reactivation upon immunosuppression. *Hepatology* 2015; **61**: 823-833 [PMID: 25418031 DOI: 10.1002/hep.27604]
- Boni C, Laccabue D, Lampertico P, Giuberti T, Viganò M, Schivazappa S, Alfieri A, Pesci M, Gaeta GB, Brancaccio G, Colombo M, Missale G, Ferrari C. Restored function of HBV-specific T cells after long-term effective therapy with nucleos(t)ide analogues. *Gastroenterology* 2012; **143**: 963-73.e9 [PMID: 22796241 DOI: 10.1053/j.gastro.2012.07.014]
- Li CZ, Hu JJ, Xue JY, Yin W, Liu YY, Fan WH, Xu H, Liang XS. Viral infection parameters not nucleoside analogue itself correlates with host immunity in nucleoside analogue therapy for chronic hepatitis B. *World J Gastroenterol* 2014; **20**: 9486-9496 [PMID: 25071343 DOI: 10.3748/wjg.v20.i28.9486]
- Jiang X, Kanda T, Wu S, Nakamura M, Miyamura T, Nakamoto S, Banerjee A, Yokosuka O. Regulation of microRNA by hepatitis B virus infection and their possible association with control of innate immunity. *World J Gastroenterol* 2014; **20**: 7197-7206 [PMID: 24966589 DOI: 10.3748/wjg.v20.i23.7197]
- Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* 2010; **140**: 805-820 [PMID: 20303872 DOI: 10.1016/j.cell.2010.01.022]
- Luangsay S, Gruffaz M, Isorce N, Testoni B, Michelet M, Faure-Dupuy S, Maadadi S, Ait-Goughoulte M, Parent R, Rivoire M, Javanbakht H, Lucifora J, Durantel D, Zoulim F. Early inhibition of hepatocyte innate responses by hepatitis B virus. *J Hepatol* 2015; **63**: 1314-1322 [PMID: 26216533 DOI: 10.1016/j.jhep.2015.07.014]
- Ma Z, Zhang E, Yang D, Lu M. Contribution of Toll-like receptors to the control of hepatitis B virus infection by initiating antiviral innate responses and promoting specific adaptive immune responses. *Cell Mol Immunol* 2015; **12**: 273-282 [PMID: 25418467 DOI: 10.1038/cmi.2014.112]
- Isogawa M, Robek MD, Furuichi Y, Chisari FV. Toll-like receptor signaling inhibits hepatitis B virus replication in vivo. *J Virol* 2005; **79**: 7269-7272 [PMID: 15890966]
- Tian T, Sun D, Wang P, Wang H, Bai X, Yang X, Wang Z, Dong M. Roles of Toll-like Receptor 7 and 8 in Prevention of Intrauterine Transmission of Hepatitis B Virus. *Cell Physiol Biochem* 2015; **37**: 445-453 [PMID: 26315138 DOI: 10.1159/000430367]
- Vincent IE, Zannetti C, Lucifora J, Norder H, Protzer U, Hainaut P, Zoulim F, Tommasino M, Trépo C, Hasan U, Chemin I. Hepatitis B virus impairs TLR9 expression and function in plasmacytoid dendritic cells. *PLoS One* 2011; **6**: e26315 [PMID: 22046272 DOI: 10.1371/journal.pone.0026315]
- Chen Z, Cheng Y, Xu Y, Liao J, Zhang X, Hu Y, Zhang Q, Wang J, Zhang Z, Shen F, Yuan Z. Expression profiles and function of Toll-like receptors 2 and 4 in peripheral blood mononuclear cells of chronic hepatitis B patients. *Clin Immunol* 2008; **128**: 400-408 [PMID: 18565796 DOI: 10.1016/j.clim.2008.04.006]
- Wu J, Meng Z, Jiang M, Pei R, Trippier M, Broering R, Bucchi A, Sowa JP, Dittmer U, Yang D, Roggendorf M, Gerken G, Lu M, Schlaak JF. Hepatitis B virus suppresses toll-like receptor-mediated innate immune responses in murine parenchymal and nonparenchymal liver cells. *Hepatology* 2009; **49**: 1132-1140 [PMID: 19140219 DOI: 10.1002/hep.22751]
- Jiang M, Broering R, Trippier M, Poggenpohl L, Fiedler M, Gerken G, Lu M, Schlaak JF. Toll-like receptor-mediated immune responses are attenuated in the presence of high levels of hepatitis B virus surface antigen. *J Viral Hepat* 2014; **21**: 860-872 [PMID: 24498958 DOI: 10.1111/jvh.12216]
- Visvanathan K, Skinner NA, Thompson AJ, Riordan SM, Sozzi V, Edwards R, Rodgers S, Kurtovic J, Chang J, Lewin S, Desmond P, Locarnini S. Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. *Hepatology* 2007; **45**: 102-110 [PMID: 17187404 DOI: 10.1002/hep.21482]
- Bagheri V, Askari A, Arababadi MK, Kennedy D. Can Toll-Like Receptor (TLR) 2 be considered as a new target for immunotherapy against hepatitis B infection? *Hum Immunol* 2014; **75**: 549-554 [PMID: 24530748 DOI: 10.1016/j.humimm.2014.02.018]
- Shi B, Ren G, Hu Y, Wang S, Zhang Z, Yuan Z. HBsAg inhibits IFN- $\alpha$  production in plasmacytoid dendritic cells through TNF- $\alpha$  and IL-10 induction in monocytes. *PLoS One* 2012; **7**: e44900 [PMID: 23024774]
- Wang S, Chen Z, Hu C, Qian F, Cheng Y, Wu M, Shi B, Chen J, Hu Y, Yuan Z. Hepatitis B virus surface antigen selectively inhibits TLR2 ligand-induced IL-12 production in monocytes/macrophages by interfering with JNK activation. *J Immunol* 2013; **190**: 5142-5151 [PMID: 23585678 DOI: 10.4049/jimmunol.1201625]
- Ayoobi F, Hassanshahi G, Zainodini N, Khorramdelazad H, Arababadi MK, Kennedy D. Reduced expression of TRIF in chronic HBV infected Iranian patients. *Clin Res Hepatol Gastroenterol* 2013; **37**: 491-495 [PMID: 23433963 DOI: 10.1016/j.clinre.2012.11.005]
- Hong Y, Zhou L, Xie H, Zheng S. Innate immune evasion by hepatitis B virus-mediated downregulation of TRIF. *Biochem Biophys Res Commun* 2015; **463**: 719-725 [PMID: 26047698 DOI: 10.1016/j.bbrc.2015.05.130]
- Lang T, Lo C, Skinner N, Locarnini S, Visvanathan K, Mansell A. The hepatitis B e antigen (HBeAg) targets and suppresses activation of the toll-like receptor signaling pathway. *J Hepatol* 2011; **55**: 762-769 [PMID: 21334391 DOI: 10.1016/j.jhep.2010.12.042]
- Wilson R, Warner N, Ryan K, Selleck L, Colledge D, Rodgers S, Li K, Revell P, Locarnini S. The hepatitis B e antigen suppresses IL-1 $\beta$ -mediated NF- $\kappa$ B activation in hepatocytes. *J Viral Hepat*

- 2011; **18**: e499-e507 [PMID: 21914069 DOI: 10.1111/j.1365-2893.2011.01484.x]
- 30 **Yu S**, Chen J, Wu M, Chen H, Kato N, Yuan Z. Hepatitis B virus polymerase inhibits RIG-I- and Toll-like receptor 3-mediated beta interferon induction in human hepatocytes through interference with interferon regulatory factor 3 activation and dampening of the interaction between TBK1/IKKepsilon and DDX3. *J Gen Virol* 2010; **91**: 2080-2090 [PMID: 20375222 DOI: 10.1099/vir.0.020552-0]
  - 31 **Zhang G**, Li N, Li Z, Zhu Q, Li F, Yang C, Han Q, Lv Y, Zhou Z, Liu Z. microRNA-4717 differentially interacts with its polymorphic target in the PD1 3' untranslated region: A mechanism for regulating PD-1 expression and function in HBV-associated liver diseases. *Oncotarget* 2015; **6**: 18933-18944 [PMID: 25895129]
  - 32 **Wang X**, Li Y, Mao A, Li C, Li Y, Tien P. Hepatitis B virus X protein suppresses virus-triggered IRF3 activation and IFN-beta induction by disrupting the VISA-associated complex. *Cell Mol Immunol* 2010; **7**: 341-348 [PMID: 20711230 DOI: 10.1038/cmi.2010.36]
  - 33 **Wei C**, Ni C, Song T, Liu Y, Yang X, Zheng Z, Jia Y, Yuan Y, Guan K, Xu Y, Cheng X, Zhang Y, Yang X, Wang Y, Wen C, Wu Q, Shi W, Zhong H. The hepatitis B virus X protein disrupts innate immunity by downregulating mitochondrial antiviral signaling protein. *J Immunol* 2010; **185**: 1158-1168 [PMID: 20554965 DOI: 10.4049/jimmunol.0903874]
  - 34 **Ebrahim M**, Mirzaei V, Bidaki R, Shabani Z, Daneshvar H, Karimi-Googheri M, Khaleghinia M, Afrooz MR, Yousefpoor Y, Arababadi MK. Are RIG-I and MDA5 Expressions Associated with Chronic HBV Infection? *Viral Immunol* 2015; **28**: 504-508 [PMID: 26485346 DOI: 10.1089/vim.2015.0056]
  - 35 **Sato S**, Li K, Kameyama T, Hayashi T, Ishida Y, Murakami S, Watanabe T, Iijima S, Sakurai Y, Watashi K, Tsutsumi S, Sato Y, Akita H, Wakita T, Rice CM, Harashina H, Kohara M, Tanaka Y, Takaoka A. The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. *Immunity* 2015; **42**: 123-132 [PMID: 25557055 DOI: 10.1016/j.immuni.2014.12.016]
  - 36 **Wang H**, Ryu WS. Hepatitis B virus polymerase blocks pattern recognition receptor signaling via interaction with DDX3: implications for immune evasion. *PLoS Pathog* 2010; **6**: e1000986 [PMID: 20657822 DOI: 10.1371/journal.ppat.1000986]
  - 37 **Dai S**, Zhuo M, Song L, Chen X, Yu Y, Tang Z, Zang G. Dendritic cell-based vaccination with lentiviral vectors encoding ubiquitinated hepatitis B core antigen enhances hepatitis B virus-specific immune responses in vivo. *Acta Biochim Biophys Sin (Shanghai)* 2015; **47**: 870-879 [PMID: 26373843 DOI: 10.1093/abbs/gmv093]
  - 38 **Wang FS**, Xing LH, Liu MX, Zhu CL, Liu HG, Wang HF, Lei ZY. Dysfunction of peripheral blood dendritic cells from patients with chronic hepatitis B virus infection. *World J Gastroenterol* 2001; **7**: 537-541 [PMID: 11819824 DOI: 10.3748/wjg.v7.i4.537]
  - 39 **van der Molen RG**, Sprengers D, Binda RS, de Jong EC, Niesters HG, Kusters JG, Kwekkeboom J, Janssen HL. Functional impairment of myeloid and plasmacytoid dendritic cells of patients with chronic hepatitis B. *Hepatology* 2004; **40**: 738-746 [PMID: 15349914]
  - 40 **Gehring AJ**, Ann D'Angelo J. Dissecting the dendritic cell controversy in chronic hepatitis B virus infection. *Cell Mol Immunol* 2015; **12**: 283-291 [PMID: 25363524 DOI: 10.1038/cmi.2014.95]
  - 41 **Tavakoli S**, Mederacke I, Herzog-Hauff S, Glebe D, Grün S, Strand D, Urban S, Gehring A, Galle PR, Böcher WO. Peripheral blood dendritic cells are phenotypically and functionally intact in chronic hepatitis B virus (HBV) infection. *Clin Exp Immunol* 2008; **151**: 61-70 [PMID: 18031557 DOI: 10.1111/j.1365-2249.2007.03547.x]
  - 42 **Op den Brouw ML**, Binda RS, van Roosmalen MH, Protzer U, Janssen HL, van der Molen RG, Woltman AM. Hepatitis B virus surface antigen impairs myeloid dendritic cell function: a possible immune escape mechanism of hepatitis B virus. *Immunology* 2009; **126**: 280-289 [PMID: 18624732 DOI: 10.1111/j.1365-2567.2008.02896.x]
  - 43 **Woltman AM**, Op den Brouw ML, Biesta PJ, Shi CC, Janssen HL. Hepatitis B virus lacks immune activating capacity, but actively inhibits plasmacytoid dendritic cell function. *PLoS One* 2011; **6**: e15324 [PMID: 21246041 DOI: 10.1371/journal.pone.0015324]
  - 44 **Martinet J**, Dufeu-Duchesne T, Bruder Costa J, Larrat S, Marlu A, Leroy V, Plumas J, Aspod C. Altered functions of plasmacytoid dendritic cells and reduced cytolytic activity of natural killer cells in patients with chronic HBV infection. *Gastroenterology* 2012; **143**: 1586-1596.e8 [PMID: 22960656 DOI: 10.1053/j.gastro.2012.08.046]
  - 45 **Moffat JM**, Cheong WS, Villadangos JA, Mintern JD, Netter HJ. Hepatitis B virus-like particles access major histocompatibility class I and II antigen presentation pathways in primary dendritic cells. *Vaccine* 2013; **31**: 2310-2316 [PMID: 23473776 DOI: 10.1016/j.vaccine.2013.02.042]
  - 46 **Lin C**, Zou H, Wang S. Hepatitis B e Antigen Seroconversion Is Related with the Function of Dendritic Cells in Chronic Hepatitis B Virus Infection. *Gastroenterol Res Pract* 2014; **2014**: 413952 [PMID: 25574162 DOI: 10.1155/2014/413952]
  - 47 **van der Molen RG**, Sprengers D, Biesta PJ, Kusters JG, Janssen HL. Favorable effect of adefovir on the number and functionality of myeloid dendritic cells of patients with chronic HBV. *Hepatology* 2006; **44**: 907-914 [PMID: 17006907 DOI: 10.1002/hep.21340]
  - 48 **Zheng PY**, Zhang DY, Lu GF, Yang PC, Qi YM, Wang BS. Effects of lamivudine on the function of dendritic cells derived from patients with chronic hepatitis B virus infection. *World J Gastroenterol* 2007; **13**: 4641-4645 [PMID: 17729422 DOI: 10.3748/wjg.v13.i34.4641]
  - 49 **Akbar SM**, Horiike N, Chen S, Michitaka K, Abe M, Hiasa Y, Matsuura B, Onji M. Mechanism of restoration of immune responses of patients with chronic hepatitis B during lamivudine therapy: increased antigen processing and presentation by dendritic cells. *J Viral Hepat* 2011; **18**: 200-205 [PMID: 20367796 DOI: 10.1111/j.1365-2893.2010.01300.x]
  - 50 **Wen YM**, Wu XH, Hu DC, Zhang QP, Guo SQ. Hepatitis B vaccine and anti-HBs complex as approach for vaccine therapy. *Lancet* 1995; **345**: 1575-1576 [PMID: 7791465 DOI: 10.1016/S0140-6736(95)91126-X]
  - 51 **Ma YJ**, He M, Han JA, Yang L, Ji XY. A clinical study of HBsAg-activated dendritic cells and cytokine-induced killer cells during the treatment for chronic hepatitis B. *Scand J Immunol* 2013; **78**: 387-393 [PMID: 23841728 DOI: 10.1111/sji.12097]
  - 52 **Martinet J**, Leroy V, Dufeu-Duchesne T, Larrat S, Richard MJ, Zoulim F, Plumas J, Aspod C. Plasmacytoid dendritic cells induce efficient stimulation of antiviral immunity in the context of chronic hepatitis B virus infection. *Hepatology* 2012; **56**: 1706-1718 [PMID: 22707082 DOI: 10.1002/hep.25879]
  - 53 **Hatipoglu I**, Ercan D, Acilan C, Basalp A, Durali D, Baykal AT. Hepatitis B virus e antigen (HBeAg) may have a negative effect on dendritic cell generation. *Immunobiology* 2014; **219**: 944-949 [PMID: 25150150 DOI: 10.1016/j.imbio.2014.07.020]
  - 54 **Untergasser A**, Zedler U, Langenkamp A, Hösel M, Quasdorff M, Esser K, Dienes HP, Tappertzhofen B, Kolanus W, Protzer U. Dendritic cells take up viral antigens but do not support the early steps of hepatitis B virus infection. *Hepatology* 2006; **43**: 539-547 [PMID: 16496321 DOI: 10.1002/hep.21048]
  - 55 **Chen L**, Zhang Z, Chen W, Zhang Z, Li Y, Shi M, Zhang J, Chen L, Wang S, Wang FS. B7-H1 up-regulation on myeloid dendritic cells significantly suppresses T cell immune function in patients with chronic hepatitis B. *J Immunol* 2007; **178**: 6634-6641 [PMID: 17475895 DOI: 10.4049/jimmunol.178.10.6634]
  - 56 **Tjwa ET**, van Oord GW, Hegmans JP, Janssen HL, Woltman AM. Viral load reduction improves activation and function of natural killer cells in patients with chronic hepatitis B. *J Hepatol* 2011; **54**: 209-218 [PMID: 21095036 DOI: 10.1016/j.jhep.2010.07.009]
  - 57 **Shabani Z**, Bagheri M, Zare-Bidaki M, Hassanshahi G, Arababadi MK, Mohammadi Nejad M, Kennedy D. NK cells in hepatitis B virus infection: a potent target for immunotherapy. *Arch Virol* 2014; **159**: 1555-1565 [PMID: 24445811 DOI: 10.1007/s00705-013-1965-3]
  - 58 **Li F**, Wei H, Wei H, Gao Y, Xu L, Yin W, Sun R, Tian Z. Blocking the natural killer cell inhibitory receptor NKG2A increases activity of human natural killer cells and clears hepatitis B virus infection



- in mice. *Gastroenterology* 2013; **144**: 392-401 [PMID: 23103614 DOI: 10.1053/j.gastro.2012.10.039]
- 59 **Li W**, Jiang Y, Wang X, Jin J, Qi Y, Chi X, Zhang H, Feng X, Niu J. Natural Killer p46 Controls Hepatitis B Virus Replication and Modulates Liver Inflammation. *PLoS One* 2015; **10**: e0135874 [PMID: 26291078 DOI: 10.1371/journal.pone.0135874]
  - 60 **Stelma F**, de Niet A, Tempelmans Plat-Sinnige MJ, Jansen L, Takkenberg RB, Reesink HW, Kootstra NA, van Leeuwen EM. Natural Killer Cell Characteristics in Patients With Chronic Hepatitis B Virus (HBV) Infection Are Associated With HBV Surface Antigen Clearance After Combination Treatment With Pegylated Interferon Alfa-2a and Adefovir. *J Infect Dis* 2015; **212**: 1042-1051 [PMID: 25791117 DOI: 10.1093/infdis/jiv180]
  - 61 **Conroy MJ**, Mac Nicholas R, Grealay R, Taylor M, Otegbayo JA, O'Dea S, Mulcahy F, Ryan T, Norris S, Doherty DG. Circulating CD56dim natural killer cells and CD56+ T cells that produce interferon- $\gamma$  or interleukin-10 are expanded in asymptomatic, E antigen-negative patients with persistent hepatitis B virus infection. *J Viral Hepat* 2015; **22**: 335-345 [PMID: 25186004 DOI: 10.1111/jvh.12299]
  - 62 **Jegaskanda S**, Ahn SH, Skinner N, Thompson AJ, Ngyuen T, Holmes J, De Rose R, Navis M, Winnall WR, Kramski M, Bernardi G, Bayliss J, Colledge D, Sozzi V, Visvanathan K, Locarnini SA, Kent SJ, Revill PA. Downregulation of interleukin-18-mediated cell signaling and interferon gamma expression by the hepatitis B virus e antigen. *J Virol* 2014; **88**: 10412-10420 [PMID: 24872585 DOI: 10.1128/JVI.00111-14]
  - 63 **Shi CC**, Tjwa ET, Biesta PJ, Boonstra A, Xie Q, Janssen HL, Woltman AM. Hepatitis B virus suppresses the functional interaction between natural killer cells and plasmacytoid dendritic cells. *J Viral Hepat* 2012; **19**: e26-e33 [PMID: 22239523 DOI: 10.1111/j.1365-2893.2011.01496.x]
  - 64 **Ratnam DT**, Sievert W, Visvanathan K. Natural killer cells display impaired responses to toll like receptor 9 that support viral persistence in chronic hepatitis B. *Cell Immunol* 2012; **279**: 109-115 [PMID: 23123793 DOI: 10.1016/j.cellimm.2012.09.005]
  - 65 **Fisicaro P**, Valdatta C, Boni C, Massari M, Mori C, Zerbini A, Orlandini A, Sacchelli L, Missale G, Ferrari C. Early kinetics of innate and adaptive immune responses during hepatitis B virus infection. *Gut* 2009; **58**: 974-982 [PMID: 19201769 DOI: 10.1136/gut.2008.163600]
  - 66 **Wang XF**, Lei Y, Chen M, Chen CB, Ren H, Shi TD. PD-1/PDL1 and CD28/CD80 pathways modulate natural killer T cell function to inhibit hepatitis B virus replication. *J Viral Hepat* 2013; **20** Suppl 1: 27-39 [PMID: 23458522 DOI: 10.1111/jvh.12061]
  - 67 **Jiang X**, Zhang M, Lai Q, Huang X, Li Y, Sun J, Abbott WG, Ma S, Hou J. Restored circulating invariant NKT cells are associated with viral control in patients with chronic hepatitis B. *PLoS One* 2011; **6**: e28871 [PMID: 22194934 DOI: 10.1371/journal.pone.0028871]
  - 68 **Zhu H**, Zhang Y, Liu H, Zhang Y, Kang Y, Mao R, Yang F, Zhou D, Zhang J. Preserved Function of Circulating Invariant Natural Killer T Cells in Patients With Chronic Hepatitis B Virus Infection. *Medicine (Baltimore)* 2015; **94**: e961 [PMID: 26091463 DOI: 10.1097/MD.0000000000000961]
  - 69 **Huang F**, Lu MH, Gong HY, Xiong ZP. Changes in peripheral blood natural killer T cells in hepatitis B e antigen-positive chronic hepatitis B patients and efficacy prediction after pegylated interferon therapy. *Genet Mol Res* 2015; **14**: 4932-4938 [PMID: 25966268 DOI: 10.4238/2015.May.11.26]
  - 70 **de Lalla C**, Galli G, Aldrighetti L, Romeo R, Mariani M, Monno A, Nuti S, Colombo M, Callea F, Porcelli SA, Panina-Bordignon P, Abrignani S, Casorati G, Dellabona P. Production of profibrotic cytokines by invariant NKT cells characterizes cirrhosis progression in chronic viral hepatitis. *J Immunol* 2004; **173**: 1417-1425 [PMID: 15240738 DOI: 10.4049/jimmunol.173.2.1417]
  - 71 **Rong YH**, Wan ZH, Song H, Li YL, Zhu B, Zang H, Zhao Y, Liu HL, Zhang AM, Xiao L, Xin SJ, You SL. Tim-3 expression on peripheral monocytes and CD3+CD16/CD56+natural killer-like T cells in patients with chronic hepatitis B. *Tissue Antigens* 2014; **83**: 76-81 [PMID: 24397461 DOI: 10.1111/tan.12278]
  - 72 **Zeissig S**, Murata K, Sweet L, Publicover J, Hu Z, Kaser A, Bosse E, Iqbal J, Hussain MM, Balschun K, Röcken C, Arlt A, Günther R, Hampe J, Schreiber S, Baron JL, Moody DB, Liang TJ, Blumberg RS. Hepatitis B virus-induced lipid alterations contribute to natural killer T cell-dependent protective immunity. *Nat Med* 2012; **18**: 1060-1068 [PMID: 22706385 DOI: 10.1038/nm.2811]
  - 73 **Sajadi SM**, Mirzaei V, Hassanshahi G, Khorramdelazad H, Daredor HY, Hosseini SM, Moogooi M, Ravary A, Arababadi MK, Kennedy D. Decreased expressions of Toll-like receptor 9 and its signaling molecules in chronic hepatitis B virus-infected patients. *Arch Pathol Lab Med* 2013; **137**: 1674-1679 [PMID: 24168509 DOI: 10.5858/arpa.2012-0415-OA]
  - 74 **Momeni M**, Zainodini N, Bidaki R, Hassanshahi G, Daneshvar H, Khaleghinia M, Ebrahim M, Karimi-Googheri M, Askari A, Arababadi MK, Kennedy D. Decreased expression of toll like receptor signaling molecules in chronic HBV infected patients. *Hum Immunol* 2014; **75**: 15-19 [PMID: 24120739 DOI: 10.1016/j.humimm.2013.09.015]
  - 75 **Jiang J**, Tang H. Mechanism of inhibiting type I interferon induction by hepatitis B virus X protein. *Protein Cell* 2010; **1**: 1106-1117 [PMID: 21213104 DOI: 10.1007/s13238-010-0141-8]
  - 76 **Chen J**, Wu M, Zhang X, Zhang W, Zhang Z, Chen L, He J, Zheng Y, Chen C, Wang F, Hu Y, Zhou X, Wang C, Xu Y, Lu M, Yuan Z. Hepatitis B virus polymerase impairs interferon- $\alpha$ -induced STA T activation through inhibition of importin- $\alpha$ 5 and protein kinase C- $\delta$ . *Hepatology* 2013; **57**: 470-482 [PMID: 22996189 DOI: 10.1002/hep.26064]
  - 77 **Christen V**, Duong F, Bernsmeier C, Sun D, Nassal M, Heim MH. Inhibition of alpha interferon signaling by hepatitis B virus. *J Virol* 2007; **81**: 159-165 [PMID: 17065208 DOI: 10.1128/JVI.01292-06]
  - 78 **Li J**, Chen F, Zheng M, Zhu H, Zhao D, Liu W, Liu W, Chen Z. Inhibition of STAT1 methylation is involved in the resistance of hepatitis B virus to Interferon alpha. *Antiviral Res* 2010; **85**: 463-469 [PMID: 19857525 DOI: 10.1016/j.antiviral.2009.10.011]
  - 79 **Wu M**, Xu Y, Lin S, Zhang X, Xiang L, Yuan Z. Hepatitis B virus polymerase inhibits the interferon-inducible MyD88 promoter by blocking nuclear translocation of Stat1. *J Gen Virol* 2007; **88**: 3260-3269 [PMID: 18024894 DOI: 10.1099/vir.0.82959-0]
  - 80 **Liu Y**, Li J, Chen J, Li Y, Wang W, Du X, Song W, Zhang W, Lin L, Yuan Z. Hepatitis B virus polymerase disrupts K63-linked ubiquitination of STING to block innate cytosolic DNA-sensing pathways. *J Virol* 2015; **89**: 2287-2300 [PMID: 25505063 DOI: 10.1128/JVI.02760-14]
  - 81 **Liu D**, Wu A, Cui L, Hao R, Wang Y, He J, Guo D. Hepatitis B virus polymerase suppresses NF- $\kappa$ B signaling by inhibiting the activity of IKKs via interaction with Hsp90 $\beta$ . *PLoS One* 2014; **9**: e91658 [PMID: 24618592 DOI: 10.1371/journal.pone.0091658]
  - 82 **Chen WN**, Liu LL, Jiao BY, Lin WS, Lin XJ, Lin X. Hepatitis B virus X protein increases the IL-1 $\beta$ -induced NF- $\kappa$ B activation via interaction with evolutionarily conserved signaling intermediate in Toll pathways (ECSIT). *Virus Res* 2015; **195**: 236-245 [PMID: 25449573 DOI: 10.1016/j.virusres.2014.10.025]
  - 83 **Wu S**, Kanda T, Imazeki F, Nakamoto S, Tanaka T, Arai M, Roger T, Shirasawa H, Nomura F, Yokosuka O. Hepatitis B virus e antigen physically associates with receptor-interacting serine/threonine protein kinase 2 and regulates IL-6 gene expression. *J Infect Dis* 2012; **206**: 415-420 [PMID: 22615316 DOI: 10.1093/infdis/jis363]
  - 84 **Bai L**, Zhang W, Tan L, Yang H, Ge M, Zhu C, Zhang R, Cao Y, Chen J, Luo Z, Ho W, Liu F, Wu K, Wu J. Hepatitis B virus hijacks CTHRC1 to evade host immunity and maintain replication. *J Mol Cell Biol* 2015; **7**: 543-556 [PMID: 26180054 DOI: 10.1093/jmcb/mjv048]
  - 85 **Tzeng HT**, Tsai HF, Chyuan IT, Liao HJ, Chen CJ, Chen PJ, Hsu PN. Tumor necrosis factor- $\alpha$  induced by hepatitis B virus core mediating the immune response for hepatitis B viral clearance in mice model. *PLoS One* 2014; **9**: e103008 [PMID: 25047809 DOI: 10.1371/journal.pone.0103008]
  - 86 **Chyuan IT**, Tsai HF, Tzeng HT, Sung CC, Wu CS, Chen PJ, Hsu PN. Tumor necrosis factor- $\alpha$  blockage therapy impairs hepatitis B viral clearance and enhances T-cell exhaustion in a mouse



model. *Cell Mol Immunol* 2015; **12**: 317-325 [PMID: 25661729 DOI: 10.1038/cmi.2015.01]

- 87 **Jan RH**, Lin YL, Chen CJ, Lin TY, Hsu YC, Chen LK, Chiang BL. Hepatitis B virus surface antigen can activate human monocyte-

derived dendritic cells by nuclear factor kappa B and p38 mitogen-activated protein kinase mediated signaling. *Microbiol Immunol* 2012; **56**: 719-727 [PMID: 22853328 DOI: 10.1111/j.1348-0421.2012.00496.x]

**P- Reviewer:** McQuillan GM, Vaughan G **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Liu SQ



Basic Study

## Anti-CD163-dexamethasone conjugate inhibits the acute phase response to lipopolysaccharide in rats

Karen Louise Thomsen, Holger Jon Møller, Jonas Heilskov Graversen, Nils E Magnusson, Søren K Moestrup, Hendrik Vilstrup, Henning Grønbæk

Karen Louise Thomsen, Hendrik Vilstrup, Henning Grønbæk, Department of Hepatology and Gastroenterology, Aarhus University Hospital, DK-8000 Aarhus C, Denmark

Holger Jon Møller, Department of Clinical Biochemistry, Aarhus University Hospital, DK-8000 Aarhus C, Denmark

Jonas Heilskov Graversen, Affinicon Aps, Incuba Science Park, DK 8200 Aarhus N, Denmark

Jonas Heilskov Graversen, Søren K Moestrup, Institute of Molecular Medicine, University of Southern Denmark, DK-5000 Odense C, Denmark

Nils E Magnusson, Department of Clinical Medicine, Faculty of Health, Medical Research Laboratory, Aarhus University, DK-8000 Aarhus C, Denmark

Søren K Moestrup, Department of Biomedicine, University of Aarhus, DK-8000 Aarhus C, Denmark

**Author contributions:** Thomsen KL, Møller HJ and Grønbæk H conceived and designed the study; Thomsen KL and Magnusson NE acquired the data and analysed the samples; Thomsen KL, Vilstrup H and Grønbæk H analysed and interpreted the data; Thomsen KL drafted the manuscript; Møller HJ, Graversen JH, Moestrup SK, Vilstrup H and Grønbæk H critically revised the manuscript for important intellectual content; all authors saw and approved the final manuscript.

**Supported by** The NOVO Nordisk foundation; the Aarhus University Research Foundation; and Clinical Institute, Aarhus University, Denmark.

**Institutional animal care and use committee statement:** The study was performed in accordance with local and national guidelines for animal welfare and reviewed and approved by the national Animal Ethics Committee, protocol No. 2010/561-1918.

**Conflict-of-interest statement:** Møller HJ, Graversen JH and Moestrup SK are inventors for the CD163-dexamethasone conjugate and minority shareholders in Affinicon Aps. All other authors have nothing to disclose.

**Data sharing statement:** Dataset is available from the corresponding author at [karethom@rm.dk](mailto:karethom@rm.dk).

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Karen Louise Thomsen, MD, PhD, Department of Hepatology and Gastroenterology, Aarhus University Hospital, 44 Nørrebrogade, DK-8000 Aarhus C, Denmark. [karethom@rm.dk](mailto:karethom@rm.dk)  
 Telephone: +45-78-463897  
 Fax: +45-78-462740

**Received:** February 22, 2016  
**Peer-review started:** February 22, 2016  
**First decision:** March 24, 2016  
**Revised:** May 4, 2016  
**Accepted:** May 31, 2016  
**Article in press:** June 2, 2016  
**Published online:** June 18, 2016

### Abstract

**AIM:** To study the effect of a new anti-CD163-dexamethasone conjugate targeting activated macrophages on the hepatic acute phase response in rats.

**METHODS:** Wistar rats were injected intravenous with either the CD163 targeted dexamethasone-conjugate (0.02 mg/kg) or free dexamethasone (0.02 or 1 mg/kg) 24 h prior to lipopolysaccharide (LPS) (2.5 mg/kg intraperitoneal). We measured plasma concentrations of

tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin 6 (IL-6) 2 h post-LPS and liver mRNAs and serum concentrations of the rat acute phase protein  $\alpha$ -2-macroglobulin ( $\alpha$ -2-M) 24 h after LPS. Also, plasma concentrations of alanine aminotransferase and bilirubin were measured at termination of the study. Spleen weight served as an indicator of systemic steroid effects.

**RESULTS:** The conjugate halved the  $\alpha$ -2-M liver mRNA ( $3.3 \pm 0.6$  vs  $6.8 \pm 1.1$ ,  $P < 0.01$ ) and serum protein ( $201 \pm 48$   $\mu$ g/mL vs  $389 \pm 67$   $\mu$ g/mL,  $P = 0.04$ ) after LPS compared to low dose dexamethasone treated animals, while none of the free dexamethasone doses had an effect on liver mRNA or serum levels of  $\alpha$ -2-M. Also, the conjugate reduced TNF- $\alpha$  ( $7208 \pm 1977$  pg/mL vs  $21583 \pm 7117$  pg/mL,  $P = 0.03$ ) and IL-6 ( $15685 \pm 3779$  pg/mL vs  $25715 \pm 4036$  pg/mL,  $P = 0.03$ ) compared to the low dose dexamethasone. The high dose dexamethasone dose decreased the spleen weight ( $421 \pm 11$  mg vs  $465 \pm 12$  mg,  $P < 0.05$ ) compared to controls, an effect not seen in any other group.

**CONCLUSION:** Low-dose anti-CD163-dexamethasone conjugate effectively decreased the hepatic acute phase response to LPS. This indicates an anti-inflammatory potential of the conjugate *in vivo*.

**Key words:** Acute phase response; Dexamethasone; Endotoxin; Hemoglobin scavenger receptor CD163; Cytokines; Inflammation; Rats

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We aimed to study the effect of a new anti-CD163-dexamethasone conjugate targeting activated macrophages on the hepatic acute phase response in rats. The central finding of the study was a reduction in liver mRNA and plasma levels of the acute phase protein  $\alpha$ -2-macroglobulin, and plasma tumour necrosis factor- $\alpha$  and interleukin 6 by administration of the conjugate prior to a lipopolysaccharide-induced inflammatory response. This anti-acute phase effect exceeded that of the therapeutic dexamethasone dose and did not cause systemic adverse effects. Thus, the antibody conjugate may be a potential candidate in future anti-inflammatory macrophage-directed therapy, *e.g.*, in liver diseases with Kupffer cells activation.

Thomsen KL, Møller HJ, Graversen JH, Magnusson NE, Moestrup SK, Vilstrup H, Grønbaek H. Anti-CD163-dexamethasone conjugate inhibits the acute phase response to lipopolysaccharide in rats. *World J Hepatol* 2016; 8(17): 726-730 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i17/726.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i17.726>

## INTRODUCTION

In conditions with macrophage proliferation and activation, CD163, a haemoglobin-haptoglobin scavenger

receptor expressed exclusively on monocytes and macrophages<sup>[1,2]</sup>, is up-regulated<sup>[3,4]</sup>. Following toll-like receptor activation by inflammatory stimuli like lipopolysaccharide (LPS), receptor shedding to circulation as soluble CD163 (sCD163) is increased, and within hours upregulated on the cell surface<sup>[5]</sup>. As an example, hepatic macrophages (Kupffer cells) are activated and sCD163 is increased in patients with liver cirrhosis who chronically experience some degree of endotoxemia and acute phase response<sup>[6,7]</sup> and this may be involved in the development of the serious cirrhosis complications<sup>[6,8]</sup>.

We have recently constructed a conjugate of CD163 antibody and the potent corticosteroid dexamethasone (anti-CD163mAb-dexa) specifically targeting dexamethasone to activated macrophages<sup>[9]</sup>. The conjugate reduces the LPS-stimulated cytokine release from activated macrophages *in vitro* and *in vivo* in rats and pigs<sup>[9,10]</sup>. The effect is obtained with very low concentration of dexamethasone, thereby minimizing steroid-induced systemic effects. A fifty-fold higher concentration of non-conjugated dexamethasone is needed to obtain the same anti-inflammatory response<sup>[9]</sup>.

Exposure to LPS is a standard method to induce an acute phase response with a large increase in pro-inflammatory cytokines and hepatic synthesis and release of acute phase proteins<sup>[11,12]</sup>. While the conjugate reduces the LPS-mediated cytokine response in rats it remains unknown whether it also inhibits the hepatic acute phase protein synthesis response.

To approach this issue we measured the gene expression in liver tissue and serum concentrations of the prevailing acute phase protein  $\alpha$ -2-macroglobulin ( $\alpha$ -2-M) 24 h post-LPS exposure in rats.  $\alpha$ -2-M is a hepatocyte-derived inhibitor of a wide range of proteinases that can be activated during inflammation<sup>[13]</sup>. Further, we compared plasma concentrations of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin 6 (IL-6) 2 h post-LPS exposure. Spleen weight served as an indicator of systemic steroid effects.

## MATERIALS AND METHODS

### Animals

The animal protocol was designed to minimize pain or discomfort to the animals. Female Wistar rats (body weight 190-210 g; Taconic M and B, Ejby, Denmark) were housed at  $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$  with a 12-h artificial light cycle. Two or three animals were housed in each cage, with free access to tap water and standard food (Altromin, Lage, Germany) and acclimatized for one week. Food intake and body weight were registered at the beginning and at the end of the experimental procedures. The study was performed in accordance with local and national guidelines for animal welfare and approved by the national Animal Ethics Committee, protocol No. 2010/561-1918.

### Design

Forty animals were allocated in 5 groups of 8: One

**Table 1** Weights, liver function tests, and cytokines.

	Controls	LPS	Anti-CD163-dexa plus LPS	High dexa plus LPS	Low dexa plus LPS
Body weight	199 ± 1	196 ± 2	207 ± 2 <sup>b</sup>	204 ± 3	206 ± 3 <sup>b</sup>
Weight loss	11 ± 1	14 ± 3	22 ± 2 <sup>a</sup>	23 ± 2 <sup>a</sup>	21 ± 1 <sup>a</sup>
Spleen weight	465 ± 12	512 ± 31	492 ± 23	421 ± 11 <sup>a</sup>	483 ± 23
ALT	42 ± 3	61 ± 16	57 ± 20	48 ± 9	77 ± 31
Bilirubin	3.0 ± 0.0	3.3 ± 0.3	3.1 ± 0.1	3.6 ± 0.4	4.0 ± 0.4
TNF- $\alpha$	0 ± 0	26817 ± 9780 <sup>a</sup>	7208 ± 1977 <sup>a,c</sup>	16891 ± 4210 <sup>a</sup>	21583 ± 7117 <sup>a</sup>
IL-6	0 ± 0	23075 ± 6758 <sup>a</sup>	15685 ± 3779 <sup>a,c,e</sup>	32964 ± 8294 <sup>a</sup>	25715 ± 4036 <sup>a</sup>

Body weight (g), body weight loss (g), spleen weight (mg), plasma alanine aminotransferase (U/L), and bilirubin ( $\mu$ mol/L) in controls ( $n = 8$ ) and in animals injected with LPS 24 h after vehicle ( $n = 8$ ), anti-CD163mAb-dexa ( $n = 8$ ), high dose ( $n = 8$ ) and low dose ( $n = 8$ ) dexamethasone at termination of study. Plasma TNF- $\alpha$  (pg/mL) and IL-6 (pg/mL) are measured 2 h after saline (controls) or LPS injection. <sup>a</sup> $P < 0.05$  vs controls; <sup>b</sup> $P < 0.05$  vs low dose free dexamethasone group; <sup>c</sup> $P < 0.05$  vs high dose free dexamethasone group; <sup>d</sup> $P < 0.05$  vs vehicle. ALT: Alanine aminotransferase; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL-6: Interleukin-6; LPS: Lipopolysaccharide.

control group receiving only vehicle (PBS pH 7.4) intravenously and four groups injected intravenously with either vehicle, anti-CD163mAb-dexa (0.02 mg/kg dexamethasone), high dose free dexamethasone (1 mg/kg) (Sigma-Aldrich, Brøndby, Denmark), or low dose free dexamethasone (0.02 mg/kg). The high ("therapeutic") dose gives maximal steroid efficacy in other rat studies<sup>[14,15]</sup> and the low dose was the same as in the anti-CD163mAb-dexa. After 24 h, 0.5 mL of saline (controls) or LPS dissolved in 0.5 mL saline (2.5 mg/kg) (from *Escherichia coli* 0111:B4 obtained from Sigma-Aldrich, Brøndby, Denmark; product No. L2630) was injected intraperitoneally. Two hours later and following anaesthesia with inhalation of isofluran 2%-3% (Forene<sup>®</sup>, Abbott Laboratories, Gentofte, Denmark), a blood sample for determination of plasma TNF- $\alpha$  and IL-6 was drawn from a retrobulbar venous plexus using heparinised micropipettes. After an overnight 12-h fast the animals were anaesthetised with a subcutaneous injection of fentanyl/fluanisone (Hypnorm<sup>®</sup>, Jansen Pharma, Birkerød, Denmark) 0.5 mL/kg and midazolam (Dormicum<sup>®</sup>, La Roche, Basel, Switzerland) 2.5 mg/kg. All blood was collected for blood analyses and approximately 200 mg of liver tissue was snap-frozen in liquid N<sub>2</sub>, and stored at -80 °C. Finally, the spleen was weighed. In all animals we measured liver mRNA levels and serum concentrations of  $\alpha$ -2-M and plasma concentrations of alanine aminotransferase and bilirubin at termination of the study.

#### Liver tissue

mRNA levels of  $\alpha$ -2-M were determined by slot blot hybridization as previously described<sup>[16]</sup>.

#### Blood analyses

The concentrations of  $\alpha$ -2-M in serum were evaluated by rat ELISA (Immunology Consultants Laboratory, Newberg, OR, United States). The plasma concentrations of TNF- $\alpha$  and IL-6 were determined by immunoassay (R and D Systems, Minneapolis, MN, United States, both). Samples were analysed in duplicate and all assays had

intra- and inter-assay coefficients of variance below 5% and 10%, respectively. Plasma concentrations of alanine aminotransferase and bilirubin were determined by standard clinical biochemical analytical methods.

#### Statistical analysis

Data were analysed using the Kruskal-Wallis One Way Analysis of Variance on Ranks; when significant, post-hoc tests were performed among groups by the Mann-Whitney rank sum test. Data are presented as the mean  $\pm$  SEM. Differences were considered significant with  $P$ -values  $< 0.05$ . A statistical review of the study was performed by a biomedical statistician.

## RESULTS

#### Body and spleen weight

LPS induced a body weight loss in all the intervention groups ( $P < 0.05$ ) (Table 1) and there was no difference among these groups. The high dose dexamethasone dose decreased the spleen weight ( $P < 0.05$ ), an effect not seen in any other group (Table 1).

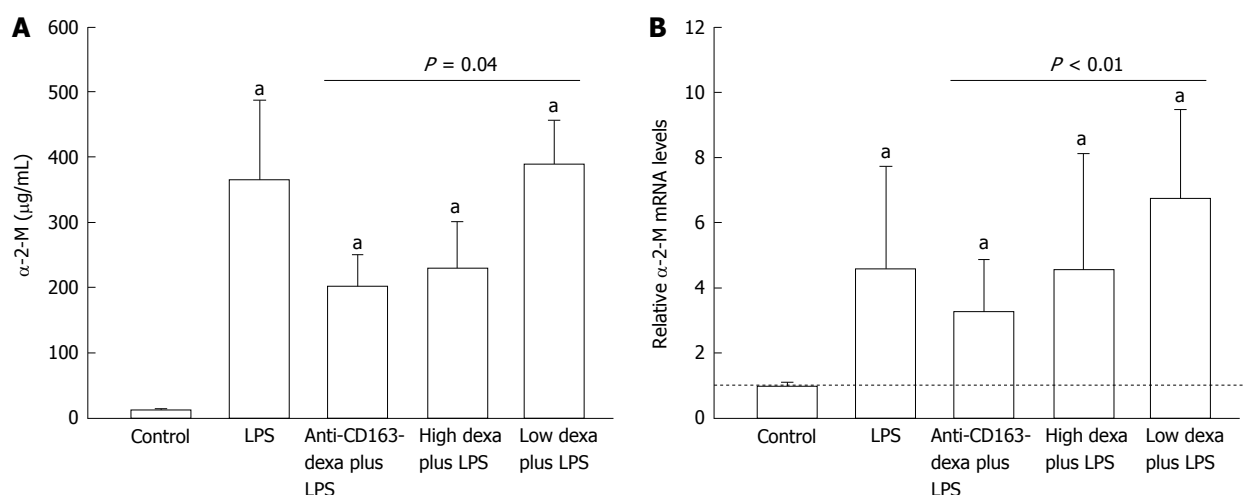
#### Acute phase protein liver mRNA and serum levels

LPS increased the liver mRNA and serum levels of  $\alpha$ -2-M several fold in all groups ( $P < 0.01$ ) (Figure 1). Anti-CD163mAb-dexa approximately halved the  $\alpha$ -2-M liver mRNA ( $P < 0.01$ ) and serum response ( $P = 0.04$ ) compared to low dose dexamethasone treated animals, while no free dexamethasone dose had any effect on liver mRNA or serum levels of  $\alpha$ -2-M compared to vehicle (Figure 1).

#### TNF- $\alpha$ and IL-6

LPS markedly increased plasma TNF- $\alpha$  and IL-6 in all groups ( $P < 0.001$ ). There was a trend for reduced TNF- $\alpha$  ( $P = 0.08$ ) after anti-CD163mAb-dexa compared to vehicle and significantly so vs the low dose dexamethasone ( $P = 0.03$ ). Also, the anti-CD163mAb-dexa decreased IL-6 compared to both dexamethasone doses ( $P < 0.05$ ). None of the free dexamethasone doses had





**Figure 1** Relative levels of serum levels (A) and liver mRNA (B) of  $\alpha$ -2-macroglobulin. Changes in serum levels ( $\mu$ g/mL) (A) and liver mRNA (% of controls) (B) of  $\alpha$ -2-macroglobulin ( $\alpha$ -2-M) in controls ( $n = 8$ ) and in animals injected with LPS 24 h after vehicle ( $n = 8$ ), anti-CD163mAb-dexa ( $n = 8$ ), high dose ( $n = 8$ ) and low dose ( $n = 8$ ) dexamethasone. mRNA results from LPS-injected animals are presented as relative levels compared to control animals. Bars represent the mean and SEM. <sup>a</sup> $P < 0.05$  vs controls. LPS: Lipopolysaccharide; SEM: Standard error of mean.

an effect on TNF- $\alpha$  or IL-6 (Table 1).

#### Plasma-alanine transferase and bilirubin

LPS had no effect on these measures at termination of the study (Table 1).

## DISCUSSION

The central finding of this study was the reduction in liver mRNA and plasma  $\alpha$ -2-M, and plasma TNF- $\alpha$  and IL-6 by the administration of the anti-CD163-dexa conjugate prior to the LPS-induced inflammatory response. This anti-acute phase effect much exceeded that of the therapeutic dexamethasone dose and did not cause systemic adverse effects, as evidenced by reduced spleen weight in the group treated with high dose free dexamethasone. This study completes the chain of evidence that the conjugate not only suppresses the LPS elicited IL signaling but also the ultimate effect on synthesis and release of hepatic acute phase proteins that effectuate the acute phase response.

The increase in plasma  $\alpha$ -2-M after LPS reflects *de novo* synthesis as almost no such protein is present under non-induced conditions<sup>[17]</sup> in contrast to conditions with ongoing low grade inflammation such as cirrhosis<sup>[18]</sup>. LPS as assumed caused a marked systemic acute phase response reflected in increased liver mRNA and plasma  $\alpha$ -2-M, TNF- $\alpha$ , and IL-6. In contrast to the equal amount of free dexamethasone, the anti-CD163mAb-dexa efficiently suppressed this response. Still, however, the acute phase response to some extent serves to restore homeostasis and one needs to be aware that suppression of the response might not be entirely beneficial entailing a potential risk using the conjugate long term.

The anti-inflammatory effects of glucocorticoids are related to a decrease in lymphocyte expansion and cell survival and also a reduction in the expression of pro-inflammatory cytokines originating from macro-

phages<sup>[19]</sup>. However, as glucocorticoids bind to the ubiquitous intracellular glucocorticoid steroid receptor present in most cell types they also exert serious systemic metabolic side effects. Thus dexamethasone causes the spleen to undergo a corticosteroid-induced weight reduction due to lymphocyte depletion<sup>[20]</sup>. Accordingly, the high dose dexamethasone in our study decreased the spleen weight as compared with the other groups reflecting systemic non-macrophages effects. In contrast, the conjugate did not affect spleen weight and was still found to exert a potent anti-inflammatory effect.

In our animal model, the conjugate was given as a pre-emptive dose prior to the induction of the acute phase response as we aimed at establishing a proof-of-concept position of the conjugate's effects. We believe our findings support further studies on interference with on-going inflammation in relevant experimental models. Such studies are also essential for monitoring of long term effects of the conjugate.

In conclusion, the anti-CD163-dexa conjugate demonstrated potent effects in reducing the acute phase proteins without evident systemic side effects during an endotoxin-induced acute phase response in rats. The effect much exceeded that of a therapeutic dose of dexamethasone. Thus, the antibody conjugate may be a potential candidate in future anti-inflammatory macrophage-directed therapy, *e.g.*, in liver diseases with Kupffer cells activation<sup>[7]</sup>.

## ACKNOWLEDGMENTS

We are indebted to Rikke Andersen, Birgitte Nielsen, and Kirsten Priisholm for their skilled technical assistance.

## COMMENTS

### Background

In conditions with macrophage proliferation and activation, CD163, a scavenger

receptor expressed exclusively on monocytes and macrophages, is up-regulated. As an example, hepatic macrophages (Kupffer cells) are activated and CD163 is increased in patients with liver cirrhosis who chronically experience some degree of endotoxemia and acute phase response.

### Research frontiers

The authors have recently constructed a conjugate of CD163 antibody and the potent corticosteroid dexamethasone (anti-CD163mAb-dexa) specifically targeting dexamethasone to activated macrophages.

### Innovations and breakthroughs

The anti-CD163-dexa conjugate exerts an anti-inflammatory effect, which is obtained with very low concentration of dexamethasone, thereby minimizing steroid-induced systemic effects.

### Applications

The antibody conjugate may be a potential candidate in future anti-inflammatory macrophage-directed therapy, *e.g.*, in liver diseases with Kupffer cells activation.

### Peer-review

This is an experimental report written by Thomsen *et al.*, which indicates an efficacy of dexamethasone-conjugated anti-CD163 against lipopolysaccharide-induced acute inflammatory reaction. The well-designed study was carried out using firm methods.

## REFERENCES

- Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, Moestrup SK. Identification of the haemoglobin scavenger receptor. *Nature* 2001; **409**: 198-201 [PMID: 11196644 DOI: 10.1038/35051594]
- Moestrup SK, Møller HJ. CD163: a regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. *Ann Med* 2004; **36**: 347-354 [PMID: 15478309 DOI: 10.1080/07853890410033171]
- Møller HJ, de Fost M, Aerts H, Hollak C, Moestrup SK. Plasma level of the macrophage-derived soluble CD163 is increased and positively correlates with severity in Gaucher's disease. *Eur J Haematol* 2004; **72**: 135-139 [PMID: 14962251 DOI: 10.1046/j.0902-4441.2003.00193.x]
- Schaer DJ, Schleiffenbaum B, Kurrer M, Imhof A, Bächli E, Fehr J, Møller HJ, Moestrup SK, Schaffner A. Soluble hemoglobin-haptoglobin scavenger receptor CD163 as a lineage-specific marker in the reactive hemophagocytic syndrome. *Eur J Haematol* 2005; **74**: 6-10 [PMID: 15613100 DOI: 10.1111/j.1600-0609.2004.00318.x]
- Hintz KA, Rassias AJ, Wardwell K, Moss ML, Morganelli PM, Pioli PA, Givan AL, Wallace PK, Yeager MP, Guyre PM. Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte hemoglobin scavenger receptor CD163. *J Leukoc Biol* 2002; **72**: 711-717 [PMID: 12377940]
- Grønbaek H, Sandahl TD, Mortensen C, Vilstrup H, Møller HJ, Møller S. Soluble CD163, a marker of Kupffer cell activation, is related to portal hypertension in patients with liver cirrhosis. *Aliment Pharmacol Ther* 2012; **36**: 173-180 [PMID: 22591184 DOI: 10.1111/j.1365-2036.2012.05134.x]
- Sandahl TD, Grønbaek H, Møller HJ, Støy S, Thomsen KL, Dige AK, Agnholt J, Hamilton-Dutoit S, Thiel S, Vilstrup H. Hepatic macrophage activation and the LPS pathway in patients with alcoholic hepatitis: a prospective cohort study. *Am J Gastroenterol* 2014; **109**: 1749-1756 [PMID: 25155228 DOI: 10.1038/ajg.2014.262]
- Mookerjee RP, Sen S, Davies NA, Hodges SJ, Williams R, Jalan R. Tumour necrosis factor alpha is an important mediator of portal and systemic haemodynamic derangements in alcoholic hepatitis. *Gut* 2003; **52**: 1182-1187 [PMID: 12865279 DOI: 10.1136/gut.52.8.1182]
- Graversen JH, Svendsen P, Dagnæs-Hansen F, Dal J, Anton G, Etzerodt A, Petersen MD, Christensen PA, Møller HJ, Moestrup SK. Targeting the hemoglobin scavenger receptor CD163 in macrophages highly increases the anti-inflammatory potency of dexamethasone. *Mol Ther* 2012; **20**: 1550-1558 [PMID: 22643864 DOI: 10.1038/mt.2012.103]
- Granfeldt A, Hvas CL, Graversen JH, Christensen PA, Petersen MD, Anton G, Svendsen P, Sølling C, Etzerodt A, Tønnesen E, Moestrup SK, Møller HJ. Targeting dexamethasone to macrophages in a porcine endotoxemic model. *Crit Care Med* 2013; **41**: e309-e318 [PMID: 23928834 DOI: 10.1097/CCM.0b013e31828a45ef]
- Millard J, Tsykin A, Thomas T, Aldred AR, Cole T, Schreiber G. Gene expression in regenerating and acute-phase rat liver. *Am J Physiol* 1990; **259**: G340-G347 [PMID: 1698035]
- Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; **340**: 448-454 [PMID: 9971870 DOI: 10.1056/NEJM199902113400607]
- Rehman AA, Ahsan H, Khan FH.  $\alpha$ -2-Macroglobulin: a physiological guardian. *J Cell Physiol* 2013; **228**: 1665-1675 [PMID: 23086799 DOI: 10.1002/jcp.24266]
- Li L, Whiteman M, Moore PK. Dexamethasone inhibits lipopolysaccharide-induced hydrogen sulphide biosynthesis in intact cells and in an animal model of endotoxic shock. *J Cell Mol Med* 2009; **13**: 2684-2692 [PMID: 19120693 DOI: 10.1111/j.1582-4934.2008.00610.x]
- Hattori Y, Murakami Y, Atsuta H, Minamino N, Kangawa K, Kasai K. Glucocorticoid regulation of adrenomedullin in a rat model of endotoxic shock. *Life Sci* 1998; **62**: PL181-PL189 [PMID: 9519804 DOI: 10.1016/S0024-3205(98)00049-6]
- Nielsen SS, Grøfte T, Tygstrup N, Vilstrup H. Synthesis of acute phase proteins in rats with cirrhosis exposed to lipopolysaccharide. *Comp Hepatol* 2006; **5**: 3 [PMID: 16968543 DOI: 10.1186/1476-5926-5-3]
- Geiger T, Andus T, Klapproth J, Hirano T, Kishimoto T, Heinrich PC. Induction of rat acute-phase proteins by interleukin 6 in vivo. *Eur J Immunol* 1988; **18**: 717-721 [PMID: 2454191 DOI: 10.1002/eji.1830180510]
- Naveau S, Poynard T, Benattar C, Bedossa P, Chaput JC. Alpha-2-macroglobulin and hepatic fibrosis. Diagnostic interest. *Dig Dis Sci* 1994; **39**: 2426-2432 [PMID: 7525168 DOI: 10.1007/BF02087661]
- McColl A, Michlewska S, Dransfield I, Rossi AG. Effects of glucocorticoids on apoptosis and clearance of apoptotic cells. *ScientificWorldJournal* 2007; **7**: 1165-1181 [PMID: 17704849 DOI: 10.1100/tsw.2007.224]
- Rungruang T, Chaweeborisuit P, Klosek SK. Effect of malaria infection and dexamethasone on spleen morphology and histology. *Southeast Asian J Trop Med Public Health* 2010; **41**: 1290-1296 [PMID: 21329300]

P- Reviewer: Ikura Y, Liu ZH, Pan JJ, Tsoulfas G, Zhu X

S- Editor: Ji FF L- Editor: A E- Editor: Liu SQ



Clinical Trials Study

# Therapeutic usability of two different fiducial gold markers for robotic stereotactic radiosurgery of liver malignancies: A pilot study

Maria Marsico, Tommaso Gabbani, Lorenzo Livi, Maria Rosa Biagini, Andrea Galli

Maria Marsico, Integrate Activity Department 1, Gastroenterology, AOU Modena University Hospital, 41121 Modena, Italy

Tommaso Gabbani, Maria Rosa Biagini, Andrea Galli, Oncology Department, Clinical Gastroenterology, AOU Careggi, Florence University Hospital, 50134 Florence, Italy

Lorenzo Livi, Oncology Department, Radiotherapy SOD, AOU Careggi, Florence University Hospital, 50134 Florence, Italy

**Author contributions:** Marsico M conceived the study, participated in the procedures for placement of the markers, wrote and reviewed the article; Gabbani T Participated in the procedures for placement of the markers, performed the statistical analysis, wrote and reviewed the article; Livi L and Galli A participated in the study design, coordinated and helped to draft the manuscript; Biagini MR participated in the study design, and in the procedures for placement of the markers and review of the article.

**Institutional review board statement:** The study was reviewed and approved by the Gastroenterology Unit, University of Florence review board.

**Informed consent statement:** All patients provided written informed consent for enrolment in the study, and inclusion in this article of information that could potentially lead to their identification.

**Conflict-of-interest statement:** The authors (Maria Marsico, MD, Tommaso Gabbani, MD, Andrea Galli, Professor, Maria Rosa Biagini, MD, Lorenzo Livi, Professor) have no conflicts to report.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at [ma.marsico@libero.it](mailto:ma.marsico@libero.it). Participants gave informed consent for data sharing, however the data presented are anonymous and risk of identification is low.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external

reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

**Correspondence to:** Maria Marsico, MD, Integrate Activity Department 1, Gastroenterology, AOU Modena University Hospital, Via del Pozzo 71, 41121 Modena, Italy. [ma.marsico@libero.it](mailto:ma.marsico@libero.it)  
 Telephone: +39-32-98051908

Received: January 31, 2016  
 Peer-review started: January 31, 2016  
 First decision: March 31, 2016  
 Revised: May 2, 2016  
 Accepted: May 31, 2016  
 Article in press: June 2, 2016  
 Published online: June 18, 2016

## Abstract

**AIM:** To assess how the application of different types of markers affects the tracking accuracy of CyberKnife's.

**METHODS:** Fifteen patients were recruited and subjected to the ultrasound-guided placement of markers. Two different type of needles 25 gauge (G) and 17 G containing two different fiducial marker, gold notched flexible anchor wire 0.28 mm × 10 mm (25 G needle) and gold cylindrical grain 1 mm × 4 mm (17 G), were used. Seven days after the procedure, a CyberKnife planning computed tomography (CT) for the simulation of radiation treatment was performed on all patients.

A binary CT score was assigned to the fiducial markers visualization. Also, the CT number was calculated for each fiducial and the values compared with a specific threshold.

**RESULTS:** For each patient from 1 to 5, intra-hepatic markers were placed (one in 2 patients, three in 8 patients, four in 3 patients, and five in 2 patients). A total of 48 needles were used (thirty-two 17 G and sixteen 25 G) and 48 gold markers were placed (32 Grain shaped markers and 16 Gold Anchor). The result showed that the CT visualization of the grain markers was better than the anchor markers ( $P = 5 \times 10^{-9}$ ). Furthermore, the grain markers were shown to present minor late complications ( $P = 3 \times 10^{-6}$ ), and the best CT threshold number ( $P = 0.0005$ ).

**CONCLUSION:** The study revealed that the Gold Anchor fiducial marker is correlated with a greater number of late minor complications and low visualization by the CT.

**Key words:** Robotic radiosurgery; Fiducial markers; Liver malignancies; CyberKnife; Radiation therapy; Stereotactic radiosurgery

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Robotic radiosurgery can employ different systems for the localization of the neoplastic targets to treat. The purpose of this study is to assess how the application of different types of markers affects the tracking accuracy of CyberKnife's. Fifteen patients have been recruited and analyzed for the study and two types of markers were used for the procedure. The computed tomography (CT) visualization of grain markers was better than anchor markers  $P = 5 \times 10^{-9}$ . Grain markers presented minor late complications of  $P = 3 \times 10^{-6}$ , and the best CT threshold number. The study revealed that the Gold Anchor fiducial marker is correlated with a greater number of late minor complication.

Marsico M, Gabbani T, Livi L, Biagini MR, Galli A. Therapeutic usability of two different fiducial gold markers for robotic stereotactic radiosurgery of liver malignancies: A pilot study. *World J Hepatol* 2016; 8(17): 731-738 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i17/731.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i17.731>

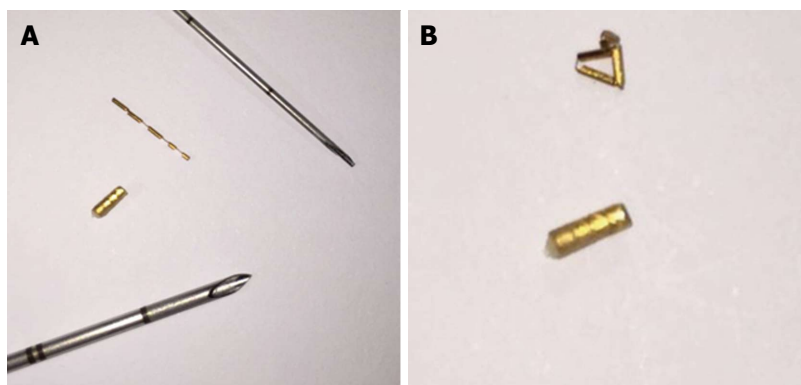
## INTRODUCTION

The stereotactic robotic radiosurgery is able to administer high-dose radiation that could reach any anatomic point with a sub-millimeter precision<sup>[1-4]</sup>. The high accuracy is achieved by the image-guidance system robotic technology and the dynamic tracking of targets,

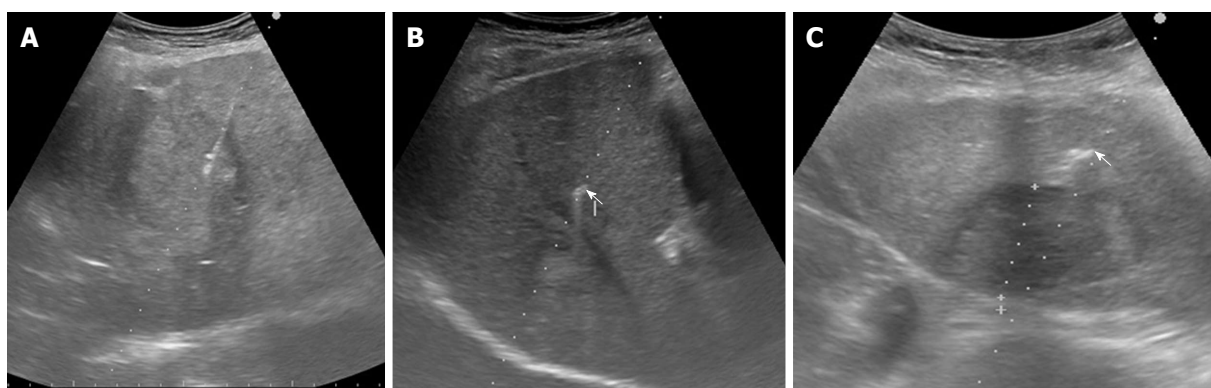
that remove the effect of breathing. The use of these techniques permits the CyberKnife system's to hit the lesion with high-dose radiation and to safeguard the surrounding critical organs which could suffer irreversible damage<sup>[5-13]</sup>. Robotic radiosurgery can employ different systems for the localization of the neoplastic targets to treat. In particular, for the treatment of the parenchymatous organ tumors, CyberKnife uses a localization system based on specific gold markers<sup>[14]</sup>. Various types of gold markers can be employed in relation to the characteristics of the lesion and the different technique of placement. In particular, the type of gold markers to use often depends on the choice of needles of different calibers and length. The choice of the needle is influenced by the type and site of the lesion to treat and its proximity to critical organs or vascular structures<sup>[15,16]</sup>. The physical characteristic (dimensions and length) of the gold markers strongly depends on the characteristics of the needle. The gold markers (Gold Anchor) contained in fine needle [25 gauge (G) and 22 G] must be smaller in dimension and longer than those contained in larger needles. Markers contained in fine needles, in order to reach an appropriate density for a normal computed tomography (CT) number and to be correctly recognized by the CyberKnife system, must assume a correct array in the parenchyma, when they are inserted. In fact, they have the advantage of being flexible and to curl up when they are pushed against the parenchyma tanks to the spindle and carried by the needle. Therefore, after their placement, the Gold Anchor reached some similar dimensions to those in grain and so, an appropriate density and a normal CT number. Therefore, if they are too crowded or shatter during their release, they do not achieve the proper density to have a normal CT number, and to be well recognized as a fiducial by the CyberKnife System. The markers (cylindrical markers) contained in larger gauge needles (17 G and 18 G) can not break and do not need to mass during their placement. Therefore they can not change their CT number (Figure 1)<sup>[17-21]</sup>. The placement of fiducial markers may be burdened by complications due to puncture or related to the gold markers. For instance, the major complications related to the gold markers could be the migration of fiducials from the positioning site and the physical alterations of the markers, like marker not deployed or shattered, that may occur during or after placement<sup>[22-26]</sup>. These complications determine the lack of fiducials recognition by CyberKnife and result to failure in targeting the lesions that prevented the execution of the treatment<sup>[27,28]</sup>.

The aim of this prospective pilot study was to assess, how the use of two different types of gold fiducial markers: Grain type and Anchor type, affects the accuracy of tracking by the CyberKnife System, and consequently, the therapeutic efficacy of the treatment of primary or metastatic liver malignancy. We also aimed to identify which type of fiducial can ensure better viability of the SRR.





**Figure 1** Types of gold fiducial markers. A: Twenty five gauge and 17 G needle and their gold markers: Grain cylindrical gold marker, 1 mm × 4 mm and flexible wire notched gold marker 0.28 mm × 10 mm, Gold Anchor Marker respectively; B: Grain cylindrical gold marker, 1 mm × 4 mm flexible wire notched gold marker 0.28 mm × 10 mm, Gold Anchor marker after massing.

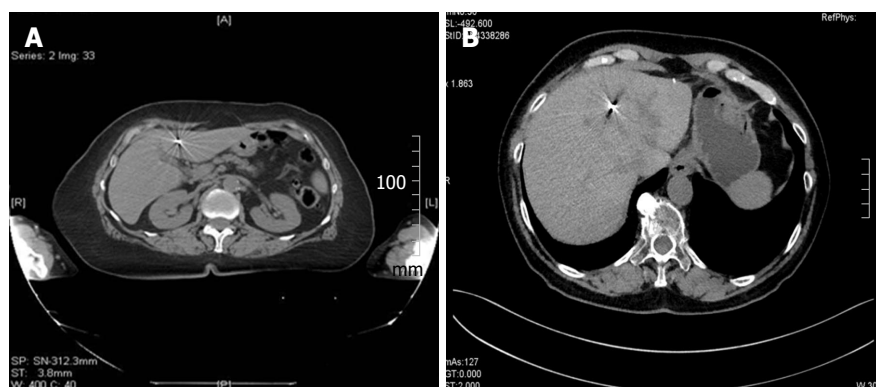


**Figure 2** Ultrasonography-guided fiducial placement. The two different gold markers are sonographically undistinguishable. A: Needle delivering fiducial into a liver mass; B: Hyperechoic flexible wire notched gold marker, 0.28 mm × 10 mm, Gold Anchor marker (arrow) near a liver mass; C: Hyperechoic Grain cylindrical gold marker, 1 mm × 4 mm (arrow) near a liver mass.

## MATERIALS AND METHODS

Fifteen consecutive patients, who were scheduled to receive robotic radiotherapy treatment for primary or metastatic liver malignancy, were recruited for percutaneous ultrasonography (US)-guided placement of intra-hepatic fiducial markers, from March 2014 to June 2014 (Figure 1). A written informed consent was obtained from the patients. Two different types of needles, 25 G and 17 G containing two different fiducial markers, gold notched flexible anchor wire of 0.28 mm × 10 mm (25 G needle) and gold cylindrical grain of 1 mm × 4 mm (17 G), were used. The needle type to use was selected according to the site of the lesion (deep or superficial liver lesion) and physical structure. The choice of the different fiducial markers depends mainly on the choice of the needle caliber. The number of fiducial markers to place was evaluated according to the acoustic window, the compliance of the patients and morphological characteristics of the lesions. The examination was performed by two expert ultrasonographers with the same echograph, ProSound Alfa7, (Hitachi-Aloka, Tokyo, Japan) with a 3.75–7.5 MHz hemispheric sound technology (HST) 91–30 Multi Frequency Convex Abdominal HST probe.

Local anesthesia was achieved *via* the subcutaneous administration of 1% lidocaine. All the gold fiducial markers were placed with US-guidance through sub or intercostals access. After confirming that the needle tip had reached the target lesion, the fiducial marker was deployed, and then the needle was removed. We placed in each patient from 1 to 5 fiducial markers, and when at least two or more fiducials were placed, it was at a distance of about 1.5–2 cm apart, in a way to occupy the perpendicular edges of a cube containing the tumor inside. The Gold Anchor markers were always placed with the same technique to take advantage of their mass effect. Fiducial positioning was confirmed with ultrasound image. A marker was usually seen as a hyperechoic structure. The two different fiducial markers used were sonographically undistinguishable (Figure 2). Technical success was defined when the implantation enables adequate treatment planning and CT simulation. Fiducial migration was defined as seed dislodgement outside the volume of the original injection site that is unusable for guiding stereotactic body radiation therapy (SBRT) as determined by planning CT. Clinical success was defined as the completion of SBRT. Seven days after the procedure, a CyberKnife planning CT for the simulation of radiation treatment was performed on all patients.



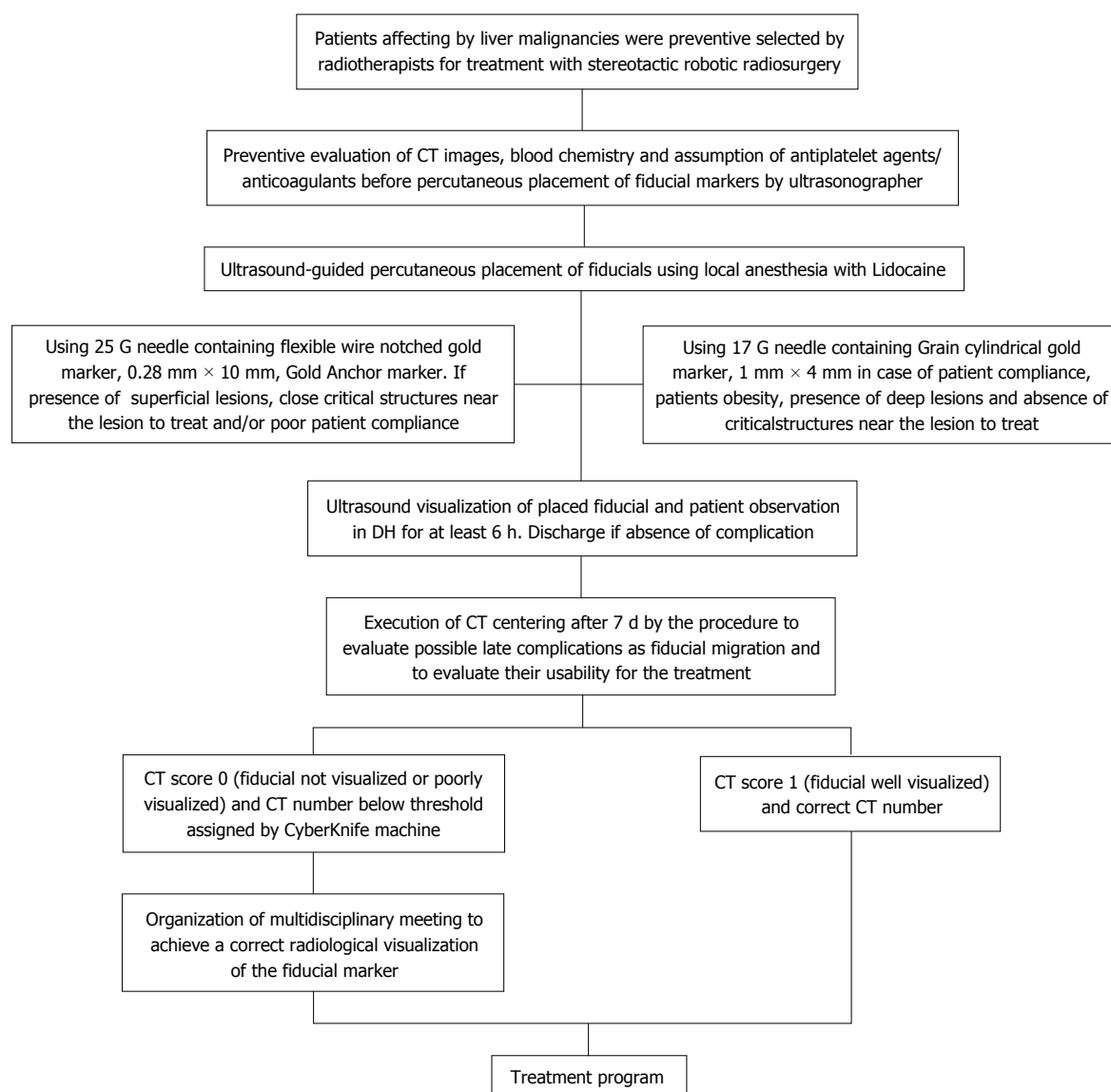
**Figure 3 Well visualized fiducial markers.** A: CT displaying of Grain cylindrical gold marker, 1 mm × 4 mm. This marker exhibits good contrast on X-ray images with typical "star effects"; B: CT displaying types of flexible wire notched gold marker, 0.28 mm × 10 mm, Gold Anchor marker. This marker exhibits good contrast on X-ray images demonstrating less artifacts. CT: Computed tomography.

A binary CT score for the fiducial markers visualization was assigned (not visualized or poorly visualized = 0; well visualized = 1) (Figure 3). In the case of CT score of zero (0) which prevented treatment, we organized a series of multidisciplinary meetings (with regards to the procedure, the physician and the radiation oncologist responsible for the radiosurgery treatment) to achieve the correct radiological visualization of the fiducial marker. Moreover, for the execution of treatment with CyberKnife, it is necessary that each fiducial reaches a CT number above a specific threshold (CT number threshold). The CT number of the fiducial is assigned in an automated manner by the CyberKnife machine (Figure 4). Database construction and data analysis were performed using Office Excel 2007, XLSTAT 2016 (microsoft) and SPSS for Windows (SPSS Inc., Chicago, United States). We examined the data with the use of appropriate parametric and non-parametric statistical tests (Student's *t*-test two-tailed and a  $\chi^2$  test according to Fischer considering  $P < 0.05$  as significant). A Lilliefors (Kolmogorov-Smirnov) test for normality has been previously performed. Statistical analysis was performed by Tommaso Gabbiani, MD, and reviewed by Principal Investigator, Maria Marsico, MD.

## RESULTS

Fifteen consecutive patients (men: 9, women: 6, mean age: 72.9 years old, range: SD ± 7.9) who had already undergone percutaneous ultrasound-guided fiducial marker implantations for CyberKnife therapy were employed for this study. Eleven patients (8 males) presented liver metastasis from a note primary neoplasm (2 right colon carcinoma, 2 sigmoid carcinoma, 2 rectum carcinoma, 1 gastric carcinoma, 1 lung carcinoma, 1 ovarian carcinoma and 2 pancreatic carcinoma). Four patients (2 males) showed liver primary malignancy [2 hepatocellular carcinoma (HCC), 1 cholangio-carcinoma, 1 hepatic cholangio-carcinoma]. Among 11 patients who presented liver metastasis, 9 patients had previously undergone radical surgery of primary neoplasm. Among these 9 patients, 4 had submitted to adjuvant therapy,

2 to metastasectomy and adjuvant therapy, 2 to neo-adjuvant and adjuvant therapy and 1 to metastasectomy without chemotherapy. Moreover among these 9 patients, 7 developed new liver metastasis during or at the end of the treatment, while 2 patients presented a metastatic recurrence. Only 2 patients of the 11 affected by liver metastasis undergone treatment by chemotherapy and not surgery, one performed a palliative chemotherapy and the other performed an effective chemotherapy with failure of treatment. In the group of patients with primary hepatic neoplasm, patients affected by HCC and hepato-cholangiocarcinoma were treated by chemoembolization, while another one affected by cholangiocarcinoma undergone chemotherapy. Patients treated with chemoembolization showed relapse of neoplasm, while the patient treated with chemotherapy showed no response to the treatment. In the group of patients with hepatic metastasis, 8 of them have a single nodule, 2 of them have two nodules, and 1 has three nodules so the total of liver lesions treated was 15. These 15 liver lesions presented a maximum diameter between 2 to 4 cm. In the group of patients with primary liver lesions, 3 patients showed a single nodule and another one presented two nodules so the total primary lesion treated was 5. Four of these measured a maximum diameter from 2 to 4 cm and only one measured a maximum diameter over 4 cm. 2 patients showed a moderate ascites at the moment of the procedure. The 20 liver lesions were localized into the VII liver segment ( $n = 7$ ), VI liver segment ( $n = 3$ ), VIII segment ( $n = 3$ ), V segment ( $n = 1$ ), IV segment ( $n = 1$ ), III segment ( $n = 1$ ), between V-VI segment ( $n = 1$ ), between V-VI-VII segments ( $n = 1$ ) and between VI-VII segments ( $n = 2$ ). Five lesions were localized close to vascular structures and 2 lesions close to critical organs. Considering the closeness of the critical organs or vascular structures and the patients' compliance, 8 patients undergone a combined placement of the two types of gold markers. Two patients presented severe compliance problems (panic attack), so they received only anchor markers (placed with fine needles). Five patients received only cylindrical grain markers. For each



**Figure 4** Flow chart of the study conducted tank to a prospective collection of data (compliance, demographic and clinic characteristics of the patients, liver lesions characteristics, type of needle and markers used, ultrasonographic and computed tomography visualization of fiducial markers, usability of the markers and immediate and late complications) and a retrospective statistical analysis. CT: Computed tomography.

patient about 1 to 5 intra-hepatic markers were placed (one in 2 patients, three in 8 patients, four in 3 patients, and five in 2 patients). A total of 48 needles were used (thirty-two 17 G and sixteen 25 G) and 48 gold markers were placed (32 Grain shaped markers and 16 Gold Anchors). In 47 cases, the gold markers were placed through subcostal access and only in a single case with an inter-costal access. Every patient received a local anesthesia with lidocaine. All fiducials placement were sonographically confirmed right after the procedure. No patient presented any major complication related to the procedure.

After the placement of markers, 14 patients underwent the planning simulation CT scan to allow fiducials to settle. One patient did not perform the CT because of a complication related to the primary tumor (hepatic failure). Removing the latter patient who was excluded from the treatment for causes not correlated to the

fiducial placement, the technical and clinical success rate was 100%. The CT scan revealed that 14 markers (11 Gold Anchors and 3 Grain shaped markers) showed late complications. Few markers showed more than one complications at the same time for a total of 27 complications. Shattered markers ( $n = 2$ ; 2 Gold Anchors), extra-hepatic migration ( $n = 4$ ; 1 Gold Anchor and 3 Grain markers), extra-hepatic migration and marker not visualized ( $n = 1$ ; 1 Gold Anchor), intra-hepatic migration ( $n = 5$ ; 5 Gold Anchors), not massed markers ( $n = 5$ ; 5 Gold Anchor). The Gold Anchor marker presented more frequent late minor complications (68.75% vs 9.375%,  $P = 3 \times 10^{-6}$ ). Moreover, 38 markers were visualized with CT score = 1 and 10 markers with CT score = 0, the markers visualized with CT score = 0 were all Gold Anchors and we demonstrated that the CT subjective visualization of Grain shaped markers was significantly higher than the CT subjective visualization for Gold

Anchor (100% vs 37.5%,  $P = 5 \times 10^{-9}$ ). For 5 patient it was necessary to organize multidisciplinary meetings to identify the correct intra-hepatic localization of the markers visualized with CT score = 0. Finally, 5 markers showed a CT number below the threshold (5 Gold Anchors). The 5 markers with the CT number below the threshold were not recognized by the CyberKnife system and so were not used for the treatment (one marker not recognized for 5 patients). Forty-three markers (32 Grain shaped markers and 11 Gold Anchors) with regular CT number were recognized by CyberKnife system and were used for the treatment. The clinical success achieved was 89.6%. We demonstrated that the Gold Anchor marker is associated with a threshold below the CT number (31.25% vs 0%,  $P = 0.0005$ ) that is not suitable for treatment. A total of 14 patients underwent radiosurgery treatment, only one patient was excluded because of a complication related to his primary tumor.

## DISCUSSION

The CyberKnife Robotic Radiosurgery System is a non-surgical option for patients who have inoperable or surgically complex tumors or who may be looking for an alternative to surgery. It is an option in the case where no response and/or relapse is observed after chemotherapy and standard radiotherapy<sup>[29-31]</sup>. In our study, we compared the therapeutic usability of the two different gold fiducial markers for robotic radiosurgery treatment of primary and metastatic liver malignancies. We used the two different gold markers according to the necessity to either use 17 or 25 G needle, depending on the patient's compliance, patient physical structure and the proximity of critical or vascular structures. This pilot trial demonstrate that the Anchor marker (0.28 mm × 10 mm) is correlated with a greater number of late minor complications that results from a frequent association with a CT number below threshold and a low subjective CT visualization, resulting in a delay or a difficulty in starting the treatment. In our opinion, the use of the Gold Anchor marker should be limited to use of the 25 G needle and in combination with the other types of markers. Only few studies have compared the use of different fiducial in the terms of efficacy and complications<sup>[32,33]</sup>. Our study differs from others because it compares the two different types of gold fiducial markers in terms of usability for CyberKnife treatment. In our study, we identified some of the factors related to the type of fiducials (the Gold Anchor) that may prevent the treatment with CyberKnife. The identification and knowledge of these factors allows us to limit the use of Gold Anchor marker type, to specific cases and preferably, in combination with the other marker types, in order to reduce the tracking problems of CyberKnife. Infact, CyberKnife tracking problems are causes of increasing costs and delay in treatment execution. Contrary to what is shown in our study, other trials have demonstrated the advantage of Gold Anchor fiducial than the other types of fiducial markers for the

treatment with CyberKnife. Nevertheless, in these other studies, inserting of the fiducial markers was executed by endoscopic ultrasonography technique to treat tumors of the pancreas and lung. Therefore, it is our opinion that the different techniques for positioning, and the different localization of the lesions may be the basis of the different results obtained in the study. Furthermore, we must consider the variable offered by the needle. The percutaneous placement of Gold Anchor (0.28 mm × 10 mm) occurred with the 25 G needle that originally contained the gold marker. In the cases of endoscopic ultrasound guided placement of fiducial gold markers (in particular, for treatment of pancreas lesion), the 25 G needle originally containing the marker, serves only as a carrier to put the fiducials inside the other needles (22 G or 19 G) usually used for the fiducial placement. The use of needles with a greater caliber (22 G and 19 G) and less flexibility than the 25 G needle, may facilitate the placement of Gold Anchor limiting the complications.

## COMMENTS

### Background

The treatment of liver malignancies has evolved over the years. Although surgery is the current standard treatment for localized surgically operable lesions. Alternative treatment approaches for unresectable liver metastasis and primary liver cancer include: Chemoembolization, radiofrequency ablation, cryotherapy, and the oral multikinase inhibitor sorafenib, chemotherapy and standard radiotherapy. The CyberKnife Robotic Radiosurgery System is a non-surgical option for patients who have inoperable or surgically complex tumors or who may be looking for an alternative to surgery. It also provides an option in the case where no response and/or relapse is observed after standard treatment.

### Research frontiers

Many points still remain unclear in literature to ameliorate the treatment by CyberKnife and a lot of them seem to correlate with the type of fiducial to be use, the technique of placement and the number of fiducial to use. Nowadays, there are many different types of gold fiducial markers with different dimensions, lengths and physical characteristics. Therefore, many other studies of fiducial comparison, like the authors', should be conducted. This is necessary to identify the basis of compliance, demographic and clinical characteristics of the patients and liver lesions characteristics, the best type of fiducial and needle to use. Percutaneous fiducial marker placement could be under computed tomography (CT) fluoroscopic guidance or ultrasonographic (US) guidance. In this study, fiducial placement was entirely conducted under US guidance demonstrating a great safety and efficacy. Cost-effectiveness studies should also be conducted to compare the CT and the US percutaneous fiducial placement to identify the best method in the terms of cost-effectiveness. Nowadays, there is yet no consensus in literatures on the exact number of fiducials necessary to effectively perform the treatment with CyberKnife. Many studies define the technical success as the ability to place more of a fiducial near the tumor target before the treatment; other studies have resulted in higher clinical success placing a unique fiducial marker for patient. In this study, the authors also demonstrated a high clinical success from using one to five fiducial for each patient, in relation to which fiducials were really recognized and used by the CyberKnife system (fiducial with correct relegated CT number). Therefore, many studies should be conducted regarding the different analysis of tracking accuracy resulting from the use of a different number of fiducial for treatment. This is important to establish the best number of fiducials to use in terms of cost effectiveness.

### Innovations and breakthroughs

The study differs from others because it compares the two different types of gold fiducial markers in terms of usability for CyberKnife treatment of liver



malignancies. The study also differs for describing other possible complications related to Gold Anchor - their wrong stacking and their break after the placement. In this study, the authors identified some factors related to the type of fiducial, (the Gold Anchor) that may prevent the treatment with CyberKnife. The identification and knowledge of these factors allows them to limit the use of Gold Anchor marker type, to specific cases and preferably in combination with other marker types, in order to reduce tracking problems of CyberKnife. In fact, CyberKnife tracking problems are causes of increasing costs and delay in treatment execution. Contrary to what is shown in the study, other trials have demonstrated the advantage of Gold Anchor fiducial over the other types of fiducial markers for the treatment with CyberKnife. Nevertheless, in these other studies, the inserting of fiducial markers was executed by endoscopic ultrasonography technique for treating tumors of the pancreas and lung. Therefore, it is the authors' opinion that the different techniques of positioning, and the different localization of the lesions and the different needles (generally of higher caliber) used for the placement of Gold Anchor may be the basis of the different results obtained in this study.

### Applications

In the authors' opinion, the use of Gold Anchor marker type has to be limited to specific cases; in order to reduce tracking problems of CyberKnife treatment which is the major cause of increasing costs and delay in treatment execution. Therefore, the authors suggest that the use of the Gold Anchor marker should be limited to the necessity to use the 25 G needle and in combination with the other type of markers. In particular, the 25 G needle should be used in the case of low patient compliance, absence of obesity and in the presence of superficial lesions at critical structure near the liver lesions.

### Terminology

Stereotactic robotic radio surgery: Ability to dispense high doses of focused radiation in a minor number of fractions respect to the standard treatment (2-5 vs 30-40). Ability to reach any point with anatomical precision and extreme sub-millimeter accuracy thanks to a target localization computerized system offered by CyberKnife system; CyberKnife: Robot with a complete autonomy characteristic with more than 1500 dispensing positions of X-ray; Variable diameter collimator; Synchrony Respiratory Tracking System to preserve the near organs from toxicity; Fiducial gold markers: Markers exploited by CyberKnife for the target localization in the treatment of parenchymatous organs lesions. This marker is made from gold, which makes it biocompatible and ensures it exhibits good contrast on X-ray images; CT number: A normalized value of the calculated X-ray absorption coefficient of a pixel (picture element) in a computed tomogram, expressed in Hounsfield units, where the CT number of air is -1000 and that of water is 0.

### Peer-review

In this work, the authors reported a comparison study of two different types of fiducial markers for robotic radiosurgery. In this study, 15 patients have been recruited, in which 48 gold markers were placed (32 Grain shaped markers and 16 Gold Anchor). All these patients except one were scanned with CT for visualization and identification of these markers. The data of these patients were analyzed and reported in this work. The work intended to address an interesting clinical issue.

## REFERENCES

- 1 **Kocher M**, Wittig A, Piroth MD, Treuer H, Seegenschmiedt H, Ruge M, Grosu AL, Guckenberger M. Stereotactic radiosurgery for treatment of brain metastases. A report of the DEGRO Working Group on Stereotactic Radiotherapy. *Strahlenther Onkol* 2014; **190**: 521-532 [PMID: 24715242 DOI: 10.1007/s00066-014-0648-7]
- 2 **Herfarth KK**. Extracranial stereotactic radiation therapy. In: Schlegel W, Bortfeld T, Grosu AL. *New Technologies in Radiation Oncology*. Berlin: Springer-Verlag, 2006: 277-288 [DOI: 10.1007/3-540-29999-8\_22]
- 3 **Seo Y**, Kim MS, Yoo S, Cho C, Yang K, Yoo H, Choi C, Lee D, Kim J, Kim MS, Kang H, Kim Y. Stereotactic body radiation therapy boost in locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2009; **75**: 1456-1461 [PMID: 19783379]
- 4 **Kalogeridi MA**, Zygogianni A, Kyrgias G, Kouvaris J, Chatziioannou S, Kelekis N, Kouloulis V. Role of radiotherapy in the management of hepatocellular carcinoma: A systematic review. *World J Hepatol* 2015; **7**: 101-112 [PMID: 25625001 DOI: 10.4254/wjh.v7.i1.101]
- 5 **Collins BT**, Vahdat S, Erickson K, Collins SP, Suy S, Yu X, Zhang Y, Subramaniam D, Reichner CA, Sarikaya I, Esposito G, Yousefi S, Jamis-Dow C, Banovac F, Anderson ED. Radical cyberknife radiosurgery with tumor tracking: an effective treatment for inoperable small peripheral stage I non-small cell lung cancer. *J Hematol Oncol* 2009; **2**: 1 [PMID: 19149899 DOI: 10.1186/1756-8722-2-1]
- 6 **Li D**, Kang J, Golas BJ, Yeung VW, Madoff DC. Minimally invasive local therapies for liver cancer. *Cancer Biol Med* 2014; **11**: 217-236 [PMID: 25610708]
- 7 **Katz AJ**, Santoro M, Diblasio F, Ashley R. Stereotactic body radiotherapy for localized prostate cancer: disease control and quality of life at 6 years. *Radiat Oncol* 2013; **8**: 118 [PMID: 23668632 DOI: 10.1186/1748-717X-8-118]
- 8 **Choi BO**, Jang HS, Kang KM, Lee SW, Kang YN, Chai GY, Choi IB. Fractionated stereotactic radiotherapy in patients with primary hepatocellular carcinoma. *Jpn J Clin Oncol* 2006; **36**: 154-158 [PMID: 16520355 DOI: 10.1093/jcco/hyi236]
- 9 **Tse RV**, Hawkins M, Lockwood G, Kim JJ, Cummings B, Knox J, Sherman M, Dawson LA. Phase I study of individualized stereotactic body radiotherapy for hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *J Clin Oncol* 2008; **26**: 657-664 [PMID: 18172187 DOI: 10.1200/JCO.2007.14.3529]
- 10 **Matsuo Y**, Onishi H, Nakagawa K, Nakamura M, Ariti T, Kumazaki Y, Shimbo M, Tohyama N, Nishio T, Okumura M, Shirato H, Hiraoka M. Guidelines for respiratory motion management in radiation therapy. *J Radiat Res* 2013; **54**: 561-568 [PMID: 23239175 DOI: 10.1093/jrr/rrs122]
- 11 **Wong JW**, Sharpe MB, Jaffray DA, Kini VR, Robertson JM, Stromberg JS, Martinez AA. The use of active breathing control (ABC) to reduce margin for breathing motion. *Int J Radiat Oncol Biol Phys* 1999; **44**: 911-919 [PMID: 10386650 DOI: 10.1016/S0360-3016(99)00056-5]
- 12 **Lohr F**, Debus J, Frank C, Herfarth K, Pastyr O, Rhein B, Bahner ML, Schlegel W, Wannenmacher M. Noninvasive patient fixation for extracranial stereotactic radiotherapy. *Int J Radiat Oncol Biol Phys* 1999; **45**: 521-527 [PMID: 10487580 DOI: 10.1016/S0360-3016(99)00190-X]
- 13 **Kubo HD**, Hill BC. Respiration gated radiotherapy treatment: a technical study. *Phys Med Biol* 1996; **41**: 83-91 [PMID: 8685260 DOI: 10.1088/0031-9155/41/1/007]
- 14 **Ohta K**, Shimohira M, Murai T, Nishimura J, Iwata H, Ogino H, Hashizume T, Shibamoto Y. Percutaneous fiducial marker placement prior to stereotactic body radiotherapy for malignant liver tumors: an initial experience. *J Radiat Res* 2016; **57**: 174-177 [PMID: 26826200 DOI: 10.1093/jrr/rrv099]
- 15 **Peiffert D**, Baumann AS, Marchesi V. Treatment of hepatic metastases of colorectal cancer by robotic stereotactic radiation (Cyberknife®). *J Visc Surg* 2014; **151** Suppl 1: S45-S49 [PMID: 24582275 DOI: 10.1016/j.jvisurg.2014.01.003]
- 16 **Koong AC**, Christofferson E, Le QT, Goodman KA, Ho A, Kuo T, Ford JM, Fisher GA, Greco R, Norton J, Yang GP. Phase II study to assess the efficacy of conventionally fractionated radiotherapy followed by a stereotactic radiosurgery boost in patients with locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2005; **63**: 320-323 [PMID: 16168826 DOI: 10.1016/j.ijrobp.2005.07.002]
- 17 **Dávila Fajardo R**, Lekkerkerker SJ, van der Horst A, Lens E, Bergman JJ, Fockens P, Bel A, van Hooft JE. EUS-guided fiducial markers placement with a 22-gauge needle for image-guided radiation therapy in pancreatic cancer. *Gastrointest Endosc* 2014; **79**: 851-855 [PMID: 24518121 DOI: 10.1016/j.gie.2013.12.027]
- 18 **Ammar T**, Coté GA, Creach KM, Kohlmeier C, Parikh PJ, Azar RR. Fiducial placement for stereotactic radiation by using EUS: feasibility when using a marker compatible with a standard 22-gauge needle. *Gastrointest Endosc* 2010; **71**: 630-633 [PMID: 20189527 DOI: 10.1016/j.gie.2009.11.023]
- 19 **DiMaio CJ**, Nagula S, Goodman KA, Ho AY, Markowitz AJ,

- Schattner MA, Gerdes H. EUS-guided fiducial placement for image-guided radiation therapy in GI malignancies by using a 22-gauge needle (with videos). *Gastrointest Endosc* 2010; **71**: 1204-1210 [PMID: 20598247 DOI: 10.1016/j.gie.2010.01.003]
- 20 **Sanders MK**, Moser AJ, Khalid A, Fasanella KE, Zeh HJ, Burton S, McGrath K. EUS-guided fiducial placement for stereotactic body radiotherapy in locally advanced and recurrent pancreatic cancer. *Gastrointest Endosc* 2010; **71**: 1178-1184 [PMID: 20362284 DOI: 10.1016/j.gie.2009.12.020]
- 21 **Pishvaian AC**, Collins B, Gagnon G, Ahlawat S, Haddad NG. EUS-guided fiducial placement for CyberKnife radiotherapy of mediastinal and abdominal malignancies. *Gastrointest Endosc* 2006; **64**: 412-417 [PMID: 16923491 DOI: 10.1016/j.gie.2006.01.048]
- 22 **Patel A**, Khalsa B, Lord B, Sandrasegaran K, Lall C. Planting the seeds of success: CT-guided gold seed fiducial marker placement to guide robotic radiosurgery. *J Med Imaging Radiat Oncol* 2013; **57**: 207-211 [PMID: 23551782 DOI: 10.1111/j.1754-9485.2012.02445.x]
- 23 **Kim JH**, Hong SS, Kim JH, Park HJ, Chang YW, Chang AR, Kwon SB. Safety and efficacy of ultrasound-guided fiducial marker implantation for CyberKnife radiation therapy. *Korean J Radiol* 2012; **13**: 307-313 [PMID: 22563268 DOI: 10.3348/kjr.2012.13.3.307]
- 24 **Shirato H**, Harada T, Harabayashi T, Hida K, Endo H, Kitamura K, Onimaru R, Yamazaki K, Kurauchi N, Shimizu T, Shinohara N, Matsushita M, Dosaka-Akita H, Miyasaka K. Feasibility of insertion/implantation of 2.0-mm-diameter gold internal fiducial markers for precise setup and real-time tumor tracking in radiotherapy. *Int J Radiat Oncol Biol Phys* 2003; **56**: 240-247 [PMID: 12694845 DOI: 10.1016/S0360-3016(03)00076-2]
- 25 **Ohta K**, Shimohira M, Sasaki S, Iwata H, Nishikawa H, Ogino H, Hara M, Hashizume T, Shibamoto Y. Transarterial Fiducial Marker Placement for Image-guided Proton Therapy for Malignant Liver Tumors. *Cardiovasc Intervent Radiol* 2015; **38**: 1288-1293 [PMID: 25366091 DOI: 10.1007/s00270-014-1013-z]
- 26 **Brook OR**, Gourtsoyianni S, Mendiratta-Lala M, Mahadevan A, Siewert B, Sheiman RR. Safety profile and technical success of imaging-guided percutaneous fiducial seed placement with and without core biopsy in the abdomen and pelvis. *AJR Am J Roentgenol* 2012; **198**: 466-470 [PMID: 22268195 DOI: 10.2214/AJR.11.6431]
- 27 **Valentine K**, Cabrera T, Roberge D. Implanting metal fiducials to guide stereotactic liver radiation: McGill experience and review of current devices, techniques and complications. *Technol Cancer Res Treat* 2014; **13**: 253-258 [PMID: 24066955]
- 28 **Sotiropoulou E**, Stathochristopoulou I, Stathopoulos K, Verigos K, Salvaras N, Thanos L. CT-guided fiducial placement for cyberknife stereotactic radiosurgery: an initial experience. *Cardiovasc Intervent Radiol* 2010; **33**: 586-589 [PMID: 19908085 DOI: 10.1007/s00270-009-9748-7]
- 29 **Scorsetti M**, Comito T, Cozzi L, Clerici E, Tozzi A, Franzese C, Navarra P, Fogliata A, Tomatis S, D'Agostino G, Iftode C, Mancosu P, Ceriani R, Torzilli G. The challenge of inoperable hepatocellular carcinoma (HCC): results of a single-institutional experience on stereotactic body radiation therapy (SBRT). *J Cancer Res Clin Oncol* 2015; **141**: 1301-1309 [PMID: 25644863 DOI: 10.1007/s00432-015-1929-y]
- 30 **Okabe T**, Kimura T, Nagata Y. [Stereotactic body radiotherapy]. *Gan To Kagaku Ryoho* 2014; **41**: 2543-2545 [PMID: 25596046]
- 31 **Casamassima F**, Cavedon C, Francescon P, Stancanella J, Avanzo M, Cora S, Scalchi P. Use of motion tracking in stereotactic body radiotherapy: Evaluation of uncertainty in off-target dose distribution and optimization strategies. *Acta Oncol* 2006; **45**: 943-947 [PMID: 16982561 DOI: 10.1080/02841860600908962]
- 32 **Machiels M**, van Hooft J, Jin P, van Berge Henegouwen MI, van Laarhoven HM, Alderliesten T, Hulshof MC. Endoscopy/EUS-guided fiducial marker placement in patients with esophageal cancer: a comparative analysis of 3 types of markers. *Gastrointest Endosc* 2015; **82**: 641-649 [PMID: 25957478 DOI: 10.1016/j.gie.2015.03.1972]
- 33 **Fuccio L**, Lami G, Guido A, Fabbri C. EUS-guided gold fiducial placement and migration rate. *Gastrointest Endosc* 2014; **80**: 533-534 [PMID: 25127952 DOI: 10.1016/j.gie.2014.03.028]

**P- Reviewer:** Chang Z, Tomuleasa C **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Liu SQ



Prospective Study

## Circulating insulin-like growth factor-binding protein 3 as prognostic biomarker in liver cirrhosis

Carina Gabriela Correa, Bruno da Silveira Colombo, Marcelo Fernando Ronsoni, Pedro Eduardo Soares e Silva, Leonardo Fayad, Telma Erotides Silva, Letícia Muraro Wildner, Maria Luiza Bazzo, Esther Buzaglo Dantas-Correa, Janaína Luz Narciso-Schiavon, Leonardo de Lucca Schiavon

Carina Gabriela Correa, Bruno da Silveira Colombo, Marcelo Fernando Ronsoni, Pedro Eduardo Soares e Silva, Leonardo Fayad, Telma Erotides Silva, Esther Buzaglo Dantas-Correa, Janaína Luz Narciso-Schiavon, Leonardo de Lucca Schiavon, Division of Gastroenterology, Federal University of Santa Catarina, Florianópolis, Santa Catarina 88.040-001, Brazil

Letícia Muraro Wildner, Maria Luiza Bazzo, Department of Clinical Analysis, Federal University of Santa Catarina, Florianópolis, Santa Catarina 88.040-970, Brazil

**Author contributions:** Narciso-Schiavon JL and Schiavon LL designed the research; Correa CG, Colombo BS, Ronsoni MF, Soares e Silva PE, Fayad L, Silva TE and Dantas-Correa EB performed the research; Wildner LM and Bazzo ML contributed with the specific laboratory analysis and sample handling; Correa CG and Schiavon LL analyzed the data and wrote the paper.

**Institutional review board statement:** The study was reviewed and approved by the Federal University of Santa Catarina Institutional Review Board.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** Nothing to report.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at [leo-jf@uol.com.br](mailto:leo-jf@uol.com.br). Participants gave informed consent for data sharing.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Dr. Leonardo de Lucca Schiavon, Division of Gastroenterology, Federal University of Santa Catarina, Rua Deputado Antonio Edu Vieira 1310, casa 217, Bairro Pantanal, Florianópolis, Santa Catarina 88.040-001, Brazil. [leo-jf@uol.com.br](mailto:leo-jf@uol.com.br)  
 Telephone: +55-48-32096854  
 Fax: +55-48-32096854

Received: February 26, 2016  
 Peer-review started: February 26, 2016  
 First decision: March 23, 2016  
 Revised: May 3, 2016  
 Accepted: May 17, 2016  
 Article in press: May 27, 2016  
 Published online: June 18, 2016

### Abstract

**AIM:** To investigate the prognostic significance of insulin-like growth factor-binding protein 3 (IGFBP-3) in patients with cirrhosis.

**METHODS:** Prospective study that included two cohorts: outpatients with stable cirrhosis ( $n = 138$ ) and patients hospitalized for acute decompensation ( $n = 189$ ). Development of complications, mortality or liver transplantation was assessed by periodical phone calls and during outpatient visits. The cohort of stable cirrhosis also underwent clinical and laboratory evaluation yearly (2013 and 2014) in predefined study visits. In patients with stable cirrhosis, IGFBP-3 levels were measured at baseline (2012) and at second re-evaluation (2014). In hospitalized subjects, IGFBP-3 levels were measured in serum samples collected in the first and in the third day after admission and stored at  $-80^{\circ}\text{C}$ . IGFBP-3 levels

were measured by immunochemiluminescence.

**RESULTS:** IGFBP-3 levels were lower in hospitalized patients as compared to outpatients (0.94 mcg/mL *vs* 1.69 mcg/mL,  $P < 0.001$ ) and increased after liver transplantation (3.81 mcg/mL *vs* 1.33 mcg/mL,  $P = 0.008$ ). During the follow-up of the stable cohort, 17 patients died and 11 received liver transplantation. Bivariate analysis showed that death or transplant was associated with lower IGFBP-3 levels (1.44 mcg/mL *vs* 1.74 mcg/mL,  $P = 0.027$ ). The Kaplan-Meier transplant-free survival probability was 88.6% in patients with IGFBP-3  $\geq 1.67$  mcg/mL and 72.1% for those with IGFBP-3  $< 1.67$  mcg/mL ( $P = 0.015$ ). In the hospitalized cohort, 30-d mortality was 24.3% and was independently associated with creatinine, INR, SpO<sub>2</sub>/FiO<sub>2</sub> ratio and IGFBP-3 levels in the logistic regression. The 90-d transplant-free survival probability was 80.4% in patients with IGFBP-3  $\geq 0.86$  mcg/mL and 56.1% for those with IGFBP-3  $< 0.86$  mcg/mL ( $P < 0.001$ ).

**CONCLUSION:** Lower IGFBP-3 levels were associated with worse outcomes in patients with cirrhosis, and might represent a promising prognostic tool that can be incorporated in clinical practice.

**Key words:** Liver cirrhosis; Acute decompensation; Insulin-like growth factor binding protein 3; Acute-on-chronic liver failure; Prognosis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Insulin-like growth factor-binding protein 3 (IGFBP-3) levels are decreased in cirrhosis and seem to correlate with the intensity of hepatic dysfunction, but its prognostic significance is uncertain. In this prospective cohort study, IGFBP-3 levels correlated with variables associated with the intensity of liver dysfunction in both outpatients with stable cirrhosis and in subjects hospitalized for acute decompensation. IGFBP-3 levels increased significantly after discharge and after liver transplantation. Lower IGFBP-3 levels were associated with poor outcomes in both outpatients with stable cirrhosis and in those hospitalized for acute decompensation, suggesting that it can be used in clinical practice as a prognostic biomarker in cirrhosis.

Correa CG, Colombo BS, Ronsoni MF, Soares e Silva PE, Fayad L, Silva TE, Wildner LM, Bazzo ML, Dantas-Correa EB, Narciso-Schiavon JL, Schiavon LL. Circulating insulin-like growth factor-binding protein 3 as prognostic biomarker in liver cirrhosis. *World J Hepatol* 2016; 8(17): 739-748 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i17/739.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i17.739>

## INTRODUCTION

Cirrhosis is a late stage progressive hepatic fibrosis

characterized by distortion of hepatic architecture and the formation of regenerative nodules. Although considered potentially reversible in some clinical scenarios, in its advanced stages, liver cirrhosis is associated with high mortality and the only treatment option may be liver transplantation<sup>[1]</sup>. Patients with cirrhosis are susceptible to a variety of complications such as variceal bleeding, ascites, spontaneous bacterial peritonitis (SBP), hepatic encephalopathy, hepatocellular carcinoma, hepatorenal syndrome, hepatopulmonary syndrome and portal vein thrombosis<sup>[1]</sup>. The prognosis of cirrhosis is highly variable because it is influenced by a number of factors including etiology, severity, presence of complications, and comorbidities. Mortality rate increases significantly once decompensation occurs<sup>[2]</sup>.

Changes in the growth hormone-insulin-like growth factor (GH-IGF) axis occur as a result of liver disease and has been reported in cirrhosis<sup>[3,4]</sup>. GH and IGF-I have important anabolic effects on the metabolism of proteins, carbohydrates and lipids<sup>[5]</sup>. GH is a peptide hormone released from anterior pituitary that stimulates growth, cell reproduction and regenerations while exerting metabolic effects on bone, cartilage, fat, muscles, heart and the immune system<sup>[6-8]</sup>.

In the liver, GH activation of GH receptors induces IGF- I gene transcription, and the subsequent synthesis and release of IGF- I to plasma<sup>[9]</sup>. However, the actions of IGF- I are tightly controlled by the binding of IGF to binding proteins (IGFBP-1 to -6). The IGFBPs carry IGFs in the serum and regulate their activity and bioavailability<sup>[10]</sup>. IGFBP-3 is the major binding protein of IGF and carries 80%-90% of circulating IGF- I. The IGFBP-3 is predominantly produced in the liver and synthesized in Kupffer cells. It forms a 150 kDa ternary complex with IGFs and the acid-labile subunit<sup>[7,9]</sup>.

Low serum concentrations in both IGF- I and IGFBP-3 have been reported in patients with cirrhosis *vs* healthy controls. This likely reflects decreased hepatic synthesis function<sup>[5,11-13]</sup>. In fact, some studies reported that IGFBP-3 serum levels are abnormally low in patients with liver cirrhosis and correlated with several variables associated with the intensity of liver dysfunction<sup>[5,7]</sup>. Even though these reports suggest the clinical utility of monitoring circulating IGFBP-3 in cirrhosis, there is little data on the prognostic significance of this biomarker. The aim of this study was to investigate the relationship between serum IGFBP-3 levels and prognosis in both outpatients with stable cirrhosis and in patients hospitalized for acute decompensation (AD).

## MATERIALS AND METHODS

### Patients

This prospective study included two cohorts of adult patients ( $\geq 18$  years of age) with liver cirrhosis in the University Hospital of the Federal University of Santa Catarina. The diagnosis of cirrhosis was established either histologically (when available) or by combination of clinical, imaging and laboratory findings in patients



with evidence of portal hypertension. The first cohort was comprised of patients with stable cirrhosis in the outpatient clinic. In this case, patients in the following situations were excluded: Diagnosis of hepatocellular carcinoma; interferon-based therapy over the last 30 d; or refusal or inability of the patient to understand the terms of the informed consent.

The second cohort included patients admitted to the emergency room due to AD of liver cirrhosis. In this group, the following exclusion criteria were adopted: Hospitalization for elective procedures; admissions not related to complications of liver cirrhosis; and hepatocellular carcinoma outside Milan criteria. Exclusion criteria for both groups also included: Pregnancy; chronic renal failure requiring hemodialysis; severe heart disease; severe chronic pulmonary disease; and active extrahepatic cancer. The study protocol complies with ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee on Human Research of the Federal University of Santa Catarina.

## Methods

This initial cohort was initially evaluated from June to October 2012. The development of complications, mortality, or liver transplantation was assessed by periodic phone calls and during outpatient visits. Patients also underwent clinical and laboratory evaluation yearly (2013 and 2014) in predefined study visits. The second cohort included subjects hospitalized for AD between January 2011 and November 2013. AD was defined by acute development of hepatic encephalopathy, large ascites, gastrointestinal bleeding, bacterial infection or any combination of these. Patients were evaluated within 24 h of admission by one of the researchers involved in the study. They were followed during their hospital stay and 30- and 90-d mortality was evaluated by phone call in case of hospital discharge. In case of more than one hospital admission during the study period-only the most recent hospitalization was considered.

The following clinical variables were collected for all patients: Age, gender, etiology of cirrhosis, history of previous decompensation, current complications of cirrhosis, active alcoholism and regular propranolol and omeprazole use. All subjects underwent laboratory evaluation including total leukocytes, serum sodium, creatinine, international normalized ratio (INR), albumin, C-reactive protein (CRP), total bilirubin and IGFBP-3. In patients hospitalized for AD of cirrhosis, blood samples were obtained on the first and third day after admission.

Active alcoholism was defined as an average overall consumption of 21 or more drinks per week for men and 14 or more drinks per week for women during the 4 wk before enrollment (one standard drink is equal to 12 g absolute alcohol)<sup>[14]</sup>.

Hospitalized individuals with a suspected infection at admission received a clinical examination to confirm this diagnosis and to establish the primary source of infection. Diagnosis of infection was made according to

the criteria of Center for Disease Control<sup>[15]</sup>. A diagnostic paracentesis was performed in all patients with ascites at admission. SBP was diagnosed when the neutrophil count in the ascitic fluid was  $\geq 250$  neutrophils/mm<sup>3</sup> in the absence of an intra-abdominal source of infection regardless of negative culture<sup>[16]</sup>. All patients with SBP received ceftriaxone plus weight-based intravenous albumin in the first and third day after diagnosis. Hepatic encephalopathy was graded according to West-Haven criteria<sup>[17]</sup>. If this was present, a precipitant event was actively investigated and lactulose was initiated, and the dose was adjusted as needed. All subjects with acute variceal bleeding received intravenous octreotide, an antibiotic (either oral quinolone or intravenous ceftriaxone), and underwent urgent therapeutic endoscopy after stabilization.

The severity of liver disease was estimated using the Child-Pugh classification system<sup>[18]</sup> and model for end-stage liver disease (MELD)<sup>[19]</sup> calculated based on laboratory tests performed at admission in the case of hospitalized patients. Acute-on-chronic liver failure (ACLF) was defined as proposed by the EASL-CLIF Consortium<sup>[20]</sup>.

## IGFBP-3 serum levels

In patients with stable cirrhosis, IGFBP-3 levels were measured at baseline (2012) and at second re-evaluation (2014). In hospitalized subjects, IGFBP-3 levels were measured in serum samples collected in the first and in the third day after admission and stored at -80 °C until use. The IGFBP-3 levels were measured by immunochemiluminescence (Immulite® 2000, Diagnostic Products Corp., Los Angeles, CA, United States). The reported analytical sensitivity of this assay is 0.50 mcg/mL.

## Statistical analysis

The normality of variable distribution was determined using the Kolmogorov-Smirnov test. The correlation between numerical variables was evaluated using Spearman's correlation coefficient. Continuous variables were compared using the Student's *t*-test in case of a normal distribution or Mann-Whitney test in the remaining cases. Categorical variables were evaluated with a  $\chi^2$  test or Fisher's exact test, as appropriate. Multiple logistic regression analysis (forward stepwise regression) was used to investigate factors independently associated with death or liver transplantation during follow-up period. The best cutoffs of IGFBP-3 for predicting mortality, in both cohorts, were chosen based on the receiver operating characteristics (ROC) curves. Survival curves were calculated using the Kaplan-Meier method and survival differences between groups were compared using a log-rank test. Wilcoxon signed rank-test was used for comparing IGFBP-3 at two times. All tests were performed using SPSS software, version 22.0 (SPSS, Chicago, IL, United States). A *P* value of less than 0.05 was considered statistically

**Table 1** Demographic, clinical and biochemical features of included patients *n* (%)

	Stable cirrhosis ( <i>n</i> = 138)	Acute decompensation ( <i>n</i> = 189)
Age; yr, mean $\pm$ SD	53.62 $\pm$ 12.52	53.58 $\pm$ 11.56
Caucasians	128 (92.8)	129 (68.6)
Male gender	97 (70.3)	138 (73.0)
Etiology of cirrhosis		
Alcohol	42 (30.4)	68 (36.0)
Hepatitis C	50 (36.2)	78 (41.3)
Hepatitis B	6 (4.3)	8 (4.2)
Cryptogenic	14 (10.1)	15 (7.9)
Other	26 (18.8)	20 (10.6)
Previous decompensation	104 (75.4)	120 (63.5)
Active alcoholism	5 (3.6)	68 (36.0)
Propranolol	87 (63.0)	74 (40.2)
PPI	69 (50.0)	43 (23.4)
Complication at evaluation		
Ascites	28 (20.3)	92 (48.7)
Hepatic encephalopathy	14 (10.1)	112 (59.3)
Gastrointestinal bleeding	0	99 (52.4)
Bacterial infection	0	50 (26.6)
ACLF	0	45 (23.8)
Laboratory data		
Leucocyte count ( $\times 10^3$ ), median	4.90	7.20
Sodium (meq/L), median	138	135
Creatinine (mg/dL), median	0.90	1.1
INR, median	1.20	1.41
Albumin (g/dL), mean $\pm$ SD	3.44 $\pm$ 0.46	2.35 $\pm$ 0.69
CRP (mg/L), median	3.5	10.05
Total bilirubin (mg/dL), median	1.00	2.10
IGFBP-3 (mcg/mL), median	1.69	0.94
Child-Pugh classification		
A	92 (66.7)	23 (12.2)
B	43 (31.2)	91 (48.1)
C	3 (2.2)	75 (39.7)
MELD score, mean $\pm$ SD	9.84 $\pm$ 2.28	16.32 $\pm$ 6.53

PPI: Proton-pump inhibitors; ACLF: Acute-on-chronic liver failure; INR: International normalized ratio; CRP: C-reactive protein; IGFBP-3: Insulin-like growth factor-binding protein 3; MELD: Model for end-stage liver disease.

significant.

## RESULTS

### Characteristics of included patients

The study included 138 patients with stable cirrhosis and 189 subjects hospitalized for AD of cirrhosis. Table 1 lists the characteristics of the included patients. In the cohort of stable cirrhosis, the mean age was 53.6  $\pm$  12.5 years, 92.8% were Caucasians with a predominance of men (70.3%). A previous history of cirrhosis decompensation was observed in 75.4% of the sample and only 3.6% of subjects reported active alcoholism during the past month. Regular propranolol and PPI use was 63.0% and 50.0% of the patients, respectively. The mean MELD score was 9.84  $\pm$  2.28 and 66.7% of subjects were Child-Pugh A.

In the group hospitalized for AD, the mean age was 53.6  $\pm$  11.6 years, 68.6% were Caucasians and 73.0% were males. Previous decompensation was

reported by 63.5% of the sample, and active alcoholism was present in 36.0% of the sample. Upon admission, upper gastrointestinal bleeding was observed in 52.4% of cases, ascites in 48.7%, hepatic encephalopathy in 59.3%, bacterial infections in 26.6% and ACLF in 23.8%. In hospitalized patients, propranolol and PPI use prior to admission was reported in 40.2% and 23.4% of the patients, respectively. The mean MELD score was 16.32  $\pm$  6.53 and 39.7% of subjects were Child-Pugh C. Patients hospitalized for AD of cirrhosis exhibited significantly lower median IGFBP-3 vs outpatients (0.94 mcg/mL vs 1.69 mcg/mL,  $P < 0.001$ ).

### IGFBP-3 in outpatients with stable cirrhosis

In patients with stable cirrhosis, IGFBP-3 levels were positively correlated with total leukocytes ( $r = 0.215$ ,  $P = 0.011$ ) and albumin levels ( $r = 0.579$ ,  $P < 0.001$ ). A negative correlation was observed between IGFBP-3 levels and INR ( $r = -0.412$ ,  $P < 0.001$ ), total bilirubin ( $r = -0.329$ ,  $P < 0.001$ ), CRP ( $r = -0.265$ ,  $P = 0.002$ ) and MELD ( $r = -0.327$ ,  $P < 0.001$ ). No significant correlations were observed between IGFBP-3 and other studied variables.

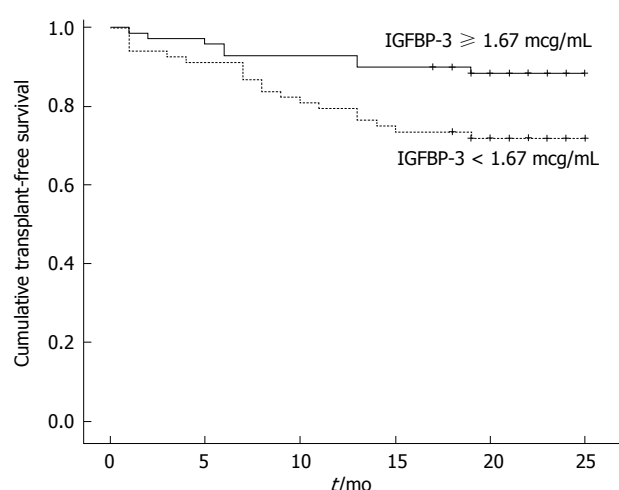
Significantly lower IGFBP-3 median levels were observed in Child-Pugh B/C patients (1.38 mcg/mL vs 1.88 mcg/mL,  $P < 0.001$ ), in those with previous decompensation of cirrhosis (1.58 mcg/mL vs 2.19 mcg/mL,  $P = 0.012$ ), hospitalization secondary to complications of cirrhosis (1.57 mcg/mL vs 1.95 mcg/mL,  $P = 0.052$ ), and those with a history of ascites (1.53 mcg/mL vs 1.85 mcg/mL,  $P = 0.024$ ). Previous variceal bleeding or hepatic encephalopathy as well as an etiology of cirrhosis had no impact on IGFBP-3 levels ( $P > 0.05$ ).

The median follow-up of patients with stable cirrhosis was 20 mo. During the study period, 17 patients died and 11 received liver transplantation. Bivariate analysis showed that progression to death or liver transplantation was associated with previous ascites (70.4% vs 42.3%,  $P = 0.009$ ), history of hospitalization for complications of cirrhosis (88.9% vs 65.8%,  $P = 0.018$ ), and Child-Pugh B/C (70.4% vs 24.3%,  $P < 0.001$ ) (Table 2). In addition, those with unfavorable outcome showed a higher median total bilirubin (1.80 mg/dL vs 0.90 mg/dL,  $P < 0.001$ ), INR (1.27 vs 1.16,  $P < 0.001$ ), CRP (4.51 mg/L vs 3.50 mg/L,  $P = 0.014$ ), MELD score (11.98  $\pm$  2.24 vs 9.32  $\pm$  1.97,  $P < 0.001$ ) and lower sodium (136.00 mEq/L vs 138.00 mEq/L,  $P = 0.022$ ), albumin (3.11  $\pm$  0.39 g/dL vs 3.52  $\pm$  0.44 g/dL,  $P < 0.001$ ) and IGFBP-3 values (1.44 mcg/mL vs 1.74 mcg/mL,  $P = 0.027$ ). A stepwise forward logistic regression analysis was performed including the following variables with  $P < 0.05$  in bivariate analysis: Previous ascites, history of hospitalization for complications of cirrhosis, albumin, INR, total bilirubin, CRP, sodium, and IGFBP-3. Multivariate analysis showed that only albumin (OR = 0.183, 95%CI: 0.053-0.631,  $P = 0.007$ ) and total bilirubin (OR = 2.482, 95%CI:

**Table 2** Factors associated with mortality or liver transplantations among patients with stable cirrhosis *n* (%)

	Survivors ( <i>n</i> = 111)	Death/liver transplantation ( <i>n</i> = 27)	<i>P</i> value
Age (yr), mean ± SD	54.33 ± 12.11	50.67 ± 13.91	0.173
Male gender	79 (71.2)	18 (66.7)	0.646
Etiology of cirrhosis			
Alcohol	34 (30.6)	8 (29.6)	0.919
Hepatitis C	42 (37.8)	8 (29.6)	0.426
Hepatitis B	4 (3.6)	2 (7.4)	0.334
Cryptogenic	10 (9.0)	4 (14.8)	0.475
Other	21 (18.9)	5 (18.5)	0.962
Previous hospitalization	73 (65.8)	24 (88.9)	0.018
Active alcoholism	3 (2.7)	2 (7.4)	0.252
Propranolol	67 (60.4)	20 (74.1)	0.185
PPI	54 (48.6)	15 (55.6)	0.520
Complication at evaluation			
Ascites	19 (17.1)	9 (33.3)	0.060
Hepatic encephalopathy	8 (7.2)	6 (22.2)	0.032
Previous ascites	47 (42.3)	19 (70.4)	0.009
Laboratory data			
Leucocyte count ( $\times 10^6$ ), median	4.93	4.83	0.548
Sodium (meq/L), median	138.00	136.00	0.022
Creatinine (mg/dL), median	0.9	0.8	0.480
INR, median	1.16	1.27	< 0.001
Albumin (g/dL), mean ± SD	3.52 ± 0.44	3.11 ± 0.39	< 0.001
CRP (mg/L), median	3.5	4.51	0.014
Total bilirubin (mg/dL), median	0.9	1.8	< 0.001
IGFBP-3 (mcg/mL), median	1.74	1.44	0.027
Child-Pugh B/C	27 (24.3)	19 (70.4)	< 0.001
MELD score, mean ± SD	9.32 ± 1.97	11.98 ± 2.24	< 0.001

PPI: Proton-pump inhibitors; INR: International normalized ratio; CRP: C-reactive protein; IGFBP-3: Insulin-like growth factor-binding protein 3; MELD: Model for end-stage liver disease.



**Figure 1** Kaplan-Meier transplant-free survival of 138 outpatients with cirrhosis stratified according to insulin-like growth factor-binding protein 3 cut-off level of 1.67 mcg/mL. Survival probability after a median follow-up of 20 mo was 88.6% for patients with IGFBP-3  $\geq$  1.67 mcg/mL and 72.1% for those with IGFBP-3 < 1.67 mcg/mL ( $P = 0.015$ ). IGFBP-3: Insulin-like growth factor-binding protein 3.

1.358-4.538,  $P = 0.003$ ) were independently associated with death or liver transplantation during follow-up. However, at the end of follow-up, Kaplan-Meier survival probability was 88.6% for patients with IGFBP-3  $\geq$  1.67 mcg/mL and 72.1% for those with IGFBP-3 <

1.67 mcg/mL. The survival was significantly shorter for those with lower IGFBP-3 values (20.24 mo, 95%CI: 18.32-22.17) as compared to the remaining subjects (23.06 mo, 95%CI: 21.72-24.40) ( $P = 0.015$ ) (Figure 1).

Twenty-seven patients developed variceal bleeding during follow-up. Those patients exhibited similar baseline IGFBP-3 levels when compared to those who did not develop this complication (1.74 mcg/mL vs 1.69 mcg/mL,  $P = 0.478$ ).

One-hundred and nine patients underwent laboratory evaluation in 2014, and 12 subjects refused blood collection. Of those who did not receive liver transplantation, IGFBP-3 significantly decreased at the second assessment (1.67 mcg/mL vs 1.74 mcg/mL,  $P = 0.013$ ). However, in patients who underwent liver transplantation, a significant increase in IGFBP-3 was observed (3.81 mcg/mL vs 1.33 mcg/mL,  $P = 0.008$ ). Actually, IGFBP-3 increased in all nine patients, and its levels were restored to normal after transplantation in five subjects.

### Prognostic significance of IGFBP-3 in patients hospitalized for AD of cirrhosis

In hospitalized patients, IGFBP-3 levels were positively correlated with sodium ( $r = 0.173$ ,  $P = 0.018$ ) and albumin levels ( $r = 0.422$ ,  $P < 0.001$ ). A negative correlation was observed between IGFBP-3 levels and

**Table 3** Factors associated with 30-d mortality among patients hospitalized for acute decompensation of cirrhosis *n* (%)

	Survivors ( <i>n</i> = 143)	Deaths ( <i>n</i> = 46)	<i>P</i> value
Age (yr), mean ± SD	53.61 ± 11.54	53.49 ± 11.75	0.952
Male gender	101 (70.6)	37 (80.4)	0.192
Etiology of cirrhosis			
Alcohol	47 (32.9)	21 (49.7)	0.116
Hepatitis C	61 (42.7)	17 (37.0)	0.495
Hepatitis B	6 (4.2)	2 (4.3)	1.000
Cryptogenic	14 (9.8)	1 (2.2)	0.123
Other	15 (10.5)	5 (10.9)	1.000
Previous decompensation	88 (61.5)	32 (69.6)	0.325
Active alcoholism	45 (31.5)	23 (50.0)	0.023
Complication at evaluation			
Ascites	57 (39.9)	35 (76.1)	< 0.001
Hepatic encephalopathy	73 (51.0)	39 (84.8)	< 0.001
Gastrointestinal bleeding	81 (56.6)	18 (39.1)	0.039
Bacterial infection	28 (19.6)	22 (48.9)	< 0.001
ACLF	16 (11.2)	29 (63.0)	< 0.001
Laboratory data			
Leucocyte count ( $\times 10^9$ ), median	6.59	8.46	0.005
Sodium (meq/L), median	136.00	134.00	0.011
Creatinine (mg/dL), median	1.00	1.80	< 0.001
INR, median	1.38	1.60	< 0.001
Albumin (g/dL), mean ± SD	2.47 ± 0.70	1.98 ± 0.51	< 0.001
CRP (mg/L), median	8.40	24.8	0.002
Total bilirubin (mg/dL), median	1.51	3.10	< 0.001
IGFBP-3 (mcg/mL), median	1.05	0.63	< 0.001
Child-Pugh C	41 (28.7)	34 (73.9)	< 0.001
MELD score, mean ± SD	14.35 ± 5.00	22.44 ± 6.95	< 0.001
Vital signs			
MAP (mmHg), mean ± SD	85.11 ± (13.95)	80.33 ± (15.27)	0.053
Heart rate (BPM), mean ± SD	81.27 ± 19.19	88.66 ± 16.40	0.022
SpO <sub>2</sub> /FiO <sub>2</sub> ratio, median	461.9	442.86	< 0.001

INR: International normalized ratio; CRP: C-reactive protein; IGFBP-3: Insulin-like growth factor-binding protein 3; MELD: Model for end-stage liver disease; MAP: Mean arterial pressure; SpO<sub>2</sub>/FiO<sub>2</sub>: Oxygen saturation to fraction of inspired oxygen ratio; ACLF: Acute-on-chronic liver failure.

INR ( $r = -0.437$ ,  $P < 0.001$ ), total bilirubin ( $r = -0.278$ ,  $P < 0.001$ ), CRP ( $r = -0.365$ ,  $P < 0.001$ ), MELD ( $r = -0.373$ ,  $P < 0.001$ ), and CLIF-SOFA ( $r = -0.410$ ,  $P < 0.001$ ). Significantly lower levels of IGFBP-3 were observed in Child-Pugh C patients (0.73 mcg/mL vs 1.13 mcg/mL,  $P < 0.001$ ), in those with ascites (0.76 mcg/mL vs 1.22 mcg/mL,  $P < 0.001$ ), hepatic encephalopathy (0.85 mcg/mL vs 1.12 mcg/mL,  $P = 0.001$ ), ACLF (0.78 mcg/mL vs 1.02 mcg/mL,  $P = 0.007$ ) and bacterial infection at admission (0.66 mcg/mL vs 1.11 mcg/mL,  $P < 0.001$ ). The etiology of cirrhosis had no impact on IGFBP-3 levels ( $P > 0.05$ ).

Overall 30-d mortality was 24.3%, and it was associated with active alcoholism in bivariate analysis (Table 3) (50.0% vs 31.5%,  $P = 0.023$ ) as well as ascites (76.1% vs 39.9%,  $P < 0.001$ ), hepatic encephalopathy (84.8% vs 51.0%,  $P < 0.001$ ), bacterial infection (48.9% vs 19.6%,  $P < 0.001$ ), Child-Pugh C (73.9% vs 28.7%,  $P < 0.001$ ), ACLF at admission (63.0% vs 11.2%,  $P < 0.001$ ), lower median SpO<sub>2</sub>/FiO<sub>2</sub> ratio (442.86 vs 461.90,  $P < 0.001$ ), and higher MELD

score (22.44 ± 6.95 vs 14.35 ± 5.00,  $P < 0.001$ ). The 30-d mortality was also related to higher median leucocyte count (8.46 × 10<sup>9</sup>/L vs 6.59 × 10<sup>9</sup>/L,  $P = 0.005$ ), creatinine (1.80 mg/dL vs 1.00 mg/dL,  $P < 0.001$ ), INR (1.60 vs 1.38,  $P < 0.001$ ), CRP (24.80 mg/L vs 8.40 mg/L,  $P = 0.002$ ), total bilirubin (3.10 mg/dL vs 1.51 mg/dL,  $P < 0.001$ ) as well as lower mean albumin (1.98 ± 0.51 g/dL vs 2.47 ± 0.70 g/dL,  $P < 0.001$ ), lower median sodium (134.00 mEq/L vs 136.00 mEq/L,  $P = 0.011$ ), and IGFBP-3 levels (0.63 mcg/mL vs 1.05 mcg/mL,  $P < 0.001$ ).

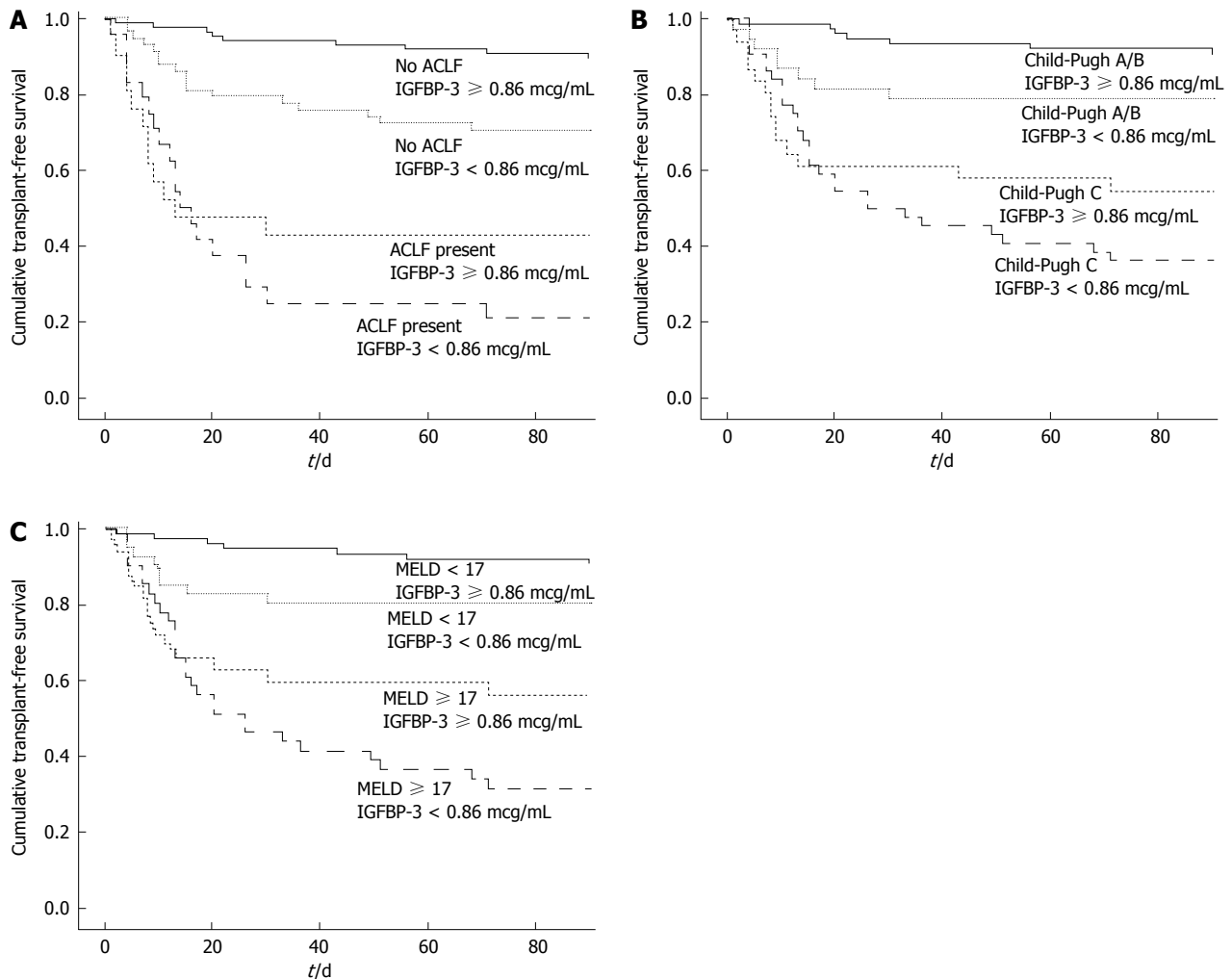
A stepwise forward logistic regression analysis was performed including the following variables with  $P < 0.010$  in the bivariate analysis: Ascites, hepatic encephalopathy, infection at admission, SpO<sub>2</sub>/FiO<sub>2</sub> ratio, leucocyte count, creatinine, INR, albumin, CRP, total bilirubin, and IGFBP-3. In this regression analysis, the 30-day mortality was independently associated with creatinine (OR = 5.331, 95%CI: 2.563-11.090,  $P < 0.001$ ), INR (OR = 5.830, 95%CI: 1.492-22.785,  $P = 0.011$ ), SpO<sub>2</sub>/FiO<sub>2</sub> ratio (OR = 0.985, 95%CI: 0.975-0.995,  $P = 0.004$ ), and IGFBP-3 (OR = 0.332, 95%CI: 0.120-0.915,  $P = 0.033$ ).

Fifty-four patients died and three subjects underwent liver transplantation during 90 d of follow-up. The Kaplan-Meier survival probability at 90-d was 80.4% in patients with IGFBP-3 ≥ 0.86 mcg/mL and 56.1% for subjects with IGFBP-3 < 0.86 mcg/mL ( $P < 0.001$ ) (Figure 2A). For the prediction of 90-d mortality, the IGFBP-3 at a cutoff of 0.86 mcg/mL showed a sensitivity of 63% and a specificity of 65%. The negative predictive value was 80% with a positive predictive value of only 44%. Figure 2A exhibited Kaplan-Meier curves for mortality during the follow-up period according to the presence of ACLF and IGFBP-3 categories. The Kaplan-Meier survival probability at 90-d was 89.5% in patients without ACLF and IGFBP-3 ≥ 0.86 mcg/mL. It was 70.7% for those with IGFBP-3 < 0.86 mcg/mL only, 42.9% for those with ACLF only, and 20.8% for patients with both ACLF and IGFBP-3 < 0.86 mcg/mL ( $P < 0.001$ , long-rank test). Similarly, the 90-d survival was 90.8% in patients with Child-Pugh A/B and with IGFBP-3 ≥ 0.86 mcg/mL, 78.9% for those with IGFBP-3 < 0.86 mcg/mL only, 54.8% for those with Child-Pugh C only, and 36.4% for Child-Pugh C patients with IGFBP-3 < 0.86 mcg/mL ( $P < 0.001$ ) (Figure 2B).

The MELD score was dichotomized as ≥ 17 and < 17 based on ROC curve. The 90-d Kaplan-Meier survival probability was 90.7% in patients with MELD < 17 and IGFBP-3 ≥ 0.86 mcg/mL, 80.5% for those with MELD < 17 and IGFBP-3 < 0.86 mcg/mL, 56.3% for those with MELD ≥ 17 and IGFBP-3 ≥ 0.86 mcg/mL, and 31.7% for patients with both MELD ≥ 17 and IGFBP-3 < 0.86 mcg/mL ( $P < 0.001$ ) (Figure 2C).

The IGFBP-3 levels were available for 163 patients at the third day of hospitalization and showed a significant decline vs admission values (0.76 mcg/mL vs 0.94 mcg/mL,  $P < 0.001$ ). However, neither the magnitude





**Figure 2** Cumulative 90-d transplant-free survival of hospitalized patients with cirrhosis according to insulin-like growth factor-binding protein 3, acute-on-chronic liver failure, Child-Pugh and model for end-stage liver disease. The 90-d survival was 89.5% in patients without ACLF and with IGFBP-3  $\geq 0.86$  mcg/mL, 70.7% for those with IGFBP-3  $< 0.86$  mcg/mL only, 42.9% for those with ACLF only and 20.8% for patients with both ACLF and IGFBP-3  $< 0.86$  mcg/mL (A:  $P < 0.001$ ). Similarly, survival probability was 90.8% in patients Child-Pugh A/B and with IGFBP-3  $\geq 0.86$  mcg/mL, 78.9% for those with IGFBP-3  $< 0.86$  mcg/mL only, 54.8% for those Child-Pugh C only and 36.4% for Child-Pugh C patients with IGFBP-3  $< 0.86$  mcg/mL (B:  $P < 0.001$ ). The 90-d Kaplan-Meier survival probability was 90.7% in patients with MELD  $< 17$  and IGFBP-3  $\geq 0.86$  mcg/mL, 80.5% for those with MELD  $< 17$  and IGFBP-3  $< 0.86$  mcg/mL, 56.3% for those with MELD  $\geq 17$  and IGFBP-3  $\geq 0.86$  mcg/mL and 31.7% for patients with both MELD  $\geq 17$  and IGFBP-3  $< 0.86$  mcg/mL (C:  $P < 0.001$ ). ACLF: Acute-on-chronic liver failure; IGFBP-3: Insulin-like growth factor-binding protein 3; MELD: Model for end-stage liver disease.

nor the occurrence of IGFBP-3 decline was related to the severity of cirrhosis or bad prognosis (data not shown). In addition, IGFBP-3 measured at the third day showed similar performance to admission levels when used at its best cutoff for prediction of 90-d mortality (0.68 mcg/mL). These metrics offered a sensitivity of 64%, specificity of 65%, negative predictive value of 83% and positive predictive value of 41%.

#### IGFBP-3 levels after hospital discharge

The 30 patients evaluated during hospitalization underwent laboratory analysis within a median 105 days after discharge. These were compared at two time points to investigate the impact of AD on IGFBP-3 levels. Vs inpatient assessment, significantly higher median IGFBP-3 levels were observed with outpatient evaluation (1.51 mcg/mL vs 1.07 mcg/mL,  $P < 0.001$ ). Likewise, an increase in IGFBP-3 levels at outpatient evaluation

were observed in 24 out of 30 patients included in this analysis (80%).

## DISCUSSION

Even though the course of cirrhosis varies according to several factors, the need for prognostic markers and scoring systems is critical to manage individuals facing different therapeutic options<sup>[21]</sup>. It was previously shown that IGFBP-3 levels in cirrhosis are related to the severity of liver dysfunction. This marker undergoes only slight influence from other factors not related to liver synthesis capacity. Therefore, IGFBP-3 is a potential and underexplored prognostic biomarker in liver cirrhosis.

Here, the IGFBP-3 levels correlated with several variables directly or indirectly associated with the intensity of liver dysfunction in both stable and decom-

pensated patients. These findings agree with previous studies, which demonstrated an association between lower IGFBP-3 levels and the severity of liver disease<sup>[5,7,10,21-24]</sup>. In a recent study including patients with AD of cirrhosis, we showed that patients with more severe liver dysfunction exhibited lower IGFBP-3 levels - these levels were not influenced by other parameters such as gender, etiology of cirrhosis and comorbidities<sup>[25]</sup>. In addition, this study showed that IGFBP-3 increased significantly after liver transplantation as well as after hospital discharge. These findings reinforce the impact of hepatic synthetic function on IGFBP-3 levels and support its utility as a potential biomarker for assessment of liver function.

In patients with stable cirrhosis, death or liver transplantation during follow-up was associated with lower IGFBP-3 levels in bivariate analysis. In addition, transplant-free survival was significantly shorter in subjects with IGFBP3 < 1.67 mcg/mL. Data about the prognostic significance of IGFBP-3 in cirrhosis are scarce. IGFBP-3 was evaluated in 354 patients with alcohol-induced liver disease from a large multicenter trial of the effect of malotilate on survival<sup>[26]</sup>. The mean follow-up period was 569 d and low IGFBP-3 levels were associated with poor prognosis, especially at a cutoff of 1.35 mcg/mL<sup>[26]</sup>. The difference between this cutoff and that in the present study probably reflects methodological issues or disparities in the severity of the disease across the cohorts. It is important to note that the European study included both patients with and without cirrhosis-no detailed analysis of only those with cirrhosis was provided<sup>[26]</sup>. In our data, IGFBP-3 was not associated with death or liver transplantation in the logistic regression analysis in stable cirrhosis. This can be explained by the relatively low number of events in this cohort. Nevertheless, these results indicate the potential of IGFBP-3 as a prognostic marker in stable cirrhosis.

In patients hospitalized for AD of cirrhosis, lower IGFBP-3 levels were associated with short-term mortality in both bivariate and multivariate analysis. There is no data about IGFBP-3 prognostic value in this setting. It is possible that suppressed IGFBP-3 reflects the acute deterioration of hepatic function as suggested by its lower levels in hospitalized cirrhosis vs outpatients. The Kaplan-Meier survival probability at 90 d was significantly worse in patients with IGFBP3 < 0.86 mcg/mL vs control subjects (56.1% vs 80.4%,  $P < 0.001$ ). This cutoff is markedly lower than the limits suggested by Møller *et al.*<sup>[26]</sup> as well as in our study for stable cirrhosis. This likely reflects the severe deterioration of hepatic function in AD of cirrhosis.

The significance of IGFBP-3 in AD of cirrhosis was evaluated according to two of the most important prognostic parameters in this setting: The presence of ACLF and Child-Pugh Classification. The ACLF definition used here was based on a modified version of the SOFA score (CLIF-SOFA) and was first proposed by the

EAS-CLIF Consortium in a large multicenter trial and subsequently validated<sup>[20,27]</sup>. Patients who presented with higher IGFBP-3 and without ACLF showed good prognosis (90-d survival approximately 90%). However, even in the absence of ACLF, low IGFBP-3 was associated with worse prognosis and a 90-d survival of 70.7%. Similarly, in the presence of ACLF, higher IGFBP-3 was associated with 90-d survival of 42.9% vs 20.8% for those with both ACLF and low IGFBP-3. These results indicate that combining IGFBP-3 and ACLF definition provided a well-defined four-level stratification for short-term prognosis in patients with cirrhosis hospitalized for AD. Similar results were observed for Child-Pugh Classification and MELD score although with a less clear stratification than that observed by using ACLF.

This study does have some limitations. The relatively small number of events in the stable cohort may have influenced the results - especially concerning regression analysis. In fact, regarding stable patients with cirrhosis there is still a need for validation of our results in larger cohorts with a longer follow-up before this biomarker is incorporated into clinical practice. Another limitation that we should highlight is the fact that we included a very heterogeneous population in distinct clinical scenarios and no specific treatment guidelines were created for the purpose of this study. Therefore, variations in the approach to specific cases are expected. In addition, the underlying liver disease status (if active or not) was not investigated. However, this issue is common in almost all studies investigating biomarkers in clinical settings. In addition, patients were evaluated according to standardized charts developed specifically for the purpose of the study and were followed both in outpatient clinic and in the ward by the same medical team. This minimized the impact of non-standardized approach.

In conclusion, in patients with cirrhosis IGFBP-3 levels correlated with several variables associated with severity of liver disease and improved significantly after discharge and after liver transplantation, indicating the impact of impaired hepatic function on its levels. Lower IGFBP-3 levels were associated with worse long-term prognosis in outpatients with stable cirrhosis and worse short-term prognosis in those hospitalized for AD. These findings suggest that measurement of circulating IGFBP-3 is of prognostic relevance and can be incorporated into clinical practice to improve the care of patients with liver cirrhosis.

## COMMENTS

### Background

Insulin-like growth factor-binding protein 3 (IGFBP-3) is the major binding protein of insulin-like growth factor (IGF) system, carrying 80%-90% of circulating IGF-1. IGFBP-3 has predominantly hepatic production and decreased IGFBP-3 serum levels have been reported in patients with cirrhosis and seem to correlate with hepatic dysfunction intensity. Although preliminary reports indicate a clinical applicability for the assessment of circulating IGFBP-3 in cirrhosis, there are very few data on prognostic significance of this biomarker

in this context.

### Research frontiers

Defining the prognosis of patients with cirrhosis is of great relevance in order to select appropriate candidates for distinct therapeutic approaches, such as liver transplantation.

### Innovations and breakthroughs

In the present study, IGFBP-3 levels correlated with several variables associated with hepatic dysfunction. Lower IGFBP-3 levels were associated with worse long-term prognosis in outpatients with stable cirrhosis and worse short-term prognosis in those hospitalized for acute decompensation.

### Applications

These data suggested that IGFBP-3 levels can be used solely or in combination with other models (including Child-Pugh, model for end-stage liver disease and acute-on-chronic liver failure definition) to evaluate the prognosis of patients with liver cirrhosis.

### Peer-review

This prospective study investigated the prognostic significance of IGFBP-3 in patients with cirrhosis. This is an interesting study, and this manuscript could provide useful information to readers.

## REFERENCES

- 1 Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet* 2014; **383**: 1749-1761 [PMID: 24480518 DOI: 10.1016/S0140-6736(14)60121-5]
- 2 Di Martino V, Weil D, Cervoni JP, Thevenot T. New prognostic markers in liver cirrhosis. *World J Hepatol* 2015; **7**: 1244-1250 [PMID: 26019739 DOI: 10.4254/wjh.v7.i9.1244]
- 3 Bonefeld K, Møller S. Insulin-like growth factor-I and the liver. *Liver Int* 2011; **31**: 911-919 [PMID: 21733081 DOI: 10.1111/j.1478-3231.2010.02428.x]
- 4 Donaghy AJ, Delhanty PJ, Ho KK, Williams R, Baxter RC. Regulation of the growth hormone receptor/binding protein, insulin-like growth factor ternary complex system in human cirrhosis. *J Hepatol* 2002; **36**: 751-758 [PMID: 12044524 DOI: 10.1016/S0168-8278(02)00049-1]
- 5 Wu YL, Ye J, Zhang S, Zhong J, Xi RP. Clinical significance of serum IGF-I, IGF-II and IGFBP-3 in liver cirrhosis. *World J Gastroenterol* 2004; **10**: 2740-2743 [PMID: 15309731 DOI: 10.3748/wjg.v10.i18.2740]
- 6 Perrini S, Laviola L, Carreira MC, Cignarelli A, Natalicchio A, Giorgino F. The GH/IGF1 axis and signaling pathways in the muscle and bone: mechanisms underlying age-related skeletal muscle wasting and osteoporosis. *J Endocrinol* 2010; **205**: 201-210 [PMID: 20197302 DOI: 10.1677/JOE-09-0431]
- 7 Colakoglu O, Tasikiran B, Colakoglu G, Kizildag S, Ari Ozcan F, Unsal B. Serum insulin like growth factor-1 (IGF-1) and insulin like growth factor binding protein-3 (IGFBP-3) levels in liver cirrhosis. *Turk J Gastroenterol* 2007; **18**: 245-249 [PMID: 18080921]
- 8 Audí L, Fernández-Cancio M, Camats N, Carrascosa A. Growth hormone deficiency: an update. *Minerva Endocrinol* 2013; **38**: 1-16 [PMID: 23435439]
- 9 Juul A. Serum levels of insulin-like growth factor I and its binding proteins in health and disease. *Growth Horm IGF Res* 2003; **13**: 113-170 [PMID: 12914749 DOI: 10.1016/S1096-6374(03)00038-8]
- 10 Donaghy A, Ross R, Gimson A, Hughes SC, Holly J, Williams R. Growth hormone, insulinlike growth factor-1, and insulinlike growth factor binding proteins 1 and 3 in chronic liver disease. *Hepatology* 1995; **21**: 680-688 [PMID: 7533122]
- 11 Ferry RJ, Cerri RW, Cohen P. Insulin-like growth factor binding proteins: new proteins, new functions. *Horm Res* 1999; **51**: 53-67 [PMID: 10352394 DOI: 10.1159/000023315]
- 12 Hwa V, Oh Y, Rosenfeld RG. The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocr Rev* 1999; **20**: 761-787 [PMID: 10605625 DOI: 10.1210/edrv.20.6.0382]
- 13 Møller S, Juul A, Becker U, Flyvbjerg A, Skakkebaek NE, Henriksen JH. Concentrations, release, and disposal of insulin-like growth factor (IGF)-binding proteins (IGFBP), IGF-I, and growth hormone in different vascular beds in patients with cirrhosis. *J Clin Endocrinol Metab* 1995; **80**: 1148-1157 [PMID: 7536200 DOI: 10.1210/jcem.80.4.7536200]
- 14 Sanyal AJ, Brunt EM, Kleiner DE, Kowdley KV, Chalasani N, Lavine JE, Ratz V, McCullough A. Endpoints and clinical trial design for nonalcoholic steatohepatitis. *Hepatology* 2011; **54**: 344-353 [PMID: 21520200 DOI: 10.1002/hep.24376]
- 15 Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. *Am J Infect Control* 1988; **16**: 128-140 [PMID: 2841893]
- 16 Runyon BA, AASLD Practice Guidelines Committee. Management of adult patients with ascites due to cirrhosis: an update. *Hepatology* 2009; **49**: 2087-2107 [PMID: 19475696 DOI: 10.1002/hep.22853]
- 17 Bajaj JS. Review article: the modern management of hepatic encephalopathy. *Aliment Pharmacol Ther* 2010; **31**: 537-547 [PMID: 20002027 DOI: 10.1111/j.1365-2036.2009.04211.x]
- 18 Angermayr B, Cejna M, Kanel F, Gschwandler M, Koenig F, Pidlich J, Mendel H, Pichler L, Wichlas M, Kreil A, Schmid M, Ferlitsch A, Lipinski E, Brunner H, Lammer J, Ferenci P, Gangl A, Peck-Radosavljevic M. Child-Pugh versus MELD score in predicting survival in patients undergoing transjugular intrahepatic portosystemic shunt. *Gut* 2003; **52**: 879-885 [PMID: 12740346]
- 19 Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: 11172350 DOI: 10.1053/jhep.2001.22172]
- 20 Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, Durand F, Gustot T, Saliba F, Domenicali M, Gerbes A, Wendon J, Alessandria C, Laleman W, Zeuzem S, Trebicka J, Bernardi M, Arroyo V. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; **144**: 1426-1437, 1437.e1-9 [PMID: 23474284 DOI: 10.1053/j.gastro.2013.02.042]
- 21 Shaarawy M, Fikry MA, Massoud BA, Lotfy S. Insulin-like growth factor binding protein-3: a novel biomarker for the assessment of the synthetic capacity of hepatocytes in liver cirrhosis. *J Clin Endocrinol Metab* 1998; **83**: 3316-3319 [PMID: 9745447 DOI: 10.1210/jcem.83.9.5082]
- 22 Scharf JG, Schmitz F, Frystyk J, Skjaerbaek C, Moeser H, Blum WF, Ramadori G, Hartmann H. Insulin-like growth factor-I serum concentrations and patterns of insulin-like growth factor binding proteins in patients with chronic liver disease. *J Hepatol* 1996; **25**: 689-699 [PMID: 8938547]
- 23 Assy N, Hochberg Z, Amit T, Shen-Orr Z, Enat R, Baruch Y. Growth hormone-stimulated insulin-like growth factor (IGF) I and IGF-binding protein-3 in liver cirrhosis. *J Hepatol* 1997; **27**: 796-802 [PMID: 9382965]
- 24 Rehem RN, El-Shikh WM. Serum IGF-1, IGF-2 and IGFBP-3 as parameters in the assessment of liver dysfunction in patients with hepatic cirrhosis and in the diagnosis of hepatocellular carcinoma. *Hepatogastroenterology* 2011; **58**: 949-954 [PMID: 21830422]
- 25 Ronsoni MF, Lazzarotto C, Fayad L, Silva MC, Nogueira CL, Bazzo ML, Narciso-Schiavon JL, Dantas-Corrêa EB, Schiavon Lde L. IGF-I and IGFBP-3 serum levels in patients hospitalized for complications of liver cirrhosis. *Ann Hepatol* 2013; **12**: 456-463 [PMID: 23619263]
- 26 Møller S, Becker U, Juul A, Skakkebaek NE, Christensen E. Prognostic value of insulinlike growth factor I and its binding protein in patients with alcohol-induced liver disease. EMALD group. *Hepatology* 1996; **23**: 1073-1078 [PMID: 8621136 DOI: 10.1002/hep.510230521]
- 27 Silva PE, Fayad L, Lazzarotto C, Ronsoni MF, Bazzo ML, Colombo BS, Dantas-Corrêa EB, Narciso-Schiavon JL, Schiavon

LL. Single-centre validation of the EASL-CLIF consortium definition of acute-on-chronic liver failure and CLIF-SOFA for

prediction of mortality in cirrhosis. *Liver Int* 2015; **35**: 1516-1523 [PMID: 24840673 DOI: 10.1111/liv.12597]

**P- Reviewer:** Bruha R, Invernizzi P, Niu ZS, Yoshida H  
**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Liu SQ







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 June 28; 8(18): 749-784





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diýarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**MINIREVIEWS**

- 749 Quality of life after liver transplantation: State of the art  
*Onghena L, Develtere W, Poppe C, Geerts A, Troisi R, Vanlander A, Berrevoet F, Rogiers X, Van Vlierberghe H, Verhelst X*

**ORIGINAL ARTICLE****Retrospective Study**

- 757 Clinical characteristics and progression of liver abscess caused by toxocara  
*Ha KH, Song JE, Kim BS, Lee CH*

**EVIDENCE-BASED MEDICINE**

- 762 Treating chronic hepatitis B virus: Chinese physicians' awareness of the 2010 guidelines  
*Wei L, Jia JD, Weng XH, Dou XG, Jiang JJ, Tang H, Ning Q, Dai QQ, Li RQ, Liu J*

**META-ANALYSIS**

- 770 Transarterial radioembolization vs chemoembolization for hepatocarcinoma patients: A systematic review and meta-analysis  
*Facciorusso A, Serviddio G, Muscatiello N*

**CASE REPORT**

- 779 Atypical presentation of a hepatic artery pseudoaneurysm: A case report and review of the literature  
*Luckhurst CM, Perez C, Collinsworth AL, Trevino JG*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Reem Al Olaby, BSc, MSc, PhD, Pharmacist, Research Associate, Research Fellow, Department of Biotechnology, The American University in Cairo, Doha 22188, Qatar

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
June 28, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjgnet.com/bpg/g_info_20160116143427.htm)

**ONLINE SUBMISSION**

<http://www.wjgnet.com/esps/>



## Quality of life after liver transplantation: State of the art

Louis Onghena, Wouter Develtere, Carine Poppe, Anja Geerts, Roberto Troisi, Aude Vanlander, Frederik Berrevoet, Xavier Rogiers, Hans Van Vlierberghe, Xavier Verhelst

Louis Onghena, Wouter Develtere, Anja Geerts, Hans Van Vlierberghe, Xavier Verhelst, Department of Hepatology and Gastroenterology, Ghent University Hospital, 9000 Ghent, East Flanders, Belgium

Carine Poppe, Roberto Troisi, Aude Vanlander, Frederik Berrevoet, Xavier Rogiers, Department of General and Hepatobiliary Surgery, Liver Transplantation Service, Ghent University Hospital and Medical School, 9000 Ghent, East Flanders, Belgium

**Author contributions:** Onghena L, Develtere W, Poppe C, Geerts A, Troisi R, Vanlander A, Berrevoet F, Rogiers X, Van Vlierberghe H and Verhelst X collected and analyzed the data and wrote the paper with equal contribution.

**Conflict-of-interest statement:** There is no conflict of interest associated with any of the senior author or other co-authors contributed their efforts in this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Xavier Verhelst, MD, Department of Hepatology and Gastroenterology, Ghent University Hospital, De Pintelaan 185 - 1K12IE, 9000 Gent, East Flanders, Belgium. [xavier.verhelst@uzgent.be](mailto:xavier.verhelst@uzgent.be)  
Telephone: +32-9-3322371  
Fax: +32-9-3324984

**Received:** February 22, 2016

**Peer-review started:** February 25, 2016

**First decision:** April 15, 2016

**Revised:** May 4, 2016

**Accepted:** June 1, 2016

**Article in press:** June 3, 2016

**Published online:** June 28, 2016

### Abstract

Quality of life (QoL) after deceased donor liver transplantation is increasingly recognized as a major outcome parameter. We reviewed recent publications in this rapidly evolving field in order to summarize recent achievements in the field and to define opportunities and perspectives for research and improvement of patient care. QoL does improve after liver transplantation according to a typical pattern. During the first year, there is a significant improvement in QoL. After one year, the improvement does stabilise and tends to decline slightly. In addition to the physical condition, different psychological parameters (such as depression, anxiety, sexual function) and socio-demographic elements (professional state, sex, marital state) seem to impact QoL. Opportunities for further research are the use of dedicated questionnaires and identification of influencing factors for QoL.

**Key words:** Epidemiologic factors; Liver transplantation; Demographic factors; Quality of life; Mental health; Sociological factors

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Quality of life (QoL) after deceased donor liver transplantation (LT) is increasingly recognized as a major outcome parameter. This review summarizes a broad spectrum of factors that influence QoL in LT and elucidates the evolution in time of physical and mental QoL after LT. Furthermore attention is given to areas for further investigation and the use of self-report QoL questionnaires in LT. This way, we want to offer a recent and complete overview in this rapidly evolving field.

Onghena L, Develtere W, Poppe C, Geerts A, Troisi R, Vanlander A, Berrevoet F, Rogiers X, Van Vlierberghe H, Verhelst X. Quality of life after liver transplantation: State of the art. *World J Hepatol* 2016; 8(18): 749-756 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i18/749.htm> DOI: <http://dx.doi.org/10.4254/>

## INTRODUCTION

In 1967, Thomas Starzl performed the first successful liver transplantation. Over the past few decades liver transplantation (LT) has become a widely accepted treatment for end-stage liver disease, acute liver failure and selected cases of hepatocellular carcinoma with excellent long-term results<sup>[1,2]</sup>. The first years after the introduction of liver transplantation were characterized by a marked increase of survival rates, due to better pre- and post-operative care, refinement of explanting techniques and organ preservation, better surgical techniques, the development of potent immunosuppressive drugs and improved patient selection. Therefore, mortality and morbidity have decreased<sup>[3,4]</sup>. Today liver transplantation has a three-month survival rate of about 91.2%, a five-year survival of about 73.3% and a ten-year survival of about 60%<sup>[3,5-7]</sup>.

Survival is the main outcome parameter after liver transplantation and a *conditio sine qua non*. However, once survival is granted, the real outcome parameter to address the success of liver transplantation on the long term is quality of life (QoL). QoL can be defined as "an overall sense of well-being, including aspects of happiness and satisfaction with life as a whole, which is measurable through mental well being, physical functioning and overall health status"<sup>[8]</sup>. The World Health Organization defines health as a "state of complete physical, mental and social well-being and not merely the absence of disease and infirmity". A shift of the focus from life expectancy to QoL can be observed in an increasing number of medical fields and is also taking place in organ transplantation research<sup>[9]</sup>. The goal of liver transplantation is to achieve a health status that is at least as good as it was before liver transplantation.

Since 2010 many authors have addressed the issue of QoL after LT. Our goal was to collect and compare these new insights and controversies in this research area.

## METHODOLOGY

We searched for articles in major databases (PubMed, Google Scholar and Science Direct) from 2009 to 2015. English, French and Dutch manuscripts were eligible.

Search terms "Quality of life" and "Liver transplantation" were used as MeSH terms or searched in the title of the article. Exclusion criteria were paediatric LT, living donor liver transplantation (LDLT) and articles published before 2009. Paediatric patients were excluded due to different interpretation of QoL, reliance on parents and difficult data collection. LDLT patients were excluded due to a different psychosocial process pretransplantation. Only articles between 2009 and 2015 were eligible for inclusion.

## STUDY RESULTS

### Description of selected manuscripts

Thirty-one publications met our criteria for the PubMed search, including 24 original articles and 7 reviews (Figure 1 and Table 1). The last search was performed September 1<sup>st</sup>, 2015.

### Components of QoL

**Overall QoL:** In general, health related QoL (HRQoL) improves and remains stable throughout the years after transplantation, but does not reach the level of the general population. This can be explained by the presence of comorbidities, the severity of the disease and the transplant procedure<sup>[3,8,10-14]</sup>.

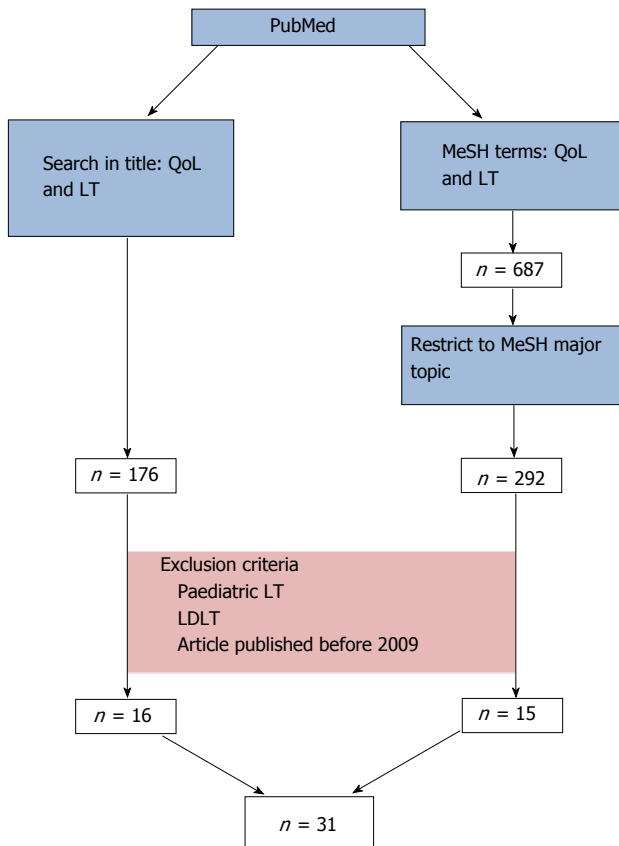
HRQoL tends to increase rapidly during the first two years, and remains stable afterwards once almost normal values have been reached<sup>[8,15]</sup>. Some authors report a more fluctuating evolution with a rapid increase of QoL during the first six months, followed by a stabilization during the remainder of the first year and a rebound effect during the second year due to adaption to certain psychosocial conditions. Patients are confronted with their new health status and can experience problems with re-enrolment in society and more particular difficulties in their professional life. The rebound effect is due to the fact that patients slowly retrieve peer acceptance and can participate in professional and social activities. After two years, in these patients, an improvement can be found until the fifth year after LT<sup>[10]</sup>.

Overall, many studies have proven significant improvements in general and mental health, vitality, social and physical functioning<sup>[1,16]</sup>.

**Physical QoL:** Overall, physical health starts improving after the first month after transplantation. This effect lasts the first six months, up to 2 years after transplantation<sup>[17]</sup>. Fluctuations are not uncommon due to the rebound effect<sup>[10,13,16]</sup>. A lower physical activity can be seen 10 to 30 years post-transplantation in comparison to the general population. This can be explained by the effect of ageing<sup>[11,14]</sup>. Due to the rapid evolution in the field of LT, older studies do not reflect the common medical practice and should be read with caution.

In summary, an improvement is seen in physical functioning after LT after the first year, if major medical complications are absent, *e.g.*, cytomegalovirus reactivation, rejection and revision<sup>[1,18-22]</sup>.

**Mental QoL:** The World Health Organisation defines mental health as "a state of well-being in which every individual realizes his or her own potential, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to her or his community"<sup>[23]</sup>. This vague definition complicates the assessment of mental health QoL after liver and impedes the comparison of different studies. Some authors assessed the mental QoL by measuring anxiety



**Figure 1** Summary of the search method. LT: Liver transplantation; LDLT: Living donor liver transplantation; QoL: Quality of life.

and depression, because of the high correlation with these mental diseases. Burra and Germani<sup>[14]</sup> showed in their systematic review an increase of depression and anxiety scores during the first year, followed by a decrease afterwards. However, another study reported a significant improvement of depression and anxiety rates especially during the first year after LT in the absence of complications such as biliary events, endocrine disorders, physical and psychiatric problems<sup>[11,15]</sup>. The relief of the stressful time-lapse awaiting transplantation combined with a better physical health status could be a logical explanation. Furthermore, differences might be related to the presence of underlying psychiatric morbidities. Affective illness, maladjustment and severe anxiety have been diagnosed in 19%-54% of patients during psychiatric evaluation. Obsessive-compulsive, somatization, anxiety and depression symptoms were frequently found. The transplantation and stay at intensive care unit have been considered as traumatic stressors that diminish QoL and can cause overall mental distress. These patients are prone to some psychiatric disorders (e.g., anxiety and affective disorders, post-traumatic stress disorder) and a low QoL after LT<sup>[21,24-31]</sup>.

The short form-36 questionnaire (SF-36) is extensively used to assess the health-related QoL, which is a reliable and standardized tool comparing as well the mental [the mental component summary score (MCS)] as the physical [physical component summary score

(PCS)] aspects of QoL. During the first month after liver transplantation, studies show a rapid improvement of the MCS; the improvement of the PCS is slower though more durable and remains at higher levels six to twelve months after transplantation<sup>[13,15]</sup>. Side effects of immunosuppressive drugs and unmet expectations after liver transplantation can also hamper the improvement of MCS<sup>[17]</sup>.

In summary an improvement is seen in the mental health status within the first months after transplantation and can be influenced by complications such as rejection, infections and biliary events. These are interesting targets for improvement<sup>[13-15,27]</sup>.

### Factors influencing QoL after transplantation

**Aetiology of liver disease:** The original liver disease leading might influence QoL afterwards. Patients transplanted for non-cholestatic liver diseases report a significantly lower QoL after LT in comparison to those with a cholestatic liver disease<sup>[12]</sup>. Patients with viral hepatitis tend to suffer more from anxiety after LT than patients with alcoholic liver disease<sup>[13]</sup>. However, others challenge these data<sup>[10,14,21]</sup>. The influence of aetiology on QoL needs further investigation, since it influences all aspects of QoL<sup>[2,14]</sup>.

**Socio-demographic factors:** The influence of gender on QoL remains a matter of debate, and data are conflicting<sup>[8,11,18]</sup>.

Relational status however, does impact QoL. Married patients have a better QoL after liver transplantation than single or non-married patients<sup>[8,19,28]</sup>. This might be explained by a better social support.

Employment after LT is a crucial factor influencing QoL. Unfortunately, only 25% will return to work after two years. Restarting an active professional life after LT generates an income, but also restores the functional role of the patient in society. In employed patients, physical functioning is also improved and this results in an overall better QoL<sup>[10,12-14,21]</sup>.

Early retirement, which is often observed in these patients, negatively impacts QoL. Patients with former alcoholic liver disease have a lower chance to return to work after transplantation, compared to other aetiologies, which can be related to the psychosocial burden present before liver transplantation. This can be explained by the psychosocial burden attached to addiction.

Professional activity before transplantation has a major impact on the general outcome after LT, which improves the activation grade after LT. As one might expect, the type of activities will determine the possibility to return to work, favouring higher educated patients compared to lower educated patients involved in physically demanding manual labour<sup>[1,3,13]</sup>.

Patients regaining professional activities after only one year had a better QoL on the long term with less emotional problems<sup>[1,3,8,10,28]</sup>. A possible bias could be that only patients in good general condition will resume

Table 1 Summary of used articles

Ref.	Title	Study design	Population (n)	Instruments used to assess QoL	Main conclusions
Masala <i>et al</i> <sup>[1]</sup>	Quality of life and physical activity in liver transplantation patients: Results of a case-control study in Italy	Case-control	45 transplant patients 108 controls	SF-36 IPAQ	Transplant recipients are more subject to psychological/emotional distress and low physical function than the general population
Lankarani <i>et al</i> <sup>[2]</sup>	Outcomes of liver transplantation for patients with acute liver failure	Retrospective cross-sectional study	12 ALF patients 20 cirrhotic patients	N/A	Liver transplantation is safe, effective and should be considered in patients diagnosed with ALF
Drent <i>et al</i> <sup>[3]</sup>	Symptom experience, nonadherence and quality of life in adult liver transplant recipients	Review	N/A	N/A	Health-related quality of life is satisfactory, but below the level of the general population
O'Mahony <i>et al</i> <sup>[4]</sup>	The future of liver transplantation	Review	N/A	N/A	Improvements in surgical techniques, postoperative care, and donor and recipient selection have all contributed to the increased success of OLT and to higher survival rates in patients with advanced liver disease
Saidi <sup>[5]</sup>	Current status of liver transplantation	Review	N/A	N/A	New problems that include severe organ shortage, recurrence of primary disease, opportunistic infections, and development of de novo malignancies are the major problems affecting further implementation of LT
Butt <i>et al</i> <sup>[6]</sup>	Quality of life, risk assessment, and safety research in liver transplantation: New frontiers in health services and outcomes research	Review	N/A	N/A	Recipient quality of life is an area that has grown in importance in the published literature, but several important questions remain unanswered in these areas that merit programmatic, interdisciplinary research
Jay <i>et al</i> <sup>[9]</sup>	A review of quality of life instruments used in liver transplantation	Review	N/A	N/A	There are no available instruments that allow for the precise and reliable assessment of the full QoL impact of liver transplantation
Wang <i>et al</i> <sup>[10]</sup>	Health-related quality of life after liver transplantation: The experience from a single Chinese center	Case-control	60 post-LT, 55 benign end-stage liver disease, 50 controls	SF-36	LT patients generally have a good HRQoL although some respects of their HRQoL remains to be improved. Lower family income and poor education are important factors relating to the poor HRQoL of LT patients
Chen <i>et al</i> <sup>[11]</sup>	Health-related quality of life of 256 recipients after liver transplantation	RCT	256	SF-36, BAI, SDS	Age > 45 yr at time of transplant, DDLT, full-time working, no complications, anxiety and depression were possible factors influencing postoperative HRQoL in liver recipients
Braun <i>et al</i> <sup>[12]</sup>	Quality of life after liver transplantation	Case-control	123 recipients, 40 patients on the waiting list and a cohort of healthy controls	EORTC QLQ C30 and a liver transplant specific module	Retransplantation was accompanied by a significant loss of QoL. Cyclosporine-treated recipients displayed a better QoL compared with those treated with tacrolimus. The influence of medical parameters, such as co-morbidity or immunosuppression, needs to be further established with reference to QoL. The global perception of quality of life increases after liver transplantation, but remains lower than in healthy subjects
Cannesson <i>et al</i> <sup>[13]</sup>	Vie quotidienne, grosseesse, qualité de vie après transplantation hépatique	Review	N/A	N/A	Liver transplantation is associated with an improvement in overall QoL. However, this improvement is lower than expected. QoL improves significantly early after liver trans-plantation, but it seems to decrease after the first year after transplantation
Burra <i>et al</i> <sup>[14]</sup>	Vie quotidienne, grosseesse, qualité de vie après transplantation hépatique	Review	N/A	N/A	High-dose steroid use for post-transplant immunosuppression in liver transplant recipients is associated with reduced physical and mental HRQoL and increased symptoms of anxiety
Zaydfudim <i>et al</i> <sup>[15]</sup>	Reduction in corticosteroids is associated with better health-related quality of life after liver transplantation	Retrospective analysis of prospective, longitudinal data	186	SF-36, BAI, and Center for Epidemiologic Studies Depression Scale	OLT improved HRQoL of end-stage liver patients and their spouses or caregivers
Sirivatanauskorn <i>et al</i> <sup>[16]</sup>	Quality of life among liver transplantation patients	Case-control	57 pre-LT 95 post-LT	SF-36, CLDQ	



Telles-Correia <i>et al</i> <sup>[17]</sup>	When does quality of life improve after liver transplantation? A longitudinal prospective study	Cohort study	60	SF-36	Our findings suggested that quality of life improved early after liver transplantation (1 mo). Between the first and the sixth months, there only was a significant improvement in the physical quality of life
Bownik <i>et al</i> <sup>[18]</sup>	When does quality of life improve after liver transplantation? A longitudinal prospective study	Review	N/A	N/A	Greater attention must be paid to patients' postoperative expectations and the effects of social influences (such as gender, education level, and socioeconomic and ethnic background)
Duffy <i>et al</i> <sup>[19]</sup>	When does quality of life improve after liver transplantation? A longitudinal prospective study	Prospective, cross-sectional study	168	SF-36, liver disease quality of life	More than 50% of LT recipients survive 20 yr, achieve important socioeconomic milestones, and report quality of life superior to patients with liver disease or other chronic conditions
Narumi <i>et al</i> <sup>[20]</sup>	Importance of awareness of perioperative social and physical situations of living donors for liver transplantation	Case-control study	31	SF-36, Hamilton's depression and anxiety scores	We must pay attention to depression and anxiety among living donors
Thiel <i>et al</i> <sup>[21]</sup>	Contributors to individual quality of life after liver transplantation	Cross-sectional study	71	SF-36, SEIQoL-DW	The five most nominated areas related to QoL are not related to health. By focusing on health, the importance of health-related factors is overrated, and the impact of non-medical effects is under-represented
Volk <i>et al</i> <sup>[22]</sup>	Organ quality and quality of life after liver transplantation	Retrospective cross-sectional study	171	SF-36	No association between organ quality and QoL after liver transplantation is found
Baranyi <i>et al</i> <sup>[24]</sup>	Overall mental distress and health-related quality of life after solid-organ transplantation: Results from a retrospective follow-up study	Retrospective follow-up	123	TERS, SCL-90-R SF-36	Transplantation recipients may face major transplantation- and treatment-related overall mental distress and impairments to their HRQoL. Further, overall mental distress is a high-risk factor in intensifying impairments to patients' overall quality of life
Jurado <i>et al</i> <sup>[23]</sup>	Coping strategies and quality of life among liver transplantation candidates	Observational	93	SF-36, MCMQ	Cirrhosis etiology is not a determinant factor of quality of life, whereas the acceptance-resignation coping strategy might lead to lower self-perception of quality of life
Lobo <i>et al</i> <sup>[26]</sup>	Care complexity, mood, and quality of life in liver pre-transplant patients	Cross-sectional	60	SF-36, HADS, INTERMED, EuroQoL	High frequency of complexity in liver transplant candidates in European hospitals, but wide between-center differences suggest that local studies in specific hospitals and/or countries may be necessary to document care needs
Martín-Rodríguez <i>et al</i> <sup>[27]</sup>	Affective status in liver transplant recipients as a function of self-perception of general health	Cross-sectional	168	SF-36, HADS	Transplant recipients with worse self-perception of general health presented the same anxiety-depressive levels as patients with severe liver disease in the pretransplantation phase
Santos <i>et al</i> <sup>[28]</sup>	Affective status in liver transplant recipients as a function of self-perception of general health	Observational, descriptive and transversal	73	SF-36, BDI, structured interviews	Psychological aspects related to transplants require psychological intervention because they can affect the recuperation process, the quality of life, and the adherence to treatment for potential transplant patients
Stille <i>et al</i> <sup>[29]</sup>	Pathways of psychosocial factors, stress, and health outcomes after liver transplantation	Longitudinal	130	N/A	A number of strong bidirectional relationships exist between coping style, self-regulatory ability, hostility, the caregiver relationship and family environment, personal and transplant-related stress over the second half of the first post-transplant year, and health (especially mental) outcomes at 12 mo post-transplant
Telles-Correia <i>et al</i> <sup>[30]</sup>	Predictors of mental health and quality of life after liver transplantation	Cross-sectional	60	SF-36	Quality of life improved early after liver transplantation (1 mo). Between the first and the sixth months, there only was a significant improvement in the physical quality of life
Telles-Correia <i>et al</i> <sup>[31]</sup>	Mental health and quality of life in alcoholic liver disease patients after liver transplantation: A prospective controlled study	Cross-sectional	45	SF-36, HADS, brief coping inventory	There is a favorable adjustment of alcoholic liver disease patients after transplantation as shown in coping mechanisms evolution, which might explain the improved mental health and quality of life dimensions

Poppe <i>et al</i> <sup>[32]</sup>	Improving quality of life in patients with chronic kidney disease: Influence of acceptance and personality	Cross-sectional	99	SF-36, ICQ, NEO-FFI	Acceptance is an important positive variable in accounting for health-related quality of life
Åberg <i>et al</i> <sup>[33]</sup>	Cost of a quality-adjusted life year in liver transplantation: The influence of the indication and the model for end-stage liver disease score	Cross-sectional	333	15D	The cost/QALY ratio for LT appears favorable, but it is dependent on the assessed time period and the severity of the liver disease
Fernández-Jiménez <i>et al</i> <sup>[34]</sup>	Comparison of quality of life between two clinical conditions with immunosuppressive therapy: Liver transplantation and multiple sclerosis	Cross-sectional	62	SF-36	Transplant recipients belong to a population that still requires special health care. Bio-psychosocial functioning is not fully restored

IPAQ: International Physical Activity Questionnaire; ALF: Acute liver failure; LT: Liver transplantation; QoL: Quality of life; HRQoL: Health related QoL; CLDQ: Chronic liver disease questionnaire; SF-36: Short form-36; OLT: Orthotopic liver transplantation; DDLT: Deceased donor liver transplantation; BAI: Beck anxiety inventory; SDS: Sheehan disability scale; EORTC QLQ: European Organization for Research and Treatment of Cancer, quality of life questionnaire; RCT: Randomized controlled trial; SEIQoL-DW: The schedule for the evaluation of individual quality of life - direct weighting; MCMQ: Medical coping modes questionnaire; SCL-90-R: Symptom checklist-90-revised; TERS: Transplant evaluation rating scale; HADS: Hospital anxiety and depression scale; BDI: Beck depression inventory; QALY: Quality-adjusted life year; ICQ: Illness cognition questionnaire; NEO-FFI: Neuroticism-extraversion-openness five-factor inventory; N/A: Not available.

work. Unemployment leads to a circulus vitiosus: Unemployed patients are less active, therefore less motivated which leads to reduced physical functioning and to lower employment. Professional reactivation should be stimulated after liver transplantation and is an interesting target for improvement of QoL.

**Depression and anxiety:** Patients with anxiety disorders or depression before LT report a lower QoL. Generally, it is assumed that mental disorders such as anxiety disorders and depression are mostly correlated with the severity of the disease pre-transplant and the occurrence of complications. However, some studies show that the acceptance of the disease is more predictive for a good or bad QoL than the severity of the disease<sup>[13,32]</sup>.

Importantly, high levels of depression may double the chances of mortality. More than half of the recipients experience at least one episode of anxiety disorder or depression within the first two years after transplantation. This negatively impacts MCS<sup>[8,11]</sup>.

**Sexual function:** An aspect of QoL that often remains taboo, is sexual function after LT. We found some conflicting results. As expected, sexual dysfunction seems to be related to old age, a positive post-transplant status and the presence of depression<sup>[14]</sup>. In a study of Cannesson *et al*<sup>[13]</sup>, 70% of the liver transplant recipients declare to have a satisfactory sexual life after LT, even though a decrease is seen in libido, sexual potency and starting of new sexual relationships, caused by bodily changes and immunosuppressive side effects. This high percentage may be caused by a reserve of patients to communicate openly about this topic. Sexual function after liver transplantation is a research area with unmet needs.

**Immunosuppressive therapy:** Intensified immunosuppressive therapy during the first six months after transplantation can cause uncomfortable side effects<sup>[5]</sup>. These side effects are common and remain a challenge on the long term<sup>[3]</sup>. Transplant recipients take a variation of immunomodulating drugs, such as mTOR and calcineurin inhibitors. Their side effects include diabetes mellitus, renal failure, hypertension, tremor, obesity and hypercholesterolemia<sup>[4,11,15]</sup>. Furthermore, corticosteroids, often used in the first 3 mo can cause insomnia, mood swings and anxiety<sup>[15]</sup>. Especially high doses of corticosteroids are associated with physical and mental health, however this correlation is not seen with low-dose corticosteroids. Corticosteroid restricting strategies can reduce long-term complications and support QoL<sup>[4,15]</sup>. Noteworthy, some observations report better QoL in patients using cyclosporines than patients using tacrolimus<sup>[8]</sup>. However, rejection and re-transplantation affect QoL, and should be avoided by proper immunosuppressive therapy<sup>[8,12,13]</sup>. We can conclude that the maximal reduction of side effects has a beneficial effect on QoL<sup>[12,33,34]</sup>.

**Waiting list:** Waiting for a liver transplantation can be long and stressful. The QoL of patients on the waiting list is significantly lower than the QoL of the general

population. Length of time spent on the waiting list has a negative impact on QoL. Thirty-eight percent of these patients are fearful (for rejection, death, recurrence of illness), 53% struggle with keeping up with their work-related functions and 23% experience social isolation. Anxiety and negative mood are known to get worse with increasing waiting time<sup>[13]</sup>. Nevertheless, some authors do not describe an increase in psychosocial stress<sup>[28]</sup>. More than half of the patients on the waiting list express a need for psychological counselling, which decreases during the waiting time<sup>[27]</sup>.

### Areas of controversy

**Areas for further investigation:** Although QoL has been extensively studied, we identified several areas of ambiguity. Identifying influencing factors of QoL is crucial to increase QoL after liver transplantation and needs further research<sup>[3,12]</sup>. In this line some interesting areas of research are: The influence of the underlying condition on QoL, gender, length of stay, immunosuppressive regimens, the influence of the recurrence of the initial liver disease, sexual function and professional reactivation. The development of more liver transplant specific outcome measures could be helpful<sup>[13,14]</sup>.

**Self-report QoL questionnaires:** For the assessment of QoL more than 50 different instruments are used, measurement is not standardized and generic health assessment questionnaires are very commonly used. The largest part of these instruments has not been designed to evaluate the health status of liver transplant patients. Consequently it is difficult to interpret the results of these questionnaires in a meaningful way<sup>[9,14]</sup>. The SF-36 is the most commonly used generic questionnaire. It offers broad-spectrum questions applicable to a variety of patient groups and enables comparison between different populations<sup>[1,9]</sup>. These questionnaires can be distributed before and after LT.

Alternative questionnaires are the Transplant Effects Questionnaire, the Positive Effects of Transplant Scale, the schedule for the evaluation of individual expects of QoL - direct weighting (SEIQoL-DW)... The SEIQoL-DW allows patients to name areas important for him/herself and weigh each area to the relative importance and fulfilment level. On the downside, it is a qualitative interview-based assessment with his inherent disadvantages<sup>[21]</sup>. This complicates its use in clinical studies and does not enable repeated questioning of the same patient. Other questionnaires are the International Physical Activity Questionnaire and the Chronic Liver Disease Questionnaire. Only 16% of the reported authors used disease-specific instruments<sup>[1,9,16]</sup>.

In conclusion, the best way to measure QoL after LT is the combination of generic questionnaires and disease-specific questionnaires, which offers a broad and thorough assessment of QoL. Jay *et al*<sup>[9]</sup> proposed the consistent use of validated, treatment-specific QoL instruments. This will result in a more accurate assessment of QoL in LT and lead to an increasing number of

studies with comparable endpoints.

## DISCUSSION

Quality of life should be a major concern for health workers involved in transplant medicine and should be the final "major outcome" to evaluate the success of liver transplantation on the long term. Fortunately, authors report a significant increase in QoL during the first year after liver transplantation, which remains stable afterwards. In general an improvement is seen both in physical and mental QoL. However, they express distinct dynamics after transplantation with a slower but more durable increase for the physical QoL compared to the mental QoL. An integrated biopsychosocial approach is the preferred model to evaluate QoL after liver transplantation.

QoL in liver transplantation is definitely influenced by numerous factors: Mental health, sociodemographic factors, underlying liver disease, immunosuppressive therapy, time on the waiting list, *etc*.

Our minireview has several limitations. Studies with different endpoints were used, since a lot of studies use different questionnaires to measure QoL. A general image of QoL in LT is given. Consequently not all aspects of QoL are reviewed in detail.

The latter could also be seen as strength of this article since we looked into almost all the aspects of QoL in liver recipients.

We can conclude that in order to further increase the QoL in LT recipients, multidisciplinary interventions of biosocial and psychological treatment are needed. An integrated approach of rehabilitation programs, psychological treatment and thorough repetitive medical follow-up seems to be helpful in these patients with physical and social problems, and stimulates the rehabilitation progress<sup>[8,10,28]</sup>. Longitudinal monitoring of QoL could increase insight into dynamics of QoL after LT and identify patients at risk for more thorough and individualized follow-up. This is a growing field of research with a lot of unanswered questions and opportunities for improvement strategies.

## REFERENCES

- 1 **Masala D**, Mannocci A, Unim B, Del Cimmuto A, Turchetta F, Gatto G, Santoro R, Ettorre GM, Boccia A, La Torre G. Quality of life and physical activity in liver transplantation patients: results of a case-control study in Italy. *Transplant Proc* 2012; **44**: 1346-1350 [PMID: 22664013 DOI: 10.1016/j.transproceed.2012.01.123]
- 2 **Lankarani KB**, Eshraghian K, Malek-Hosseini SA, Janghorban P, Geramizadeh B, Eshraghian A. Outcomes of liver transplantation for patients with acute liver failure. *Arch Iran Med* 2013; **16**: 64-67 [PMID: 23360625]
- 3 **Drent G**, De Geest S, Dobbels F, Kleibeuker JH, Haagsma EB. Symptom experience, nonadherence and quality of life in adult liver transplant recipients. *Neth J Med* 2009; **67**: 161-168 [PMID: 19581664]
- 4 **O'Mahony CA**, Goss JA. The future of liver transplantation. *Tex Heart Inst J* 2012; **39**: 874-875 [PMID: 23304042]
- 5 **Saidi RF**. Current status of liver transplantation. *Arch Iran Med* 2012; **15**: 772-776 [PMID: 23199251]

- 6 **United Network for Organ Sharing.** Richmond, VA (United States of America), 2014. [accessed 2014 Dec]. Available from: URL: <http://www.unos.org>
- 7 **European Liver Transplant Registry.** Villejuif (France), 2014. [accessed 2014 Dec]. Available from: URL: <http://www.eltr.org/spip.php?article4>
- 8 **Butt Z**, Parikh ND, Skaro AI, Ladner D, Cella D. Quality of life, risk assessment, and safety research in liver transplantation: new frontiers in health services and outcomes research. *Curr Opin Organ Transplant* 2012; **17**: 241-247 [PMID: 22476225 DOI: 10.1097/MOT.0b013e32835365c6]
- 9 **Jay CL**, Butt Z, Ladner DP, Skaro AI, Abecassis MM. A review of quality of life instruments used in liver transplantation. *J Hepatol* 2009; **51**: 949-959 [PMID: 19775771 DOI: 10.1016/j.jhep.2009.07.010]
- 10 **Wang GS**, Yang Y, Li H, Jiang N, Fu BS, Jin H, Yang JX, Chen GH. Health-related quality of life after liver transplantation: the experience from a single Chinese center. *Hepatobiliary Pancreat Dis Int* 2012; **11**: 262-266 [PMID: 22672819]
- 11 **Chen PX**, Yan LN, Wang WT. Health-related quality of life of 256 recipients after liver transplantation. *World J Gastroenterol* 2012; **18**: 5114-5121 [PMID: 23049223 DOI: 10.3748/wjg.v18.i36.5114]
- 12 **Braun F**, Teren K, Wilms P, Günther R, Allmann J, Broering DC, Küchler T. Quality of life after liver transplantation. *Transplant Proc* 2009; **41**: 2564-2566 [PMID: 19715975 DOI: 10.1016/j.transproceed.2009.06.030]
- 13 **Cannesson A**, Boleslawski E, Declerck N, Mathurin P, Pruvot FR, Dharancy S. [Daily life, pregnancy, and quality of life after liver transplantation]. *Presse Med* 2009; **38**: 1319-1324 [PMID: 19586750 DOI: 10.1016/j.lpm.2009.06.003]
- 14 **Burra P**, Germani G. Long-term quality of life for transplant recipients. *Liver Transpl* 2013; **19** Suppl 2: S40-S43 [PMID: 23960031 DOI: 10.1002/lt.23725]
- 15 **Zaydfudim V**, Feurer ID, Landman MP, Moore DE, Wright JK, Pinson CW. Reduction in corticosteroids is associated with better health-related quality of life after liver transplantation. *J Am Coll Surg* 2012; **214**: 164-173 [PMID: 22137824 DOI: 10.1016/j.jamcollsurg.2011.10.006]
- 16 **Sirivatanauskorn Y**, Dumronggittigule W, Limsrichamrern S, Iramaneerat C, Kolladarungkri T, Kositamongkol P, Mahawithitwong P, Asavakarn S, Tovikkai C. Quality of life among liver transplantation patients. *Transplant Proc* 2012; **44**: 532-538 [PMID: 22410064 DOI: 10.1016/j.transproceed.2011.12.056]
- 17 **Telles-Correia D**, Barbosa A, Mega I, Mateus E, Monteiro E. When does quality of life improve after liver transplantation? A longitudinal prospective study. *Transplant Proc* 2009; **41**: 904-905 [PMID: 19376385 DOI: 10.1016/j.transproceed.2009.01.051]
- 18 **Bownik H**, Saab S. Health-related quality of life after liver transplantation for adult recipients. *Liver Transpl* 2009; **15** Suppl 2: S42-S49 [PMID: 19876941 DOI: 10.1002/lt.21911]
- 19 **Duffy JP**, Kao K, Ko CY, Farmer DG, McDiarmid SV, Hong JC, Venick RS, Feist S, Goldstein L, Saab S, Hiatt JR, Busuttil RW. Long-term patient outcome and quality of life after liver transplantation: analysis of 20-year survivors. *Ann Surg* 2010; **252**: 652-661 [PMID: 20881772 DOI: 10.1097/SLA.0b013e3181f5f23a]
- 20 **Narumi S**, Umehara M, Toyoki Y, Ishido K, Kudo D, Kimura N, Kobayashi T, Sugai M, Hakamada K. Importance of awareness of perioperative social and physical situations of living donors for liver transplantation. *Transplant Proc* 2012; **44**: 328-331 [PMID: 22410008 DOI: 10.1016/j.transproceed.2012.01.049]
- 21 **Thiel C**, Landgrebe K, Knubben E, Nadalin S, Ladurner R, Grasshoff C, Königsrainer A, Schenk M, Thiel K. Contributors to individual quality of life after liver transplantation. *Eur J Clin Invest* 2013; **43**: 11-19 [PMID: 23078202 DOI: 10.1111/eci.12007]
- 22 **Volk ML**, Hagan M. Organ quality and quality of life after liver transplantation. *Liver Transpl* 2011; **17**: 1443-1447 [PMID: 21898767 DOI: 10.1002/lt.22425]
- 23 **World Health Organisation.** Copenhagen (Denmark), 2014. [accessed 2014 Dec]. Available from: URL: <http://www.who.int/features/qa/62/en/>
- 24 **Baranyi A**, Krauseneck T, Rothenhäusler HB. Overall mental distress and health-related quality of life after solid-organ transplantation: results from a retrospective follow-up study. *Health Qual Life Outcomes* 2013; **11**: 15 [PMID: 23391215 DOI: 10.1186/1477-7525-11-15]
- 25 **Jurado R**, Morales I, Taboada D, Denia F, Mingote JC, Jiménez MÁ, Palomo T, Rubio G. Coping strategies and quality of life among liver transplantation candidates. *Psicothema* 2011; **23**: 74-79 [PMID: 21266145]
- 26 **Lobo E**, Stiefel F, Söllner W, Santabarbara J, Lobo A, Huyse F, Marcos G, Michaud L, Hohenberger W, Ludwig G. Care complexity, mood, and quality of life in liver pre-transplant patients. *Clin Transplant* 2013; **27**: 417-425 [PMID: 23488869 DOI: 10.1111/ctr.12104]
- 27 **Martín-Rodríguez A**, Pérez-San-Gregorio MA, Domínguez-Cabello E, Fernández-Jiménez E, Pérez Bernal J. Affective status in liver transplant recipients as a function of self-perception of general health. *Transplant Proc* 2012; **44**: 2619-2621 [PMID: 23146474 DOI: 10.1016/j.transproceed.2012.09.052]
- 28 **Santos GG**, Gonçalves LC, Buzzo N, Mendes TA, Dias TP, da Silva RC, da Silva RF, de Felicio HC, Santos Júnior R, Miyazaki MC. Quality of life, depression, and psychosocial characteristics of patients awaiting liver transplants. *Transplant Proc* 2012; **44**: 2413-2415 [PMID: 23026609 DOI: 10.1016/j.transproceed.2012.07.046]
- 29 **Stillely CS**, Flynn WB, Sereika SM, Stimer ED, DiMartini AF, deVera ME. Pathways of psychosocial factors, stress, and health outcomes after liver transplantation. *Clin Transplant* 2012; **26**: 216-222 [PMID: 21518004 DOI: 10.1111/j.1399-0012.2011.01467.x]
- 30 **Telles-Correia D**, Barbosa A, Mega I, Monteiro E. Predictors of mental health and quality of life after liver transplantation. *Psychother Psychosom* 2011; **80**: 60-61 [PMID: 21088450 DOI: 10.1159/000317539]
- 31 **Telles-Correia D**, Barbosa A, Mega I, Monteiro E, Barroso E. Mental health and quality of life in alcoholic liver disease patients after liver transplantation: a prospective controlled study. *Transplant Proc* 2011; **43**: 184-186 [PMID: 21335183 DOI: 10.1016/j.transproceed.2011.01.002]
- 32 **Poppe C**, Crombez G, Hanouille I, Vogelaers D, Petrovic M. Improving quality of life in patients with chronic kidney disease: influence of acceptance and personality. *Nephrol Dial Transplant* 2013; **28**: 116-121 [PMID: 22822093 DOI: 10.1093/ndt/gfs151]
- 33 **Åberg F**, Mäklin S, Räsänen P, Roine RP, Sintonen H, Koivusalo AM, Höckerstedt K, Isoniemi H. Cost of a quality-adjusted life year in liver transplantation: the influence of the indication and the model for end-stage liver disease score. *Liver Transpl* 2011; **17**: 1333-1343 [PMID: 21770017 DOI: 10.1002/lt.22388]
- 34 **Fernández-Jiménez E**, Pérez-San-Gregorio MA, Martín-Rodríguez A, Domínguez-Cabello E, Navarro-Mascarell G, Bernardos-Rodríguez A. Comparison of quality of life between two clinical conditions with immunosuppressive therapy: liver transplantation and multiple sclerosis. *Transplant Proc* 2012; **44**: 2609-2611 [PMID: 23146471 DOI: 10.1016/j.transproceed.2012.09.051]

**P- Reviewer:** Feier FH, Gutierrez JA, Mittal PK, Srivastava M  
**S- Editor:** Gong XM **L- Editor:** A **E- Editor:** Li D





Retrospective Study

# Clinical characteristics and progression of liver abscess caused by toxocara

Kyung Ho Ha, Jung Eun Song, Byung Seok Kim, Chang Hyeong Lee

Kyung Ho Ha, Department of Internal Medicine, Daegu Medical Center, Daegu 41845, South Korea

Jung Eun Song, Byung Seok Kim, Chang Hyeong Lee, Department of Internal Medicine, Division of Gastroenterology and Hepatology, Daegu Catholic University, Daegu 42472, South Korea

**Author contributions:** Ha KH searched literature, drafted the manuscript, incorporated corrections by coauthors into final manuscript and organized details for submission of manuscript; Song JE and Kim BS supervised the manuscript; Lee CH contributed to writing the manuscript, reviews and corrections, final approval and submissions.

**Institutional review board statement:** The study was reviewed and approved by the Institutional Review Board of Daegu Catholic University.

**Informed consent statement:** Because of retrospective and anonymous character of this study, the need for informed consent was waived by the institutional review board.

**Conflict-of-interest statement:** The authors have no conflict of interest related to this publication.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Chang Hyeong Lee, MD, Professor, Department of Internal Medicine, Division of Gastroenterology and Hepatology, Daegu Catholic University, 33, Duryugongwon-ro 17-gil, Nam-gu, Daegu 42472, South Korea. [chlee1@cu.ac.kr](mailto:chlee1@cu.ac.kr)

Telephone: +82-53-6503067  
 Fax: +82-53-6284050

Received: February 15, 2016  
 Peer-review started: February 16, 2016  
 First decision: March 24, 2016  
 Revised: April 7, 2016  
 Accepted: June 1, 2016  
 Article in press: June 3, 2016  
 Published online: June 28, 2016

## Abstract

**AIM:** To evaluate the clinical characteristics and progression of liver abscess caused by toxocara.

**METHODS:** We retrospectively reviewed the medical records of patients with serum IgG antibody to *Toxocara canis* and liver abscess diagnosed using abdominal computed tomography between February 2010 and February 2015. Among 84 patients exhibiting serum IgG antibody to *Toxocara canis*, 34 patients were diagnosed with liver abscess and treated with albendazole. A follow-up period of 1 year was conducted.

**RESULTS:** Mean patient age was 53 (34-79) years, with 26 (76.5%) patients being male. Twenty-one (61.7%) patients were moderate or heavy drinkers, 23 (67.6%) patients had a history of eating raw meat or liver and 6 (17.6%) patients owned pet dogs or cats. Main patient symptoms consisted of right upper quadrant pain, fever, and fatigue; 18 (52.9%) patients, however, presented with no symptoms. Lung involvement was detected in 444 (11.7%) patients. The eosinophil count increased in 29 (85.3%) patients at initial diagnosis, and decreased in most patients after albendazole treatment. The initial serum IgE level increased in 25 (73.5%) patients, but exhibited various response levels after albendazole treatment. Liver abscess formation improved in all patients.

**CONCLUSION:** The liver abscess was improved with albendazole treatment.

**Key words:** Toxocariasis; Liver abscess; Eosinophilia

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This is a retrospective study to evaluate the clinical characteristics and progression of liver abscess caused by toxocara. Eating uncooked food was a more common route of infection than contact with pet animals. Alcohol consumption, sex (male), and ingestion of raw meat or liver were considered to be significant risk factors for toxocariasis. Patients can present with no specific symptoms, eosinophilia, and/or increased levels of serum IgE. Liver abscess caused by toxocara has characteristic radiologic findings. Even if a few patients experience relapse or migration of abscess posttreatment, a good prognosis exists for the overall clinical course.

Ha KH, Song JE, Kim BS, Lee CH. Clinical characteristics and progression of liver abscess caused by toxocara. *World J Hepatol* 2016; 8(18): 757-761 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i18/757.htm> DOI: <http://dx.doi.org/10.4254/wjch.v8.i18.757>

## INTRODUCTION

Toxocariasis is a parasitic infection caused by *Toxocara canis* or *Toxocara cati*. It is known as a main cause of eosinophilia<sup>[1]</sup>. Clinical manifestations of toxocariasis range from asymptomatic infection to involvement of various organs. Visceral larva migrans (VLM) means toxocara infection associated with various internal organs of the body<sup>[2]</sup>. Liver abscess represent one type of VLM, which differs from pyogenic liver abscess in displaying specific histologic and radiologic findings<sup>[3,4]</sup>. Hepatic VLM or liver abscess caused by toxocara can occasionally be detected as an abnormal finding at ultrasonography screening and therefore be misdiagnosed as a malignancy in patients with chronic liver disease or a history of other cancer(s)<sup>[5]</sup>. A thorough understanding of the clinical characteristics and progression of hepatic VLM or liver abscess caused by toxocara is necessary in order to determine potential factors that may help improve diagnosis, as well as avoid unnecessary testing and improper disease treatment.

## MATERIALS AND METHODS

We retrospectively reviewed the medical records of patients with serum IgG antibody to *Toxocara canis* and liver abscess diagnosed by abdominal computed tomography (CT) at Daegu Catholic University Hospital between February 23, 2010 and February 24, 2015. We investigated patients about a history of moderate

or heavy alcohol consumption, raw meat or cow's liver ingestion, and owning pet dogs or cats. We obtained liver transaminase levels, peripheral blood eosinophil counts, serum IgE levels, hepatitis B surface antigen and antibody and anti-hepatitis C virus antibody results. In addition, we obtained any history of underlying disease and other organ(s) involvement. Eosinophilia was defined as an absolute peripheral blood eosinophil count  $\geq 500/\mu\text{L}$ . Elevated serum levels of IgE were defined as IgE levels  $\geq 100$  IU/mL. We treated patients with liver abscess with 400 mg orally twice daily for 5 d. The follow-up protocol consisted of obtaining repeat eosinophil counts and serum IgE levels, as well as performing abdominal CT scans at various intervals for 1 year.

## RESULTS

Among a total of 84 patients exhibiting serum IgG antibody to *Toxocara canis*, 34 patients were diagnosed with liver abscess. Mean patient age was 53 years, with serum IgG antibody to *Toxocara canis* being three times more prevalent in men than in women (Table 1). Twenty-three (67.6%) patients had a history of eating raw meat or liver and 6 (17.6%) patients owned pet dogs or cats (Table 1). Four patients had no specific history of eating uncooked food or owning pet animals. Main patient symptoms consisted of right upper quadrant pain, fever, and fatigue. Eighteen (52.9%) patients were asymptomatic (Table 1). Five patients revealed involvement of other organs including the lung, a leg muscle, and the brain in addition to liver involvement (Table 1). One of four patients with lung involvement demonstrated concomitant brain involvement. Aspartate transaminase and alanine transaminase levels were normal in all patients except one who had alcoholic hepatitis. Twenty-nine (85.3%) patients initially presented with eosinophilia. Among these 29 patients, 17 had mild eosinophilia, 7 had moderate eosinophilia, and 5 had severe eosinophilia (Table 1). Twenty-five of 26 patients who had repeat serum IgE levels had initially increased IgE levels (Table 1). The remaining patient demonstrated an upper normal serum IgE level of 99 IU/mL. Liver abscess on dynamic CT included multiple lesions in 19 patients and a single lesion in 15 patients (Table 1). The lesions were seen as ill-defined, low-attenuating, oval nodules. They were faintly seen on arterial and equilibrium phase images and best seen on the portal venous phase. All of these 34 patients were treated with albendazole. After treatment, the eosinophil count was normal in 16 patients, decreased in 8, and remained the same in one (Table 2). The eosinophilic response pattern was divided into two groups: Continuously decreasing (15 patients, 62.5%) and fluctuating (9 patients, 37.5%). We were unable to evaluate the eosinophilic response in 9 patients. Among those patients, 5 had normal eosinophil counts at initial diagnosis and 4 did not participate in the follow-up protocol posttreatment. Fifteen of 24 patients who showed an eosinophilic response did so within 1 mo posttreatment. Six months after treatment, the serum

**Table 1** Baseline characteristics of patients with liver abscess caused by toxocara

Patients, <i>n</i>	34
Mean age, yr	53
Male, <i>n</i> (%)	26 (76.5)
Underlying disease, <i>n</i> (%)	
Hypertension	9 (26.5)
Diabetes	4 (11.8)
Tuberculosis	3 (8.8)
Liver cirrhosis	2 (5.9)
Chronic viral hepatitis	2 (5.9)
Cancer history	2 (5.9)
None	9 (26.5)
Alcohol drinking, <i>n</i> (%)	
Heavy drinking	14 (41.2)
Moderate drinking	7 (20.6)
No drinking	9 (26.5)
Unknown	4 (11.8)
Transmission, <i>n</i> (%)	
Eating raw meat or liver	23 (67.6)
Keeping pet dogs or cats	6 (17.6)
No specific history	4 (11.8)
Unknown	7 (20.6)
Symptoms, <i>n</i> (%)	
Asymptomatic	18 (52.9)
RUQ pain	6 (17.6)
Fever	4 (11.8)
Fatigue	4 (11.8)
Anorexia	2 (5.9)
Cough	2 (5.9)
Weakness of legs	2 (5.9)
Involvement of other organs, <i>n</i> (%)	
Lung	4 (11.8)
Muscle of legs	1 (2.9)
CNS	1 (2.9)
Mean AST/ALT, IU/L	31/31
Eosinophilia, <i>n</i> (%)	
Normal (< 500/ $\mu$ L)	5 (14.7)
Mild (500-1500/ $\mu$ L)	17 (50.0)
Moderate (1500-5000/ $\mu$ L)	7 (20.6)
Severe (> 5000/ $\mu$ L)	5 (14.7)
Serum IgE, <i>n</i> (%)	
Normal (< 100 IU/mL)	1 (2.9)
Mild elevated (100-500 IU/mL)	10 (29.4)
Severe elevated (> 500 IU/mL)	15 (44.1)
Unknown	8 (23.5)
Liver abscess, <i>n</i> (%)	
Single	15 (44.1)
Multiple	19 (55.9)

Liver abscess caused by toxocara was related to sex (male), alcohol drinking, eating raw meat or liver. Laboratory characteristics showed normal liver enzymes, peripheral blood eosinophilia, and elevated level of serum IgE. RUQ: Right upper quadrant; CNS: Central nervous system; AST: Alanine aminotransferase; ALT: Aspartate aminotransferase.

IgE level increased in 7 patients, decreased in 8, and remained the same in 3 (Table 2). We were unable to evaluate the serum IgE response in 16 patients. Among these patients, 8 patients did not undergo check serum IgE level tests initially and the other 8 patients did not participate in the follow-up protocol. A follow-up CT was performed for 22 patients. Among these patients, 15 demonstrated disappearance of liver abscess within 3 mo and 21 within 6 mo. Relapse or migration of liver abscess was observed in 3 patients.

**Table 2** Therapeutic response after treatment with albendazole *n* (%)

Eosinophilia ( <i>n</i> = 25)	
Normalized	16 (64)
Decreased	8 (32)
No change	1 (4)
Serum IgE ( <i>n</i> = 18)	
Increased	7 (38.9)
Decreased	8 (44.4)
No change	3 (16.7)
Liver abscess ( <i>n</i> = 22)	
Improved	22 (100)
Not improved	0

Most eosinophil counts were normalized or decreased and all of the abscesses were improved on computed tomography after 1 year, but the levels of serum IgE showed variable response after 6 mo.

## DISCUSSION

Toxocariasis is a worldwide disease. The overall seroprevalence of toxocariasis has been reported as 13.9% in United States<sup>[6]</sup>, 2%-5% and 14%-37% respectively in the urban and rural areas of France<sup>[7]</sup>, 18% in China, 20% in Malaysia, 68% in Indonesia, and 81% in Nepal<sup>[8]</sup>. In South Korea the seroprevalence has been reported as 5% in Gangwon-do<sup>[9]</sup>, 6% in Seoul, and 11% in Gyeongsangnam-do<sup>[10]</sup>. The seroprevalence of toxocariasis in patients with eosinophilia has been reported as 64.9%-86.7% in Seoul<sup>[8,11]</sup>, 50.5% in Chungcheongnam-do<sup>[12]</sup>, and 62% in Pohang<sup>[13]</sup>. These reports confirm that toxocariasis is known to be a main cause of eosinophilia. Toxocara infection is caused by ingestion of embryonated eggs from the soil and pet animals or by ingestion of encapsulated larva while eating uncooked paratenic hosts<sup>[14]</sup>. In this study, eating uncooked food was a more common route of infection than contact with pet animals (67.6% vs 17.6%). Choi *et al*<sup>[8]</sup> suggested that ingestion of raw cow liver was related to an increased risk of toxocariasis, but not ingestion of raw meat or animal blood and owning dogs. Based on the results of our epidemiologic study, demonstrating that men are three times more susceptible to toxocariasis than women, and revealing that approximately 60% of the patients consumed alcohol, we consider sex (male) and alcohol consumption as risk factors for toxocariasis.

The most commonly utilized serologic test for toxocariasis is the detection of the serum IgG antibody to the toxocara excretory/secretory antigen (TES Ag) with a toxocara ELISA kit (Bordier Affinity Products, Crissier, Switzerland)<sup>[15]</sup>. Eosinophilia, elevated serum IgE levels or increased eosinophil cationic protein is helpful for the diagnosis of active toxocariasis<sup>[16-18]</sup>. Although the toxocara ELISA test possesses high sensitivity and specificity<sup>[15]</sup>, the test cannot differentiate present from past infections<sup>[16]</sup> and may produce cross-reactivity with other parasites such as *Clonorchis sinensis*, *Sparganum*, *Fasciola hepatica*, and *Paragonimus westermani*<sup>[2]</sup>. Infected larva can penetrate the intestinal wall through vessels and invade various organs such as the liver, lung, muscle,

eye, heart and central nervous system, etc<sup>[19,20]</sup>. We investigated other organ(s) involvement in 84 patients who had the IgG antibody for *Toxocara canis*, and found the organs involved, displayed in order of frequency, were the liver (40.5%), lung (27.4%), eye (8.3%), skin (3.6%), muscle (1.2%), and brain (1.2%). Lung involvement occurred in 11.8% of patients with liver abscess (Table 1). This occurrence, therefore, creates the necessity of assessing the possibility of lung involvement in patients with liver abscess caused by toxocariasis. TES Ag secreted from the epicuticle of the moving larva causes an immune reaction, which produces increased serum IgE and eosinophilia<sup>[21]</sup>. Liver abscess, histologically described as eosinophilic abscess or granuloma, results from eosinophilic inflammation which develops when larva remain in the liver<sup>[22]</sup>.

Approximately 50% of patients were asymptomatic. A small number of patients had right upper quadrant pain, fever, and fatigue (Table 1). It remains impossible to rule out liver abscess in patients with toxocariasis using only symptom information. In contrast to patients with hepatic visceral larva migrans or liver abscess caused by toxocara, approximately 90% of patients with pyogenic liver abscess have fever and approximately 70% have abdominal pain<sup>[23]</sup>. The possibility of abscess caused by toxocara must be considered if liver abscess is inadvertently detected upon abdominal ultrasonography during routine medical exams. CT findings of liver abscess caused by toxocara usually include lesions that measure approximately 1-1.5 cm in diameter; possess an oval shape, obscure margin, multiplicity, and hypodensity<sup>[3]</sup>. In contrast to hepatocellular carcinoma, CT findings for liver abscess include lesions that are regular, not round, and striking at portal venous phase<sup>[24]</sup>. We can confirm the CT findings cited in this study; however, one finding that differs from the previous study<sup>[24]</sup> is that data from this study reveal that a relatively high number of patients with single abscess existed.

Toxocariasis is a self-limiting disease; therefore, patients with mild symptoms do not necessarily require medication<sup>[2]</sup>. However, if patients have moderate or severe symptoms due to visceral larva migrans, they should be treated with albendazole<sup>[24]</sup>. Previous literature has recommended treating liver abscess regardless of symptom status because, compared with the control group, the albendazole group demonstrated accelerated liver abscess healing<sup>[5]</sup>. Twenty-four of 25 patients who presented with eosinophilia and had been treated with albendazole displayed decreased eosinophil counts. Fifteen of these patients (62.5%) had checked their eosinophil count within 1 mo posttreatment. All patients experienced decreased eosinophil counts. Previous literature has reported an eosinophilic response occurred 1 mo posttreatment<sup>[16]</sup>; therefore, we concluded that the eosinophilic response could be evaluated 1 mo posttreatment. In addition, if the eosinophilic count initially decreases, but continually increases during the posttreatment period, relapse or migration of lesions should be considered. Transient eosinophil count fluctuations, which can occur among eosinophilic response patients

as observed in this study, must also be considered. Abdominal CT and repeat eosinophil counts at follow-up can help distinguish relapse or migration from eosinophilic fluctuation. Repeat serum IgE levels at follow-up provides an inadequate evaluation measurement of treatment response because serum IgE responds unpredictably<sup>[16]</sup>. CT follow-up was performed within 3 mo for 68.2% of patients and liver abscess disappeared in all of them. We therefore concluded that CT scan results could be evaluated 3 mo posttreatment. We encountered relapse or migration of lesions in three patients at 4, 6 and 8 mo posttreatment. Eosinophilia developed in only one of these patients, while the other two experienced continuously decreasing eosinophil levels despite relapse or migration of lesions. Two of these three patients were retreated with albendazole, while the other was only observed. Subsequently, all lesions of all three patients disappeared. If this phenomenon is observed posttreatment, the possibility of reinfection also needs to be considered. No evidence exists confirming that patients who are experiencing relapse or migration of liver abscess, regardless of clinical symptoms or eosinophilia, should be retreated with albendazole. It is reasonable, however, to retreat relapsed or migrated lesions with albendazole because albendazole is inexpensive, easily available over the counter and has no significant side effects. Existence of toxocara-specific IgG antibody can persist for years after the disappearance of liver abscess. One study reported that the mean duration of IgG antibody existence in the body was 2.7 years<sup>[25]</sup>. IgG antibody detection was conducted at 3, 9, 18, 24 mo and 5 years each in five patients from this study. All patients persistently displayed IgG antibody during the follow-up period, therefore, excluding detection of serum IgG antibody testing from the follow-up protocol<sup>[16]</sup>.

The study has limitations as a retrospective study, so our recommendations about the evaluation measurement of treatment response are based on the literature data and not on the results of this study.

Liver abscess caused by toxocara is a disease resulting from the ingestion of uncooked food, which causes an immune reaction in the liver. Patients can present with no specific symptoms, eosinophilia, and/or increased levels of serum IgE. Toxocariasis has characteristic radiologic findings and may involve other organs such as the lung. Treatment of toxocariasis consists of taking albendazole for 5 d. After treatment, the eosinophil count starts to decrease within 1 mo and the abscess begins to disappear within 3 mo as displayed on CT scan. Complete disappearance of liver abscess can occur after 1 year. Even if a few patients experience relapse or migration of abscess posttreatment, a good prognosis exists for the overall clinical course of this disease.

## COMMENTS

### Background

Toxocariasis is a parasitic infection caused by *Toxocara canis* or *Toxocara cati*. It is known as a main cause of eosinophilia. Clinical manifestations of



toxocarasis range from asymptomatic infection to involvement of various organs. Liver abscess caused by toxocara can occasionally be detected as an abnormal finding at ultrasonography screening and therefore be misdiagnosed as a malignancy in patients with chronic liver disease or a history of other cancer(s). The authors evaluated the clinical characteristics and progression of liver abscess caused by toxocara.

### Research frontiers

This study contributes to determining potential factors that may help improve diagnosis of liver abscess caused by toxocara, as well as avoid unnecessary testing and improper treatment.

### Innovations and breakthroughs

In this study, all patients (62.5%) who had checked their eosinophil count within 1 mo posttreatment experienced decreased eosinophil counts. And all patients (68.2%) who had checked computed tomography (CT) follow-up within 3 mo posttreatment experienced disappearance of liver abscess. Therefore, the authors concluded that the eosinophilic response could be evaluated 1 mo posttreatment and CT scan could be evaluated 3 mo posttreatment.

### Applications

Human toxocarasis can clinically present as liver abscess. If a patient with a history of eating raw meat or liver presents peripheral eosinophilia and abnormal liver imaging, liver abscess caused by toxocara should be considered for diagnosis.

### Terminology

Toxocarasis: An infection transmitted from animals to humans caused by the parasitic roundworms commonly found in the intestine of dogs (*Toxocara canis*) and cats (*Toxocara cati*).

### Peer-review

Studies exploring toxocarasis in liver abscess have been infrequent. The author of this paper evaluated the clinical characteristics and progression of liver abscess caused by toxocara. This study is useful for diagnosing and monitoring the disease in the clinical practice.

## REFERENCES

- 1 **Kwon NH**, Oh MJ, Lee SP, Lee BJ, Choi DC. The prevalence and diagnostic value of toxocarasis in unknown eosinophilia. *Ann Hematol* 2006; **85**: 233-238 [PMID: 16463154 DOI: 10.1016/j.jaci.2005.12.320]
- 2 **Pawlowski Z**. Toxocarasis in humans: clinical expression and treatment dilemma. *J Helminthol* 2001; **75**: 299-305 [PMID: 11818044 DOI: 10.1017/S0022149X01000464]
- 3 **Lim JH**. Toxocarasis of the liver: visceral larva migrans. *Abdom Imaging* 2008; **33**: 151-156 [PMID: 17924161 DOI: 10.1007/s00261-007-9325-y]
- 4 **Rahimian J**, Wilson T, Oram V, Holzman RS. Pyogenic liver abscess: recent trends in etiology and mortality. *Clin Infect Dis* 2004; **39**: 1654-1659 [PMID: 15578367 DOI: 10.1086/425616]
- 5 **Jang EY**, Choi MS, Gwak GY, Koh KC, Paik SW, Lee JH, Paik YH, Yoo BC. Enhanced resolution of eosinophilic liver abscess associated with toxocarasis by albendazole treatment. *Korean J Gastroenterol* 2015; **65**: 222-228 [PMID: 25896156 DOI: 10.4166/kjg.2015.65.4.222]
- 6 **Won KY**, Kruszon-Moran D, Schantz PM, Jones JL. National seroprevalence and risk factors for Zoonotic *Toxocara* spp. infection. *Am J Trop Med Hyg* 2008; **79**: 552-557 [PMID: 18840743]
- 7 **Magnaval JF**, Michault A, Calon N, Charlet JP. Epidemiology of human toxocarasis in La Réunion. *Trans R Soc Trop Med Hyg* 1994; **88**: 531-533 [PMID: 7992328 DOI: 10.1016/0035-9203(94)90148-1]
- 8 **Choi D**, Lim JH, Choi DC, Paik SW, Kim SH, Huh S. Toxocarasis and ingestion of raw cow liver in patients with eosinophilia. *Korean J Parasitol* 2008; **46**: 139-143 [PMID: 18830052 DOI: 10.3347/kjp.2008.46.3.139]
- 9 **Park HY**, Lee SU, Huh S, Kong Y, Magnaval JF. A seroepidemiological survey for toxocarasis in apparently healthy residents in Gangwon-do, Korea. *Korean J Parasitol* 2002; **40**: 113-117 [PMID: 12325440 DOI: 10.3347/kjp.2002.40.3.113]
- 10 **Kim HS**, Jin Y, Choi MH, Kim JH, Lee YH, Yoon CH, Hwang EH, Kang H, Ahn SY, Kim GJ, Hong ST. Significance of serum antibody test for toxocarasis in healthy healthcare examinees with eosinophilia in Seoul and Gyeongsangnam-do, Korea. *J Korean Med Sci* 2014; **29**: 1618-1625 [PMID: 25469060 DOI: 10.3346/jkms.2014.29.12.1618]
- 11 **Kim YH**, Huh S, Chung YB. Seroprevalence of toxocarasis among healthy people with eosinophilia. *Korean J Parasitol* 2008; **46**: 29-32 [PMID: 18344674 DOI: 10.3347/kjp.2008.46.1.29]
- 12 **Seo M**, Yoon SC. A seroepidemiological survey of toxocarasis among eosinophilia patients in Chungcheongnam-do. *Korean J Parasitol* 2012; **50**: 249-251 [PMID: 22949755 DOI: 10.3347/kjp.2012.50.3.249]
- 13 **Ryu BH**, Park JS, Jung YJ. Clinical and Serological Findings in Patients with Toxocarasis in the Pohang Region: The Features of Toxocarasis in Pohang. *Korean J Med* 2013; **84**: 203-210
- 14 **Morris PD**, Katerndahl DA. Human toxocarasis. Review with report of a probable case. *Postgrad Med* 1987; **81**: 263-267 [PMID: 3543902]
- 15 **Jacquier P**, Gottstein B, Stingelin Y, Eckert J. Immunodiagnosis of toxocarosis in humans: evaluation of a new enzyme-linked immunosorbent assay kit. *J Clin Microbiol* 1991; **29**: 1831-1835 [PMID: 1774303]
- 16 **Magnaval JF**, Glickman LT, Dorchie P, Morassin B. Highlights of human toxocarasis. *Korean J Parasitol* 2001; **39**: 1-11 [PMID: 11301585 DOI: 10.3347/kjp.2001.39.1.1]
- 17 **Fillaux J**, Magnaval JF. Laboratory diagnosis of human toxocarasis. *Vet Parasitol* 2013; **193**: 327-336 [PMID: 23318165 DOI: 10.1016/j.vetpar.2012.12.028]
- 18 **Magnaval JF**, Berry A, Fabre R, Morassin B. Eosinophil cationic protein as a possible marker of active human *Toxocara* infection. *Allergy* 2001; **56**: 1096-1099 [PMID: 11703226 DOI: 10.1034/j.1398-9995.2001.00284.x]
- 19 **Marx C**, Lin J, Masruha MR, Rodrigues MG, da Rocha AJ, Vilanova LC, Gabbai AA. Toxocarasis of the CNS simulating acute disseminated encephalomyelitis. *Neurology* 2007; **69**: 806-807 [PMID: 17709716 DOI: 10.1212/01.wnl.0000267664.53595.75]
- 20 **Enko K**, Tada T, Ohgo KO, Nagase S, Nakamura K, Ohta K, Ichiba S, Ujike Y, Nawa Y, Maruyama H, Ohe T, Kusano KF. Fulminant eosinophilic myocarditis associated with visceral larva migrans caused by *Toxocara canis* infection. *Circ J* 2009; **73**: 1344-1348 [PMID: 19122304 DOI: 10.1253/circj.CJ-08-0334]
- 21 **Lee S**. Pathophysiology of hypersensitivity in human by *Toxocara canis* larval infection. Chung-Ang University Doctoral Thesis, 2001
- 22 **Despommier D**. Toxocarasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clin Microbiol Rev* 2003; **16**: 265-272 [PMID: 12692098 DOI: 10.1128/CMR.16.2.265-272.2003]
- 23 **Chang S**, Lim JH, Choi D, Park CK, Kwon NH, Cho SY, Choi DC. Hepatic visceral larva migrans of *Toxocara canis*: CT and sonographic findings. *AJR Am J Roentgenol* 2006; **187**: W622-W629 [PMID: 17114516]
- 24 **Stürchler D**, Schubarth P, Gualzata M, Gottstein B, Oettli A. Thiabendazole vs. albendazole in treatment of toxocarasis: a clinical trial. *Ann Trop Med Parasitol* 1989; **83**: 473-478 [PMID: 2694978]
- 25 **Jeanneret JP**. Épidémiologie de la toxocarose dans la région jurassienne: Université de Neuchâtel, 1991

**P- Reviewer:** Akyuz U, Ferraioli G, He JY, Romero MR, Zielinski J  
**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Li D



## Treating chronic hepatitis B virus: Chinese physicians' awareness of the 2010 guidelines

Lai Wei, Ji-Dong Jia, Xin-Hua Weng, Xiao-Guang Dou, Jia-Ji Jiang, Hong Tang, Qin Ning, Qing-Qing Dai, Run-Qin Li, Jie Liu

Lai Wei, Department of Hepatology, Peking University People's Hospital, Beijing 100044, China

Ji-Dong Jia, Department of Hepatology, Beijing Friendship Hospital, Capital Medical University, Beijing 100029, China

Xin-Hua Weng, Department of Infectious Disease, Shanghai Huashan Hospital, Shanghai 200040, China

Xiao-Guang Dou, Department of Infectious Disease, Shengjing Hospital of China Medical University, Shenyang 110022, Liaoning Province, China

Jia-Ji Jiang, Department of Hepatology, the First Affiliated Hospital of Fujian Medical University, Fuzhou 350005, Fujian Province, China

Hong Tang, Department of Infectious Disease, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

Qin Ning, Department of Infectious Disease, Affiliated Tongji Hospital of Tongji Medical College, Wuhan 430030, Hubei Province, China

Qing-Qing Dai, SmithStreet, Shanghai 200040, China

Run-Qin Li, Jie Liu, Bristol-Myers Squibb, Shanghai 200240, China

**Author contributions:** Wei L, Jia JD, Weng XH, Dou XG, Jiang JJ, Tang H and Ning Q (ranking reference their input) analyzed the data, interpreted data in final review, and final approval of the manuscript; Dai QQ designed the survey, completed data acquisition, analysis and interpretation, and final approval of the manuscript; Li RQ and Liu J designed the survey, analyzed and interpreted data, drafted the paper and revised it critically for important intellectual content and final approval of the manuscript.

Supported by Bristol-Myers Squibb.

**Conflict-of-interest statement:** Lai Wei has received research support and/or consulting fees from Bristol-Myers Squibb,

Roche and Novartis. Ji-Dong Jia has received research support, consulting fees and speaker fees from Bristol-Myers Squibb, consulting fees and speaker fees from Gilead, and research support from Novartis. Xin-Hua Weng has received consulting fees and speaker fees from Novartis, Roche and Bristol-Myers Squibb, and speaker fees from Pfizer and AstraZeneca. Xiao-Guang Dou and Jia-Ji Jiang have no conflicts of interest to declare. Hong Tang has received research support, consulting fees and speaker fees from Bristol-Myers Squibb, Novartis and Roche. Qin Ning has received research support and consulting fees from Novartis, Bristol-Myers Squibb and Roche, and consulting fees from GlaxoSmithKline. Qing-Qing Dai is an employee of SmithStreet. Run-Qin Li and Jie Liu are employees of Bristol-Myers Squibb.

**Data sharing statement:** The survey participants gave consent for information to be shared. The data was collected by on-line survey or off-line survey (post or fax). No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Lai Wei, MD, PhD, Department of Hepatology, Peking University People's Hospital, 11 Xizhimen South Street, Xicheng District, Beijing 100044, China. [weilai@pkuph.edu.cn](mailto:weilai@pkuph.edu.cn)  
**Telephone:** +86-10-88325566  
**Fax:** +86-10-66515490

**Received:** December 9, 2015  
**Peer-review started:** December 10, 2015  
**First decision:** January 18, 2016  
**Revised:** March 17, 2016  
**Accepted:** April 5, 2016  
**Article in press:** April 6, 2016  
**Published online:** June 28, 2016

## Abstract

**AIM:** To investigate Chinese physicians' awareness of the 2010 guidelines on the treatment of chronic hepatitis B virus (HBV) infection.

**METHODS:** This was a quantitative survey that investigated the characteristics and practices of physicians who were treating patients with hepatitis B, the profile of their patients and physician practices regarding the diagnosis and treatment of HBV at the time of the survey. Participants were randomly selected from available databases of Chinese physicians and requested to complete either an online or paper-based survey. Data from the survey responses were analysed. For data validation and interpretation, qualitative in-depth interviews were conducted with 39 of the respondents.

**RESULTS:** Five-hundred completed surveys, from 663 physicians were available for analysis. A mean of 175 chronic hepatitis B (CHB) patients was seen by each physician every month, of whom 85 (49%) were treated in line with therapeutic indications stated in the 2010 guidelines. A total of 444 (89%) physicians often (> 60% of the time) adhered to the guidelines. Most physicians used antiviral medications as recommended. For patients with compensated and decompensated cirrhosis, 342 (68%) and 336 (67%) of physicians, respectively, often followed the recommendation to use potent nucleos(t)ide analogues with a high genetic barrier to resistance, using the appropriate treatment more than 60% of the time. Physicians from infectious disease or liver disease departments were better informed than those from gastrointestinal or other departments.

**CONCLUSION:** The majority of Chinese physicians often adhere to Chinese 2010 CHB guidelines and are well-informed about the use of antiviral medications for hepatitis B.

**Key words:** Chronic hepatitis B; Practice guidelines; Awareness; China; Physicians

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In general, the majority of Chinese physicians often adhere to Chinese 2010 chronic hepatitis B guidelines and they are well-informed about the use of antiviral medications for hepatitis B. Most of the physicians who participated in our survey used antiviral medications as recommended. For patients with compensated and decompensated cirrhosis, more than two-thirds of physicians, often followed the recommendation to use potent nucleos(t)ide analogues with a high genetic barrier to resistance. Our survey also showed that physicians from infectious disease or liver disease departments were better informed than those from gastrointestinal or other departments.

Q, Dai QQ, Li RQ, Liu J. Treating chronic hepatitis B virus: Chinese physicians' awareness of the 2010 guidelines. *World J Hepatol* 2016; 8(18): 762-769 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i18/762.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i18.762>

## INTRODUCTION

The clinical management of chronic hepatitis B (CHB) has undergone dramatic changes over the past two decades following the registration worldwide of several antiviral agents that effectively suppress hepatitis B virus (HBV) loads<sup>[1-4]</sup>. Currently, the primary aim of CHB treatment is the permanent suppression of HBV replication to decrease viral infectivity and pathogenicity<sup>[5]</sup>. Two different classes of drug are used to treat HBV: Conventional interferon (IFN) or pegylated IFN, and oral nucleos(t)ide analogues (NAs). Nucleoside analogues include lamivudine, telbivudine, clevudine and entecavir, while nucleotide analogues include adefovir dipivoxil and tenofovir dipivoxil fumarate<sup>[5]</sup>. Guidelines have been developed to help standardise the prevention, diagnosis and treatment of CHB. Key clinical practice guidelines have been developed by the Asian Pacific Association for the Study of the Liver (APASL; 2012 update)<sup>[6]</sup>, the European Association for the Study of the Liver (EASL; 2012 update)<sup>[6]</sup> and the American Association for the Study of Liver Diseases (AASLD; 2009 update)<sup>[7]</sup>. In March 2015, the World Health Organization issued its first-ever guidance for the treatment of CHB<sup>[8]</sup>.

Chinese CHB guidelines were first developed in 2005 by the Chinese Society of Hepatology, Chinese Medical Association and Chinese Society of Infectious Diseases<sup>[9]</sup>, and updated in 2010<sup>[10]</sup>. The 2010 guidelines state that no antiviral treatment is recommended for chronic and inactive HBV carriers, although regular diagnostic tests should be performed to ensure criteria for antiviral therapy are not met. For hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients with CHB, both IFNs and NAs are recommended as first-line treatments. However, due to these patients' need for long-term treatment, it is recommended that those with HBeAg-negative CHB or CHB with cirrhosis (compensated or decompensated) receive treatment with NAs that have a high genetic barrier to resistance<sup>[10]</sup>.

It is known that the implementation of treatment guidelines in clinical practice can improve the outcome of patients, but despite wide promulgation, many guidelines are not readily accepted by physicians or incorporated in clinical management strategies<sup>[11]</sup>. There is some evidence of poor adherence to HBV treatment guidelines among healthcare providers in the United States who treat HBV/human immunodeficiency virus (HIV) co-infected patients<sup>[12]</sup>, but in general, real-world clinical practice with CHB guidelines is not well understood. In China, the prevalence of HBV is high<sup>[13,14]</sup> and physician adherence to CHB treatment guidelines could potentially have an important impact on the long-term outcome of a large

proportion of the CHB population. To date, there are limited available data on how CHB is treated in real-world clinical practice in China and whether physicians adhere to available guidelines. Therefore, the aim of this study was to investigate Chinese physicians' awareness of the updated 2010 Chinese CHB treatment guidelines<sup>[10]</sup>, to improve understanding of guideline use in clinical practice, and to assist with the development of future CHB clinical practice guideline updates.

## MATERIALS AND METHODS

### Study design

This was a quantitative survey to investigate the characteristics of physicians who treat patients with HBV, the profile of their patients and physician practices regarding the diagnosis and treatment of HBV. Three study dimensions were therefore included. The first captured physician gender, location, affiliation and professional position. The second captured number of CHB patients treated, proportion of HBeAg-positive and HBeAg-negative patients and the proportion of patients with cirrhosis. The third captured physician reference to the Chinese 2010 CHB guidelines<sup>[12]</sup>, as well as specific data on physician prescription, treatment and follow-up practices.

### Participants

Participants were randomly selected from internal databases of Chinese physicians, belonging to either SmithStreet (Shanghai, China) or Bristol-Myers Squibb (BMS; Shanghai, China). SmithStreet has proven experience in healthcare survey development, a focus on China growth strategies, and relevant experience in consumer health and prescription medicines. For survey data capture, physicians working in Chinese grade III hospitals and in liver disease or infectious disease departments were targeted, with appropriate segmentation to obtain an even distribution of physicians by region, city tier and professional position. In China, the Ministry of Health grades hospitals according to a three-grade system, which assesses a hospital's ability to provide medical care and medical education, and to conduct medical research. In general, grade III hospitals are considered the highest level in China. These hospitals are able to provide high quality, specialized care in well-equipped facilities. Considering the availability of medical education and specialized care at grade III hospitals, in general, physicians at these hospitals are thought to be at the forefront of clinical medicine.

Participants were approached by SmithStreet *via* phone or email, and also face-to-face for qualitative follow-up questions. There was a target sample size of 500 respondents, which was deemed sufficient to allow nationwide representation of the data. Participants were required to be physicians currently treating patients with HBV, but there were no other pre-specified eligibility criteria or screening questions prior to enrolment.

### Research setting

Survey questions were developed by SmithStreet. Contributions were made by leading physicians, who reviewed and provided feedback on the questions prior to survey initiation. Data were collected remotely online, *via* the SurveyMonkey® platform (<https://www.surveymonkey.com>). Physicians who were unable to complete the survey online were provided an offline survey by SmithStreet, which was distributed by post or fax; offline surveys were subsequently returned by email to SmithStreet for data collection and analysis.

### Data collection

Instruction guides for online and offline surveys were provided to participating physicians. A pilot online survey was tested on five randomly selected representative physicians outside of the target pool to obtain feedback on language, content and interface structure. Participant responses were captured by the SurveyMonkey® platform. For consistency during data extraction and analysis, data from the offline surveys were entered into SurveyMonkey® by SmithStreet. Respondents who completed the survey offline were given the opportunity to review their responses.

### Data analyses

All coding and data analyses were conducted by SmithStreet. Responses were reviewed to eliminate repeat submissions and incomplete responses. Follow-up phone calls were conducted with 25 randomly selected online respondents to verify their participation. Responses that were deemed invalid or defective were eliminated from the analyses. To assist with data validation and interpretation, qualitative in-depth interviews were conducted with 39 respondents of the quantitative survey. The statistical methods of this study were reviewed by Qing-Qing Dai of SmithStreet. No statistical tests were performed. The data are presented as percentages and described.

## RESULTS

### Characteristics of physicians

The first participant was screened on 21 March 2013 and the last participant completed the survey on 1 September 2013. Participant flow through the study is shown in Figure 1. Of 663 physicians who completed the survey, 500 were available for analysis (472 from online surveys); of these, 194 were recruited through BMS' physician database and 306 were recruited *via* SmithStreet's physician database. Demographics and background characteristics of the responding physicians are shown in Table 1. The majority were female, from South, North or East China, held attending level positions or above, and worked in an infectious diseases department (Table 1).

### Characteristics of treated HBV patients

Segmentation of physicians by number of CHB patients



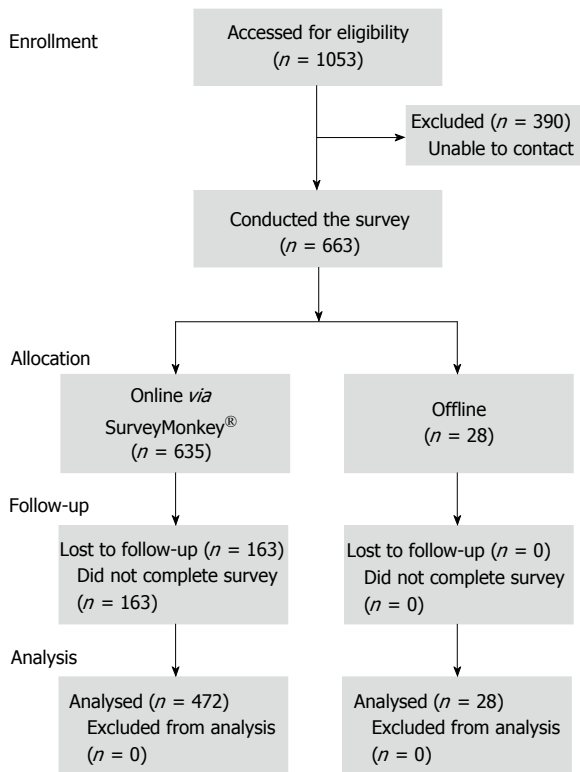


Figure 1 Participant flow through the study.

seen per month is shown in Figure 2. A mean of 175 CHB patients was seen by each physician every month, of whom 85 (49%) were treated with antiviral therapy in line with the therapeutic indications as stated in the Chinese 2010 CHB guidelines<sup>[12]</sup> and 46 (26%) had cirrhosis (27 with compensated cirrhosis). Among treatment-naïve CHB patients, 25 (14%) were HBeAg-positive and 21 (12%) were HBeAg-negative. The number of patients seen each month varied by physician rank, hospital grade, city tier and region; however, the number of treatment-naïve HBeAg-positive and HBeAg-negative patients seen by physicians each month was similar across different regions and city tiers. (In China, cities are ranked into tiers (tier I through IV) according to size and economic development, with tier I cities generally the largest economical hubs).

#### Physician awareness of guidelines

A total of 444 (89%) surveyed physicians indicated that they "often" (defined as more than 60% of the time) adhered to the Chinese 2010 CHB guidelines. In particular, more than 90% of physicians from infectious disease or liver disease departments often adhered to the guidelines (Figure 3).

Most physicians used antiviral medications consistent with guideline recommendations. However, in patients older than 40 years, who were HBV DNA-positive (but with  $< 1 \times 10^4$  copies/mL), and with alanine aminotransferase (ALT) levels above the upper limit of normal (ULN), 196 (39%) of the surveyed physicians did not consider antiviral medication necessary (Figure 4). The guideline recommends that in these patients, the

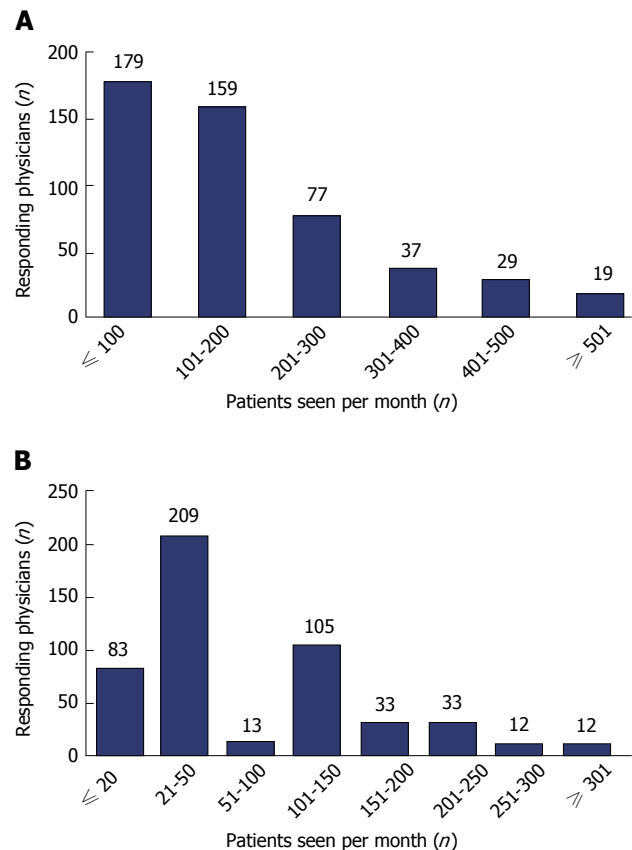
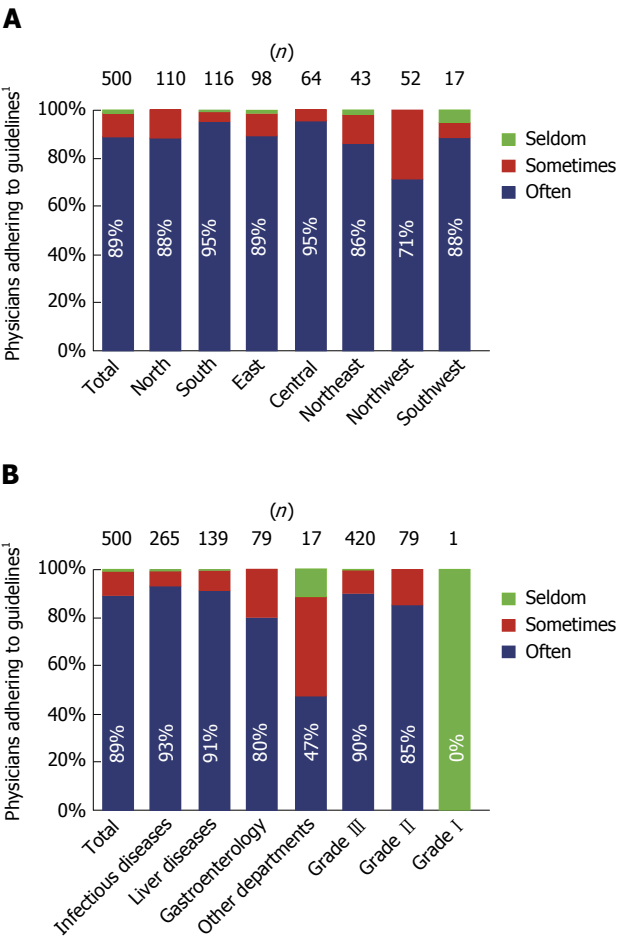


Figure 2 Segmentation of physicians. Segmentation of physicians by number of chronic hepatitis B (CHB) patients seen per month (A) in total, and (B) according to indications stated in the Chinese 2010 CHB guidelines<sup>[12]</sup>. A: Physicians ( $n = 500$ ) were asked, "how many CHB patients do you treat per month?"; B: Physicians ( $n = 500$ ) were asked, "amongst the CHB patients that you treat per month, how many of them are in line with the therapeutic indications as stated in the (Chinese 2010 CHB) guidelines?".

presence of liver fibrosis (as judged by the physician), should be an indication for antiviral therapy.

A total of 354 (71%) physicians could identify the distractor (HBeAg-positive, HBV DNA  $\geq 10^5$  copies/mL,  $1 \times \text{ULN} < \text{ALT} < 2 \times \text{ULN}$ ), including 194 (73%) of those from infectious disease departments, 106 (76%) from liver disease departments, 49 (62%) from gastroenterology departments, and five (29%) from other departments.

When asked for a response regarding the reasonable treatment course for antiviral medications, 422 (84%) physicians considered that more than 12 mo (responses for "12 to 18 mo" and "more than 18 mo") of consolidation treatment was needed for HBeAg-positive patients following serological conversion. However, this proportion was lower among physicians from gastroenterology ( $n = 54$ ; 68% of all physicians from gastroenterology departments) or other ( $n = 11$ ; 65%) departments, than among physicians from infectious disease ( $n = 236$ ; 89%) or liver disease ( $n = 121$ ; 87%) departments. For HBeAg-negative patients following serological conversion, 302 (60%) physicians considered that more than 18 mo of consolidation treatment was needed. However, this proportion was lower among physicians from gas-



**Figure 3 Physician adherence to the Chinese 2010 chronic hepatitis B guidelines.** Proportion of physicians adhering to Chinese 2010 chronic hepatitis B (CHB) guidelines<sup>[12]</sup> by (A) region, or (B) by hospital department or grade. "Often" defined as > 60% of the time, "sometimes" defined as > 30% to < 60% of the time and "seldom" defined as < 30% of the time.

troenterology ( $n = 40$ ; 51% of all physicians from gastroenterology departments) or other ( $n = 8$ ; 47%) departments, than among physicians from infectious disease ( $n = 169$ ; 64%) or liver disease ( $n = 85$ ; 61%) departments.

For patients with compensated and decompensated cirrhosis, 342 (68%) and 336 (67%) physicians, respectively, followed guidelines recommending the use of potent NAs with a high genetic barrier to resistance, using the appropriate treatment more than 60% of the time. This recommendation was followed most frequently by physicians from liver disease departments for patients with compensated and decompensated cirrhosis (both  $n = 100$ ; 72%). A low proportion of physicians did not follow this recommendation (use of the appropriate treatment less than 30% of the time) for compensated cirrhosis ( $n = 17$ ; 3%) and decompensated cirrhosis patients ( $n = 20$ ; 4%).

## DISCUSSION

Our survey results show that the majority of Chinese physicians often adhered to Chinese 2010 CHB guide-

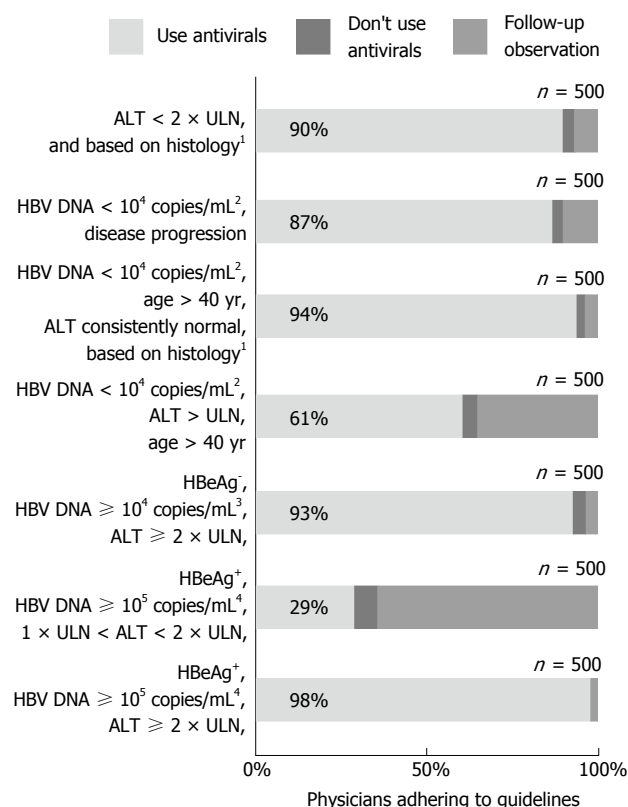
**Table 1 Characteristics of responding physicians**

Characteristic	Physician response (n = 500)
Female physicians, n (%)	282 (56)
Location within China, n (%)	
North	110 (22)
South	116 (23)
East	98 (20)
Central	64 (13)
Northeast	43 (9)
Northwest	52 (10)
Southwest	17 (3)
City tier, n (%)	
I	110 (22)
II	171 (34)
III	143 (29)
IV	46 (9)
V	30 (6)
Hospital grade, n (%)	
I	1 (0)
II	79 (16)
III	420 (84)
Hospital affiliation, n (%)	
Gastroenterology	79 (16)
Infectious disease	265 (53)
Liver disease	139 (28)
Other	17 (3)
Hospital position, n (%)	
Chief	151 (30)
Associate chief	90 (18)
Attending	147 (29)
Resident	112 (22)

lines<sup>[10]</sup>. Physicians from liver disease and infectious disease departments were most familiar with the guidelines, but physicians from other departments adhered to the guidelines less frequently, indicating that access to, or awareness of CHB treatment guidelines in China could be improved.

The physicians in this survey saw a mean of 175 CHB patients every month. This was below the number expected, especially for physicians working in tier I cities and grade III hospitals (210 and 184 patients per month, respectively; data not shown). We found 26% of patients to have cirrhosis, 29% to be HBeAg-positive and 23% to be HBeAg-negative. Nearly half (49%) of patients were treated in line with guidelines for various indications, and nearly nine out of 10 (89%) responding physicians often adhered to the guidelines; based on our clinical experience, this number is higher than expected.

In China, most patients with HBV are treated in infectious disease or liver disease departments, with physicians working in these departments generally regarded as specialists. Accordingly, when our survey results were analysed by hospital department, we found that adherence was greatest among physicians from infectious disease or liver disease departments. Adherence was slightly lower among physicians working in gastroenterology departments, but much lower among physicians from other departments. In Northwest China, the percentage of physicians who adhered to the guideline was noticeably lower than in other regions. Although



**Figure 4 Physician familiarity with various indications for antiviral medication.** Physician familiarity with the different indications for antiviral medication as described in the Chinese chronic hepatitis B (CHB) guidelines<sup>[12]</sup> is depicted here. The various indications are listed on the left. Physicians were asked, “does antiviral medication apply to the following cases?” and the proportion of physicians who would consider antivirals, no antivirals, and follow-up for each indication, is depicted in each horizontal bar. According to the Chinese 2010 CHB guidelines, general indications for antiviral treatment include: (1) hepatitis B e antigen (HBeAg)-positive and hepatitis B virus (HBV) DNA ≥ 105 copies/mL, or HBeAg-negative and HBV DNA ≥ 104 copies/mL; (2) alanine aminotransferase (ALT) ≥ 2 × upper limit of normal (ULN); or (3) ALT < 2 × ULN, but hepatic histology show Knodell histology activity index (HAI) ≥ 4, inflammation necrosis grade ≥ 2, or fibrosis stage ≥ 2. If HBV DNA is consistently positive but the above general indications cannot be reached, then antiviral treatment should be considered under the following circumstances: (1) ALT > ULN and age > 40 years; (2) ALT consistently normal and age > 40 years (can be closely monitored, but liver biopsy is recommended; antiviral treatment is indicated when Knodell HAI ≥ 4, or inflammation necrosis grade ≥ 2, or fibrosis stage ≥ 2); or (3) evidence of disease progression following dynamic observation (hepatic histology examination is recommended and antiviral treatment should be administered as necessary). For the distractor (HBeAg-positive, HBV DNA ≥ 105 copies/mL, 1 × ULN < ALT < 2 × ULN), liver biopsy tests are also needed to determine whether antiviral treatment is required. <sup>1</sup>Hepatic histology shows Knodell HAI ≥ 4, or inflammatory necrosis grade ≥ 2, or fibrosis stage ≥ 2; <sup>2</sup>HBV DNA < 104 copies/mL was considered HBV positive; <sup>3</sup>Equivalent to 2000 IU/mL; <sup>4</sup>Equivalent to 20000 IU/mL.

speculative, this may be due to a higher proportion of respondents who were residents, and a lower proportion of respondents who were chief physicians in Northwest China compared with other regions (data not shown). In addition, Northwest China had the second highest proportion of respondents from other departments (6%) and the second lowest combined proportion of respondents who were specialists (48% from infectious disease departments plus 23% from liver disease departments; data not shown).

Previous publications have demonstrated inadequacy of CHB management and need for improved education among Chinese physicians<sup>[15,16]</sup>. Even with the availability of CHB guidelines from key international associations, continual advancement in our understanding of CHB and availability of new data can create ongoing challenges regarding who should be treated and for how long<sup>[17]</sup>. Chao *et al.*<sup>[16]</sup> (2010) previously reported that there is a lack of basic knowledge surrounding HBV natural history, prevention and transmission. They identified critical gaps in HBV knowledge; in particular, 34% of physicians surveyed in their study did not know that CHB is often asymptomatic, 29% did not know that CHB infection confers a high risk of cirrhosis, liver cancer and premature death, and only 31% knew the recommended protocol for testing liver function and screening for liver cancer in CHB<sup>[16]</sup>.

In contrast, in our study, the majority of physicians were found to be well-informed about the importance of antiviral medication and were educated on the appropriate indications for their use. However, there was some inconsistency among physicians regarding the use of antiviral treatment for patients older than 40 years, who present with HBV DNA of < 1 × 10<sup>4</sup> copies/mL, and ALT levels above the ULN. The guideline recommends that these patients be initiated on antiviral therapy if they have liver fibrosis (as judged by the physician). For these patients, liver biopsy is clearly important when making treatment decisions.

As expected, and in line with the proportion of physicians who adhered to the Chinese 2010 CHB guidelines, physicians from infectious disease and liver disease departments were better able to identify the distractor, followed by those from gastroenterology departments and physicians from other departments.

According to the Chinese 2010 CHB guidelines, the endpoint for antiviral treatment in HBeAg-positive patients should be HBV DNA levels below the lower limit of detection, normalisation of ALT levels, HBeAg seroconversion, at least 1 year of consolidation therapy and a total treatment duration of at least 2 years. In HBeAg-negative patients, these criteria are the same for HBV DNA and ALT levels, but at least 1.5 years of consolidation therapy and a total treatment duration of at least 2.5 years is recommended<sup>[10]</sup>. We found that 84% and 60% of physicians often followed recommendations for consolidation therapy for HBeAg-positive and HBeAg-negative patients, respectively. Physicians from infectious disease or liver disease departments again showed greatest awareness of the guidelines in this context. For HBeAg-negative patients, the optimal duration of NA treatment is unknown unless hepatitis B surface antigen seroclearance has occurred, and the decision to stop therapy can be based upon clinical response and severity of the underlying liver disease<sup>[5]</sup>; this flexibility may explain the lower adherence to recommended endpoints for HBeAg-negative patients compared with HBeAg-positive patients.

As CHB requires long-term treatment, the Chinese

2010 CHB guidelines recommend that HBeAg-negative CHB patients and CHB patients with cirrhosis (compensated or decompensated) receive treatment with NAs that have a high genetic barrier to resistance<sup>[10]</sup>. We found that, in both patients with compensated and decompensated cirrhosis, over two-thirds of physicians often followed guidelines recommending the use of potent NAs with a high genetic barrier to resistance. While this finding is promising for the appropriate antiviral treatment of patients with CHB, this alignment with the guidelines was higher than expected.

Although the majority of Chinese physicians in our survey often adhered to Chinese 2010 CHB guidelines, our results also showed that there is a need to improve physicians' awareness and knowledge of CHB guidelines. This was particularly evident among non-specialists, where a need for education on CHB and its treatment was confirmed. Although physicians from gastroenterology departments were relatively well-informed about CHB, their awareness of CHB guidelines was lower than that of specialist physicians from infectious disease or liver disease departments, implying that Chinese gastroenterologists may require additional training on HBV antiviral recommendations. The delay and reduction of liver cirrhosis is one goal of CHB treatment<sup>[5]</sup> and the Chinese 2010 CHB guidelines provide specific recommendations for antiviral treatment and follow-up in CHB patients with cirrhosis<sup>[10]</sup>. Since patients with cirrhosis are frequently referred to gastroenterologists, consensus statements for treatment of cirrhotic HBV patients with antiviral therapy should help educate Chinese gastroenterologists moving forward.

Other than general limitations associated with the acquisition of data using a survey design, the respondent pool in our survey could be considered a potential limitation leading to over- or underestimation of guideline awareness and uptake. In particular, our survey included only one grade I hospital, the majority of physicians came from grade III hospitals and physicians from Southwest China or other departments may have been under-represented. In addition, this survey was based upon the latest update to the Chinese CHB guidelines; because this update was published in 2010, and this survey was completed at the end of 2013, this survey may not reflect changes in physician attitudes or education in the past few years.

This survey has shown that the majority of Chinese physicians often adhered to Chinese 2010 CHB guidelines and were well-informed about the use of antiviral medication for HBV. However, there is a need to further educate non-specialist physicians who treat patients with CHB and to promote physician adherence to future CHB guidelines or updates.

The majority of Chinese physicians often adhere to Chinese 2010 CHB guidelines and they are well-informed about the use of antiviral medications for hepatitis B. In general, the physicians who participated in our survey used antiviral medications as recommended. For patients with compensated and decompensated cirrhosis, more

than two-thirds of physicians, often followed the recommendation to use potent nucleos(t)ide analogues with a high genetic barrier to resistance. We found that physicians from infectious disease or liver disease departments were better informed than those from gastrointestinal or other departments.

## ACKNOWLEDGMENTS

The survey that formed the basis of this study was sponsored by Bristol-Myers Squibb. The survey was executed by SmithStreet. Editorial support for this manuscript was provided by Manette Williams, PhD, from Nucleus Global.

## COMMENTS

### Background

The implementation of treatment guidelines in clinical practice can improve the outcome of patients, but despite wide promulgation, many guidelines are not readily accepted by physicians or incorporated in clinical management strategies. In China, the prevalence of hepatitis B virus (HBV) is high and physician adherence to chronic hepatitis B (CHB) treatment guidelines could potentially have an important impact on the long-term outcome of a large proportion of the CHB population. There are limited available data on how CHB is treated in real-world clinical practice in China and whether physicians adhere to available guidelines. The authors investigated Chinese physicians' awareness of the updated 2010 Chinese CHB treatment guidelines.

### Research frontiers

CHB patients require long-term treatment and it is recommended that those with hepatitis B e antigen negative CHB or CHB with cirrhosis (compensated or decompensated) receive treatment with nucleos(t)ide analogues that have a high genetic barrier to resistance. Despite these recommendations, many treatment naïve CHB patients do not receive appropriate treatment because physicians do not adhere to the treatment guidelines. The authors assessed how well Chinese physicians adhere to the Chinese CHB treatment guidelines, released in 2010.

### Innovations and breakthroughs

The authors show that in China, the majority of Chinese physicians often adhere to Chinese 2010 chronic hepatitis B guidelines and they are well-informed about the use of antiviral medications for hepatitis B.

### Applications

Despite high adherence rates, there was some inconsistency among physicians regarding the use of antiviral treatment for patients older than 40 years, who present with HBV DNA of  $< 1 \times 10^4$  copies/mL, and alanine aminotransferase levels above the upper limit of normal. The guideline recommends that these patients be initiated on antiviral therapy if they have liver fibrosis (as judged by the physician). For these patients, liver biopsy is clearly important when making treatment decisions.

### Terminology

HBV DNA levels, measure in copies/mL indicates the rate of viral replication. Low or undetectable levels (about 300 copies/mL) indicate "inactive infection", whereas higher levels indicate "active infection".

### Peer-review

It is a well-designed study and provides useful information to physicians especially treating patients with hepatitis B about the trends in the treatment of CHB.

## REFERENCES

- 1 Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH. Risk of hepatocellular carcinoma across a biological



- gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65-73 [PMID: 16391218 DOI: 10.1001/jama.295.1.65]
- 2 **Chen G**, Lin W, Shen F, Iloeje UH, London WT, Evans AA. Chronic hepatitis B virus infection and mortality from non-liver causes: results from the Haimen City cohort study. *Int J Epidemiol* 2005; **34**: 132-137 [PMID: 15659459 DOI: 10.1093/ije/dyh339]
  - 3 **Iloeje UH**, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; **130**: 678-686 [PMID: 16530509 DOI: 10.1053/j.gastro.2005.11.016]
  - 4 **Liang X**, Bi S, Yang W, Wang L, Cui G, Cui F, Zhang Y, Liu J, Gong X, Chen Y, Wang F, Zheng H, Wang F, Guo J, Jia Z, Ma J, Wang H, Luo H, Li L, Jin S, Hadler SC, Wang Y. Epidemiological serosurvey of hepatitis B in China--declining HBV prevalence due to hepatitis B vaccination. *Vaccine* 2009; **27**: 6550-6557 [PMID: 19729084 DOI: 10.1016/j.vaccine.2009.08.048]
  - 5 **Liaw YF**, Kao JH, Piratvisuth T, Chan HL, Chien RN, Liu CJ, Gane E, Locarnini S, Lim SG, Han KH, Amarapurkar D, Cooksley G, Jafri W, Mohamed R, Hou JL, Chuang WL, Lesmana LA, Sollano JD, Suh DJ, Omata M. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. *Hepatol Int* 2012; **6**: 531-561 [PMID: 26201469 DOI: 10.1007/s12072-012-9365-4]
  - 6 **European Association for the Study of the Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]
  - 7 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]
  - 8 **World Health Organization**. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection, 2015 [PMID: 26225396]
  - 9 **Chinese Society of Hepatology and Chinese Society of Infectious Diseases, Chinese Medical Association**. Guideline on prevention and treatment of chronic hepatitis B in China (2005). *Chin Med J (Engl)* 2007; **120**: 2159-2173 [PMID: 18167196]
  - 10 **Chinese Society of Hepatology and Chinese Society of Infectious Diseases, Chinese Medical Association**. [The guideline of prevention and treatment for chronic hepatitis B (2010 version)]. *Zhonghua Gan Zang Bing Za Zhi* 2011; **19**: 13-24 [PMID: 21272453 DOI: 10.3760/cma.j.issn.1007-3418.2011.01.007]
  - 11 **Cabana MD**, Rand CS, Powe NR, Wu AW, Wilson MH, Abboud PA, Rubin HR. Why don't physicians follow clinical practice guidelines? A framework for improvement. *JAMA* 1999; **282**: 1458-1465 [PMID: 10535437 DOI: 10.1001/jama.282.15.1458]
  - 12 **Jain MK**, Opio CK, Osuagwu CC, Pillai R, Keiser P, Lee WM. Do HIV care providers appropriately manage hepatitis B in coinfecting patients treated with antiretroviral therapy? *Clin Infect Dis* 2007; **44**: 996-1000 [PMID: 17342656 DOI: 10.1086/512367]
  - 13 **Lu FM**, Li T, Liu S, Zhuang H. Epidemiology and prevention of hepatitis B virus infection in China. *J Viral Hepat* 2010; **17** Suppl 1: 4-9 [PMID: 20586928 DOI: 10.1111/j.1365-2893.2010.01266.x]
  - 14 **Lesmana LA**. Hepatitis B: overview of the burden of disease in the Asia-Pacific region. *Liver Int* 2006; **26**: 3-10 [DOI: 10.1111/j.1478-3231.2006.01370.x]
  - 15 **Ning LH**, Hao J, Liao ZL, Zhou YY, Guo H, Zhao XY. A survey on the current trends in the management of hepatitis B in China. *Eur J Gastroenterol Hepatol* 2012; **24**: 884-889 [PMID: 22569081 DOI: 10.1097/MEG.0b013e32835447fa]
  - 16 **Chao J**, Chang ET, So SK. Hepatitis B and liver cancer knowledge and practices among healthcare and public health professionals in China: a cross-sectional study. *BMC Public Health* 2010; **10**: 98 [PMID: 20184740 DOI: 10.1186/1471-2458-10-98]
  - 17 **Ahn SH**, Chan HL, Chen PJ, Cheng J, Goenka MK, Hou J, Lim SG, Omata M, Piratvisuth T, Xie Q, Yim HJ, Yuen MF. Chronic hepatitis B: whom to treat and for how long? Propositions, challenges, and future directions. *Hepatol Int* 2010; **4**: 386-395 [PMID: 20305758 DOI: 10.1007/s12072-010-9163-9]

**P- Reviewer:** Akbar SMF, Coban M, He JY, Toyoda T

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D



## Transarterial radioembolization vs chemoembolization for hepatocarcinoma patients: A systematic review and meta-analysis

Antonio Facciorusso, Gaetano Serviddio, Nicola Muscatiello

Antonio Facciorusso, Nicola Muscatiello, Gastroenterology Unit, Department of Medical Sciences, University of Foggia, 71100 Foggia, Italy

Gaetano Serviddio, Internal Medicine Unit, University of Foggia, 71100 Foggia, Italy

**Author contributions:** Facciorusso A designed the study, performed the statistical analysis and wrote the paper; Serviddio G and Muscatiello N revised the paper; Muscatiello N collected the data.

**Conflict-of-interest statement:** None of the authors have received fees for serving as a speaker or are consultant/advisory board member for any organizations; None of the authors have received research funding from any organizations; None of the authors are employees of any organizations; None of the authors own stocks and/or share in any organizations; None of the authors own patents.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Antonio Facciorusso, MD, Gastroenterology Unit, Department of Medical Sciences, University of Foggia, Viale L. Pinto, 1, 71100 Foggia, Italy. [antonio.facciorusso@virgilio.it](mailto:antonio.facciorusso@virgilio.it)  
 Telephone: +39-0881-732154  
 Fax: +39-0881-732135

Received: January 30, 2016

Peer-review started: January 30, 2016

First decision: April 15, 2016

Revised: May 4, 2016

Accepted: May 31, 2016

Article in press: June 2, 2016

Published online: June 28, 2016

### Abstract

**AIM:** To compare the efficacy and safety of yttrium-90 radioembolization (Y90RE) and transarterial chemoembolization (TACE) in hepatocellular carcinoma patients.

**METHODS:** Bibliographic research was conducted on main scientific databases. When there was no statistically significant heterogeneity, pooled effects were calculated using a fixed-effects model by means of Mantel-Haenszel test, otherwise, a random-effects model was used with DerSimonian and Laird test. Summary estimates were expressed in terms of odds ratios (ORs) and 95%CI. The probability of publication bias was assessed using funnel plots and with Begg and Mazumdar's test. Sensitivity analysis was finally conducted using the method of excluding extreme data.

**RESULTS:** A total of 10 studies were analyzed, of which 2 randomized controlled trials. Survival rate (SR) assessed at 1 year showed an absolute similarity between the two treatment groups (OR = 1.01, 95%CI: 0.78-1.31,  $P = 0.93$ ). As long as time elapsed since the treatment, ORs for survival rate tended to significantly increase, thus meaning better long-term outcomes in patients who underwent Y90RE (2-year SR: OR = 1.43, 1.08-1.89,  $P = 0.01$ ; 3-year SR: OR = 1.48, 1.03-2.13,  $P = 0.04$ ). Meta-analysis of plotted hazard ratios (HRs) determined a non-significant overall estimate in favor of Y90RE (HR = 0.91, 0.80-1.04,  $P = 0.16$ ). Y90RE showed a statistically significant benefit as compared to TACE in terms of higher progression-free survival rate

assessed at 1 year (OR = 1.67; 95%CI: 1.10-2.55;  $P = 0.02$ ). Pooled analyses do not revealed a statistically significant increase in OR for tumor objective responses after Y90RE with respect to TACE (OR = 1.22, 95%CI: 0.69-2.16,  $P = 0.50$ ). A non-significant trend in favor of Y90RE was observed according to adverse event rate (OR = 0.70, 0.38-1.30,  $P = 0.26$ ).

**CONCLUSION:** Our meta-analysis reveals that Y90RE and TACE show similar effects in terms of survival, response rate and safety profile, although tumor progression is delayed after radioembolization.

**Key words:** Yttrium-90 radioembolization; Transarterial chemoembolization; Hepatocellular carcinoma; Survival; Prognosis; Recurrence

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** A clear evidence in support of the superiority of yttrium-90 radioembolization (Y90RE) over chemoembolization (TACE) in hepatocellular carcinoma patients is still lacking. Results of our meta-analysis reveal that Y90RE and TACE show similar effects in terms of survival, response rate and safety profile, although tumor progression is delayed after radioembolization. Similar results were found as for objective response rate and safety profile. The sole statistical difference was with regard to 1-year progression-free survival, which resulted significantly in favor of Y90RE (OR = 1.67,  $P = 0.02$ ).

Facciorusso A, Serviddio G, Muscatiello N. Transarterial radioembolization vs chemoembolization for hepatocarcinoma patients: A systematic review and meta-analysis. *World J Hepatol* 2016; 8(18): 770-778 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i18/770.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i18.770>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is a global health problem, representing the third most common cause of cancer-related death and the leading cause of mortality among patients with cirrhosis<sup>[1,2]</sup>. Thanks to the recent improvements in surveillance protocols, diagnostic tools and therapeutic armamentaria, nowadays early HCC diagnosis is feasible in 30%-60% of cases in developed countries<sup>[3]</sup>. However, a substantial proportion of patients develop tumoral portal vein thrombosis (PVT) or a multifocal pattern as a result of HCC recurrence or progression, leading to an advanced disease stage not amenable to curative treatments.

Transarterial chemoembolization (TACE) is the most widely used primary treatment for unresectable HCC and the recommended first line-therapy for patients in intermediate stage<sup>[2,4,5]</sup>. The rationale for TACE is that

intra-arterial infusion of a cytotoxic agent followed by embolization of the tumor-feeding blood vessels will result in a strong cytotoxic and ischemic effect<sup>[4,5]</sup>.

A novel technique in the field of loco-regional treatments for HCC is called transarterial radioembolization with yttrium-90 (Y90RE), which induces tumor necrosis by means of injection of glass or resin microsphere loaded with yttrium-90<sup>[6,7]</sup>. Y90RE, which is in fact a novel form of liver-directed brachytherapy, has already demonstrated its efficacy in HCC patients leading to delayed time to progression (TTP) and prolonged overall survival (OS)<sup>[8,9]</sup>. Commonly adopted Y90-loaded microspheres present usually a small size (< 40  $\mu\text{m}$ ), therefore due to their microembolic effect they can be used even in patients with portal vein occlusion. Furthermore, because of absence of flow obstruction, in the case of Y90RE there is no hypoxia-initiated cascade and therefore typical post-TACE sequelae as post-embolization syndrome are less common<sup>[6,7]</sup>.

Although several studies comparing the two loco-regional techniques have been recently published, whether there is a clear superiority of one treatment over the other is still debated.

In this study, we performed a meta-analysis to compare the efficacy of Y90RE and TACE in treating patients with unresectable HCC considering as main endpoints survival rate (SR), progression-free survival (PFS) and adverse events rate. We think that the comparison of these two procedures could help to better define the treatment strategy in intermediate/advanced HCC patients.

## MATERIALS AND METHODS

### Inclusion and exclusion criteria

This meta-analysis only included studies meeting the following criteria: (1) studies comparing Y90RE and TACE in HCC patients; (2) studies published in English; and (3) articles reporting at least one of the following data: TTP, survival and adverse events.

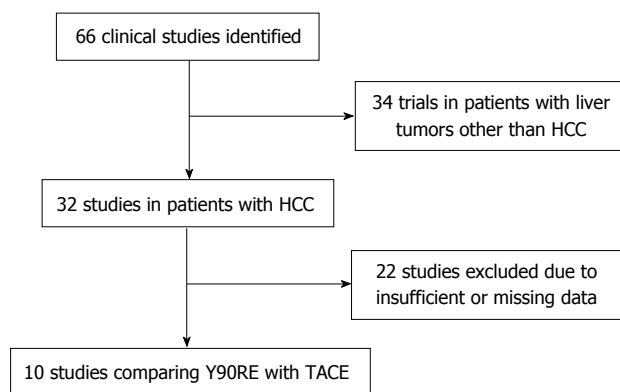
### Search strategy

Figure 1 reports the search strategy followed in the meta-analysis.

Bibliographic research was conducted on PubMed, EMBASE, Cochrane Library and Embase including all studies fulfilling inclusion criteria published until January 2016. Keywords used included "transcatheter arterial chemoembolization", "TACE", "transcatheter arterial radioembolization", "TARE", "liver cancer", "hepatocellular carcinoma" and "HCC". Relevant reviews and meta-analyses of loco-regional treatments in unresectable HCC were examined for potential suitable studies. Authors of included studies were contacted to obtain full text or further information when needed.

### Data extraction and management

Data extraction was conducted by two reviewers (Facciorusso A and Muscatiello N) using a standardized



**Figure 1** Flow chart summarizing study selection. HCC: Hepatocellular carcinoma; TACE: Transarterial chemoembolization; Y90RE: Yttrium-90 radioembolization.

approach (PRISMA Statement)<sup>[10]</sup>. Data on publication details (year of publication, name of first author and country), study characteristics (patients' age and sex, study design, sample size, Child-Pugh stage, interventions, follow-up duration), OS, TTP, and 1-year SR were gathered. Case reports and abstracts or studies with insufficient data were excluded. In case of repetitive publications from the same population, only most recent and complete articles were included.

The quality of the included studies was assessed by two authors independently (Facciorusso A and Muscatiello N) according to the currently accepted criteria described elsewhere<sup>[11,12]</sup>.

Disagreements were resolved by discussion and following a third opinion (Serviddio G).

### Statistical analysis

$\chi^2$  and  $I^2$  tests were used for across studies comparison of the percentage of variability attributable to heterogeneity beyond chance.  $P < 0.10$  for  $\chi^2$  test and  $I^2 < 25\%$  were interpreted as low-level heterogeneity.

When there was no statistically significant heterogeneity, pooled effects were calculated using a fixed-effects model by means of Mantel-Haenszel test; otherwise, a random-effects model was used with DerSimonian and Laird test. Summary estimates were expressed in terms of odds ratios (ORs) and 95%CI.

Probability of publication bias was assessed using funnel plots and with Begg and Mazumdar's test. To explore eventual sources of heterogeneity, we compared summary results obtained from subsets of studies grouped according to their design or quality. Sensitivity analysis was finally conducted using the method of excluding extreme data (the maximum or the minimum).

All statistical analyses were conducted using RevMan version 5 from the Cochrane collaboration. For all calculations a two-tailed  $P$  value of less than 0.05 was considered statistically significant.

## RESULTS

### Selection of studies

After initial screening, 66 potentially relevant articles were identified; 34 were excluded because dealing with non-HCC patients and 22 due to missing or incomplete data. Clinical data of 1557 patients from 10 studies were finally pooled to compare Y90RE and TACE (Figure 1).

### Characteristics of included articles

A total of 10 studies published from 2005 to 2015 were analyzed, which included 461 HCC patients treated with Y90RE and 1096 who underwent TACE<sup>[13-22]</sup> (Table 1).

Among these articles, 8 were retrospective studies<sup>[13-18,20,22]</sup> and 2 were randomized controlled trials (RCTs)<sup>[19,21]</sup>. Overall, 5 studies (of which 1 RCT) were judged high quality<sup>[14,16-19]</sup> and 5 (of which 1 RCT) moderate quality<sup>[13,15,20-22]</sup> (Table 2). In all the studies, the two treatment cohorts were well-balanced in terms of either clinical parameters and tumoral stage (Table 2).

### Survival

Data on overall survival were available for 1481 patients enrolled in 9 studies<sup>[14-22]</sup>, which estimated this outcome by means of Kaplan-Meier curves and compared the two groups using log-rank test. Table 3 describes summary estimates for SR at three consecutive time-points, specifically at 1, 2 and 3 years. SR at 1 year was reported in all the aforementioned nine studies and showed an absolute similarity between the two treatment groups (OR = 1.01, 95%CI: 0.78-1.31,  $P = 0.93$ ). As long as time elapsed since the treatment, ORs for survival rate tended to significantly increase, thus meaning better long-term outcomes in patients who underwent Y90RE (2-year SR: OR = 1.43, 1.08-1.89,  $P = 0.01$ ; 3-year SR: OR = 1.48, 1.03-2.13,  $P = 0.04$ ). Notably, the number of studies reporting long-term outcomes tended to decrease with 7 studies assessing 2-year SR<sup>[15-20,22]</sup> and only 5 reporting 3-year SR<sup>[15,16,18-20]</sup>. No evidence of heterogeneity was found at any time points (Table 3).

In order to obtain a more robust and reliable estimate of patient survival, we performed a meta-analysis of plotted hazard ratios (HRs) from 7 studies which provided data to calculate this parameter<sup>[14-20]</sup>, obtaining as result a non-significant overall estimate in favor of Y90RE (HR = 0.91, 0.80-1.04,  $P = 0.16$ ; Figure 2). There was only a low level of heterogeneity among studies [ $\chi^2 = 7.32$ ,  $df = 6$  ( $P = 0.29$ ),  $I^2 = 18\%$ ] and no publication bias was detected by using funnel plot (Figure 3) and performing Begg and Mazumdar's test ( $P = 0.51$ ). Subgroup analysis retrieving separately results of the only RCT and observational studies did not alter the final findings of our meta-analysis ( $P = 0.77$  and 0.16, respectively). Sensitivity analysis was also performed by restricting analysis to high-quality



**Table 1** Characteristics of the included studies

Ref.	Arm	Sample size	Recruitment period	Study design	Region	CP (A/B/C)	BCLC (A/B/C/D)	Quality
Ahmad <i>et al</i> <sup>[13]</sup>	Y90RE	24	1990-2003	R	United States	NA	NA	M
	TACE	52						
Kooby <i>et al</i> <sup>[14]</sup>	Y90RE	27	1996-2006	R	United States	13/14/0	NA	H
	TACE	44				22/22/0		
Carr <i>et al</i> <sup>[15]</sup>	Y90RE	99	1992-2005	R	United States	NA	NA	M
	TACE	691						
Salem <i>et al</i> <sup>[16]</sup>	Y90RE	123	1999-2008	R	United States	67/54/2	43/65/13/2	H
	TACE	122				67/53/2	47/61/12/2	
Lance <i>et al</i> <sup>[17]</sup>	Y90RE	38	2008-2010	R	United States	31/7/0	NA	H
	TACE	35				24/11/0		
Moreno-Luna <i>et al</i> <sup>[18]</sup>	Y90RE	61	1998-2008	R	United States	53/8/0	12/35/14/0	H
	TACE	55				44/11/0	23/13/19/0	
Pitton <i>et al</i> <sup>[19]</sup>	Y90RE	12	2010-2012	RCT	Germany	10/2/0	0/12/0/0	H
	TACE	12				9/3/0	1/11/0/0	
El Fouly <i>et al</i> <sup>[20]</sup>	Y90RE	44	2009-2011	R	Egypt/Germany	37/7/0	NA	M
	TACE	42				33/9/0		
Kolligs <i>et al</i> <sup>[21]</sup>	Y90RE	13	2009-2012	RCT	Germany/Spain	9/3/1	5/5/3/0	M
	TACE	15				9/4/2	4/8/3/0	
Akinwande <i>et al</i> <sup>[22]</sup>	Y90RE	20	2007-2013	R	United States	7/11/2	0/0/20/0	M
	TACE	28				14/13/1	0/0/28/0	

CP: Child-Pugh; BCLC: Barcelona Clinic Liver Cancer; Y90RE: Yttrium-90 radioembolization; TACE: Transarterial chemoembolization; R: Retrospective; RCT: Randomized controlled trial; NA: Not available; H: High; M: Moderate.

**Table 2** Risk of bias assessment and quality of included studies

	Selection		Comparability		Outcome		Overall quality	
Observational studies <sup>1</sup>								
Ahmad <i>et al</i> <sup>[13]</sup>		++		+		++		5
Kooby <i>et al</i> <sup>[14]</sup>		+++		++		++		7
Carr <i>et al</i> <sup>[15]</sup>		++		++		++		6
Salem <i>et al</i> <sup>[16]</sup>		++++		++		+++		9
Lance <i>et al</i> <sup>[17]</sup>		+++		++		++		7
Moreno-Luna <i>et al</i> <sup>[18]</sup>		++++		++		+++		9
El Fouly <i>et al</i> <sup>[20]</sup>		++		+		+++		6
Akinwande <i>et al</i> <sup>[22]</sup>		++		+		++		5
Randomized controlled trials <sup>2</sup>								
	1	2	3	4	5	6	7	
Pitton <i>et al</i> <sup>[19]</sup>	L	L	L	U	L	L	L	H
Kolligs <i>et al</i> <sup>[21]</sup>	L	H	U	U	H	L	L	M

<sup>1</sup>Study quality assessment performed by means of Newcastle/Ottawa scale (each asterisk represents if the respective criterion within the subsection was satisfied); <sup>2</sup>Cochrane Collaboration's tool for assessing the risk of bias across 7 domains: (1) random sequence generation; (2) allocation concealment; (3) blinding of participants and personnel; (4) blinding of outcome assessment; (5) incomplete outcome data; (6) selective reporting; and (7) other bias. L: Low; H: High; U: Unclear; M: Moderate.

**Table 3** Odds ratios and heterogeneity of 1-year, 2-year and 3-year survival rate

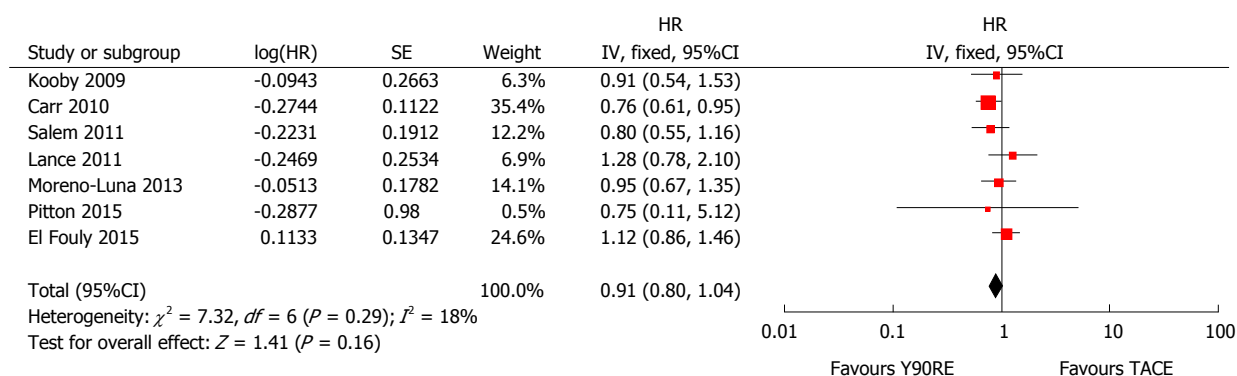
Survival estimate	No. of studies	No. of patients	OR (95%CI)	P-value	Heterogeneity	
					I <sup>2</sup>	P
1-yr SR	9	1481	1.01 (0.78-1.31)	0.93	0%	0.71
2-yr SR	7	1382	1.43 (1.08-1.89)	0.01	0%	0.93
3-yr SR	5	1261	1.48 (1.03-2.13)	0.04	0%	0.44

SR: Survival rate; OR: Odds ratio.

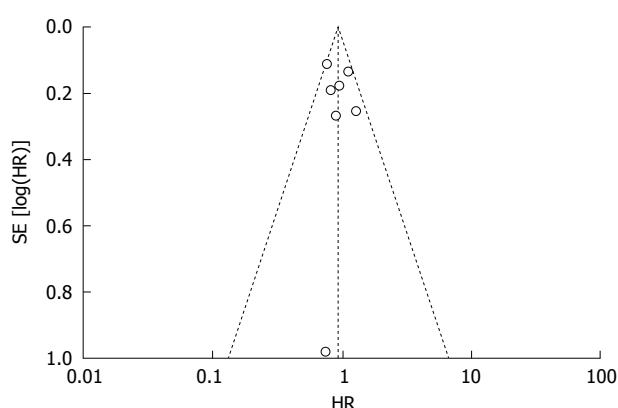
studies and by excluding each article once per time and it showed that the outcome effect was coherent (data not shown).

### PFS

Data on tumor progression was available in 4 studies<sup>[16,19-21]</sup>. Y90RE showed a statistically significant



**Figure 2 Forest plot of hazard ratios for overall survival after yttrium-90 radioembolization and transarterial chemoembolization.** Overall estimate was non-significantly in favor of Y90RE (HR = 0.91, 0.80-1.04,  $P = 0.16$ ). There was only a low level of heterogeneity among studies [ $\chi^2 = 7.32$ ,  $df = 6$  ( $P = 0.29$ ),  $I^2 = 18\%$ ]. Y90RE: Yttrium-90 radioembolization; TACE: Transarterial chemoembolization; HR: Hazard ratio.



**Figure 3 Funnel plot for detection of publication bias with regard to overall survival.** No evidence of publication bias was detected. HR: Hazard ratio.

benefit in terms of higher PFS rate assessed at 1 year (OR = 1.67; 95%CI: 1.10-2.55;  $P = 0.02$ ) (Figure 4). There was no evidence of heterogeneity among individual studies ( $P = 0.66$ ;  $I^2 = 0\%$ ), hence a fixed model was used. Furthermore, there was no publication bias detected using funnel plot and Begg and Mazumdar's test was not significant ( $P = 0.304$  and  $P = 0.412$ , respectively). A low sensitivity to individual studies resulted after performing sensitivity analysis.

### Objective response rate

There were eight studies containing information about objective response rate<sup>[13-16,18,20-22]</sup> enrolling 407 and 1041 patients treated with Y90RE and TACE, respectively. Pooled analyses did not reveal a statistically significant increase in OR for tumor objective responses after Y90RE with respect to TACE (OR = 1.22, 95%CI: 0.69-2.16,  $P = 0.50$ ) (Figure 5). There was, however, evidence of heterogeneity across these studies ( $P = 0.004$ ;  $I^2 = 67\%$ ), therefore we performed a subgroup analysis in order to explore the cause of this heterogeneity, which was mainly due to some outlier studies<sup>[13,15,20,21]</sup>. In fact, tumor response assessment is dependent on a number of variables, such as radiologic criteria adopted, local expertise, imaging technique used (whether computed tomography-scan or magnetic

resonance imaging) and time of response evaluation. Unfortunately, conducting a meta-regression analysis taking into account all these variables was not possible due to the small number of studies. No evidence of publication bias was detected.

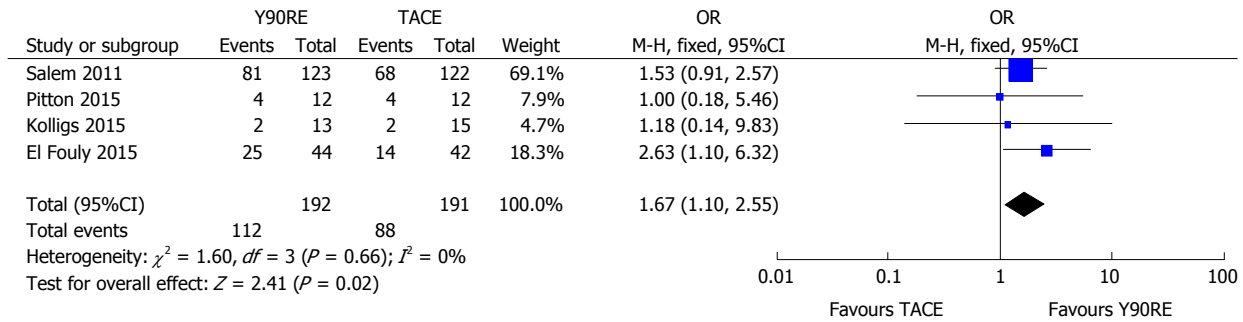
### Toxicity

Seven studies reported toxicity data of their treated patients<sup>[13,14,16-18,21,22]</sup>. A non-significant trend in favor of Y90RE was observed (OR = 0.70, 0.38-1.30,  $P = 0.26$ ), but with high evidence of heterogeneity ( $I^2 = 52\%$ ,  $P = 0.05$ ) (Figure 6). Major responsible of heterogeneity was the study by Salem *et al.*<sup>[16]</sup>, since  $I^2$  dropped to 13% after exclusion of this article. No evidence of publication bias was detected.

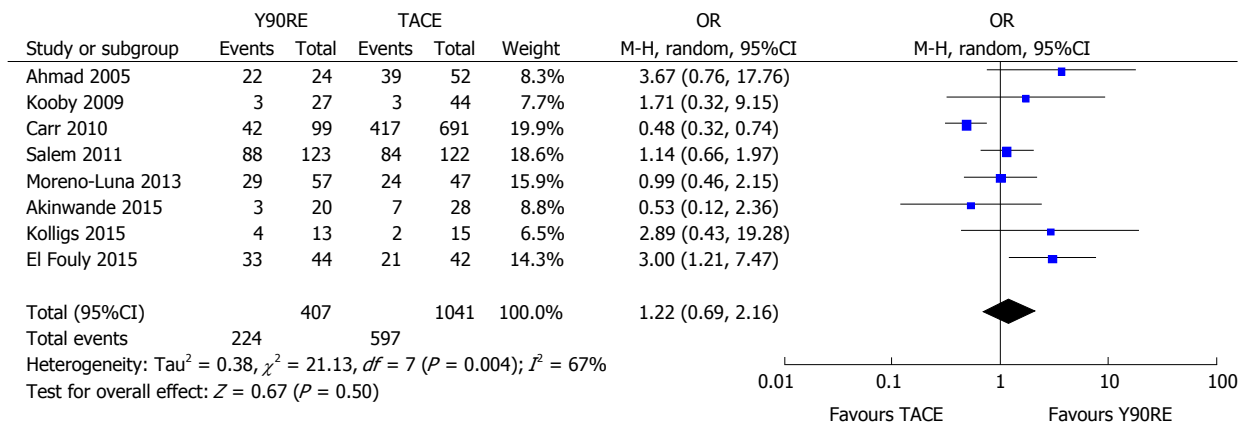
## DISCUSSION

TACE is actually the recommended first-line therapy for patients with unresectable intermediate HCC<sup>[2-4]</sup>. In this setting where curative surgical or ablative treatments are not feasible, palliation with TACE has been found to improve survival as compared to best supportive care<sup>[23,24]</sup>. The pressing need for novel therapeutic regimens able to improve response rates and survival while reducing treatment-related complications led to the development of new drug-delivery systems, such as drug-eluting beads (DEBs)<sup>[4,25]</sup>. Although whether there is a clear superiority of DEB-TACE over conventional TACE using lipiodol is still matter of debate<sup>[12,26,27]</sup>, increasing interest has been recently raised on smaller DEBs which seem able to induce wider necrosis of the target lesion since they achieve a more distal embolization, thus also obstructing collateral channels<sup>[28]</sup>. As a consequence, survival estimates after TACE has considerably improved in the last years, reaching more than 40 mo of median OS in two recent studies<sup>[29,30]</sup>.

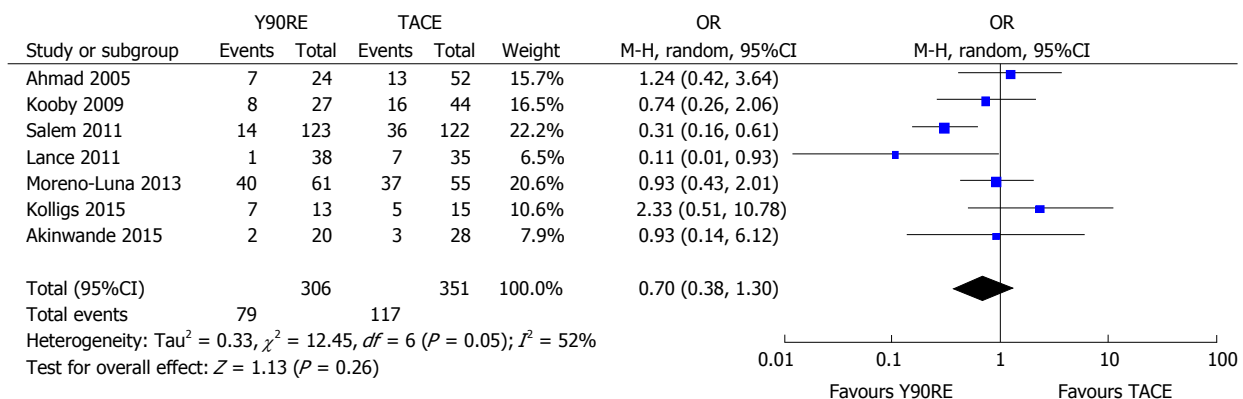
On the other hand, Y90 transarterial radioembolization is a form of brachytherapy in which intra-arterially injected 90Y-loaded microspheres serve as sources for internal radiation purposes, with no significant vessel occlusion thus rendering this treatment



**Figure 4 Forest plot for 1-year progression-free survival after yttrium-90 radioembolization and transarterial chemoembolization.** Y90RE showed a statistically significant benefit in terms of higher progression-free survival rate assessed at 1 year (OR = 1.67, 1.10-2.55,  $P = 0.02$ ). There was no evidence of heterogeneity among individual studies ( $P = 0.66$ ;  $I^2 = 0\%$ ). Y90RE: Yttrium-90 radioembolization; TACE: Transarterial chemoembolization; OR: Odds ratio.



**Figure 5 Forest plot for objective response rate after yttrium-90 radioembolization and transarterial chemoembolization.** Pooled analyses do not revealed a statistically significant increase in odds ratio for tumor objective responses after Y90RE with respect to TACE (OR = 1.22, 95%CI: 0.69-2.16,  $P = 0.50$ ). There was, however, evidence of heterogeneity across these studies ( $P = 0.004$ ;  $I^2 = 67\%$ ), mainly due to some outlier studies. Y90RE: Yttrium-90 radioembolization; TACE: Transarterial chemoembolization; OR: Odds ratio.



**Figure 6 Forest plot for serious adverse event rate after yttrium-90 radioembolization and transarterial chemoembolization.** A non-significant trend in favor of Y90RE was observed (OR = 0.70, 0.38-1.30,  $P = 0.26$ ), but with a high evidence of heterogeneity ( $I^2 = 52\%$ ;  $P = 0.05$ ). Y90RE: Yttrium-90 radioembolization; TACE: Transarterial chemoembolization; OR: Odds ratio.

feasible even in patients with PVT, which is a well-known contraindication to TACE<sup>[6,7]</sup>.

In particular radioembolization produces average disease control rates above 80% and is usually very well tolerated. Main complications do not result from the microembolic effect, even in patients with portal vein occlusion, but rather from excessive irradiation of non-target liver tissue<sup>[6,7,9]</sup>.

Although several studies comparing the two therapies have been published so far, a clear evidence in support of the superiority of one technique over the other in terms of OS is still lacking. Therefore, given the similarity in survival outcomes between the two procedures, post-hoc analyses indicated that a randomized study with > 1000 patients would be required to establish equivalence of survival times between patients treated with Y90RE

and TACE<sup>[16]</sup>.

Aim of our meta-analysis was hence to compare these two trans-arterial treatments in terms of overall survival, progression-free survival, objective response rate and safety profile in unresectable HCC patients.

A total of 10 studies were identified and statistically analyzed, which included 461 HCC patients treated with Y90RE and 1096 treated with chemoembolization. Of note, all the included studies were from the West and in particular 70% from United States. Average quality was moderate-high.

Survival rate assessed at 1 year was similar between the two therapeutic groups (OR = 1.01,  $P = 0.93$ ), but as long as time elapsed since the treatment ORs for survival rate tended to significantly increase, thus meaning better long-term outcomes in patients who underwent Y90RE ( $P = 0.01$  and  $0.04$  at 2 and 3 years, respectively). Notably, the number of studies reporting long-term outcomes tended to decrease from 10 (all the included studies) which reported 1-year SR to only 5 reporting 3-year SR<sup>[15,16,18-20]</sup> (Table 3).

In order to obtain a more robust and reliable estimate of patient survival, we performed a meta-analysis of plotted HRs, obtaining as result a non-significant overall estimate in favor of Y90RE (HR = 0.91, 0.80-1.04,  $P = 0.16$ ; Figure 2).

Therefore, our analysis seem to confirm the non-superiority of one treatment over the other as found in previous papers<sup>[16,18]</sup>.

Unfortunately, subgroup analysis performed on the basis of baseline tumor stage or other clinical prognostic factors known to influence HCC patients' survival, such as ferritin level<sup>[31]</sup> or drug therapy used<sup>[32]</sup>, was not feasible due to the low number of available studies and the absence of outcomes stratification in most of them.

In the above cited study by Salem *et al.*<sup>[16]</sup>, time to progression was significantly longer after Y90RE (13.3 mo vs 8.4 mo,  $P = 0.023$ ), but it did not translate directly into improved survival. Such a finding was confirmed in our meta-analysis where OR for 1-year PFS resulted significantly in favor of Y90RE (OR = 1.67,  $P = 0.02$ ) (Figure 4).

No significant difference according to the other two analyzed outcomes (response rate and major adverse event rate) was found.

The apparent discrepancy between the significant benefit of Y90RE in terms of progression-free survival and the non-significant trends found with regard to the other outcomes are likely due to the complex multistep pathogenesis of HCC and the different course of underlying liver cirrhosis<sup>[33-35]</sup>. In this regard, a post-progression survival analysis would be interesting and represents in our opinion one of the most important targets of incoming clinical research in hepato-oncology<sup>[36,37]</sup>.

There are some limitations to our study. First, we included both RCTs and observational studies, thus resulting in greater heterogeneity (as seen with regard to response rate analysis) and higher risk of

selection and reporting bias. This, in addition to the aforementioned lack of data stratification and subgroup analysis, calls for a carefully interpretation of our findings. Moreover, technical details varied widely throughout the included studies either in TACE cohorts (for instance some studies adopted conventional TACE whereas others DEB-TACE) and in Y90RE arms (for instance as for dosimetry protocol)<sup>[38]</sup>.

In conclusion, our meta-analysis reveals that Y90RE and TACE show similar effects in unresectable HCC patients in terms of OS, response rate and safety profile, although tumor progression is delayed after radioembolization. Further properly sized RCTs are warranted in order to confirm these results.

## COMMENTS

### Background

Transarterial chemoembolization (TACE) is the most widely used primary treatment for unresectable hepatocellular carcinoma (HCC) and the recommended first line-therapy for patients in intermediate stage. A novel technique in the field of loco-regional treatments for HCC is called transarterial radioembolization with yttrium-90 (Y90RE), which induces tumor necrosis by means of injection of glass or resin microsphere loaded with yttrium-90. Although several studies comparing the two loco-regional techniques have been recently published, whether there is a clear superiority of one treatment over the other is still debated.

### Research frontiers

This meta-analysis reveals that Y90RE and TACE show similar effects in unresectable HCC patients in terms of overall survival, response rate and safety profile, although tumor progression is delayed after radioembolization. Further properly sized randomized controlled trials are warranted in order to confirm these results.

### Innovations and breakthroughs

The authors' findings stand for a similarity in treatment effects between Y90RE and TACE in HCC patients. Their meta-analysis constitutes the most up-to-date overview of studies comparing the two techniques.

### Applications

The present report allows understanding the role of two transarterial treatments in HCC patients.

### Terminology

TACE: Transarterial treatment whose rationale is that the intra-arterial infusion of a cytotoxic agent followed by embolization of the tumor-feeding blood vessels will result in a strong cytotoxic and ischemic effect; Y90RE: Novel form of liver-directed brachytherapy which induces tumor necrosis by means of injection of glass or resin microsphere loaded with yttrium-90.

### Peer-review

This meta-analysis aims to compare the efficacy and safety of Y90RE and TACE in HCC. It is a nicely written manuscript. The analysis is well performed.

## REFERENCES

- 1 **El-Serag HB.** Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]
- 2 **European Association For The Study Of The Liver;** European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]



- 3 **Llovet JM**, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. *J Hepatol* 2008; **48** Suppl 1: S20-S37 [PMID: 18304676 DOI: 10.1016/j.jhep.2008.01.022]
- 4 **Facciorusso A**, Licinio R, Muscatiello N, Di Leo A, Barone M. Transarterial chemoembolization: Evidences from the literature and applications in hepatocellular carcinoma patients. *World J Hepatol* 2015; **7**: 2009-2019 [PMID: 26261690 DOI: 10.4254/wjh.v7.i16.2009]
- 5 **Bolondi L**, Burroughs A, Dufour JF, Galle PR, Mazzaferro V, Piscaglia F, Raoul JL, Sangro B. Heterogeneity of patients with intermediate (BCLC B) Hepatocellular Carcinoma: proposal for a subclassification to facilitate treatment decisions. *Semin Liver Dis* 2012; **32**: 348-359 [PMID: 23397536 DOI: 10.1055/s-0032-1329906]
- 6 **Salem R**, Mazzaferro V, Sangro B. Yttrium 90 radioembolization for the treatment of hepatocellular carcinoma: biological lessons, current challenges, and clinical perspectives. *Hepatology* 2013; **58**: 2188-2197 [PMID: 23512791 DOI: 10.1002/hep.26382]
- 7 **Sangro B**, Iñarrairaegui M, Bilbao JI. Radioembolization for hepatocellular carcinoma. *J Hepatol* 2012; **56**: 464-473 [PMID: 21816126 DOI: 10.1016/j.jhep.2011.07.012]
- 8 **Salem R**, Lewandowski RJ, Mulcahy MF, Riaz A, Ryu RK, Ibrahim S, Atassi B, Baker T, Gates V, Miller FH, Sato KT, Wang E, Gupta R, Benson AB, Newman SB, Omary RA, Abecassis M, Kulik L. Radioembolization for hepatocellular carcinoma using Yttrium-90 microspheres: a comprehensive report of long-term outcomes. *Gastroenterology* 2010; **138**: 52-64 [PMID: 19766639 DOI: 10.1053/j.gastro.2009.09.006]
- 9 **Mazzaferro V**, Sposito C, Bhoori S, Romito R, Chiesa C, Morosi C, Maccauro M, Marchianò A, Bongini M, Lanocita R, Civelli E, Bombardieri E, Camerini T, Spreafico C. Yttrium-90 radioembolization for intermediate-advanced hepatocellular carcinoma: a phase 2 study. *Hepatology* 2013; **57**: 1826-1837 [PMID: 22911442 DOI: 10.1002/hep.26014]
- 10 **Mohr D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* 2010; **8**: 336-341 [PMID: 20171303 DOI: 10.1016/j.ijsu.2010.02.007]
- 11 **Facciorusso A**, Di Maso M, Muscatiello N. Microwave ablation versus radiofrequency ablation for the treatment of hepatocellular carcinoma: A systematic review and meta-analysis. *Int J Hypertension* 2016; **32**: 339-344 [PMID: 26794414]
- 12 **Facciorusso A**, Di Maso M, Muscatiello N. Drug-eluting beads versus conventional chemoembolization for the treatment of unresectable hepatocellular carcinoma: A meta-analysis. *Dig Liver Dis* 2016; **48**: 571-577 [PMID: 26965785 DOI: 10.1016/j.dld.2016.02.005]
- 13 **Ahmad J**, Rhee J, Carr BI. The effects of hepatic artery chemotherapy on viral hepatitis in patients with hepatocellular carcinoma. *Dig Dis Sci* 2005; **50**: 331-335 [PMID: 15745096 DOI: 10.1007/s10620-005-1606-0]
- 14 **Kooby DA**, Egnatashvili V, Srinivasan S, Chamsuddin A, Delman KA, Kauh J, Staley CA, Kim HS. Comparison of yttrium-90 radioembolization and transcatheter arterial chemoembolization for the treatment of unresectable hepatocellular carcinoma. *J Vasc Interv Radiol* 2010; **21**: 224-230 [PMID: 20022765 DOI: 10.1016/j.jvir.2009.10.013]
- 15 **Carr BI**, Kondragunta V, Buch SC, Branch RA. Therapeutic equivalence in survival for hepatic arterial chemoembolization and yttrium 90 microsphere treatments in unresectable hepatocellular carcinoma: a two-cohort study. *Cancer* 2010; **116**: 1305-1314 [PMID: 20066715 DOI: 10.1002/cncr.24884]
- 16 **Salem R**, Lewandowski RJ, Kulik L, Wang E, Riaz A, Ryu RK, Sato KT, Gupta R, Nikolaidis P, Miller FH, Yaghami V, Ibrahim SM, Senthilnathan S, Baker T, Gates VL, Atassi B, Newman S, Memon K, Chen R, Vogelzang RL, Nemcek AA, Resnick SA, Chrisman HB, Carr J, Omary RA, Abecassis M, Benson AB, Mulcahy MF. Radioembolization results in longer time-to-progression and reduced toxicity compared with chemoembolization in patients with hepatocellular carcinoma. *Gastroenterology* 2011; **140**: 497-507.e2 [PMID: 21044630 DOI: 10.1053/j.gastro.2010.10.049]
- 17 **Lance C**, McLennan G, Obuchowski N, Cheah G, Levitin A, Sands M, Spain J, Srinivas S, Shrikanthan S, Aucejo FN, Kim R, Menon KV. Comparative analysis of the safety and efficacy of transcatheter arterial chemoembolization and yttrium-90 radioembolization in patients with unresectable hepatocellular carcinoma. *J Vasc Interv Radiol* 2011; **22**: 1697-1705 [PMID: 21983055 DOI: 10.1016/j.jvir.2011.08.013]
- 18 **Moreno-Luna LE**, Yang JD, Sanchez W, Paz-Fumagalli R, Harnois DM, Mettler TA, Gansen DN, de Groen PC, Lazaridis KN, Narayanan Menon KV, Larusso NF, Alberts SR, Gores GJ, Fleming CJ, Slettedahl SW, Harmsen WS, Thorneau TM, Wiseman GA, Andrews JC, Roberts LR. Efficacy and safety of transarterial radioembolization versus chemoembolization in patients with hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 2013; **36**: 714-723 [PMID: 23093355 DOI: 10.1007/s00270-012-0481-2]
- 19 **Pitton MB**, Kloeckner R, Ruckes C, Wirth GM, Eichhorn W, Wörns MA, Weinmann A, Schreckenberger M, Galle PR, Otto G, Duebner C. Randomized comparison of selective internal radiotherapy (SIRT) versus drug-eluting bead transarterial chemoembolization (DEB-TACE) for the treatment of hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 2015; **38**: 352-360 [PMID: 25373796 DOI: 10.1007/s00270-014-1012-0]
- 20 **El Fouly A**, Ertle J, El Dorry A, Shaker MK, Dechène A, Abdella H, Mueller S, Barakat E, Lauenstein T, Bockisch A, Gerken G, Schlaak JF. In intermediate stage hepatocellular carcinoma: radioembolization with yttrium 90 or chemoembolization? *Liver Int* 2015; **35**: 627-635 [PMID: 25040497 DOI: 10.1111/liv.12637]
- 21 **Kolligs FT**, Bilbao JI, Jakobs T, Iñarrairaegui M, Nagel JM, Rodriguez M, Haug A, D'Avola D, op den Winkel M, Martinez-Cuesta A, Trumm C, Benito A, Tatsch K, Zech CJ, Hoffmann RT, Sangro B. Pilot randomized trial of selective internal radiation therapy vs. chemoembolization in unresectable hepatocellular carcinoma. *Liver Int* 2015; **35**: 1715-1721 [PMID: 25443863 DOI: 10.1111/liv.12750]
- 22 **Akinwande O**, Kim D, Edwards J, Brown R, Philips P, Scoggins C, Martin RC. Is radioembolization ((90)Y) better than doxorubicin drug eluting beads (DEBDOX) for hepatocellular carcinoma with portal vein thrombosis? A retrospective analysis. *Surg Oncol* 2015; **24**: 270-275 [PMID: 26133576 DOI: 10.1016/j.sur-onc.2015.06.008]
- 23 **Llovet JM**, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J; Barcelona Liver Cancer Group. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862]
- 24 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794]
- 25 **Varela M**, Real MI, Burrell M, Forner A, Sala M, Brunet M, Ayuso C, Castells L, Montaña X, Llovet JM, Bruix J. Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. *J Hepatol* 2007; **46**: 474-481 [PMID: 17239480]
- 26 **Lammer J**, Malagari K, Vogl T, Pilleul F, Denys A, Watkinson A, Pitton M, Sergent G, Pfammatter T, Terraz S, Benhamou Y, Avajon Y, Gruenberger T, Pomoni M, Langenberger H, Schuchmann M, Dumortier J, Mueller C, Chevallier P, Lencioni R. Prospective randomized study of doxorubicin-eluting-bead embolization in the treatment of hepatocellular carcinoma: results of the PRECISION V study. *Cardiovasc Intervent Radiol* 2010; **33**: 41-52 [PMID: 19908093 DOI: 10.1007/s00270-009-9711-7]
- 27 **Facciorusso A**, Mariani L, Sposito C, Spreafico C, Bongini M, Morosi C, Cascella T, Marchianò A, Camerini T, Bhoori S, Brunero F, Barone M, Mazzaferro V. Drug-eluting beads versus conventional chemoembolization for the treatment of unresectable hepatocellular carcinoma. *J Gastroenterol Hepatol* 2016; **31**: 645-653 [PMID: 26331807 DOI: 10.1111/jgh.13147]
- 28 **Spreafico C**, Cascella T, Facciorusso A, Sposito C, Rodolfo

- L, Morosi C, Civelli EM, Vaiani M, Bhoori S, Pellegrinelli A, Marchianò A, Mazzaferro V. Transarterial chemoembolization for hepatocellular carcinoma with a new generation of beads: clinical-radiological outcomes and safety profile. *Cardiovasc Intervent Radiol* 2015; **38**: 129-134 [PMID: 24870698 DOI: 10.1007/s00270-014-0907-0]
- 29 **Burrel M**, Reig M, Forner A, Barrufet M, de Lope CR, Tremosini S, Ayuso C, Llovet JM, Real MI, Bruix J. Survival of patients with hepatocellular carcinoma treated by transarterial chemoembolisation (TACE) using Drug Eluting Beads. Implications for clinical practice and trial design. *J Hepatol* 2012; **56**: 1330-1335 [PMID: 22314428 DOI: 10.1016/j.jhep.2012.01.008]
- 30 **Malagari K**, Pomoni M, Moschouris H, Bouma E, Koskinas J, Stefanidou A, Marinis A, Kelekis A, Alexopoulou E, Chatziioannou A, Chatzimichael K, Dourakis S, Kelekis N, Rizos S, Kelekis D. Chemoembolization with doxorubicin-eluting beads for unresectable hepatocellular carcinoma: five-year survival analysis. *Cardiovasc Intervent Radiol* 2012; **35**: 1119-1128 [PMID: 22614031]
- 31 **Facciorusso A**, Del Prete V, Antonino M, Neve V, Crucinio N, Di Leo A, Carr BI, Barone M. Serum ferritin as a new prognostic factor in hepatocellular carcinoma patients treated with radiofrequency ablation. *J Gastroenterol Hepatol* 2014; **29**: 1905-1910 [PMID: 24731153 DOI: 10.1111/jgh.12618]
- 32 **Facciorusso A**, Del Prete V, Crucinio N, Muscatiello N, Carr BI, Di Leo A, Barone M. Angiotensin receptor blockers improve survival outcomes after radiofrequency ablation in hepatocellular carcinoma patients. *J Gastroenterol Hepatol* 2015; **30**: 1643-1650 [PMID: 25974743 DOI: 10.1111/jgh.12988]
- 33 **Facciorusso A**. The influence of diabetes in the pathogenesis and the clinical course of hepatocellular carcinoma: recent findings and new perspectives. *Curr Diabetes Rev* 2013; **9**: 382-386 [PMID: 23845075]
- 34 **Facciorusso A**, Antonino M, Del Prete V, Neve V, Scavo MP, Barone M. Are hematopoietic stem cells involved in hepatocarcinogenesis? *Hepatobiliary Surg Nutr* 2014; **3**: 199-206 [PMID: 25202697 DOI: 10.3978/j.issn.2304-3881.2014.06.02]
- 35 **Facciorusso A**, Licinio R, Carr BI, Di Leo A, Barone M. MEK 1/2 inhibitors in the treatment of hepatocellular carcinoma. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 993-1003 [PMID: 25915713 DOI: 10.1586/17474124.2015.1040763]
- 36 **Reig M**, Rimola J, Torres F, Darnell A, Rodriguez-Lope C, Forner A, Llach N, Ríos J, Ayuso C, Bruix J. Postprogression survival of patients with advanced hepatocellular carcinoma: rationale for second-line trial design. *Hepatology* 2013; **58**: 2023-2031 [PMID: 23787822 DOI: 10.1002/hep.26586]
- 37 **Facciorusso A**, Del Prete V, Antonino M, Crucinio N, Neve V, Di Leo A, Carr BI, Barone M. Post-recurrence survival in hepatocellular carcinoma after percutaneous radiofrequency ablation. *Dig Liver Dis* 2014; **46**: 1014-1019 [PMID: 25085684 DOI: 10.1016/j.dld.2014.07.012]
- 38 **Chiesa C**, Mira M, Maccauro M, Spreafico C, Romito R, Morosi C, Camerini T, Carrara M, Pellizzari S, Negri A, Aliberti G, Sposito C, Bhoori S, Facciorusso A, Civelli E, Lanocita R, Padovano B, Migliorisi M, De Nile MC, Seregni E, Marchianò A, Crippa F, Mazzaferro V. Radioembolization of hepatocarcinoma with (90)Y glass microspheres: development of an individualized treatment planning strategy based on dosimetry and radiobiology. *Eur J Nucl Med Mol Imaging* 2015; **42**: 1718-1738 [PMID: 26112387 DOI: 10.1007/s00259-015-3068-8]

**P-Reviewer:** Al-Gayyar MMH, Eskens FALM, Wong GLH

**S-Editor:** Gong XM **L-Editor:** A **E-Editor:** Liu SQ



## Atypical presentation of a hepatic artery pseudoaneurysm: A case report and review of the literature

Casey M Luckhurst, Chelsey Perez, Amy L Collinsworth, Jose G Trevino

Casey M Luckhurst, Chelsey Perez, Jose G Trevino, Department of Surgery, Colleges of Medicine, University of Florida Health Science Center, Gainesville, FL 32610, United States

Amy L Collinsworth, Department of Pathology, Colleges of Medicine, University of Florida Health Science Center, Gainesville, FL 32610, United States

**Author contributions:** All authors have read, approved the final manuscript and agree to be accountable for all aspects of the manuscript ensuring the accuracy and integrity of this manuscript; Luckhurst CM and Perez C participated in the acquisition of data, design and drafting of the manuscript; Collinsworth AL participated in the acquisition and interpretation of data; Trevino JG participated in the conception and design of this manuscript and give final approval of this version of the manuscript to be published.

**Institutional review board statement:** This manuscript was designed and drafted with approval from University of Florida Institutional Review Board (UF IRB).

**Informed consent statement:** Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

**Conflict-of-interest statement:** All the authors declare they have no competing interests.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Jose G Trevino, MD, Assistant Professor, Department of Surgery, Colleges of Medicine, University of Florida Health Science Center, Room R6116, Shands Hospital,

1600 SW Archer Rd, Gainesville, FL 32610, United States. [jose.trevino@surgery.ufl.edu](mailto:jose.trevino@surgery.ufl.edu)  
 Telephone: +1-352-2737967  
 Fax: +1-352-2650889

Received: March 30, 2016  
 Peer-review started: March 31, 2016  
 First decision: May 17, 2016  
 Revised: May 27, 2016  
 Accepted: June 1, 2016  
 Article in press: June 3, 2016  
 Published online: June 28, 2016

### Abstract

Classically, hepatic artery pseudoaneurysms (HAPs) arise secondary to trauma or iatrogenic causes. With an increasing prevalence of laparoscopic procedures of the hepatobiliary system the risk of inadvertent injury to arterial vessels is increased. Pseudoaneurysm formation post injury can lead to serious consequences of rupture and subsequent hemorrhage, therefore intervention in all identified visceral pseudoaneurysms has been advocated. A variety of interventional methods have been proposed, with surgical management becoming the last step intervention when minimally invasive therapies have failed. The authors present a case of a HAP in a 56-year-old female presenting with jaundice and pruritis suggestive of a Klatskin's tumor. This presentation of HAP in a patient without any significant past medical or surgical intervention is atypical when considering that the majority of HAP cases present secondary to iatrogenic causes or trauma. Multiple minimally invasive approaches were employed in an attempt to alleviate the symptomology which included jaundice and associated inflammatory changes. Ultimately, a right hepatic trisegmentectomy was required to adequately relieve the mass effect on biliary outflow obstruction and definitively address the HAP. The presentation of a HAP masquerading as a malignancy with jaundice and pruritis, rather than the classic symptoms of abdominal

pain, anemia, and melena, is unique. This presentation is only further complicated by the absent history of either trauma or instrumentation. It is important to be aware of HAPs as a potential cause of jaundice in addition to the more commonly thought of etiologies. Furthermore, given the morbidity and mortality associated with pseudoaneurysm rupture, intervention in identifiable cases, either by minimally invasive or surgical interventions, is recommended.

**Key words:** Klatskin tumor; Cholangitis; Hepatic artery pseudoaneurysm; Biliary obstruction; Trisegmentectomy

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatic artery pseudoaneurysms (HAPs) typically arise from secondary trauma or iatrogenic causes. Most of HAPs are asymptomatic but can be complicated with rupture and bleeding. Biliary obstruction due to HAPs is a rare phenomenon and can present clinically as Quinke's triad (hematobilia, abdominal pain, and jaundice). Most cases can be managed with non-operative vascular and endoscopic interventions. This case report presents an atypical presentation of HAP with a multidisciplinary approach to a complex problem.

Luckhurst CM, Perez C, Collinsworth AL, Trevino JG. Atypical presentation of a hepatic artery pseudoaneurysm: A case report and review of the literature. *World J Hepatol* 2016; 8(18): 779-784 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i18/779.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i18.779>

## INTRODUCTION

Hepatic artery pseudoaneurysms (HAPs), or false aneurysms, classically arise secondary to trauma or iatrogenic causes and pose a serious risk of exsanguinating hemorrhage and subsequent death<sup>[1,2]</sup>. Numerous case reports have been published addressing the occurrence of HAPs subsequent to a variety of interventional procedures including cholecystectomy, pancreaticoduodenectomy, and orthotopic liver transplant. It has been hypothesized that with the increasing frequency of laparoscopic procedures, such as cholecystectomy, which can result in inadvertent injury to the right hepatic artery, the overall incidence of HAPs will continue to rise<sup>[1,3]</sup>. Due to the increased risk of rupture compared with true aneurysms, some have advocated intervention in all cases of identifiable visceral pseudoaneurysm<sup>[4]</sup>. It has been reported that HAPs have been successfully treated using a variety of interventional methods, including endovascular embolization, coiling embolization, and arterial stent grafting<sup>[1,2]</sup>. Because of the increased morbidity associated with surgery in this setting, surgical management is the final treatment option when these subsequent methods have failed. Although in most cases interventional technologies can quench the risk of hemorrhagic rupture of HAP and

alleviate biliary obstruction<sup>[5]</sup>, surgical management of impairment to adjacent structures such as the bile duct and portal vein have to our knowledge ever been reported.

Here we provide a unique case of HAP, initially presenting as a Klatskin tumor (tumor at the confluence of the right and left hepatic biliary confluence) mimic and with no identifiable cause. Furthermore, the aforementioned treatment options were inadequate to address this persistent and recurrent chronic HAP, ultimately requiring surgical intervention.

## CASE REPORT

A 65-year-old female with no significant past medical history was transferred from an outside institution for further management of painless jaundice and pruritis, with suspicion for a Klatskin's tumor. At this outside institution, non-contrast computed tomography (CT) imaging studies revealed a 5 cm dense lesion within the hilar region of the liver and intrahepatic biliary duct dilatation. Subsequent endoscopic retrograde cholangiopancreatography showed a hilar obstructive lesion that was concerning for cholangiocarcinoma/Klatskin's tumor. A temporary hepatic duct stent was placed and sphincterotomy was performed. Cytology results from this procedure were negative. On the day of her transfer, she was afebrile but was placed on empiric antibiotic coverage. Her total and direct bilirubin levels were elevated (total bilirubin 24.3 mg/dL, direct bilirubin 18.6 mg/dL), as was her serum alkaline phosphatase (432 U/L), liver transaminases (AST 132 U/L, ALT 148 U/L), and CA 19-9 (891 U/mL). Other pertinent labs included a positive ANA and CEA within the reference range.

At transfer to our institution, a triphasic computed tomography angiography demonstrated a right branch HAP measuring 2.1 cm, a suspected hematoma measuring 5 cm in diameter (Figure 1A and B) and significant biliary duct dilatation (Figure 1C). Further studies including esophagogastroduodenoscopy and colonoscopy demonstrated hemobilia with the 2 cm biliary stent protruding into the lumen of the small bowel. Therapeutic strategies to control pseudoaneurysmal bleeding included embolization procedures encompassing percutaneous ultrasound and fluoroscopic guided thrombin injections into the HAP (Figure 2). Multiple sub-centimeter smaller pseudoaneurysms were noted to be extending off of the right hepatic artery. Due to lack of trauma or interventions that could be a likely etiology for pseudoaneurysm, an autoimmune work up, including C-ANCA, P-ANCA, anti-smooth muscle antibodies, double-stranded DNA antibody, anti-smooth muscle antibody, anti-RNP antibody, to establish etiology of these multiple pseudoaneurysms was performed and was largely negative. Rheumatology was consulted and ruled out systemic vasculitides including polyarteritis nodosa (PAN), systemic lupus erythematosus, and cryoglobulinemia. The patient continued to have a complicated long hospital course and even after attempts to alleviate





**Figure 1** Computerized tomography scan demonstrating pseudoaneurysm and significant biliary ductal dilatation. A: Triple contrast computerized tomography scan of abdomen demonstrates hepatic artery pseudoaneurysm; B: With significant thrombus formation adjacent to aneurysm; C: Significant biliary ductal dilatation in the right and left hepatic ducts.

obstructive jaundice with stent procedures, the patient had recurrent episodes of cholangitis with septic shock. Blood cultures demonstrated pan-sensitive *Klebsiella pneumoniae* likely from biliary source.

Non-operative strategies included fluoroscopic and ultrasound-guided embolization of re-emergent right HAP with multiple coils, gelfoam, and thrombin. During this procedure it was noted that a portion of thrombosed aneurysm was exerting significant mass effect, effectively occluding multiple right biliary branches and compressing vascular flow in her right portal vein thereby potentially rendering her right lobe ischemic. It was determined that surgical intervention was required for inability to completely address the obstructing nature of this pseudoaneurysm. The patient underwent right hepatic trisegmentectomy with Roux-en-Y hepaticojejunostomy.

Pathology demonstrated the presence of a porta hepatis hematoma (5.0 cm) and organized thrombus dissecting into the hepatic artery wall (Figure 3). Although the liver specimen was negative for malignancy, the liver was microscopically consistent with centrilobular cholestasis (Figure 4A) and bile ductular reaction with bile plugs (Figure 4B) all of which were consistent with chronic biliary obstruction.

The patient was discharged home without any events. She is three years from surgery with no complaints, doing well.

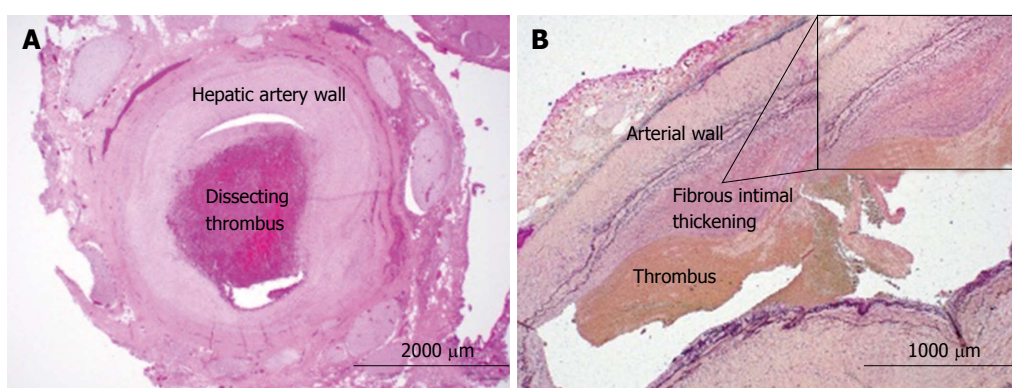
## DISCUSSION

While visceral artery aneurysms and pseudoaneurysms (VAP) may be a rare finding in the general population, with a reported incidence of approximately 0.1%-2%, timely diagnosis is imperative because of the risk of rupture and subsequent mortality<sup>[6]</sup>. In their retrospective review, Tulsyan *et al*<sup>[7]</sup> reported that of the 28 patients found to have VAPs between 1997 and 2005 at the Cleveland Clinic Foundation, 39% involved the celiac axis or its branches, 39% arose from the hepatic arteries, 18% from the splenic artery and 4% from the superior mesenteric artery. In this review, the majority of VAPs, including HAPs arose secondary to arterial trauma, intrabdominal or retroperitoneal inflammation or malignancy, and manipulation of the biliary tract<sup>[7]</sup>. Other non-iatrogenic causes of HAPs include trauma, acute and chronic pancreatitis, arteriosclerosis, PAN, necrotizing vasculitis, infection and hepatocellular carcinoma<sup>[2,3,8-10]</sup>.

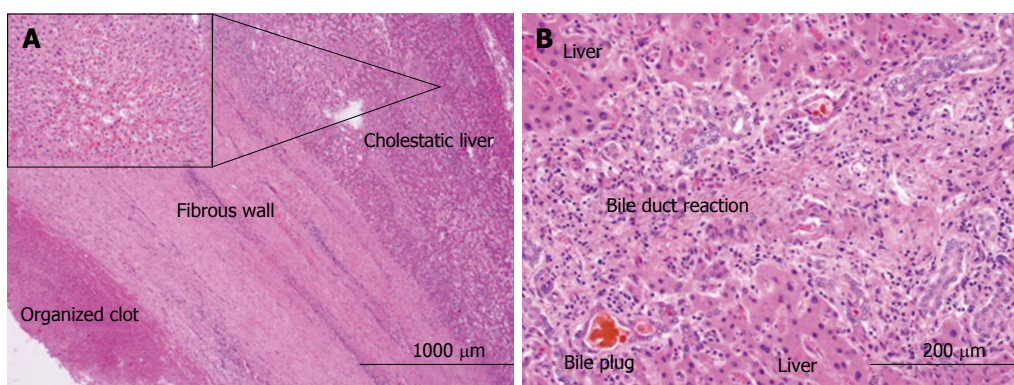
Recent increases in the incidence of HAPs have been attributed to a rise in the number of liver transplantations, percutaneous liver and gallbladder interventions and the use of laparoscopic surgery<sup>[1,4,9,11]</sup>. Advances in imaging techniques have enhanced the detection rate of asymptomatic HAPs<sup>[1]</sup>. While HAPs may be an incidental finding in an asymptomatic patient, more commonly patients present with abdominal pain, anemia, hemobilia, melena, and can present as life-threatening hemorrhage following rupture<sup>[2,8,11]</sup>. Although we advocate interventional attempts such as angioembolization or stenting to stop hemorrhage immediately, failures in these attempts or chronic sequel on adjacent structures such as the biliary system and portal vein can require further investigations with possible surgical intervention. We advocate the use of non-invasive assessments, such as CT and/or MRI-MRCP radiographic imaging, to determine the possible etiology of biliary obstruction with then focused therapies toward alleviating the problem. To our knowledge, this is the first presentation of a patient presenting with classic picture of a Klatskin's tumor, specifically jaundice and pruritis secondary to biliary system compression by HAP that could not be managed with a non-operative approach. This unique presentation and atypical history for HAP initially masked the diagnosis. Despite a thorough diagnostic workup, no identifiable cause was uncovered for this patient's HAP,



**Figure 2** Interventional attempts to embolize the hepatic artery pseudoaneurysm. A: Angiogram of common hepatic artery demonstrates pseudoaneurysmal formation off right hepatic artery; B and C: Interventional fluoroscopic and ultrasound-guided embolization of right hepatic artery pseudoaneurysm with multiple coils, gelfoam, and thrombin.



**Figure 3** Histochemical analyses of resected hepatic artery. A: H and E of hepatic artery with organized thrombus dissecting into the vessel wall, 40 × magnification; B: Verhoeff-Van Gieson (VVG) staining of the hepatic artery with eccentric fibrous intimal thickening with adherent thrombus, 20 × magnification (B, inset). Internal elastic lamina is highlighted by the VVG stain with notable fibrous intimal thickening, 100 × magnifications.



**Figure 4** Histologic analyses of hepatic artery pseudoaneurysm and adjacent liver. A: H and E of fibrous capsule of the contained pseudoaneurysmal rupture adjacent to liver, 20 × magnification (A, inset). Liver with centrilobular cholestasis, 200 × magnification; B: Liver with bile duct reaction and bile plugs consistent with biliary obstruction, 200 × magnification.

which further adds to the complexity and atypical nature of the case.

While HAPs can thrombose and resolve without intervention, the risk of rupture and subsequent hemorrhage are high enough that the general consensus has been in favor of intervention<sup>[1,2,4]</sup>. At this time, numerous minimally invasive techniques have proven to be successful, including coiling, covered stent exclusion, thrombin injection,

gelfoam injection, plug deployment, polyvinyl alcohol injection, and surgical intervention<sup>[1,3,4]</sup>. The literature has supported all of these techniques as viable options for management of HAPs. Nagaraja *et al*<sup>[12]</sup> retrospectively analyzed 29 patients with HAP were successfully managed with angioembolization not surprisingly resulting in more rapid bleeding control, shorter hospital stay, and lower transfusion requirements although a 14%



mortality rate in the angioembolization group with no mortality in the surgical group. Currently, minimally invasive management is favored, although indications still exist for surgical intervention. Of note, recurrence of HAPs following embolization has been reported to be significant, and therefore current recommendations involve follow-up imaging with potential need for secondary intervention<sup>[9]</sup>. In the case of our patient, standard minimally invasive endovascular (HAP) and biliary (obstructive jaundice) management was attempted on several occasions with the use of multiple modalities, but ultimately failed to palliate her symptoms. This specific case presented multiple complications including inability to decompress the right biliary system secondary to mass effect and subsequent ischemia as portal vein. Therefore, surgical intervention, specifically right hepatic trisegmentectomy and bile duct resection, was required to address the biliary outflow obstruction and the inherent risk of rupture and hemorrhage of the HAP itself.

In conclusion, we present a unique case of HAP of unknown etiology, initially presenting with jaundice and pruritus. While the presenting symptoms were inconsistent with the typical presentation of HAPs, it is important to be aware of this as a potential cause of jaundice in addition to the more commonly thought of etiologies. The risk of morbidity and mortality secondary to HAP rupture and subsequent hemorrhage requires immediate identification and intervention. Currently, the literature supports the use of minimally invasive endovascular, endoscopic, and percutaneous approaches in the initial management of HAPs, with the potential need for future open surgical intervention if these techniques are inadequate.

## COMMENTS

### Case characteristics

A 65-year-old female with no significant past medical history with a history of painless jaundice and pruritis.

### Clinical diagnosis

Computed tomography imaging studies revealed a 5 cm dense lesion within the hilar region of the liver and intrahepatic biliary duct dilatation.

### Differential diagnosis

Klatskin's tumor, extrahepatic cholangiocarcinoma, pancreatic cancer.

### Laboratory diagnosis

Total and direct bilirubin levels were elevated (total bilirubin 24.3 mg/dL, direct bilirubin 18.6 mg/dL), as was her serum alkaline phosphatase (432 U/L), liver transaminases (AST 132 U/L, ALT 148 U/L), and CA 19-9 (891 U/mL). Other pertinent labs included a positive ANA and CEA within the reference range.

### Imaging diagnosis

Triphasic computed tomography angiography demonstrated a right branch hepatic artery pseudoaneurysm (HAP) measuring 2.1 cm, a suspected hematoma measuring 5 cm in diameter and significant biliary duct dilatation.

### Pathological diagnosis

Right hepatic trisegmentectomy pathology specimen demonstrated the presence

of a porta hepatis hematoma (5.0 cm) and organized thrombus dissecting into the hepatic artery wall, negative for malignancy and microscopically consistent with centrilobular cholestasis and bile ductular reaction with bile plugs all of which were consistent with chronic biliary obstruction.

### Treatment

Complete surgical excision of locally involved right hepatic artery pseudoaneurysm.

### Related reports

HAPs classically arise secondary to trauma or iatrogenic causes and pose a serious risk of exsanguinating hemorrhage and subsequent death. Numerous case reports have been published addressing the occurrence of HAPs subsequent to a variety of interventional procedures including cholecystectomy, pancreaticoduodenectomy, and orthotopic liver transplant. Due to the increased risk of rupture compared with true aneurysms, HAPs have been successfully treated using a variety of interventional methods. Because of the increased morbidity associated with surgery in this setting, surgical management is the final treatment option.

### Term explanation

HAPs are "false" aneurysms that do not have the presences of layers of the arterial wall, communicates with the vessel in question and has blood confined by the surrounding tissues.

### Experiences and lessons

HAPs are typically managed with a variety of interventional methods such as endovascular embolization, coiling embolization, and arterial stent grafting. Because of the increased morbidity associated with surgery in this setting, surgical management is the final treatment when impairment to adjacent structures such as the bile duct and portal vein cannot be alleviated.

### Peer-review

This manuscript presents a rare case of pseudoaneurysm of hepatic artery. The management of the case is informative, and useful for the readers.

## REFERENCES

- 1 Lü PH, Zhang XC, Wang LF, Chen ZL, Shi HB. Stent graft in the treatment of pseudoaneurysms of the hepatic arteries. *Vasc Endovascular Surg* 2013; **47**: 551-554 [PMID: 24052448 DOI: 10.1177/1538574413488460]
- 2 Finley DS, Hinojosa MW, Paya M, Imagawa DK. Hepatic artery pseudoaneurysm: a report of seven cases and a review of the literature. *Surg Today* 2005; **35**: 543-547 [PMID: 15976950 DOI: 10.1007/s00595-005-2987-6]
- 3 Krokidis ME, Hatzidakis AA. Acute hemobilia after bilioplasty due to hepatic artery pseudoaneurysm: treatment with an ePTFE-covered stent. *Cardiovasc Intervent Radiol* 2009; **32**: 605-607 [PMID: 19093147 DOI: 10.1007/s00270-008-9486-2]
- 4 Fankhauser GT, Stone WM, Naidu SG, Oderich GS, Ricotta JJ, Bjarnason H, Money SR. The minimally invasive management of visceral artery aneurysms and pseudoaneurysms. *J Vasc Surg* 2011; **53**: 966-970 [PMID: 21216559 DOI: 10.1016/j.jvs.2010.10.071]
- 5 Julianov A, Georgiev Y. Hepatic artery aneurysm causing obstructive jaundice. *Quant Imaging Med Surg* 2014; **4**: 294-295 [PMID: 25202666 DOI: 10.3978/j.issn.2223-4292.2014.06.02]
- 6 Hossain A, Reis ED, Dave SP, Kerstein MD, Hollier LH. Visceral artery aneurysms: experience in a tertiary-care center. *Am Surg* 2001; **67**: 432-437 [PMID: 11379643]
- 7 Tulsyan N, Kashyap VS, Greenberg RK, Sarac TP, Clair DG, Pierce G, Ouriel K. The endovascular management of visceral artery aneurysms and pseudoaneurysms. *J Vasc Surg* 2007; **45**: 276-283; discussion 283 [PMID: 17264002 DOI: 10.1016/j.jvs.2006.10.049]
- 8 Harvey J, Dardik H, Impeduglia T, Woo D, DeBernardis F. Endovascular management of hepatic artery pseudoaneurysm hemorrhage complicating pancreaticoduodenectomy. *J Vasc*

- Surg* 2006; **43**: 613-617 [PMID: 16520182 DOI: 10.1016/j.jvs.2005.11.031]
- 9 **Spiliopoulos S**, Sabharwal T, Karnabatidis D, Broutzos E, Katsanos K, Krokidis M, Gkoutzios P, Siablis D, Adam A. Endovascular treatment of visceral aneurysms and pseudoaneurysms: long-term outcomes from a multicenter European study. *Cardiovasc Intervent Radiol* 2012; **35**: 1315-1325 [PMID: 22146976 DOI: 10.1007/s00270-011-0312-x]
  - 10 **Rencuzogullari A**, Okoh AK, Akcam TA, Roach EC, Dalci K, Ulku A. Hemobilia as a result of right hepatic artery pseudoaneurysm rupture: An unusual complication of laparoscopic cholecystectomy. *Int J Surg Case Rep* 2014; **5**: 142-144 [PMID: 24531018 DOI: 10.1016/j.ijscr.2014.01.005]
  - 11 **Yao CA**, Arnell TD. Hepatic artery pseudoaneurysm following laparoscopic cholecystectomy. *Am J Surg* 2010; **199**: e10-e11 [PMID: 20103061 DOI: 10.1016/j.amjsurg.2009.03.014]
  - 12 **Nagaraja R**, Govindasamy M, Varma V, Yadav A, Mehta N, Kumaran V, Gupta A, Nundy S. Hepatic artery pseudoaneurysms: a single-center experience. *Ann Vasc Surg* 2013; **27**: 743-749 [PMID: 23711970 DOI: 10.1016/j.avsg.2012.08.018]

**P- Reviewer:** Palmucci S, Panic N, Tomizawa M

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 July 8; 8(19): 785-826





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vysloulzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**Stephen DH Malnick, *Rehovot***Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**Kamal E Bani-Hani, *Zarqa***Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**Angela Peltec, *Chishinev***Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**CA Asabamaka Onyekwere, *Lagos***Pakistan**Bikha Ram Devrajani, *Jamshoro***Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**Jacek Zielinski, *Gdansk***Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**Reem Al Olaby, *Doha***Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**Ser Yee Lee, *Singapore***South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



## Contents

Three issues per month Volume 8 Number 19 July 8, 2016

### EDITORIAL

- 785 Sofosbuvir/velpatasvir: A promising combination  
*Bonaventura A, Montecucco F*

### MINIREVIEWS

- 790 Could there be light at the end of the tunnel? Mesocaval shunting for refractory esophageal varices in patients with contraindications to transjugular intrahepatic portosystemic shunt  
*Davis J, Chun AK, Borum ML*

### ORIGINAL ARTICLE

#### Basic Study

- 796 Assembly and release of infectious hepatitis C virus involving unusual organization of the secretory pathway  
*Triyatni M, Berger EA, Saunier B*

#### Prospective Study

- 815 Is neutrophil gelatinase associated lipocalin a useful marker in hepatitis C virus infection?  
*Strazzulla A, Coppolino G, Di Fatta C, Giancotti F, D'Onofrio G, Postorino MC, Mazzitelli M, Mammone SV, Gentile I, Rivoli L, Palella E, Gravina T, Costa C, Pisani V, De Maria V, Barreca GS, Marascio N, Focà A, Fuiano G, Gulletta E, Torti C*

### LETTERS TO THE EDITOR

- 825 Lot to give, got to live - the restless minds of the "Liver on Tour" project  
*Macedo G, Peixoto A, Lopes S*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Sandro Vento, MD, Professor, Department of Medicine, School of Medicine, Nazarbayev University, Astana 010000, Kazakhstan

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: editorialoffice@wjgnet.com  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
July 8, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_20160116143427.htm](http://www.wjgnet.com/bpg/g_info_20160116143427.htm)

**ONLINE SUBMISSION**

<http://www.wjgnet.com/esps/>



## Sofosbuvir/velpatasvir: A promising combination

Aldo Bonaventura, Fabrizio Montecucco

Aldo Bonaventura, Fabrizio Montecucco, First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa School of Medicine, IRCCS Azienda Ospedaliera Universitaria San Martino-IST Istituto Nazionale per la Ricerca sul Cancro, 16132 Genoa, Italy

**Author contributions:** Bonaventura A and Montecucco F contributed equally to this paper.

**Conflict-of-interest statement:** Bonaventura A and Montecucco F declare no conflict of interest related to this publication.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Fabrizio Montecucco, MD, PhD, Assistant Professor, First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa School of Medicine, IRCCS Azienda Ospedaliera Universitaria San Martino-IST Istituto Nazionale per la Ricerca sul Cancro, 6 viale Benedetto XV, 16132 Genoa, Italy. [fabrizio.montecucco@unige.it](mailto:fabrizio.montecucco@unige.it)  
 Telephone: +39-10-3538694  
 Fax: +39-10-3538686

Received: March 14, 2016  
 Peer-review started: March 16, 2016  
 First decision: May 17, 2016  
 Revised: May 23, 2016  
 Accepted: June 14, 2016  
 Article in press: June 16, 2016  
 Published online: July 8, 2016

### Abstract

Hepatitis C virus (HCV) affects 3% of the world population. It represents the main cause of chronic liver

disease and is responsible for extra-hepatic complications, such as type 2 diabetes and cardiovascular diseases. HCV includes 7 genotypes differing in the nucleotide sequence variability, the geographic distribution, the rates of viral clearance, the risk of progression to liver fibrosis and to hepatocellular carcinoma, and the response to therapy. Last years have seen remarkable advances in the field of HCV infection with the approval of direct antiviral agents (DAAs) targeting key viral proteins involved in the HCV replication. Several oral regimens combining DAAs from different families have been developed and these regimens showed increased and sustained virological response rates to above 90% reducing the treatment duration to 12 wk or less. In particular, sofosbuvir, a nucleotide analogue nonstructural (NS)5B polymerase inhibitor, and velpatasvir, a NS5A inhibitor, have been tested in two phase 3 trials, the ASTRAL-2 (against HCV genotype 2) and the ASTRAL-3 (against HCV genotype 3), demonstrating to be effective, safe, and well tolerated in patients who were 18 years of age or older and had at least a 6-mo history of HCV infection with a compensated liver disease.

**Key words:** Hepatitis C virus; Sofosbuvir; Velpatasvir; NS5A inhibitor; NS5B inhibitor

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatitis C virus (HCV) spread all over the world. In the last years, new therapies with direct antiviral agents draw a great revolution thanks to several oral regimens combining different drugs of this class. The present editorial provides a brief overview on the association between two direct antiviral agents, sofosbuvir and velpatasvir, and their implication in the treatment of HCV infection.

Bonaventura A, Montecucco F. Sofosbuvir/velpatasvir: A promising combination. *World J Hepatol* 2016; 8(19): 785-789 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i19/785>.

## INTRODUCTION

Hepatitis C virus (HCV) infects approximately 3% of the world population and leads to cirrhosis and hepatocellular carcinoma<sup>[1]</sup>. HCV is a member of the Flaviviridae family of RNA viruses and is classified into 7 genotypes; each genotype is different from the others in its nucleotide sequence. This remarkable genetic diversity represents a challenge for the development of new therapies<sup>[2]</sup>. HCV genotypes differ not only in the nucleotide sequence variability, but also in their geographic distribution, rates of viral clearance, risk of progression to liver fibrosis and to hepatocellular carcinoma, and response to therapy<sup>[3]</sup>.

In last years, HCV treatment has undergone substantial advances. Direct antiviral agents (DAAs), when combined with pegylated interferon (PegIFN) and ribavirin (RVR), demonstrated increased rates of cure in chronic HCV infections when compared to PegIFN and RVR alone<sup>[4]</sup>. The first class of DAAs to be approved for treatment of HCV was that of protease inhibitors targeting the nonstructural protein (NS)3/4A serine protease, responsible for the processing of the nascent viral polyprotein<sup>[4]</sup>. The search for new key viral targets continued and compounds against two additional targets - NS5A replication scaffold and the NS5B RNA-dependent RNA polymerase (RdRp) - have been generated<sup>[5]</sup>. Drugs inhibiting NS5B include two subclasses: Nucleos(t)ide inhibitors (NIs) and non-nucleoside inhibitors (NNIs). NS5B is strictly required during the HCV replication both to copy the RNA genome and to transcribe messenger RNA: These steps are essential and the inhibition of NS5B is able to block viral propagation (Figure 1).

Many phase 2 trials have been conducted with promising results. Two phase 3 trials have done encouraging results with the combination of the nucleotide polymerase inhibitor sofosbuvir and the NS5A inhibitor velpatasvir in patients chronically infected with HCV genotype 2 and 3<sup>[6]</sup>.

## A BRIEF PRESENTATION OF SOFOSBUVIR AND VELPATASVIR

### Sofosbuvir

Sofosbuvir (SOF) (formerly known as GS-7977; Gilead Sciences, Foster City, CA, United States) is a NS5B NI<sup>[7]</sup>. It is converted into a pharmacologically active form (GS-461203) within hepatocytes, inhibits RdRp activity by competing with uridine, and blocks RNA synthesis by acting as "chain terminator"<sup>[8]</sup>. Given the high conservation of the catalytic site of the NS5B protein, this drug is believed to have pangenotypic activity<sup>[8]</sup>. The combination of SOF and RVR achieved sustained virologic response (SVR) rates of 100% for genotype 2 infection and 91% for genotype 3 infection<sup>[9]</sup>. On December 6, 2013, the United States Food and Drug Administration (FDA) approved SOF (Sovaldi™) for the

treatment of chronic HCV, genotypes 1, 2, 3 and 4, in combination with Peg-IFN and RVR or with RVR alone. SOF is also highly effective in HCV patients who are co-infected with human immunodeficiency virus (HIV). It should not be administered with potent inducers of intestinal P-glycoprotein, such as rifampin and Saint John's wort, resulting in reduced absorption<sup>[10]</sup>. Moreover, co-administration of SOF with select anticonvulsants, rifabutin, rifapentine, or tipranavir/ritonavir is not recommended<sup>[5]</sup>. SOF showed no clinically significant drug interactions with many of the common medications metabolized by CYP3A enzymes, such as tacrolimus, cyclosporine, and methadone, as well as with the common combination therapies for HIV<sup>[5]</sup>.

### Velpatasvir

Velpatasvir (VEL) (formerly GS-5816; Gilead Sciences; Foster City, CA, United States) is a new NS5A protein inhibitor with pan-genotypic activity *in vitro*<sup>[11]</sup>. In phase 2 trials, it demonstrated high rates of SVR in patients with HCV genotypes 2 and 3 in combination with sofosbuvir for a period of 12 wk of treatment<sup>[12,13]</sup>. Regimens including an NS5A inhibitor have demonstrated high tolerability and high antiviral efficacy in phase 3 studies<sup>[11]</sup>.

## SOF AND VEL IN PHASE 2 AND 3 STUDIES

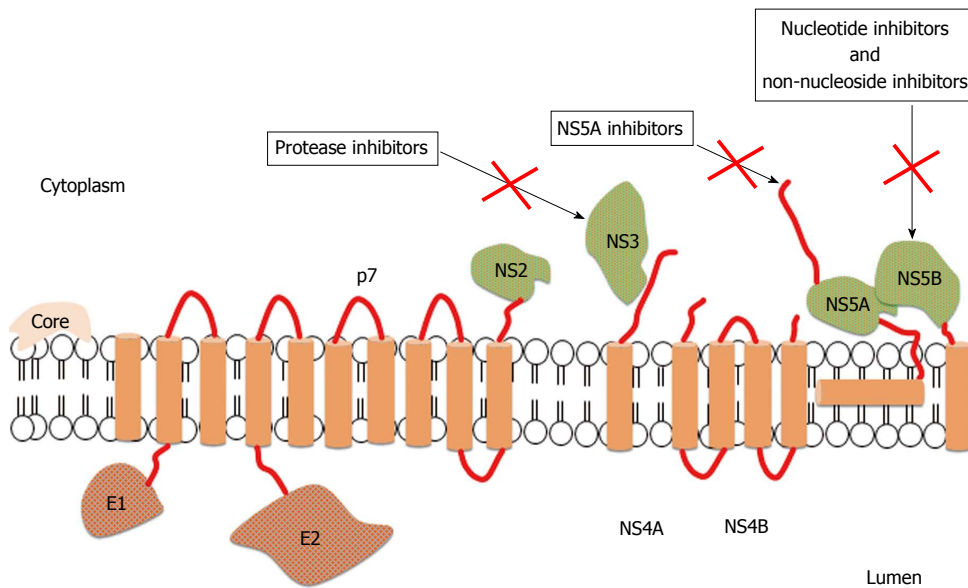
### The phase 2 studies

In phase 2 studies, the SOF/VEL combination administered for 12 wk showed high rates of SVR and was well tolerated in all HCV genotype infections<sup>[12,13]</sup>.

In the phase 2 study by Everson *et al.*<sup>[12]</sup>, treatment-naïve non-cirrhotic patients achieved high rates of SVR at 12 wk with SOF/VEL, independently of HCV genotypes. Virologic failure was rare; the nonresponse of 1 patient with HCV genotype 3 infection and the relapse in a patient with HCV genotype 1 infection, both receiving 25 mg of VEL, may suggest that the 100 mg dose could have a clinical advantage over the 25 mg one. In general, the therapy with SOF/VEL with or without RVR was well tolerated. In the other phase 2 study by Pianko *et al.*<sup>[13]</sup>, SOF/VEL demonstrated to be safe, effective, and well-tolerated for treatment-experienced patients with HCV infection genotype 1 or 3, including those with compensated cirrhosis, which are the most common genotypes accounting for approximately 46% and 22% of all global infections, respectively<sup>[14]</sup>. In particular, the SVR at 12 wk in patients with genotype 3 HCV infection under VEL 100 mg compared favorably with those previously reported for other regimens<sup>[15-17]</sup>.

### The ASTRAL-2 and the ASTRAL-3 trials

The ASTRAL program aimed to evaluate the safety and the efficacy of the association SOF/VEL in patients with HCV genotype 1-6 infection including patients with decompensated liver disease. ASTRAL-2 and ASTRAL-3 trials are two randomized, controlled, phase 3 trials, in which a fixed-dose combination tablet of SOF/VEL for



**Figure 1 Structure of hepatitis C virus and mechanisms of action of direct antiviral agents<sup>[29]</sup>.** The open reading frame of hepatitis C virus (HCV) encodes 11 proteins: 3 structural proteins (core, E1, and E2); 6 nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B); the p7 protein; and the frameshift (F) protein (not illustrated). The core protein has a role in viral capsid formation and can directly interact with many cellular proteins and pathways implicated in the HCV lifecycle. Envelope glycoproteins, E1 and E2, are fundamental components of the virion envelope and are essential for HCV entry and fusion; in particular, E2 has a central role in the early steps of infection because it functions as a host receptor binding protein and mediates the attachment to host cells. The F protein is probably produced during viral infection and could be involved in viral persistence, but the exact role has not been fully elucidated. The p7 protein is an integral membrane protein belonging to the viroporin family and maybe acts as a calcium ion channel, but further studies are needed to confirm its function. NS2 is a non-glycosylated transmembrane protein having a protease activity, which may interact with host cell proteins. NS3 is a multi-functional protein owning a serine protease domain and a helicase/nucleoside triphosphatase domain, while NS4A is a cofactor of NS3 protease activity. The 2 proteins can interact with host cell pathways and proteins involved in HCV lifecycle and for this reason they are an appealing viral target for anti-HCV therapies. NS4 is an integral membrane protein serving as a membrane anchor for the replication complex; moreover, it can inhibit cellular synthesis and modulate the HCV RdRp activity. NS5A is a zinc-metalloprotein playing a role in virus replication, cell growth replication, and in mediating interferon-resistance, even if some of these functions need to be still clarified. NS5B belongs to the class of tail-anchored proteins. Its crystal structure showed that the RdRp has a "fingers, palm and thumb" structure; interactions between the fingers and thumb subdomains create a fully surrounded catalytic site ensuring both HCV RNA strand synthesis. For this reason, the RdRp is an attractant target for new anti-HCV drugs.

12 wk was compared to standard treatment with SOF plus RVR for 12 or 24 wk in patients already treated for HCV genotype 2 and 3 infection and in those who had not received this treatment, including the ones with decompensated cirrhosis<sup>[6]</sup>.

The two studies shared the same eligibility criteria, except for HCV genotype. Patients who were 18 years of age or older and with at least a 6-mo history of HCV infection could enter the study, whilst patients with clinical evidence of hepatic decompensation were excluded. In the two trials, patients with chronic HCV infection were randomly assigned to receive a fixed-dose combination tablet containing 400 mg of SOF and 100 mg of VEL once daily for 12 wk or 400 mg of SOF plus RVR for 12 wk (for patients with HCV genotype 2) or 24 wk (for patients with HCV genotype 3). RVR was administered orally twice daily, with body weight-determined doses. The primary endpoint was a SVR, defined as an HCV RNA level of less than 15 IU per milliliter at 12 wk after the end of treatment.

In the ASTRAL-2 trial, patients who had received SOF/VEL met the primary endpoint with no virologic failures. In the ASTRAL-3 trial, patients who were administered with SOF/VEL gained a SVR, but 4% of them showed virologic failure after the end of treatment. Overall, the rate of SVR was higher among patients without cirrhosis and who did not receive any previous treatment.

### Safety

In both trials, rates of adverse events (AEs) were lower among patients who received SOF/VEL than those who received SOF/RVR. In both trials, only 1 patient receiving SOF/VEL stopped the treatment prematurely because of an AE (anxiety, headache, and difficulty in concentrating). Common AEs, such as headache, fatigue, and nausea have been reported in 10%-38% of SOF/VEL patients, together with insomnia, irritability, pruritus, nasopharyngitis, cough, and dyspnea<sup>[6]</sup>. Serious AEs in the SOF/VEL group of the ASTRAL-2 included enteritis, pneumonia, and abdominal pain, whereas in the ASTRAL-3 acute myocardial infarction, acute cholecystitis, food poisoning, hematochezia, intracranial aneurysm, and rupture of ovarian cyst have been described<sup>[6]</sup>. In both trials, only 1 patient receiving SOF/VEL stopped the treatment prematurely because of an AE (anxiety, headache, and difficulty of concentration). Death after treatment occurred in two patients in the ASTRAL-2 (1 from cardiac arrest and one from metastatic lung cancer complications); in the ASTRAL-3, two deaths occurred during treatment (1 from unknown cause and 1 from gunshot wounds) and one in the post-treatment period (from unknown cause).

### Comparison between NIs and NNIs

NIs inhibit the RdRp by mimicking NS5B protein sub-

strate leading to the termination of the new viral RNA chain; they possess a high-resistance barrier, are highly effective, and own a pan-genotypic activity<sup>[18]</sup>. NNIs behave as allosteric inhibitors by binding to the RdRp blocking polymerase function through conformational change; this results in a lower barrier to resistance and lower anti-viral activity with respect to NIs<sup>[18]</sup>.

SOF is the only NI approved and was associated with high SVR rates in all kind of patients. The addition of SOF to PegIFN and RVR demonstrated to be the most effective IFN-containing regimen in HCV patients with compensated cirrhosis. In the NEUTRINO and FISSION studies, patients receiving SOF had rapid and substantial decreases in serum HCV RNA levels. Moreover, AEs were uncommon among patients receiving SOF regimens as well as severe AEs were few in all study groups<sup>[19]</sup>. In IFN-free regimens, SOF was tested in combination with the first generation NS5A inhibitor ledispavir both in treatment-naïve and pre-treated patients and showed good response in terms of SVR<sup>[20,21]</sup>. Nonetheless, among all patients, the majority had at least one, mild-to-moderate AE (fatigue, headache, insomnia, and nausea), but also serious AEs occurred in 6%-8% of patients of the 24 wk-SOF regimens<sup>[20,21]</sup>. AEs were higher and more serious in the groups concomitantly treated with RVR. Finally, some mild modifications of the haemochrome were present, whilst the increase in bilirubin and transaminases were more frequently in the group treated with RVR.

Among NNI, dasabuvir is the only drug approved and is usually administered in combination with ritonavir/paritaprevir and ombitasvir. Dasabuvir is mainly effective against HCV genotype 1. When used in combination, these three drugs showed excellent SVR rates at 12 wk in patients with HCV-1-compensated cirrhosis. Mild AEs were recorded in nearly 80% of patients, especially when treated with RVR and included nausea, fatigue, pruritus, and headache. A modest decrease of hemoglobin was reported, sometimes reaching the lower limit of the normal range<sup>[22-26]</sup>. Serious AEs were rare. However, in the post-marketing surveillance many cirrhotic were found developing hepatic decompensation and/or liver failure. For this reason, the United States FDA issued a warning in which it was noted that this treatment could cause serious liver injury in cirrhotic patients<sup>[27]</sup>.

## CONCLUSION

The development of DAAs revolutionized the therapeutic weapons against HCV infection. These advances have been possible thanks to the increased knowledge of the structures of HCV protease and HCV polymerase, which permitted to design structure-based drug inhibiting key viral enzymes. Indeed, HCV drug development was fast for different reasons, such as a shorter treatment duration, no need for control arms, and the short period to evaluate the SVR (12-24 wk)<sup>[28]</sup>.

Since 2014, many drugs were approved: The first one was SOF, followed by simeprevir and daclatasvir. In 2015, ledipasvir in combination with SOF, and paritaprevir in combination with ombitasvir and dasabuvir were

approved in several countries. In 2016/2017, three other combinations could be approved, such as grazoprevir in combination with elbasvir, SOF with VEL, and ABT-493 plus ABT-530 combination therapy<sup>[28]</sup>.

The combination SOF/VEL was extensively studied in phase 2 and 3 studies. In the ASTRAL-2 and ASTRAL-3 studies, SOF/VEL showed great efficacy against HCV infections genotype 2 and 3 (except for a virologic failure after the end of treatment for 11 patients in the ASTRAL-3) and a reasonable safety<sup>[6]</sup>. The treatment of patients with HCV genotype 3 is still a challenge, even in the era of DAAs and further researches are needed to increase the rate of SVR in this subtype of patients. As the same researchers of ASTRAL-2 and ASTRAL-3 state, the generalizability of the results are limited by the small number of black patients included in the trials, considering that genotype 2 is present in sub-Saharan Africa<sup>[14]</sup>. Moreover, the exclusion of patients with decompensated cirrhosis represents another pivotal point to cope with, given the huge importance of HCV in the natural history of cirrhosis and hepatocellular carcinoma, never forgiving the extrahepatic complications, such as type 2 diabetes mellitus and cardiovascular diseases.

Further trials evaluating new DAAs should focus on the prolongation of the SVR and the inclusion of patients such as those abovementioned, which can get a real benefit from these new therapies, if safe, effective, and well tolerated.

## REFERENCES

- Cholongitas E**, Pipili C, Papatheodoridis G. Interferon-free regimens for the treatment of hepatitis C virus in liver transplant candidates or recipients. *World J Gastroenterol* 2015; **21**: 9526-9533 [PMID: 26327760 DOI: 10.3748/wjg.v21.i32.9526]
- Smith DB**, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. *Hepatology* 2014; **59**: 318-327 [PMID: 24115039 DOI: 10.1002/hep.26744]
- Eslam M**, George J. Is hepatitis C subtyping still relevant in the era of direct-acting antiviral therapy? *Hepatol Int* 2015; **9**: 5-8 [PMID: 25788373 DOI: 10.1007/s12072-014-9600-2]
- Chae HB**, Park SM, Youn SJ. Direct-acting antivirals for the treatment of chronic hepatitis C: open issues and future perspectives. *ScientificWorldJournal* 2013; **2013**: 704912 [PMID: 23844410 DOI: 10.1155/2013/704912]
- McQuaid T**, Savini C, Seyedkazemi S. Sofosbuvir, a Significant Paradigm Change in HCV Treatment. *J Clin Transl Hepatol* 2015; **3**: 27-35 [PMID: 26357632 DOI: 10.14218/JCTH.2014.00041]
- Foster GR**, Afdhal N, Roberts SK, Bräu N, Gane EJ, Pianko S, Lawitz E, Thompson A, Shiffman ML, Cooper C, Townner WJ, Conway B, Ruane P, Bourlière M, Asselah T, Berg T, Zeuzem S, Rosenberg W, Agarwal K, Stedman CA, Mo H, Dvory-Sobol H, Han L, Wang J, McNally J, Osinusi A, Brainard DM, McHutchison JG, Mazzotta F, Tran TT, Gordon SC, Patel K, Reau N, Mangia A, Sulkowski M. Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection. *N Engl J Med* 2015; **373**: 2608-2617 [PMID: 26575258 DOI: 10.1056/NEJMoa1512612]
- Gane EJ**, Stedman CA, Hyland RH, Ding X, Svarovskaia E, Symonds WT, Hinds RG, Berrey MM. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. *N Engl J Med* 2013; **368**: 34-44 [PMID: 23281974 DOI: 10.1056/NEJMoa1208953]
- Zopf S**, Kremer AE, Neurath MF, Siebler J. Advances in hepatitis C therapy: What is the current state - what comes next? *World J Hepatol* 2016; **8**: 139-147 [PMID: 26839638 DOI: 10.4254/wjh.v8.i3.139]



- 9 **Zhang X.** Direct anti-HCV agents. *Acta Pharm Sin B* 2016; **6**: 26-31 [PMID: 26904396 DOI: 10.1016/j.apsb.2015.09.008]
- 10 **Rodríguez-Torres M.** Sofosbuvir (GS-7977), a pan-genotype, direct-acting antiviral for hepatitis C virus infection. *Expert Rev Anti Infect Ther* 2013; **11**: 1269-1279 [PMID: 24215243 DOI: 10.1586/14787210.2013.855126]
- 11 **Lawitz E,** Freilich B, Link J, German P, Mo H, Han L, Brainard DM, McNally J, Marbury T, Rodríguez-Torres M. A phase 1, randomized, dose-ranging study of GS-5816, a once-daily NS5A inhibitor, in patients with genotype 1-4 hepatitis C virus. *J Viral Hepat* 2015; **22**: 1011-1019 [PMID: 26183611 DOI: 10.1111/jvh.12435]
- 12 **Everson GT,** Townner WJ, Davis MN, Wyles DL, Nahass RG, Thuluvath PJ, Etzkorn K, Hineostroza F, Tong M, Rabinovitz M, McNally J, Brainard DM, Han L, Doehle B, McHutchison JG, Morgan T, Chung RT, Tran TT. Sofosbuvir With Velpatasvir in Treatment-Naïve Noncirrhotic Patients With Genotype 1 to 6 Hepatitis C Virus Infection: A Randomized Trial. *Ann Intern Med* 2015; **163**: 818-826 [PMID: 26551051 DOI: 10.7326/M15-1000]
- 13 **Pianko S,** Flamm SL, Shiffman ML, Kumar S, Strasser SI, Dore GJ, McNally J, Brainard DM, Han L, Doehle B, Mogalian E, McHutchison JG, Rabinovitz M, Townner WJ, Gane EJ, Stedman CA, Reddy KR, Roberts SK. Sofosbuvir Plus Velpatasvir Combination Therapy for Treatment-Experienced Patients With Genotype 1 or 3 Hepatitis C Virus Infection: A Randomized Trial. *Ann Intern Med* 2015; **163**: 809-817 [PMID: 26551263 DOI: 10.7326/M15-1014]
- 14 **Gower E,** Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol* 2014; **61**: S45-S57 [PMID: 25086286 DOI: 10.1016/j.jhep.2014.07.027]
- 15 **Zeuzem S,** Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]
- 16 **Foster GR,** Pianko S, Brown A, Forton D, Nahass RG, George J, Barnes E, Brainard DM, Massetto B, Lin M, Han B, McHutchison JG, Subramanian GM, Cooper C, Agarwal K. Efficacy of sofosbuvir plus ribavirin with or without peginterferon-alfa in patients with hepatitis C virus genotype 3 infection and treatment-experienced patients with cirrhosis and hepatitis C virus genotype 2 infection. *Gastroenterology* 2015; **149**: 1462-1470 [PMID: 26248087 DOI: 10.1053/j.gastro.2015.07.043]
- 17 **Nelson DR,** Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R, Oguchi G, Thuluvath PJ, Ortiz-Lasanta G, Rabinovitz M, Bernstein D, Bennett M, Hawkins T, Ravendran N, Sheikh AM, Varunok P, Kowdley KV, Hennicken D, McPhee F, Rana K, Hughes EA. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology* 2015; **61**: 1127-1135 [PMID: 25614962 DOI: 10.1002/hep.27726]
- 18 **Majumdar A,** Kitson MT, Roberts SK. Systematic review: current concepts and challenges for the direct-acting antiviral era in hepatitis C cirrhosis. *Aliment Pharmacol Ther* 2016; **43**: 1276-1292 [PMID: 27087015 DOI: 10.1111/apt.13633]
- 19 **Lawitz E,** Mangia A, Wyles D, Rodríguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
- 20 **Afdhal N,** Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P; ION-2 Investigators. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]
- 21 **Afdhal N,** Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P; ION-1 Investigators. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]
- 22 **Andreone P,** Colombo MG, Enejosa JV, Koksai I, Ferenci P, Maieron A, Müllhaupt B, Horsmans Y, Weiland O, Reesink HW, Rodrigues L, Hu YB, Podsadecki T, Bernstein B. ABT-450, ritonavir, ombitasvir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. *Gastroenterology* 2014; **147**: 359-365.e1 [PMID: 24818763 DOI: 10.1053/j.gastro.2014.04.045]
- 23 **Feld JJ,** Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, Weiland O, Aguilar H, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1594-1603 [PMID: 24720703 DOI: 10.1056/NEJMoa1315722]
- 24 **Ferenci P,** Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A, Box TD, Younes Z, Enayati P, Green S, Baruch Y, Bhandari BR, Caruntu FA, Sepe T, Chulanov V, Janczewska E, Rizzardini G, Gervain J, Planas R, Moreno C, Hassanein T, Xie W, King M, Podsadecki T, Reddy KR. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *N Engl J Med* 2014; **370**: 1983-1992 [PMID: 24795200 DOI: 10.1056/NEJMoa1402338]
- 25 **Kowdley KV,** Lawitz E, Poordad F, Cohen DE, Nelson DR, Zeuzem S, Everson GT, Kwo P, Foster GR, Sulkowski MS, Xie W, Pilot-Matias T, Liossis G, Larsen L, Khatri A, Podsadecki T, Bernstein B. Phase 2b trial of interferon-free therapy for hepatitis C virus genotype 1. *N Engl J Med* 2014; **370**: 222-232 [PMID: 24428468 DOI: 10.1056/NEJMoa1306227]
- 26 **Sulkowski MS,** Eron JJ, Wyles D, Trinh R, Lalezari J, Wang C, Slim J, Bhatti L, Gathe J, Ruane PJ, Elion R, Bredeek F, Brennan R, Blick G, Khatri A, Gibbons K, Hu YB, Fredrick L, Schnell G, Pilot-Matias T, Tripathi R, Da Silva-Tillmann B, McGovern B, Campbell AL, Podsadecki T. Ombitasvir, paritaprevir co-dosed with ritonavir, dasabuvir, and ribavirin for hepatitis C in patients co-infected with HIV-1: a randomized trial. *JAMA* 2015; **313**: 1223-1231 [PMID: 25706092 DOI: 10.1001/jama.2015.1328]
- 27 **Food and Drug Administration.** FDA Drug Safety Communication: FDA warns of serious liver injury risk with hepatitis C treatments Viekira Pak and Technivie, 2015. Available from: URL: <http://www.fda.gov/Drugs/DrugSafety/ucm468634>.
- 28 **Asselah T,** Boyer N, Saadoun D, Martinot-Peignoux M, Marcellin P. Direct-acting antivirals for the treatment of hepatitis C virus infection: optimizing current IFN-free treatment and future perspectives. *Liver Int* 2016; **36** Suppl 1: 47-57 [PMID: 26725897 DOI: 10.1111/liv.13027]
- 29 **Chevaliez S,** Pawlotsky JM. HCV Genome and Life Cycle. In: Tan SL, editor. *Hepatitis C Viruses: Genomes and Molecular Biology*. Chapter 1. Norfolk (UK): Horizon Bioscience; 2006. Available from: URL: <http://www.ncbi.nlm.nih.gov/books/NBK1630/>

**P- Reviewer:** Kim SR, Kuramitsu Y, Pellicano R  
**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Li D



# Could there be light at the end of the tunnel? Mesocaval shunting for refractory esophageal varices in patients with contraindications to transjugular intrahepatic portosystemic shunt

Jessica Davis, Albert K Chun, Marie L Borum

Jessica Davis, Department of Internal Medicine, George Washington University, Washington, DC 20037, United States

Albert K Chun, Department of Radiology, George Washington University, Washington, DC 20037, United States

Marie L Borum, Division of Gastroenterology and Liver Diseases, George Washington University, Washington, DC 20037, United States

**Author contributions:** Davis J wrote the manuscript; Chun AK provided fluoroscopic images; Borum ML provided endoscopic images; Chun AK and Borum ML revised the manuscript.

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Marie L Borum, MD, EdD, MPH, Director of the Division of Gastroenterology and Liver Disease, George Washington University, 2150 Pennsylvania Avenue, NW Suite 3-405, Washington, DC 20037, United States. [mborum@mfa.gwu.edu](mailto:mborum@mfa.gwu.edu)  
 Telephone: +1-202-7412160  
 Fax: +1-202-7412169

Received: March 25, 2016  
 Peer-review started: March 25, 2016  
 First decision: May 17, 2016  
 Revised: May 19, 2016  
 Accepted: June 14, 2016

Article in press: June 16, 2016

Published online: July 8, 2016

## Abstract

Cirrhotic patients with recurrent variceal bleeds who have failed prior medical and endoscopic therapies and are not transjugular intrahepatic portosystemic shunt candidates face a grim prognosis with limited options. We propose that mesocaval shunting be offered to this group of patients as it has the potential to decrease portal pressures and thus decrease the risk of recurrent variceal bleeding. Mesocaval shunts are stent grafts placed by interventional radiologists between the mesenteric system, most often the superior mesenteric vein, and the inferior vena cava. This allows flow to bypass the congested hepatic system, reducing portal pressures. This technique avoids the general anesthesia and morbidity associated with surgical shunt placement and has been successful in several case reports. In this paper we review the technique, candidate selection, potential pitfalls and benefits of mesocaval shunt placement.

**Key words:** Portal hypertension; Surgical portacaval shunt; Gastrointestinal hemorrhage; Esophageal and gastric varices; Transjugular intrahepatic portosystemic shunt

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Cirrhotic patients who have recurrent variceal hemorrhage despite medical and endoscopic therapy have limited options if they are not transjugular intrahepatic portosystemic shunting candidates. One promising new method to decrease portal pressures while avoiding

surgical shunt placement is mesocaval shunt placement with fluoroscopic guidance. In this paper we review the technique, candidate selection, potential pitfalls and benefits of mesocaval shunt placement.

Davis J, Chun AK, Borum ML. Could there be light at the end of the tunnel? Mesocaval shunting for refractory esophageal varices in patients with contraindications to transjugular intrahepatic portosystemic shunt. *World J Hepatol* 2016; 8(19): 790-795 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i19/790.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i19.790>

## INTRODUCTION

Patients with cirrhosis and recurrent variceal bleeding face a high mortality, 20% in the first year vs 5.4% for compensated patients<sup>[1]</sup>. Current standard of care for variceal bleeding includes three primary modalities: Medical therapy with beta blockade, endoscopic therapy with ligation of varices and shunt therapy with transjugular intrahepatic portosystemic shunting (TIPS). Each of these have been shown to improve rebleeding rates and mortality<sup>[2-4]</sup>.

For an unfortunate cohort of patients with varices who fail medical and endoscopic therapy and are not TIPS candidates, there are limited options in the face of a grim prognosis. Historically, these patients have been offered surgical shunt approaches, however, mortality of surgical shunt placements is high - 20%-50% if emergent - and many patients may not be suitable surgical candidates<sup>[5]</sup>. First described in 1996 by Nyman *et al*<sup>[6]</sup>, mesocaval shunting may provide an alternate route to alleviate portal hypertension in these challenging patients. This paper will review the technique, candidate selection, potential pitfalls and benefits of mesocaval shunting. While there are not enough data to comment on a mortality benefit, we believe that mesocaval shunting is a feasible procedure for the prevention of variceal bleeding. It will likely be most useful for patients whose anatomy prohibits TIPS to provide a bridge to transplant.

## TECHNIQUE

Mesocaval shunting involves the creation of a shunt from the mesenteric vasculature, typically the superior mesenteric vein (SMV), into the inferior vena cava (IVC). Similar to TIPS, this provides relief of portal pressures by allowing blood to bypass the congested hepatic vasculature. Shunt placement is performed by interventional radiologists. There have been both femoral and transabdominal approaches reported (Table 1)<sup>[6-9]</sup>.

Fluoroscopy from a recent case of refractory variceal bleeding in a patient with a portal vein thrombus (PVT) from our institution will be used to graphically illustrate the basic technique (Figure 1). Our patient had cirrhosis and prior medical and endoscopic attempts to control her varices were limited by significant chest pain attributed

to her banding procedures that required inpatient admission. Her PVT prohibited TIPS placement and she consented to undergo endovascular mesocaval shunt placement.

In our patient, and, in general, first, a needle is directed, in our case transabdominally, through the SMV, or, in this instance, a portal vein remnant, at a target placed *via* internal jugular (IJ) access (Figure 2A). Then, a wire is threaded from this needle through the IVC and out the IJ access so that, when the needle is removed, the distal tip of the the wire is in the splenic vein and its proximal end functions as a guidewire exiting the IJ access (Figure 2B). Finally, a stent graft, in our case a covered VIATORR stent, is passed over the guidewire *via* IJ access using Seldinger technique and placement is confirmed with fluoroscopic guidance (Figure 2C).

In the initial case report<sup>[6]</sup>, contrast-enhanced computed tomography (CT) was first performed to define cross-sectional anatomy. The patient underwent bowel preparation pre-procedurally and was given prophylactic antibiotics as a transcolonic approach was anticipated. Using CT and fluoroscopic guidance, a needle was inserted through and through the transverse colon and SMV into the IVC to a retrieval basket. The retrieval basket had been placed in the IVC *via* the right internal jugular vein. A guide wire was then passed from the abdominal access through the SMV to IVC and jugular access. A stent was placed under angiographic guidance from the internal jugular access across the IVC to SMV and the wire was removed.

Another case report, by Moriarty *et al*<sup>[9]</sup>, used similar methodology but opted for a transgastric rather than transcolonic approach to reduce the risk of infection. Interestingly, the case reported by Moriarty *et al*<sup>[9]</sup> required a cardiac transseptal needle to puncture the IVC as attempts made with a Rosch-Uchida TIPS needle were unsuccessful. The final published percutaneous approach to date was remarkable for the ability to avoid luminal puncture; Bercu *et al*<sup>[8]</sup> were able to approach transabdominally without perforating the bowel and relied on fluoroscopic rather than CT-guidance for visualization of the patient's anatomy during the procedure.

Hong *et al*<sup>[7]</sup> reported an interesting series of three cases in which they were able to place mesocaval shunts but avoid a transabdominal approach. Using techniques similar to direct intra-hepatic portosystemic shunt (DIPS) placement, they describe a series of cases in which they relied on intravascular ultrasound to avoid transabdominal puncture to access the SMV. The stent itself is extra-hepatic (and thus distinct from DIPS) and possible in patients who are not candidates for TIPS or DIPS given portal vein thrombi. In short, sheaths were placed both femorally and in the internal jugular vein. A guide wire was used to couple the jugular and femoral sheaths. Following guide wire placement, a longitudinal side-firing intravascular ultrasound (IVUS), akin to those used in placement of DIPS, was introduced through the femoral sheath so that the SMV could be cannulated using a needle introduced at the jugular access. In this

**Table 1** Summary of published mesocaval shunt placements

Ref.	Case history	Details and outcomes	
Nyman <i>et al</i> <sup>[6]</sup> 1996	37-year-old male with history of recurrent massive variceal bleeds attributed to congenital PVT and failed prior surgical shunt attempt	Visualization Approach Duration of follow-up Thrombosis Recurrent bleeding Hepatic encephalopathy	CT angiography Transcolonic 5, 12 and 14 mo Yes <sup>1</sup> No NR
Moriarty <i>et al</i> <sup>[9]</sup> 2012	57-year-old male with history of metastatic CRC and extrahepatic PVT who failed prior TIPs and was thought not to be surgical candidate	Visualization Approach Duration of follow-up Thrombosis Recurrent bleeding Hepatic encephalopathy	CT and fluoroscopy Transgastric 3 mo Yes <sup>2</sup> Yes <sup>2</sup> NR
Bercu <i>et al</i> <sup>[8]</sup> 2015	58-year-old female with history of HCV cirrhosis, PVT with recurrent ascites who failed prior TIPs attempt and was a poor surgical candidate	Visualization Approach Duration of follow-up Thrombosis Recurrent bleeding Hepatic encephalopathy	Fluoroscopy Transabdominal 3 and 6 mo No No Yes <sup>3</sup>
Hong <i>et al</i> <sup>[7]</sup> 2012	16-year-old female with history of chronic PVT and hematemesis who was felt to have high surgical risk	Visualization Approach Duration of follow-up Thrombosis Recurrent bleeding Hepatic encephalopathy	Fluoroscopy and IVUS Endovascular 1 mo No No NR
Hong <i>et al</i> <sup>[7]</sup> 2012	60-year-old female with history of HBV, HCV, HCC with thrombus obliterating PV	Visualization Approach Duration of follow-up Thrombosis Recurrent bleeding Hepatic encephalopathy	Fluoroscopy and IVUS Endovascular 2 and 10 mo No No NR
Hong <i>et al</i> <sup>[7]</sup> 2012	53-year-old male with history of pancreatic teratoma treated with Whipple with clot at SMV and splenic veins	Visualization Approach Duration of follow-up Thrombosis Recurrent bleeding Hepatic encephalopathy	Fluoroscopy and IVUS Endovascular 1 and 3 mo No No NR

<sup>1</sup>The shunt was found to be thrombosed on POD #1 so the patient underwent ballooning of his stent and directed thrombolysis and was started on a therapeutic heparin. His hematocrit began to fall on the heparin but stabilized when the anticoagulation was held; <sup>2</sup>The shunt was found to be thrombosed on POD #2 and on POD #3 the patient had a recurrent upper gastrointestinal hemorrhage. A new shunt was placed and the patient had no further bleeding; <sup>3</sup>The patient had no encephalopathy at a 3-mo follow-up visit but was noted by an outside hospital to have encephalopathy 6 mo after shunt placement when the patient was hospitalized for concern for partial small bowel obstruction. The patient's home lactulose and rifaximin had been held; when these medicines were resumed her encephalopathy resolved. NR: Not reported; PVT: Portal vein thrombus; CRC: Colorectal cancer; TIPs: Transjugular intrahepatic portosystemic shunting; HCV: Hepatitis C virus; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; SMV: Superior mesenteric vein; PV: Portal vein; CT: Computed tomography.

way, they were able to avoid a percutaneous transabdominal approach altogether. It should be noted that the third patient included in this series was not a cirrhotic patient but rather had portal and SMV clots due to a pancreatic tumor; we chose to include this patient in our review to demonstrate the feasibility of the procedure but appreciate that his underlying pathophysiology may be different from the others presented.

## SELECTION OF CANDIDATES

Candidates likely to benefit from mesocaval shunting include those with recurrent variceal bleeds who have failed prior medical and endoscopic therapies. Traditionally, TIPS has been employed in these patients to alleviate portal pressures. We propose that mesocaval shunting be offered to patients who are not TIPS can-

didates, particularly the group awaiting transplant, as there are not yet mortality data for mesocaval shunting and the mortality benefit of other portosystemic shunts, including TIPs, has been questioned<sup>[10]</sup>.

As in our illustrative case, PVT, for example, are known to make TIPS more difficult and result in lower success rates, ranging from 40%-75%<sup>[11]</sup>. In some cases, when PVT is chronic, TIPS is not only difficult but actually technically impossible as in order to re-establish flow, there must be functional vessels surrounding the planned recanalized clot segment. This intact vasculature is often absent in those with chronic PVT as intrahepatic vessels may have atrophied while extrahepatic vessels form collaterals at high risk of bleeding<sup>[6]</sup>. Given the relatively high prevalence of PVT in cirrhotics, up to 5% to 16% of patients at the time of liver transplantation, mesocaval shunting has the potential to offer a therapy



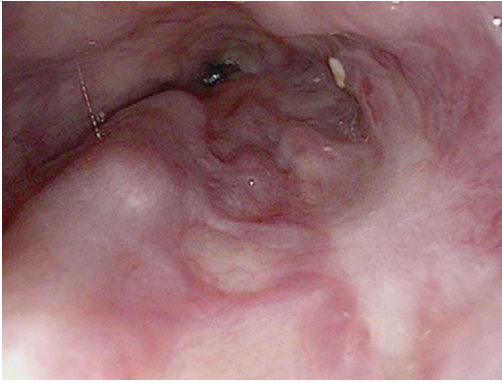


Figure 1 Grade III varices in distal esophagus on pre-procedure esophagogastroduodenoscopy.

to a large group of patients who were previously thought to be without options, particularly in those patients whose PVT prohibits them from receiving a liver transplant<sup>[12]</sup>.

Prior to endovascular placement of mesocaval shunts, the other option for patients in this scenario was surgical shunt placement. Historically, surgically placed portosystemic shunts have had high mortality<sup>[5]</sup>. While experienced centers are reporting improved operative mortality<sup>[13,14]</sup>, the ability to replicate these lower mortality rates at smaller, less experienced centers remains to be seen. Furthermore, several of the patients in published percutaneous mesocaval shunt cases to date were thought to be poor surgical shunt candidates due to a history of prior abdominal surgeries and/or anatomy of their PVT<sup>[6,8,9]</sup>.

If a patient is felt to be appropriate for consideration of mesocaval shunt placement, assessment of cross-sectional anatomy should be undertaken with computed tomography or magnetic resonance imaging of the abdomen to assist in procedural planning. For successful shunt placement, the IVC and SMV should be aligned and proximal in an anatomic window without any significant vasculature or viscera interposed between the two vessels<sup>[8]</sup>. The IVC and SMV (or a large collateral) must be patent for shunt placement.

## POTENTIAL PITFALLS

There are a few potential pitfalls we consider with placement of a mesocaval shunt. First, similar to the surgical expertise required for safe surgical shunt placement, institutional interventional radiologic expertise will be required to safely recommend this procedure and this may not be available at all centers. This procedure, unlike TIPS, has not been reported to be performed in the setting of active variceal bleeding and thus there are no data to support its safety in that setting.

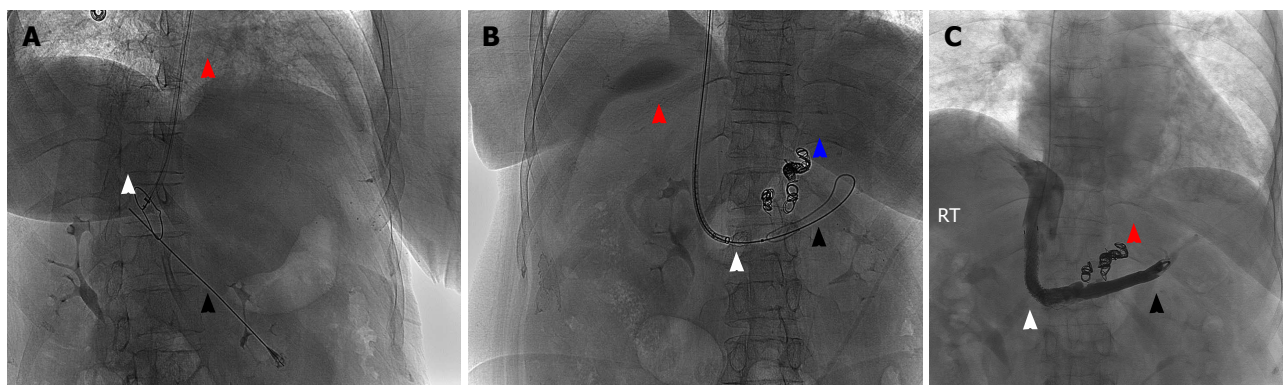
The most serious risk is that of procedure-related hemorrhage due to puncture of proximal vasculature. As noted by Hong *et al.*<sup>[7]</sup> both the SMV and infrahepatic IVC lack any surrounding solid organs that could provide tamponade during shunt placement, creating a risk of major hemorrhage. Of the published cases to date, one noted intraabdominal hemorrhage-multiple small

bowel hematomas - which was successfully treated conservatively with intravenous fluid, transfusion support and discontinuation of anticoagulation<sup>[6]</sup>. If the cannulated vessels require predilation prior to shunt placement, this risk of bleeding is likely increased<sup>[7]</sup>. Finally, while they did not experience intrabdominal hemorrhage despite use of an uncovered stent, Moriarty *et al.*<sup>[9]</sup> note that use of an uncovered stent certainly increases risk of bleeding and recommend using covered stents and/or balloons to minimize this risk. In addition to procedural technique, we anticipate that, similar to other invasive procedures in cirrhotic patients, platelet counts influence the risk of hemorrhage. The cases reviewed here unfortunately do not provide patient platelet counts or other measures of clotting function.

In addition to the potential for vessel perforation, depending on each individual patient's anatomy, there are risks of perforation of different structures. If an intestinal perforation is created, risk of sepsis, hemorrhage and/or abscess formation will certainly be increased<sup>[8]</sup>. In two reported cases, the transabdominal approach necessitated intestinal puncture<sup>[6,9]</sup>. In one case the track was transcolonic while the other approach was transgastric. Bowel preparation and antibiotic prophylaxis were administered in the transcolonic case and neither case resulted in sepsis. Although there were no reported infectious complications in the cases we reviewed, this risk should be underscored as it is likely not negligible in cirrhotic patients with impaired immunity. In addition to the risk of intestinal perforation, other nearby viscera are at risk of puncture as well. If the pancreas is punctured, both hemorrhage and pancreatitis are potential risks<sup>[8]</sup>. One published case to date notes pancreatic bisection and reports that serum amylase levels were within the normal range for at least five days post-operatively<sup>[7]</sup>.

In the six cases reviewed, two cases reported subsequent shunt occlusion and need for further revision, a rate of 33% in our, appreciably, small series. Shunt thrombosis is an important outcome as it presumably puts the patient at risk for further portal hypertension, variceal formation and variceal bleeding. In the first of the two cases complicated by occlusion, lack of flow through the shunt was noted on both Doppler and CT on POD #2<sup>[6]</sup>. The patient underwent repeat angiography and had ballooning and directed thrombolysis of his stent with subsequent patency on 5 mo follow-up angiogram. The stent was again noted to be occluded on 12 mo follow-up angiography but he had no further gastrointestinal bleeding and no attempt to revise the shunt further was made. In the second case complicated by shunt occlusion, lack of flow through the shunt was noted on POD #2 on CT<sup>[9]</sup>. This was attributed to the severe angle of the initial shunt placement and its proximity to the wall of the IVC. This patient experienced upper gastrointestinal bleeding on POD #3 and underwent repeat angiography with stent replacement and had a patent stent and no further bleeding at 3 mo follow-up.

Finally, as with any form of portosystemic shunting, we anticipate that these patients will have higher rates



**Figure 2** Intra-procedure steep oblique fluoroscopy of upper abdomen during mesocaval shunt placement. First, a needle is inserted percutaneously (black arrowhead) and directed through the portal vein remnant at a target snare placed in the inferior vena cava (white arrowhead) via internal jugular (IJ) access sheath (red arrowhead) (A). The wire is then threaded from its original percutaneous entry via the needle through the IJ sheath (red arrowhead) so that it extends from the IJ and is seen coiling in the splenic vein (black arrowhead) (B). The unexpanded stent graft (white arrowhead) is passed over the wire using Seldinger technique with fluoroscopic guidance (B). Coils are placed in varices (blue arrowhead) (B). Shuntogram with contrast 22 mo post-procedure shows functioning mesocaval shunt (white arrowhead) with tip in the splenic vein (black arrowhead) and absent varices (C). Previous coils in the varices are still visible (red arrowhead).

of hepatic encephalopathy (HE) than patients without portosystemic shunting. Given the limited numbers of patients who have undergone percutaneous or endovascular mesocaval shunt placement, there are no data to evaluate rates of HE with these shunts vs TIPS or surgical shunt creation but presumably the rate is similar, around 30%<sup>[15,16]</sup>. In the cases reviewed above, only one case reported on the presence or absence of encephalopathy. In that case, the patient was noted to have no encephalopathy during index hospitalization or at 3 mo follow-up but was noted to be encephalopathic 6 mo post-operatively when her lactulose and rifaximin were held at an outside hospital for partial small bowel obstruction<sup>[8]</sup>. Her encephalopathy reportedly resolved with resumption of these medications.

## BENEFITS

As above, mesocaval shunting offers a treatment for bleeding varices for patients who otherwise face a high mortality with virtually no options. It can be offered to patients with PVT who cannot undergo TIPS and may be best utilized as a bridge to transplant. Furthermore, if an IVUS is utilized, vessels are directly visualized, avoiding the blind puncture method used in TIPS<sup>[7]</sup>. As with other similar endovascular procedures, we anticipate a lower mortality with this less invasive approach vs surgical shunt placement. Regardless, the majority of the published patients to date were not felt to be surgical candidates<sup>[6,7,9]</sup>.

In the six adult cases published to date, two stents thrombosed within two days post-operatively while the remaining four remained patent<sup>[6,9]</sup>. Of the two patients with shunt thrombosis, one had a recurrent upper gastrointestinal bleed. In this case, it was postulated that the severe angle of the initial stent placement may have contributed to turbulence and subsequent thrombosis<sup>[9]</sup>. In both cases, subsequent shunt revision was performed and the revised shunts remained open during five and

three months follow-up respectively. One shunt ultimately lacked patency at 12 mo follow-up but the patient had no further bleeding up to 14 mo follow-up. In summary, all reported cases have follow-up ranging from 1 to 14 mo in which, with the exception of the post-operative day 2 bleed noted above, there were no further variceal bleeding episodes. These are promising results in light of the known 60% 1-year risk of rebleeding and 33% 1-year mortality in patients who survive an episode of variceal hemorrhage<sup>[17]</sup>.

In addition to offering a rescue therapy for a group of patients with minimal options, mesocaval shunting has an advantage compared to local variceal therapy, it will result in lower portal pressures and thus will reduce recurrent ascites as well reducing the risk of variceal bleeding. As noted by Garcia-Tsao and Bosch in a recent review, judgment of treatment success of varices should include mindfulness about the impact of variceal treatment on other complications of portal hypertension—ascites, jaundice, encephalopathy—rather than artificially isolating the treatment's impact on variceal bleeding alone<sup>[17]</sup>. Finally, given that the presence of portal vein thrombosis is no longer thought to be an absolute contraindication to transplant<sup>[18]</sup>, for those patients that are transplant eligible, placement of a mesocaval shunt may enable survival to the operating room table for transplant, a pressing concern given that our most recent national statistics are dire. In 2014, 1821 patients died while awaiting transplant and an additional 1290 were removed from the waiting list as they were felt to be “too sick” for transplant<sup>[19]</sup>.

## CONCLUSION

As reviewed above, endovascular mesocaval shunting is a feasible procedure that offers a promising intervention to a patient population with few options and one-year mortality as high as 20%<sup>[1]</sup>. TIPS has been shown to be an effective intervention to prevent recurrent variceal

bleeding<sup>[2]</sup> and mesocaval shunting provides similar physiologic relief of portal pressure in patients who are not TIPS candidates. Like TIPS, mesocaval shunting avoids major surgery and may require less anesthesia than a surgical shunt approach. Furthermore, it can be offered to patients who are not surgical candidates. Mesocaval shunting alleviates portal hypertension, a key component of reducing the rate of variceal bleeding, and one that will potentially reduce recurrent ascites as well. The patient who stands to gain the most from this procedure has recurrent variceal bleeds, has failed endoscopic and medical therapies, cannot undergo TIPS due to anatomy and needs a bridge to transplant to minimize the chance of further decompensating while awaiting an organ. In order to have successful shunt placement, these patients must have alignment between IVC and SMV or SMV collaterals. Potential procedural complications include perforation of nearby vessels or viscera which could result in hemorrhage, sepsis, pancreatitis or abscess formation as well as stent thrombosis. Placement of a portosystemic shunt will also increase the risk of hepatic encephalopathy although there is little data to compare mesocaval shunts to surgical shunts or TIPS. To date, several approaches and imaging techniques have been utilized by reporting groups, notably including one approach that avoids transabdominal puncture<sup>[7]</sup>. In the cases reported, all have prevented rebleeding for the post-procedural monitoring period after initial shunt or initial shunt revision<sup>[6,8,9]</sup>. Further research should be performed to better assess outcomes - variceal bleeding, hepatic encephalopathy rates and mortality - in these patients compared to standard-of-care controls so that the benefits of this promising technique may be maximized.

## ACKNOWLEDGMENTS

Authors Jessica Davis, Albert K Chun and Marie L Borum have all contributed to this manuscript and endorse the data and conclusions within.

## REFERENCES

- 1 **Zipprich A**, Garcia-Tsao G, Rogowski S, Fleig WE, Seufferlein T, Dollinger MM. Prognostic indicators of survival in patients with compensated and decompensated cirrhosis. *Liver Int* 2012; **32**: 1407-1414 [PMID: 22679906 DOI: 10.1111/j.1478-3231.2012.02830.x]
- 2 **Rössle M**, Haag K, Ochs A, Sellinger M, Nöldge G, Perarnau JM, Berger E, Blum U, Gabelmann A, Hauenstein K. The transjugular intrahepatic portosystemic stent-shunt procedure for variceal bleeding. *N Engl J Med* 1994; **330**: 165-171 [PMID: 8264738 DOI: 10.1056/NEJM199401203300303]
- 3 **Hayes PC**, Davis JM, Lewis JA, Bouchier IA. Meta-analysis of value of propranolol in prevention of variceal haemorrhage. *Lancet* 1990; **336**: 153-156 [PMID: 1973480 DOI: 10.1016/0140-6736(90)91668-Z]
- 4 **Laine L**, Cook D. Endoscopic ligation compared with sclerotherapy for treatment of esophageal variceal bleeding. A meta-analysis. *Ann Intern Med*. 1995; **123**: 280-287 [PMID: 7611595 DOI: 10.7326/0003-4819-123-4-199508150-00007]
- 5 **Villeneuve JP**, Pomier-Layrargues G, Duguay L, Lapointe R, Tanguay S, Marleau D, Willems B, Huet PM, Infante-Rivard C, Lavoie P. Emergency portacaval shunt for variceal hemorrhage. A prospective study. *Ann Surg* 1987; **206**: 48-52 [PMID: 3496860 DOI: 10.1097/0000658-198707000-00007]
- 6 **Nyman UR**, Semba CP, Chang H, Hoffman C, Dake MD. Percutaneous creation of a mesocaval shunt. *J Vasc Interv Radiol* 1996; **7**: 769-773 [PMID: 8897349]
- 7 **Hong R**, Dhanani RS, Louie JD, Sze DY. Intravascular ultrasound-guided mesocaval shunt creation in patients with portal or mesenteric venous occlusion. *J Vasc Interv Radiol* 2012; **23**: 136-141 [PMID: 22221479 DOI: 10.1016/j.jvir.2011.09.029]
- 8 **Bercu ZL**, Sheth SB, Noor A, Lookstein RA, Fischman AM, Nowakowski FS, Kim E, Patel RS. Percutaneous Mesocaval Shunt Creation in a Patient with Chronic Portal and Superior Mesenteric Vein Thrombosis. *Cardiovasc Intervent Radiol* 2015; **38**: 1316-1319 [PMID: 25189666 DOI: 10.1007/s00270-014-0989-8]
- 9 **Moriarty JM**, Kokabi N, Kee ST. Transvenous creation of a mesocaval shunt: report of use in the management of extrahepatic portal vein occlusion. *J Vasc Interv Radiol* 2012; **23**: 565-567 [PMID: 22464719 DOI: 10.1016/j.jvir.2011.09.023]
- 10 **Khan S**, Tudur Smith C, Williamson P, Sutton R. Portosystemic shunts versus endoscopic therapy for variceal rebleeding in patients with cirrhosis. *Cochrane Database Syst Rev* 2006; **(2006)**: CD000553 [PMID: 17054131 DOI: 10.1002/14651858.CD000553.pub2]
- 11 **Wils A**, van der Linden E, van Hoek B, Pattynama PM. Transjugular intrahepatic portosystemic shunt in patients with chronic portal vein occlusion and cavernous transformation. *J Clin Gastroenterol* 2009; **43**: 982-984 [PMID: 19417681 DOI: 10.1097/MCG.0b013e31819706a4]
- 12 **Stine JG**, Shah NL, Argo CK, Pelletier SJ, Caldwell SH, Northup PG. Increased risk of portal vein thrombosis in patients with cirrhosis due to nonalcoholic steatohepatitis. *Liver Transpl* 2015; **21**: 1016-1021 [PMID: 25845711 DOI: 10.1002/lt.24134]
- 13 **Kokudo T**, Bonard E, Gillet M, Kokudo N, Halkic N. Reappraisal of shunt surgery for extrahepatic portal vein obstruction in adults: Report of a single-center case series. *Hepatol Res* 2015; **45**: 1307-1311 [PMID: 25731583 DOI: 10.1111/hepr.12512]
- 14 **Orloff MJ**. Fifty-three years' experience with randomized clinical trials of emergency portacaval shunt for bleeding esophageal varices in Cirrhosis: 1958-2011. *JAMA Surg* 2014; **149**: 155-169 [PMID: 24402314 DOI: 10.1001/jamasurg.2013.4045]
- 15 **Sanyal AJ**, Freedman AM, Shiffman ML, Purdum PP, Luketic VA, Cheatham AK. Portosystemic encephalopathy after transjugular intrahepatic portosystemic shunt: results of a prospective controlled study. *Hepatology* 1994; **20**: 46-55 [PMID: 8020904]
- 16 **Riggio O**, Merlli M, Pedretti G, Servi R, Meddi P, Lionetti R, Rossi P, Bezzi M, Salvatori F, Ugolotti U, Fiaccadori F, Capocaccia L. Hepatic encephalopathy after transjugular intrahepatic portosystemic shunt. Incidence and risk factors. *Dig Dis Sci* 1996; **41**: 578-584 [PMID: 8617139]
- 17 **Garcia-Tsao G**, Bosch J. Varices and Variceal Hemorrhage in Cirrhosis: A New View of an Old Problem. *Clin Gastroenterol Hepatol* 2015; **13**: 2109-2117 [PMID: 26192141 DOI: 10.1016/j.cgh.2015.07.012]
- 18 **Jamieson NV**. Changing perspectives in portal vein thrombosis and liver transplantation. *Transplantation* 2000; **69**: 1772-1774 [PMID: 10830208]
- 19 **Kim WR**, Lake JR, Smith JM, Skeans MA, Schladt DP, Edwards EB, Harper AM, Wainright JL, Snyder JJ, Israni AK, Kasiske BL. Liver. *Am J Transplant* 2016; **16** Suppl 2: 69-98 [PMID: 26755264 DOI: 10.1111/ajt.13668]

P- Reviewer: Contini S, Hoff DAL S- Editor: Qi Y  
L- Editor: A E- Editor: Li D





Basic Study

# Assembly and release of infectious hepatitis C virus involving unusual organization of the secretory pathway

Miriam Triyatni, Edward A Berger, Bertrand Saunier

Miriam Triyatni, Roche Innovation Center, Roche Pharma Research and Early Development, F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland

Miriam Triyatni, Edward A Berger, Bertrand Saunier, Molecular Structure Section, Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD 20892-3210, United States

Bertrand Saunier, Unité de Virologie Structurale, Institut Pasteur and CNRS UMR 3569, 75724 Paris, France

**Author contributions:** Triyatni M designed and performed experiments and analyzed the data; Berger EA provided facilities and resources for research, analyzed the data, and contributed to editing the manuscript; Saunier B designed most and performed some experiments, analyzed the data and wrote the manuscript.

**Supported by** Intramural Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases (Project No. 1 ZIA AI000733-15: Enveloped Virus Glycoprotein/ Receptor Interactions) to Edward A Berger, PhD (MSS, LVD, DIR, NIAID); and ORISE Senior Fellow award (Award No. 1238-1238-03: Department of Energy/Oak Ridge Institute for Science and Education) to Bertrand Saunier, MD, PhD.

**Institutional review board statement:** Approved the use of serum IgG of a patient cured from an HCV infection for *in vitro* studies (Hôpital and Institut Cochin, Paris).

**Institutional animal care and use committee statement:** Not applicable.

**Conflict-of-interest statement:** Saunier B, Triyatni M and Berger EA are co-inventors on NIH-owned patent #US 9052321 B2 on the HCV particle production system.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on

different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Bertrand Saunier, MD, PhD, Unité de Virologie Structurale, Institut Pasteur and CNRS UMR 3569, Centre François Jacob, 28 rue du Docteur Roux, 75724 Paris, France. [bsaunier@pasteur.fr](mailto:bsaunier@pasteur.fr)  
**Telephone:** +33-01-45688855  
**Fax:** +33-01-45688993

**Received:** January 31, 2016

**Peer-review started:** February 1, 2016

**First decision:** March 25, 2016

**Revised:** May 16, 2016

**Accepted:** June 1, 2016

**Article in press:** June 3, 2016

**Published online:** July 8, 2016

## Abstract

**AIM:** To determine if calnexin (CANX), RAB1 and alpha-tubulin were involved in the production of hepatitis C virus (HCV) particles by baby hamster kidney-West Nile virus (BHK-WNV) cells.

**METHODS:** Using a siRNA-based approach complemented with immuno-fluorescence confocal microscope and Western blot studies, we examined the roles of CANX, RAB1 and alpha-tubulin in the production of HCV particles by permissive BHK-WNV cells expressing HCV structural proteins or the full-length genome of HCV genotype 1a. Immuno-fluorescence studies in producer cells were performed with monoclonal antibodies against HCV structural proteins, as well as immunoglobulin from the serum of a patient recently cured from an HCV infection of same genotype. The cellular compartment stained by the serum immunoglobulin was also observed



in thin section transmission electron microscopy. These findings were compared with the JFH-1 strain/Huh-7.5 cell model.

**RESULTS:** We found that CANX was necessary for the production of HCV particles by BHK-WNV cells. This process involved the recruitment of a subset of HCV proteins, detected by immunoglobulin of an HCV-cured patient, in a compartment of rearranged membranes bypassing the endoplasmic reticulum-Golgi intermediary compartment and surrounded by mitochondria. It also involved the maturation of N-linked glycans on HCV envelope proteins, which was required for assembly and/or secretion of HCV particles. The formation of this specialized compartment required RAB1; upon expression of HCV structural genes, this compartment developed large vesicles with viral particles. RAB1 and alpha-tubulin were required for the release of HCV particles. These cellular factors were also involved in the production of HCVcc in the JFH-1 strain/Huh-7.5 cell system, which involves HCV RNA replication. The secretion of HCV particles by BHK-WNV cells presents similarities with a pathway involving caspase-1; a caspase-1 inhibitor was found to suppress the production of HCV particles from a full-length genome.

**CONCLUSION:** Prior activity of the WNV subgenomic replicon in BHK-21 cells promoted re-wiring of host factors for the assembly and release of infectious HCV in a caspase-1-dependent mechanism.

**Key words:** Membrane rearrangements; Hepatitis C virus; Flavivirus replicon; Virus assembly and secretion; Host cellular factors

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Our system for production of authentic infectious hepatitis C virus (HCV) in non-humanized, non-hepatic cells involves the rearrangement of inner cellular membranes triggered by the replication of flaviviruses. The present results suggest that this feature relies on the re-wiring of host factors that usually contribute to the secretion of glycoproteins to generate an unusual secretory pathway. This model offers a new way to study the properties of free HCV particles, *i.e.*, independently from lipoproteins.

Triyatni M, Berger EA, Saunier B. Assembly and release of infectious hepatitis C virus involving unusual organization of the secretory pathway. *World J Hepatol* 2016; 8(19): 796-814 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i19/796.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i19.796>

## INTRODUCTION

Hepatitis C virus (HCV) genotype 1 has accounted for up to 70% of HCV infections worldwide and is more often

resistant than the other genotypes to the combination of pegylated interferon plus ribavirin, the standard of care until recently<sup>[1]</sup>. New antiviral drugs specifically target either the viral protease NS3, the protein NS5A or the RNA polymerase NS5B and are able to clear HCV infections in a higher number of individuals with a shorter duration of treatment than the standard of care<sup>[2]</sup>. However, a significant proportion of patients requiring treatment still cannot be cured<sup>[3]</sup>, does not have access to treatment<sup>[4]</sup> and/or will need the development of a vaccine<sup>[5]</sup>. Experimental models are being developed<sup>[6]</sup>, but may not cover all aspects of the pathogenesis of hepatitis C, such as the mechanism by which antibodies prevent the spread of infection<sup>[7]</sup>.

In an effort to develop alternative systems, we have established a model in which infectious HCV production is independent from its replicon-mediated RNA replication<sup>[8]</sup>, hence circumventing limitations inherent to existing cell culture models<sup>[9]</sup>. For instance, the replication of HCV genotype 1 isolates in hepatocellular carcinoma cell lines is inefficient or generates adaptive mutations interfering with viral fitness *in vivo*. Briefly, our system relies on the amplification provided by a dual bacteriophage RNA polymerase plasmid system (referred to as P2B) that generates large amounts of HCV RNA transcripts from a T7 promoter-driven plasmid in the cytoplasm of baby hamster kidney (BHK)-21 cells conditioned by a lineage II West Nile virus (WNV) subgenomic replicon<sup>[8]</sup>. We observed that the WNV replicon in this cell line created an environment permissive for the assembly and release of infectious HCV of various genotypes, including virions of strains H77 (genotype 1a)<sup>[10]</sup> or Con1 (genotype 1b)<sup>[11]</sup>; these virions infected human liver slices *ex vivo*<sup>[8]</sup>.

BHK-WNV cells produce infectious HCV particles independently from lipoprotein biosynthesis. The fact that these particles retain the possibility to interact with lipoproteins *in vitro*<sup>[8]</sup> is in line with previous results<sup>[12-14]</sup> and supports the view that HCV particles may interact with lipoproteins in a second step (*e.g.*,<sup>[15,16]</sup>) and not necessarily co-assemble with them (*e.g.*,<sup>[17]</sup>). Potential mechanisms for WNV-conditioned BHK cells producing highly infectious HCV virions could relate to common genomic features between the *Flavivirus* and *Hepacivirus* genera within the *Flaviviridae* family<sup>[18]</sup>. In addition, several flaviviruses infect hepatocytes<sup>[19,20]</sup> and may use similar host factors as HCV for their production<sup>[21-25]</sup>.

Our previous results showed that after curing BHK cells from the WNV subgenomic replicon, the production and release of infectious HCV particles were still observed for a while<sup>[8]</sup>. In addition, although recombinant expression of HCV structural genes in cultured cells, including in human hepatocytes<sup>[26]</sup>, usually leads to their retention in the endoplasmic reticulum (ER)<sup>[27]</sup>, BHK-WNV cells released infectious HCV particles even in the absence of HCV non-structural genes<sup>[8]</sup>. These findings suggested that, while the viral replication machineries played no direct role in the secretion process, the reorganization of intracellular membranes induced by the WNV subgenomic replicon contributed to the permissiveness

of BHK cells.

In mammalian cells, conventional protein traffic from the ER to the Golgi complex passes through the membrane clusters of the ER-Golgi intermediate compartment (ERGIC), the marker of which is the lectin ERGIC-protein of 53 kDa (ERGIC-53). ER-derived cargo is first shuttled to the ERGIC in a coat protein (COP) II-dependent step and subsequently to the Golgi apparatus in a second vesicular transport step involving COP I-coated vesicles, RAB and ARF GTPases, as well as cytoskeletal networks; incoming vesicles can also be recycled to the ER in a COP I-mediated process<sup>[28]</sup>. The ERGIC contributes to the concentration, folding, and quality control of newly synthesized proteins and is required for the production of several viral pathogens<sup>[29]</sup>. N-linked glycosyl antenna are matured by Golgi-resident enzymes along with glycoproteins' progression from the proximal to the distal Golgi *cisternae*, then across the plasma membrane for their secretion, *via* the *trans*-Golgi/endosomal network.

In the present work, we studied the potential involvement of components of this secretory machinery in the production of HCV particles by BHK-WNV cells and compared this model with the JFH-1 strain/Huh-7.5 cells model. We show that, upon expression of an HCV genome of genotype 1a in these cells, a subpopulation of HCV proteins were recruited through calnexin (CANX) to a cytoplasmic compartment of rearranged membranes. The small GTPase RAB1 was involved in the formation of this compartment. The secretion of HCV particles produced from a full-length genome required also the N-linked glycosylation of HCV envelope glycoproteins and  $\alpha$ -tubulin ( $\alpha$ -TUB), a component of microtubules and, surprisingly, the activity of cysteine protease caspase-1. As our understanding of the HCV virus life cycle has recently widened to alternative routes of transmission, elucidating mechanisms at work in BHK-WNV cells could shed some light on the production of HCV *in vivo*.

## MATERIALS AND METHODS

### Cell cultures

BHK-21 cells were grown in E-MEM supplemented with 10% fetal bovine serum (FBS; HyClone, United States), Glutamax-I (Gibco, Life Technologies, United States); BHK cells harboring WNV lineage II SG-replicon encoding *Renilla* luciferase<sup>[30]</sup>, herein called BHK-WNV cells, were propagated in D-MEM supplemented with 10% FBS, Glutamax-I and 5  $\mu$ g/mL blasticidin. Huh-7.5 cells were maintained in D-MEM supplemented with 10% FBS, Glutamax-I, non-essential amino acid mix (Gibco, Life Technologies, United States).

### Plasmid constructs

A previously described system of two plasmids (P2B = dual phage RNA polymerases plasmid system for generation of T7 RNA polymerase in the cytoplasm) was used to amplify the cytoplasmic transcription of a plasmid encoding HCV bicistronic particles (HCVbp) under the control of bacteriophage T7 DNA-dependent

RNA polymerase's cognate promoter<sup>[8]</sup>; a sequence encoding an HDV antigenomic ribozyme<sup>[31]</sup> was added at its C termini; as a consequence, HCV transcripts were uncapped and have correct 5'- and 3'-ends. p90 HCVcon-FLlongpU<sup>[10]</sup> and pH-SGNeo (L + I) encoding a SG-replicon of the same strain with cell-culture adaptive mutations<sup>[32]</sup> were used as templates to construct HCVbp-coding plasmids. pHCV STp7 is a pcDNA3.1(+)-based plasmid (Life Technologies, United States) encoding the structural genes (*core*, *E1*, *E2*) plus *p7* of HCV genotype 1a linked to the human cytomegalovirus (CMV) immediate early promoter. HCVbp-4cys are HCVbp particles with a sequence encoding a tetracysteine tag<sup>[33]</sup> inserted in the part of their genome encoding NS5A<sup>[8]</sup>.

### Antibodies and cellular markers

Anti-E2 (ALP98 and AP33)<sup>[34]</sup>, and anti-E1 (A4) monoclonal antibodies were used for Western blot (WB) analysis. Conformational AP33 monoclonal antibody, human serum from an HCV patient (genotype 1a) that recognizes conformational HCV core and E2 protein subspecies by WB and anti-HCV core peptides 9-21 (C1) or 7-50 (Thermo Scientific, United States) monoclonal antibodies were used for confocal microscopy analysis. Neutralizing monoclonal antibody E16 recognizes WNV E by WB<sup>[35]</sup>. Antibodies against various cellular proteins are as followed: ERGIC-53 (Alexis Biochemicals, United States), GDI (Life Technologies, United States); RAB1 and atlastin (Santa Cruz Biotechnology, United States); CANX and GM130 (Abcam, United States); and calreticulin (Cell Signaling Technology, United States). For immunofluorescence analysis, the secondary antibodies used were Alexa Fluor 488-, 594- or 635-conjugated goat anti-mouse and anti-human antibodies, and Alexa Fluor 594-, 635- or -680 conjugated goat anti-rabbit antibodies from Molecular Probes (Life Technologies, United States). Mito Tracker Orange CMTMRos, TC-ReASH II In-Cell Tetracysteine Tag detection kit and Paclitaxel (Taxol) Oregon Green® 488 Conjugate were obtained from Molecular Probes (Life Technologies, United States). Nuclei were counterstained with DAPI (blue). The cells were observed with a laser-scanning confocal microscope and the pictures were deconvoluted. Bar scales = 5  $\mu$ m.

### Production of HCV particles in mammalian cells

One day before transfection, BHK-WNV cells were seeded at a density of  $6 \times 10^6$  cells per 162 cm<sup>2</sup> flask. Plasmids encoding the HCV sequence under the control of the CMV early promoter or the bacteriophage T7 promoter were transfected using Lipofectamine® LTX with Plus™ Reagent according to the manufacturer's protocol (Life Technologies, United States). Culture medium after transfection was D-MEM supplemented with 10% FBS, non-essential amino acid mix, Glutamax-I, 25 mmol/L Hepes and 3.7 g/L sodium bicarbonate. Cells were incubated at 37 °C for 3 d in an incubator with a 95% air/5% CO<sub>2</sub> atmosphere saturated in humidity. Culture media were harvested, centrifuged at 30000  $\times$  g for 30

min at 4 °C to remove cell debris; then clarified supernatants (SN) were filtered with 0.45 µm PVDF membrane (Millipore, United States) and centrifuged at 100000 × g for 3 h at 4 °C. Pellets were suspended in ice-cold Tris-buffered saline solution (TBS; Quality Biologicals, United States) containing protease inhibitor cocktail (Roche, United States). HCVcc (Huh-7.5-produced JFH-1) was obtained by electroporating IVT RNA into Huh-7.5 cells as described<sup>[36]</sup>; the SN was concentrated, and aliquots of the virus stock were stored at -80 °C.

### Gene knockdown in BHK-WNV cells using siRNAs

CANX, melanocortin 5 receptor (MC5R), α-TUB were analyzed for their effects on HCV release. BHK-WNV cells were treated with the corresponding siRNA (2 siRNAs per target gene; Dharmacon, United States) for 2 d, re-seeded and transfected the next day with HCV-coding plasmid. Cells and supernatants were harvested 48 h later, and analyzed by WB. To verify the effects of these genes on HCV release, CANX and α-TUB cDNAs were synthesized from BHK-WNV mRNA, and cloned into pTracer-CMV/Bsd (Invitrogen, United States). The corresponding pTracer plasmid was co-transfected with HCV-coding plasmid in siRNA-treated BHK-WNV cells to show the specificity of their knockdown.

### Effect of RAB1 on HCV production by BHK-WNV cells

To study co-localization of HCV and RAB1 in the producer cells, BHK and BHK-WNV cells were transfected with HCVbp-coding plasmid, and the following day, re-seeded on 8-well chambered coverglass (5 × 10<sup>3</sup> cells/well). Two days later, cells were fixed and permeabilized as above, then incubated with serum from an HCV-infected patient (HCV genotype 1a) and anti-RAB1 antibodies. BHK-WNV cells were then treated with siRNA against RAB1 (2 siRNA per target gene; Dharmacon, United States) for 2 d, re-seeded, and the following day were transfected with HCVbp-coding plasmid. Cells and SN were harvested 3 d after transfection; cell lysates and particles released into SN were analyzed by WB.

### Electron microscopy

BHK-WNV cells seeded in a 6-well plate (2.5 × 10<sup>5</sup> cells) were transfected with HCVbp-coding plasmid. Three days later, cells were fixed in 2% glutaraldehyde in 0.1 mol/L sodium cacodylate for 1 h at RT, then at 4 °C, overnight. Cells were subsequently processed for transmission electron microscopy (TEM) as described<sup>[37]</sup>.

### Effects of siRNAs on HCVcc production and release in Huh-7.5 cells

Cells were treated with the siRNAs (CANX, α-TUB, RAB1A, RAB1B, or non-target) for 48 h; the same number of cells was reseeded on 24-well plates, and the next day was inoculated with HCVcc at MOI 0.5. Cells were harvested daily, total RNA was extracted using lysis buffer of TaqMan Gene Expression Cells-to-ct kit (Life Technologies, United States) and HCV RNA was analyzed by RT-TaqMan<sup>TM</sup> PCR with a StepOne Plus

thermocycler (Applied Biosystems, United States).

### IF experiments

For live staining after inoculation with HCVbp-4cys, Huh-7.5 cells were incubated with two cell-permeant reagents: The arsenical ReASH-ethane dithiol (Life Technologies) that fluoresces upon binding a tetra-cysteine tag<sup>[33]</sup>, and Oregon Green 488 paclitaxel bis-acetate (TubulinTracker Green Reagent; Life Technologies, United States) that binds to polymerized alpha tubulin. Immuno-fluorescence studies on fixed cells were performed on stacks of images (a dozen cells per IF condition); co-localization was analyzed using Coloc 2 Plugin ([http://imagej.net/Coloc\\_2](http://imagej.net/Coloc_2)) with ImageJ software (<https://imagej.nih.gov/ij/>).

### Experimental reproducibility

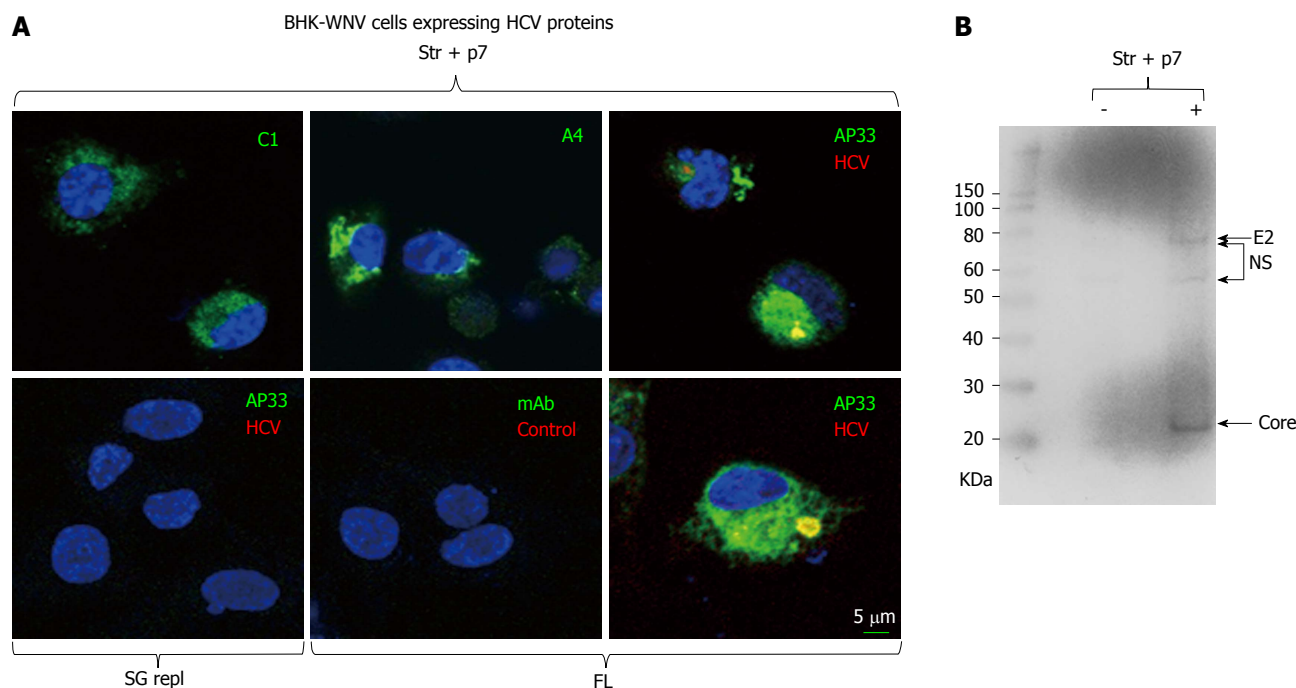
Unless specified otherwise, all shown results are representative of at least three independent experiments.

## RESULTS

### Identification of a compartment possibly linked to the assembly of HCV particles in the cytoplasm of BHK-WNV cells

To gain insight regarding the mechanisms by which BHK-WNV cells produce HCV particles, we transfected the cells with either of three different plasmids based on HCV genotype 1a (HCV1a): (1) HCV structural and p7 genes<sup>[38]</sup> linked to a CMV promoter (Str + p7); (2) HCV subgenomic replicon (SG repl); or (3) full-length HCV genome (FL) linked to the bacteriophage T7 promoter; for (2) and (3), the cells were also co-transfected with the P2B (dual phage RNA polymerases) plasmid system for generation of T7 RNA polymerase in the cytoplasm. The cells were then permeabilized and incubated with monoclonal antibodies (mAbs) against HCV1a core (C1), E1 (A4) and E2 (AP33) or a control mAb. Like in hepatic cells expressing a HCV genome<sup>[22]</sup>, we observed that the three HCV1a mAbs stained a large and heterogeneous area in the cytoplasm of BHK-WNV cells expressing the structural genes, while the control mAb did not stain these cells (Figure 1A). AP33 is a neutralizing mAb that targets a linear epitope within a flexible region of E2<sup>[34]</sup> and, as expected, did not stain cells not expressing HCV1a structural genes (Figure 1A). In contrast, a neutralizing IgG from the serum of a patient (HCV1a sIgG) who had been cured from hepatitis C of this genotype<sup>[8]</sup> stained a more limited and heterogeneously shaped compartment of the cytoplasm of BHK-WNV cells expressing a full-length genome of HCV1a, which was also stained by AP33 but not by control human sIgG (Figure 1A); of note, HCV1a sIgG did not stain BHK-WNV cells expressing a HCV1a subgenomic replicon. A mitochondrion-specific staining surrounded this compartment (Figure 2A). By WB, beside non-specific bands (NS), HCV1a sIgG specifically recognized bands with apparent molecular weights of 21 kDa and 75 kDa (Figure 1B) whose sizes coincided with those reported for HCV core and E2, both known to





**Figure 1** Immunodetection of hepatitis C virus proteins of genotype 1a expressed in baby hamster kidney-West Nile virus cells. **A:** A plasmid encoding Str + p7 of HCV strain H77 (genotype 1a) from an early human cytomegalovirus promoter or a system of plasmids (P2B) expressing a subgenomic replicon or genome (FL) of same genotype in the cytoplasm (8) were transfected in BHK-WNV cells; after 2 d, IF study was performed with monoclonal antibodies (green) targeting core 9-21 (C1), envelope E1 (A4) and E2 (AP33) (28) glycoproteins of strain H77 (all IgG1a), or anti-rabbit Ig mouse Ab (mAb) of same isotype; and with human serum IgG (red) obtained from a patient recently cured from an infection of same genotype (HCV) or uninfected (control). Nuclei were counterstained with DAPI (blue) and cells were observed with a laser-scanning confocal microscope; **B:** BHK-WNV cells were either mock transfected (-) or transfected with a system of plasmids expressing the genome of H77 strain (+); after 3 d, cell lysates were prepared and human anti-HCV IgG tested in (a) were used as a Western blot probe. The scale on the left shows molecular weight markers. HCV: Hepatitis C virus; BHK-WNV: Baby hamster kidney-West Nile virus; Str + p7: Structural and p7 genes.

display most HCV epitopes recognized by human B cell (<http://www.iedb.org/>).

To further characterize the cytoplasmic compartment stained by HCV1a sIgG, thin sections of BHK-WNV cells expressing HCV genome of genotype 1a were observed by TEM. The sub-compartment was easily identified due to the presence of mitochondria in its periphery (Figure 2B)<sup>[39]</sup>. As previously, it was comprised of large vesicles containing viral-like particles - not observed in cells expressing an HCV1a subgenomic replicon, *i.e.*, without structural genes - as well as membranous web with vesicle packets, both typical of membrane rearrangements triggered by WNV replication<sup>[8]</sup>. This time, we also observed a mix of rearranged cellular organelles (ER, Golgi *cisternae*) with a few additional viral-like particles (Figure 2B). Altogether, these results support the view that HCV particles assembled in BHK-WNV cells were specifically recognized by sIgG from a cured patient.

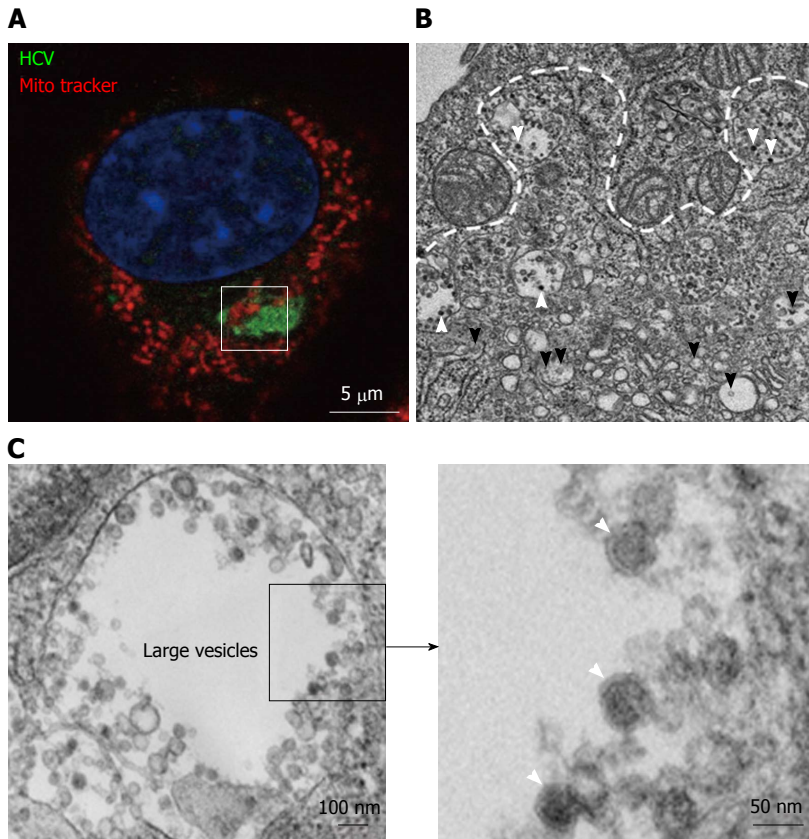
#### **CANX recruits a subset of HCV proteins into a compartment of BHK-WNV cells for the release of HCV particles**

Classically, the secretion of glycoproteins involves lectins of the ER, CANX and calreticulin (CRT), microtubule-dependent ER-to-Golgi vesicular traffic<sup>[40,41]</sup>, maturation of their carbohydrates in the Golgi apparatus and the secretion machinery of the trans-Golgi network<sup>[42]</sup>. We

had previously observed that brefeldin A prevented the secretion of HCV by BHK-WNV cells, while it did not affect that of exosomes<sup>[8]</sup>. Brefeldin A is an inhibitor of GBF1 that activates small-sized GTPase ARF1 and, in turn, COPI-dependent traffic<sup>[43]</sup>. The effect of brefeldin A was consistent with HCV particles being released *via* a classical secretion path. Two components of the classical secretion path, CANX and microtubules, have been previously implicated in HCV life cycle in other systems<sup>[27,44,45]</sup>. Since what appeared to be the assembly compartment displayed major membrane rearrangements, we explored whether a classical secretion path was involved in the production of HCV particles by BHK-WNV cells.

In BHK-WNV cells expressing an HCV1a genome, most CANX was unexpectedly detected in the same compartment as HCV proteins stained by HCV1a sIgG (Figure 3A, left panels). After a treatment of BHK-WNV cells by CANX siRNA, which decreased the level of CANX (Figure 3A, right panels, and Figure 3C, top panels), HCV1a sIgG staining was no longer localized in this compartment but, instead, displayed a scattered pattern in the cytoplasm (Figure 3A, right panels). The effect of knocking down CANX and  $\alpha$ -TUB expression on HCV production was analyzed. Figure 3B shows WB analysis of E2 glycoprotein in particles purified from the supernatants and cell lysates as a marker for HCV production. Treatment of BHK-WNV cells by control or





**Figure 2** Transmission electron microscopy view of a hepatitis C virus compartment forming in baby hamster kidney-West Nile virus cells. BHK-WNV cells were transfected with a mix of HCVbp-expressing and P2B plasmids (8). A: IF analysis showing a compartment recognized by human serum HCV IgG (green) surrounded by mitochondria (red); nucleus is counterstained with DAPI (blue). The white square delimits an area similar to that displayed in the next panel; B: Thin section observed by transmission electron microscopy: White arrowheads: Electron-dense viral particles in large vesicles; black arrowheads: Nascent viral particles in traffic vesicles; white dotted line: Limit of mitochondria surrounding the compartment containing viral particles (cf. area within white square in previous panel); C: Left panel: Example of large vesicle that develops in the cytoplasm of permissive BHK-WNV cells upon expression of full-length HCV genome; Right panel: Magnification of viral particles. HCV: Hepatitis C virus; BHK-WNV: Baby hamster kidney-West Nile virus.

MC5R siRNA did not alter the production or release of HCV (Figure 3B). In contrast, siRNA-mediated down-regulation of CANX or  $\alpha$ -TUB significantly reduced the secretion of HCV (Figure 3B). Albeit incomplete, the siRNA effect on CANX expression was sufficient to abolish BHK-WNV permissiveness for HCV production (Figure 3C, top panels).

Hsp70 or Hsp90 were chosen as controls since they associate with exosomes<sup>[46,47]</sup>, which are particulate materials secreted by BHK cells yet distinct from HCV particles<sup>[8]</sup>. After a treatment of BHK-WNV cells with  $\alpha$ -TUB siRNA, the releases of Hsp70- and Hsp90-containing particles in the supernatant decreased (Figure 3B and C, bottom panels), in accordance with the requirement for a functional cellular traffic to release exosomes and consistent with an accumulation of Hsp70 in the producer cells. In contrast, HCV E2 did not accumulate in BHK-WNV cells (Figure 3B).

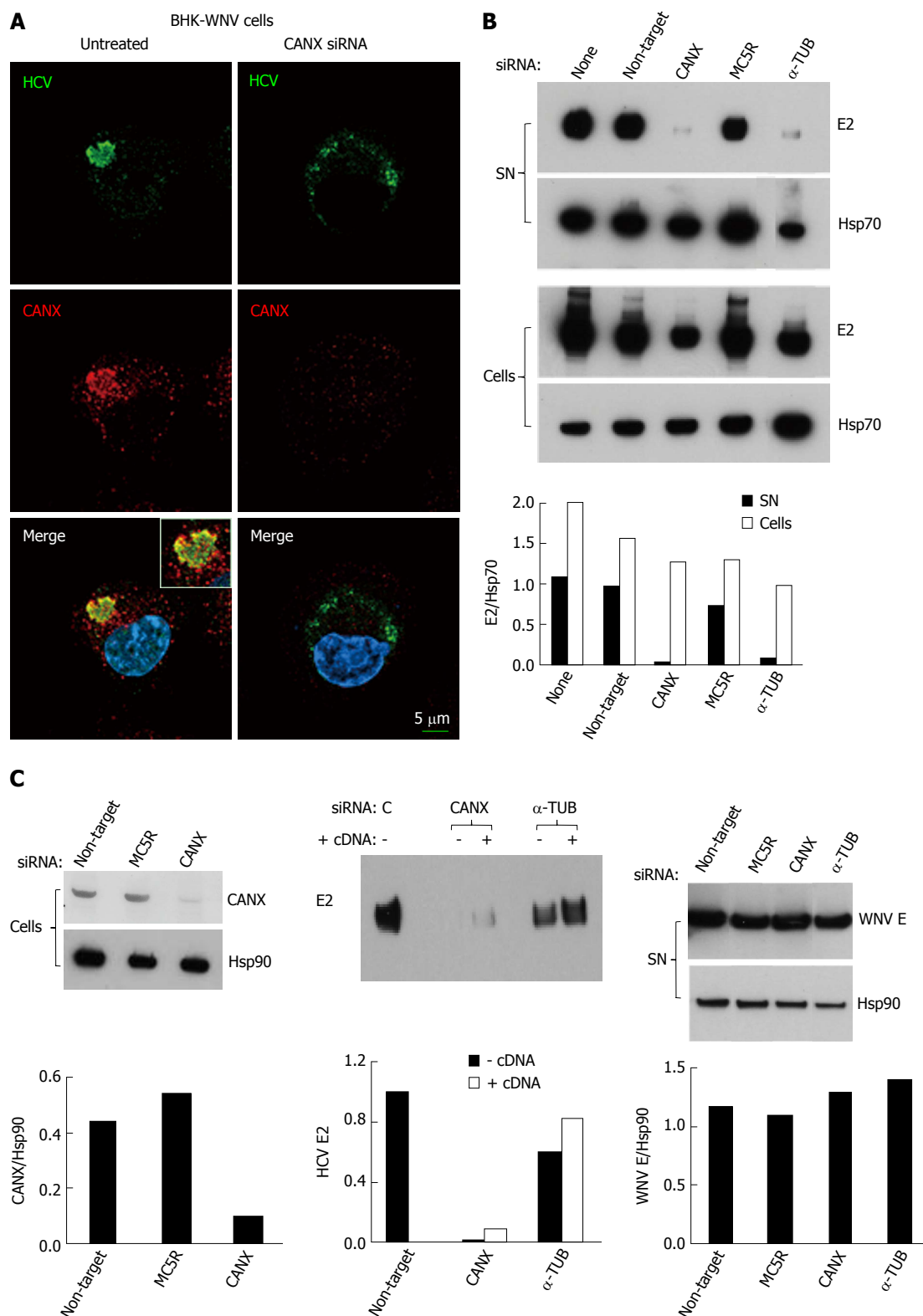
To evaluate whether inhibition of HCV release could result from an off-target effect, CANX and  $\alpha$ -TUB cDNAs cloned from BHK-WNV cells were inserted into mammalian expression plasmids. In BHK-WNV cells treated by CANX siRNA, expression of recombinant CANX gene partially restored both CANX levels (Figure 4) and HCV

secretion (Figure 3C, middle panels). A similar result was observed with  $\alpha$ -TUB cDNA expressed in  $\alpha$ -TUB siRNA-treated BHK-WNV cells (Figure 3C, middle panels). The release of WNV particles upon expression of WNV structural genes was minimally affected by either siRNA treatment (Figure 3C, bottom panels). These results show that HCV production in BHK-WNV cells specifically involved CANX and  $\alpha$ -TUB.

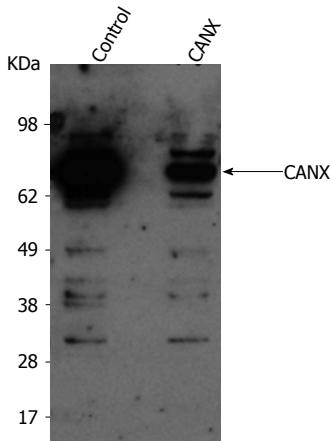
#### ***N-glycans of HCV envelope proteins are required for the non-classical secretion of HCV particles by BHK-WNV cells***

Together with CANX, CRT usually ensures the proper conformation of nascent glycoproteins in the ER lumen. However, CRT expression was lower in BHK-WNV cells than in parental cells (Figure 5A) and did not co-localize with HCV proteins detected by the immune sIgG (Figure 5B). An additional C-type lectin, ERGIC-53, transports properly folded glycoproteins from the ER to the Golgi apparatus. Its expression was also slightly lower in BHK-WNV cells (Figure 5A) and did not co-localize with this pool of HCV proteins (Figure 5B).

Treatment of producer cells with deoxynojirimycin (DNJ), which prevents the binding of nascent glyco-



**Figure 3** Involvement of calnexin and alpha-tubulin in the release of hepatitis C virus particles by baby hamster kidney-West Nile virus cells. A: BHK-WNV cells were treated, or not, with CANX siRNA and, three days later, transfected with the HCV-coding and P2B plasmids. Two days later, IF was performed with anti-HCV serum of Figure 1 (green) and anti-CANX antibody (red) followed by confocal microscopy analysis; nucleus were counterstained with DAPI (blue); B: Top panels: BHK-WNV cells were treated with the indicated siRNA for 2 d, then transfected with a plasmid encoding full length HCV (HCVbp) in the cytoplasm. Contents in E2 envelope protein of both supernatant (SN) and cell lysate (Cells) were analyzed 2 d later by Western blot (WB); Bottom panel: Densitometry analysis; C: Left panels: BHK-WNV cells were treated with the indicated siRNA for 2 d and content in CANX was analyzed by WB; Hsp90 was used as a control; Middle panel: BHK-WNV cells were transfected with the indicated siRNA for 2 d and cells were then reseeded and transfected the next day with the HCV-coding plasmid together with a control plasmid (-) or one expressing the cDNA of the knocked-down transcript (+). Two days later, HCV materials released in SN were analyzed by WB; Right panels: BHK-WNV treated as in (B) were transfected with a plasmid encoding West Nile virus (WNV) structural genes (core, prM and E). Two days later, materials released in the SN were analyzed by WB with an antibody recognizing WNV E (29); Hsp90 was used as a control; Bottom panels: Densitometry analyses. CANX: Calnexin;  $\alpha$ -TUB: Alpha-tubulin; HCV: Hepatitis C virus; BHK-WNV: Baby hamster kidney-West Nile virus; C: Control siRNA.



**Figure 4** Baby hamster kidney-West Nile virus cells treated with the indicated siRNA (on top). After 2 d, cells from both conditions were reseeded and transfected the next day with either a control plasmid or plasmid expressing CANX cDNA, respectively in control or CANX siRNA-treated BHK-WNV cells. The following day, content in CANX was analyzed by Western blot in cell lysates. CANX: Calnexin; BHK-WNV: Baby hamster kidney-West Nile virus.

proteins to CANX<sup>[42]</sup>, modified the glycosylation and stability of HCV envelope proteins in BHK-WNV cells (Figure 5C, bottom panel), which was accompanied by a lower amount of HCV particles released (Figure 5C, top panels). As expected, DNJ treatment resulted in decreased resistance of carbohydrates on E1 to a digestion by endo- $\beta$ -N-acetylglucosaminidase H (Endo-H). In contrast, O-glycosylation<sup>[48]</sup> inhibitors PAG and ALL did not display any effect on HCV production or E1 glycosylation (Figure 5C, middle panels). A similar pattern was observed with BHK cells bearing a Dengue 2 subgenomic replicon (Figure 6). Therefore, the decreased production of HCV in the presence of DNJ resulted from a lack of maturation of N-linked glycosyl antenna on HCV envelope proteins, consistent with the effect of CANX siRNA observed.

#### **RAB1 and conformational HCV protein subspecies co-localize within a compartment of reorganized ER and Golgi components**

We looked for additional cellular factors that could contribute to the secretion of HCV particles. RAB1 exerts a key control on the ER-to-Golgi traffic. In parental BHK-21 cells, RAB1 was spread in the cytoplasm, but co-localized with HCV proteins expressed after transfection (Figure 7A). The WNV subgenomic replicon enhanced RAB1 staining, coalescing into a few membranous-like spots within which most HCV proteins detected by HCV1a sIgG were localized (Figure 7B). Treatment of BHK-WNV cells with RAB1 siRNA greatly reduced HCV protein expression (Figure 7D); concomitantly, fewer HCV particles were released (Figure 7D).

Although maturation of HCV envelope glycoproteins did not involve a classical secretory pathway, some carbohydrate residues at the surface of released HCV particles became resistant to a treatment by Endo-H<sup>[42]</sup>. This indicates that the ER-to-Golgi machinery as well as the Golgi apparatus contributed to HCV production in BHK-WNV cells. Interestingly, in these cells, the

expression of RAB1 GDP dissociation inhibitor (GDI)<sup>[49]</sup> was enhanced and co-localized with HCV proteins in a pattern reminiscent of RAB1's (Figure 8). In addition, the expression of Atlastin 1, a high molecular weight GTPase that is involved in maintenance of the ER compartment<sup>[50]</sup> and fusion of ER tubules<sup>[51]</sup>, as well as in ER-to-Golgi trafficking<sup>[52]</sup>, was up-regulated in BHK-WNV cells compared to parental BHK-21 cells (Figure 9). We detected its presence within the CANX-enriched compartment formed in BHK-WNV cells (Figure 10), as well as that of p115/USO1<sup>[53]</sup>, required for membrane fusion of ERGIC vesicles with the Golgi apparatus. We also detected the presence of GM130/GOLGA2 (Figure 11), which contributes to the progression of glycoproteins between Golgi cisternae<sup>[41]</sup>, which in turn correlates with the maturation of their carbohydrate residues<sup>[54]</sup>.

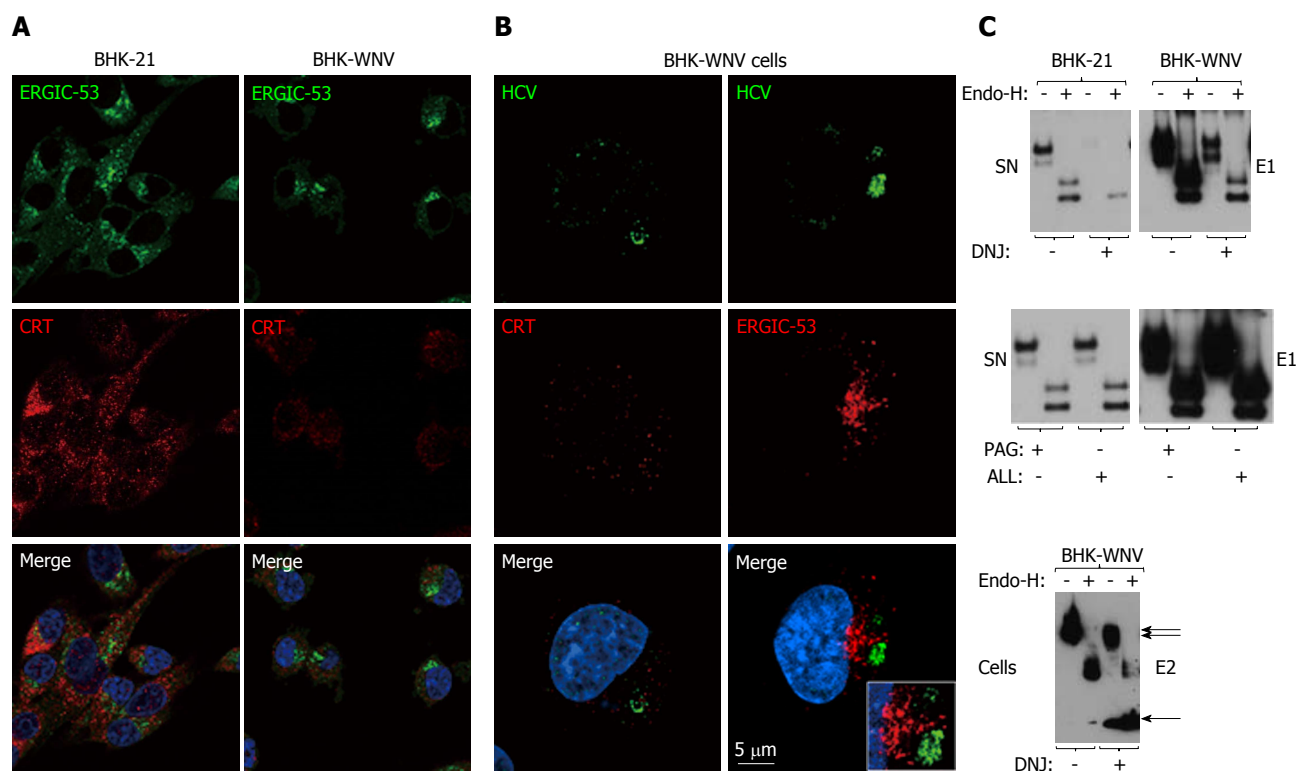
#### **Similarities between HCV productions in BHK-WNV and Huh-7.5 cells**

We next tested whether host factors assisting HCV production in BHK-WNV cells also play a role in the JFH-1 strain of genotype 2a/Huh-7.5 hepatocarcinoma cell paradigm. Mock transfection and non-target siRNA slightly decreased HCV production in Huh-7.5 cells infected with HCVcc. All other siRNAs tested further decreased the amount of HCV RNA, the inhibition ranging between 1 and 3 logs; HCV particle release in the supernatant was diminished as well (Figure 12A).

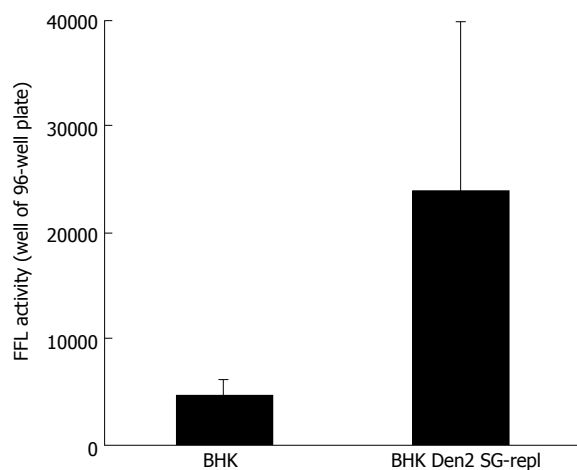
The human HCV serum used in BHK-WNV cells was of a different genotype than JFH-1; hence, for our IF studies we used a different antibody recognizing HCV core 7-50 peptide. There was not obvious co-localization of the staining it elicited in Huh-7.5 cells inoculated with HCVcc and that of the studied cellular factors. It has been reported that the localization of HCV proteins changed with time<sup>[55]</sup>. Therefore, we examined Huh-7.5 cells infected with the JFH-1 strain for over two weeks. HCV core staining was more widely spread within the cytoplasm and several hot spots were observed, similar to HCV proteins detected with the immune human serum in BHK-WNV cells. Core preferentially co-localized with CANX and RAB1 (Figure 12B); it did less so with tubulin.

Huh-7.5 cells were then inoculated with HCVbp-4cys particles (encoding a tetra-cysteine tag on NS5A) of genotype 1a produced in BHK-WNV cells (Figure 12C). In a live-cell immunofluorescence study, the pattern of NS5A-4cys was reminiscent of that observed with the same construct expressed in BHK-WNV cells<sup>[8]</sup>. However, this pattern was different from the staining of NS5A (without a tag) detected with an in-house antibody against a 48 aa-long peptide<sup>[8]</sup>, suggesting that distinct NS5A protein species were observed with two detection methods; each species could differentially affect HCV particle assembly and secretion. Interestingly, in Huh-7.5 cells, NS5A-4cys closely associated with microtubules (Figure 12C)<sup>[56]</sup>.

With minor differences, these results suggest that cellular factors involved in permissiveness of BHK-WNV cells are also required for long term HCV production in Huh-7.5 cells.



**Figure 5** Calnexin and N-linked glycosylation are involved in the release of hepatitis C virus particles via a non-classical secretion path in baby hamster kidney-West Nile virus cells. A: BHK-21 and BHK-WNV cells were transfected with the HCV expression plasmid system. IF was performed three days later with anti-ERGIC-53 (green) and anti-CRT (red) antibodies, followed by confocal microscopy analysis; B: Same protocol as in (A) with BHK-WNV cells transfected with the HCV-coding plasmids; C: Twelve hours after transfection with the HCV expression plasmid mix, parental BHK cells or BHK-WNV cells were treated, or not, with N- (DNJ) or O- (PAG, ALL) glycosylation inhibitors; materials released in SN (top and middle panels) or cell lysates (bottom panel) were collected, incubated with or without Endo-H and analyzed by Western blot. HCV: Hepatitis C virus; BHK-WNV: Baby hamster kidney-West Nile virus; ERGIC-53: Endoplasmic reticulum-Golgi intermediate compartment-protein of 53 kDa; CRT: Calreticulin; SN: Supernatants; DNJ: Deoxynojirimycin; Endo-H: Endo- $\beta$ -N-acetylglucosaminidase H.



**Figure 6** Huh-7.5 cells were incubated with hepatitis C virus reporter particles produced either in parental baby hamster kidney cells or in baby hamster kidney cells chronically replicating a dengue 2 subgenomic replicon (similar to the West Nile virus's). Infectivity was measured in target cells with a Firefly luciferase (FFL)-based reporter system, as previously described<sup>[6]</sup>. Error bars represent the SD in a representative experiment. BHK: Baby hamster kidney.

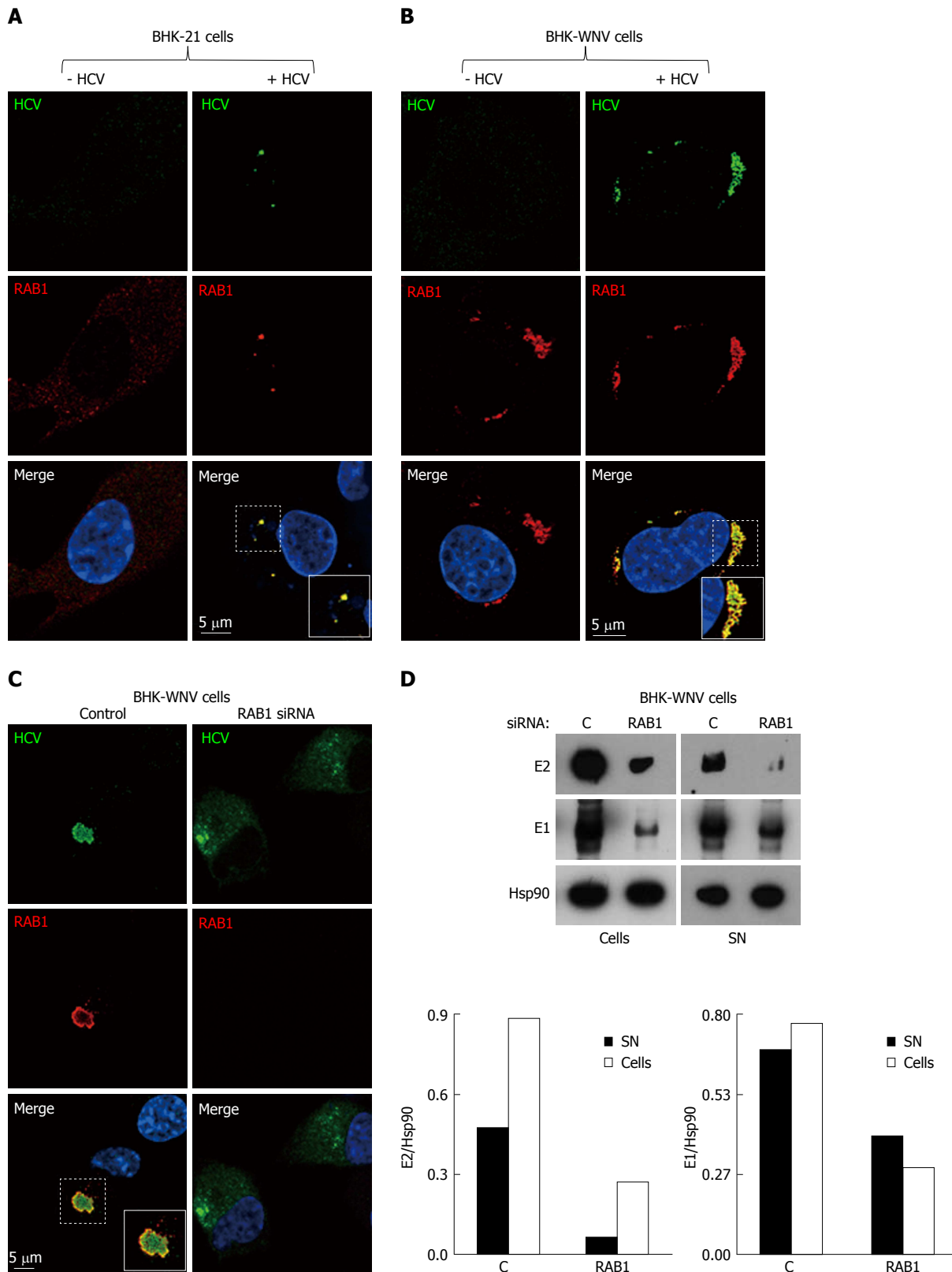
### Caspase-1 is required for the release of HCV particles via interplay with viral non-structural genes

Caspase-1 is a cysteine protease and the common denominator of some twenty NLR/ALR inflammasome

complexes identified so far and that contribute to host defenses against infections<sup>[57]</sup>. Cleavage sites for caspase-1 are present on HCV E2, NS2 and NS3 ([http://web.expasy.org/peptide\\_cutter/](http://web.expasy.org/peptide_cutter/)). They are well conserved between genotypes, but those on E2 and NS2 are generally missing in genotypes 6 and 2, respectively. Additional sites on E1, NS3, NS4B or NS5A are found only in particular HCV strains/isolates. Overall, an average of 3 (2 to 5) cleavage sites are found in HCV polyproteins.

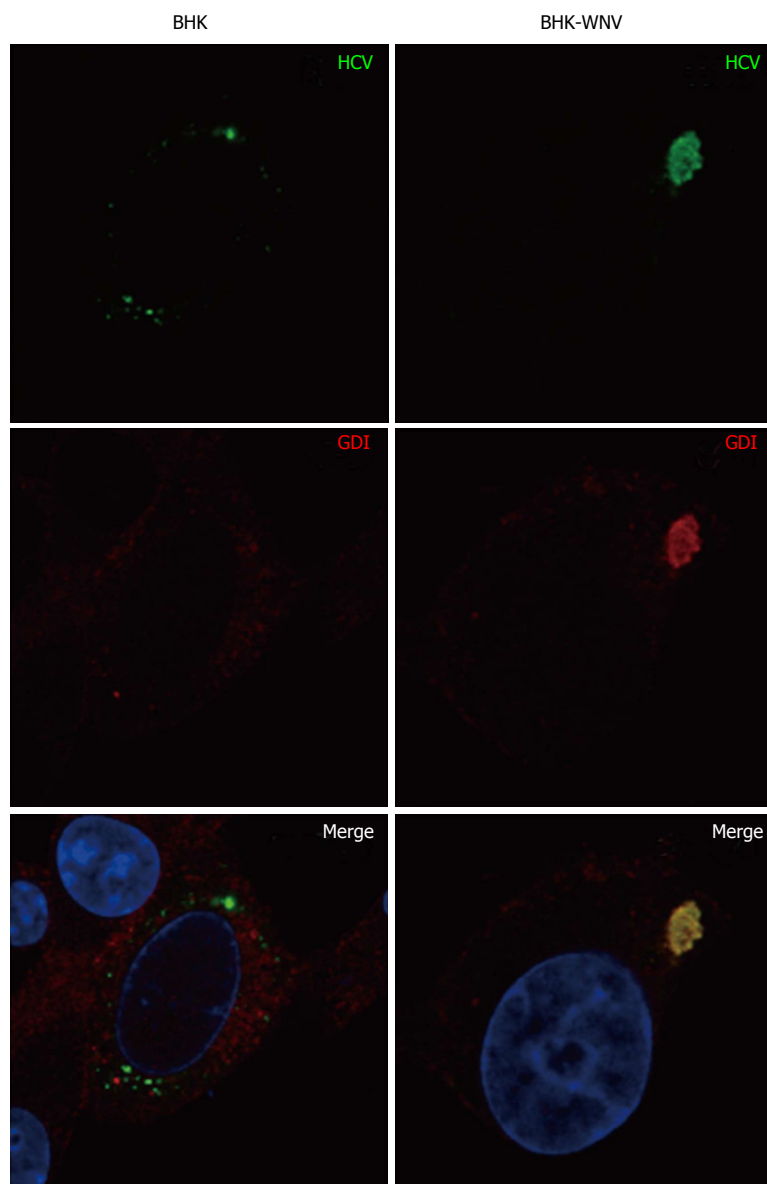
The HCV strain H77 polyprotein displays cleavage sites for caspase-1 at aspartate residues 728 (E2), 989 (NS2) and 1302 (NS3), but none for other caspases. This prompted us to test whether this protease could interfere with the production of HCV particles by BHK-WNV cells. We tested the effect of an inhibitor preferentially targeting caspase-1 (ZVAD-FMK) on the production of this strain of HCV by BHK-WNV cells. While neither production of envelope E1 protein nor its release by parental BHK cells changed with the inhibitor, the release of HCVbp particles by BHK-WNV cells was dramatically reduced (Figure 13A). This was associated with a different processing of the core protein, with a predominant size of 21 kDa in treated vs 23 kDa in untreated cells (Figure 13A). This observation is reminiscent of the shorter core protein in HCVcc released by Huh7.5.1 cells, compared to that of HCV progenies produced in human liver slices or primary





**Figure 7** Release of hepatitis C virus particles by baby hamster kidney-West Nile virus cells requires RAB1 in a cytoplasmic subcompartment. Three days after transfection with the HCV-coding and P2B plasmids, or not, IF of BHK-21 (A) or BHK-WNV (B) cells was performed with anti-HCV serum (green) and anti-RAB1 antibody (red), followed by confocal microscopy analysis; C: BHK-WNV cells treated (right panels) or not (left panels) with RAB1 siRNA were transfected with the HCV expression plasmid system; IF was performed as in (B); A-C: Nuclei were counterstained with DAPI (blue); D: BHK-WNV cells treated with RAB1 siRNA were transfected with the HCV expression plasmid system. Cells and SN were harvested 3 d later, and analyzed by Western blot; Hsp90 = control; bottom: Densitometry analysis. HCV: Hepatitis C virus; BHK-WNV: Baby hamster kidney-West Nile virus; SN: Supernatants.

hepatocytes<sup>[58]</sup>. In contrast, no clear effect of the inhibitor was observed on HCV production with BHK-WNV cells



**Figure 8** Parental or baby hamster kidney-West Nile virus cells were transfected to express hepatitis C virus genome in the cytoplasm and IF experiment was performed and analyzed as described throughout the manuscript. HCV: Hepatitis C virus; BHK-WNV: Baby hamster kidney-West Nile virus; GDI: GDP dissociation inhibitor.

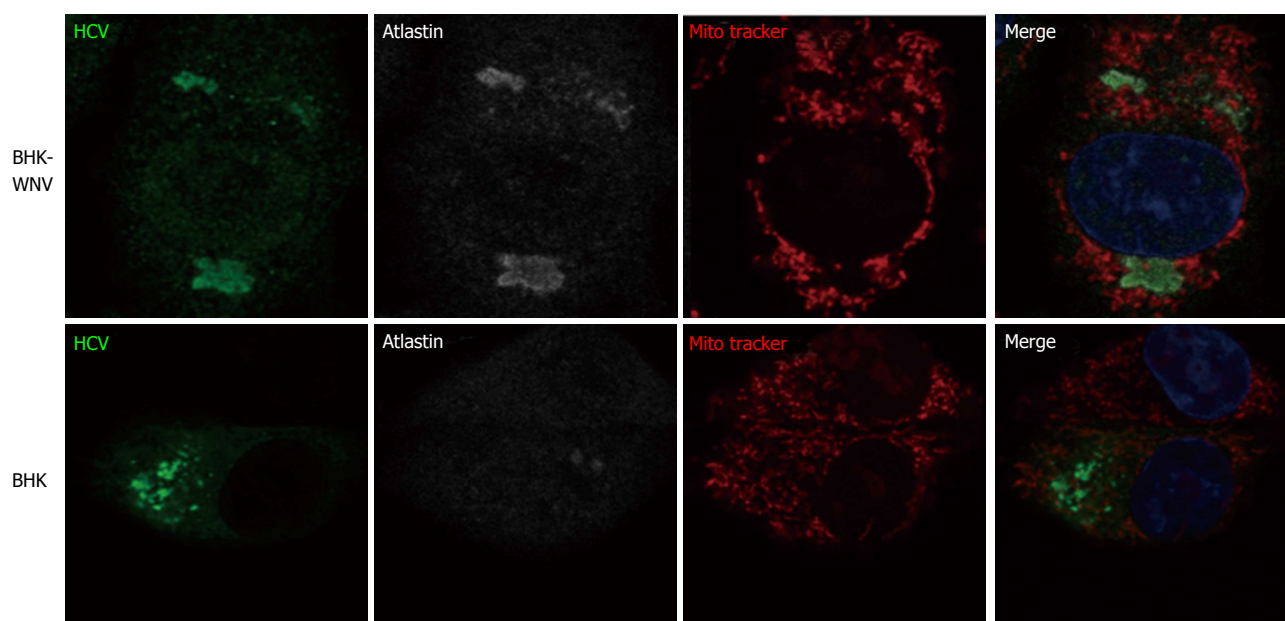
expressing only HCV structural genes, with or without p7 (Figure 13B).

## DISCUSSION

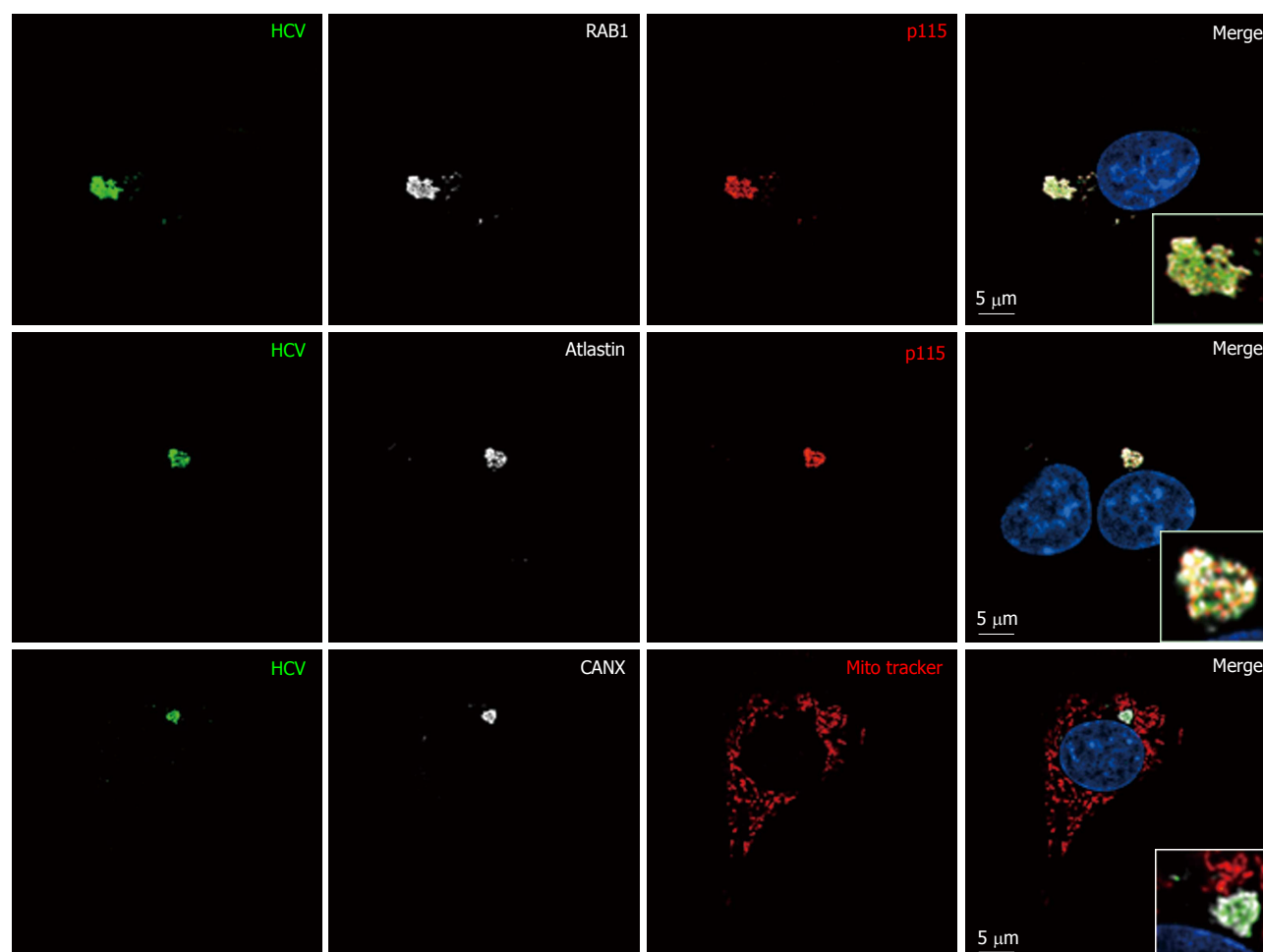
A classical secretion pathway has been implicated in the release of HCV virions<sup>[59]</sup> that are associated with apolipoprotein E (apoE) in Huh-7.5 cells<sup>[60]</sup> or other cell types<sup>[61]</sup>. In spite of a fully functional secretion pathway, parental BHK-21 cells, which do not express apoE, released only trace amounts of HCV particles<sup>[8]</sup>. Lack of apoE was overcome by the prior reorganization of intracellular trafficking by the replication of WNV (or dengue virus) for the production of infectious HCV particles (Figure 14). Epitopes detected by IgG from a patient cured of an infection of same genotype as the HCV strain used in this study (H77), are likely to be

amongst those displayed on native HCV virions. This pool of HCV protein isoforms/conformers represented only a fraction of the HCV structural proteins stained by monoclonal antibodies. It overlapped with a compartment of rearranged membranes. The presence of virus particles in this compartment supports the hypothesis that it could be the HCV assembly site in permissive BHK-WNV cells.

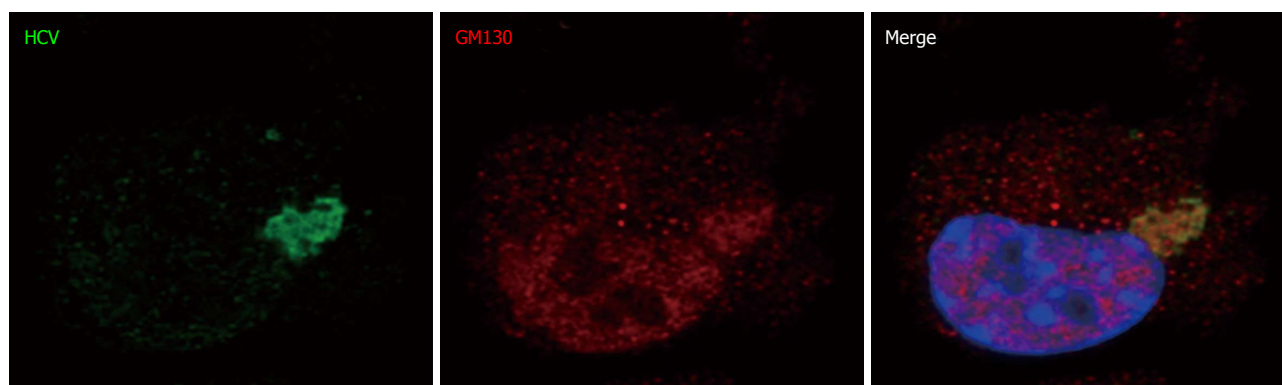
Some arguments are consistent with the involvement of a conventional secretion pathway in the production of HCV particles by BHK-WNV cells; other arguments suggest otherwise. On one hand, an involvement of the ERGIC implies a requirement for RAB1, which was the case for the formation of the assembly compartment, as well as for the production of HCV particles. In accordance, RAB1 and p115/USO1 were co-enriched in this compartment<sup>[43,62]</sup>. GBF1, an additional component of



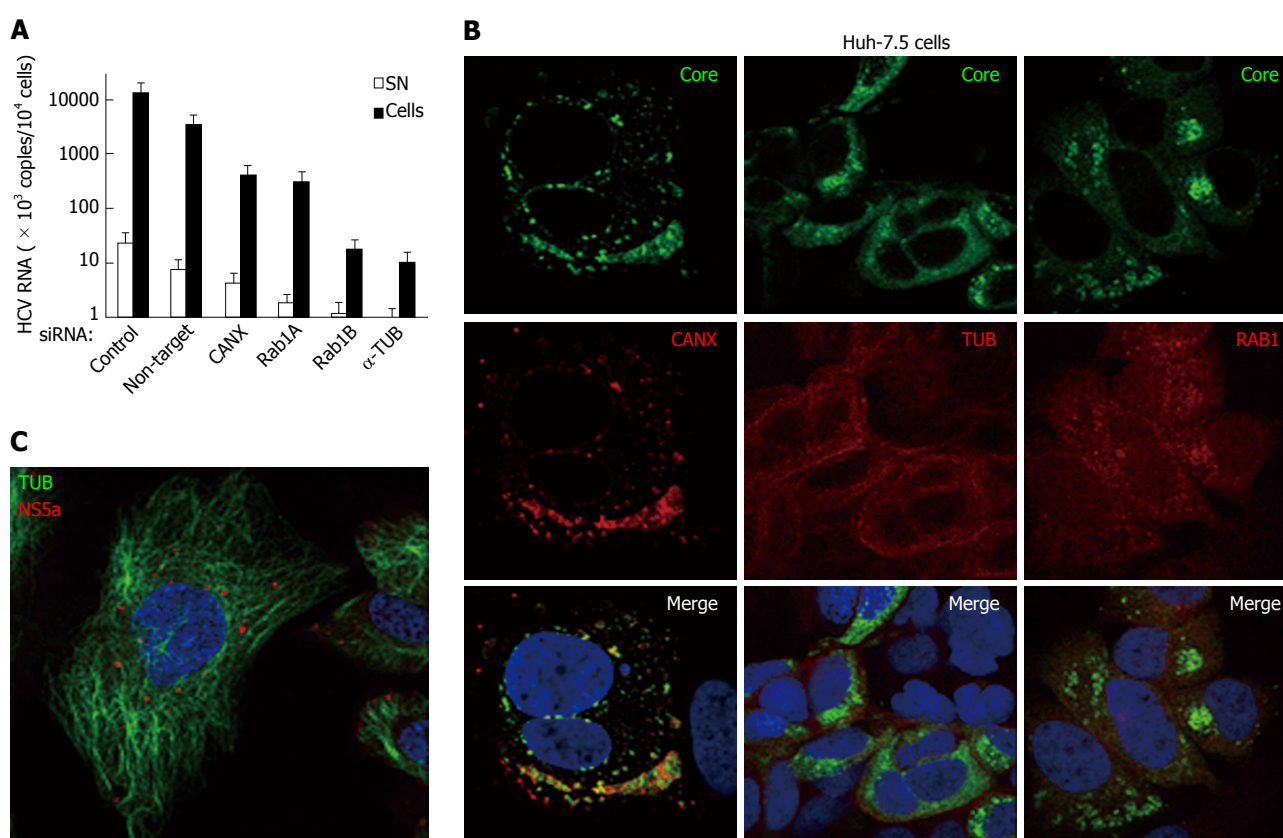
**Figure 9** Parental or baby hamster kidney-West Nile virus cells were transfected to express hepatitis C virus genome in the cytoplasm and IF experiment was performed and analyzed as described throughout the manuscript. HCV: Hepatitis C virus; BHK-WNV: Baby hamster kidney-West Nile virus.



**Figure 10** Endoplasmic reticulum-Golgi membrane remodelers (RAB1, p115 and atlantin) are recruited into the hepatitis C virus assembly compartment in baby hamster kidney-West Nile virus cells. Three days after transfection of BHK-WNV cells with the HCV expression plasmid system, IF was performed with anti-HCV serum (green) and anti-p115 (red), RAB1, Atlantin-1 or CANX (white) antibodies. Mitochondria were labeled with Mito-Tracker-Orange-CMTMRos (red). HCV: Hepatitis C virus; BHK-WNV: Baby hamster kidney-West Nile virus; CANX: Calnexin.



**Figure 11** This experiment was performed with baby hamster kidney-West Nile virus cells transfected to express hepatitis C virus genome in the cytoplasm and IF experiment was performed and analyzed as described throughout the manuscript (anti-GM130 monoclonal antibodies have been reported to cross-react with an unidentified protein of lower molecular weight). HCV: Hepatitis C virus.

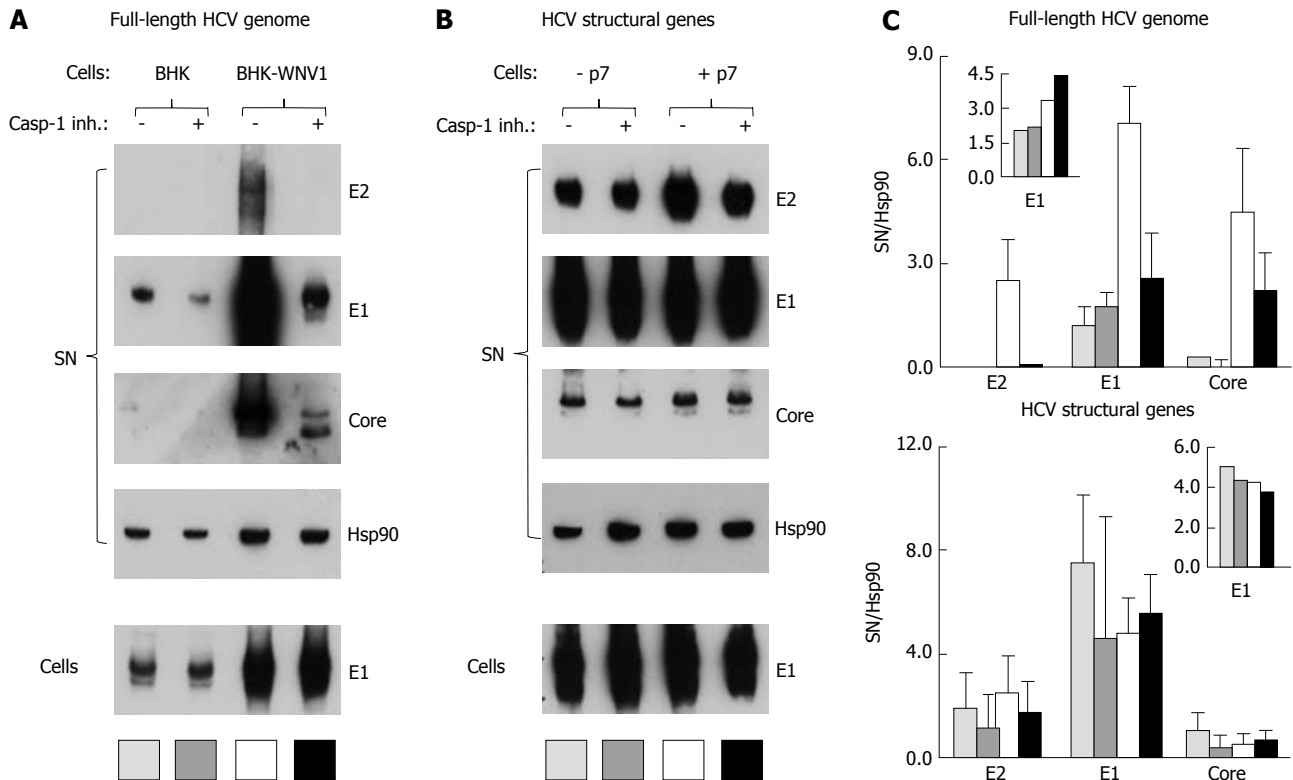


**Figure 12** Hepatitis C virus produced in cultured human hepatic cells involves same cellular factors as those enhancing hepatitis C virus production in baby hamster kidney-West Nile virus cells. **A:** Huh-7.5 cells were seeded on 24-well plates and the next day were transfected, or not, with siRNA, as indicated. After 2 d, cells were reseeded into 24-well plates and, the next day, incubated at a MOI = 0.5 with HCVcc produced with the JFH-1 strain in Huh-7.5 cells. At 3 dpi, HCV RNA contents were determined in the cells (closed bars) and SN (open bars) by RT-qPCR (TaqMan). Results are plotted on a log-scale; errors bars represent maximum variations observed in this assay; **B:** Huh-7.5 cells were electroporated with *in vitro*-transcribed genome of the JFH-1 strain and passaged for two weeks, then were seeded onto coverslips. Two days later, IF was performed with anti-HCV core 7-50 (green) and anti-human CANX, tubulin (TUB) or RAB1 antibodies (red); **C:** Huh-7.5 cells were inoculated with HCVbp-4cys produced in permissive BHK-WNV cells (8); 2 d later, the cells were incubated with both ReASH (red) and Taxol fluorophore conjugate (green); result representative of two independent experiments; **B** and **C:** Nuclei were counterstained with DAPI and cells were observed by confocal microscopy. CANX: Calnexin; HCV: Hepatitis C virus; BHK-WNV: Baby hamster kidney-West Nile virus; SN: Supernatants.

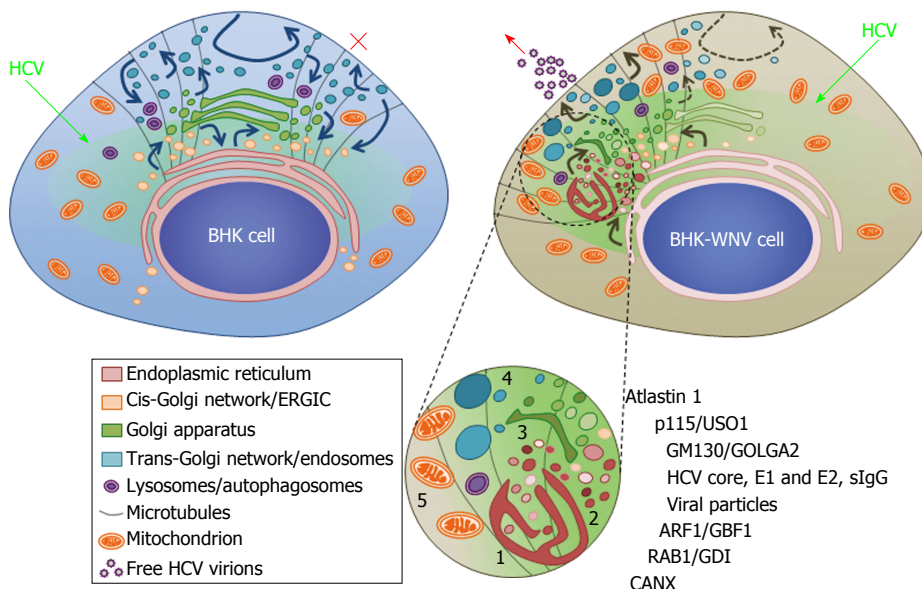
the ERGIC that activates small-sized GTPase ARF1 and, in turn, COPI-dependent traffic<sup>[43]</sup>, was also involved in the production of HCV particles<sup>[8]</sup>. On the other hand, another feature of the ERGIC, the lectin ERGIC-53, was excluded from the assembly compartment. As observed in TEM, the latter directly brought together membranes

from the ER and the Golgi apparatus, which was consistent with an enrichment of this compartment in, respectively, Atlastin 1<sup>[50-52]</sup> and GM130/GOLGA2<sup>[41]</sup>, both reported to reorganize and/or maintain membranes of their organelle. In addition, an enrichment in both RAB1 and its GDI, like what was observed in the assembly





**Figure 13** Caspase-1 inhibitor conditionally inhibits the secretion of hepatitis C virus particles by baby hamster kidney-West Nile virus cells. **A:** BHK-21 and BHK-WNV cells were transfected with a mix of HCVbp-coding and P2B plasmids; the next day, a caspase-1 inhibitor was added in the culture medium and the cells were incubated for 2 more days; cell lysates and HCV particles were harvested and analyzed by Western blot (WB); **B:** BHK-WNV cells were transfected with a plasmid coding for the structural (*core*, *E1*, *E2*) genes of HCV H77 strain, plus (+) or minus (-) p7, then were analyzed as in (A); **C:** Quantification of WBs: Top panels for (A) and bottom panels for (B); bar inside patterns are displayed underneath corresponding data; error bars represent standard deviations; inserts = results in cells. HCV: Hepatitis C virus; BHK-WNV: Baby hamster kidney-West Nile virus.



**Figure 14** Model of a hepatitis C virus assembly compartment in baby hamster kidney-West Nile virus cells. Schematic organization of cell traffic in parental BHK-21 (top left) and BHK-WNV (top right) cells; curved arrows represent cell traffic; HCV genome is produced by the P2B plasmid system (green arrows) and expressed (greenish areas) in the cytoplasm of BHK cells. Bottom, sketches' legend and close up of a HCV assembly site (cf. also Figure 2B): (1) convoluted membranes; (2) vesicular packets; (3) Golgi cisternae; (4) large vesicles filled with viral particles; and (5) mitochondrion; host and viral factors identified within this compartment. sIgG: Antibodies from the serum of a cured HCV patient; CANX: Calnexin; HCV: Hepatitis C virus; BHK-WNV: Baby hamster kidney-West Nile virus; ERGIC: Endoplasmic reticulum-Golgi intermediate compartment; GDI: GDP dissociation inhibitor.

compartment, has been reported to initiate a cascade of events involving GBF1, ARF1 and phosphatidylinositol

4-kinase III alpha (PI4KIII $\alpha$ )<sup>[43]</sup>, which may increase the local proportion of phosphatidylinositol 4-phosphate<sup>[63]</sup>

and subvert endocytic trafficking<sup>[64]</sup>. In Huh-7.5 cells, this role is normally devoted to HCV NS5A that brings together PI4KIII $\alpha$  and TBC1D20, an activator of RAB1<sup>[65]</sup>. Therefore, replication of the genomic RNA of flaviviruses in BHK-21 cells may pre-position components - or their equivalent - HCV establishes by itself in the cells in which it usually replicates. Although excluding ERGIC as we know it in the classical secretion pathway, such reorganization of the secretion machinery in BHK-21 cells (Figure 14) could explain why a BFA treatment inhibited HCV particle production.

Finally, if CANX was required for the production of HCV particles, most of it shuttled a pool of HCV protein subspecies to the assembly compartment of BHK-WNV cells, instead of co-localizing with CRT for the proper folding of glycoproteins. How CANX really assists these cells to produce HCV virions is not fully understood. CANX still probably requires binding N-linked glycans on the HCV envelope proteins. The reported slow dissociation rate between CANX and HCV envelope proteins<sup>[27]</sup> could contribute to unusual involvements of CANX, such as what is observed with proteins targeted to mitochondria-ER associated membranes (MAMs) for the formation of inflammasome and autophagy compartments<sup>[66]</sup>. HCV has previously been shown to target MAMs in hepatocytes<sup>[67]</sup>. However, mitochondria were excluded from the assembly compartment in BHK-WNV cells. And, upon expression of HCV structural genes, this coincided with the appearance within the assembly compartment of large vesicles containing particles<sup>[8]</sup>.

Oxidative stress, inflammation and/or infection can lead to the appearance in the cytoplasm of large vesicles, often indiscriminately referred to as multivesicular bodies, releasing extracellular particles of very different compositions depending on their origin. They are formed by the fusion of late endosomal vesicles with membranes from lysosomal and/or autophagosomal compartments, or originating from the periphery of the ER-Golgi complex assembling - independently from a COP II - and COP I -mediated membrane transport - into a compartment of unconventional protein secretion<sup>[68]</sup>. Unconventional pathways are used by intracellular pathogens reorganizing the vesicular traffic to secrete proteins (*e.g.*,<sup>[69]</sup>). The reorganization of the secretion machinery required for the production of HCV particles by BHK-WNV cells displayed similar features.

Knocking down  $\alpha$ -TUB expression not only resulted in a lesser release of HCV particles, but also of particulate materials containing Hsp70 and Hsp90, a feature of exosomes. Such extracellular vesicles have been shown to participate in the transfer of viral materials<sup>[46]</sup>. Recently, exosomes have also been implicated in the propagation/dissemination of HCV genome, proteins and replication complexes, although in the former case with a weaker effectiveness than lipoprotein-associated virions<sup>[70,71]</sup>. However, the production of HCV particles by BHK-WNV cells cannot be reduced to the secretion of exosomes. We

had previously shown that, albeit overlapping, the peaks of particles containing HCV structural and heat-shock proteins released by BHK-WNV cells did not coincide after ultracentrifugation on a density gradient<sup>[8]</sup>. In addition, while the secretion of HCV particles was blocked, the release of heat-shock proteins was insensitive to a treatment by brefeldin A (BFA), an inhibitor of GBF1<sup>[8]</sup>. In this work, the secretions of Hsp70 and Hsp90-containing particles and HCV particles displayed different patterns. Therefore, the dual effect of  $\alpha$ -TUB siRNA on the secretions of exosomes and HCV particles probably reflects separate needs for microtubules taking place at different steps. For exosomes, it could relate to effects on the BFA-insensitive *trans*-Golgi network, which is consistent with an accumulation of hsp70 inside producer cells following knockdown of  $\alpha$ -TUB expression. Conversely, the production of HCV particles could be inhibited at the level of a GBF1/ARF1-dependent traffic, within the assembly compartment, or later during their secretion.

Treatment of BHK-WNV cells with a caspase-1 inhibitor abolished the release of HCV particles. Caspase-1 has been shown to activate IL-1 $\beta$  and IL-18 proinflammatory cytokine precursors<sup>[57]</sup>. It has long remained unknown how their matured species translocate across the plasma membrane. Recent results support the idea that their secretion involves a cytosolic compartment containing vesicles and their exocytosis<sup>[72]</sup>. It could be the mechanism by which a caspase-1-dependent secretory pathway contributed to the production of HCV particles by BHK-WNV cells. However, inhibition of HCV production with the caspase inhibitor occurred only in the context of a full-length genome, suggesting the existence of interaction(s) between caspase-1 and HCV non-structural genes, here NS2 and/or NS3. Although the catalytic activity of NS2 is dispensable, this viral cysteine protease is required for the production of HCV particles<sup>[73]</sup>, as is the helicase/serine protease NS3<sup>[74]</sup>. Therefore, the cleavage(s) of NS2/3 by caspase-1, in addition, could prevent the maturation of non-structural proteins required for RNA replication and/or remove interactions, either of them or both being detrimental to the formation and secretion of HCV particles.

At variance with the JFH-1/Huh-7.5 cell model<sup>[59,75]</sup>, infectious HCV particles released by BHK-WNV cells did not require lipoproteins or exosomes. It is still unclear whether discrepancies between models<sup>[9]</sup> could not also relate to active HCV replication in a non-physiological environment<sup>[76]</sup>, differences between viral genotypes<sup>[12]</sup> and/or differential processing of some HCV proteins<sup>[58]</sup>. Nevertheless, host factors involved in BHK-WNV cells were also required in Huh-7.5 cells that, over time, developed a cytoplasmic compartment<sup>[26]</sup> enriched in HCV core, CANX and RAB1. Infectious HCV particles are assembled in the cytoplasm of Huh-7.5 cells<sup>[13]</sup> before their association to lipoproteins<sup>[8,77]</sup> or exosomes<sup>[75,78]</sup>. The associations to lipoprotein particles and extracellular particles have been proposed as mechanisms to regulate

vertebrate Hedgehog dispersion during development<sup>[79]</sup>. In addition, ApoE<sup>[25,80]</sup> and extracellular particles<sup>[47]</sup> are suspected to contribute also to the pathogenesis of viruses unrelated to HCV. Therefore, rather than having evolved a unique mechanism of viral propagation, HCV may have instead subverted existing cellular processes. It could involve intrinsic properties of its envelope proteins, since HCV E1 interacts with apoE<sup>[81]</sup> and possibly lipids<sup>[82]</sup>, which is believed to help the virus escape from immune recognition<sup>[83]</sup>.

The release of HCV particles by BHK-WNV cells, instead, involved an unusual, if not unconventional secretion pathway. The present data suggest that their mechanism of assembly and egress could be a mean by which HCV circumvents intracellular defenses. These infectious particles are not those usually observed as such in infected patients. Therefore, as for other HCV particles produced *in vitro*<sup>[9]</sup>, we are not sure of their relationship to the normal viral cycle. Are they merely a precursor for the main HCV species, which will eventually associate to lipoproteins, or do they represent different viral species of higher buoyant density? In patients, the proportion of circulating HCV particles associated to lipoproteins varies between individuals and during the course of infection. Denser viral species can amount up to half of the circulating HCV genome<sup>[84]</sup>; they are usually opsonized by IgG in the serum of infected patients, making the study of free virions difficult. At least during the assembly process in BHK-WNV cells, HCV particles exposed epitopes that were recognized by neutralizing sIgG from a patient previously chronically infected by HCV of same genotype. We propose that the BHK-WNV cell model could be useful to study the structure of free HCV virions and their immunological properties.

## ACKNOWLEDGMENTS

We thank Charles M Rice (Center for the Study of Hepatitis C, the Rockefeller University) for his generosity in providing plasmids encoding HCV RNA of genotype 1a (wild type and adaptive mutants) and Huh-7.5 cells; Theodore C Pierson (LVD, NIAID, NIH) for kindly provided BHK-21 cells bearing a WNV subgenomic replicon as well as a plasmid encoding WNV structural genes; Takaji Wakita (Department of Virology, National Institute of Infectious Diseases, Tokyo) provided plasmids encoding HCV RNA of genotype 2a; anti-HCV antibodies were generous gifts from Arvind H Patel (anti-E2; MRC Virology Unit, Institute of Virology, University of Glasgow), Ramsey C Cheung (anti-E1; Division of Gastroenterology and Hepatology, Stanford University School of Medicine), Stanislas Pol (HCV serum; Hôpital and Institut Cochin, Paris). We are indebted to the staff of the Biological Imaging Section (RTB, NIAID, NIH) for their assistance with the confocal microscopy and Kunio Nagashima (SAIC/NCI, NIH Frederick) for the EM analyses.

## COMMENTS

### Background

The production of infectious hepatitis C virus (HCV) particles by model human hepatocellular carcinoma Huh-7.5 cells is believed to involve a classical secretion pathway; it has also been reported to involve lipoproteins and exosomes. The authors previously demonstrated that the intracellular environment generated by a West-Nile virus (WNV) subgenomic replicon rendered non-human, non-hepatic mammalian cells [here referred to as baby hamster kidney-WNV (BHK-WNV) cells] permissive for assembly and release of infectious HCV particles of various genotypes wherein the HCV genome is generated in the cytoplasm independently of its replication machinery. HCV production did not require ongoing activity of the WNV replicon, but instead was associated with persisting replicon-induced changes in the cellular environment. Since secretion of HCV particles by BHK-WNV cells neither involved lipoproteins nor exosomes, could it still follow a conventional pathway? Activity of the small GTPase ARF1 and the maturation of carbohydrates on envelope proteins of released HCV particles were both required for the production of infectious HCV by permissive BHK-WNV cells, suggesting that, indeed, it followed a classical secretion pathway. The authors, therefore, examined the possibility that the endoplasmic reticulum (ER)-bound lectin calnexin (CANX), the small GTPase RAB1 and microtubule-associated alpha-tubulin, which all contribute to the secretion of several glycoproteins by BHK-21 cells, were involved in the production of HCV particles by BHK-WNV cells. Surprisingly, the results show that secretion of HCV particles went through a re-organized and re-wired pathway bypassing the conventional ER-to-Golgi intermediary compartment and involving components of the inflammasome.

### Research frontiers

The structure of HCV virions/progenies remains elusive. The fact that infectious HCV particles are produced independently from lipoprotein biosynthesis, yet retain the possibility to interact with lipoproteins *in vitro* supports the view that, *in vivo*, HCV particles may interact with lipoproteins in a second step and not necessarily co-assemble with them. This has probably important consequences regarding the way HCV interacts with producer cells and the immune system.

### Innovations and breakthroughs

The authors' results suggest that the HCV production made possible by the prior WNV replication in BHK cells could be related to that observed in human hepatocytes. It is unclear whether this reflects an organization of HCV production more complex than expected in hepatocytes or the existence of additional route(s) of HCV secretion.

### Applications

Although no immediate application of these findings is foreseen, the production of free HCV virions could pave the way for identifying the most important epitopes for HCV neutralization and the development of an effective vaccine.

### Terminology

Subgenomic replicon: Fragment of a viral RNA genome encoding the non structural genes required for its own replication in the cytoplasm, but not the structural genes required for the formation of virions.

### Peer-review

In this manuscript, the authors aimed to examine whether the cellular factors including CANX, RAB1 and alpha-tubulin were involved in the production of HCV particles by BHK-WNV cells. Moreover, it brings an interesting contribution to the current literature.

## REFERENCES

- 1 Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; **5**: 558-567 [PMID: 16122679 DOI: 10.1016/S1473-3099(05)70216-4]
- 2 Webster DP, Klenerman P, Dusheiko GM. Hepatitis C. *Lancet* 2015; **385**: 1124-1135 [PMID: 25687730 DOI: 10.1016/S0140-6736(14)62401-6]
- 3 Solund C, Krarup H, Ramirez S, Thielsen P, Røge BT, Lunding

- S, Barfod TS, Madsen LG, Tarp B, Christensen PB, Gerstoft J, Laursen AL, Bukh J, Weis N. Nationwide experience of treatment with protease inhibitors in chronic hepatitis C patients in Denmark: identification of viral resistance mutations. *PLoS One* 2014; **9**: e113034 [PMID: 25438153 DOI: 10.1371/journal.pone.0113034]
- 4 **Feeney ER**, Chung RT. Antiviral treatment of hepatitis C. *BMJ* 2014; **348**: g3308 [PMID: 25002352 DOI: 10.1136/bmj.g3308]
- 5 **Baumert TF**, Fauvel C, Chen DY, Lauer GM. A prophylactic hepatitis C virus vaccine: a distant peak still worth climbing. *J Hepatol* 2014; **61**: S34-S44 [PMID: 25443345 DOI: 10.1016/j.jhep.2014.09.009]
- 6 **de Jong YP**, Rice CM, Ploss A. New horizons for studying human hepatotropic infections. *J Clin Invest* 2010; **120**: 650-653 [PMID: 20179350 DOI: 10.1172/JCI42338]
- 7 **Mina MM**, Luciani F, Cameron B, Bull RA, Beard MR, Booth D, Lloyd AR. Resistance to hepatitis C virus: potential genetic and immunological determinants. *Lancet Infect Dis* 2015; **15**: 451-460 [PMID: 25703062 DOI: 10.1016/S1473-3099(14)70965-X]
- 8 **Triyatni M**, Berger EA, Saunier B. A new model to produce infectious hepatitis C virus without the replication requirement. *PLoS Pathog* 2011; **7**: e1001333 [PMID: 21533214 DOI: 10.1371/journal.ppat.1001333]
- 9 **Saunier B**, Triyatni M, Berger EA. Culturing HCV: challenges and progress. *Future Virol* 2011; **6**: 1169-1178 [DOI: 10.2217/FVL.11.95]
- 10 **Kolykhalov AA**, Agapov EV, Blight KJ, Mihalik K, Feinstone SM, Rice CM. Transmission of hepatitis C by intrahepatic inoculation with transcribed RNA. *Science* 1997; **277**: 570-574 [PMID: 9228008 DOI: 10.1126/science.277.5325.570]
- 11 **Lohmann V**, Körner F, Koch J, Herian U, Theilmann L, Bartenschlager R. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* 1999; **285**: 110-113 [PMID: 10390360 DOI: 10.1126/science.285.5424.110]
- 12 **Pietschmann T**, Zayas M, Meuleman P, Long G, Appel N, Koutsoudakis G, Kallis S, Leroux-Roels G, Lohmann V, Bartenschlager R. Production of infectious genotype 1b virus particles in cell culture and impairment by replication enhancing mutations. *PLoS Pathog* 2009; **5**: e1000475 [PMID: 19521536 DOI: 10.1371/journal.ppat]
- 13 **Gastaminza P**, Kapadia SB, Chisari FV. Differential biophysical properties of infectious intracellular and secreted hepatitis C virus particles. *J Virol* 2006; **80**: 11074-11081 [PMID: 16956946 DOI: 10.1128/JVI.01150-06]
- 14 **Felmlee DJ**, Sheridan DA, Bridge SH, Nielsen SU, Milne RW, Packard CJ, Caslake MJ, McLauchlan J, Toms GL, Neely RD, Bassendine MF. Intravascular transfer contributes to postprandial increase in numbers of very-low-density hepatitis C virus particles. *Gastroenterology* 2010; **139**: 1774-1783, 1783.e1-e6 [PMID: 20682323 DOI: 10.1053/j.gastro.2010.07.047]
- 15 **Lindenbach BD**, Rice CM. The ins and outs of hepatitis C virus entry and assembly. *Nat Rev Microbiol* 2013; **11**: 688-700 [PMID: 24018384 DOI: 10.1038/nrmicro3098]
- 16 **Grassi G**, Di Caprio G, Fimia GM, Ippolito G, Tripodi M, Alonzi T. Hepatitis C virus relies on lipoproteins for its life cycle. *World J Gastroenterol* 2016; **22**: 1953-1965 [PMID: 26877603 DOI: 10.3748/wjg.v22.i6.1953]
- 17 **Bartenschlager R**, Penin F, Lohmann V, André P. Assembly of infectious hepatitis C virus particles. *Trends Microbiol* 2011; **19**: 95-103 [PMID: 21146993 DOI: 10.1016/j.tim.2010.11.005]
- 18 **Murray CL**, Jones CT, Rice CM. Architects of assembly: roles of Flaviviridae non-structural proteins in virion morphogenesis. *Nat Rev Microbiol* 2008; **6**: 699-708 [PMID: 18587411 DOI: 10.1038/nrmicro1928]
- 19 **Fredericksen BL**, Smith M, Katze MG, Shi PY, Gale M. The host response to West Nile Virus infection limits viral spread through the activation of the interferon regulatory factor 3 pathway. *J Virol* 2004; **78**: 7737-7747 [PMID: 15220448 DOI: 10.1128/JVI.78.14.7737-7747.2004]
- 20 **Welsch S**, Miller S, Romero-Brey I, Merz A, Bleck CK, Walther P, Fuller SD, Antony C, Krijnse-Locker J, Bartenschlager R. Composition and three-dimensional architecture of the dengue virus replication and assembly sites. *Cell Host Microbe* 2009; **5**: 365-375 [PMID: 19380115 DOI: 10.1016/j.chom.2009.03.007]
- 21 **Ye J**. Reliance of host cholesterol metabolic pathways for the life cycle of hepatitis C virus. *PLoS Pathog* 2007; **3**: e108 [PMID: 17784784 DOI: 10.1371/journal.ppat.0030108]
- 22 **Miyazaki Y**, Atsuzawa K, Usuda N, Watahi K, Hishiki T, Zayas M, Bartenschlager R, Wakita T, Hijikata M, Shimotohno K. The lipid droplet is an important organelle for hepatitis C virus production. *Nat Cell Biol* 2007; **9**: 1089-1097 [PMID: 17721513 DOI: 10.1038/ncb1631]
- 23 **Mackenzie JM**, Khromykh AA, Parton RG. Cholesterol manipulation by West Nile virus perturbs the cellular immune response. *Cell Host Microbe* 2007; **2**: 229-239 [PMID: 18005741 DOI: 10.1016/j.chom.2007.09.003]
- 24 **Samsa MM**, Mondotte JA, Iglesias NG, Assunção-Miranda I, Barbosa-Lima G, Da Poian AT, Bozza PT, Gamarnik AV. Dengue virus capsid protein usurps lipid droplets for viral particle formation. *PLoS Pathog* 2009; **5**: e1000632 [PMID: 19851456 DOI: 10.1371/journal.ppat.1000632]
- 25 **Faustino AF**, Martins IC, Carvalho FA, Castanho MA, Maurer-Stroh S, Santos NC. Understanding Dengue Virus Capsid Protein Interaction with Key Biological Targets. *Sci Rep* 2015; **5**: 10592 [PMID: 26161501 DOI: 10.1038/srep10592]
- 26 **Rouillé Y**, Helle F, Delgrange D, Roingeard P, Voisset C, Blanchard E, Belouzard S, McKeating J, Patel AH, Maertens G, Wakita T, Wychowski C, Dubuisson J. Subcellular localization of hepatitis C virus structural proteins in a cell culture system that efficiently replicates the virus. *J Virol* 2006; **80**: 2832-2841 [PMID: 16501092 DOI: 10.1128/JVI.80.6.2832-2841.2006]
- 27 **Dubuisson J**, Rice CM. Hepatitis C virus glycoprotein folding: disulfide bond formation and association with calnexin. *J Virol* 1996; **70**: 778-786 [PMID: 8551615]
- 28 **Dodonova SO**, Diestelkoetter-Bachert P, von Appen A, Hagen WJ, Beck R, Beck M, Wieland F, Briggs JA. VESICULAR TRANSPORT. A structure of the COPI coat and the role of coat proteins in membrane vesicle assembly. *Science* 2015; **349**: 195-198 [PMID: 26160949 DOI: 10.1126/science.aab1121]
- 29 **Klaus JP**, Eisenhauer P, Russo J, Mason AB, Do D, King B, Taatjes D, Cornillez-Ty C, Boyson JE, Thali M, Zheng C, Liao L, Yates JR, Zhang B, Ballif BA, Botten JW. The intracellular cargo receptor ERGIC-53 is required for the production of infectious arenavirus, coronavirus, and filovirus particles. *Cell Host Microbe* 2013; **14**: 522-534 [PMID: 24237698 DOI: 10.1016/j.chom.2013.10.010]
- 30 **Pierson TC**, Sánchez MD, Puffer BA, Ahmed AA, Geiss BJ, Valentine LE, Altamura LA, Diamond MS, Doms RW. A rapid and quantitative assay for measuring antibody-mediated neutralization of West Nile virus infection. *Virology* 2006; **346**: 53-65 [PMID: 16325883 DOI: 10.1016/j.virol.2005.10.030]
- 31 **Wadkins TS**, Been MD. Ribozyme activity in the genomic and antigenomic RNA strands of hepatitis delta virus. *Cell Mol Life Sci* 2002; **59**: 112-125 [PMID: 11846024 DOI: 10.1007/s00018-002-8409-7]
- 32 **Blight KJ**, McKeating JA, Marcotrigiano J, Rice CM. Efficient replication of hepatitis C virus genotype 1a RNAs in cell culture. *J Virol* 2003; **77**: 3181-3190 [PMID: 12584342 DOI: 10.1128/JVI.77.5.3181-3190.2003]
- 33 **Griffin BA**, Adams SR, Tsien RY. Specific covalent labeling of recombinant protein molecules inside live cells. *Science* 1998; **281**: 269-272 [PMID: 9657724 DOI: 10.1126/science.281.5374.269]
- 34 **Owsianka A**, Clayton RF, Loomis-Price LD, McKeating JA, Patel AH. Functional analysis of hepatitis C virus E2 glycoproteins and virus-like particles reveals structural dissimilarities between different forms of E2. *J Gen Virol* 2001; **82**: 1877-1883 [PMID: 11457993 DOI: 10.1099/0022-1317-82-8-1877]
- 35 **Oliphant T**, Engle M, Nybakken GE, Doane C, Johnson S, Huang L, Gorlatov S, Mehlhop E, Marri A, Chung KM, Ebel GD, Kramer LD, Fremont DH, Diamond MS. Development of a humanized monoclonal antibody with therapeutic potential against West Nile virus. *Nat Med* 2005; **11**: 522-530 [PMID: 15852016 DOI: 10.1038/nm1240]



- 36 **Wakita T**, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Kräusslich HG, Mizokami M, Bartenschlager R, Liang TJ. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 2005; **11**: 791-796 [PMID: 15951748 DOI: 10.1038/nm1268]
- 37 **Dussupt V**, Javid MP, Abou-Jaoudé G, Jadwin JA, de La Cruz J, Nagashima K, Bouamr F. The nucleocapsid region of HIV-1 Gag cooperates with the PTAP and LYPXnL late domains to recruit the cellular machinery necessary for viral budding. *PLoS Pathog* 2009; **5**: e1000339 [PMID: 19282983 DOI: 10.1371/journal.ppat.1000339]
- 38 **Saunier B**, Triyatni M, Ulianich L, Maruvada P, Yen P, Kohn LD. Role of the asialoglycoprotein receptor in binding and entry of hepatitis C virus structural proteins in cultured human hepatocytes. *J Virol* 2003; **77**: 546-559 [PMID: 12477859 DOI: 10.1128/JVI.77.1.546-559.2003]
- 39 **de Castro IF**, Volonté L, Risco C. Virus factories: biogenesis and structural design. *Cell Microbiol* 2013; **15**: 24-34 [PMID: 22978691 DOI: 10.1111/cmi.12029]
- 40 **Tomás M**, Martínez-Alonso E, Ballesta J, Martínez-Menárguez JA. Regulation of ER-Golgi intermediate compartment tubulation and mobility by COPI coats, motor proteins and microtubules. *Traffic* 2010; **11**: 616-625 [PMID: 20136777 DOI: 10.1111/j.1600-0854.2010.01047.x]
- 41 **Rivero S**, Cardenas J, Bornens M, Rios RM. Microtubule nucleation at the cis-side of the Golgi apparatus requires AKAP450 and GM130. *EMBO J* 2009; **28**: 1016-1028 [PMID: 19242490 DOI: 10.1038/emboj.2009.47]
- 42 **Helenius A**, Aeby M. Roles of N-linked glycans in the endoplasmic reticulum. *Annu Rev Biochem* 2004; **73**: 1019-1049 [PMID: 15189166 DOI: 10.1146/annurev.biochem.73.011303.073752]
- 43 **Dumaresq-Doiron K**, Savard MF, Akam S, Costantino S, Lefrançois S. The phosphatidylinositol 4-kinase PI4KIIIalpha is required for the recruitment of GBF1 to Golgi membranes. *J Cell Sci* 2010; **123**: 2273-2280 [PMID: 20530568 DOI: 10.1242/jcs.055798]
- 44 **de Chassey B**, Navratil V, Tafforeau L, Hiet MS, Aublin-Gex A, Agaugué S, Meiffren G, Pradezynski F, Faria BF, Chantier T, Le Breton M, Pellet J, Davoust N, Mangeot PE, Chaboud A, Penin F, Jacob Y, Vidalain PO, Vidal M, André P, Rabourdin-Combe C, Lotteau V. Hepatitis C virus infection protein network. *Mol Syst Biol* 2008; **4**: 230 [PMID: 18985028 DOI: 10.1038/msb.2008.66]
- 45 **Roohvand F**, Maillard P, Lavergne JP, Boulant S, Walic M, Andréo U, Goueslain L, Helle F, Mallet A, McLauchlan J, Budkowska A. Initiation of hepatitis C virus infection requires the dynamic microtubule network: role of the viral nucleocapsid protein. *J Biol Chem* 2009; **284**: 13778-13791 [PMID: 19269968 DOI: 10.1074/jbc.M807873200]
- 46 **Lancaster GI**, Febbraio MA. Exosome-dependent trafficking of HSP70: a novel secretory pathway for cellular stress proteins. *J Biol Chem* 2005; **280**: 23349-23355 [PMID: 15826944 DOI: 10.1074/jbc.M502017200]
- 47 **Chahar HS**, Bao X, Casola A. Exosomes and Their Role in the Life Cycle and Pathogenesis of RNA Viruses. *Viruses* 2015; **7**: 3204-3225 [PMID: 26102580 DOI: 10.3390/v7062770]
- 48 **Bräutigam J**, Scheidig AJ, Egge-Jacobsen W. Mass spectrometric analysis of hepatitis C viral envelope protein E2 reveals extended microheterogeneity of mucin-type O-linked glycosylation. *Glycobiology* 2013; **23**: 453-474 [PMID: 23242014 DOI: 10.1093/glycob/cws171]
- 49 **Chen CY**, Balch WE. The Hsp90 chaperone complex regulates GDI-dependent Rab recycling. *Mol Biol Cell* 2006; **17**: 3494-3507 [PMID: 16687576 DOI: 10.1091/mbc.E05-12-1096]
- 50 **Orso G**, Pendin D, Liu S, Toso J, Moss TJ, Faust JE, Micaroni M, Egorova A, Martinuzzi A, McNew JA, Daga A. Homotypic fusion of ER membranes requires the dynamin-like GTPase atlastin. *Nature* 2009; **460**: 978-983 [PMID: 19633650 DOI: 10.1038/nature08280]
- 51 **Morin-Leisk J**, Saini SG, Meng X, Makhov AM, Zhang P, Lee TH. An intramolecular salt bridge drives the soluble domain of GTP-bound atlastin into the postfusion conformation. *J Cell Biol* 2011; **195**: 605-615 [PMID: 22065636 DOI: 10.1083/jcb.201105006]
- 52 **Hu J**, Shibata Y, Zhu PP, Voss C, Rismanchi N, Prinz WA, Rapoport TA, Blackstone C. A class of dynamin-like GTPases involved in the generation of the tubular ER network. *Cell* 2009; **138**: 549-561 [PMID: 19665976 DOI: 10.1016/j.cell.2009.05.025]
- 53 **Allan BB**, Moyer BD, Balch WE. Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion. *Science* 2000; **289**: 444-448 [PMID: 10903204 DOI: 10.1126/science.289.5478.444]
- 54 **Rothman JE**, Fine RE. Coated vesicles transport newly synthesized membrane glycoproteins from endoplasmic reticulum to plasma membrane in two successive stages. *Proc Natl Acad Sci USA* 1980; **77**: 780-784 [PMID: 6244586 DOI: 10.1073/pnas.77.2.780]
- 55 **Boson B**, Granio O, Bartenschlager R, Cosset FL. A concerted action of hepatitis C virus p7 and nonstructural protein 2 regulates core localization at the endoplasmic reticulum and virus assembly. *PLoS Pathog* 2011; **7**: e1002144 [PMID: 21814513 DOI: 10.1371/journal.ppat.1002144]
- 56 **Wölk B**, Büchele B, Moradpour D, Rice CM. A dynamic view of hepatitis C virus replication complexes. *J Virol* 2008; **82**: 10519-10531 [PMID: 18715913 DOI: 10.1128/JVI.00640-08]
- 57 **de Zoete MR**, Palm NW, Zhu S, Flavell RA. Inflammasomes. *Cold Spring Harb Perspect Biol* 2014; **6**: a016287 [PMID: 25324215 DOI: 10.1101/cshperspect.a016287]
- 58 **Lagaye S**, Shen H, Saunier B, Nascimbeni M, Gaston J, Bourdoncle P, Hannoun L, Massault PP, Vallet-Pichard A, Mallet V, Pol S. Efficient replication of primary or culture hepatitis C virus isolates in human liver slices: a relevant ex vivo model of liver infection. *Hepatology* 2012; **56**: 861-872 [PMID: 22454196 DOI: 10.1002/hep.25738]
- 59 **Goueslain L**, Alsaleh K, Horellou P, Roingeard P, Descamps V, Duverlie G, Ciczora Y, Wychowski C, Dubuisson J, Rouillé Y. Identification of GBF1 as a cellular factor required for hepatitis C virus RNA replication. *J Virol* 2010; **84**: 773-787 [PMID: 19906930 DOI: 10.1128/JVI.01190-09]
- 60 **Steinmann E**, Whitfield T, Kallis S, Dwek RA, Zitzmann N, Pietschmann T, Bartenschlager R. Antiviral effects of amantadine and iminosugar derivatives against hepatitis C virus. *Hepatology* 2007; **46**: 330-338 [PMID: 17599777 DOI: 10.1002/hep.21686]
- 61 **Hueging K**, Doepke M, Vieyres G, Bankwitz D, Frentzen A, Doerrbecker J, Gumz F, Haid S, Wölk B, Kaderali L, Pietschmann T. Apolipoprotein E codetermines tissue tropism of hepatitis C virus and is crucial for viral cell-to-cell transmission by contributing to a postenvelopment step of assembly. *J Virol* 2014; **88**: 1433-1446 [PMID: 24173232 DOI: 10.1128/JVI.01815-13]
- 62 **Guo Y**, Linstedt AD. Binding of the vesicle docking protein p115 to the GTPase Rab1b regulates membrane recruitment of the COPI vesicle coat. *Cell Logist* 2013; **3**: e27687 [PMID: 25332841 DOI: 10.4161/cl.27687]
- 63 **Dorobantu CM**, Albulescu L, Harak C, Feng Q, van Kampen M, Strating JR, Gorbalenya AE, Lohmann V, van der Schaar HM, van Kuppeveld FJ. Modulation of the Host Lipid Landscape to Promote RNA Virus Replication: The Picornavirus Encephalomyocarditis Virus Converges on the Pathway Used by Hepatitis C Virus. *PLoS Pathog* 2015; **11**: e1005185 [PMID: 26406250 DOI: 10.1371/journal.ppat.1005185]
- 64 **Berger KL**, Cooper JD, Heaton NS, Yoon R, Oakland TE, Jordan TX, Mateu G, Grakoui A, Randall G. Roles for endocytic trafficking and phosphatidylinositol 4-kinase III alpha in hepatitis C virus replication. *Proc Natl Acad Sci USA* 2009; **106**: 7577-7582 [PMID: 19376974 DOI: 10.1073/pnas.0902693106]
- 65 **Nevo-Yassaf I**, Yaffe Y, Asher M, Ravid O, Eizenberg S, Henis YI, Nahmias Y, Hirschberg K, Sklan EH. Role for TBC1D20 and Rab1 in hepatitis C virus replication via interaction with lipid droplet-bound nonstructural protein 5A. *J Virol* 2012; **86**: 6491-6502 [PMID: 22491470 DOI: 10.1128/JVI.00496-12]
- 66 **Marchi S**, Patergnani S, Pinton P. The endoplasmic reticulum-mitochondria connection: one touch, multiple functions. *Biochim Biophys Acta* 2014; **1837**: 461-469 [PMID: 24211533 DOI: 10.1016/j.bba.2014.05.011]

- 10.1016/j.bbabo.2013.10.015]
- 67 **Horner SM**, Liu HM, Park HS, Briley J, Gale M. Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus. *Proc Natl Acad Sci USA* 2011; **108**: 14590-14595 [PMID: 21844353 DOI: 10.1073/pnas.1110133108]
- 68 **Nickel W**, Rabouille C. Mechanisms of regulated unconventional protein secretion. *Nat Rev Mol Cell Biol* 2009; **10**: 148-155 [PMID: 19122676 DOI: 10.1038/nrm2617]
- 69 **Shoji JY**, Kikuma T, Kitamoto K. Vesicle trafficking, organelle functions, and unconventional secretion in fungal physiology and pathogenicity. *Curr Opin Microbiol* 2014; **20**: 1-9 [PMID: 24835421 DOI: 10.1016/j.mib.2014.03.002]
- 70 **Bukong TN**, Momen-Heravi F, Kodys K, Bala S, Szabo G. Exosomes from hepatitis C infected patients transmit HCV infection and contain replication competent viral RNA in complex with Ago2-miR122-HSP90. *PLoS Pathog* 2014; **10**: e1004424 [PMID: 25275643 DOI: 10.1371/journal.ppat.1004424]
- 71 **Longatti A**, Boyd B, Chisari FV. Virion-independent transfer of replication-competent hepatitis C virus RNA between permissive cells. *J Virol* 2015; **89**: 2956-2961 [PMID: 25505060 DOI: 10.1128/JVI.02721-14]
- 72 **Monteleone M**, Stow JL, Schroder K. Mechanisms of unconventional secretion of IL-1 family cytokines. *Cytokine* 2015; **74**: 213-218 [PMID: 25922276 DOI: 10.1016/j.cyto.2015.03.022]
- 73 **Jones CT**, Murray CL, Eastman DK, Tassello J, Rice CM. Hepatitis C virus p7 and NS2 proteins are essential for production of infectious virus. *J Virol* 2007; **81**: 8374-8383 [PMID: 17537845]
- 74 **Ma Y**, Yates J, Liang Y, Lemon SM, Yi M. NS3 helicase domains involved in infectious intracellular hepatitis C virus particle assembly. *J Virol* 2008; **82**: 7624-7639 [PMID: 18508894 DOI: 10.1128/JVI.00724-08]
- 75 **Gastaminza P**, Dryden KA, Boyd B, Wood MR, Law M, Yeager M, Chisari FV. Ultrastructural and biophysical characterization of hepatitis C virus particles produced in cell culture. *J Virol* 2010; **84**: 10999-11009 [PMID: 20686033 DOI: 10.1128/JVI.00526-10]
- 76 **Lindenbach BD**, Meuleman P, Ploss A, Vanwolleghem T, Syder AJ, McKeating JA, Lanford RE, Feinstone SM, Major ME, Leroux-Roels G, Rice CM. Cell culture-grown hepatitis C virus is infectious in vivo and can be recultured in vitro. *Proc Natl Acad Sci USA* 2006; **103**: 3805-3809 [PMID: 16484368]
- 77 **Triyatni M**, Saunier B, Maruvada P, Davis AR, Ulianich L, Heller T, Patel A, Kohn LD, Liang TJ. Interaction of hepatitis C virus-like particles and cells: a model system for studying viral binding and entry. *J Virol* 2002; **76**: 9335-9344 [PMID: 12186916]
- 78 **Masciopinto F**, Giovani C, Campagnoli S, Galli-Stampino L, Colombatto P, Brunetto M, Yen TS, Houghton M, Pileri P, Abrignani S. Association of hepatitis C virus envelope proteins with exosomes. *Eur J Immunol* 2004; **34**: 2834-2842 [PMID: 15368299]
- 79 **Vyas N**, Walvekar A, Tate D, Lakshmanan V, Bansal D, Lo Cicero A, Raposo G, Palakodeti D, Dhawan J. Vertebrate Hedgehog is secreted on two types of extracellular vesicles with different signaling properties. *Sci Rep* 2014; **4**: 7357 [PMID: 25483805 DOI: 10.1038/srep07357]
- 80 **Zhang L**, Yesupriya A, Chang MH, Teshale E, Teo CG. Apolipoprotein E and protection against hepatitis E viral infection in American non-Hispanic blacks. *Hepatology* 2015; **62**: 1346-1352 [PMID: 26096528 DOI: 10.1002/hep.27938]
- 81 **Mazumdar B**, Banerjee A, Meyer K, Ray R. Hepatitis C virus E1 envelope glycoprotein interacts with apolipoproteins in facilitating entry into hepatocytes. *Hepatology* 2011; **54**: 1149-1156 [PMID: 21735466 DOI: 10.1002/hep.24523]
- 82 **El Omari K**, Iourin O, Kadlec J, Sutton G, Harlos K, Grimes JM, Stuart DI. Unexpected structure for the N-terminal domain of hepatitis C virus envelope glycoprotein E1. *Nat Commun* 2014; **5**: 4874 [PMID: 25224686 DOI: 10.1038/ncomms5874]
- 83 **Wahid A**, Dubuisson J. Virus-neutralizing antibodies to hepatitis C virus. *J Viral Hepat* 2013; **20**: 369-376 [PMID: 23647953 DOI: 10.1111/jvh.12094]
- 84 **André P**, Komurian-Pradel F, Deforges S, Perret M, Berland JL, Sodoyer M, Pol S, Bréchet C, Paranhos-Baccalà G, Lotteau V. Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. *J Virol* 2002; **76**: 6919-6928 [PMID: 12072493]

**P- Reviewer:** Melhem NM, Temel HE, Uyanik M  
**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D



Prospective Study

## Is neutrophil gelatinase associated lipocalin useful in hepatitis C virus infection?

Alessio Strazzulla, Giuseppe Coppolino, Concetta Di Fatta, Francesca Giancotti, Giuseppina D'Onofrio, Maria Concetta Postorino, Maria Mazzitelli, Selma Valerie Mammone, Innocenza Gentile, Laura Rivoli, Eleonora Palella, Tiziana Gravina, Chiara Costa, Vincenzo Pisani, Vincenzo De Maria, Giorgio Settimo Barreca, Nadia Marascio, Alfredo Focà, Giorgio Fuiano, Elio Gulletta, Carlo Torti

Alessio Strazzulla, Maria Concetta Postorino, Maria Mazzitelli, Selma Valerie Mammone, Chiara Costa, Vincenzo Pisani, Carlo Torti, Infectious Diseases Unit, Department of Medical and Surgical Sciences, "Magna Graecia" University, 88100 Catanzaro, Italy

Giuseppe Coppolino, Giuseppina D'Onofrio, Nephrology and Dialysis Unit, "Pugliese-Ciaccio" Hospital, 88100 Catanzaro, Italy

Concetta Di Fatta, Innocenza Gentile, Eleonora Palella, Elio Gulletta, Clinical Pathology Unit, Department of Health Sciences, "Magna Graecia" University, 88100 Catanzaro, Italy

Francesca Giancotti, Tiziana Gravina, Vincenzo De Maria, Hepatology Unit, "Mater Domini" Teaching Hospital, 88100 Catanzaro, Italy

Laura Rivoli, Giorgio Fuiano, Nephrology Unit, Department of Medical and Surgical Sciences, "Magna Graecia" University, 88100 Catanzaro, Italy

Giorgio Settimo Barreca, Nadia Marascio, Alfredo Focà, Microbiology Unit, Department of Health Sciences, "Magna Graecia" University, 88100 Catanzaro, Italy

**Author contributions:** Strazzulla A, Rivoli L and Torti C drafted the article; Coppolino G, Focà A, Fuiano G, Gulletta E and Torti C participated in oversight the study; Di Fatta C made NGAL measurements; Giancotti F, D'Onofrio G, Mazzitelli M, Mammone SV, Gravina T, Costa C, Pisani V, De Maria V and Marascio N were involved in data collection; Postorino MC and Rivoli L performed statistical analysis; Gentile I and Palella E were involved in storing samples; Barreca GS performed microbiological analysis; all authors approved the final version to be published.

**Institutional review board statement:** The study was approved

by the IRB of "Mater Domini" Teaching Hospital in the session of January 2014.

**Informed consent statement:** All study participants provided written consent prior to study enrollment.

**Conflict-of-interest statement:** All authors declare that they do not have any conflicts of interest for the content of this paper.

**Data sharing statement:** There are no additional data available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Alessio Strazzulla, Infectious Diseases Unit, Department of Medical and Surgical Sciences, "Magna Graecia" University, Unità Operativa Malattie Infettive, Policlinico "Mater Domini", viale Europa, 88100 Catanzaro, Italy. [alessiostrazzulla@yahoo.it](mailto:alessiostrazzulla@yahoo.it)  
**Telephone:** +39-961-3647203  
**Fax:** +39-961-3647544

**Received:** January 26, 2016

**Peer-review started:** January 27, 2016

**First decision:** March 23, 2016

**Revised:** May 22, 2016

**Accepted:** June 14, 2016

**Article in press:** June 16, 2016

**Published online:** July 8, 2016

## Abstract

**AIM:** To evaluate neutrophil gelatinase associated lipocalin (NGAL) in patients infected by hepatitis C virus (HCV) before and during treatment with directly acting antivirals (DAAs).

**METHODS:** NGAL was measured in a group of patients with chronic HCV infection ranked, at baseline, by age, gender, anti-hypertensive therapy, HCV viral load, liver fibrosis stage and, either at baseline or after 1 year, estimated glomerular filtration rate (eGFR). Then, NGAL and eGFR evolutions were monitored in a subgroup of patients who started antiviral therapy with DAAs. Differences of median NGAL levels were evaluated through Wilcoxon-Mann-Whitney test for non-parametric data. Differences in dichotomous variables were evaluated through  $\chi^2$  test. At baseline, a univariate regression analysis was conducted to verify if NGAL values correlated with other quantitative variables [age, fibrosis four (FIB-4), AST to platelet ratio index (APRI), and eGFR].

**RESULTS:** Overall, 48 patients were enrolled, 8 of them starting HCV treatment. At baseline, statistically significant differences were found in median NGAL values only between patients with eGFR < 60 mL/min *vs* patients with eGFR  $\geq$  90 mL/min. Differences in NGAL were not significant among patients ranked by HCV viral load, FIB-4 score and APRI, when patients with NGAL > 118.11 ng/dL were compared with those of NGAL  $\leq$  118.11 ng/dL, not statistically significant differences were present for age, gender, chronic kidney disease classification and liver fibrosis ( $P > 0.05$ ). Linear correlation was found between NGAL and both age ( $P = 0.0475$ ) and eGFR ( $P = 0.0282$ ) values. Not statistically significant predictions of NGAL at baseline were demonstrated for eGFR evolution 1 year later. Interestingly, in the 8 patients treated with DAAs, median NGAL significantly increased at week 12 compared to baseline ( $P = 0.0239$ ).

**CONCLUSION:** Our results suggest that NGAL should be further evaluated as an adjunct marker of kidney function in these patients.

**Key words:** Directly acting antivirals; Hepatitis C virus; Inflammation; Neutrophil gelatinase lipocalin; Tubular impairment

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** For the first time, we evaluated the evolution of neutrophil gelatinase associated lipocalin (NGAL), a novel biomarker of renal impairment, in patients infected by hepatitis C virus before and during treatment with directly acting antivirals (DAAs). In our study, we documented a significant increase of NGAL during the first 12 wk of therapy with DAAs and a correlation of NGAL with both age and estimated glomerular filtration

rate before starting treatment. In a context of paucity of information about safety of the new DAAs, we believe that these data are both informative and novel, provoking urgent investigations.

Strazzulla A, Coppolino G, Di Fatta C, Giannotti F, D'Onofrio G, Postorino MC, Mazzitelli M, Mammone SV, Gentile I, Rivoli L, Palella E, Gravina T, Costa C, Pisani V, De Maria V, Barreca GS, Marascio N, Focà A, Fuiano G, Gulletta E, Torti C. Is neutrophil gelatinase associated lipocalin a useful marker in hepatitis C virus infection? *World J Hepatol* 2016; 8(19): 815-824 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i19/815.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i19.815>

## INTRODUCTION

Estimated prevalence of renal insufficiency is increased by 40% in patients infected by hepatitis C virus (HCV) compared to the HCV negative population<sup>[1]</sup>. Renal disease, ranging from mild to end-stage renal disease (ESRD), often complicates prognosis and treatment of HCV infection with haemodialysis being required in some cases. Moreover, in the context of liver disease, kidney function is one of the key predictors of death and serum creatinine, is a component of both King's college criteria and model for end-stage liver disease scoring systems that are used for prognostic stratification in patients with acute and chronic liver failure<sup>[2]</sup>.

Virus related kidney diseases mainly show up as a glomerular impairment, predominantly a membranoproliferative glomerulonephritis with type 2 crioglobulinaemia and sub-endothelial or intra luminal deposits of IgG, IgM and complement components<sup>[3]</sup>. Moreover, HCV core proteins were isolated in both glomerular and tubular tissues, suggesting the presence of a parallel tubular-interstitial damage<sup>[4]</sup>.

Treatment of HCV infection in patients with renal impairment has always been challenging due to the side effects of the past treatment used as standard, an association of pegylated-interferon (PEG-IFN) and ribavirin (RBV), which was more difficult to manage in patients with renal impairment than in patients with normal renal function. In 2014, with the advent of first generation directly acting antivirals (DAAs), efficacy of anti HCV treatment was substantially improved. However, the first generation DAAs, boceprevir and telaprevir (TVR), were not recommended in patients with severe renal impairment or ESRD because these patients were excluded from registrational trials<sup>[5]</sup>. Moreover, in real-life patients without renal impairment, an increase of serum creatinine was observed during treatment with TVR, even if it was not associated with more severe renal impairment<sup>[6]</sup>. In March 2015, second generation DAAs were available in Italy for the treatment of HCV. Even if second generation DAAs are better tolerated, there is a paucity of information about the possible impact of these



drugs on renal function<sup>[7]</sup>. Therefore, the evidence of kidney impairment may make clinicians less comfortable to begin an antiviral treatment.

Estimated glomerular filtration rate (eGFR) can be calculated by creatinine values, however in circumstances such as toxic drug dosage, kidney disease improving global outcome (KDIGO) guidelines suggest to add at least another biomarker. In fact, GFR estimating equation alone is biased in many situations, such as acute kidney disease (AKI), high GFR, non-GFR determinants of serum creatinine and interferences with creatinine assays<sup>[8]</sup>. Additionally, creatinine-based measures are even more limited in cirrhotic patients due to a decrease in hepatic synthesis of creatinine, reduced skeletal muscle mass and increased tubular secretion<sup>[9]</sup>.

Among novel kidney biomarkers, one of the most promising is neutrophil gelatinase associated lipocalin (NGAL) either in urine and serum (or plasma)<sup>[10]</sup>. This is a small glycoprotein in three different forms: A monomer (25 kDa), a homodimer (45 kDa) and a heterodimer (135 kDa). It is secreted by many human cells, such as epithelial cells (liver, kidney, lungs) and blood cells (neutrophils, monocytes and macrophages), filtered in the glomerulus and reabsorbed by the proximal tubules<sup>[11,12]</sup>. It is removed by hemodialysis<sup>[13]</sup>. In general, NGAL urinary levels increase after a tubular injury and reveal a kidney damage earlier than the increased levels of creatinine<sup>[14]</sup>. On the other hand, plasmatic or serum NGAL are more extensively adopted in AKI contexts, because their measurement is less limited by availability of samples when patients are anuric<sup>[15]</sup>. Remarkably, an increase of plasmatic NGAL is considered to be an early predictor of AKI in various critical settings such as cardiac surgery, septic shock, contrast induced nephropathy, renal and liver transplantation<sup>[16-22]</sup>. However, NGAL is increased also in case of epithelial damage or inflammation outside the kidney<sup>[10]</sup>.

In cirrhotic patients, NGAL is a marker of AKI and urinary NGAL that can help distinguish among different causes of renal impairment<sup>[10]</sup>. Recently, a significant difference in plasmatic NGAL levels amongst HCV positive patients with cirrhosis and eGFR < 60 mL/min vs ≥ 60 mL/min has been demonstrated<sup>[23]</sup>. Also, recent data showed that NGAL is a good marker of renal damage due to drug toxicity. For example, urinary NGAL is a good predictor of tacrolimus induced AKI in liver transplanted patients and nonsteroidal anti-inflammatory drug (NSAID) associated AKI in cirrhotic patients<sup>[24,25]</sup>. To our best knowledge, no published data on NGAL during HCV treatment with DAAs are available so far.

The objectives of this study were to explore: (1) whether there is a difference in plasmatic NGAL between HCV positive patients and HCV negative people; (2) whether there is a difference in plasmatic NGAL among HCV positive patients ranked by age, gender, viral load, eGFR and liver fibrosis stage; (3) whether NGAL levels at baseline correlate with modification of eGFR after 1 year; and (4) the evolution of renal function in patients

treated with DAA including regimens.

## MATERIALS AND METHODS

### Recruitment of patients and data collection

A prospective study was conducted. Patients with chronic hepatitis C who attended the Outpatient Service of the Infectious Diseases Unit and the Hepatology Unit of the "Mater Domini" Teaching Hospital in Catanzaro (Italy) from February 1, 2014 to April 30, 2014 were included in this study. Exclusion criteria included: Leukocytosis (leukocyte count higher than 12000 cells/ $\mu$ L), variceal bleeding, primary kidney diseases (glomerular nephropathy), KDIGO classification of chronic kidney disease (CKD) ≥ G4 (eGFR < 30 mL/min), ongoing HCV therapy (with or without interferon or DAA). Approval from local ethical committee was obtained. All enrolled patients signed an informed consent.

All patients underwent physical examination and history taking at baseline. The following blood tests were collected: AST, ALT, total and fractioned bilirubin, albumin,  $\gamma$ GT, alkaline phosphatase, prothrombin time, total blood cell count (including neutrophil and platelet count) and urea. Serum creatinine levels were measured at baseline and after 1 year. For patients who started anti-viral therapy, serum creatinine levels and GFR were studied at week 4 and week 12 after baseline.

Glomerular filtration rate was estimated through Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI, 2009) since CKD-EPI is less biased and more accurate for eGFR ≥ 60 mL/min than MDRD (Modification of Diet in Renal Disease) and Cockcroft-Gault formulas<sup>[7]</sup>. The following formula was used:  $141 \times \min(\text{SCr}/k, 1)^a \times \max(\text{SCr}/k, 1)^{-1.209} \times 0.993^{\text{Age}}$  × (1.018 if female or 1.159 if black), where SCr is serum creatinine (in mg/dL), k is 0.7 for females and 0.9 for males, a is 0.329 for females and 0.411 for males, min is the minimum of SCr/k or 1, and max is the maximum of SCr/k or 1.

Liver fibrosis was estimated at baseline by either fibrosis four (FIB-4) score or AST to platelet ratio index (APRI) which are the most used formulas for estimating stage of liver disease. FIB-4 has a negative predictive value of 94.7% to exclude severe fibrosis with a sensitivity of 74.3% when < 1.45 and a positive predictive value to confirm the existence of a significant fibrosis (F3-F4) of 82.1% with a specificity of 98.2% when ≥ 3.25. The following formula was used:  $\text{Age} \times \text{AST}/(\text{platelets} \times \sqrt{\text{ALT}})$  where AST and ALT were measured as IU/L, platelets were measured as number × 10<sup>6</sup>/ $\mu$ L and age was measured in years<sup>[26]</sup>. An APRI value ≤ 0.5 rules out significant fibrosis and cirrhosis while values ≥ 1.5 indicates significant fibrosis<sup>[27]</sup>. More specifically, when APRI score is greater than 1.0 it has a sensitivity of 76% and a specificity of 72% for predicting cirrhosis<sup>[28]</sup>. The following formula was used:  $[(\text{AST}/\text{AST upper normal limit})/\text{platelets}] \times 100$  where AST was measured as IU/L, platelets were measured as number

$\times 10^6/\mu\text{L}$  and AST upper normal limit was fixed at 35 IU/L.

### NGAL measurement

Peripheral venous blood samples were taken from each patient at baseline and then processed for NGAL measurement. Plasmatic NGAL was measured in all patients at baseline. For patients who started anti-viral therapy, NGAL was measured at different endpoints (baseline, week 4 and 12). NGAL assay was performed using human NGAL Rapid Elisa Kit (BioPorto Diagnostic). A 96-well microtiter plate coated with purified anti-human NGAL monoclonal antibody was used. In each well 50  $\mu\text{L}$  of each sample, diluted 1:100 with sample diluting buffer, undiluted calibrators and controls were added; then 50  $\mu\text{L}$  of horseradish peroxidase -conjugated NGAL antibodies were added. During this first step of incubation at room temperature for 30 min, NGAL bind either the specific antibody adsorbed to microwells or the second antibody; an aspiration-washing step (3 times with 300  $\mu\text{L}$  of wash solution) was performed to remove excess and unbounded reagents; 100  $\mu\text{L}$  of tetramethyl-benzidine solution were added to each well and a second step of incubation performed at room temperature for 15 min; at end of incubation 100  $\mu\text{L}$  of stop solution was added and the color developed in each well was measured at 450 nm. The values of samples were determined on the basis of a standard curve. The methodology was performed using a Triturus automatic analyzer. The commercial kit, that is validated by Pedersen *et al.*<sup>[29]</sup> is used in our study. The reference range (41.19-118.11 ng/mL) had been previously validated in our laboratory on a group of healthy volunteers.

### Groups of patients

Differences of NGAL values were evaluated in patients ranked by age (< 65 years vs  $\geq$  65 years), gender (female vs male), anti-hypertensive therapy (present vs absent), HCV viral load (< 1000000 copies/mL vs  $\geq$  1000000 copies/mL), FIB-4 score ( $\leq$  1.45 vs 1.45 to 3.25 vs  $\geq$  3.25), APRI ( $\leq$  0.5 vs 0.5 to 1.5 vs  $\geq$  1.5), and eGFR ( $\geq$  90 mL/min vs  $\geq$  60 mL/min to < 90 mL/min vs < 60 mL/min). Patients were ranked by eGFR worsening vs stable/improved with respect to baseline values after 1 year. Any reduction of eGFR was considered as worsening. Viral load cut-off was set at 1000000 HCV RNA copies/mL because this value is commonly considered to be high<sup>[30]</sup>. Cut-off for age was set at 65 years because that is the threshold discriminating adulthood and elderly life in many western countries<sup>[31]</sup>. We analyzed eGFR as a continuous measure and did not consider any specific-cut off for change of eGFR.

Differences in dichotomous variables (gender, FIB-4  $\geq$  3.25, APRI  $\geq$  1.5, KDIGO CKD classification  $\geq$  G3a, KDIGO CKD classification  $\geq$  G2, age  $\geq$  65 year) were evaluated in patients ranked at baseline by NGAL values (> 118.11 ng/dL vs  $\leq$  118.11 ng/dL).

Evolution of NGAL at different time points (baseline, week 4 and week 12) was evaluated separately in

patients who started antiviral treatment with DAA containing regimens during the first twelve weeks of therapy.

### Statistical analysis

Differences of distribution NGAL levels from baseline to one year in the overall population and differences of NGAL and eGFR distribution in patients prescribed HCV therapy (evaluated at baseline, week 4 and week 12) were evaluated through Wilcoxon-Mann-Whitney test for non parametric data. Also, at baseline, a Spearman correlation analysis was conducted to verify if NGAL values correlated with other quantitative variables (age, FIB-4, APRI, and eGFR). Differences in dichotomous variables (age, gender, CKD classification and liver fibrosis) in patients with NGAL > 118.11 ng/dL or NGAL  $\leq$  118.11 ng/dL were evaluated through  $\chi^2$  test. Statistical analysis was performed using Graphpad Prism 6.01 (GraphPad Software, La Jolla, CA, United States). Nominal statistical significance was set at  $P < 0.05$ .

## RESULTS

### Patient characteristics at baseline

Forty-eight HCV RNA positive patients were enrolled with median NGAL of 68.5 ng/dL (range: 136-27). Main characteristics of the population are summarized in Table 1. Seventeen (35%) patients were < 65 years old, 25 (52%) patients were females, 10 (21%) patients suffered from blood hypertension. Median NGAL was: 63 ng/dL (111-27) for patients < 65 years old and 77 ng/dL (136-28) for patients  $\geq$  65 years old ( $P = 0.1353$ ); 70 ng/dL (132-27) for males and 63 ng/dL (111-27) for females ( $P = 0.7822$ ); 72.5 ng/dL (102-28) for patients with hypertension and 66.5 ng/dL (136-27) for patients with normal blood pressure ( $P = 0.7756$ ).

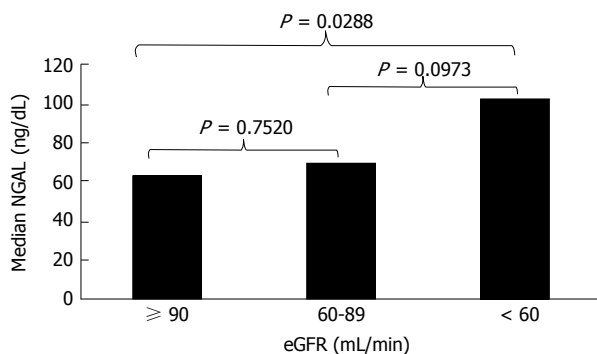
Differences in NGAL were not significant among patients ranked by HCV viral load, FIB-4 score and APRI. Quantitative HCV RNA was available in 36 patients, 19 (53%) of them having HCV RNA < 1000000 copies/mL (*i.e.*, low HCV RNA group). Median NGAL at baseline was 70 ng/dL (range: 132-27) vs 63 ng/dL (121-28), respectively. For FIB-4, 8 (17%) patients with FIB-4  $\leq$  1.45 had median NGAL of 60.5 ng/dL (111-27), 16 (33%) with FIB-4 from 1.45 to 3.25 had median NGAL of 82 ng/dL (136-36) and 24 (50%) with FIB-4  $\geq$  3.25 had median NGAL of 74.5 ng/dL (132-28). Regarding APRI, 10 (21%) patients were  $\leq$  0.5, 22 (46%) were between 0.5 and 1.5, and 16 (33%) were  $\geq$  1.5. Median NGAL values were 60.5 ng/dL (range: 136-27), 74.5 ng/dL (124-36) ng/dL and 80 ng/dL (132-28) in the three APRI groups, respectively.

According to eGFR ranking, 25 (52%) patients had eGFR  $\geq$  90 mL/min, 19 (40%) had eGFR  $\geq$  60 but less than 90 mL/min and 4 (8%) had eGFR < 60 mL/min. Median NGAL was 63 ng/dL (range: 136-36), 70 ng/dL (124-27) and 102.5 ng/dL (132-88) in the three groups, respectively. Statistical analysis showed significant

**Table 1** Characteristics of the population at baseline *n* (%)

Patients' characteristics	Overall population ( <i>n</i> = 48)	Patients treated with DAAs ( <i>n</i> = 8)
Qualitative variables		
Gender		
Female	25 (52)	1 (12)
Male	23 (48)	7 (88)
HCV RNA genotype		
1a	3 (6)	0 (0)
1b	29 (61)	8 (100)
2a/2c	4 (8)	0 (0)
3	1 (2)	0 (0)
4	2 (4)	0 (0)
Not available	9 (19)	0 (0)
Quantitative variables, median (range)		
Age (yr)	67.0 (36.0-84.0)	63.5 (51.0-69.0)
eGFR (mL/min)	90.0 (30.0-111.7)	93.0 (77.0-109.0)
FIB-4	3.0 (0.5-18.3)	3.7 (1.5-9.0)
AST (IU/L)	44.0 (17.0-180.0)	70.0 (28.0-103.0)
ALT (IU/L)	45.0 (12.0-268.0)	53.5 (35.0-171.0)
Albumin (mg/dL)	4.2 (3.0-4.8)	4.3 (3.3-4.6)
Total bilirubin (mg/dL)	0.8 (0.3-2.2)	0.6 (0.3-1.0)
PLT (n/μL)	141000 (13500-312000)	132000 (71000-229000)
WBC (cells/μL)	5560 (2220-11500)	6245 (2790-9200)
HCV-RNA (copies/mL)	776500 (90100-19500000)	2330000 (793000-3250000)

HCV: Hepatitis C virus; eGFR: Estimated glomerular filtration rate; FIB-4: Fibrosis four index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; PLT: Platelet; WBC: White blood cell; DAAs: Directly acting antivirals.



**Figure 1** Plasmatic neutrophil gelatinase associated lipocalin at baseline according to estimated glomerular filtration rate ranking. eGFR: Estimated glomerular filtration rate; NGAL: Neutrophil gelatinase associated lipocalin.

differences in median NGAL values only in patients with eGFR < 60 mL/min vs patients with eGFR ≥ 90 mL/min (Figure 1;  $P = 0.0288$ ).

Spearman correlation analysis showed statistically significant positive correlation between NGAL and age (Spearman  $r = 0.341$ ; 95%CI: 0.05450-0.5759; two-tailed  $P = 0.0088$ ) and a negative correlation between NGAL and eGFR values (Spearman  $r = -0.257$ ; 95%CI: -0.5164 to 0.04421; two-tailed  $P = 0.0419$ ) (Figure 2). Not statistically significant results were obtained when NGAL was correlated FIB-4 score ( $P = 0.413$ ) or APRI ( $P = 0.7430$ ).

In 6 (12.5%) patients, NGAL exceeded the upper limit of the reference interval (NGAL > 118.11 ng/mL). When patients with NGAL > 118.11 ng/dL were compared with patients with NGAL ≤ 118.11 ng/dL, no statistically significant differences were present for age,

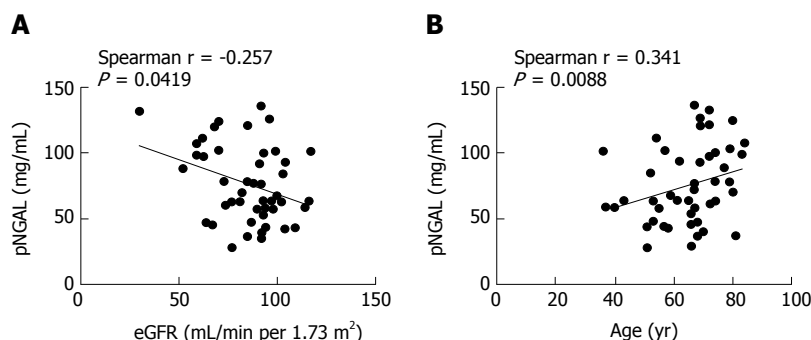
gender, CKD classification and liver fibrosis ( $P > 0.05$ ). In fact: 6/6 (100%) patients with NGAL > 118.11 ng/mL vs 25/42 (60%) patients with NGAL ≤ 118.11 ng/mL were ≥ 65 years old; 3/6 (50%) patients with NGAL > 118.11 ng/mL vs 20/42 (48%) patients with NGAL ≤ 118.11 ng/mL were males; 1/6 (17%) patients with NGAL > 118.11 ng/mL vs 7/42 (17%) patients with NGAL ≤ 118.11 ng/mL had a KDIGO CKD ≥ G3a; 4/6 (67%) patients with NGAL > 118.11 ng/mL vs 26/42 (62%) patients with NGAL ≤ 118.11 ng/mL had a KDIGO CKD classification ≥ G2; 4/6 (67%) patients with NGAL > 118.11 ng/mL vs 19/42 (45%) patients with NGAL ≤ 118.11 ng/mL had a FIB-4 ≥ 3.25; 3/6 (50%) patients with NGAL > 118.11 ng/mL vs 13/42 (31%) patients with NGAL ≤ 118.11 ng/mL had an APRI ≥ 1.5.

#### Evaluation of renal parameters after one year

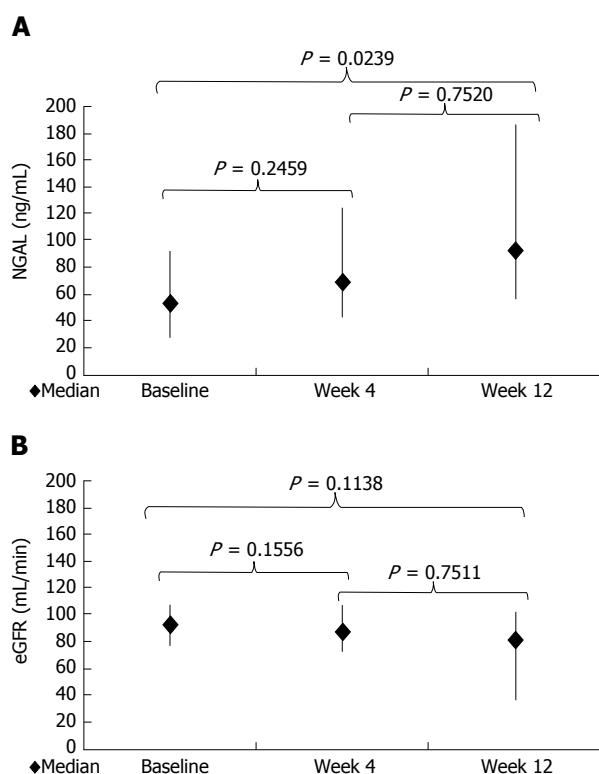
Serum creatinine and eGFR were collected after 1 year for 40 patients, while the remaining 8 were analyzed separately because they started HCV treatment in the meanwhile. No statistically significant differences were demonstrated in median NGAL values at baseline between patients with worsening eGFR vs patients with stable/improved eGFR at year 1. Indeed, median NGAL at baseline was 71.5 ng/dL (range: 136-36) in patients with worsening eGFR after 1 year, while it was 73.5 ng/dL (132-27) in patients with stable/improved eGFR after 1 year ( $P = 0.4898$ ).

#### Evolution of renal parameters during HCV treatment

A separate prospective analysis was conducted in the 8 patients who started with antiviral therapy. Baseline



**Figure 2** Univariate linear correlations between plasmatic neutrophil gelatinase associated lipocalin (NGAL) at baseline and estimated glomerular filtration rate (A) and NGAL at baseline and age (B). Data are represented as a scatter plot in which each point represents a patient. Correlation coefficient, Pearson  $r$  values, and statistical significance are indicated.  $n = 48$  patients. eGFR: Estimated glomerular filtration rate; NGAL: Neutrophil gelatinase associated lipocalin.



**Figure 3** Evolution of plasmatic neutrophil gelatinase associated lipocalin (A) and estimated glomerular filtration rate (B) during the first 12 wk of hepatitis C virus therapy with directly acting antiviral containing regimens. eGFR: Estimated glomerular filtration rate; NGAL: Neutrophil gelatinase associated lipocalin.

characteristics of these patients are summarized in Table 1. Two patients started simeprevir (SMV) + daclatasvir (DCV) for 24 wk, while 6 patients started PEG-IFN + RBV + TVR for 12 wk followed by PEG-IFN + RBV for other 24 wk (36 wk overall). Three patients were relapser to previous treatments with PEG-IFN + RBV, while 5 were partial responders. At week 4, 2 patients had negative but detectable HCV RNA ( $\leq 15$  UI/mL) while 6 had undetectable HCV RNA. At week 12, HCV RNA was undetectable in all patients. Median NGAL was 53 ng/dL (range: 92-28) at baseline, 69 ng/dL (125-43) at week 4, and 93 ng/dL (186-56) at week 12 ( $P = 0.0239$  compared with baseline value) (Figures 3A and 4A).

Median eGFR was 93 mg/dL (109-77) at baseline, 86.5 mg/dL (108-72) at week 4, and 82.5 mg/dL (103-72) at week 12; no statistically significant differences were found for eGFR along the time points of the study (Figures 3B and 4B).

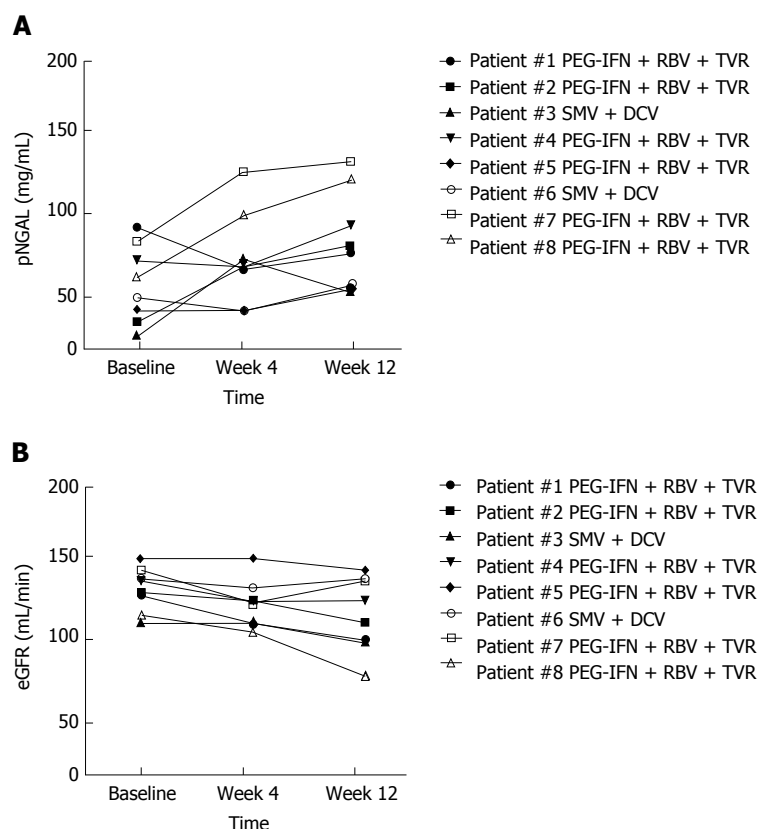
## DISCUSSION

This is the first study which investigated NGAL, both before and during the first twelve weeks of therapy with DAA including regimens. Until now, other investigators evaluated NGAL but only in HCV positive patients with cirrhosis and before treatment with DAA containing regimens<sup>[23]</sup>.

We found that, in patients not exposed to DAA, eGFR was below normality around 50%, while NGAL was in the range of normality in most individuals. Moreover, among the six patients with increased NGAL, two cases had eGFR normal. These results suggest that a discordance between the two methods exists when interpretation is "categorical". Despite these findings, in the overall population plasmatic NGAL was statistically correlated with eGFR. Particularly, NGAL was significantly higher in patients with eGFR  $< 60$  mL/min than those with eGFR  $\geq 90$  mL/min. It is difficult to explain apparent discrepancies with the current literature data because Alhaddad *et al.*<sup>[23]</sup> previously demonstrated that HCV positive cirrhotic patients with eGFR  $< 60$  mL/min had a significantly lower plasmatic NGAL than HCV positive cirrhotic patients with eGFR  $\geq 60$  mL/min. Inclusion of both cirrhotic and non-cirrhotic patients in our study could be an explanation. In conclusion, we think that further studies should evaluate the rate of concordance between the two methods in diverse stages of liver disease. Also, it has to be seen whether these markers provide useful insights on the glomerular (especially detected by e-GFR) or tubular (especially detected by NGAL) damage in these conditions.

We were not able to find any correlations explaining the variability in NGAL values apart from age and in contrast with Bolignano *et al.*<sup>[11]</sup> who, however, selected patients with non-terminal CKD of various etiologies. The correlation found in our study (increasing NGAL with increase in age) was consistent with the inverse





**Figure 4** Evolution of neutrophil gelatinase associated lipocalin (A) and estimated glomerular filtration rate (B) in 8 patients during the first 12 week of hepatitis C virus therapy with directly acting antiviral containing regimens. Number of patients and respective treatment for hepatitis C virus infection are specified. PEG-IFN: Pegylated-interferon; RBV: Ribavirin; TVR: Telaprevir; SMV: Simeprevir; DCV: Daclatasvir; eGFR: Estimated glomerular filtration rate; NGAL: Neutrophil gelatinase associated lipocalin.

and expected correlation between eGFR and age. Importantly, NGAL was not correlated with stage of liver disease or burden of HCV RNA, suggesting that further mechanisms are implicated. Similarly, in their study, Gungor *et al*<sup>[22]</sup> did not find differences between cirrhotic patients and healthy controls but, at the same time, they found that a high plasmatic NGAL was associated with a higher risk of mortality in cirrhotic patients. Our data support the fact that NGAL is not influenced by HCV RNA, suggesting that glomerular and tubular damages have different pathogenic and clinical significances.

We did not find any signals pointing to a predictive role of NGAL for eGFR evolution during the follow-up. Indeed, contrary to this hypothesis, patients with a reduction of eGFR at 1 year had lower NGAL than patients with an improvement of eGFR at 1 year. Bolognani *et al*<sup>[11]</sup> found that patients with non-advanced CKD who progressed in their kidney disease during the follow-up period (median 18.5 mo, range 1-20) had a significantly higher urinary and serum NGAL levels at baseline than patients with non-advanced CKD who did not progress. Our patients did not suffer from significant CKD and renal function did not decrease significantly during the follow-up. These considerations, together with the small sample size and limited length of follow-up may have limited our possibility to demonstrate a significant prediction in this study.

We measured NGAL for the first time during therapy with DAA. Importantly, NGAL increased significantly from baseline to week 12, whereas eGFR did not change significantly, suggesting that tubular damage induced by drugs is not accompanied by glomerular impairment. An alternative hypothesis could be that NGAL increase is induced by a pro-inflammatory status, so this increase in NGAL would be a consequence, at least in part, of inflammation due to activity of antiviral drugs and consequent reduction of HCV RNA. Until now, data about relationship of HCV RNA and inflammation have been controversial. Indeed, some studies pointed to a pro-inflammatory role of HCV RNA, highlighting the enhanced activity of tumor necrosis factor- $\alpha$  and the consequent increase of some pro-inflammatory interleukins (ILs), such as IL-10, in presence of detectable HCV RNA<sup>[32,33]</sup>. Instead, other studies showed that a higher HCV RNA load was correlated with a lower value of C-reactive protein (CRP) and that levels of other ILs, such as IL-6, were correlated with liver fibrosis rather than with HCV RNA load<sup>[34]</sup>. Lastly, successful IFN-free regimens resulted in improved functional responses by natural killer cells (such as degranulation and TRAIL expression) to *in vitro* stimulation with IFN $\alpha$ <sup>[35]</sup>. Further studies are necessary to understand evolutions of immunologic or inflammatory markers (including NGAL) after DAA treatment and the underlying mechanisms.

According to this hypothesis that increase of NGAL during HCV treatment is due to drug toxicity, it is likely that our results were scarcely affected by the use of PEG-IFN and RBV. In fact, these two molecules did not demonstrate significant nephrotoxicity, while the reverse may be true because renal impairment reduces excretion of both PEG-IFN and RBV, leading to an increased risk of side effects, such as hemolytic anaemia due to intra-erythrocyte accumulation of RBV<sup>[5,36]</sup>.

All regimens analyzed in our study included a NS3/4A protease inhibitor (*i.e.*, TVR or SMV). Currently, far more data are available about renal safety of TVR than renal safety of SMV or other DAAs. The available data showed that TVR causes a significant but reversible reduction of eGFR which may be associated with an increase of side effect related to the drug<sup>[37,38]</sup>. Our study showed that, aside reduction of eGFR, there was also an increase of NGAL which could be a consequence of tubular damage. Currently, there is a paucity of data about renal safety of interferon free regimens including DAAs different from TVR. Particularly, it is unknown if second or third generation DAAs have a direct nephrotoxic effect. In our study, both patients treated with SMV + DCV experienced an increase of NGAL with a concomitant eGFR decrease during the first 12 week of treatment. Our results need to be confirmed by further and larger studies, however.

Several limitations may affect the study conclusions: (1) sample size was small and length of follow-up was limited; (2) correlations of NGAL with other factors which could have impaired renal function, such as diabetes, cryoglobulinaemia, treatment with non NSAIDs or diuretics were not evaluated; and (3) we did not measure urinary NGAL or inflammatory molecules such as CRP and ILs which could confirm or disprove the hypotheses above. Notwithstanding these limitations, we feel that our results are important and should provoke further investigations. In particular, we hypothesize that NGAL reveals kidney damage earlier than eGFR during DAA containing regimens.

However, many questions still remain to be answered. Indeed, it has to be established whether a clinical cut-off of NGAL may guide clinical decisions (*e.g.*, dosage modification or stopping of the offending drug). Also, it has to be evaluated whether NGAL could predict AKI during HCV treatment, especially in most-at-risk patients such as those with advanced cirrhosis and a high risk of renal complications (*e.g.*, hepato-renal syndrome). Lastly, cost-effectiveness studies need to be conducted to verify the hypothesis that NGAL should be routinely used to monitor kidney function during HCV treatment instead of (or in addition to) creatinine.

## COMMENTS

### Background

Neutrophil gelatinase associated lipocalin (NGAL) is a novel biomarker of renal impairment but also a marker of inflammation. For the first time, the authors evaluated the evolution of NGAL in patients infected by hepatitis C virus (HCV)

before and during treatment with directly acting antivirals (DAAs).

### Research frontiers

Kidney toxicity of new DAAs has not been established until now. The authors' results suggest that NGAL could provide information complementary to estimated glomerular filtration rate in monitoring the kidney toxicity of DAAs.

### Innovations and breakthroughs

The literature suggests that NGAL is a good marker of acute kidney injury but no data are available about NGAL in HCV positive patients. This article adds to literature data about evolution of NGAL before and during HCV treatment with DAA containing regimens.

### Applications

This study serves as an additional evidence supporting the fact that NGAL can reveal the presence of a kidney impairment before creatinine.

### Terminology

NGAL is a small glycoprotein secreted by multiple human cells, such as epithelial cells (liver, kidney, lungs) and blood cells (neutrophils, monocytes and macrophages), filtered in the glomerulus and reabsorbed by the proximal tubules. An increase of plasmatic NGAL is considered to be an early predictor of acute kidney disease in various critical settings.

### Peer-review

This study examined the measures of a novel biomarker for kidney function, NGAL, before and after HCV treatment with direct acting antivirals among 48 patients. The authors have performed a good study, the manuscript is interesting.

## REFERENCES

- 1 Ozer Etik D, Ocal S, Boyacioglu AS. Hepatitis C infection in hemodialysis patients: A review. *World J Hepatol* 2015; 7: 885-895 [PMID: 25937865 DOI: 10.4254/wjh.v7.i6.885]
- 2 Singal AK, Kamath PS. Model for End-stage Liver Disease. *J Clin Exp Hepatol* 2013; 3: 50-60 [PMID: 25755471 DOI: 10.1016/j.jceh.2012.11.002]
- 3 Cacoub P, Gragnani L, Comarmond C, Zignego AL. Extrahepatic manifestations of chronic hepatitis C virus infection. *Dig Liver Dis* 2014; 46 Suppl 5: S165-S173 [PMID: 25458776 DOI: 10.1016/j.dld.2014.10.005]
- 4 Sansonno D, Lauletta G, Montrone M, Grandaliano G, Schena FP, Dammacco F. Hepatitis C virus RNA and core protein in kidney glomerular and tubular structures isolated with laser capture microdissection. *Clin Exp Immunol* 2005; 140: 498-506 [PMID: 15932511 DOI: 10.1111/j.1365-2249.2005.02778.x]
- 5 Bunchorntavakul C, Maneerattanaporn M, Chavalitdharmrong D. Management of patients with hepatitis C infection and renal disease. *World J Hepatol* 2015; 7: 213-225 [PMID: 25729476 DOI: 10.4254/wjh.v7.i2.213]
- 6 Matsui K, Kamijo-Ikemori A, Sugaya T, Ikeda H, Okuse C, Shibagaki Y, Yasuda T, Kimura K. Does elevation of serum creatinine in patients with chronic hepatitis C under therapy of telaprevir mean renal impairment? *Nephrology (Carlton)* 2015; 20: 843-848 [PMID: 25998031 DOI: 10.1111/nep.12517]
- 7 Fabrizi F, Messa P. Therapy of hepatitis C by direct-acting antivirals: the end of HCV in dialysis population? *Expert Rev Clin Pharmacol* 2015; 8: 785-793 [PMID: 26365524 DOI: 10.1586/17512433.2015.1086266]
- 8 Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl* 2013; 3: 1-150
- 9 Davenport A, Cholongitas E, Xirouchakis E, Burroughs AK. Pitfalls in assessing renal function in patients with cirrhosis--

- potential inequity for access to treatment of hepatorenal failure and liver transplantation. *Nephrol Dial Transplant* 2011; **26**: 2735-2742 [PMID: 21690201 DOI: 10.1093/ndt/gfr354]
- 10 **Firu SG**, Streba CT, Firu D, Tache DE, Rogoveanu I. Neutrophil Gelatinase Associated Lipocalin (NGAL) - a biomarker of renal dysfunction in patients with liver cirrhosis: Do we have enough proof? *J Med Life* 2015; **8** Spec Issue: 15-20 [PMID: 26361506]
  - 11 **Bolignano D**, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR, Nicocia G, Buemi M. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin J Am Soc Nephrol* 2009; **4**: 337-344 [PMID: 19176795 DOI: 10.2215/CJN.03530708]
  - 12 **Bolignano D**, Coppolino G, Aloisi C, Romeo A, Nicocia G, Buemi M. Effect of a single intravenous immunoglobulin infusion on neutrophil gelatinase-associated lipocalin levels in proteinuric patients with normal renal function. *J Investig Med* 2008; **56**: 997-1003 [PMID: 18955901 DOI: 10.231/JIM.0b013e31818e7e95]
  - 13 **Bolignano D**, Coppolino G, Romeo A, Lacquaniti A, Buemi M. Neutrophil gelatinase-associated lipocalin levels in chronic haemodialysis patients. *Nephrology (Carlton)* 2010; **15**: 23-26 [PMID: 20377767 DOI: 10.1111/j.1440-1797.2009.01163.x]
  - 14 **Bolignano D**, Donato V, Coppolino G, Campo S, Buemi A, Lacquaniti A, Buemi M. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. *Am J Kidney Dis* 2008; **52**: 595-605 [PMID: 18725016 DOI: 10.1053/j.ajkd.2008.01.020]
  - 15 **Schley G**, Köberle C, Manuilova E, Rutz S, Forster C, Weyand M, Formentini I, Kientsch-Engel R, Eckardt KU, Willam C. Comparison of Plasma and Urine Biomarker Performance in Acute Kidney Injury. *PLoS One* 2015; **10**: e0145042 [PMID: 26669323 DOI: 10.1371/journal.pone.0145042]
  - 16 **Mishra J**, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, Ruff SM, Zahedi K, Shao M, Bean J, Mori K, Barasch J, Devarajan P. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. *Lancet* 2005; **365**: 1231-1238 [PMID: 15811456 DOI: 10.1016/S0140-6736(05)74811-X]
  - 17 **Hirsch R**, Dent C, Pfriem H, Allen J, Beekman RH, Ma Q, Dastrala S, Bennett M, Mitsnefes M, Devarajan P. NGAL is an early predictive biomarker of contrast-induced nephropathy in children. *Pediatr Nephrol* 2007; **22**: 2089-2095 [PMID: 17874137 DOI: 10.1007/s00467-007-0601-4]
  - 18 **Ling W**, Zhaohui N, Ben H, Leyi G, Jianping L, Huili D, Jiaqi Q. Urinary IL-18 and NGAL as early predictive biomarkers in contrast-induced nephropathy after coronary angiography. *Nephron Clin Pract* 2008; **108**: c176-c181 [PMID: 18287807 DOI: 10.1159/000117814]
  - 19 **Wheeler DS**, Devarajan P, Ma Q, Harmon K, Monaco M, Cvijanovich N, Wong HR. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. *Crit Care Med* 2008; **36**: 1297-1303 [PMID: 18379258]
  - 20 **Parikh CR**, Jani A, Mishra J, Ma Q, Kelly C, Barasch J, Edelstein CL, Devarajan P. Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation. *Am J Transplant* 2006; **6**: 1639-1645 [PMID: 16827865 DOI: 10.1111/j.1600-6143.2006.01352.x]
  - 21 **Portal AJ**, McPhail MJ, Bruce M, Coltart I, Slack A, Sherwood R, Heaton ND, Shawcross D, Wendon JA, Heneghan MA. Neutrophil gelatinase-associated lipocalin predicts acute kidney injury in patients undergoing liver transplantation. *Liver Transpl* 2010; **16**: 1257-1266 [PMID: 21031541 DOI: 10.1002/lt.22158]
  - 22 **Gungor G**, Ataseven H, Demir A, Solak Y, Gaipov A, Biyik M, Ozturk B, Polat I, Kiyici A, Cakir OO, Polat H. Neutrophil gelatinase-associated lipocalin in prediction of mortality in patients with hepatorenal syndrome: a prospective observational study. *Liver Int* 2014; **34**: 49-57 [PMID: 23799980 DOI: 10.1111/liv.12232]
  - 23 **Alhaddad OM**, Alsebaey A, Amer MO, El-Said HH, Salman TA. Neutrophil Gelatinase-Associated Lipocalin: A New Marker of Renal Function in C-Related End Stage Liver Disease. *Gastroenterol Res Pract* 2015; **2015**: 815484 [PMID: 26221137 DOI: 10.1155/2015/815484]
  - 24 **Tsuchimoto A**, Shinke H, Uesugi M, Kikuchi M, Hashimoto E, Sato T, Ogura Y, Hata K, Fujimoto Y, Kaide T, Kishimoto J, Yanagita M, Matsubara K, Uemoto S, Masuda S. Urinary neutrophil gelatinase-associated lipocalin: a useful biomarker for tacrolimus-induced acute kidney injury in liver transplant patients. *PLoS One* 2014; **9**: e110527 [PMID: 25329716 DOI: 10.1371/journal.pone.0110527]
  - 25 **Elia C**, Graupera I, Barreto R, Solà E, Moreira R, Huelin P, Ariza X, Solé C, Pose E, Baiges A, Fabrellas N, Poch E, Fernández J, Arroyo V, Ginès P. Severe acute kidney injury associated with non-steroidal anti-inflammatory drugs in cirrhosis: A case-control study. *J Hepatol* 2015; **63**: 593-600 [PMID: 25872166 DOI: 10.1016/j.jhep.2015.04.004]
  - 26 **Vallet-Pichard A**, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**: 32-36 [PMID: 17567829 DOI: 10.1002/hep.21669]
  - 27 **Loaeza-del-Castillo A**, Paz-Pineda F, Oviedo-Cárdenas E, Sánchez-Avila F, Vargas-Vorácková F. AST to platelet ratio index (APRI) for the noninvasive evaluation of liver fibrosis. *Ann Hepatol* 2008; **7**: 350-357 [PMID: 19034235]
  - 28 **Lin ZH**, Xin YN, Dong QJ, Wang Q, Jiang XJ, Zhan SH, Sun Y, Xuan SY. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatology* 2011; **53**: 726-736 [PMID: 21319189 DOI: 10.1002/hep.24105]
  - 29 **Pedersen KR**, Ravn HB, Hjortdal VE, Nørregaard R, Povlsen JV. Neutrophil gelatinase-associated lipocalin (NGAL): validation of commercially available ELISA. *Scand J Clin Lab Invest* 2010; **70**: 374-382 [PMID: 20509756 DOI: 10.3109/00365513.2010.486868]
  - 30 **Saracco G**, Ciancio A, Ghisetti V, Rocca G, Cariti G, Andreoni M, Tabone M, Roffi L, Calleri G, Ballarè M, Terreni N, Sartori M, Tappero GF, Traverso A, Poggio A, Orani A, Maggi G, Di Napoli A, Arrigoni A, Rizzetto M. Treatment with interferon-alpha2b of naive non-cirrhotic patients with chronic hepatitis C according to viraemia and genotype. Results of a randomized multicentre study. The North West Italian Hepatological Group. *Eur J Gastroenterol Hepatol* 2001; **13**: 149-155 [PMID: 11246614 DOI: 10.1097/00042737-200102000-00010]
  - 31 **Delanaye P**, Glasscock RJ, Pottel H, Rule AD. An Age-Calibrated Definition of Chronic Kidney Disease: Rationale and Benefits. *Clin Biochem Rev* 2016; **37**: 17-26 [PMID: 27057075]
  - 32 **Vanis N**, Mehmedović A, Mesihović R. Use of serum levels of proinflammatory cytokine IL-1α in chronic hepatitis C. *Coll Antropol* 2015; **39**: 75-79 [PMID: 26040073]
  - 33 **Pircher J**, Czermak T, Merkle M, Mannell H, Krötz F, Ribeiro A, Vielhauer V, Nadjiri J, Gaitzsch E, Niemeyer M, Porubsky S, Gröne HJ, Wörmlle M. Hepatitis C virus induced endothelial inflammatory response depends on the functional expression of TNFα receptor subtype 2. *PLoS One* 2014; **9**: e113351 [PMID: 25419735 DOI: 10.1371/journal.pone.0113351]
  - 34 **Shah S**, Ma Y, Scherzer R, Huhn G, French AL, Plankey M, Peters MG, Grunfeld C, Tien PC. Association of HIV, hepatitis C virus and liver fibrosis severity with interleukin-6 and C-reactive protein levels. *AIDS* 2015; **29**: 1325-1333 [PMID: 25870985 DOI: 10.1097/QAD.0000000000000654]
  - 35 **Serti E**, Park H, Keane M, O'Keefe AC, Rivera E, Liang TJ, Ghany M, Rehrmann B. Rapid decrease in hepatitis C viremia by direct acting antivirals improves the natural killer cell response to IFNα. *Gut* 2016; Epub ahead of print [PMID: 26733671 DOI: 10.1136/gutjnl-2015-310033]
  - 36 **Jain AB**, Eghtesad B, Venkataramanan R, Fontes PA, Kashyap R, Dvorchik I, Shakil AO, Kingery L, Fung JJ. Ribavirin dose modification based on renal function is necessary to reduce hemolysis in liver transplant patients with hepatitis C virus infection. *Liver Transpl* 2002; **8**: 1007-1013 [PMID: 12424713 DOI: 10.1053/jlts.2002.36241]
  - 37 **Kozielewicz D**, Dybowska D, Karwowska K, Wietlicka-Piszc M.

Renal impairment in patients with chronic hepatitis C treated with first generation protease inhibitors. *Expert Opin Drug Saf* 2015; **14**: 1815-1825 [PMID: 26513231 DOI: 10.1517/14740338.2015.1102882]

- 38 **Loustaud-Ratti V**, Carrier P, Vong C, Essig M. Renal impairment is frequent in chronic hepatitis C patients under triple therapy with telaprevir or boceprevir. *Hepatology* 2014; **59**: 2426 [PMID: 24002912 DOI: 10.1002/hep.26730]

**P- Reviewer:** Grant JL, Leidner AJ **S- Editor:** Gong ZM  
**L- Editor:** A **E- Editor:** Li D







## Lot to give, got to live - the restless minds of the "Liver on Tour" project

Guilherme Macedo, Armando Peixoto, Susana Lopes

Guilherme Macedo, Armando Peixoto, Susana Lopes, Centro Hospitalar de São João - Gastroenterology Department, 4200 Porto, Portugal

Guilherme Macedo, Armando Peixoto, Susana Lopes, Faculty of Medicine, University of Porto, 4200 Porto, Portugal

Guilherme Macedo, Armando Peixoto, Susana Lopes, WGO Training Center, 4200 Porto, Portugal

Author contributions: Macedo G wrote the letter; Peixoto A and Lopes S revised the letter.

Conflict-of-interest statement: The authors have no conflicts of interest to state.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Armando Peixoto, MD, Centro Hospitalar de São João - Gastroenterology Department, Alameda Hernâni Monteiro, 4200 Porto, Portugal. [armandoafp5@gmail.com](mailto:armandoafp5@gmail.com)  
Telephone: +351-225-512100  
Fax: +351-225-512101

Received: March 8, 2016

Peer-review started: March 10, 2016

First decision: April 15, 2016

Revised: May 27, 2016

Accepted: June 14, 2016

Article in press: June 16, 2016

Published online: July 8, 2016

### Abstract

The Liver on Tour was a special project devoted to increase the public awareness on Liver Health and Liver Diseases that the Portuguese Association for the Study of Liver Diseases launched throughout the country in 2010.

**Key words:** Liver disease; Hepatology; Hepatitis; Public health

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The Liver on Tour was a special Project devoted to increase the public awareness on Liver Health and Liver Diseases that the Portuguese Association for the Study of Liver Diseases launched throughout the country, between mid-April and mid-June 2010, and consisted of a road show, travelling through and reaching all 18 district capitals of Portugal. It was mainly focused on giving simple, reliable and practical information about liver problems with emphasis in messages trying to raise public attention on Liver Health. In a ten million people country where there is evidence of more than 10% being affected with some form of liver diseases, all efforts need to be made to enroll everyone in this never ending battle. High priority to education, gathering on board committed doctors with expertise in mass communication and engage reluctant policymakers (pressured by well-informed voters): A formula that could be a brighter way to raise the standards in prevention, detection and management of liver diseases.

Macedo G, Peixoto A, Lopes S. Lot to give, got to live - the restless minds of the "Liver on Tour" project. *World J Hepatol* 2016; 8(19): 825-826 Available from: URL: <http://www.wjgnet.com>

## TO THE EDITOR

The Liver on Tour (LOT) was a special Project devoted to increase the public awareness on Liver Health and Liver Diseases that the Portuguese Association for The Study of Liver Diseases (APEF) launched throughout the country, between mid-April and mid-June 2010, and consisted of a road show, travelling through and reaching all 18 district capitals of Portugal. It was mainly focused on giving simple, reliable and practical information about liver problems with emphasis in messages trying to raise public attention on Liver Health. "Meet the liver", "Care for your Liver", "Mind the Liver", were repeatedly announced sound bites, and had a professional media support so that liver problems reached the level of widespread lay public interest and, of course, politicians rule makers, while members of the APEF Board were literally on the road, claiming for help and protection for the liver.

Although pointing out the need for understanding and for taking care of the Liver, the deep goal was directed to the knowledge of the social and individual threats of Hepatitis, Alcoholic Liver Disease and The Obesity Epidemics. Hepatitis B and Hepatitis C were major players in these topics, and all efforts were made to promote public to decide to look (individually) for serological markers of infection to, give referral and guidance to liver specialists, creating an environment of highly awareness and concern on liver problems.

We believe that policy makers, in these times of funding constraints, will only be sensible to these matters if the public itself realizes how deep and broad impact liver diseases may cause either individually or collectively.

The scientific responsibility of all the pedagogical messages and contents of this Road Show (which included small conferences and media interviews) was from the Board of Direction of APEF.

It was one of our tasks to show how liver diseases may lead to social exclusion and how social exclusion can promote liver diseases. The stigmata and inadequate understanding and misperception often associated with liver diseases are truly cumbersome and there is an urgent need to overcome these misconceptions: Liver diseases were shown not to be caused only by self-inflicted deviated behaviors, previous drugs use and alcohol abuse, but instead that there is a wide spectrum

of causes, chances and risk factors, that may affect each and any one of us, regardless social, cultural or, economic status.

Our itinerant road show, located in central areas of the main Portuguese towns, addressed precisely these topics, stressed the fact why liver diseases are generally referred as "silent killers", how the liver itself copes with inflammation and regeneration, which ubiquitous factors challenge our livers. The hepatotropic viruses of course, alcohol consumption, excess weight and medical conditions as diabetes that cooperate to damage liver cells, were object of description, thorough but simple, clear enough to raise the public curiosity and awareness without frightening but with plenty of hope and need for global commitment.

Several thousands of people, from teens to elderly, walked through the exhibition displayed in the truck, reading carefully all the texts and pictures, from historical trivia to the most advanced virtuosity of liver technology. A small questionnaire was distributed, trying to promote the understanding of eventual individual risk factors for liver diseases and to evaluate a possible previous perception of the exposed topics.

The media came along and had different approaches depending on the places and towns we visited. Local radios interviewed liver specialists, national TVs broadcasted several messages regarding liver health. Underage drinking and alcohol marketing were actively challenged. Our presence in several students' parties, the recruitment of local doctors to get themselves to be available for questioning and even for some counselling, were extremely important to bring everyone together. We were able to gather thousands of in loco measurements of body mass index, and thousands of leaflets with simple knowledge on viral hepatitis were widely distributed.

In a ten million people country where there is evidence of more than 10% being affected with some form of liver diseases<sup>[1]</sup>, all efforts need to be made to enroll everyone in this never ending battle. High priority to education, gathering on board committed doctors with expertise in mass communication and engage reluctant policymakers (pressured by well-informed voters): A formula that could be a brighter way to raise the standards in prevention, detection and management of liver diseases.

## REFERENCES

- 1 Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol* 2013; **58**: 593-608 [PMID: 23419824 DOI: 10.1016/j.jhep.2012.12.005]

P- Reviewer: Balaban Y, Romero MR, Sazci A, Vento S  
S- Editor: Kong JX L- Editor: A E- Editor: Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 July 18; 8(20): 827-862







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



**TOPIC HIGHLIGHT**

- 827 Genetics of non-alcoholic fatty liver disease: From susceptibility and nutrient interactions to management  
*Ravi Kanth VV, Sasikala M, Sharma M, Rao PN, Reddy DN*

**ORIGINAL ARTICLE****Basic Study**

- 838 Lipogenesis in Huh7 cells is promoted by increasing the fructose: Glucose molar ratio  
*Windemuller F, Xu J, Rabinowitz SS, Hussain MM, Schwarz SM*

**Retrospective Study**

- 844 Aluminum potassium sulfate and tannic acid sclerotherapy for Goligher Grades II and III hemorrhoids: Results from a multicenter study  
*Miyamoto H, Hada T, Ishiyama G, Ono Y, Watanabe H*

**Clinical Trials Study**

- 850 Transjugular intrahepatic portosystemic shunt combined with esophagogastric variceal embolization in the treatment of a large gastroduodenal shunt  
*Jiang Q, Wang MQ, Zhang GB, Wu Q, Xu JM, Kong DR*

**CASE REPORT**

- 858 Hepatitis C virus cures after direct acting antiviral-related drug-induced liver injury: Case report  
*Hasin Y, Shteingart S, Dahari H, Gafanovich I, Floru S, Braun M, Shlomai A, Verstandig A, Dery I, Uprichard SL, Cotler SJ, Lurie Y*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Andres F Carrion, MD, Assistant Professor, Texas Tech University Health Sciences Center El Paso, Paul L Foster School of Medicine, El Paso, TX 79905, United States

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
July 18, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

<http://www.wjgnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**

<http://www.wjgnet.com/esps/>

**2016 Nonalcoholic Fatty Liver Disease: Global view**

# Genetics of non-alcoholic fatty liver disease: From susceptibility and nutrient interactions to management

Vishnubhotla Venkata Ravi Kanth, Mitnala Sasikala, Mithun Sharma, Padaki Nagaraja Rao,  
 Duvvuru Nageshwar Reddy

Vishnubhotla Venkata Ravi Kanth, Mitnala Sasikala, Asian Healthcare Foundation, a Research Wing of Asian Institute of Gastroenterology, Hyderabad 500082, Telangana, India

Mithun Sharma, Padaki Nagaraja Rao, Duvvuru Nageshwar Reddy, Asian Institute of Gastroenterology, Hyderabad 500082, Telangana, India

**Author contributions:** Ravi Kanth VV wrote the manuscript; Sasikala M revised the manuscript; Sharma M, Rao PN and Reddy DN participated in reviewing the manuscript content.

**Conflict-of-interest statement:** No conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Mitnala Sasikala, Head of Research Labs, Asian Healthcare Foundation, a Research Wing of Asian Institute of Gastroenterology, 6-3-661, Somajiguda, Hyderabad 500082, Telangana, India. [aigres.mit@gmail.com](mailto:aigres.mit@gmail.com)  
 Telephone: +91-40-23378888-701  
 Fax: +91-40-23324255

Received: March 28, 2016

Peer-review started: April 1, 2016

First decision: April 18, 2016

Revised: May 4, 2016

Accepted: June 14, 2016

Article in press: June 16, 2016

Published online: July 18, 2016

## Abstract

Genetics plays an important role in determining the susceptibility of an individual to develop a disease. Complex, multi factorial diseases of modern day (diabetes, cardiovascular disease, hypertension and obesity) are a result of disparity between the type of food consumed and genes, suggesting that food which does not match the host genes is probably one of the major reasons for developing life style diseases. Non-alcoholic fatty liver is becoming a global epidemic leading to substantial morbidity. While various genotyping approaches such as whole exome sequencing using next generation sequencers and genome wide association studies have identified susceptibility loci for non-alcoholic fatty liver disease (NAFLD) including variants in patatin-like phospholipase domain containing 3 and transmembrane 6 superfamily member 2 genes apart from others; nutrient based studies emphasized on a combination of vitamin D, E and omega-3 fatty acids to manage fatty liver disease. However majority of the studies were conducted independent of each other and very few studies explored the interactions between the genetic susceptibility and nutrient interactions. Identifying such interactions will aid in optimizing the nutrition tailor made to an individual's genetic makeup, thereby aiding in delaying the onset of the disease and its progression. The present topic focuses on studies that identified the genetic susceptibility for NAFLD, nutritional recommendations, and their interactions for better management of NAFLD.

**Key words:** Transmembrane 6 superfamily member 2 gene; Patatin-like phospholipase domain containing 3 gene; Genotyping; Nutrient interactions; Non-alcoholic fatty liver disease; Genetic susceptibility

© **The Author(s) 2016.** Published by Baishideng Publishing

Group Inc. All rights reserved.

**Core tip:** Various genome wide association and replication studies across ethnicities have consistently associated variants in patatin-like phospholipase domain containing 3 gene with a higher risk of non-alcoholic fatty liver disease (NAFLD). More recently a variant in transmembrane 6 superfamily member 2 gene was also associated with susceptibility to the disease. Functional studies have established the role of these genes in NAFLD. Gene and nutrient interactions should be the focus of future research in the management of NAFLD.

Ravi Kanth VV, Sasikala M, Sharma M, Rao PN, Reddy DN. Genetics of non-alcoholic fatty liver disease: From susceptibility and nutrient interactions to management. *World J Hepatol* 2016; 8(20): 827-837 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i20/827.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i20.827>

## INTRODUCTION

Genetic susceptibility carried by an individual determines the risk of developing a disease. However, not all individuals who carry the risk manifest with the disease, suggesting that, most of the complex multifactorial diseases are the result of interactions between genes and environment. Disease occurrence (onset) or severity may differ in individuals with same genotype exposed to different environmental conditions or vice versa, reiterating the fact that phenotype is the consequence of genotype and environment interactions (Figure 1). Diet, life style, exposure to chemicals and toxins form the major part of environmental risks. Majority of the modern day life style diseases such as diabetes, cardiovascular disease, hypertension, obesity are typically inherited with multifactorial mode of inheritance. It refers to a complex pattern of inheritance where a combination of both genetic and other factors including environmental are involved. Multifactorial conditions do not always manifest despite the fact that the individual carries a genetic variant that increases the risk of disease, putting the emphasis on favorable environment.

Non-alcoholic fatty liver disease (NAFLD) is one of the most important lifestyle based complex and multifactorial diseases. The prevalence of the disease varies markedly in various populations. It ranges between 20%-30% in the Western countries<sup>[1]</sup>, 20%-30% in Europeans<sup>[2]</sup>, 8% in Japanese<sup>[3]</sup> and 25%-30% in Indians<sup>[4]</sup>. The spectrum of the disease ranges between steatosis alone on one hand and non-alcoholic steatohepatitis (NASH)/cirrhosis/hepatocellular carcinoma on the other. However, the progression through the spectrum involves multiple risks including genetic and environmental interactions, in addition to other risk factors (Obesity, advancing age, diabetes, hypertension, and hypertriglyceridemia).

Therefore, understanding genetic susceptibility has been the major focus of recent research in addition to alterations in dietary habits and life style modifications which have been demonstrated to benefit the patients and aid in better management of the disease.

It is imperative for organisms from bacteria to humans to regulate their metabolism vis a vis availability of nutrients for better survival of the species. Nutrient-gene interactions have therefore been an ancient and omnipresent mechanism across species. However research started to explore these interactions only lately and the topic has been of prime importance in the context of disease including NAFLD. This review focuses on the genetic susceptibility identified till date employing various approaches (Exome sequencing, GWAs, candidate gene) thus far and the nutrient risks and the interactions between the two wherever studies are available.

## WHOLE EXOME SEQUENCING AND NAFLD

Recent advances in sequencing the human genome have transformed methods of identifying genetic susceptibility for complex, multifactorial diseases. With whole exome sequencing studies, it is now possible to sequence protein coding regions of the genome and identify genetic susceptibility for complex diseases in an unbiased manner. Although very few studies are available that exploited this technology, important loci have been identified. A quick search on PubMed revealed a single whole exome sequencing study in morbidly obese patients of Caucasian origin with NAFLD, that revealed novel damaging mutations in Bardet-Biedl syndrome 1 gene and Melanocortin 3 receptor gene (*MC3R*). *MC3R* gene encodes MC3 a G-protein coupled receptor for melanocyte stimulating hormone and adrenocorticotrophic hormone. Studies have identified that mice deficient for this gene product have increased fat and play a critical role in weight regulation ("Entrez gene: *MC3R* melanocortin 3 receptor"). Further another patient with NAFLD-related cirrhosis was compound heterozygous for rare and damaging mutations in patatin-like phospholipase domain containing 3 (*PNPLA3*)<sup>[5]</sup>. It is only recently that researchers have started to harness NGS technology to identify genetic susceptibility for complex diseases. In future by exploiting this technology many more important loci may be identified for NAFLD that may aid in better management of the disease.

## GENOME WIDE ASSOCIATION STUDIES AND NAFLD

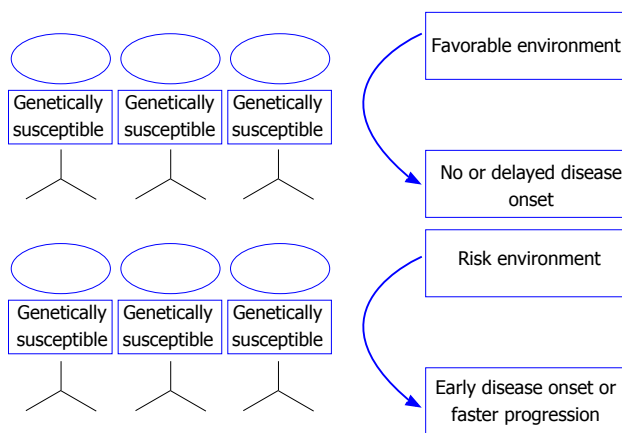
Genome wide association studies (GWAS) are employed to identify genetic susceptibility for complex diseases in an unbiased way. One of the first GWA studies for NAFLD was performed by Romeo *et al.*<sup>[6]</sup> that used a custom chip of approximately 9000 non-synonymous variants across



**Table 1** List of genome/exome wide association studies and loci identified for non-alcoholic fatty liver disease

Ref.	Phenotype associated with	Ancestry of samples included	Genotyping platform	Discovery sample size	Replication sample size	Genes
Romeo <i>et al</i> <sup>[6]</sup> , 2008	Increased hepatic fat levels and inflammation	Hispanic, African American and European American individuals	Perlegen Sciences Custom array (12138 NS variation)	2111 individuals with MRS measured hepatic steatosis	None	<i>PNPLA3</i>
Chalasani <i>et al</i> <sup>[15]</sup> , 2010	Features of hepatic histology	Non-Hispanic white women	Illumina (324,623 SNPs)	236, non-Hispanic white women	None	<i>FDFT1</i> , rs343062 ( <i>Chr 7</i> ), <i>COL13A1</i> , rs6591182 ( <i>Chr 11</i> ), <i>EFCAB4B</i> , rs2499604 ( <i>chr 1</i> ), <i>PZP</i> , rs1421201 ( <i>Chr 18</i> ) rs2710833 ( <i>Chr 4</i> )
Speliotes <i>et al</i> <sup>[16]</sup> , 2011	CT measured hepatic steatosis	European american including Amish	Affymetrix, Illumina	7126 with CT measured hepatic steatosis	592/1405	<i>PNPLA3</i> , <i>NCAN</i> , <i>PPP1R3B</i> , <i>GCKR</i> , <i>LYPLAL1</i>
Kawaguchi <i>et al</i> <sup>[3]</sup> , 2012	NAFLD	Japanese	Illumina	529 patients consisting of four NAFLD subgroups (Matteoni's classification)	None	<i>PNPLA3</i> , <i>SAMM50</i> , <i>PARVB</i> , <i>HS3ST1</i> - <i>HSP90AB2P</i> , <i>YIPF1</i>
Kitamoto <i>et al</i> <sup>[66]</sup> , 2013	NAFLD	Japanese	Illumina	392 NAFLD and 934 controls	172 NAFLD and 1012 controls	<i>PNPLA3</i> , <i>SAMM50</i> , <i>PARVB</i> gene
Kozlitina <i>et al</i> <sup>[19]</sup> , 2014	MRS measured hepatic steatosis	Hispanic, African, American and European	Illumina	2,736	None	<i>PNPLA3</i> and <i>TM6SF2</i>

NAFLD: Non-alcoholic fatty liver disease; CT: Computed tomography; SNPs: Single nucleotide polymorphisms; MRS: Magnetic resonance spectroscopy.

**Figure 1** Genetic and environmental interactions to produce a phenotype.

the genome. The sample included patients with and without NAFLD of various ethnicities including European, Hispanic and African-American. The liver fat was measured by proton magnetic resonance spectroscopy. One variant (rs738409), a G allele encoding I148M in *PNPLA3* gene was associated with increased fat level in the liver across all the ethnicities. A list of various GWA studies and the variants identified are given in Table 1. Subsequently various groups have replicated the association of this variant in different ethnicities including Japanese<sup>[3,7]</sup>, Indian<sup>[8,9]</sup>, Chinese<sup>[10,11]</sup>. Further the variant was also associated with higher levels of ALT, histologic NAFLD including steatosis<sup>[7,8]</sup>.

A meta-analysis of 24 studies that included 9915

patients from different ethnicities, identified that *PNPLA3* rs738409 variant was associated with fibrosis severity (OR = 1.32, 95%CI: 1.20-1.45)<sup>[12]</sup>. Another meta-analysis of 16 studies<sup>[13]</sup>, showed that rs738409 had a strong influence on liver fat accumulation. Individuals with GG homozygous genotype showed 77% higher lipid fat content compared to CC genotype and were susceptible to 3.24 fold aggressive disease and NASH. Further, when the risk associated with heterozygosity was evaluated for the variant, additive genetic model was better at explaining the effect of the variant on the susceptibility to develop NAFLD. However the analysis suggested that carrying two G alleles did not seem to increase the risk of severe histological features. Also, meta-regression showed a negative correlation between male sex and the effect of rs738409 on liver fat content (slope:  $-2.45 \pm 1.04$ ;  $P < 0.02$ ). Importantly, the rs738409 GG genotype vs the CC genotype was associated with a 28% increase in serum alanine aminotransferase levels. Xu *et al*<sup>[14]</sup> recent meta analysis of the rs738409 variant that included 23 case-control studies (6071 NAFLD and 10366 controls) showed a significant association of the variant with NAFLD, NASH. The subgroup and sensitivity analysis revealed that the changes were not influenced by the ethnicities and age of the subjects.

A GWA study conducted<sup>[15]</sup> in 236 non-Hispanic white woman who were genotyped for 3,24,623 single nucleotide polymorphisms (SNPs) on the Illumina platform and were assessed for various histologic parameters revealed that NAFLD activity score was associated with

rs2645424 in farnesyl diphosphate farnesyl transferase 1. Further analysis revealed that degree of fibrosis was associated with rs343062, lobular inflammation with rs1227756 in COL13A1), rs6591182, and rs887304 in EFCAB4B. SNPs associated with serum levels of alanine aminotransferase included rs2499604, rs6487679, rs1421201 and rs2710833. However, no significant associations were found between genotypes and steatosis, ballooning degeneration, portal inflammation, or other features of NAFLD.

A meta-analysis<sup>[16]</sup> carried out across four groups of European ancestry and one of the largest GWA studies for NAFLD was tested for associations with computed tomography (CT) measured steatosis initially in the 4 groups independently followed by a meta-analysis. The study involved 7176 individuals that were controlled for age, gender and all the principal components. Variants in or near *PNPLA3*, *LYPLAL1*, *PPP1R3B*, *NCAN*/transmembrane 6 superfamily member 2 (*TM6SF2*) and *GCKR* genes were found to be associated with hepatic steatosis. These above variants but for *PPP1R3B* were also associated with NASH and fibrosis.

Few GWA studies identified loci associated with the associated parameters of NAFLD and most importantly the liver function tests. Two such studies<sup>[17,18]</sup> have identified four loci namely SNPs in or near *PNPLA3* (rs2281135, rs738409), *SAMM50* (rs2143571, *CPN1-ERLIN1-CHUK* gene cluster (rs10883437, rs11597390, rs11591741, rs11597086), *TRIB1* (rs2954021) and near *HSD17B13/MAPK10* (rs6834314) that were associated with elevated levels of ALT.

Our pooled genetic study<sup>[8]</sup>, where 19 variants were selected that were associated with NAFLD from 4 GWA studies conducted until 2013 and replicated in patients with and without ultrasound detected NAFLD in Indians. The study identified variants in *PNPLA3*, *PZP*, *SAMM50* and *PARVB* were associated with NAFLD. Furthermore, the haplotype data suggested that variants in *PNPLA3*, *SAMM50* and *PARVB* on chromosome 22 were linked, suggesting that this loci is very important in Indian context. Studies from Japan<sup>[3]</sup> also associated these loci with NAFLD suggesting that it is an important loci conferring susceptibility in Asian population.

## WHOLE EXOME ASSOCIATION STUDY AND NAFLD

Two studies published during the same time reported the association of a variant (rs58542926) in *TM6SF2* gene with susceptibility to NAFLD<sup>[19]</sup> and influencing total cholesterol and myocardial infarction risk<sup>[20]</sup>. The first study identified that the variant in *TM6SF2* gene was associated with hepatic triglyceride content (HTGC) and is a adenine to guanine substitution in coding nucleotide 499, replacing glutamate with lysine at position 167 (c.499A > G; p.Glu167Lys). The frequency of this variant was higher in three ancestries studied (European, African-American and Hispanics). The study suggested

that the variant carriers had elevated mean and median HTGC in European and African-American ancestries. The study also identified that there was a reduction in the expression of recombinant protein in cultured hepatocytes by almost 50% by the Glu167Lys *TM6SF2* variant compared to the wild type. Further knockdown of the gene by Adeno-associated virus-mediated short hairpin RNA in mice increased the liver triglyceride content by threefold and decreased very-low-density lipoprotein (VLDL) secretion by half. Based on the above, the study suggested that *TM6SF2* activity may be required for normal VLDL secretion and that impaired function of the *TM6SF2* gene causally contributes to NAFLD<sup>[19]</sup>. Further, the function of the gene was also clearly established, where it is now known to be a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content<sup>[21]</sup>. The second study<sup>[20]</sup>, systematically assessed coding variants at the genome-wide level to identify novel lipid genes and also evaluate whether low frequency variants with large effect exist, identified a coding variant (p.Glu167Lys) in *TM6SF2* gene that modifies total cholesterol levels and further was associated with myocardial infarction. The functional role of few of the genes identified employing GWAS and exome wide association studies are as given in Table 2.

In an ongoing study at our center, we have replicated these variants (rs58542926 in *TM6SF2* and rs2281135 in *PNPLA3* genes) in 220 patients with NAFLD and 185 controls to date. Both the variants are significantly associated with the disease (*TM6SF2*  $P = 0.00008$ ; *PNPLA3*  $P = 0.002$ ) with a higher risk of the disease (Odds - 2.17, 95%CI: 1.34-3.52 and 1.85, 95%CI: 1.24-2.76). Further both the variants were significantly associated ( $P > 0.05$ ) with higher ALT and AST levels (data unpublished).

## CANDIDATE GENE STUDIES (OTHER GENES) AND NAFLD

Based on the two hit hypothesis as discussed earlier<sup>[22]</sup>, studies have explored genes that have an important role in mechanisms related to lipid metabolism, insulin signaling, oxidative stress, inflammation and fibrogenesis.

While *APCO3* gene is the major gene studied for its role in lipid metabolism (association with higher triglyceride levels), *MTP* gene was studied for its role in regulating synthesis, storage and export of hepatic triglyceride content. A loci on the long arm of chromosome 11 (11q23) harbors genes coding for apolipoproteins, including apolipoprotein A1 (*APOA1*), A4 (*APOA4* and *APOC3*<sup>[23]</sup>). Two polymorphisms T455C (rs2854117) and C482T (rs2854116) in the *APOC3* gene either singly or in combination had 30% higher levels of fasting plasma *APOC3* and triglyceride levels as compared to the wild type<sup>[24]</sup>. Subsequent studies failed to replicate these associations<sup>[25,26]</sup>, including our own study<sup>[27]</sup>. However we found that the SNPs were associated with higher triglyceride levels. *MTP* gene (microsomal triglyceride

Table 2 Functional role of major genes associated with non-alcoholic fatty liver disease identified by genome/exome wide association studies

Gene	Protein	Cellular location <sup>1</sup>	Function <sup>1</sup>	Chromosome location <sup>2</sup>	No. of exons and size <sup>2</sup>	Pathway/biologic function <sup>1</sup>	Tissues expressed <sup>1</sup>
<i>PNPLA3</i>	Patatin-like phospholipase domain-containing protein 3	Lipid droplets	Triacyl glycerol lipase and acylglycerol O-acyltransferase activities	Chromosome 22: 43,923,739-43,964,488 (forward strand)	9 (2805 bp)	Triacyl glycerol degradation and in glycerol-lipid metabolism	Liver, gall bladder, kidney, exocrine pancreas, seminal vesicles, intestine and salivary glands
<i>TM6SF2</i>	Transmembrane 6 superfamily member 2	ER and the ER-golgi intermediate compartment	Regulation of fat in liver influencing triglyceride secretion and lipid droplet content	Chromosome 19: 19,264,364-19,273,391 (reverse strand)	10 (1505 bp)	Promotes very low density lipoprotein export	Liver and intestine
<i>SAMM50</i>	Sorting and assembly machinery component 50 homolog	Outer mitochondrial membrane	Assembly of beta-barrel proteins	Chromosome 22: 43,955,421-44,010,531 (forward strand)	15 (1717)	Transport to the Golgi and subsequent modification and mitochondrial protein import	Liver, muscle, skeletal, lung adipocyte, colon and other tissues
<i>PARVB</i>	Parvin, beta	Cytoplasm	Involved in the reorganization of the actin cytoskeleton and formation of lamellipodia	Chromosome 22: 44,024,277-44,172,949 (forward strand)	13 (5429 bp)	ERK signaling and focal adhesion	Liver, muscle, skeletal, lung adipocyte, colon and other tissues
<i>NCAN</i>	Neurocan	Extracellular, Golgi lumen	Modulates neuronal adhesion and neurite growth during development	Chromosome 19: 19,211,973-19,252,233 (forward strand)	15 (6387 bp)	Developmental	Liver, muscle, skeletal, lung adipocyte, colon and other tissues
<i>PPP1R3B</i>	Protein phosphatase 1, regulatory subunit 3B	Glycogen granule	Acts as glycogen targeting subunit for phosphatase PP1	Chromosome 8: 9,136,255-9,151,574 (reverse strand)	2 (5548 bp)	Regulating glycogen synthesis	Liver, skeletal muscle
<i>GCKR</i>	GCKR	Cytoplasm, nucleus	Inhibits glucokinase by forming an inactive complex with this enzyme. The affinity of GCKR for GK is modulated by fructose metabolites	Chromosome 2: 27,496,842-27,523,684 (forward strand)	19 (2186 bp)	Carbohydrate metabolism	Liver, pancreas, colon and other tissues
<i>LYPLAL1</i>	Lysophospholipase-like 1	Cytoplasm	Depalmitoylating activity	Chromosome 1: 219,173,844-219,212,865 (forward strand)	5 (1898 bp)	Negative regulation of golgi to plasma membrane protein transport	Liver, muscle, skeletal, lung adipocyte, colon and other tissues

<sup>1</sup>Data extracted from <http://www.genecards.org/>; <sup>2</sup>Data extracted from ENSEMBL <http://asia.ensembl.org/index.html>; ER: Endoplasmic reticulum; PP1: Protein phosphatase 1; GCKR: Glucokinase (Hexokinase 4) regulator; GK: Glucokinase; ERK: Extracellular signal-regulated kinase.

transfer protein), located at 4q24<sup>[28]</sup> is critical for the synthesis and secretion of VLDL (very low density lipoprotein) in the liver. A meta-analysis<sup>[29]</sup> of most studied polymorphism -493G > T (rs1800591 G > T) in the *MTP* gene suggested that the SNP was significantly associated with higher risk of NAFLD.

Genes influencing inflammation and immune responses are known to modify susceptibility to NAFLD. Cytokines not only play an active role in the development of disease, but also in the progression by regulating the inflammatory process<sup>[30]</sup>. Studies identified a positive correlation between increasing degree of liver fibrosis and levels of TNF- $\alpha$ <sup>[31-33]</sup> including pediatric NAFLD<sup>[34]</sup>. Further, polymorphism studies associated a promoter SNP (-238G > A) in *TNFr*- $\alpha$  gene with susceptibility to NAFLD in Chinese

population<sup>[35]</sup>. transforming growth factor-beta (TGF- $\beta$ ), known to regulate cell death and lipid metabolism<sup>[36]</sup>, has been shown to be up-regulated and is considered an early event in steatohepatitis that is progressive.

Expression of interleukin-6 (IL-6), a major pro-inflammatory cytokine was shown to be increased in animal models of NAFLD, while in mice, sustained selective up-regulation in the liver resulted in systemic insulin resistance<sup>[37]</sup>. This was subsequently confirmed in humans<sup>[38]</sup>. Further, a positive correlation was observed between the expression levels and degree of inflammation and stage of fibrosis. A study identified that -174G/C in the IL-6 gene was involved in inflammation and insulin resistance and associated with NASH<sup>[39]</sup>, chronic liver disease and Hepatocellular carcinoma<sup>[40]</sup>.

Interleukin-10 (IL-10), an anti-inflammatory cytokine coded by IL-10 gene<sup>[41]</sup> has a role in regulating inflammation and its anti-inflammatory properties are well known<sup>[42]</sup>. T cell, monocyte and macrophage mediated functions are inhibited by IL-10. Different types of the cells in liver including stellate cells, hepatocytes and kupffer cells have shown the presence of IL-10. Few studies that explored the role of the gene, identified the protective role of endogenous role of IL-10 against hepatic steatosis, however they suggested that it does not improve hepatic or whole body insulin sensitivity during high-fat feeding<sup>[43]</sup>. Furthermore, in an animal model of diet-induced fatty liver disease, inhibition of IL-10 promoted increased expression of inflammatory cytokines, worsened insulin signaling and activated gluconeogenic and lipogenic pathways<sup>[44]</sup>.

Hepatic insulin resistance is associated with NAFLD and is one of the contributory factors in the pathogenesis of metabolic syndrome. Genetic screening of insulin signaling cascade identified a substitution (Glycine-Arginine) at codon 972 of the insulin receptor substrate-1 gene with a prevalence of approximately 9% in Caucasians that was associated with reduced insulin sensitivity. Furthermore, obese individuals heterozygous for this mutation have 50% reduced insulin sensitivity as compared to wild type obese subjects<sup>[45]</sup>. This variant is known to affect insulin receptor activity predisposing to liver damage and decreased hepatic insulin signaling in patients with NAFLD. It is suggested that insulin signaling might play a causal role in the progression of liver damage in NAFLD<sup>[46]</sup>.

NAFLD pathogenesis is a complex mechanism with involvement of free fatty acid (FFA) oxidation<sup>[47,48]</sup> and genes encoding proteins that are involved in the oxidation process of FFAs influence the oxidation load in individuals with obesity, insulin resistance and metabolic syndrome<sup>[22]</sup>. Genes harboring polymorphisms involved in generation and degradation of reactive oxygen species play a crucial role that could be due to excessive oxidation of FFA leading to oxidative stress causing apoptosis and liver injury<sup>[49]</sup>. Namikawa *et al.*<sup>[50]</sup> reported that the TT genotype in the *MnSOD* gene, the main ROS scavenger in mitochondria, leads to decreased efficiency

in the transport of MnSOD to the mitochondria and therefore confers susceptibility for NAFLD. Apart from the *MnSOD* gene, substantial evidence is now available on the role of polymorphisms in genes namely *GSTM1*, *GSTT1* and *GSTP1* genes that are involved in the generation or degradation of ROS. These genes are known to be involved in the progression to cirrhosis<sup>[51]</sup>.

## NUTRITIONAL RECOMMENDATIONS

Currently in clinical practice, a combination of vitamin D, vitamin E and omega-3 fatty acids have shown promise in the treatment of NAFLD and seem to be beneficial in patients with NAFLD. Studies further suggests that apart from nutritional counseling that includes a multi-disciplinary team (dietician, psychologist, and physical activity supervisor) aerobic exercises, gradual weight loss, management of NAFLD associated conditions namely diabetes, obesity and metabolic syndrome, nutritional recommendations namely use of 400-800 IU/d vitamin E, 1000 IU/d vitamin D, 1 g/d omega-3 fatty acids, and olive oil containing omega-9 fatty acids seem to benefit in reducing the severity of NAFLD. Also, restricting calorie intake to less than 30 kcal/kg per day and including a balanced diet with low levels of saturated and trans fats and simple sugars, avoiding soft drinks with high fructose corn syrup, fast food (trans fats, and reduce red and processed meats), and genetically modified crops seem to be beneficial<sup>[52]</sup>.

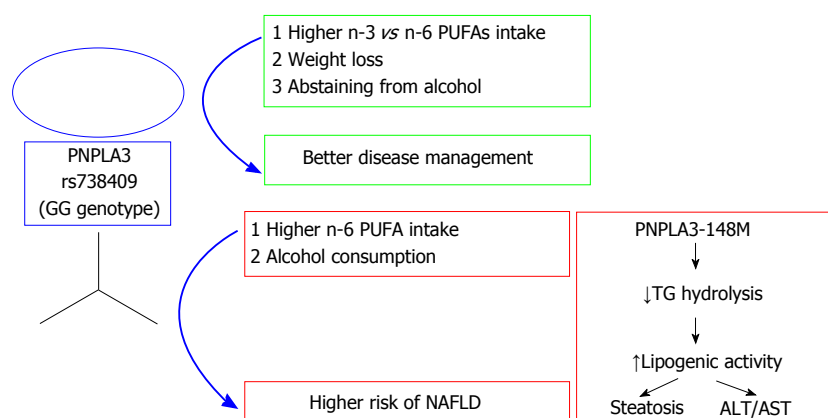
## MANAGEMENT OF NAFLD

Lifestyle modification usually by way of weight reduction through diet and exercises is currently the only proven strategy for managing NAFLD. As obesity is a strong risk and influencing factor for NAFLD, weight loss ( $\geq 8\%$  of body weight) is effective and is also the first line of therapy. A low caloric diet with reduction in the intake of total fat, saturated fatty acids, trans fatty acids and fructose, increase in physical activity and abstaining from smoking is advantageous and the patients are encouraged to follow these. Antioxidants, anti-inflammatory, insulin sensitizers, lipid lowering agents apart from wide range of drugs and supplements have been evaluated in various studies, both animal and human, however none of these have efficacy on long term use<sup>[53,54]</sup>.

## GENETIC AND NUTRIENT INTERACTIONS

It is almost imperative that all living organisms either simple or complex multicellular, regulate their metabolism vis a vis nutrient availability. Thus, interactions between nutrition and gene are widespread and an ancient feature across species. However, this aspect has not been explored and it is only recently that research started to uncover the mechanism. In view of the rapid advances made in sequencing human genome enormous amount of genetic data is being generated, particularly with





**Figure 2** Genotype (rs738409) in patatin-like phospholipase domain containing 3 gene and its interactions. PNPLA3: Patatin-like phospholipase domain containing 3; PUFA: Polyunsaturated fatty acid; NAFLD: Non-alcoholic fatty liver disease; ALT/AST: Alanine aminotransferase/aspartate aminotransferase.

respect to common multigenic, multifactorial conditions including obesity, diabetes, NAFLD, *etc.* It is becoming more and more obvious that an individual's susceptibility to lifestyle disease represents a complex interaction between genetics and environmental interactions. Food and nutrient intake are the important environmental factors and their interactions with genes play a key role in the pathogenesis and progression of polygenic diseases. Therefore, research should be focused on identifying such interactions of genes with nutrients and identifying susceptible genotypes to particular nutrients. This will help us optimize nutrient/diet intake (personalized nutrition) to reduce disease risk<sup>[55]</sup>. However, there are challenges in analyzing these interactions in the form of genetic heterogeneity and complex nature of human genome, complexity of environmental factors including diet, *etc.*<sup>[56]</sup>.

NAFLD is considered to be the hepatic manifestation of the metabolic syndrome<sup>[57]</sup>. The "thrifty genotype" a possible explanation for the steep increase in obesity and diabetes, where periods of famine in the history of modern humans has exerted natural selection in favor of selecting genes favorable for fat storage and this is likely mediated through fertility and not viability selection<sup>[58]</sup>.

Hepatic lipase gene (*HL*) is a lipolytic enzyme that regulates triglyceride levels. Insulin is known to up-regulate the activity of HL through the insulin-responsive elements in the promoter region. It is suggested that higher intake of total and saturated fat is associated with higher activity of *HL* gene. A study reported that this activity is influenced by the -514 C > T polymorphism in the *HL* gene, with significantly stronger associations noted between total dietary fat intake and HL activity in individuals with CT and TT genotypes as compared to the wild type (CC)<sup>[59]</sup>. Another study<sup>[60]</sup> that explored epidemiologic genotype-nutrient interactions in obesity, where a total of 42 SNPs in 26 candidate genes were genotyped identified an interaction between -514 C > T in *HL* gene and fiber intake. Further they also suggested that the -681 C > G polymorphism in *PPARG3* gene might interact with the percentage of energy derived

from fat in the diet for the development of obesity. However, this was a case-only study with only adult obese women as part of the analysis. A study<sup>[61]</sup> that examined the interactions between the -514 C > T in *HL* gene, dietary fat and HDL-related measures in 1020 men and 1110 women from the farmingham study reported that individuals with the "TT" genotype may have an impaired adaptation to higher animal fat diets. Furthermore, they suggested that dietary fat intake modifies the effect of the polymorphism in *HL* gene on HDL-C concentrations and subclasses, where the T allele was significantly associated with greater HDL-C concentrations only in subjects consuming < 30% of energy from fat and the reverse is true when total fat intake was  $\geq$  30% of energy.

It is well established that apolipoprotein A5 (*APOA5*) gene is an important determinant of plasma triglyceride levels. Further it is a component of several lipoprotein fractions including high density lipoprotein (HDL), VLDL<sup>[62]</sup>. A study<sup>[63]</sup>, investigated the interaction between variants in *APOA5* gene and dietary fat in determining plasma fasting triglycerides, remnant-like particle concentrations and lipoprotein particle size in 1001 men and 1147 women from farmingham heart study reported significant gene-diet interactions between the -1131T > C polymorphism in *APOA5* gene and polyunsaturated fatty acid (PUFA) intake were found that determined fasting TGs, RLP concentrations and particle size. However, these interactions were not found for the other polymorphism (56C > G). Further they noted that the -1131C allele was associated with higher fasting TGs and RLP concentrations in only individuals who consumed a high-PUFA diet with > 6% of total energy. The study concluded that individuals who are carriers of -1131C polymorphism in *APOA5* gene and take higher n-6 but not n-3 PUFA, have increased fasting TGs, RLP concentrations, and VLDL size and decreased LDL size, suggesting a more atherogenic lipid profile in these individuals because of the n-6 PUFA-rich diet.

A study<sup>[64]</sup> that recruited 8 subjects with homozygous genotype for the rs738409G allele in *PNPLA3* gene and

10 with C allele, explored the influence of the variant on the ability to lose weight thereby reducing liver fat or change insulin sensitivity. The study identified that the fasting serum insulin and C-peptide concentrations were significantly lower in rs738409G as compared to rs738409C group. Although weight loss was not significantly different between the groups (approximately 3.1 kg), liver fat decreased by 45% in rs738409G as compared to 18% in the rs738409C group, suggesting weight loss is more effective in decreasing liver fat in rs738409G carriers. Another study<sup>[65]</sup> that explored the influence of PNPLA3 (rs738409) genotype on hepatic fat and modulation by dietary factors such as PUFAs identified that the ratio of n-6 to n-3 PUFAs interacted with the GG genotype to promote hepatic steatosis (Figure 2).

## FUTURE TRENDS

In future, testing for variants that pre-dispose to NAFLD along with their nutrient interactions would help identify the type of nutrition to be taken based on individual's genetic makeup thereby minimizing the risk of fatty infiltration.

## CONCLUSION

Among all the loci identified thus far, there is a compelling evidence of the association of variants in PNPLA3 with NAFLD and functional role of TM6SF2 in the regulation of liver fat metabolism and hepatic lipid droplet content. It may be prudent to genotype these well characterized variants (PNPLA3) as part of the diagnostic workup for NAFLD, to assess the risk of an individual. Further genotyping in asymptomatic individuals will help in making lifestyle based recommendations, including nutrition to minimize the risk of future disease. As the genetic susceptibility risk cannot be changed, it is important to identify the risk at an early age and manage/lower the other modifiable risks/modifiable triggers to efficiently manage the disease without progressing to subsequent pathologies. Finally, the genetic information along with personalized environment exposures will help in stratifying risk of NAFLD in an individual.

## REFERENCES

- 1 **Bellentani S**, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 155-161 [PMID: 20460905 DOI: 10.1159/000282080]
- 2 **Blachier M**, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol* 2013; **58**: 593-608 [PMID: 23419824 DOI: 10.1016/j.jhep.2012.12.005]
- 3 **Kawaguchi T**, Sumida Y, Umemura A, Matsuo K, Takahashi M, Takamura T, Yasui K, Saibara T, Hashimoto E, Kawanaka M, Watanabe S, Kawata S, Imai Y, Kokubo M, Shima T, Park H, Tanaka H, Tajima K, Yamada R, Matsuda F. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. *PLoS One* 2012; **7**: e38322 [PMID: 22719876 DOI: 10.1371/journal.pone.0038322]
- 4 **Amarapurkar D**, Kamani P, Patel N, Gupte P, Kumar P, Agal S, Baijal R, Lala S, Chaudhary D, Deshpande A. Prevalence of non-alcoholic fatty liver disease: population based study. *Ann Hepatol* 2007; **6**: 161-163 [PMID: 17786142]
- 5 **Gerhard GS**, Chu X, Wood GC, Gerhard GM, Benotti P, Petrick AT, Gabrielsen J, Strodel WE, Still CD, Argyropoulos G. Next-generation sequence analysis of genes associated with obesity and nonalcoholic fatty liver disease-related cirrhosis in extreme obesity. *Hum Hered* 2013; **75**: 144-151 [PMID: 24081230 DOI: 10.1159/000351719]
- 6 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]
- 7 **Akuta N**, Kawamura Y, Arase Y, Suzuki F, Sezaki H, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Suzuki Y, Ikeda K, Kumada H. Relationships between Genetic Variations of PNPLA3, TM6SF2 and Histological Features of Nonalcoholic Fatty Liver Disease in Japan. *Gut Liver* 2016; **10**: 437-445 [PMID: 26610348 DOI: 10.5009/gnl15163]
- 8 **Kanth VV**, Sasikala M, Rao PN, Steffie Avanthi U, Rao KR, Nageshwar Reddy D. Pooled genetic analysis in ultrasound measured non-alcoholic fatty liver disease in Indian subjects: A pilot study. *World J Hepatol* 2014; **6**: 435-442 [PMID: 25018854 DOI: 10.4254/wjh.v6.i6.435]
- 9 **Bhatt SP**, Nigam P, Misra A, Guleria R, Pandey RM, Pasha MA. Genetic variation in the patatin-like phospholipase domain-containing protein-3 (PNPLA-3) gene in Asian Indians with nonalcoholic fatty liver disease. *Metab Syndr Relat Disord* 2013; **11**: 329-335 [PMID: 23734760 DOI: 10.1089/met.2012.0064]
- 10 **Zhang Y**, Cai W, Song J, Miao L, Zhang B, Xu Q, Zhang L, Yao H. Association between the PNPLA3 I148M polymorphism and non-alcoholic fatty liver disease in the Uyghur and Han ethnic groups of northwestern China. *PLoS One* 2014; **9**: e108381 [PMID: 25290313 DOI: 10.1371/journal.pone.0108381]
- 11 **Peng XE**, Wu YL, Lin SW, Lu QQ, Hu ZJ, Lin X. Genetic variants in PNPLA3 and risk of non-alcoholic fatty liver disease in a Han Chinese population. *PLoS One* 2012; **7**: e50256 [PMID: 23226254 DOI: 10.1371/journal.pone.0050256]
- 12 **Singal AG**, Manjunath H, Yopp AC, Beg MS, Marrero JA, Gopal P, Waljee AK. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 325-334 [PMID: 24445574 DOI: 10.1038/ajg.2013.476]
- 13 **Sookoian S**, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 1883-1894 [PMID: 21381068 DOI: 10.1002/hep.24283]
- 14 **Xu R**, Tao A, Zhang S, Deng Y, Chen G. Association between patatin-like phospholipase domain containing 3 gene (PNPLA3) polymorphisms and nonalcoholic fatty liver disease: a HuGE review and meta-analysis. *Sci Rep* 2015; **5**: 9284 [PMID: 25791171 DOI: 10.1038/srep09284]
- 15 **Chalasani N**, Guo X, Loomba R, Goodarzi MO, Haritunians T, Kwon S, Cui J, Taylor KD, Wilson L, Cummings OW, Chen YD, Rotter JI. Genome-wide association study identifies variants associated with histologic features of nonalcoholic Fatty liver disease. *Gastroenterology* 2010; **139**: 1567-1576, 1576.e1-e6 [PMID: 20708005 DOI: 10.1053/j.gastro.2010.07.057]
- 16 **Speliotes EK**, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, Gudnason V, Eiriksdottir G, Garcia ME, Launer LJ, Nalls MA, Clark JM, Mitchell BD, Shuldiner AR, Butler JL, Tomas M, Hoffmann U, Hwang SJ, Massaro JM, O'Donnell CJ, Sahani DV, Salomaa V, Schadt EE, Schwartz SM, Siscovick DS, Voight BF, Carr JJ, Feitosa MF, Harris TB, Fox CS, Smith AV, Kao WH, Hirschhorn JN, Borecki IB. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet* 2011; **7**:

- e1001324 [PMID: 21423719 DOI: 10.1371/journal.pgen.1001324]
- 17 **Yuan X**, Waterworth D, Perry JR, Lim N, Song K, Chambers JC, Zhang W, Vollenweider P, Stirnadel H, Johnson T, Bergmann S, Beckmann ND, Li Y, Ferrucci L, Melzer D, Hernandez D, Singleton A, Scott J, Elliott P, Waeber G, Cardon L, Frayling TM, Kooner JS, Mooser V. Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. *Am J Hum Genet* 2008; **83**: 520-528 [PMID: 18940312 DOI: 10.1016/j.ajhg.2008.09.012]
- 18 **Chambers JC**, Zhang W, Sehmi J, Li X, Wass MN, Van der Harst P, Holm H, Sanna S, Kavousi M, Baumeister SE, Coin LJ, Deng G, Gieger C, Heard-Costa NL, Hottenga JJ, Kühnel B, Kumar V, Lagou V, Liang L, Luan J, Vidal PM, Mateo Leach I, O'Reilly PF, Peden JF, Rahmioglu N, Soininen P, Speliotes EK, Yuan X, Thorleifsson G, Alizadeh BZ, Atwood LD, Borecki IB, Brown MJ, Charoen P, Cucca F, Das D, de Geus EJ, Dixon AL, Döring A, Ehret G, Eyjolfsson GI, Farrall M, Forouhi NG, Friedrich N, Goessling W, Gudbjartsson DF, Harris TB, Hartikainen AL, Heath S, Hirschfeld GM, Hofman A, Homuth G, Hyppönen E, Janssen HL, Johnson T, Kangas AJ, Kema IP, Kühn JP, Lai S, Lathrop M, Lerch MM, Li Y, Liang TJ, Lin JP, Loos RJ, Martin NG, Moffatt MF, Montgomery GW, Munroe PB, Musunuru K, Nakamura Y, O'Donnell CJ, Olafsson I, Penninx BW, Pouta A, Prins BP, Prokopenko I, Puls R, Ruokonen A, Savolainen MJ, Schlessinger D, Schouten JN, Seedorf U, Sen-Chowdhry S, Siminovich KA, Smit JH, Spector TD, Tan W, Teslovich TM, Tukiainen T, Uitterlinden AG, Van der Klauw MM, Vasan RS, Wallace C, Wallaschowski H, Wichmann HE, Willemsen G, Würtz P, Xu C, Yerges-Armstrong LM; Alcohol Genome-wide Association (AlcGen) Consortium; Diabetes Genetics Replication and Meta-analyses (DIAGRAM+) Study; Genetic Investigation of Anthropometric Traits (GIANT) Consortium; Global Lipids Genetics Consortium; Genetics of Liver Disease (GOLD) Consortium; International Consortium for Blood Pressure (ICBP-GWAS); Meta-analyses of Glucose and Insulin-Related Traits Consortium (MAGIC), Abecasis GR, Ahmadi KR, Boomsma DI, Caulfield M, Cookson WO, van Duijn CM, Froguel P, Matsuda K, McCarthy MI, Meisinger C, Mooser V, Pietiläinen KH, Schumann G, Snieder H, Sternberg MJ, Stolk RP, Thomas HC, Thorsteinsdottir U, Uda M, Waeber G, Wareham NJ, Waterworth DM, Watkins H, Whitfield JB, Witteman JC, Wolffenbuttel BH, Fox CS, Ala-Korpela M, Stefansson K, Vollenweider P, Völzke H, Schadt EE, Scott J, Järvelin MR, Elliott P, Kooner JS. Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. *Nat Genet*. 2011; **43**: 1131-1138 [PMID: 22001757 DOI: 10.1038/ng.970]
- 19 **Kozlitina J**, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjaerg-Hansen A, Vogt TF, Hobbs HH, Cohen JC. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014; **46**: 352-356 [PMID: 24531328 DOI: 10.1038/ng.2901]
- 20 **Holmen OL**, Zhang H, Fan Y, Hovelson DH, Schmidt EM, Zhou W, Guo Y, Zhang J, Langhammer A, Löchen ML, Ganesh SK, Vatten L, Skorpén F, Dalen H, Zhang J, Pennathur S, Chen J, Platou C, Mathiesen EB, Wilsgaard T, Njølstad I, Boehnke M, Chen YE, Abecasis GR, Hveem K, Willer CJ. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nat Genet* 2014; **46**: 345-351 [PMID: 24633158 DOI: 10.1038/ng.2926]
- 21 **Mahdessian H**, Taxiarchis A, Popov S, Silveira A, Franco-Cereceda A, Hamsten A, Eriksson P, van't Hooft F. TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. *Proc Natl Acad Sci USA* 2014; **111**: 8913-8918 [PMID: 24927523 DOI: 10.1073/pnas.1323785111]
- 22 **Day CP**. From fat to inflammation. *Gastroenterology* 2006; **130**: 207-210 [PMID: 16401483 DOI: 10.1053/j.gastro.2005.11.017]
- 23 **Karathanasis SK**, Oettgen P, Haddad IA, Antonarakis SE. Structure, evolution, and polymorphisms of the human apolipoprotein A4 gene (APOA4). *Proc Natl Acad Sci USA* 1986; **83**: 8457-8461 [PMID: 3095836]
- 24 **Petersen KF**, Dufour S, Hariri A, Nelson-Williams C, Foo JN, Zhang XM, Dziura J, Lifton RP, Shulman GI. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *N Engl J Med* 2010; **362**: 1082-1089 [PMID: 20335584 DOI: 10.1056/NEJMoa0907295]
- 25 **Kozlitina J**, Boerwinkle E, Cohen JC, Hobbs HH. Dissociation between APOC3 variants, hepatic triglyceride content and insulin resistance. *Hepatology* 2011; **53**: 467-474 [PMID: 21274868 DOI: 10.1002/hep.24072]
- 26 **TG and HDL Working Group of the Exome Sequencing Project**, National Heart, Lung, and Blood Institute, Crosby J, Peloso GM, Auer PL, Crosslin DR, Stitzel NO, Lange LA, Lu Y, Tang ZZ, Zhang H, Hindy G, Masca N, Stirrups K, Kanoni S, Do R, Jun G, Hu Y, Kang HM, Xue C, Goel A, Farrall M, Duga S, Merlini PA, Asselta R, Girelli D, Olivieri O, Martinelli N, Yin W, Reilly D, Speliotes E, Fox CS, Hveem K, Holmen OL, Nikpay M, Farlow DN, Assimes TL, Franceschini N, Robinson J, North KE, Martin LW, DePristo M, Gupta N, Escher SA, Jansson JH, Van Zuydam N, Palmer CN, Wareham N, Koch W, Meitinger T, Peters A, Lieb W, Erbel R, König IR, Krupp J, Degenhardt F, Gottesman O, Bottinger EP, O'Donnell CJ, Psaty BM, Ballantyne CM, Abecasis G, Ordovas JM, Melander O, Watkins H, Orho-Melander M, Ardisson D, Loos RJ, McPherson R, Willer CJ, Erdmann J, Hall AS, Samani NJ, Deloukas P, Schunkert H, Wilson JG, Kooperberg C, Rich SS, Tracy RP, Lin DY, Altshuler D, Gabriel S, Nickerson DA, Jarvik GP, Cupples LA, Reiner AP, Boerwinkle E, Kathiresan S. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med* 2014; **371**: 22-31 [PMID: 24941081 DOI: 10.1056/NEJMoa1307095]
- 27 **Ravikanth VV**, Mitnala S, Avanthi US, Manjeera B, Aswini R, Mukherjee RM, Rao PN, Reddy DN. PNPLA3 gene polymorphism but not APOC3 enhances risk for nonalcoholic fatty liver disease in Indian subjects. *J Clin Exp Hepatol* 2013; **3**: S24 [DOI: 10.1016/j.jceh.2013.03.049]
- 28 **Narcsi TM**, Shoulders CC, Chester SA, Read J, Brett DJ, Harrison GB, Grantham TT, Fox MF, Povey S, de Bruin TW. Mutations of the microsomal triglyceride-transfer-protein gene in abetalipoproteinemia. *Am J Hum Genet* 1995; **57**: 1298-1310 [PMID: 8533758]
- 29 **Li L**, Wang SJ, Shi K, Chen D, Jia H, Zhu J. Correlation between MTP -493G> T polymorphism and non-alcoholic fatty liver disease risk: a meta-analysis. *Genet Mol Res* 2014; **13**: 10150-10161 [PMID: 25501226 DOI: 10.4238/2014.December.4.9]
- 30 **Braunersreuther V**, Viviani GL, Mach F, Montecucco F. Role of cytokines and chemokines in non-alcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 727-735 [PMID: 22371632 DOI: 10.3748/wjg.v18.i8.727]
- 31 **Lesmana CR**, Hasan I, Budihusodo U, Gani RA, Krisnuhoni E, Akbar N, Lesmana LA. Diagnostic value of a group of biochemical markers of liver fibrosis in patients with non-alcoholic steatohepatitis. *J Dig Dis* 2009; **10**: 201-206 [PMID: 19659788 DOI: 10.1111/j.1751-2980.2009.00386.x]
- 32 **Hui JM**, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004; **40**: 46-54 [PMID: 15239085 DOI: 10.1002/hep.20280]
- 33 **Crespo J**, Cayón A, Fernández-Gil P, Hernández-Guerra M, Mayorga M, Domínguez-Diez A, Fernández-Escalante JC, Pons-Romero F. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology* 2001; **34**: 1158-1163 [PMID: 11732005 DOI: 10.1053/jhep.2001.29628]
- 34 **Manco M**, Marcellini M, Giannone G, Nobili V. Correlation of serum TNF-alpha levels and histologic liver injury scores in pediatric nonalcoholic fatty liver disease. *Am J Clin Pathol* 2007; **127**: 954-960 [PMID: 17509993 DOI: 10.1309/6VJ4DWGYDU0XYJ8Q]
- 35 **Hu ZW**, Luo HB, Xu YM, Guo JW, Deng XL, Tong YW, Tang X. Tumor necrosis factor--alpha gene promoter polymorphisms in Chinese patients with nonalcoholic fatty liver diseases. *Acta*



- Gastroenterol Belg* 2009; **72**: 215-221 [PMID: 19637776]
- 36 **Stärkel P**, Sempoux C, Leclercq I, Herin M, Deby C, Desager JP, Horsmans Y. Oxidative stress, KLF6 and transforming growth factor-beta up-regulation differentiate non-alcoholic steatohepatitis progressing to fibrosis from uncomplicated steatosis in rats. *J Hepatol* 2003; **39**: 538-546 [PMID: 12971963 DOI: 10.1016/S0168-8278(03)00360-X]
  - 37 **Nieto-Vazquez I**, Fernández-Veledo S, de Alvaro C, Lorenzo M. Dual role of interleukin-6 in regulating insulin sensitivity in murine skeletal muscle. *Diabetes* 2008; **57**: 3211-3221 [PMID: 18796617 DOI: 10.2337/db07-1062]
  - 38 **Wieckowska A**, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol* 2008; **103**: 1372-1379 [PMID: 18510618 DOI: 10.1111/j.1572-0241.2007.01774.x]
  - 39 **Carulli L**, Canedi I, Rondinella S, Lombardini S, Ganazzi D, Fargion S, De Palma M, Lonardo A, Ricchi M, Bertolotti M, Carulli N, Loria P. Genetic polymorphisms in non-alcoholic fatty liver disease: interleukin-6-174G/C polymorphism is associated with non-alcoholic steatohepatitis. *Dig Liver Dis* 2009; **41**: 823-828 [PMID: 19403348 DOI: 10.1016/j.dld.2009.03.005]
  - 40 **Giannitrapani L**, Soresi M, Balasus D, Licata A, Montalto G. Genetic association of interleukin-6 polymorphism (-174 G/C) with chronic liver diseases and hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 2449-2455 [PMID: 23674845 DOI: 10.3748/wjg.v19.i16.2449]
  - 41 **Eskdale J**, Kube D, Tesch H, Gallagher G. Mapping of the human IL10 gene and further characterization of the 5' flanking sequence. *Immunogenetics* 1997; **46**: 120-128 [PMID: 9162098 DOI: 10.1007/s002510050250]
  - 42 **Couper KN**, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol* 2008; **180**: 5771-5777 [PMID: 18424693 DOI: 10.4049/jimmunol.180.9.5771]
  - 43 **den Boer MA**, Voshol PJ, Schröder-van der Elst JP, Korshe-ninnikova E, Ouwens DM, Kuipers F, Havekes LM, Romijn JA. Endogenous interleukin-10 protects against hepatic steatosis but does not improve insulin sensitivity during high-fat feeding in mice. *Endocrinology* 2006; **147**: 4553-4558 [PMID: 16709607 DOI: 10.1210/en.2006-0417]
  - 44 **Cintra DE**, Pauli JR, Araújo EP, Moraes JC, de Souza CT, Milanski M, Morari J, Gambero A, Saad MJ, Velloso LA. Interleukin-10 is a protective factor against diet-induced insulin resistance in liver. *J Hepatol* 2008; **48**: 628-637 [PMID: 18267346 DOI: 10.1016/j.jhep.2007.12.017]
  - 45 **Pedersen O**. Genetics of insulin resistance. *Exp Clin Endocrinol Diabetes* 1999; **107**: 113-118 [PMID: 10320051 DOI: 10.1055/s-0029-1212085]
  - 46 **Dongiovanni P**, Valenti L, Rametta R, Daly AK, Nobili V, Mozzi E, Leathart JB, Pietrobattista A, Burt AD, Maggioni M, Fracanzani AL, Lattuada E, Zappa MA, Roviato G, Marchesini G, Day CP, Fargion S. Genetic variants regulating insulin receptor signalling are associated with the severity of liver damage in patients with non-alcoholic fatty liver disease. *Gut* 2010; **59**: 267-273 [PMID: 20176643 DOI: 10.1136/gut.2009.190801]
  - 47 **Sanyal AJ**, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; **120**: 1183-1192 [PMID: 11266382 DOI: 10.1053/gast.2001.23256]
  - 48 **Day CP**. Pathogenesis of steatohepatitis. *Best Pract Res Clin Gastroenterol* 2002; **16**: 663-678 [PMID: 12406438 DOI: 10.1053/bega.2002.0333]
  - 49 **Wang K**. Molecular mechanisms of hepatic apoptosis. *Cell Death Dis* 2014; **5**: e996 [PMID: 24434519 DOI: 10.1038/cddis.2013.499]
  - 50 **Namikawa C**, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y, Ono M, Akisawa N, Saibara T, Hiroi M, Enzan H, Onishi S. Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. *J Hepatol* 2004; **40**: 781-786 [PMID: 15094225 DOI: 10.1016/j.jhep.2004.01.028]
  - 51 **Ghobadloo SM**, Yaghmaei B, Bakayev V, Goudarzi H, Noorinayer B, Rad FH, Samiy S, Aghabozorgi S, Zali MR. GSTP1, GSTM1, and GSTT1 genetic polymorphisms in patients with cryptogenic liver cirrhosis. *J Gastrointest Surg* 2004; **8**: 423-427 [PMID: 15120366 DOI: 10.1016/j.gassur.2004.02.005]
  - 52 **Assy N**. Nutritional recommendations for patients with non-alcoholic fatty liver diseases. *World J Gastroenterol* 2011; **17**: 3375-3376 [PMID: 21876629 DOI: 10.3748/wjg.v17.i29.3375]
  - 53 **Eslamparast T**, Eghtesad S, Poustchi H, Hekmatdoost A. Recent advances in dietary supplementation, in treating non-alcoholic fatty liver disease. *World J Hepatol* 2015; **7**: 204-212 [PMID: 25729475 DOI: 10.4254/wjh.v7.i2.204]
  - 54 **Dongiovanni P**, Lanti C, Riso P, Valenti L. Nutritional therapy for nonalcoholic fatty liver disease. *J Nutr Biochem* 2016; **29**: 1-11 [PMID: 26895659 DOI: 10.1016/j.jnutbio.2015.08.024]
  - 55 **Phillips CM**, Tierney AC, Roche HM. Gene-nutrient interactions in the metabolic syndrome. *J Nutrigenet Nutrigenomics* 2008; **1**: 136-151 [PMID: 19776623 DOI: 10.1159/000112461]
  - 56 **Wise C**, Kaput J. A strategy for analyzing gene-nutrient interactions in type 2 diabetes. *J Diabetes Sci Technol* 2009; **3**: 710-721 [PMID: 20144318 DOI: 10.1177/1932296809000300416]
  - 57 **Kim CH**, Younossi ZM. Nonalcoholic fatty liver disease: a manifestation of the metabolic syndrome. *Cleve Clin J Med* 2008; **75**: 721-728 [PMID: 18939388 DOI: 10.3949/ccjm.75.10.721]
  - 58 **Prentice AM**, Hennig BJ, Fulford AJ. Evolutionary origins of the obesity epidemic: natural selection of thrifty genes or genetic drift following predation release? *Int J Obes (Lond)* 2008; **32**: 1607-1610 [PMID: 18852700 DOI: 10.1038/ijo.2008.147]
  - 59 **Bos G**, Snijder MB, Nijpels G, Dekker JM, Stehouwer CD, Bouter LM, Heine RJ, Jansen H. Opposite contributions of trunk and leg fat mass with plasma lipase activities: the Hoorn study. *Obes Res* 2005; **13**: 1817-1823 [PMID: 16286530 DOI: 10.1038/oby.2005.221]
  - 60 **Santos JL**, Boutin P, Verdich C, Holst C, Larsen LH, Toubro S, Dina C, Saris WH, Blaak EE, Hoffstedt J, Taylor MA, Polak J, Clement K, Langin D, Astrup A, Froguel P, Pedersen O, Sorensen TI, Martinez JA. Genotype-by-nutrient interactions assessed in European obese women. A case-only study. *Eur J Nutr* 2006; **45**: 454-462 [PMID: 17080261 DOI: 10.1007/s00394-006-0619-6]
  - 61 **Ordovas JM**, Corella D, Demissie S, Cupples LA, Couture P, Coltell O, Wilson PW, Schaefer EJ, Tucker KL. Dietary fat intake determines the effect of a common polymorphism in the hepatic lipase gene promoter on high-density lipoprotein metabolism: evidence of a strong dose effect in this gene-nutrient interaction in the Framingham Study. *Circulation* 2002; **106**: 2315-2321 [PMID: 12403660 DOI: 10.1161/01.CIR.0000036597.52291.C9]
  - 62 **Nilsson SK**, Christensen S, Raarup MK, Ryan RO, Nielsen MS, Olivecrona G. "Endocytosis of apolipoprotein A-V by members of the low density lipoprotein receptor and the VPS10p domain receptor families". *The Journal of Biological Chemistry* 2008; **283**: 25920-25927 [PMID: 18603531 DOI: 10.1074/jbc.M802721200]
  - 63 **Lai CQ**, Corella D, Demissie S, Cupples LA, Adiconis X, Zhu Y, Parnell LD, Tucker KL, Ordovas JM. Dietary intake of n-6 fatty acids modulates effect of apolipoprotein A5 gene on plasma fasting triglycerides, remnant lipoprotein concentrations, and lipoprotein particle size: the Framingham Heart Study. *Circulation* 2006; **113**: 2062-2070 [PMID: 16636175 DOI: 10.1161/CIRCULATIONAHA.105.577296]
  - 64 **Sevastianova K**, Kotronen A, Gastaldelli A, Perttilä J, Hakkarainen A, Lundbom J, Suojanen L, Orho-Melander M, Lundbom N, Ferrannini E, Rissanen A, Olkkonen VM, Yki-Järvinen H. Genetic variation in PNPLA3 (adiponutrin) confers sensitivity to weight loss-induced decrease in liver fat in humans. *Am J Clin Nutr* 2011; **94**: 104-111 [PMID: 21525193 DOI: 10.3945/ajcn.111.012369]
  - 65 **Santoro N**, Savoye M, Kim G, Marotto K, Shaw MM, Pierpont B, Caprio S. Hepatic fat accumulation is modulated by the interaction between the rs738409 variant in the PNPLA3 gene and the dietary omega6/omega3 PUFA intake. *PLoS One* 2012; **7**: e37827 [PMID:



22629460 DOI: 10.1371/journal.pone.0037827]

- 66 **Kitamoto T**, Kitamoto A, Yoneda M, Hyogo H, Ochi H, Nakamura T, Teranishi H, Mizusawa S, Ueno T, Chayama K, Nakajima A, Nakao K, Sekine A, Hotta K. Genome-wide scan revealed that

polymorphisms in the PNPLA3, SAMM50, and PARVB genes are associated with development and progression of nonalcoholic fatty liver disease in Japan. *Hum Genet* 2013; **132**: 783-792 [PMID: 23535911 DOI: 10.1007/s00439-013-1294-3]

**P- Reviewer:** Ampuero J, Higuera-de la Tijera MF, Joven J  
**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Li D



Basic Study

## Lipogenesis in Huh7 cells is promoted by increasing the fructose: Glucose molar ratio

Fernando Windemuller, Jiliu Xu, Simon S Rabinowitz, M Mahmood Hussain, Steven M Schwarz

Fernando Windemuller, Jiliu Xu, Simon S Rabinowitz, Steven M Schwarz, Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition, State University of New York Downstate Medical Center, Brooklyn, NY 11203, United States

M Mahmood Hussain, Department of Cell Biology, State University of New York Downstate Medical Center, Brooklyn, NY 11203, United States

**Author contributions:** Windemuller F performed all the experiments and prepared the initial manuscript draft; Xu J and Rabinowitz SS assisted in study design and interpretation of data, as well as review of the final document; all experiments were performed in the laboratory of Hussain MM, under his guidance; Schwarz SM provided the initial study design, conducted all statistical analyses and wrote the final version of the manuscript.

**Institutional review board statement:** N/A.

**Informed consent statement:** N/A.

**Conflict-of-interest statement:** The authors have no personal, professional or financial relationships relevant to this report.

**Data sharing statement:** All technical and statistical documents, as well as all data sets, are available from the corresponding author at [steven.schwarz@downstate.edu](mailto:steven.schwarz@downstate.edu). No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Steven M Schwarz, MD, Department of Pediatrics, Division of Gastroenterology, Hepatology and

Nutrition, State University of New York Downstate Medical Center, 445 Lenox Rd, Box 49, Brooklyn, NY 11203, United States. [steven.schwarz@downstate.edu](mailto:steven.schwarz@downstate.edu)  
**Telephone:** +1-718-2708968  
**Fax:** +1-718-2701985

**Received:** February 20, 2016  
**Peer-review started:** February 22, 2016  
**First decision:** March 25, 2016  
**Revised:** March 28, 2016  
**Accepted:** June 14, 2016  
**Article in press:** June 16, 2016  
**Published online:** July 18, 2016

### Abstract

**AIM:** To determine whether hepatocyte lipogenesis, in an *in vitro* cell culture model, is modulated by adjusting culture media monosaccharide content and concentration.

**METHODS:** Hepatocytes (Huh7), demonstrating glucose and fructose uptake and lipid biosynthesis, were incubated in culture media containing either glucose alone (0.65-0.72 mmol/L) or isosmolar monosaccharide (0.72 mmol/L) comprising fructose:glucose (F:G) molar ratios ranging from 0.58-0.67. Following a 24-h incubation, cells were harvested and analyzed for total protein, triglyceride (TG) and cholesterol (C) content. Significant differences ( $P < 0.05$ ) among groups were determined using analysis of variance followed by Dunnett's test for multiple comparisons.

**RESULTS:** After a 24 h incubation period, Huh7 cell mass and viability among all experimental groups were not different. Hepatocytes cultured with increasing concentrations of glucose alone did not demonstrate a significant change either in C or in TG content. However, when the culture media contained increasing F: G molar ratios, at a constant total monosaccharide

concentration, synthesis both of C and of TG increased significantly [F:G ratio = 0.58, C/protein ( $\mu\text{g}/\mu\text{g}$ ) = 0.13; F:G = 0.67, C/protein = 0.18,  $P < 0.01$ ; F:G ratio = 0.58, TG/protein ( $\mu\text{g}/\mu\text{g}$ ) = 0.06; F:G ratio = 0.67, TG/protein = 0.11,  $P < 0.01$ ].

**CONCLUSION:** In an *in vitro* hepatocyte model, glucose or fructose plus glucose support total cell mass and lipogenic activity. Increasing the fructose:glucose molar ratio (but not glucose alone) enhances triglyceride and cholesterol synthesis. These investigations demonstrate fructose promotes hepatocellular lipogenesis, and they provide evidence supporting future, *in vivo* studies of fructose's role in the development of hepatic steatosis and non-alcoholic fatty liver disease.

**Key words:** Hepatocytes; Cholesterol; Triglycerides; Fructose; Glucose

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Employing an *in vitro* hepatocyte culture model, these data demonstrate fructose promotes intracellular synthesis both of cholesterol and of triglyceride. The results support the requirement for future, *in vivo* investigations to determine whether diets high in fructose are risk factors for hepatic steatosis and development of non-alcoholic fatty liver disease.

Windemuller F, Xu J, Rabinowitz SS, Hussain MM, Schwarz SM. Lipogenesis in Huh7 cells is promoted by increasing the fructose: Glucose molar ratio. *World J Hepatol* 2016; 8(20): 838-843 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i20/838.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i20.838>

## INTRODUCTION

Fructose, a five-carbon monosaccharide, comprises an increasing component of the Western diet, particularly in the form of high fructose corn syrup (HFCS). In the United States, this sweetener is both readily available from domestically grown corn and inexpensive, when compared to imported, granulated sugar. It was introduced in the 1960's, with subsequent expansion into a vast array of foods and beverages. HFCS is made from corn starch, processed by glucose isomerase to convert a portion of its glucose fraction into fructose. HFCS preparations contain approximately 25% water, fructose (up to 55% of the water-free fraction), glucose and 0%-5% unprocessed glucose oligomers. HFCS's use as a commercial sweetener has doubled in the last decade and, as a consequence, fructose intake in developed countries has increased five-fold<sup>[1]</sup>.

In several human studies to date, increased fructose intake has been linked both to abnormal plasma lipid profiles and to the development of non-alcoholic fatty liver disease (NAFLD)<sup>[2-4]</sup>. However, because the effects

of fructose, *per se*, are difficult to distinguish from the influences of other dietary carbohydrates and fats, current clinical evidence does not establish the precise contribution of fructose-containing food and beverage products to the etiology and/or the exacerbation of specific biochemical, clinical and histopathologic abnormalities. Further, since the causes of dietary carbohydrate and lipid-related problems (e.g., hyperlipidemia, type II diabetes, NAFLD, metabolic syndrome) are multifactorial and also related to age, gender and lifestyle, additional investigations are required to determine the relative contributions of dietary and other co-factors.

In attempting to identify the importance of fructose in hepatocellular lipid metabolism, available *in vitro* studies indicate fructose may preferentially promote *de novo* lipogenesis<sup>[5]</sup>. Since fructose bypasses the glycolytic rate-limiting enzyme phosphofructokinase, it is metabolized efficiently and provides a readily available substrate for hepatic lipid synthesis<sup>[6]</sup>. In addition, fructose has been shown to inhibit peroxisome proliferator-activated receptor (PPAR)-alpha mediated hepatocellular fatty acid beta-oxidation and lipid clearance<sup>[7]</sup>.

The potential lipogenic role of fructose also may be related to its relationship to endogenous insulin activity. Insulin insensitivity has been implicated in the pathogenesis and progression of NAFLD<sup>[8]</sup>. Impaired insulin responsiveness to circulating carbohydrate is associated both with increased adipocyte lipolysis and with increased levels of circulating free fatty acids (FFA). Thus, insulin resistance promotes lipolysis, particularly in intra-abdominal, white adipose tissue. This phenomenon occurs as a consequence of dysregulation of lipid regulatory transcription factors (e.g., PPAR-gamma), instability of adipocyte lipid and impaired lipogenesis. Released FFA lead to upregulation of inflammatory cytokines and chemokines<sup>[8,9]</sup>. Subsequently, liberated adipocyte FFA are taken-up by the liver and re-esterified to triglyceride. As fructose does not promote insulin secretion, it may therefore exacerbate the effects of insulin resistance on hepatic lipid synthesis, steatosis and the development of NAFLD<sup>[10]</sup>. Further evidence of fructose' role in the development of NAFLD derives from a recently reported rat model of hepatic steatosis. A high fructose-containing diet promoted not only lipogenesis leading to steatosis, but also increased expression of lipocalin-2, a ubiquitous glycoprotein involved in the response to inflammation and oxidative stress<sup>[11]</sup>.

Despite evidence linking fructose to the development of altered lipid dynamics, hepatic steatosis and NAFLD, a recent meta-analysis concluded that studies examining the hepatocellular effects of fructose were confounded by the co-stimulation of lipogenesis resulting from increased total energy intake<sup>[12]</sup>. This observation suggests the deleterious effects of HFCS are merely a reflection of overall calorie excess in the Western diet.

In light of the above clinical and experimental data, the present study seeks to establish the influence of fructose on hepatocyte lipogenesis and provide a basis for future, translational investigations of fructose-

**Table 1** Hepatocellular protein content

Glucose (mmol/L)	0.65	0.68	0.72
Protein (μg/mL)	635.0 ± 52.7	608.6 ± 35.3	543.2 ± 126.6
Fructose:glucose (mmol/L: mmol/L)	0.58:1 <sup>1</sup>	0.61:1 <sup>1</sup>	0.67:1 <sup>1</sup>
Protein (μg/mL)	626.6 ± 95.2	610.2 ± 30.6	521.9 ± 38.7

<sup>1</sup>Total monosaccharide concentration = 0.72 mmol/L, Huh7 cell homogenates, means ± SD after 24 h of incubation in culture media containing only glucose or glucose plus fructose as the nutrient monosaccharide(s). All experiments comprise samples at a fixed volume of 10 mL/plate.

mediated lipid biosynthesis. These experiments employ an established, immortal and metabolically active human hepatocellular carcinoma cell line, Huh7, used extensively in studies of hepatocyte metabolism<sup>[13-15]</sup>. Since facilitated uptake of glucose and fructose by the transmembrane GLUT2 transporter is demonstrated in Huh7 cells<sup>[16]</sup>, these cells provide an excellent model for studies of carbohydrate-induced lipogenesis. Accordingly, the studies herein were carried out to determine whether hepatocyte lipogenesis, in an *in vitro* cell culture model, is modulated by adjusting culture media monosaccharide content and concentration.

## MATERIALS AND METHODS

### Cell culture

Huh7 cells (American Type Culture Collection, Manassas, VA) were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS), 1% penicillin-streptomycin and 1% L-glutamine, in a 37 °C, 5% CO<sub>2</sub> cell culture incubator on 100 mm × 200 mm tissue culture dishes (BD Falcon Durham, NC). Once 80% confluence was achieved (cell count -  $1 \times 10^5$ ), cells were incubated for 24 h in culture media (DMEM) with 10% FBS containing either glucose alone (0.65, 0.68 and 0.72 mmol/L) or isosmolar monosaccharide (0.72 mmol/L) comprising fructose:glucose (F:G) molar ratios of 0.58, 0.61, and 0.67. For all experiments, total media osmolality was 400 mOsm/L. All incubations were performed in triplicate. Following the 24 h incubation, as described above, culture media was removed, plates were washed with phosphate buffered saline, and cells were lysed and collected in 750 microliters isopropanol, as previously described<sup>[17]</sup>. The cell lysates were kept at 4 °C for 12 h. Each sample was then centrifuged at 4 °C for 10 min at 10000 rpm. Supernatants were removed for lipid studies and the remaining cellular precipitate was re-suspended in 0.1N NaOH for protein quantification, employing a previously validated method<sup>[17]</sup>.

### Protein and lipid analyses

Cholesterol and triglyceride were measured utilizing samples from the isopropanol supernatant<sup>[18]</sup>. The samples were placed in a 96 well plate for lipid quantification, using established spectrophotometric methods<sup>[19,20]</sup>. Spectrophotometric absorbancies of each cell lysate sample were compared to known lipid standards, and the concentrations of cholesterol and triglyceride

were calculated from the derived measurements<sup>[19,20]</sup>. Protein was quantified in triplicate utilizing samples from the NaOH suspension, employing standard spectrophotometric methods<sup>[21]</sup>.

### Cell mass determination

For these cell culture studies, estimates of cell mass in all experimental groups were made using total protein measurements in Huh7 lysates<sup>[22]</sup>. All single cell-line incubations were performed concurrently, under the same environmental conditions, thereby synchronizing cell cycles among experimental groups. Accordingly, as previously described, calculation of total cell protein content was employed to estimate relative cell biomass among incubations<sup>[23]</sup>.

### Statistical analysis

Differences among all experimental groups were assessed by analysis of variance, followed by Dunnett's test for multiple comparisons. A *P*-value of < 0.05 was considered significant.

## RESULTS

### Cell mass

Cultured Huh7 cells incubated in glucose-only supplemented media (0.65, 0.68, and 0.72 mmol/L) and in media containing varying F:G molar ratios (total monosaccharide concentration 0.72 mmol/L), did not show any statistically significant differences in protein content among all study groups. These data indicate total cell mass was not affected by varying the monosaccharide concentration or distribution (Table 1).

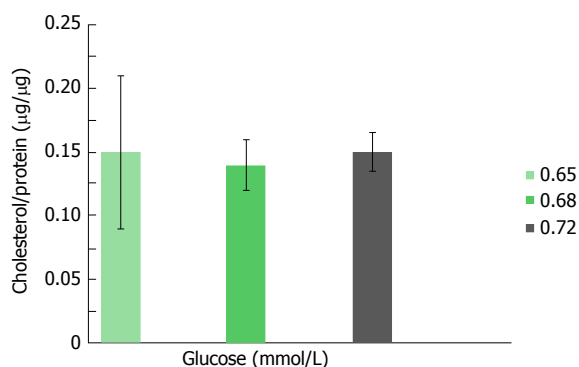
### Glucose-mediated lipogenesis

As shown in Figures 1 and 2, triglyceride and cholesterol content (μg/mg cell protein) did not differ significantly among Huh7 cells incubated for 24 h in media containing 0.65, 0.68 or 0.72 mmol/L glucose per plate. Further increases (> 0.72 mmol/L) in glucose molar concentration did not result in any additional enhancement in cellular lipid content (data not shown).

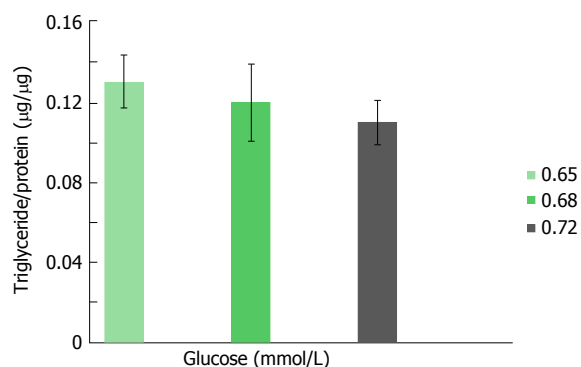
### Fructose-mediated lipogenesis

For these experiments, all cells were incubated in media containing 0.72 mmol/L monosaccharide (total media osmolality = 400 mOsm/L), the maximum sugar concentration employed in the "glucose-only" experiments, described above. To determine the effects of fructose on lipogenesis, cells were incubated in the presence of increasing molar ratios of F:G (0.58, 0.61 and 0.67). Following a 24 h incubation, Huh7 cell cholesterol content (μg/μg cell protein) increased significantly at a F:G molar ratio of 0.67:1 (Figure 3), compared both to a 0.61:1 ratio (0.18 μg/μg vs 0.14 μg/μg protein, *P* < 0.05) and to a 0.58:1 ratio (0.18 μg/μg vs 0.13 μg/μg protein, *P* < 0.01). Triglyceride analyses (Figure 4) demonstrated fructose-mediated Huh7 triglyceride synthesis also increased significantly in step-wise fashion. Thus, increased triglyceride content was

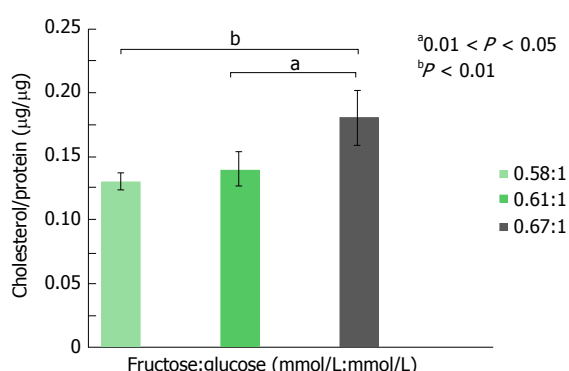




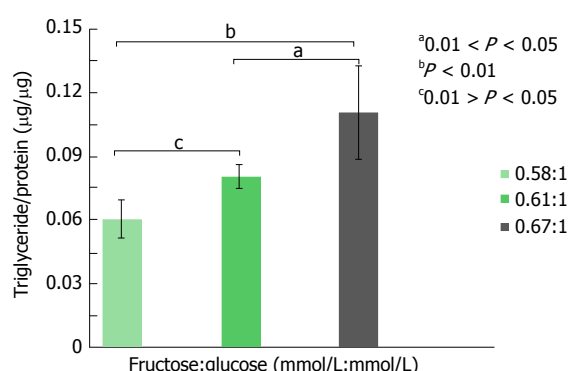
**Figure 1** Cholesterol content of Huh7 cells ( $\mu\text{g}/\mu\text{g}$  cellular protein) following a 24-h incubation, in culture media containing 0.65, 0.68 and 0.72 mmol/L glucose.



**Figure 2** Triglyceride content of Huh7 cells ( $\mu\text{g}/\mu\text{g}$  cellular protein) following a 24-h incubation, in culture media containing 0.65, 0.68 and 0.72 mmol/L glucose.



**Figure 3** Cholesterol content of Huh7 cells ( $\mu\text{g}/\mu\text{g}$  cellular protein) following a 24-h incubation, in culture media containing fructose and glucose, at fructose:glucose molar ratios of 0.58:1, 0.61:1 and 0.67:1. All incubations were carried out in media containing 0.72 mmol/L total monosaccharide.



**Figure 4** Triglyceride content of Huh7 cells ( $\mu\text{g}/\mu\text{g}$  cellular protein) following a 24-h incubation in culture media containing fructose and glucose, at fructose:glucose molar ratios of 0.58:1, 0.61:1 and 0.67:1. All incubations were carried out in media containing 0.72 mmol/L total monosaccharide.

noted at a F:G ratio of 0.61:1 (compared to the ratio of 0.58:1,  $0.08 \mu\text{g}/\mu\text{g}$  vs  $0.06 \mu\text{g}/\mu\text{g}$  protein,  $P < 0.05$ ) and at a ratio of 0.67:1 (compared to the ratio of 0.61:1,  $0.11 \mu\text{g}/\mu\text{g}$  vs  $0.08 \mu\text{g}/\mu\text{g}$  protein,  $P < 0.05$ ; to 0.67:1,  $0.11 \mu\text{g}/\mu\text{g}$  vs  $0.06 \mu\text{g}/\mu\text{g}$  protein,  $P < 0.01$ ).

## DISCUSSION

The results presented in this report demonstrate Huh7 cells, an immortal hepatocellular carcinoma cell line, grown in standard culture media containing increasing glucose concentrations (up to 0.72 mmol/L), exhibit no differences in relative cellular TG and C, as a consequence of increased media glucose content. However, when the nutrient monosaccharides comprise fructose plus glucose (at increasing F:G molar ratios), significant promotion of lipogenesis is demonstrated by increased hepatocellular TG and C concentrations. For these studies, the cell culture media monosaccharide content of 0.72 mmol/L (glucose alone or glucose plus fructose) was found to maximize hepatocellular lipogenesis. This molar amount was determined following a series of experiments, employing a step-wise increase in sugar content and based on previous human studies showing serum total monosaccharide concentrations of approximately 0.50 mmol/L following a fructose-rich

meal<sup>[24]</sup>. Higher amounts of monosaccharide ( $> 0.72$  mmol/L) in the Huh7 incubating media did not yield statistically significant increases either in cellular TG or in cellular C content, while further increases ( $> 400$  mmol/L) in media osmolality resulted in decreased cell viability.

These results are consistent with a prior clinical report, suggesting hepatic fat (estimated by magnetic resonance imaging) in subjects fed fructose, in addition to a specific weight maintenance diet, was increased compared to a study group fed the same diet supplemented with glucose alone<sup>[4]</sup>. Conversely, another report failed to demonstrate any promotion of lipogenesis following four weeks of a fructose supplemented diet. As stated previously, a recent meta-analysis concluded the potential association between fructose and NAFLD was confounded by the concurrent consumption of hypercaloric diets<sup>[12]</sup>. The influence of fructose on lipogenesis, as a consequence of excess energy intake, may be mediated by this monosaccharide's direct attenuation of post-prandial ghrelin suppression<sup>[24]</sup>. On the other hand, another study examining the effects of dietary sucrose vs HFCS on endogenous hormone levels, failed to demonstrate any significant differences in serum insulin, leptin and ghrelin levels<sup>[25]</sup>. These combined *in vivo* studies therefore suggest the amount of carbohydrate taken up by hepatocytes is increased (thus leading

to enhanced lipogenesis) as a consequence of total calorie intake, rather than resulting directly from the lipogenic effects of any specific dietary substrate.

In contrast to available clinical data examining fructose mediated steatosis (often derived from imprecise imaging techniques), considerable biochemical evidence supports the role of fructose in promoting *de novo* lipogenesis. Since fructose is more efficiently utilized intracellularly, as compared with glucose, it may provide a more readily available substrate for lipid synthesis<sup>[26]</sup>. Interestingly, one recent study failed to demonstrate any effects of fructose on regulating hepatocellular lipogenic genes, thus providing further, indirect evidence linking the lipogenic role of this monosaccharide to its ability to provide increased amounts of carbon fragments for lipid synthesis<sup>[27]</sup>. The present results extend this observation by demonstrating that lipogenesis, in cultured hepatocytes, is not solely related to the provision of carbon precursors, since all incubations contained similar total monosaccharide concentrations. Thus, fructose, in this experimental model, appears to exert a direct effect on promotion of lipogenesis, and this effect is independent of carbon supply.

Because fructose metabolism is insulin-independent and does not stimulate pancreatic insulin secretion<sup>[28]</sup>, this monosaccharide was previously thought to be a superior dietary substrate, as compared with other sugars<sup>[29]</sup>. However, more recent experimental data, while confirming these metabolic characteristics, also demonstrate fructose promotion of insulin resistance<sup>[12]</sup>. Fructose stimulates Jun-N-terminal kinase-1 (JNK-1), an intracellular mitogen activated protein kinase. JNK-1 in turn phosphorylates insulin receptor substrate-1 resulting in suppression of cellular glucose uptake, increased blood glucose levels and increased insulin secretion<sup>[30-32]</sup>. These findings, therefore, suggest another potential pathway for fructose-mediated steatosis.

Despite results presented in this report and published previously, the precise mechanisms by which fructose increases hepatic TG are not completely understood. Carbohydrate responsive element binding protein (ChREBP) and sterol regulatory element binding protein 1c (SREBP1c) are two transcription regulators of hepatic lipogenesis induced by glucose and insulin, respectively. Animal knockdown studies of peroxisome proliferator-activated receptor gamma co-activator-1 beta, a transcriptional co-activator of SREBP1c, have demonstrated improved hepatic lipid profiles in fructose fed rats. In a separate study<sup>[7]</sup>, fructose fed rats showed increased expression of ChREBP hepatic mRNA, compared with a glucose fed group. Because recent data shows fetal bovine serum (FBS) contains factors that promote cellular lipogenesis, independent of insulin<sup>[33]</sup>, and because FBS concentrations were held constant in our studies, stimulation of either SREBP1c or ChREBP represents an unlikely contributor to the observation of fructose-mediated *de novo* lipogenesis.

Our preliminary study certainly has several important limitations. These observations, in an *in vitro* cell culture model, cannot accurately predict the metabolic fate of

fructose, in terms of its ability to enter lipogenic pathways. While the data clearly suggest direct effects of fructose on hepatocellular synthesis of triglyceride and cholesterol, they do not provide further elucidation of the underlying mechanisms leading to these findings. Nevertheless, the observation that fructose exerts a promoting influence on lipid synthesis, confirms prior studies suggesting the significant role of this monosaccharide in the development and/or exacerbation of hepatic steatosis. These data, therefore, warrant further investigations into the mechanisms and extent of fructose-mediated, *de novo* hepatic lipogenesis.

## COMMENTS

### Background

Available studies, both *in vitro* and in clinical trials, indicate the dietary monosaccharide, fructose, may be an important substrate for hepatic lipid synthesis, and may promote the development of hepatic steatosis. However, whether fructose-associated lipogenesis is related to dietary intake of fructose, *per se*, or merely reflects excess total energy consumption, remains unclear. The present study seeks to establish the effects of fructose on hepatocyte lipogenesis and provide a basis for future, translational investigations of fructose-mediated lipid biosynthesis. These experiments employ an established, immortal and metabolically active human hepatocellular carcinoma cell line, Huh7, used extensively in studies of hepatocyte metabolism. The studies herein were carried out to determine whether hepatocyte lipogenesis, in an *in vitro* cell culture model, is modulated by adjusting culture media monosaccharide content and concentration.

### Innovations and breakthroughs

The results of these experiments clearly demonstrate, in a stable, *in vitro* hepatocyte culture model, at constant monosaccharide concentrations (glucose  $\pm$  fructose), by increasing the culture medium fructose to glucose molar ratio, but not by increasing glucose alone, significant enhancement of lipogenesis.

### Applications

The observation that fructose exerts a promoting influence on lipid synthesis, confirms prior studies suggesting the significant role of this monosaccharide in the development and/or exacerbation of hepatic steatosis. These studies, therefore, support the need for further investigations into the mechanisms and extent of fructose-mediated, *de novo* hepatic lipogenesis. Most important, these results provide a basis for future, clinical studies of fructose's role in development of hepatic steatosis and non-alcoholic liver disease.

### Terminology

Huh7 cells, an immortal, stable hepatocyte line, derived from human hepatocellular carcinoma. These cells take-up both glucose and fructose, and have been used extensively in studies of hepatic metabolism.

### Peer-review

This is an interesting study that reveals fructose is linked to lipogenesis in a concentration dependent way.

## REFERENCES

- 1 **Lustig RH.** Fructose: metabolic, hedonic, and societal parallels with ethanol. *J Am Diet Assoc* 2010; **110**: 1307-1321 [PMID: 20800122 DOI: 10.1016/j.jada]
- 2 **Ouyang X, Cirillo P, Sautin Y, McCall S, Bruchette JL, Diehl AM, Johnson RJ, Abdelmalek MF.** Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *J Hepatol* 2008; **48**: 993-999 [PMID: 18395287 DOI: 10.1016/j.jhep]
- 3 **Silbernagel G, Machann J, Unmuth S, Schick F, Stefan N, Häring HU, Fritsche A.** Effects of 4-week very-high-fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. *Br J Nutr* 2011; **106**: 79-86 [PMID: 21396140]

- DOI: 10.1017/S000711451000574X]
- 4 **Lecoultrre V**, Egli L, Carrel G, Theytaz F, Kreis R, Schneiter P, Boss A, Zwygart K, Lê KA, Bortolotti M, Boesch C, Tappy L. Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. *Obesity* (Silver Spring) 2013; **21**: 782-785 [PMID: 23512506 DOI: 10.1002/oby.20377]
  - 5 **Spence JT**, Pitot HC. Induction of lipogenic enzymes in primary cultures of rat hepatocytes. Relationship between lipogenesis and carbohydrate metabolism. *Eur J Biochem* 1982; **128**: 15-20 [PMID: 6293823 DOI: 10.1111/j.1432-1033.1982.tb06924.x]
  - 6 **Basciano H**, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab* (Lond) 2005; **2**: 5 [PMID: 15723702 DOI: 10.1186/1743-7075-2-5]
  - 7 **Nagai Y**, Yonemitsu S, Erion DM, Iwasaki T, Stark R, Weismann D, Dong J, Zhang D, Jurczak MJ, Löffler MG, Cresswell J, Yu XX, Murray SF, Bhanot S, Monia BP, Bogan JS, Samuel V, Shulman GI. The role of peroxisome proliferator-activated receptor gamma coactivator-1 beta in the pathogenesis of fructose-induced insulin resistance. *Cell Metab* 2009; **9**: 252-264 [PMID: 19254570 DOI: 10.1016/j.cmet.2009.01.011]
  - 8 **Delarue J**, Magnan C. Free fatty acids and insulin resistance. *Curr Opin Clin Nutr Metab Care* 2007; **10**: 142-148 [PMID: 17285001 DOI: 10.1097/MCO.0b013e328042ba90]
  - 9 **Shah A**, Mehta N, Reilly MP. Adipose inflammation, insulin resistance, and cardiovascular disease. *JPEN J Parenter Enteral Nutr* 2008; **32**: 638-644 [PMID: 18974244 DOI: 10.1177/0148607108325251]
  - 10 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844-1850 [PMID: 11473047 DOI: 10.2337/diabetes.50.8.1844]
  - 11 **Alwahsh SM**, Xu M, Seyhan HA, Ahmad S, Mihm S, Ramadori G, Schultze FC. Diet high in fructose leads to an overexpression of lipocalin-2 in rat fatty liver. *World J Gastroenterol* 2014; **20**: 1807-1821 [PMID: 24587658 DOI: 10.3748/wjg.v20.i7.1807]
  - 12 **Bremer AA**, Mietus-Snyder M, Lustig RH. Toward a unifying hypothesis of metabolic syndrome. *Pediatrics* 2012; **129**: 557-570 [PMID: 22351884 DOI: 10.1542/peds.2011-2912]
  - 13 **Chung M**, Ma J, Patel K, Berger S, Lau J, Lichtenstein AH. Fructose, high-fructose corn syrup, sucrose, and nonalcoholic fatty liver disease or indexes of liver health: a systematic review and meta-analysis. *Am J Clin Nutr* 2014; **100**: 833-849 [PMID: 25099546 DOI: 10.3945/ajcn.114.086314]
  - 14 **Goto D**, Okimoto T, Ono M, Shimotsu H, Abe K, Tsujita Y, Kuwano M. Upregulation of low density lipoprotein receptor by gemfibrozil, a hypolipidemic agent, in human hepatoma cells through stabilization of mRNA transcripts. *Arterioscler Thromb Vasc Biol* 1997; **17**: 2707-2712 [PMID: 9409246 DOI: 10.1161/01.ATV.17.11.2707]
  - 15 **Yao H**, Ye J. Long chain acyl-CoA synthetase 3-mediated phosphatidylcholine synthesis is required for assembly of very low density lipoproteins in human hepatoma Huh7 cells. *J Biol Chem* 2008; **283**: 849-854 [PMID: 18003621 DOI: 10.1074/jbc.M706160200]
  - 16 **Zhao Y**, Chen YQ, Bonacci TM, Bredt DS, Li S, Bensch WR, Moller DE, Kowala M, Konrad RJ, Cao G. Identification and characterization of a major liver lysophosphatidylcholine acyltransferase. *J Biol Chem* 2008; **283**: 8258-8265 [PMID: 18195019 DOI: 10.1074/jbc.M710422200]
  - 17 **Tuch BE**, Szymanska B, Yao M, Tabiin MT, Gross DJ, Holman S, Swan MA, Humphrey RK, Marshall GM, Simpson AM. Function of a genetically modified human liver cell line that stores, processes and secretes insulin. *Gene Ther* 2003; **10**: 490-503 [PMID: 12621453 DOI: 10.1038/sj.gt.3301911]
  - 18 **Egusa G**, Brady DW, Grundy SM, Howard BV. Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. *J Lipid Res* 1983; **24**: 1261-1267 [PMID: 6631250]
  - 19 **Cramp DG**. Lipid methodology. *J Clin Pathol Suppl* (Assoc Clin Pathol) 1973; **5**: 17-21 [PMID: 4354843 DOI: 10.1136/jcp.s1-5.1.17]
  - 20 **Roesschlau P**, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum. *Z Klin Chem Klin Biochem* 1974; **12**: 226 [PMID: 4440114]
  - 21 **Fossati P**, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* 1982; **28**: 2077-2080 [PMID: 6812986]
  - 22 **Spector T**. Refinement of the coomassie blue method of protein quantitation. A simple and linear spectrophotometric assay for less than or equal to 0.5 to 50 microgram of protein. *Anal Biochem* 1978; **86**: 142-146 [PMID: 655375 DOI: 10.1016/0003-2697(78)90327-5]
  - 23 **Butler M**, Spearman M, Braasch K. Monitoring cell growth, viability, and apoptosis. *Methods Mol Biol* 2014; **1104**: 169-192 [PMID: 24297416]
  - 24 **Teff KL**, Grudziak J, Townsend RR, Dunn TN, Grant RW, Adams SH, Keim NL, Cummings BP, Stanhope KL, Havel PJ. Endocrine and metabolic effects of consuming fructose- and glucose-sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. *J Clin Endocrinol Metab* 2009; **94**: 1562-1569 [PMID: 19208729 DOI: 10.1210/jc.2008-2192]
  - 25 **Melanson KJ**, Zukley L, Lowndes J, Nguyen V, Angelopoulos TJ, Rippe JM. Effects of high-fructose corn syrup and sucrose consumption on circulating glucose, insulin, leptin, and ghrelin and on appetite in normal-weight women. *Nutrition* 2007; **23**: 103-112 [PMID: 17234503 DOI: 10.1016/j.nut.2006.11.001]
  - 26 **Basaranoglu M**, Basaranoglu G, Sabuncu T, Sentürk H. Fructose as a key player in the development of fatty liver disease. *World J Gastroenterol* 2013; **19**: 1166-1172 [PMID: 23482247 DOI: 10.3748/wjg.v19.i8.1166]
  - 27 **Hirahatake KM**, Meissen JK, Fiehn O, Adams SH. Comparative effects of fructose and glucose on lipogenic gene expression and intermediary metabolism in HepG2 liver cells. *PLoS One* 2011; **6**: e26583 [PMID: 22096489 DOI: 10.1371/journal.pone.0026583]
  - 28 **Curry DL**. Effects of mannose and fructose on the synthesis and secretion of insulin. *Pancreas* 1989; **4**: 2-9 [PMID: 2654926 DOI: 10.1097/00006676-198902000-00002]
  - 29 **Schultz A**, Neil D, Aguila MB, Mandarim-de-Lacerda CA. Hepatic adverse effects of fructose consumption independent of overweight/obesity. *Int J Mol Sci* 2013; **14**: 21873-21886 [PMID: 24196354 DOI: 10.3390/ijms141121873]
  - 30 **Hirosumi J**, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. *Nature* 2002; **420**: 333-336 [PMID: 12447443 DOI: 10.1038/nature01137]
  - 31 **Lim JS**, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 251-264 [PMID: 20368739]
  - 32 **Havel PJ**. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutr Rev* 2005; **63**: 133-157 [PMID: 15971409 DOI: 10.1111/j.1753-4887.2005.tb00132.x]
  - 33 **Berenguer M**, Martinez L, Giorgetti-Peraldi S, Le Marchand-Brustel Y, Govers R. A serum factor induces insulin-independent translocation of GLUT4 to the cell surface which is maintained in insulin resistance. *PLoS One* 2010; **5**: e15560 [PMID: 21187969 DOI: 10.1371/journal.pone.0015560]

**P- Reviewer:** de F Higuera-de la Tijera M, Galvao FHF, Peltec A, Ratnasari N

**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Li D



Retrospective Study

# Aluminum potassium sulfate and tannic acid sclerotherapy for Goligher Grades II and III hemorrhoids: Results from a multicenter study

Hidenori Miyamoto, Takenori Hada, Gentaro Ishiyama, Yoshito Ono, Hideo Watanabe

Hidenori Miyamoto, Department of Digestive and Pediatric Surgery, Institute of Health Bioscience, University of Tokushima Graduate School, Tokushima 770-8503, Japan

Hidenori Miyamoto, Department of Proctologic Surgery, Shiseikai Miyamoto Hospital, Anan, Tokushima 779-1105, Japan

Takenori Hada, Division of Proctologic Surgery, Onaka Clinic Oshiri Center, Hachioji, Tokyo 192-0083, Japan

Gentaro Ishiyama, Department of Anorectal Surgery, Sapporo Ishiyama Hospital, Sapporo, Hokkaido 064-0915, Japan

Yoshito Ono, Hideo Watanabe, Department of Surgery, Watanabe Hospital, Matsuyama, Ehime 791-0054, Japan

**Author contributions:** Miyamoto H designed the study; Miyamoto H, Hada T, Ishiyama G, Ono Y and Watanabe H acquired, analyzed and interpreted the data, and drafted the manuscript; Hada T contributed to statistical advice.

**Institutional review board statement:** This study was reviewed and approved by the Ethics Committee of the Miyamoto Hospital.

**Informed consent statement:** All study participants or their legal guardian provided informed consent written consent about personal and medical data collection prior to study enrolment.

**Conflict-of-interest statement:** All the authors have no conflict of interest related to the manuscript.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Hidenori Miyamoto, MD, PhD, Department of Digestive and Pediatric Surgery, Institute of Health Bioscience, University of Tokushima Graduate School, 3-18-15 Kuramoto, Tokushima 770-8503, Japan. [hanna@diamond.nmt.ne.jp](mailto:hanna@diamond.nmt.ne.jp)  
Telephone: +81-88-6337137  
Fax: +81-88-6319698

Received: March 16, 2016  
Peer-review started: March 18, 2016  
First decision: April 18, 2016  
Revised: May 29, 2016  
Accepted: June 14, 2016  
Article in press: June 16, 2016  
Published online: July 18, 2016

## Abstract

**AIM:** To show that aluminum potassium sulfate and tannic acid (ALTA) sclerotherapy has a high success rate for Grade II and III hemorrhoids.

**METHODS:** This study was based on the clinical data of 604 patients with hemorrhoids who underwent ALTA sclerotherapy between January 2009 and February 2015. The objective of this study was to assess the efficacy of this treatment for Grades II and III hemorrhoids. Preoperative and postoperative symptoms, complications and success rate were all assessed retrospectively. Follow-up consisted of a simple questionnaire, physical examination and an anoscopy. Patients were followed-up at one day, one week, two weeks, one month, one year, two years, three years, four years and five years after the ALTA sclerotherapy.

**RESULTS:** One hundred and sixty-nine patients were diagnosed with Grade II hemorrhoids and 435 patients were diagnosed with Grade III hemorrhoids. The one year, three year and five year cumulative success rates of ALTA sclerotherapy for Grades II and III hemo-



rrhoids were 95.9% and 93.1%; 89.3% and 83.7%; and 89.3% and 78.2%, respectively. No significant differences were observed in the cumulative success rates after ALTA sclerotherapy between Grades II and III hemorrhoids ( $P = 0.09$ ). There were forty-seven post-operative complications (low grade fever; anal pain; urinary retention; rectal ulcer; and others). No serious or life-threatening complications occurred and all cases improved through conservative treatment. At univariate analysis there were no predictive factors of failure.

**CONCLUSION:** ALTA sclerotherapy has had a high success rate for Grade II and III hemorrhoids during five years of post-operative treatment. However, additional studies are needed to evaluate the efficacy of this ALTA sclerotherapy in the management of hemorrhoidal disease.

**Key words:** Sclerotherapy; Aluminum potassium sulfate and tannic acid; Goligher grade III; Minimally invasive treatment; Hemorrhoid

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Since 2000, aluminum potassium sulfate and tannic acid (ALTA) sclerotherapy has been frequently performed in Japan for internal hemorrhoids as a minimally invasive treatment. This study affirms that ALTA sclerotherapy is an effective and safety treatment for Goligher Grades II and III hemorrhoids because of the high cumulative success rate and no serious complications during post-operative treatment.

Miyamoto H, Hada T, Ishiyama G, Ono Y, Watanabe H. Aluminum potassium sulfate and tannic acid sclerotherapy for Goligher Grades II and III hemorrhoids: Results from a multicenter study. *World J Hepatol* 2016; 8(20): 844-849 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i20/844.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i20.844>

## INTRODUCTION

Hemorrhoids are the most common anorectal disease world-wide. The term "hemorrhoidal disease" can be employed when hemorrhoidal tissue gives rise to symptoms such as bleeding, prolapse, or pruritus<sup>[1]</sup>. Etiologic factors are multi-factorial and include prolonged straining, irregular bowel habits and heredity. Supporting connective tissue degenerates and hemorrhoidal cushions slide as a consequence.

Conservative treatment based on lifestyle changes such as dietary and exercise and laxatives can help the majority of patients, and rubber band ligation, sclerotherapy and phlebotonic drugs can effectively treat Grades I and II hemorrhoids<sup>[2]</sup>. Surgical treatment is required for the most advanced stages, Grade III or IV and bleeding. There are several methods of surgical treatment for hemorrhoids: Conventional hemorrhoidectomy

(CH), stapled hemorrhoidopexy (PPH), and trans-anal hemorrhoidal dearterialization (THD)<sup>[1-4]</sup>.

Among them, PPH and THD aim to correct the underlying pathophysiological mechanisms involved in the etiology of hemorrhoids. These treatments are painless treatment, because of the sparing of the anoderm. Although an increased risk of recurrence is the price to pay for these minimally invasive treatments, a rapid return to normal life without pain are greatly appreciated by patients<sup>[5]</sup>.

On the other hand, new and effective sclerosant named aluminum potassium sulfate and tannic acid (ALTA) has been developed in Japan. ALTA sclerotherapy has been frequently performed in Japan for internal hemorrhoids as a minimally invasive treatment<sup>[6-9]</sup>. Nowadays in Japan, ALTA sclerotherapy for internal hemorrhoids has been performed in over 300000 cases.

In this report, we present effective results of ALTA sclerotherapy for Grades II and III hemorrhoids, according to the five year follow-up for this multi-center study.

## Indication for ALTA sclerotherapy

ALTA sclerotherapy was performed on all patients with internal hemorrhoids except for the following: Patients with associated acute inflammatory internal hemorrhoids and acute irreducible hemorrhoids; patients with serious cardiac, hepatic, renal, and hematological diseases; pregnant women or women who may be pregnant; nursing mothers; and patients with a past history of hypersensitivity to local anesthetics.

## MATERIALS AND METHODS

The medical records of 604 patients with hemorrhoidal disease who underwent ALTA sclerotherapy at four institutions from January 2009 to February 2015 were analyzed. All patients underwent clinical evaluation and physical examination including digital examination and proctoscopy for diagnosis of hemorrhoid engorgement and easy-bleeding, prolapsing hemorrhoids. The severity of hemorrhoidal disease was graded according to Goligher's classification.

Patients assumed the lithotomy position or Sims' position. Zero point five percent Lidocaine 10 mL was injected around sphincter muscle. The concentration of ALTA solution is set by, Mitsubishi Tanabe Pharma Corporation, Osaka, Japan. Procedures were undertaken under local anesthesia using the Z-type proctoscope (ARAKAWA SEISAKUJO, Tokyo, Japan) with a distally opening window that allowed for the application of an injection into the rectal mucosa. An ALTA four-step injection was performed<sup>[7]</sup>. The method used for the ALTA four-step injection was to inject four times, once into each of the following parts of the primary hemorrhoid: The superior part; the central deep and slight parts; and the inferior part above the dentate line.

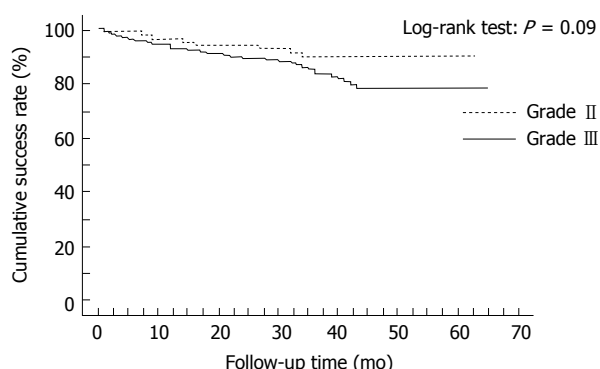
## Assessment and post-operative follow-up

All data were retrospectively collected from medical

**Table 1** The characteristics of patients with Grades II and III hemorrhoids

Goligher classification	Grade II	Grade III	Statistical significance
Number	169	435	
Gender			
Male	103	231	NS
Female	66	204	
Age (yr)	58 ± 18	59 ± 16	NS
Injection dose of ALTA (mL)	13.4 ± 5.2	21.5 ± 6.8	$P < 0.0001$
Operative time	14 ± 8	16 ± 7	$P = 0.0020$

NS: Not significant; ALTA: Aluminum potassium sulfate and tannic acid.

**Figure 1** The cumulative success rate of aluminum potassium sulfate and tannic acid sclerotherapy. At one year, three years and five years after ALTA sclerotherapy, the cumulative success rate of Grades II and III were 95.9% and 93.1%; 89.3% and 83.7%; and 89.3% and 78.2%, respectively. ALTA: Aluminum potassium sulfate and tannic acid.

record. Patients took oral analgesia (loxoprofen or acetaminophen) at three days post operation. They were followed-up at one day, one week, two weeks, one month, one year, two years, three years, four years and five years after the ALTA sclerotherapy. Follow-up consisted of a simple questionnaire, physical examination and an anoscopy. Effects were defined as follows: (1) cure (after bowel movement there is no prolapse of the hemorrhoids, hemorrhage or other discomfort; and on examination with an anoscope, atrophied internal hemorrhoids have disappeared); (2) improvement (after bowel movement, some hemorrhoids prolapse but return into the anal canal spontaneously; occasional blood or hemorrhage with bowel movements; and an anoscopic examination reveals some internal hemorrhoids still visible); and (3) failure (no improvement, or some hemorrhoids prolapsed and did not return into the anal canal spontaneously).

### The action mechanism of ALTA

ALTA compounds with aluminum potassium sulfate and tannic acid. The aluminum ion induces a strong local inflammatory reaction, resulting in fibrosis<sup>[10,11]</sup>. Tannic acid has a strong astringent effect on tissue, promoting protein coagulation and the contraction of blood vessels, while reducing exudation into tissue from the inflammatory reaction<sup>[10,11]</sup>. These actions tend to prevent tissue necrosis, and promote sclerosis, adhesion of hemorrhoidal

**Table 2** Prognostic factors associated with the recurrence of hemorrhoids after aluminum potassium sulfate and tannic acid sclerotherapy

Prognostic factor	Relative risk (95%CI)	P value
Age	0.997 (0.981-1.012)	0.6567
Gender	0.800 (0.469-1.365)	0.4124
Goligher grade	1.768 (0.897-3.483)	0.0997
Operation time	0.974 (0.941-1.010)	0.1542
ALTA injection dose	0.976 (0.941-1.012)	0.1901
Complication	1.293 (0.469-3.564)	0.6189

ALTA: Aluminum potassium sulfate and tannic acid.

tissue and immediate hemostasis and are also effective for the prolapse and bleeding of internal hemorrhoids early after injection.

## RESULTS

The characteristics of the patients involved in this study are shown in Table 1. From January 2009 until February 2015 ALTA sclerotherapy was performed on 604 patients with second or third degree hemorrhoids. There were three 334 men and 270 women. The age range (mean ± SD) of the patients with Grades II and III hemorrhoids was 58 ± 18 years and 59 ± 16 years, respectively. Overall, 169 patients had Grade II hemorrhoids and 435 had Grade III hemorrhoids. The total injection dose of ALTA (mean ± SD) for Grades II and III was 13.4 ± 5.2 mL and 21.5 ± 6.8 mL, respectively. The operative time (mean ± SD) of Grades II and III was 14 ± 8 min and 16 ± 7 min, respectively. All the operations were performed under local anesthesia during either day surgery or a one day hospital stay.

Prolapses and bleeding disappeared immediately after ALTA sclerotherapy. All cases with bleeding or prolapses were cured or there was improvement after the first post-operative month. At one year after treatment, the rate of successful resolution of bleeding or prolapse of Grades II and III was 95.9% and 93.1%, respectively. The three year and five year cumulative success rates of ALTA sclerotherapy for Grades II and III hemorrhoids were 89.3% and 83.7%; and 89.3% and 78.2%, respectively. At one year after treatment, the rate of failure of Grades II and III was 4.1% and 6.9%, respectively. The three year and five year cumulative failure rates of ALTA sclerotherapy for Grades II and III hemorrhoids were 10.7% and 16.3%; and 10.7% and 21.8%, respectively. No significant differences were observed in the cumulative success rates after ALTA sclerotherapy between Grades II and III hemorrhoids ( $P = 0.09$ ) (Figure 1). There were 47 post-operative complications (low grade fever; anal pain; urinary retention; rectal ulcer; and others). No serious or life-threatening complications occurred and all cases improved through conservative treatment. At univariate analysis there were no predictive factors of failure (Table 2). However, the only factor associated with a recurrence may be the grade of hemorrhoids ( $P = 0.09$ ).

**Table 3** Scheme of recurrence rate and/or additional operation rate of each hemorrhoid treatment

Ref.	Grade	Technique	n	Follow-up	Recurrence rate and/or additional surgery rate (%)
Giordano <i>et al</i> <sup>[4]</sup>	II, III	THD	28	3 yr	14.00
		SH	24		13.00
Giordano <i>et al</i> <sup>[17]</sup>	IV	THD + targeted mucopexy	31	32 mo	6.40
Ratto <i>et al</i> <sup>[18]</sup>	II, III, IV	THD	170	11.5 mo	4.10
Ratto <i>et al</i> <sup>[19]</sup>	II, III, IV	THD	803	11.1 mo	10.20
Zampieri <i>et al</i> <sup>[20]</sup>	III, IV	THD	46	1-6 mo	0
		Ligasure	68		4.00
Theodoropoulos <i>et al</i> <sup>[21]</sup>	III, IV	DGHAL + RAR	147	15 mo	4.00
Walega <i>et al</i> <sup>[22]</sup>	III, IV	DGHAL + RAR	29	3 mo	10.34
Gravié <i>et al</i> <sup>[23]</sup>		SH	63	2 yr	7.50
		MMH	63		1.80
Ammaturo <i>et al</i> <sup>[24]</sup>	III	SH	39	2 yr	13.00
		MMH	40		0
Hachiro <i>et al</i> <sup>[8]</sup>	III, IV	ALTA	448	29 mo	3.60
Takano <i>et al</i> <sup>[6]</sup>	III, IV	ALTA (OC-108)	80	1 yr	16.00
		CH	85		2.00
Miyamoto <i>et al</i> <sup>[7]</sup>	II, III, IV	ALTA	28	5 mo	10.70

THD: Trans-anal hemorrhoidal dearterialization; DGHAL: Doppler-guided hemorrhoidal arterial ligation; SH: Stapled hemorrhoidopexy; RAR: Rectoanal repair; CH: Conventional hemorrhoidectomy; ALTA: Aluminum potassium sulfate and tannic acid.

## DISCUSSION

The ideal operation for hemorrhoids should be effective with a low rate of recurrence; minimal post-operative pain to allow early return to normal activities; and safe with minimal morbidity<sup>[12]</sup>. The treatment for internal hemorrhoids is gradually shifting to minimally invasive surgery. CH has been used to be the most widely performed surgical procedure till now. Although CH was very effective, it was painful and potentially affected the mechanism of anal continence. Over the years, alternative minimally invasive techniques have been developed including stapled hemorrhoidopexy, also known as PPH, and trans-anal hemorrhoidal dearterialization, also known as THD<sup>[1,3-5]</sup>. In Japan, new sclerosant, ALTA, has been developed. Takano *et al*<sup>[6]</sup> reported that ALTA (OC-108) sclerotherapy was effective for Grades II, III, and IV internal hemorrhoids. In recent years in Japan, ALTA sclerotherapy has become gradually recognized as minimally invasive treatment for internal hemorrhoids.

In Japan, ALTA sclerotherapy is popular with patients with symptomatic internal hemorrhoids, because unpleasant symptoms disappear immediately on the first post-operative day in almost all cases<sup>[7]</sup>, and patients experience little post-operative pain and no serious complications<sup>[6-9]</sup>. ALTA sclerotherapy could be performed on patients with Grades II and III hemorrhoids in an outpatient clinic. Patients are highly satisfied with ALTA sclerotherapy as a treatment for internal hemorrhoids. Our previous study demonstrated that overall patient satisfaction at one month and one year after ALTA sclerotherapy was 97.7%<sup>[13]</sup>. In the Japanese literature, Matsuda *et al*<sup>[14]</sup> reported that the overall satisfaction of ALTA sclerotherapy was over 90% and concluded that ALTA sclerotherapy matched the needs of patients with symptomatic internal hemorrhoids. Minimally invasive treatments such as PPH, THD, or Doppler-guided hemorrhoidal arterial ligation (DGHAL) are acceptable

treatments. But, if recurrence is the main consideration, CH is still considered the best<sup>[15]</sup>. A recent systematic review of 27 randomized controlled trials demonstrated that, compared with CH, PPH had less pain, shorter operative time, and quicker patient's recovery of patient, but a significantly higher rate of prolapse and reintervention for prolapse<sup>[16]</sup>. The recurrence rate and additional operation rate related to each technique were summarized in Table 3. Although the follow-up time was different, the recurrence rate and/or additional operation rate of CH, stapled hemorrhoidopexy, THD, DGHAL with rectoanal repair and ALTA was 0%-2%, 7.5%-13%, 0%-14%, 4%-10.34% and 3.6%-16%, respectively<sup>[4-7,17-24]</sup>. In this study, the one year cumulative rate for the successful resolution of bleeding or prolapse of Grades II and III was 95.9% and 93.1%, respectively. The five year cumulative success rate of ALTA sclerotherapy for Grades II and III hemorrhoids was 89.3% and 78.2%, respectively. ALTA sclerotherapy has had a high success rate for Grades II and III hemorrhoids during the five years post treatment.

Vidal *et al*<sup>[25]</sup> reported a new concept for the treatment of hemorrhoids with arterial embolization. Fourteen patients with disabling chronic rectal bleeding were treated using the emborrhoid technique. Although 10 patients had coagulation disorders (anticoagulants or cirrhosis), coil embolization of the superior rectal arteries is technically feasible, safe and well tolerated. Yano *et al*<sup>[26]</sup> reported that among patients with hemorrhoids receiving anticoagulant, ALTA sclerotherapy was recommended for those in whom it was difficult to discontinue anticoagulant. Miyamoto *et al*<sup>[27]</sup> reported the efficacy and safety of ALTA sclerotherapy for hemorrhoidal patients with liver cirrhosis.

In conclusion, ALTA sclerotherapy is an effective treatment for Grades II or III hemorrhoids. ALTA sclerotherapy might revolutionize the present state of hemo-

hemorrhoid treatment and be the ideal method for symptomatic internal hemorrhoids needed surgery. However, additional studies are needed to evaluate the efficacy of this ALTA sclerotherapy in the management of hemorrhoidal disease.

## COMMENTS

### Background

Aluminum potassium sulfate and tannic acid (ALTA) sclerotherapy is intended to shrink and harden internal hemorrhoids to eliminate hemorrhoidal prolapse and bleeding. ALTA sclerotherapy has been recognized as minimally invasive treatment for symptomatic internal hemorrhoid in Japan. The effectiveness of ALTA sclerotherapy, which shrinks and hardens internal hemorrhoids, is permanent.

### Research frontiers

There are few reports of clarifying long time follow-up after ALTA sclerotherapy. The results of this study contribute to clarifying the effectiveness of ALTA sclerotherapy for Grade II or III hemorrhoids with five-year follow-up.

### Innovations and breakthroughs

In this study, ALTA sclerotherapy is an effective treatment for Grades II or III hemorrhoids. The one-year, three-year and five-year cumulative success rates of ALTA sclerotherapy for Grades II and III hemorrhoids were 95.9% and 93.1%; 89.3% and 83.7%; and 89.3% and 78.2%, respectively. ALTA is an epoch-making sclerosant. ALTA sclerotherapy might be an effective sclerotherapy for symptomatic internal hemorrhoids needed surgery.

### Applications

This study suggests that ALTA sclerotherapy is an effective treatment for Grades II or III hemorrhoids. If a patient is diagnosed with Grade II or III hemorrhoids, ALTA sclerotherapy can be chosen.

### Peer-review

This is an interesting manuscript describing a relatively new minimally invasive treatment of hemorrhoids. The paper has a good number of cases and it is well designed.

## REFERENCES

- 1 Festen S, van Hoogstraten MJ, van Geloven AA, Gerhards MF. Treatment of grade III and IV haemorrhoidal disease with PPH or THD. A randomized trial on postoperative complications and short-term results. *Int J Colorectal Dis* 2009; **24**: 1401-1405 [PMID: 19798507 DOI: 10.1007/s00384-009-0803-2]
- 2 Acheson AG, Scholefield JH. Management of haemorrhoids. *BMJ* 2008; **336**: 380-383 [PMID: 18276714 DOI: 10.1136/bmj.39465.674745.80]
- 3 Dal Monte PP, Tagariello C, Sarago M, Giordano P, Shafi A, Cudazzo E, Franzini M. Transanal haemorrhoidal dearterialisation: nonexcisional surgery for the treatment of haemorrhoidal disease. *Tech Coloproctol* 2007; **11**: 333-338; discussion 338-339 [PMID: 18060529 DOI: 10.1007/s10151-007-0376-4]
- 4 Giordano P, Nastro P, Davies A, Gravante G. Prospective evaluation of stapled haemorrhoidopexy versus transanal haemorrhoidal dearterialisation for stage II and III haemorrhoids: three-year outcomes. *Tech Coloproctol* 2011; **15**: 67-73 [PMID: 21318581 DOI: 10.1007/s10151-010-0667-z]
- 5 Altomare DF, Giuratrabocchetta S. Conservative and surgical treatment of haemorrhoids. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 513-521 [PMID: 23752820 DOI: 10.1038/nrgastro.2013.91]
- 6 Takano M, Iwadare J, Ohba H, Takamura H, Masuda Y, Matsuo K, Kanai T, Ieda H, Hattori Y, Kurata S, Koganezawa S, Hamano K, Tsuchiya S. Sclerosing therapy of internal hemorrhoids with a novel sclerosing agent. Comparison with ligation and excision. *Int J Colorectal Dis* 2006; **21**: 44-51 [PMID: 15843937 DOI: 10.1007/s00384-005-0771-0]
- 7 Miyamoto H, Asanoma M, Miyamoto H, Shimada M. ALTA injection sclerosing therapy: non-excisional treatment of internal hemorrhoids. *Hepatogastroenterology* 2012; **59**: 77-80 [PMID: 22260824 DOI: 10.5754/hge11089]
- 8 Hachiro Y, Kunimoto M, Abe T, Kitada M, Ebisawa Y. Aluminum potassium sulfate and tannic acid (ALTA) injection as the mainstay of treatment for internal hemorrhoids. *Surg Today* 2011; **41**: 806-809 [PMID: 21626327 DOI: 10.1007/s00595-010-4386-x]
- 9 Tokunaga Y, Sasaki H, Saito T. Evaluation of sclerotherapy with a new sclerosing agent and stapled hemorrhoidopexy for prolapsing internal hemorrhoids: retrospective comparison with hemorrhoidectomy. *Dig Surg* 2010; **27**: 469-472 [PMID: 21063123 DOI: 10.1159/000320321]
- 10 Ono T, Goto K, Takagi S, Iwasaki S, Komatsu H. Sclerosing effect of OC-108, a novel agent for hemorrhoids, is associated with granulomatous inflammation induced by aluminum. *J Pharmacol Sci* 2005; **99**: 353-363 [PMID: 16314689 DOI: 10.1254/jphs.FPJ05026X]
- 11 Ono T, Nakagawa H, Fukunari A, Hashimoto T, Komatsu H. Hemostatic action of OC-108, a novel agent for hemorrhoids, is associated with regional blood flow arrest induced by acute inflammation. *J Pharmacol Sci* 2006; **102**: 314-320 [PMID: 17072100 DOI: 10.1254/jphs.FPJ060583]
- 12 Yeo D, Tan KY. Hemorrhoidectomy - making sense of the surgical options. *World J Gastroenterol* 2014; **20**: 16976-16983 [PMID: 25493010 DOI: 10.3748/wjg.v20.i45.16976]
- 13 Miyamoto H. ALTA sclerotherapy: the new sclerotherapy for curing advanced internal hemorrhoids, in *Sclerotherapy: Procedures, Potential Complications and Clinical Outcomes* (editor Brown ER), Nova Science Publishers, Inc. New York, USA, 2014: 149-163
- 14 Matsuda Y, Kawakami K, Nakai K, Asano M, Tanaka S, Nonaka M, Yano T, Ishimaru K, Yano Y, Ishii S, Kimura K. Recent evidence for operation versus non-operation of the anal treatment. *Geka* 2010; **72**: 1515-1520
- 15 Giordano P, Gravante G, Sorge R, Ovens L, Nastro P. Long-term outcomes of stapled hemorrhoidopexy vs conventional hemorrhoidectomy: a meta-analysis of randomized controlled trials. *Arch Surg* 2009; **144**: 266-272 [PMID: 19289667 DOI: 10.1001/archsurg.2008.591]
- 16 Burch J, Epstein D, Sari AB, Weatherly H, Jayne D, Fox D, Woolacott N. Stapled haemorrhoidopexy for the treatment of haemorrhoids: a systematic review. *Colorectal Dis* 2009; **11**: 233-243; discussion 243 [PMID: 18637932 DOI: 10.1111/j.1463-1318.2008.01638.x]
- 17 Giordano P, Tomasi I, Pascariello A, Mills E, Elahi S. Transanal dearterialization with targeted mucopexy is effective for advanced haemorrhoids. *Colorectal Dis* 2014; **16**: 373-376 [PMID: 24460621 DOI: 10.1111/codi.12574]
- 18 Ratto C, Donisi L, Parello A, Litta F, Doglietto GB. Evaluation of transanal hemorrhoidal dearterialization as a minimally invasive therapeutic approach to hemorrhoids. *Dis Colon Rectum* 2010; **53**: 803-811 [PMID: 20389215 DOI: 10.1007/DCR.0b013e3181cdafa7]
- 19 Ratto C, Parello A, Veronese E, Cudazzo E, D'Agostino E, Pagano C, Cavazzoni E, Brugnano L, Litta F. Doppler-guided transanal haemorrhoidal dearterialization for haemorrhoids: results from a multicentre trial. *Colorectal Dis* 2015; **17**: O10-O19 [PMID: 25213152 DOI: 10.1111/codi.12779]
- 20 Zampieri N, Castellani R, Andreoli R, Geccherle A. Long-term results and quality of life in patients treated with hemorrhoidectomy using two different techniques: Ligasure versus transanal hemorrhoidal dearterialization. *Am J Surg* 2012; **204**: 684-688 [PMID: 23140829 DOI: 10.1016/j.amjsurg.2012.01.014]
- 21 Theodoropoulos GE, Sevrisianos N, Papaconstantinou J, Panousopoulos SG, Dardamanis D, Stamopoulos P, Bramis K, Spiliotis J, Datsis A, Leandros E. Doppler-guided haemorrhoidal artery ligation, rectoanal repair, sutured haemorrhoidopexy and minimal mucocutaneous excision for grades III-IV haemorrhoids:



- a multicenter prospective study of safety and efficacy. *Colorectal Dis* 2010; **12**: 125-134 [PMID: 19055522 DOI: 10.1111/j.1463-1318.2008.01739.x]
- 22 **Walega P**, Krokowicz P, Romaniszyn M, Kenig J, Salówka J, Nowakowski M, Herman RM, Nowak W. Doppler guided haemorrhoidal arterial ligation with recto-anal-repair (RAR) for the treatment of advanced haemorrhoidal disease. *Colorectal Dis* 2010; **12**: e326-e329 [PMID: 19674029 DOI: 10.1111/j.1463-1318.2009.02034.x]
  - 23 **Gravié JF**, Lehur PA, Hutten N, Papillon M, Fantoli M, Descottes B, Pessaux P, Arnaud JP. Stapled hemorrhoidopexy versus milligan-morgan hemorrhoidectomy: a prospective, randomized, multicenter trial with 2-year postoperative follow up. *Ann Surg* 2005; **242**: 29-35 [PMID: 15973098 DOI: 10.1097/01.sla.0000169570.64579.31]
  - 24 **Ammaturo C**, Tufano A, Spiniello E, Sodano B, Iervolino EM, Brillantino A, Braccio B. Stapled haemorrhoidopexy vs. Milligan-Morgan haemorrhoidectomy for grade III haemorrhoids: a randomized clinical trial. *G Chir* 2012; **33**: 346-351 [PMID: 23095566]
  - 25 **Vidal V**, Sapoval M, Sielezneff Y, De Parades V, Tradi F, Louis G, Bartoli JM, Pellerin O. Emborrhoid: a new concept for the treatment of hemorrhoids with arterial embolization: the first 14 cases. *Cardiovasc Intervent Radiol* 2015; **38**: 72-78 [PMID: 25366092 DOI: 10.1007/s00270-014-1017-8]
  - 26 **Yano T**, Nogaki T, Asano M, Tanaka S, Kawakami K, Matsuda Y. Outcomes of case-matched injection sclerotherapy with a new agent for hemorrhoids in patients treated with or without blood thinners. *Surg Today* 2013; **43**: 854-858 [PMID: 23052752 DOI: 10.1007/s00595-012-0365-8]
  - 27 **Miyamoto H**, Nakagawa T, Miyamoto H, Takata A. Sclerotherapy Using Aluminum Potassium Sulfate and Tannic Acid (ALTA) for Haemorrhoids in Patients With Liver Cirrhosis. *Ann Colorectal Res* 2015; **3**: e32980 [DOI: 10.19975/acr-32980]

**P- Reviewer:** Milone M, Parellada CM, Tarazov PG  
**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Li D



Clinical Trials Study

# Transjugular intrahepatic portosystemic shunt combined with esophagogastric variceal embolization in the treatment of a large gastroduodenal shunt

Qin Jiang, Ming-Quan Wang, Guo-Bing Zhang, Qiong Wu, Jian-Ming Xu, De-Run Kong

Qin Jiang, Department of Gastroenterology, 161 Hospital of Chinese People's Liberation Army, Wuhan 430000, Hubei Province, China

Qin Jiang, Qiong Wu, Jian-Ming Xu, De-Run Kong, Department of Gastroenterology, First Affiliated Hospital of Anhui Medical University, Hefei 230022, Anhui Province, China

Ming-Quan Wang, Guo-Bing Zhang, Department of Intervention, First Affiliated Hospital of Anhui Medical University, Hefei 230022, Anhui Province, China

**Author contributions:** Jiang Q was involved in analysis and interpretation of data, as well as drafting the manuscript; Jiang Q and Kong DR designed the research; Jiang Q and Wu Q performed the research; Wang MQ, Zhang GB, and Xu JM provided TIPS technical support and were involved in study supervision; Xu JM and Kong DR were involved in study design, analysis and interpretation of data, critical revision of the manuscript, and study supervision.

**Supported by** National Natural Science Foundation of China, Nos. 81070337 and 81271736.

**Institutional review board statement:** The study was approved by the Ethics Committee of Anhui Medical University.

**Informed consent statement:** All involved patients gave their informed written consent prior to study inclusion.

**Conflict-of-interest statement:** We declare that we have no financial or personal relationships with other individuals or organizations that can inappropriately influence our work. We have no professional or personal interests of any nature related to any product, service, and/or company that could be construed as influencing the position presented within, or the review of, the entitled manuscript.

**Data sharing statement:** Technical appendix, statistical code, and dataset are available from the corresponding author at [kdr168@sohu.com](mailto:kdr168@sohu.com). Participants gave informed consent for data sharing.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** De-Run Kong, MD, Department of Gastroenterology, First Affiliated Hospital of Anhui Medical University, Jixi Road 218, Hefei 230022, Anhui Province, China. [kdr168@sohu.com](mailto:kdr168@sohu.com)  
**Telephone:** +86-551-62922039  
**Fax:** +86-551-65120742

**Received:** March 27, 2016  
**Peer-review started:** March 28, 2016  
**First decision:** April 19, 2016  
**Revised:** May 5, 2016  
**Accepted:** May 31, 2016  
**Article in press:** June 2, 2016  
**Published online:** July 18, 2016

## Abstract

**AIM:** To evaluate the efficacy and safety of transjugular intrahepatic portosystemic shunt (TIPS) combined with stomach and esophageal variceal embolization (SEVE) in cirrhotic patients with a large gastroduodenal vessel shunt (GRVS).

**METHODS:** Eighty-one cirrhotic patients with gastric variceal bleeding (GVB) associated with a GRVS were enrolled in the study and accepted TIPS combined with SEVE (TIPS + SEVE), by which portosystemic pressure

gradient (PPG), biochemical, TIPS-related complications, shunt dysfunction, rebleeding, and death were evaluated.

**RESULTS:** The PPGs before TIPS were greater than 12 mmHg in 81 patients. TIPS + SEVE treatment caused a significant decrease in PPG (from  $37.97 \pm 6.36$  mmHg to  $28.15 \pm 6.52$  mmHg,  $t = 19.22$ ,  $P < 0.001$ ). The percentage of reduction in PPG was greater than 20% from baseline. There were no significant differences in albumin, alanine aminotransferase, aspartate aminotransferase, bilirubin, prothrombin time, or Child-Pugh score before and after operation. In all patients, rebleeding rates were 3%, 6%, 12%, 18%, and 18% at 1, 3, 6, 12, and 18 mo, respectively. Five patients (6.2%) were diagnosed as having hepatic encephalopathy. The rates of shunt dysfunction were 0%, 4%, 9%, 26%, and 26%, at 1, 3, 6, 12, and 18 mo, respectively. The cumulative survival rates in 1, 3, 6, 12, and 18 mo were 100%, 100%, 95%, 90%, and 90%, respectively.

**CONCLUSION:** Our preliminary results indicated that the efficacy and safety of TIPS + SEVE were satisfactory in cirrhotic patients with GVB associated with a GRVS (GVB + GRVS).

**Key words:** Transjugular intrahepatic portosystemic shunt; Cirrhosis; Gastric varices; Variceal embolization; Gastroduodenal shunt

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The optimal treatment of gastric variceal bleeding (GVB) + gastroduodenal vessel shunt (GRVS) remains uncertain. Transjugular intrahepatic portosystemic shunt (TIPS) alone cannot be widely used in the treatment of GVB + GRVS. Some studies have evaluated the short-term outcomes of cirrhosis treated with TIPS combined with variceal embolization. In this study, we found that the efficacy and safety of TIPS + stomach and esophageal variceal embolization were satisfactory for patients with GVB + GRVS.

Jiang Q, Wang MQ, Zhang GB, Wu Q, Xu JM, Kong DR. Transjugular intrahepatic portosystemic shunt combined with esophagogastric variceal embolization in the treatment of a large gastroduodenal shunt. *World J Hepatol* 2016; 8(20): 850-857 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i20/850.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i20.850>

## INTRODUCTION

Although the rate of gastric variceal bleeding (GVB) is significantly lower than that of esophageal variceal bleeding (EVV)<sup>[1,2]</sup>, it is usually more severe, requires more transfusions, and is associated with higher mortality than EVV<sup>[1-3]</sup>. Currently, the optimal treatment of GVB re-

mains a difficult issue for clinicians. In terms of recommended therapy for gastric varices, there are various primary options, including surgery, endoscopic variceal obturation with tissue adhesive, Transjugular intrahepatic portosystemic shunt (TIPS) placement, and balloon-occluded retrograde transvenous obliteration<sup>[4,5]</sup>. First-line therapies for gastric varices are endoscopically administered tissue adhesives and TIPS placement.

GVB is often associated with a gastroduodenal vessel shunt (GRVS)<sup>[6]</sup>. The safety of endoscopically-administered tissue adhesives in patients with GVB + GRVS is controversial, due to potential cerebral or pulmonary embolism secondary to migration of cyanoacrylate into the systemic circulation through GRVS<sup>[7]</sup>. TIPS placement has been widely accepted as an effective and safe treatment for GVB in cirrhotic patients<sup>[4,8]</sup>. However, because the portosystemic pressure gradient (PPG) in patient with GVB + GRVS is lower than that in patient with EV, TIPS placement alone is seldom used in the treatment of GVB + GRVS<sup>[9-13]</sup>.

Recent years, some studies have shown that TIPS combined with variceal embolization prevented recurrent variceal bleeding and improved liver function<sup>[14,15]</sup>. However, there are no similar studies to evaluate the effectiveness of a combination of these two methods for patients with GVB + GRVS. The aim of this study was therefore to evaluate the efficacy and safety of TIPS + SEVE for patients with GVB + GRVS.

## MATERIALS AND METHODS

### Patients

Between October 2013 and December 2015, a total of 107 patients in whom TIPS + SEVE had been successfully performed in our hospital were recruited for this study. Inclusion criteria were as follow: (1) age > 18 years; (2) history of cirrhosis and GVB (based on findings of histological or typical cross-sectional imaging, such as ultrasound, endoscopy, computed tomography, or magnetic resonance imaging); and (3) patients was diagnosed as having GRVS by computed tomography angiography (CTA). Exclusion criteria were: (1) hepatocellular carcinoma or other malignancies; (2) chronic renal failure; (3) portal vein thrombosis; (4) infection; and (5) coagulation disorder. Of the 107 patients, 26 with EVB or GVB without GRVS were excluded from this study. Thus, the final population for study consisted of 81 patients. The main clinical and biochemical characteristics of these 81 patients are presented in Table 1. All patients provided their informed written consent. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Anhui Medical University.

### Procedural protocol

Procedures were performed with general anesthesia in the angiography suite. The procedure of TIPS + SEVE has been described previously<sup>[14-16]</sup>. Briefly, before cath-

**Table 1** Characteristics of the 81 patients treated with transjugular intrahepatic portosystemic shunt + stomach and esophageal variceal embolization

No. of patients	81 (%)
Men	63 (77.8)
Female	9 (22.2)
Age (yr)	
Mean $\pm$ SD	50.9 $\pm$ 10.9
Range	25-76
Cause of liver disease	<i>n</i> (%)
Viral	61 (75.4)
Alcoholic	7 (8.7)
Viral and alcoholic	1 (1.2)
Primary biliary cirrhosis	4 (4.9)
Autoimmune hepatitis	1 (1.2)
Cryptogenic	7 (8.6)
Child-Pugh class	<i>n</i> (%)
A	15 (18.5)
B	47 (58.0)
C	19 (23.5)
Endoscopic findings	
IGV1	25 (30.9)
GOV1	10 (12.3)
GOV2	46 (56.8)
Pre-PPG (mmHg)	
Mean $\pm$ SD	38.0 $\pm$ 6.4
Range	26.0-48.0
Follow-up (mo)	
Mean $\pm$ SD	7.87 $\pm$ 5.57
Range	1-18

PPG: Portosystemic pressure gradient.

terization of the hepatic vein was performed through the right internal jugular vein, inferior vena cava pressure was measured when the tip of the catheter floated in the inferior vena cava at the junction with the hepatic vein. A needle and guide-wire were advanced through the liver parenchyma into a branch of the portal vein with fluoroscopic guidance, which was then followed by direct portography and measurement of portal vein pressure. A catheter was passed into the gastroesophageal collateral vessels and embolization of the collateral vessels was initiated, which formed coils of varying diameters and resulted in the disappearance of varices at post-embolization angiography. The catheter was then exited *via* the liver parenchyma. After the parenchymal tract between the hepatic vein and portal vein was dilated with an angioplasty balloon catheter, the patency of the TIPS was facilitated by deployment of a covered stent (8 mm in diameter, BARD E LUMINEXX Vascular Stent, France). The PPG was determined *via* the difference between the portal vein pressure and inferior vena cava pressure. The mid-chest was used as the external zero reference. Pressure tracings must remain stable for at least 30 s to be considered satisfactory. The mean value of two PPG measurements was used for analysis.

All patients received intravenous antibiotic prophylaxis 1 d before the procedure. Intravenous heparin was given as an anti-coagulate during the procedure and for 1 wk post-procedure, which then changed to oral aspirin and warfarin for 1 year. Oral lactulose was used to prevent

hepatic encephalopathy (HE).

### Follow-up

All patients were asked to enroll in the follow-up protocol. PPG, biochemical examination, TIPS-related complications, post-HE, primary patency, rebleeding, and death were recorded respectively. Patients were examined during follow-up with Doppler ultrasound, endoscopy, and CTA at 1, 3, 6, and 12 mo after TIPS placement and then every 6 mo thereafter. Patients suffering from HE, rebleeding, or any other severe complications were invited to our TIPS unit at any time. Liver functions were assessed by testing albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, prothrombin time (PT) levels, and Child-Pugh score at 1 wk before and 1 mo after TIPS. TIPS patency could be assessed by Doppler ultrasonography. Endoscopy confirmed sources of bleeding and variceal disappearance. CTA was used to define the GRVS. Patients were followed until death or liver transplantation, while first rebleeding, first HE, and first shunt insufficiency were followed-up on to a maximum of 2 years after the procedure (closure date: December 31, 2015).

### Definitions

The following definitions were used: (1) rebleeding: Any subsequent hematemesis or melena confirmed endoscopically; (2) HE: Diagnosis of HE was made according to the final report of the 1998 Working Party at the 11<sup>th</sup> World Congress of Gastroenterology in Vienna<sup>[17]</sup>, and patients with clinical evidence of HE were classified according to the West Haven criteria grades: HE  $\geq$  grade I; (3) shunt dysfunction<sup>[18]</sup>: Doppler criteria for shunt insufficiency was that maximal flow velocity was less than 50 cm/s or that there was an absence of flow within the shunt. Suspected shunt dysfunction was confirmed by portography that showed shunt stenosis > 50%; (4) primary patency: The absence of shunt insufficiency without intervention during TIPS surveillance; and (5) endoscopic findings of esophagogastric varices were recorded as proposed by the Japanese Society for portal hypertension<sup>[19]</sup>.

### Statistical analysis

The data were expressed as means  $\pm$  SD. Quantitative variables were compared using Student's *t* test. The rates of primary patency, HE, survival, and variceal rebleeding were analyzed using Kaplan-Meier analyses. A statistically significant difference was assessed for any of the analyses with results of *P* < 0.05. Analyses were performed using the SPSS 10.0 software package.

## RESULTS

### Basic data

Table 2 summarizes the basic clinical and biochemical characteristics of patients. As shown, the PPG before TIPS placement was greater than 12 mmHg in all patients. The mean PPG dropped from 37.97  $\pm$  6.36 mmHg to



**Table 2** Comparison of main biochemical data and porto-systemic pressure gradient before and after the transjugular intrahepatic portosystemic shunt + stomach and esophageal variceal embolization

	Before TIPS	After TIPS	P
Albumin (mg/dL)	32.24 ± 5.88	33.90 ± 7.26	0.199
ALT (u/L)	30.00 ± 17.51	30.85 ± 20.60	0.806
AST (u/L)	38.00 ± 25.95	41.88 ± 24.03	0.318
Bilirubin (mg/dL)	1.41 ± 0.76	1.45 ± 0.65	0.561
PT (%)	52 ± 14	51 ± 15	0.903
Creatinine (mg/dL)	1 ± 0.3	1 ± 0.4	0.58
Child-Pugh score	6.91 ± 1.44	6.79 ± 1.34	0.563
PPG (mmHg)	38.0 ± 6.4	28.2 ± 6.5	< 0.001

PPG: Portosystemic pressure gradient; TIPS: Transjugular intrahepatic portosystemic shunt; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; PT: Prothrombin time.

28.15 ± 6.52 mmHg after TIPS ( $t = 19.22$ ,  $P < 0.001$ ), with reductions in PPG greater than 20% from baseline. There were no significant differences in albumin, ALT, AST, bilirubin, PT, or Child-Pugh score 1 wk before or 1 mo after operation.

### Rebleeding

Rebleeding from the upper gastrointestinal tract occurred in ten patients (12.3%) after TIPS placement. One patient had 4 U of blood transfused within 24 h after the TIPS procedure, with no symptoms of rebleeding observed thereafter. The cumulative rates of rebleeding (Kaplan-Meier estimation) after 1, 3, 6, 12, and 18 mo were 3%, 6%, 12%, 18%, and 18%, respectively. The actual probability of rebleeding is presented in Figure 1. One patient underwent tissue adhesive administration 6 mo after TIPS implantation and is, at the time of writing, alive and free of rebleeding. One patient was found to have portal hypertensive gastropathy, which resulted in rebleeding. The other rebleeding patients were found to have shunt stenosis or obstruction.

### Survival

Five patients died within the follow-up period because of procedure-related complications. In one patient, a shunt obstruction was observed 6 mo after TIPS placement; the patient refused intervention treatment and died seven months after TIPS due to recurrent bleeding. The other four patients died 5 to 12 mo after TIPS placement. The cumulative rates of survival (Kaplan-Meier estimation) after 1, 3, 6, 12, and 18 mo were 100%, 100%, 95%, 90%, and 90%, respectively. Survival curves are shown in Figure 2.

### HE

Five patients experienced HE sometimes before the operation and were also diagnosed as having HE after TIPS placement. A protein-restricted diet and/or lactulose treatment were given to prevent the recurrence of HE. The cumulative rates of HE (Kaplan-Meier estimation) after 1, 3, 6, 12, and 18 mo were 9%, 13%, 18%, 18%,

and 18%, respectively (Figure 3).

### Primary shunt patency

The cumulative rates of primary shunt patency (Kaplan-Meier estimation) after 1, 3, 6, 12, and 18 mo were 100%, 96%, 91%, 74%, and 74%, respectively (Figure 4). During the follow-up period, 10 (12.3%) patients were diagnosed as having shunt stenosis or obstruction, of which 8 patients successfully underwent shunt recanalization with balloon angioplasty. Although one patient with shunt obstruction died 7 mo after TIPS (as previously mentioned), at the time of writing, the remaining patients are alive and well, albeit with one patient who had to receive anticoagulant therapy.

### Other complications

During the follow-up period, the rare complication of hepatic myelopathy (HM) occurred in two patients 6 to 8 mo after the TIPS procedure, which exerted a significant impact on their mobility and quality of life. Due to economic factors, the patients received conservative medical treatment and are, at the time of writing, alive.

## DISCUSSION

The rate of GVB is significantly lower than that of EVB<sup>[1,2]</sup>, but is usually more severe, requires more transfusions, and is associated with higher mortality than EVB<sup>[1-3]</sup>. Currently, the optimal treatment of GVB remains a difficult issue for clinicians.

Variceal embolotherapy has been recognized as an efficient method for preventing bleeding caused by portal hypertension<sup>[19,20]</sup>, while TIPS is used worldwide for the prevention of variceal bleeding<sup>[4,5,8]</sup>. Previous studies have advocated TIPS combined with variceal embolization in the prevention of recurrent variceal bleeding and improvement of liver function<sup>[14,15]</sup>. However, there are no similar studies evaluating the combination of these two methods in patients with GVB + GRVS.

In the current study, we found that the PPG before TIPS placement was greater than 12 mmHg in patients with GVB + GRVS. All included patients had previously experienced at least one instance of bleeding. Ou *et al*<sup>[21]</sup> found that 35% (14/40) of patients with GVB had a PPG ≤ 12 mmHg at the time of TIPS<sup>[22]</sup>. The differing results may be related to the number of cases and the size of spontaneous GRVS in our study, despite previous studies illustrating that PPG appears to correlate inversely with the presence and size of spontaneous GRVS<sup>[6,21]</sup>; to date, there have been no attempts to measure the size of GRVS and the definition of GRVS size remains as yet undetermined.

It has been reported that patients with strong GVB have a lower PPG than those with EV, which may be a result of GRVS development<sup>[6,23]</sup>. Several studies have found that decompressive methods such as TIPS do not seem to confer much of a benefit for GVB + GRVS<sup>[9-13]</sup>. Our results suggest that the rebleeding rate after TIPS was 12% after 1 year, which was similar to the typically

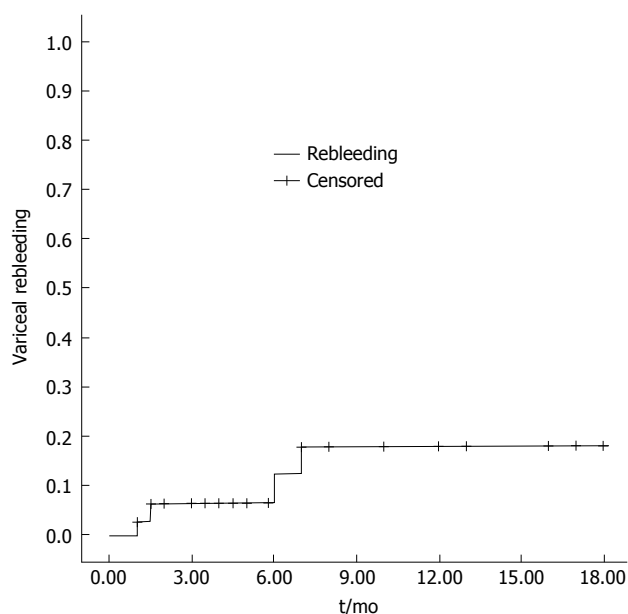


Figure 1 Graph of Kaplan-Meier estimation of cumulative percentages of rebleeding.

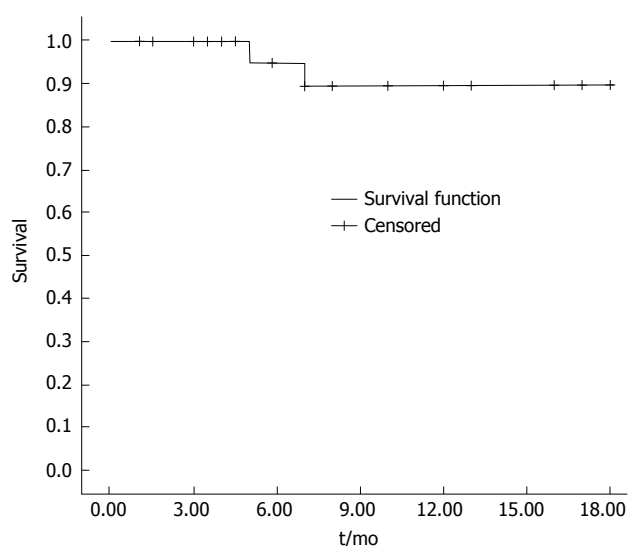


Figure 2 Kaplan-Meier plot shows the rates of survival after transjugular intrahepatic portosystemic shunt placement.

reported result of between 10% to 40%<sup>[24,25]</sup>, while the reduction in PPG was greater than 20% from the baseline. Moreover, we noticed that TIPS + SEVE may reduce the risk of rebleeding. It should be noted that previous studies of TIPS differed from our own in that they used bare stents with TIPS alone placement or did not limit the stent diameter. In our study, all patients underwent decompressive operation and embolotherapy *via* coil, as well as the embolization of extensive collateral circulation (such as that of the short or posterior gastric vein), which may contribute to the occlusion of GRVS. All covered stents were dilated to 8 mm, which may be regarded as limited shunts that accord with natural hemodynamic features.

Survival is usually regarded as the strongest evidence

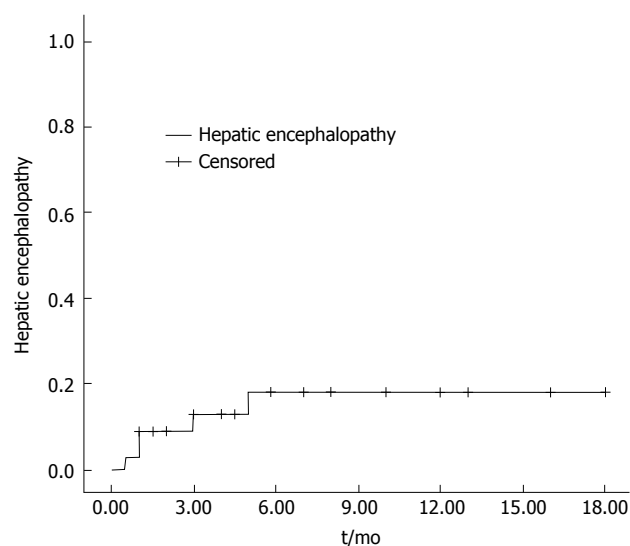


Figure 3 Actuarial probability of hepatic encephalopathy in 81 patients treated with transjugular intrahepatic portosystemic shunt + stomach and esophageal variceal embolization.

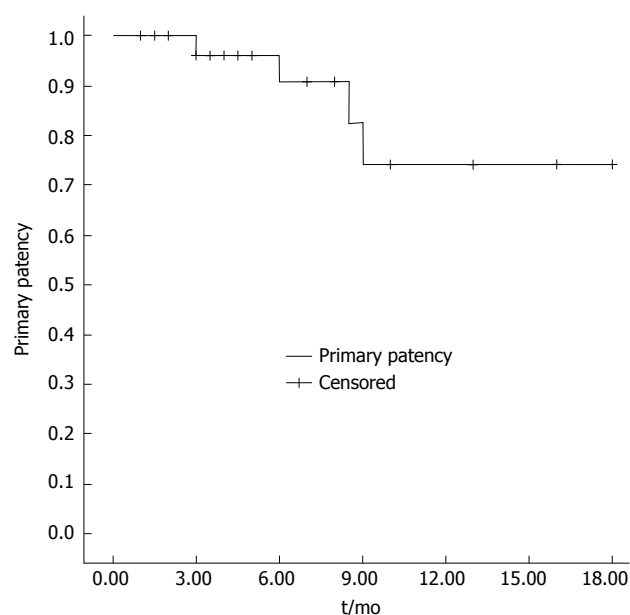


Figure 4 Graph of Kaplan-Meier estimation of cumulative percentages of patients with primary shunt patency in all patients undergoing transjugular intrahepatic portosystemic shunt + stomach and esophageal variceal embolization.

for evaluating the effectiveness of a therapy. In previous studies, total survival 1-year post-TIPS ranged from 58% to 80% and depended mainly on the severity of the underlying liver disease<sup>[25,26]</sup>. The survival rate was 94% at 1 year in our study; such a high rate may be related to the patients' liver function (76.5% patients with Child-Pugh class A or B). Although our results support patients with Child-Pugh class C as well, TIPS placement should be used with extreme caution. Taken together, improving liver function before TIPS may increase the survival rates.

TIPS has been extensively used within the last 20 years. Previous studies showed that TIPS increases the

incidence of HE without improving survival<sup>[27-29]</sup>, which may be the reason why it is currently only recommended as a rescue therapy. HE has been reported to occur in 16%-31% of patients who receive a TIPS in the presence of GVB + GRS<sup>[30]</sup>. Our results indicated that 15% of our patients were diagnosed as having HE after TIPS placement, which is very similar to the reports of other studies, and that only one patient required admission. Importantly, our results were attributed to three effective improvements. First, oral lactulose was used to prevent HE after operation. Second, the left portal vein could be successfully punctured in 58% patients. As we know, the left portal vein receives blood from the splenic vein and inferior mesenteric vein, which have fewer digestive products but more electrolytes. Most recent studies have illustrated that introducing TIPS to the left portal vein instead of the right portal vein could decrease the risk of HE<sup>[31-33]</sup>. Third, 8 mm stents were used in patients. It has been previously reported in the literature that the incidence of portosystemic HE increased with increasing diameter of the stent<sup>[31]</sup>.

It has been shown that occlusion and stenosis are the main disadvantages of TIPS. Studies have demonstrated that stent insufficiency occurs in 14% to 82% of patients by 1 year post-TIPS<sup>[25,33]</sup>. Our findings suggest that 12% of patients in our study were diagnosed as having stenosis or obstruction one year after TIPS; our results therefore showed higher patency rates when compared to historical data. It was reported that the routine administration of anticoagulants and the use of covered stents play important roles in the improved patency rate<sup>[34-36]</sup>. Thus, the higher patency rate of our patients was partially attributed to the use of covered stents and anticoagulant therapy. Other possible reasons for our results are that patients were regularly followed-up on and that TIPS was placed in the left portal vein.

During the follow-up period, two patients were diagnosed with HM, in which the spontaneous shunt found by CTA was not completely closed. Embolization only with coils may be an insufficient embolization factor that was thought to be secondary to the increased systemic circulation of shunting portal venous toxins from the hypoperfusion and ischemia of the hepatocytes. Studies showed that a liver transplant could fully reverse the effects of HM in patients with early stage disease<sup>[37,38]</sup>, however, due to economic factors, patients only received conservative medical treatment. Despite previous studies advocating TIPS combined with variceal embolization to improve liver function<sup>[15,39]</sup>, there were no significant differences in liver functions before and after TIPS placement in our study.

In spite of these results, we may conclude that PPG before TIPS placement may be greater than 12 mmHg in patients with GVB + GRVS, and that the efficacy and safety of TIPS + SEVE were satisfactory in these patients.

## ACKNOWLEDGMENTS

The authors thank all patients involved in this study. We

would also like to thank Professor Keyang Chen, from the Temple University School of Medicine, for language assistance.

## COMMENTS

### Background

The optimal treatment for gastric variceal bleeding (GVB) + gastroduodenal vessel shunt (GRVS) is still controversial. Transjugular intrahepatic portosystemic shunt (TIPS) alone cannot be widely used in the treatment for GVB + GRVS. Previous studies have advocated TIPS combined with variceal embolization in order to prevent recurrent variceal bleeding and improve liver function. However, the efficacy and safety of TIPS + stomach and esophageal variceal embolization (SEVE) in patients with GVB + GRVS was unclear.

### Research frontiers

In recent years, more and more patients have undergone TIPS procedure to prevent variceal bleeding. For the use of the TIPS procedure, the research hot spot is how to increase the patient survival rate and reduce complications by bettering the patient selection and improving techniques. Interestingly, TIPS + SEVE may decrease portal pressure and embolize extensive collateral circulation, thereby potentially reducing the risk of rebleeding.

### Innovations and breakthroughs

Most GVB is associated with a GRVS. The efficacy of tissue adhesives in patients with GVB + GRVS is controversial, due to the potential for systemic embolism secondary to migration of cyanoacrylate into the systemic circulation through a GRVS. TIPS alone cannot be widely used in the treatment of GVB + GRVS. In the study, all patients underwent TIPS + SEVE with via coil, with extensive collateral circulation, such as short or posterior gastric vein, potentially contributing to the occlusion of GRVS. In this study, the authors found that the efficacy and safety of TIPS + SEVE were satisfactory in patients with GVB + GRVS.

### Applications

The results suggest that the efficacy and safety of TIPS + SEVE were satisfactory in patients with GVB + GRVS. Additional studies with long-term follow-up are needed to confirm the results.

### Peer-review

The authors have provided a well-designed study that shows the satisfactory efficacy and safety of combination TIPS + SEVE in cirrhotic patients with gastric variceal bleeding associated with a gastroduodenal vessel shunt.

## REFERENCES

- 1 **Sarin SK**, Lahoti D, Saxena SP, Murthy NS, Makwana UK. Prevalence, classification and natural history of gastric varices: a long-term follow-up study in 568 portal hypertension patients. *Hepatology* 1992; **16**: 1343-1349 [PMID: 1446890 DOI: 10.1002/hep.1840160607]
- 2 **Kim T**, Shijo H, Kokawa H, Tokumitsu H, Kubara K, Ota K, Akiyoshi N, Iida T, Yokoyama M, Okumura M. Risk factors for hemorrhage from gastric fundal varices. *Hepatology* 1997; **25**: 307-312 [PMID: 9021939 DOI: 10.1002/hep.510250209]
- 3 **Thakeb F**, Salem SA, Abdallah M, el Batanouny M. Endoscopic diagnosis of gastric varices. *Endoscopy* 1994; **26**: 287-291 [PMID: 8076547 DOI: 10.1055/s-2007-1008969]
- 4 **Boyer TD**, Haskal ZJ. The Role of Transjugular Intrahepatic Portosystemic Shunt (TIPS) in the Management of Portal Hypertension: update 2009. *Hepatology* 2010; **51**: 306 [PMID: 19902484 DOI: 10.1002/hep.23383]
- 5 **de Franchis R**. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2010; **53**: 762-768 [PMID: 20638742 DOI: 10.1016/j.jhep.2010.06.004]
- 6 **Watanabe K**, Kimura K, Matsutani S, Ohto M, Okuda K. Portal hemodynamics in patients with gastric varices. A study in 230

- patients with esophageal and/or gastric varices using portal vein catheterization. *Gastroenterology* 1988; **95**: 434-440 [PMID: 3391371]
- 7 **Irisawa A**, Obara K, Sato Y, Saito A, Orikasa H, Ohira H, Sakamoto H, Sasajima T, Rai T, Odajima H, Abe M, Kasukawa R. Adherence of cyanoacrylate which leaked from gastric varices to the left renal vein during endoscopic injection sclerotherapy: a histopathologic study. *Endoscopy* 2000; **32**: 804-806 [PMID: 11068842 DOI: 10.1055/s-2000-7702]
  - 8 **García-Pagán JC**, Caca K, Bureau C, Laleman W, Appenrodt B, Luca A, Abalde JG, Nevens F, Vinel JP, Mössner J, Bosch J. Early use of TIPS in patients with cirrhosis and variceal bleeding. *N Engl J Med* 2010; **362**: 2370-2379 [PMID: 20573925 DOI: 10.1056/NEJMoa0910102]
  - 9 **Caldwell S**. Gastric varices: is there a role for endoscopic cyanoacrylates, or are we entering the BRTO era? *Am J Gastroenterol* 2012; **107**: 1784-1790 [PMID: 23211846 DOI: 10.1038/ajg.2012.160]
  - 10 **Matsumoto A**, Matsushita M, Sugano Y, Takimoto K, Yasuda M, Inokuchi H. Limitations of transjugular intrahepatic portosystemic shunt for management of gastric varices. *Gastroenterology* 2004; **126**: 380-381 [PMID: 14753222 DOI: 10.1053/j.gastro.2003.07.021]
  - 11 **Ryan BM**, Stockbrugger RW, Ryan JM. TIPS for gastric varices. *Gut* 2003; **52**: 772; author reply 772 [PMID: 12692074 DOI: 10.1136/gut.52.5.772]
  - 12 **Sanyal AJ**, Freedman AM, Luketic VA, Purdum PP, Shiffman ML, DeMeo J, Cole PE, Tisnado J. The natural history of portal hypertension after transjugular intrahepatic portosystemic shunts. *Gastroenterology* 1997; **112**: 889-898 [PMID: 9041251 DOI: 10.1053/gast.1997.v112.pm9041251]
  - 13 **Choi YH**, Yoon CJ, Park JH, Chung JW, Kwon JW, Choi GM. Balloon-occluded retrograde transvenous obliteration for gastric variceal bleeding: its feasibility compared with transjugular intrahepatic portosystemic shunt. *Korean J Radiol* 2003; **4**: 109-116 [PMID: 12845306 DOI: 10.3348/kjr.2003.4.2.109]
  - 14 **Tesdal IK**, Filser T, Weiss C, Holm E, Dueber C, Jaschke W. Transjugular intrahepatic portosystemic shunts: adjunctive embolotherapy of gastroesophageal collateral vessels in the prevention of variceal rebleeding. *Radiology* 2005; **236**: 360-367 [PMID: 15955858 DOI: 10.1148/radiol.2361040530]
  - 15 **Chen S**, Li X, Wei B, Tong H, Zhang MG, Huang ZY, Cao JW, Tang CW. Recurrent variceal bleeding and shunt patency: prospective randomized controlled trial of transjugular intrahepatic portosystemic shunt alone or combined with coronary vein embolization. *Radiology* 2013; **268**: 900-906 [PMID: 23657891 DOI: 10.1148/radiol.13120800]
  - 16 **Rössle M**, Haag K, Ochs A, Sellinger M, Nöldge G, Perarnau JM, Berger E, Blum U, Gabelmann A, Hauenstein K. The transjugular intrahepatic portosystemic stent-shunt procedure for variceal bleeding. *N Engl J Med* 1994; **330**: 165-171 [PMID: 8264738 DOI: 10.1056/NEJM199401203300303]
  - 17 **Ferenci P**, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy--definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002; **35**: 716-721 [PMID: 11870389 DOI: 10.1053/jhep.2002.31250]
  - 18 **Han G**, Qi X, He C, Yin Z, Wang J, Xia J, Yang Z, Bai M, Meng X, Niu J, Wu K, Fan D. Transjugular intrahepatic portosystemic shunt for portal vein thrombosis with symptomatic portal hypertension in liver cirrhosis. *J Hepatol* 2011; **54**: 78-88 [PMID: 20932597 DOI: 10.1016/j.jhep.2010.06.029]
  - 19 **Tajiri T**, Yoshida H, Obara K, Onji M, Kage M, Kitano S, Kokudo N, Kokubu S, Sakaida I, Sata M, Tajiri H, Tsukada K, Nonami T, Hashizume M, Hirota S, Murashima N, Moriyasu F, Saigenji K, Makuuchi H, Oho K, Yoshida T, Suzuki H, Hasumi A, Okita K, Futagawa S, Idezuki Y. General rules for recording endoscopic findings of esophagogastric varices (2nd edition). *Dig Endosc* 2010; **22**: 1-9 [PMID: 20078657 DOI: 10.1111/j.1443-1661]
  - 20 **Kwok AC**, Wang F, Maher R, Harrington T, Gananadha S, Hugh TJ, Samra JS. The role of minimally invasive percutaneous embolisation technique in the management of bleeding stomal varices. *J Gastrointest Surg* 2013; **17**: 1327-1330 [PMID: 23546560 DOI: 10.1007/s11605-013-2180-y]
  - 21 **Ou HY**, Huang TL, Chen TY, Tsang LL, Concejero AM, Chen CL, Cheng YF. Emergency splenic arterial embolization for massive variceal bleeding in liver recipient with left-sided portal hypertension. *Liver Transpl* 2005; **11**: 1136-1139 [PMID: 16123955 DOI: 10.1002/lt.20543]
  - 22 **Tripathi D**, Therapondos G, Jackson E, Redhead DN, Hayes PC. The role of the transjugular intrahepatic portosystemic stent shunt (TIPSS) in the management of bleeding gastric varices: clinical and haemodynamic correlations. *Gut* 2002; **51**: 270-274 [PMID: 12117893 DOI: 10.1136/gut.51.2.270]
  - 23 **Ohnishi K**, Nakayama T, Koen H, Saito M, Saito M, Chin N, Terabayashi H, Iida S, Nomura F, Okuda K. Interrelationship between type of spontaneous portal systemic shunt and portal vein pressure in patients with liver disease. *Am J Gastroenterol* 1985; **80**: 561-564 [PMID: 4014107]
  - 24 **Chao Y**, Lin HC, Lee FY, Wang SS, Tsai YT, Hsia HC, Lin WJ, Lee SD, Lo KJ. Hepatic hemodynamic features in patients with esophageal or gastric varices. *J Hepatol* 1993; **19**: 85-89 [PMID: 8301048 DOI: 10.1016/S0168-8278(05)80180-1]
  - 25 **García-Pagán JC**, Barrufet M, Cardenas A, Escorsell A. Management of gastric varices. *Clin Gastroenterol Hepatol* 2014; **12**: 919-928.e1; quiz e51-e52 [PMID: 23899955 DOI: 10.1016/j.cgh.2013.07.015]
  - 26 **Ryan BM**, Stockbrugger RW, Ryan JM. A pathophysiologic, gastroenterologic, and radiologic approach to the management of gastric varices. *Gastroenterology* 2004; **126**: 1175-1189 [PMID: 15057756 DOI: 10.1053/j.gastro.2004.01.058]
  - 27 **Berry K**, Lerrigo R, Liou IW, Ioannou GN. Association between Transjugular Intrahepatic Portosystemic Shunt and Survival in Patients with Cirrhosis. *Clin Gastroenterol Hepatol* 2016; **14**: 118-123 [PMID: 26192147 DOI: 10.1016/j.cgh.2015.06.042]
  - 28 **Papathodoridis GV**, Goulis J, Leandro G, Patch D, Burroughs AK. Transjugular intrahepatic portosystemic shunt compared with endoscopic treatment for prevention of variceal rebleeding: A meta-analysis. *Hepatology* 1999; **30**: 612-622 [PMID: 10462365 DOI: 10.1002/hep.510300316]
  - 29 **Khan S**, Tudur Smith C, Williamson P, Sutton R. Portosystemic shunts versus endoscopic therapy for variceal rebleeding in patients with cirrhosis. *Cochrane Database Syst Rev* 2006; **(4)**: CD000553 [PMID: 17054131]
  - 30 **Escorsell A**, Bañares R, García-Pagán JC, Gilabert R, Moitinho E, Piqueras B, Bru C, Echenagusia A, Granados A, Bosch J. TIPS versus drug therapy in preventing variceal rebleeding in advanced cirrhosis: a randomized controlled trial. *Hepatology* 2002; **35**: 385-392 [PMID: 11826413 DOI: 10.1053/jhep.2002.30418]
  - 31 **Sabri SS**, Abi-Jaoudeh N, Swee W, Saad WE, Turba UC, Caldwell SH, Angle JF, Matsumoto AH. Short-term rebleeding rates for isolated gastric varices managed by transjugular intrahepatic portosystemic shunt versus balloon-occluded retrograde transvenous obliteration. *J Vasc Interv Radiol* 2014; **25**: 355-361 [PMID: 24468043 DOI: 10.1016/j.jvir.2013.12.001]
  - 32 **Xue H**, Yuan J, Chao-Li Y, Palikhe M, Wang J, Shan-Lv L, Qiao W. Follow-up study of transjugular intrahepatic portosystemic shunt in the treatment of portal hypertension. *Dig Dis Sci* 2011; **56**: 3350-3356 [PMID: 21643741 DOI: 10.1007/s10620-011-1744-5]
  - 33 **Chen L**, Xiao T, Chen W, Long Q, Li R, Fang D, Wang R. Outcomes of transjugular intrahepatic portosystemic shunt through the left branch vs. the right branch of the portal vein in advanced cirrhosis: a randomized trial. *Liver Int* 2009; **29**: 1101-1109 [PMID: 19386025 DOI: 10.1111/j.1478-3231.2009.02016.x]
  - 34 **Bai M**, He CY, Qi XS, Yin ZX, Wang JH, Guo WG, Niu J, Xia JL, Zhang ZL, Larson AC, Wu KC, Fan DM, Han GH. Shunting branch of portal vein and stent position predict survival after transjugular intrahepatic portosystemic shunt. *World J Gastroenterol* 2014; **20**: 774-785 [PMID: 24574750 DOI: 10.3748/wjg.v20.i3.774]



- 35 **Bureau C**, Garcia-Pagan JC, Otal P, Pomier-Layrargues G, Chabbert V, Cortez C, Perreault P, Péron JM, Abralles JG, Bouchard L, Bilbao JJ, Bosch J, Rousseau H, Vinel JP. Improved clinical outcome using polytetrafluoroethylene-coated stents for TIPS: results of a randomized study. *Gastroenterology* 2004; **126**: 469-475 [PMID: 14762784 DOI: 10.1053/j.gastro.2003.11.016]
- 36 **Yang Z**, Han G, Wu Q, Ye X, Jin Z, Yin Z, Qi X, Bai M, Wu K, Fan D. Patency and clinical outcomes of transjugular intrahepatic portosystemic shunt with polytetrafluoroethylene-covered stents versus bare stents: a meta-analysis. *J Gastroenterol Hepatol* 2010; **25**: 1718-1725 [PMID: 21039832 DOI: 10.1111/j.1440-1746.2010.06400.x]
- 37 **Sauer P**, Theilmann L, Herrmann S, Bruckner T, Roeren T, Richter G, Stremmel W, Stiehl A. Phenprocoumon for prevention of shunt occlusion after transjugular intrahepatic portosystemic stent shunt: a randomized trial. *Hepatology* 1996; **24**: 1433-1436 [PMID: 8938176 DOI: 10.1002/hep.510240622]
- 38 **Baccarani U**, Zola E, Adani GL, Cavalletti M, Schiff S, Cagnin A, Poci C, Merkel C, Amodio P, Montagnese S. Reversal of hepatic myelopathy after liver transplantation: fifteen plus one. *Liver Transpl* 2010; **16**: 1336-1337 [PMID: 21031552 DOI: 10.1002/lt.22149]
- 39 **Weissenborn K**, Tietge UJ, Bokemeyer M, Mohammadi B, Bode U, Manns MP, Caselitz M. Liver transplantation improves hepatic myelopathy: evidence by three cases. *Gastroenterology* 2003; **124**: 346-351 [PMID: 12557140 DOI: 10.1053/gast.2003.50062]

**P- Reviewer:** Garbuzenko DV, Nakamura S **S- Editor:** Ji FF  
**L- Editor:** Rutherford A **E- Editor:** Li D



## Hepatitis C virus cures after direct acting antiviral-related drug-induced liver injury: Case report

Yaakov Hasin, Shimon Shteingart, Harel Dahari, Inna Gafanovich, Sharon Floru, Marius Braun, Amir Shlomai, Anthony Verstandig, Ilana Dery, Susan L Uprichard, Scott J Cotler, Yoav Lurie

Yaakov Hasin, Shimon Shteingart, Inna Gafanovich, Ilana Dery, Yoav Lurie, Liver Unit, Digestive Disease Institute, Shaare Zedek Medical Center, Jerusalem 9103102, Israel

Harel Dahari, Susan L Uprichard, Scott J Cotler, the Program for Experimental and Theoretical Modeling, Division of Hepatology, Department of Medicine, Loyola University Medical Center, Maywood, IL 60153, United States

Sharon Floru, "Bat Yamon" Internal Medicine Day Care, Clalit Health Care Organization, Bat Yam 5962025, Israel

Marius Braun, Amir Shlomai, Liver Institute, Rabin Medical Center, Beilinson Hospital, Petah-Tiqwa, Affiliated with the Sackler Faculty of Medicine, Tel Aviv 4941492, Israel

Anthony Verstandig, Invasive Radiology Unit, Shaare Zedek Medical Center, Jerusalem 9103102, Israel

**Author contributions:** All authors contributed to the acquisition of data, writing, and revision of this manuscript.

Supported by NIH, No. R01-AI078881.

**Institutional review board statement:** This case report was exempt from the Institutional Review Board standards and from informed consent by the institutional review board in "Shaare Zedek" medical center.

**Informed consent statement:** This case report was exempt from providing informed consent by the institutional review board in "Shaare Zedek" medical center.

**Conflict-of-interest statement:** All the authors have no conflicts of interests to declare.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

licenses/by-nc/4.0/

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Dr. Yoav Lurie, Liver Unit, Digestive Disease Institute, Shaare Zedek Medical Center, Shmu'el Bait St 12, Jerusalem 9103102, Israel. [yoavtalitami@gmail.com](mailto:yoavtalitami@gmail.com)  
 Telephone: +972-2-6555035  
 Fax: +972-2-6555359

**Received:** February 28, 2016

**Peer-review started:** February 29, 2016

**First decision:** April 15, 2016

**Revised:** June 1, 2016

**Accepted:** June 27, 2016

**Article in press:** June 29, 2016

**Published online:** July 18, 2016

### Abstract

The United States Food and Drug Administration recently warned that the direct acting antiviral (DAA) combination hepatitis C virus (HCV) treatment of Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir, and Ribavirin (PODr + R) can cause severe liver injury in patients with advanced liver disease. Drug induced liver injury was observed in a small number of patients with decompensated cirrhosis treated with other DAAs, but has not been reported in patients with compensated cirrhosis. We report a case of a 74-year-old woman with chronic HCV and Child-Pugh class A cirrhosis (compensated cirrhosis) treated with PODr + R. The patient presented on day 14 of PODr + R therapy with jaundice and new-onset ascites. Her total bilirubin level increased to 23 mg/dL and international normalized ratio rose to 1.65, while aminotransferase levels remained relatively stable. Hepatitis C treatment was discontinued on day 24 and she gradually recovered. Follow-up testing showed that she achieved a sustained virologic response. In conclusion, hepatic decompensation developed within two weeks of starting treatment with

PODr + R in a patient with Child-Pugh class A cirrhosis and was characterized by jaundice and ascites with stable aminotransferase levels. Careful monitoring is warranted in patients with HCV-related cirrhosis treated with PODr + R.

**Key words:** Direct antiviral agent; Drug-induced liver injury; Hepatitis C; Mathematical modeling; Sustained virological response; Viral kinetics

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** To the best of our knowledge, this is the first report of hepatic decompensation in a hepatitis C patient with Child Pugh class A cirrhosis due to treatment with Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir and Ribavirin (PODr + R). Liver aminotransferase levels did not increase prior to decompensation, depriving us of our usual alarm signs heralding hepatic decompensation. The patient achieved sustained virologic response despite very early discontinuation of therapy (day 24).

Hasin Y, Shteingart S, Dahari H, Gafanovich I, Floru S, Braun M, Shlomai A, Verstandig A, Dery I, Uprichard SL, Cotler SJ, Lurie Y. Hepatitis C virus cures after direct acting antiviral-related drug-induced liver injury: Case report. *World J Hepatol* 2016; 8(20): 858-862 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i20/858.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i20.858>

## INTRODUCTION

Globally, it was estimated that in 2005, more than 185 million people were anti-hepatitis C virus (HCV) seropositive (prevalence 2.8%)<sup>[1]</sup>. End-stage liver disease due to HCV is a leading indication for liver transplantation and accounts for almost 500000 deaths annually<sup>[2]</sup>. The growing proportion of patients with chronic HCV infection and cirrhosis has the highest priority for treatment, but are at risk for complications of liver disease<sup>[3]</sup>.

The interferon-free, 12-wk Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir and Ribavirin (PODr + R) regimen is approved for the treatment of patients with Child's-Pugh class A cirrhosis and was shown to achieve a sustained virologic response (SVR) rate exceeding 90% in such patients<sup>[4]</sup>. However, on 10/22/15, an Food and Drug Administration (FDA) Drug Safety Communication warned that PODr + R can cause severe drug-induced liver injury (DILI) in patients with advanced liver disease<sup>[5]</sup>. Since December 2014, 26 cases of severe liver injury in patients with advanced liver disease were reported to the FDA Adverse Event Reporting System database that were considered probably or possibly related to PODr + R. Ten patients developed liver failure resulting in death or liver transplantation. The pattern of liver injury consisted of an acute increase in bilirubin level without alanine aminotransferase (ALT) elevations. The FDA

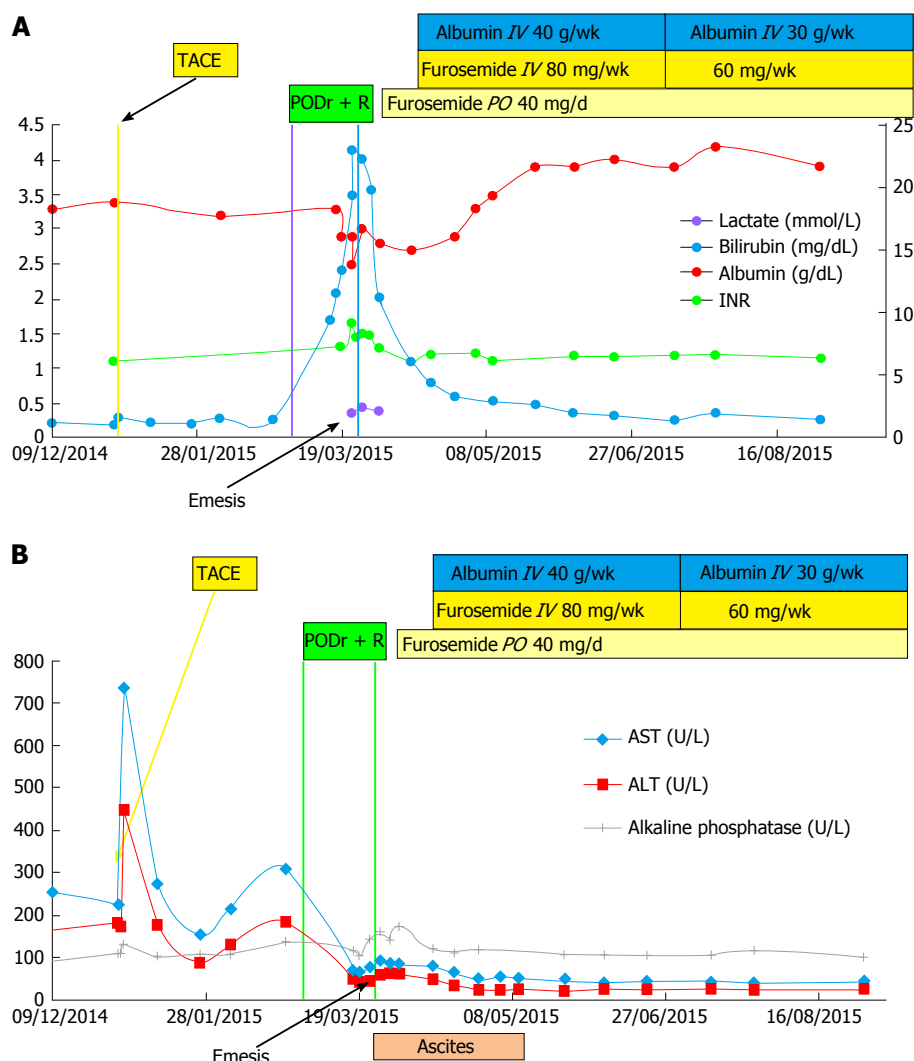
Drug Safety Communication emphasized that PODr + R is contraindicated in patients with Child's class B and C cirrhosis. More recently, four cases of severe DILI were identified in patients who had decompensated cirrhosis at pre-treatment baseline who were treated with sofosbuvir and a NS5A inhibitor, with or without Ribavirin<sup>[6-8]</sup>. Three of the patients were human immunodeficiency virus-co-infected and were receiving anti-retroviral therapy. To date, severe DILI has not been reported in patients with compensated cirrhosis who receive direct acting antiviral (DAA).

## CASE REPORT

A 74-year-old woman presented to Shaare Zedek Medical Center in Jerusalem on 12/30/14 for management of HCV genotype 1b infection with compensated cirrhosis (Child's Pugh class A-score 6, MELD score 7), and a 5.6 cm × 6.8 cm right lobe liver lesion with diagnostic features of hepatocellular carcinoma (HCC) on ultrasonography and contrast-enhanced computed tomography. Her baseline laboratory data are shown in Figure 1.

On 1/1/15 she underwent transcatheter arterial chemoembolization (TACE) as treatment for HCC. Her ALT and aspartate transaminase levels increased transiently and then declined to baseline (Figure 1B). She showed no signs of hepatic decompensation before or after the procedure. Treatment with Ombitasvir, Paritaprevir, Ritonavir (2 tablets daily), Dasabuvir (250 mg twice daily, and Ribavirin (400 mg, twice daily) was initiated on 3/1/15, two months after TACE. The HCV RNA level declined from a pre-treatment baseline of 2000000 IU/mL to 474 IU/mL in 7 d (Figure 2). However, on day 14 of treatment, she presented with nausea and increased abdominal girth. Her medications were limited to PODr + R and Ramipril for hypertension, which she had taken for four months. She reported taking no other prescription, over the counter, or alternative medications during the four months prior to the onset of liver injury. She did not drink alcohol. On physical examination, she had icteric sclera and moderate ascites. She developed vomiting and anorexia. Serial laboratories showed a rising total bilirubin (maximum 23 mg/dL) and international normalized ratio (maximum 1.65) levels and her albumin level decreased to 2.5 g/dL (Figure 1A). Of note, her aminotransferase levels remained stable (Figure 1B). An abdominal ultrasound with Doppler study showed flow in the portal and hepatic veins and no new mass lesions in the liver.

Antiviral therapy was discontinued on treatment day 24 (3/24/15) for suspected DILI. The patient was hospitalized on 4/1/15 for management of ascites. After hospital discharge, she was treated with weekly intravenous albumin and Furosemide until November 2015. Her ascites slowly improved and her liver biochemistries normalized (Figure 1). Seven months after discontinuation of antiviral therapy, she has recovered and her HCV RNA remains undetectable (Figure 2). She



**Figure 1** Change in laboratories in relation to antiviral therapy (A) and in aminotransferase and Alkaline Phosphate levels over time (B). A: Change in laboratories in relation to antiviral therapy. Shortly after initiation of hepatitis C treatment, and after 4 mo of stable levels, laboratory values deteriorated indicating a decline in hepatic synthetic function; peak bilirubin - 23 mg/dL, PT/INR - 1.65, lactate-2.44 mg/dL, lowest albumin - 2.5 g/L. Shortly after treatment discontinuation, laboratory values slowly returned to baseline; B: Change in aminotransferase and alkaline phosphate levels over time. There was a spike in aminotransferase levels after the TACE procedure. Following HCV treatment initiation, aminotransferase levels declined over time, reaching plateau levels of AST = 40 U/L and ALT = 25 U/L. Aminotransferase levels remained stable when total bilirubin and PT/INR levels increased (shown in Figure 1A above). AST: Aspartate transaminase; ALT: Alanine aminotransferase; TACE: Transcatheter arterial chemoembolization; INR: International normalized ratio; PODr + R: Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir, and Ribavirin; IV: Intravenous; PO: By mouth.

has no evidence of viable HCC by magnetic resonance imaging.

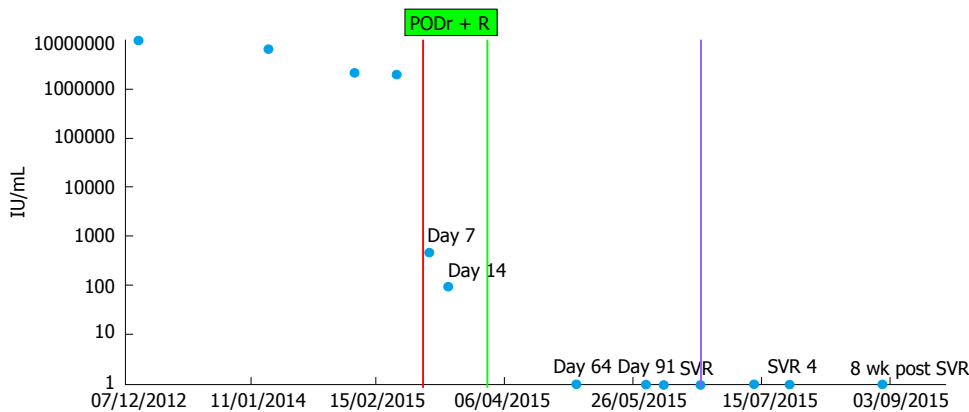
The time to reach cure, or SVR, was previously defined as the time to reach less than one hepatitis C virion in the extracellular fluid volume (approximately  $13.5\text{--}15\text{ L}$ )<sup>[9–12]</sup>. Thus a value of approximately  $3 \times 10^5$  IU/mL is the threshold for viral clearance (termed here cure boundary). The fact that the patient achieved SVR despite a very short course of therapy (24 d) was striking since her viral load level 10 d before DAA therapy stopped (3/15/15) was 97 IU/mL, which is several log IU/mL higher than the cure boundary. To estimate, retrospectively, when the patient reached cure we used the standard biphasic HCV treatment model<sup>[11,12]</sup> and the multiscale HCV treatment model<sup>[13,14]</sup>. Both these models predicted that cure occurred 3 to 6 wk after therapy was stopped (not shown).

## DISCUSSION

Herein we detail hepatic decompensation in a patient treated with PODr + R. The patient had Child's-Pugh class A cirrhosis prior to initiation of treatment and presented with jaundice and ascites 14 d after DAA therapy was started. Treatment was discontinued on day 24. She had a prolonged recovery and gradually returned to her baseline condition. She achieved SVR despite a truncated course of therapy. No causes of liver injury other than DAA were identified in the present case and the available data are consistent with the minimal elements for reporting DILI<sup>[15]</sup>.

The patient's presentation is consistent with the pattern of PODr + R-related liver injury reported by the FDA with an early onset of hyperbilirubinemia without a rise in ALT level<sup>[5]</sup>. Similarly, two cases of severe liver





**Figure 2** Hepatitis C virus RNA measurements over time. HCV RNA levels (circles) declined rapidly during treatment reaching not detected levels by the first measurement after treatment discontinuation and the patient achieved a sustained virologic response (SVR) defined as no detectable viral RNA 12 wk post treatment (purple line). HCV: Hepatitis C virus; PODr + R: Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir, and Ribavirin.

injury in patients with decompensated cirrhosis treated with sofosbuvir and a NS5A inhibitor were characterized by a rising bilirubin level with stable ALT levels<sup>[6]</sup>. Aminotransferase levels were not reported in the other two sofosbuvir/NS5A inhibitor DILI cases<sup>[7,8]</sup>. It is critical that clinicians be aware that aminotransferase levels do not tend to rise prior to, or in parallel with bilirubin levels in patients with DAA-related DILI, as failure to recognize this pattern could delay recognition of severe liver injury and discontinuation of therapy.

The most novel and important feature of the present case is that the patient had well compensated liver disease before starting treatment with a Child Pugh score of 6 and a MELD score of 7. In contrast, the FDA Drug Safety Communication emphasized that PODr + R is contraindicated in patients with Child's Pugh class B and C cirrhosis. The current case provides evidence that patients with Child's Pugh class A cirrhosis are at risk for severe DILI with PODr + R.

Of interest, HCV was eradicated with only 24 d of treatment. Both the standard biphasic and the multiscale models of HCV kinetics during therapy suggest that cure might have occurred between 3 to 6 wk after therapy was stopped. While it is not feasible that the drugs had a prolonged direct antiviral effect, in theory the ongoing liver injury (*i.e.*, cell loss) and/or resulting inflammation might have exerted an immune mediated antiviral effect. Alternatively, cure might have occurred by the end of DAA therapy if the DAAs affected the ratio between non-infectious and infectious viral particles (*i.e.*, viral infectivity) as previously observed during HCV DAA treatment in cell culture<sup>[16]</sup>. That is, DAAs may reduce the infectivity of the virus particles produced such that only a small fraction of the viral RNA detected 10 d before drug cessation was infectious. This alternative explanation is also consistent with reports in which some patients treated with DAAs were documented to achieve SVR despite having detectable HCV RNA at end of treatment<sup>[17,18]</sup>. Further experimental and modeling efforts are needed to provide insights into the biological and/or immunological aspects that gave rise to HCV cure

after such short durations of DAA therapy<sup>[19]</sup>.

Our study has several limitations. Blood samples during therapy are not available for further measurements to test for superimposed acute viral infections such as hepatitis A or B or to measure paritaprevir concentration at the time of the rise in bilirubin. However, the temporal relation between PODr + R therapy and hepatic decompensation and recovery following discontinuation of therapy provides strong evidence for DILI.

Careful laboratory and clinical monitoring, beginning early in the course of therapy, is prudent in patients with compensated cirrhosis treated with PODr + R.

## COMMENTS

### Case characteristics

Patient suffered from jaundice, nausea and vomiting 14 d following treatment with Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir, and Ribavirin (PODr + R).

### Clinical diagnosis

Aminotransferase levels did not increase. Despite that, Nausea, Vomiting, Ascites, Jaundice, hypoalbuminemia and coagulopathy occurred.

### Differential diagnosis

Ribavirin induced hemolysis, possible drug induced liver injury, recurrence of hepatocellular carcinoma (HCC), or vascular causes for hepatic failure. The authors performed liver ultrasonography to exclude Recurrence of HCC and vascular causes. Blood tests to exclude hemolysis as cause for jaundice were performed.

### Laboratory diagnosis

The authors witnessed increase in bilirubin levels, decrease in albumin levels and impaired coagulation tests with no increase in aminotransferase levels. Despite the above, eradication of hepatitis C virus was achieved (Figures 1 and 2).

### Imaging diagnosis

The authors performed liver ultrasonography to rule out recurrence of HCC or vascular causes as the cause of decompensation. Eventually magnetic resonance imaging scan was also performed as follow-up on HCC.

### Treatment

The main treatment in this case was discontinuation of PODr + R treatment. Albumin infusions and IV furosemide were administered.

## Term explanation

All acronyms are explained in the main text.

## Experiences and lessons

The new direct acting antiviral have an excellent safety record. Still, careful clinical and laboratory monitoring, beginning early in the course of therapy, is warranted especially in compensated cirrhotic patients treated with PODr + R because decompensation can occur without aminotransferase flareup.

## Peer-review

The author presents an interesting and valuable case report.

## REFERENCES

- 1 **Mohd Hanafiah K**, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]
- 2 **Holtzman D**. Hepatitis C 2016. CDC Health Information for International Travel. [updated 2015 Jul 10]. Available from: URL: <http://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/hepatitis-c#4627>
- 3 **Panel AIHG**. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology* 2015; **62**: 932-954 [PMID: 26111063 DOI: 10.1002/hep.27950]
- 4 **Poordad F**, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, Shiffman ML, Wedemeyer H, Berg T, Yoshida EM, Fornis X, Lovell SS, Da Silva-Tillmann B, Collins CA, Campbell AL, Podsadecki T, Bernstein B. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med* 2014; **370**: 1973-1982 [PMID: 24725237 DOI: 10.1056/NEJMoa1402869]
- 5 **FDA Drug Safety Communication**. FDA warns of serious liver injury risk with hepatitis C treatments Viekira Pak and Technivie. 2015. Available from: URL: <http://www.fda.gov/Drugs/DrugSafety/ucm468634.htm>
- 6 **Dyson JK**, Hutchinson J, Harrison L, Rotimi O, Tiniakos D, Foster GR, Aldersley MA, McPherson S. Liver toxicity associated with sofosbuvir, an NS5A inhibitor and ribavirin use. *J Hepatol* 2016; **64**: 234-238 [PMID: 26325535 DOI: 10.1016/j.jhep.2015.07.041]
- 7 **Dyson JK**, McPherson S. Reply to "Liver failure in human immunodeficiency virus - Hepatitis C virus coinfection treated with sofosbuvir, ledipasvir and antiretroviral therapy". *J Hepatol* 2016; **64**: 753-754 [PMID: 26682725 DOI: 10.1016/j.jhep.2015.11.038]
- 8 **Marchan-Lopez A**, Dominguez-Dominguez L, Kessler-Saiz P, Jarrin-Estupiñan ME. Liver failure in human immunodeficiency virus - Hepatitis C virus coinfection treated with sofosbuvir, ledipasvir and antiretroviral therapy. *J Hepatol* 2016; **64**: 752-753 [PMID: 26682727 DOI: 10.1016/j.jhep.2015.10.033]
- 9 **Snoeck E**, Chanu P, Lavielle M, Jacqmin P, Jonsson EN, Jorga K, Goggin T, Grippo J, Jumble NL, Frey N. A comprehensive hepatitis C viral kinetic model explaining cure. *Clin Pharmacol Ther* 2010; **87**: 706-713 [PMID: 20463660 DOI: 10.1038/clpt.2010.35]
- 10 **Guedj J**, Perelson AS. Second-phase hepatitis C virus RNA decline during telaprevir-based therapy increases with drug effectiveness: implications for treatment duration. *Hepatology* 2011; **53**: 1801-1808 [PMID: 21384401 DOI: 10.1002/hep.24272]
- 11 **Dahari H**, Shteingart S, Gafanovich I, Cotler SJ, D'Amato M, Pohl RT, Weiss G, Ashkenazi YJ, Tichler T, Goldin E, Lurie Y. Sustained virological response with intravenous silybinin: individualized IFN-free therapy via real-time modelling of HCV kinetics. *Liver Int* 2015; **35**: 289-294 [PMID: 25251042 DOI: 10.1111/liv.12692]
- 12 **Dahari H**, Canini L, Graw F, Uprichard SL, Araújo ES, Penaranda G, Coquet E, Chiche L, Riso A, Renou C, Bourliere M, Cotler SJ, Halfon P. HCV kinetic and modeling analyses indicate similar time to cure among sofosbuvir combination regimens with daclatasvir, simeprevir or ledipasvir. *J Hepatol* 2016; **64**: 1232-1239 [PMID: 26907973 DOI: 10.1016/j.jhep.2016.02.022]
- 13 **Rong L**, Guedj J, Dahari H, Coffield DJ, Levi M, Smith P, Perelson AS. Analysis of hepatitis C virus decline during treatment with the protease inhibitor danoprevir using a multiscale model. *PLoS Comput Biol* 2013; **9**: e1002959 [PMID: 23516348 DOI: 10.1371/journal.pcbi.1002959]
- 14 **Guedj J**, Dahari H, Rong L, Sansone ND, Nettles RE, Cotler SJ, Layden TJ, Uprichard SL, Perelson AS. Modeling shows that the NS5A inhibitor daclatasvir has two modes of action and yields a shorter estimate of the hepatitis C virus half-life. *Proc Natl Acad Sci USA* 2013; **110**: 3991-3996 [PMID: 23431163 DOI: 10.1073/pnas.1203110110]
- 15 **Barritt AS**, Lee J, Hayashi PH. Detective work in drug-induced liver injury: sometimes it is all about interviewing the right witness. *Clin Gastroenterol Hepatol* 2010; **8**: 635-637 [PMID: 20363371 DOI: 10.1016/j.cgh.2010.03.020]
- 16 **Sansone N**, Dahari H, Subramanya G, Perelson AS, Uprichard SL. Modeling HCVcc infection reveals new insights into the dynamics that maintain the in vitro HCV steady state and the mechanisms of action of the NS5A inhibitor daclatasvir. *Hepatology* 2014; **60**: 4 (Suppl): 1165A
- 17 **Kohli A**, Osinusi A, Sims Z, Nelson A, Meissner EG, Barrett LL, Bon D, Marti MM, Silk R, Kotb C, Gross C, Jolley TA, Sidharthan S, Petersen T, Townsend K, Egerson D, Kapoor R, Spurlin E, Sneller M, Proschan M, Herrmann E, Kwan R, Teferi G, Talwani R, Diaz G, Kleiner DE, Wood BJ, Chavez J, Abbott S, Symonds WT, Subramanian GM, Pang PS, McHutchison J, Polis MA, Fauci AS, Masur H, Kottlil S. Virological response after 6 week triple-drug regimens for hepatitis C: a proof-of-concept phase 2A cohort study. *Lancet* 2015; **385**: 1107-1113 [PMID: 25591505 DOI: 10.1016/S0140-6736(14)61228-9]
- 18 **Harrington PR**, Deming DJ, Komatsu TE, Naeger LK. Hepatitis C Virus RNA Levels During Interferon-Free Combination Direct-Acting Antiviral Treatment in Registrational Trials. *Clin Infect Dis* 2015; **61**: 666-667 [PMID: 26002846 DOI: 10.1093/cid/civ402]
- 19 **Meissner EG**, Nelson A, Marti M, Masur H, Osinusi A, Kottlil S. Sustained Virologic Response for Chronic Hepatitis C Infection after 27 Days of Treatment with Sofosbuvir and Ribavirin. *Open Forum Infect Dis* 2014; **1**: 013 [PMID: 25411655 DOI: 10.1093/ofid/ofu013]

**P- Reviewer:** Jiang W, Preda CM, Sirin G, Silva LD

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 July 28; 8(21): 863-914







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*





## Contents

Three issues per month Volume 8 Number 21 July 28, 2016

### EDITORIAL

- 863 New horizon for radical cure of chronic hepatitis B virus infection  
*Tajiri K, Shimizu Y*

### THERAPEUTIC ADVANCES

- 874 Hepatocellular carcinoma beyond Milan criteria: Management and transplant selection criteria  
*Elshamy M, Aucejo F, Menon KVN, Eghtesad B*

### TOPIC HIGHLIGHT

- 881 Contribution of alpha-fetoprotein in liver transplantation for hepatocellular carcinoma  
*Charrière B, Maulat C, Suc B, Muscari F*

### REVIEW

- 891 Acute renal injury after partial hepatectomy  
*Peres LAB, Bredt LC, Cipriani RFF*

### ORIGINAL ARTICLE

#### Basic Study

- 902 Anti-hepatitis C virus potency of a new autophagy inhibitor using human liver slices model  
*Lagaye S, Brun S, Gaston J, Shen H, Stranska R, Camus C, Dubray C, Rousseau G, Massault PP, Courcambeck J, Bassisi F, Halfon P, Pol S*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Giovan Giuseppe Di Costanzo, Director, Division of Hepatology, AORN A Cardarelli, 80131 Napoli, Italy

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjnet.com](mailto:editorialoffice@wjnet.com)  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjnet.com](mailto:bpgoffice@wjnet.com)  
Help Desk: <http://www.wjnet.com/esps/helpdesk.aspx>  
<http://www.wjnet.com>

PUBLICATION DATE  
July 28, 2016

**COPYRIGHT**

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**

<http://www.wjnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**

<http://www.wjnet.com/esps/>

## New horizon for radical cure of chronic hepatitis B virus infection

Kazuto Tajiri, Yukihiro Shimizu

Kazuto Tajiri, the Third Department of Internal Medicine, Toyama University Hospital, Toyama 930-0194, Japan

Yukihiro Shimizu, Gastroenterology Center, Nanto Municipal Hospital, Toyama 932-0211, Japan

**Author contributions:** Tajiri K and Shimizu Y wrote this paper; Shimizu Y conducted this work.

**Conflict-of-interest statement:** Tajiri K and Shimizu Y declare there is no conflict of interest related to this publication.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Yukihiro Shimizu, MD, PhD, Gastroenterology Center, Nanto Municipal Hospital, 938 Inami, Toyama 932-0211, Japan. [rsf14240@nifty.com](mailto:rsf14240@nifty.com)  
 Telephone: +81-76-3821475  
 Fax: +81-76-3821853

Received: March 27, 2016  
 Peer-review started: March 28, 2016  
 First decision: May 17, 2016  
 Revised: May 28, 2016  
 Accepted: June 27, 2016  
 Article in press: June 29, 2016  
 Published online: July 28, 2016

### Abstract

About 250 to 350 million people worldwide are chronically infected with hepatitis B virus (HBV), and about 700000 patients per year die of HBV-related cirrhosis

or hepatocellular carcinoma (HCC). Several anti-viral agents, such as interferon and nucleos(t)ide analogues (NAs), have been used to treat this disease. NAs especially have been shown to strongly suppress HBV replication, slowing the progression to cirrhosis and the development of HCC. However, reactivation of HBV replication often occurs after cessation of treatment, because NAs alone cannot completely remove covalently-closed circular DNA (cccDNA), the template of HBV replication, from the nuclei of hepatocytes. Anti-HBV immune responses, in conjunction with interferon- $\gamma$  and tumor necrosis factor- $\alpha$ , were found to eliminate cccDNA, but complete eradication of cccDNA by immune response alone is difficult, as shown in patients who recover from acute HBV infection but often show long-term persistence of small amounts of HBV-DNA in the blood. Several new drugs interfering with the life cycle of HBV in hepatocytes have been developed, with drugs targeting cccDNA theoretically the most effective for radical cure of chronic HBV infection. However, the safety of these drugs should be extensively examined before application to patients, and combinations of several approaches may be necessary for radical cure of chronic HBV infection.

**Key words:** Covalently-closed circular DNA; Genome editing technology; Immune response; Immunotherapy; Program death-1; Interferon- $\gamma$ ; Tumor necrosis factor- $\alpha$

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Among the agents used to treat chronic hepatitis B virus (HBV) infection are nucleos(t)ide analogues, which have been shown to strongly suppress HBV replication. HBV replication, however, may be reactivated after cessation of treatment, because complete removal of covalently-closed circular DNA (cccDNA) from hepatocyte nuclei is extremely difficult. Immune responses have been shown to destroy cccDNA, but immune response alone is insufficient for complete eradication of template DNA. Several drugs were

recently developed to block the HBV life cycle in hepatocytes, with drugs targeting cccDNA being, at least theoretically, the most effective for radical cure of chronic HBV infection. The safety of these agents should be extensively examined before their use in patients. Combinations of two or more classes of agent may be necessary for radical cure of chronic HBV infection.

Tajiri K, Shimizu Y. New horizon for radical cure of chronic hepatitis B virus infection. *World J Hepatol* 2016; 8(21): 863-873 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i21/863.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i21.863>

## INTRODUCTION

About 250 to 350 million people worldwide are chronically infected with hepatitis B virus (HBV)<sup>[1,2]</sup>, with about 700000 patients per year dying from HBV-related cirrhosis or hepatocellular carcinoma (HCC)<sup>[3]</sup>. Several anti-viral agents, including interferons and nucleos(t)ide analogues (NAs), have been shown effective, with NA-based treatment strongly suppressing the replication of HBV-DNA and normalizing serum alanine aminotransferase activity, resulting in little or no progression of liver disease<sup>[4-6]</sup>. NAs target the viral reverse transcriptase, effectively reducing serum HBV-DNA concentrations. However, intrahepatic HBV-DNA, such as converted covalently closed circular DNA (cccDNA), is not a direct target of NAs. cccDNA is a template for all viral RNAs and HBV-DNA replication can be induced to start from residual cccDNA after cessation of treatment with NAs<sup>[7]</sup>. Small amounts of HBV-DNA can be found in serum long after patients recover from acute HBV infection, suggesting that cccDNA may persist for decades<sup>[8]</sup>. Thus, cccDNA is difficult to eradicate once infection is established, and should be the main target for the complete eradication of HBV infection. However, measuring intrahepatic cccDNA concentrations is difficult in a clinical setting<sup>[9]</sup>. The cccDNA levels in HBV-infected human hepatocytes are low, ranging from 1 to 50 copies per hepatocyte<sup>[10]</sup>. Real-time polymerase chain reaction (PCR) amplification with specific primers for cccDNA or Southern blotting can be used for the detection. However, PCR amplification may be hampered by other co-extracted viral DNA and Southern blotting needs much time and effort. Moreover, the form of cccDNA may be changed during the DNA extraction procedure. Therefore, further investigation should be required to establish the precise evaluation of intrahepatic cccDNA. As an alternative, the reduction in HBV surface antigen (HBsAg) concentration has been reported to partly reflect the decrease in intrahepatic cccDNA, with the goal of treatment for chronic HBV infection being the complete disappearance of HBsAg<sup>[11]</sup>. Fewer than 10% of patients receiving interferon-based therapy<sup>[4-6]</sup>, and few patients treated with NAs<sup>[12,13]</sup>, achieve complete loss of HBsAg. Various trials have tested agents targeting the life cycle of HBV in hepatocytes, including the elimination of cccDNA.

This review summarizes and discusses the radical cure (Table 1) of chronic HBV infection, mainly focusing on the elimination of cccDNA.

## HBV REPLICATION CYCLE AND THE PRODUCTION OF HBV-RELATED PROTEINS

### HBV replication cycle

HBV is a DNA virus that belongs to the family *Hepadnaviridae*, with a 3.2 kb-long partially double-stranded relaxed circular DNA (rcDNA) genome<sup>[14]</sup>. The life cycle of HBV is shown in Figure 1. HBV virions are thought to enter hepatocytes through a high-affinity interaction between the myristoylated preS1 region of HBV and the surface structures of hepatocytes, including sodium taurocholate cotransporting polypeptide (NTCP)<sup>[15-17]</sup>. After entry into hepatocytes, uncoated rcDNA is released into the cytoplasm and then enters the nucleus, where it is converted to cccDNA. The cccDNA remains for a long time in the nucleus, where it serves as a template for the transcription of viral mRNA<sup>[17,18]</sup>. All viral RNAs, pregenomic RNAs (pgRNA) and RNAs encoding the surface proteins, precore and HBx of HBV, are transcribed from cccDNA, with efficient transcription regulated by liver-specific transcription factors<sup>[19]</sup> and the HBx protein itself<sup>[20]</sup>. Epigenetic control of cccDNA transcriptional activity, such as acetylation, methylation or phosphorylation, appears to occur<sup>[21]</sup>. Cytoplasmic pgRNA and polymerase protein are subsequently packaged into envelope proteins, with rcDNA produced from the reverse transcription of pgRNA. Nucleocapsids packaging rcDNA are encapsulated by HBsAg as the envelope protein and released from hepatocytes as virions. The precise understanding of these processes is important for the development of new strategies for the radical cure of chronic HBV infection.

### HBV-related proteins and their roles in hepatocarcinogenesis

The HBV-related proteins translated from cccDNA consist not only of the envelope, core and polymerase proteins of HBV, but may play a role in hepatocarcinogenesis itself.

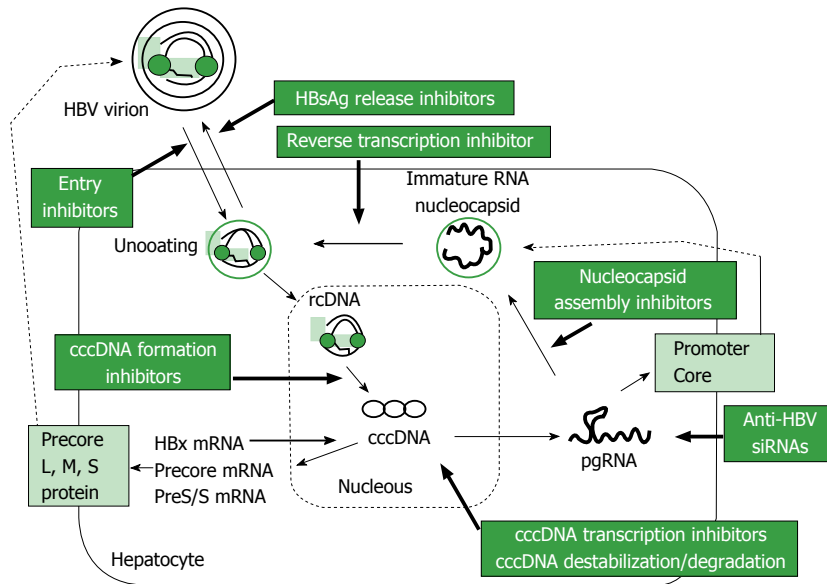
Studies analyzing the role of HBx proteins in hepatocellular transformation and HCC progression have found that low levels of HBx protein are present in non-tumor tissues of HBV-infected liver, whereas high levels of HBx protein are present in HCCs arising in HBV infected individuals, suggesting that this protein has an oncogenic function<sup>[22,23]</sup>. Moreover, HBx transgenic mice often develop liver cancer<sup>[24,25]</sup>, and HBx protein has been found to accumulate in hepatocytes, affecting the expression of genes associated with signal transduction, cell cycle control, transcription, and immune response<sup>[23,26]</sup>. Expression of genes on the X-chromosome is regulated epigenetically, including by DNA and histone methyltransferases<sup>[27,28]</sup>, and by microRNAs<sup>[29,30]</sup>.



**Table 1** Cure status of hepatitis B virus infection

	Serum HBV-DNA	Serum HBsAg	Intraheptic cccDNA	HBV-DNA-integrated hepatocytes
Functional cure (clinical cure)	Low	(-)(-++)	(+)	(-)(+)
Radical cure (virological cure)	(-)	(-)	(-)	(-)

HBV: Hepatitis B virus; cccDNA: Covalently-closed circular DNA; HBsAg: Hepatitis B virus surface antigen.



**Figure 1** Simplified schema of the hepatitis B virus life cycle and possible targets of therapy. HBV: Hepatitis B virus; cccDNA: Covalently-closed circular DNA; HBsAg: Hepatitis B virus surface antigen; rcDNA: Relaxed circular DNA; siRNAs: Small interfering RNAs; pgRNA: Pregenomic RNAs.

HBx is not only involved in carcinogenesis but in the progression of HCC. HBx has been shown to increase beta-catenin signaling through epigenetic control or micro-RNA<sup>[31,32]</sup> and to be an independent predictor of survival after HCC resection<sup>[33]</sup>.

HBsAg is also involved in hepatocarcinogenesis. The ground glass appearance of hepatocytes was shown to be a typical histological finding in HBV-infected livers, with this ground glass appearance resulting from the accumulation of HBsAg with preS mutations<sup>[34-36]</sup>. PreS-mutated HBsAg, especially large HBsAg, was found to accumulate in cytoplasm, leading to the induction of ER stress and oxidative DNA damage<sup>[35-37]</sup>. Furthermore preS mutations upregulated intracellular signaling *via* hepatocyte proliferation<sup>[35,38]</sup>. High serum HBsAg levels showed a definite correlation with HCC development in patients with controlled HBV-DNA<sup>[39-41]</sup>. Like HBV-related proteins, spliced HBV proteins were found to activate intracellular signaling *via* hepatocyte proliferation<sup>[42,43]</sup>. These findings suggest that not only HBV replication, but the production of HBV-related proteins, should be suppressed to efficiently prevent hepatocarcinogenesis.

## IMMUNE RESPONSE AGAINST HBV INFECTION

Immune responses against HBV are involved in both the pathogenesis and control of HBV infection<sup>[44-47]</sup>. There-

fore, understanding the immune response against HBV may result in better control of HBV infection.

### Acute infection

Analysis of immune responses that occur during acute HBV infection may provide valuable information on strategies by which immune responses control HBV infection.

A mouse model of acute viral hepatitis B was established by injecting HBsAg-specific T-cell clones into HBV transgenic mice<sup>[48]</sup>. Although HBsAg-specific T-cells were found to kill small numbers of HBV-replicating hepatocytes, these T cell clones destroyed intracellular HBV-RNA and HBV-DNA in most infected hepatocytes without killing these cells. This effect was found to be due to interferon (IFN)- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$ <sup>[40,49-51]</sup>. Because HBV transgenic mice do not have cccDNA<sup>[52]</sup>, the effects of these cytokines on cccDNA were unclear. In cccDNA-expressing cultured cells, however, IFN- $\gamma$  and TNF- $\alpha$  inhibited HBV replication and reduced cccDNA in an additive manner<sup>[53]</sup>. Moreover, the decay of cccDNA was found to require activation of APOBEC3 deaminases<sup>[53]</sup>, which are expressed in liver tissues of individuals with acute, but not chronic, HBV infection. These observations indicate that HBV-specific T-cell activation followed by treatment with anti-viral cytokines, such as IFN- $\gamma$  and TNF- $\alpha$ , could eradicate HBV without cytotoxicity.

In a chimpanzee model, cccDNA was found to dis-

appear during the course of acute hepatitis B, and HBV-DNA was found to be susceptible to noncytolytic control by cytokines<sup>[54]</sup>. Moreover, HBV-DNA titers in these livers were reduced before T-cell influx, suggesting that non-T-cells, possibly natural killer cells, may have an important role in the noncytolytic destruction of HBV-DNA in liver during early phases of acute HBV infection<sup>[54]</sup>.

Broad and vigorous CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses have been reported in patients with acute hepatitis B<sup>[55]</sup>. Moreover, HBV-specific T-cell responses were observed during the incubation period of acute hepatitis, with HBV-DNA reduced before alanine aminotransferase concentration peaked, indicating that noncytolytic eradication of HBV also occurs in acute hepatitis B in humans<sup>[56]</sup>. However, recovery from acute hepatitis B does not imply complete eradication of HBV, as small amounts of HBV-DNA can be detected in the blood for a long time after resolution of acute hepatitis B<sup>[8]</sup>. T-cell responses are therefore not sufficient to completely eradicate cccDNA from infected livers, even in acute hepatitis B.

### Chronic infection

Immune responses in patients chronically infected with HBV were found to consist of four phases: The immunotolerant, immune-active, inactive carrier, and reactivation phases<sup>[57]</sup>. Although the exact mechanism by which HBV induces immune tolerance is unclear, it may arise from central deletion or peripheral non-recognition of HBV-specific T-cells<sup>[58]</sup>. Immune tolerance may be broken after several decades by as yet undetermined mechanisms, but these may involve the maturation of dendritic cell (DC) function<sup>[59]</sup>. Breaking immune tolerance to HBV can lead to the immune-active phase, resulting in some degree of hepatitis. During this phase, suppression of HBV replication is observed in 85% to 90% of patients, leading to an inactive carrier state. Most patients in an inactive carrier state do not need antiviral treatments, but cccDNA may be present in their livers. The cccDNA persisting in inactive carriers may be a template for reactivation of HBV replication. The 10% to 15% of patients who remain in the immune-active phase continue to experience liver inflammation with active replication of HBV, and may be at high risk for progression to liver cirrhosis and the development of HCC. The number of HBV-specific CD8<sup>+</sup> T-cells was found to be the same in livers with low HBV replication and little hepatitis and in livers with high HBV replication and severe hepatitis<sup>[60]</sup>. These findings suggest that HBV replication is suppressed by immune surveillance of HBV-specific T-cells in the liver and that these T-cells are important in controlling HBV replication in a noncytolytic manner in inactive carriers. In contrast, HBV-specific immune responses are thought to be dysregulated in livers with active hepatitis, and several possible mechanisms have been proposed.

### Impairment of innate immune response

Innate immune system such as pattern recognition receptors, macrophages, DCs, natural killer cells or natural killer T cells are involved in the pathogenesis of HBV

infection especially at an early stage of infection<sup>[61,62]</sup>. HBV has been shown to alter the function of macrophages by modulating the secretion of cytokines<sup>[63,64]</sup> or type-1 IFN gene expression<sup>[64]</sup>. Hepatitis B e antigen was shown to directly suppress toll-like receptor (TLR) signaling via interaction with Toll/IL-1 receptor-containing proteins such as TRAM and Mal<sup>[65]</sup>. HBV has been shown to downregulate TLR-2 expression in patients with chronic HBV infection<sup>[66]</sup>. Thus, innate immunity alteration plays a role, at least in part, in the pathogenesis of chronic HBV infection and TLR-7 agonists have been applied as immune-modulatory components<sup>[67,68]</sup>. On the other hand, the effect of IFN- $\alpha$  on intrahepatic cccDNA has been recently explored<sup>[69]</sup>, and IFN- $\alpha$  in addition to lymphotoxin- $\beta$  receptor (LT $\beta$ R) activation has been shown to induce cccDNA degradation through upregulation of nuclear APOBEC3 deaminases<sup>[70]</sup>. APOBEC3 can deaminate double-stranded DNA cytidines to uridines<sup>[71]</sup> and induce cccDNA degradation. IFN- $\gamma$  and TNF- $\alpha$  produced from T-cells can induce deamination of cccDNA without cytolysis, supporting the essential role of APOBEC3 in reduction of cccDNA<sup>[53]</sup>. Collectively, type-1 IFN-mediated effects, especially APOBEC3 upregulation, will be a key subject for development of new therapeutics.

### Dysfunction of dendritic cells

DCs are the most potent antigen-presenting cells, stimulating both T- and B-cells. In patients with chronic hepatitis, the cytokine-induced maturation of circulating myeloid DCs is impaired, possibly by exposure to high amounts of HBV or HBsAg<sup>[72,73]</sup>. Dysfunctional DCs may act as tolerogenic antigen-presenting cells, resulting in a failure to induce HBV-specific immune responses.

### Alteration of the hierarchy of epitope-specific CD8<sup>+</sup> T-cell responses

In acute hepatitis B, the CD8<sup>+</sup> T-cell response to the immunogenic epitope HBc18-27 (HLA-A2 restricted epitope) is dominant. In contrast, HBc18-27-specific CD8<sup>+</sup> T-cell responses are low and CD8<sup>+</sup> T-cell responses against less immunogenic envelope (183-191) are dominant in chronic hepatitis B<sup>[74]</sup>. Although the mechanisms underlying changes in the major epitope to CD8<sup>+</sup> T-cell response are not yet known, they may account, at least in part, for the different CD8<sup>+</sup> T-cell responses observed in patients with acute and chronic hepatitis.

### Regulatory T-cells

Regulatory T-cells (Tregs) expressing the forkhead family transcription factor, Foxp3, are specialized cells that have a major role in the maintenance of immunological self-tolerance by suppressing self-reactive cells<sup>[75]</sup>. Tregs express CD25 [interleukin (IL)-2 receptor  $\alpha$ -chain] and/or cytotoxic T-lymphocyte antigen-4 (CTLA-4), which are excellent inhibitors of IL-2 production or downregulation of CD80 and CD86 on DCs by a CTLA-4-dependent mechanism<sup>[76]</sup>.

The numbers of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> Tregs were higher in the livers of patients with chronic hepatitis B,

suggesting that these cells suppress intrahepatic HBV-specific T-cell responses, leading to insufficient immune control of HBV replication in the liver<sup>[77]</sup>.

### **Inhibitory receptors**

Program death (PD)-1 is a surface receptor critical for the regulation of T-cell function<sup>[78,79]</sup>. Binding of the ligand PD-L1 to PD-1 on T-cells results in the antigen-specific inhibition of T-cell proliferation, with a molecule related to T-cell exhaustion found in the livers of patients with chronic hepatitis B. T-cell exhaustion is characterized by poor cytotoxic activity and cytokine production, as well as by the expression of inhibitory receptors, including not only PD-1 but lymphocyte activation gene-3, CTLA-4, T-cell immunoglobulin domain and mucin domain-3, and CD244<sup>[66]</sup>. These inhibitory receptors are thought to be induced by persistent exposure of intrahepatic T-cells to HBV or HBV-related proteins<sup>[80]</sup>. Exhaustion of T-cells could also account for impaired T-cell responses in the livers of patients with chronic hepatitis B, and blockade of these receptors could be therapeutic.

Patients with high serum HBV-DNA concentration have been reported likely to progress to cirrhosis and eventually HCC<sup>[81]</sup>. Transition of immune-active patients to an inactive state with low HBV-DNA replication by the direct stimulation of HBV-specific T-cells or removal of immunosuppressive factors, may be sufficient to inhibit progression to cirrhosis or HCC. Inactive HBV carriers may not require specific treatment, because spontaneous HBsAg develops at a rate of 1% to 1.9%/year in these patients, making the development of HCC rare<sup>[82]</sup>. Therefore, an inactive HBV carrier may be regarded as in a state of functional cure (Table 1). However, HBV replication may be reactivated, either spontaneously or during treatment with an immunosuppressive or anticancer agent, resulting in a higher risk of hepatocarcinogenesis than in the general population<sup>[83]</sup>. The rate of HCC development was recently reported to be greater in patients with high than with low serum HBsAg concentrations, even in inactive HBV carriers with low serum HBV-DNA concentrations<sup>[36,37]</sup>.

Collectively, these results suggest that induction of immune control against HBV infection may result in functional cure of HBV infection. Functional cure, however, may be an unstable condition, allowing progression to cirrhosis or HCC under various conditions. Although radical cure (Table 1) is desirable, it is problematic because of the difficulty in eliminating HBV cccDNA from the liver.

## **THERAPEUTIC STRATEGIES FOR HBV INFECTION**

### **Immunotherapy**

Radical cure of HBV infection could be achieved by both the elimination of cccDNA in the liver and the destruction of HBV-DNA-integrated hepatocytes. The primary goals of immunotherapy in HBV-infected individuals include the induction or stimulation of HBV-specific immune re-

sponses, leading to the killing of infected cells or the degradation of HBV-RNA and HBV-DNA in a noncytolytic manner, inhibiting progression to liver cirrhosis and hepatocarcinogenesis. Although immune responses involving cytokines such as IFN- $\gamma$  and TNF- $\alpha$  can eliminate cccDNA<sup>[50,53]</sup>, cccDNA is not completely eliminated even after resolution of acute hepatitis B<sup>[8]</sup>, suggesting that immune responses alone may be insufficient to achieve radical cure of HBV infection.

### **Induction or stimulation of HBV-specific immune responses**

Efforts to stimulate HBV-specific T-cells have included immunizations with HBV-peptides, viral proteins, DCs, and DNA, as well as treatment with cytokines<sup>[84]</sup>. Because HBV-specific T-cells in patients with chronic hepatitis B are exhausted by long-term exposure to high levels of HBV-related antigens, activation of those cells by immunization would be ineffective without functional restoration of the cells by blocking the inhibitory signals responsible for T-cell exhaustion. Blockade of PD-1, CTLA-4 or Tim-3 has been shown to restore exhausted HBV-specific T-cells<sup>[80]</sup>, suggesting that the combination of immunization and blockade of inhibitory signals would be effective in activating HBV-specific T-cells.

Other immunotherapeutic approaches to HBV infection include administration of cytokines, such as IFN- $\gamma$ , IL-6, IL-1 $\beta$ , LT $\beta$ R-agonists and/or TLR-7 agonist, as well as IFN- $\gamma$  and TNF- $\alpha$  which were shown to cause silencing or degradation of cccDNA<sup>[67]</sup>. This strategy may be more effective in the complete eradication of HBV infection than strategies involving the activation of HBV-specific cells, suggesting that only cytokine administration results in the elimination of cccDNA.

### **Elimination of HBV-infected hepatocytes by a novel approach**

A novel approach to eliminate HBV-core containing hepatocytes<sup>[85]</sup> was based on findings showing that elimination of HBV is impaired by cellular inhibitor of apoptosis proteins (cIAPs), which inhibit the TNF- $\alpha$ -mediated death of HBV-infected cells<sup>[86]</sup>. This led to testing the effects of inhibitors of cIAPs, including birinapant and other Smac mimetics, on HBV-infected hepatocytes. These inhibitors of cIAPs resulted in the rapid reduction in serum HBV-DNA and HBsAg concentrations, possibly by eliminating HBV-core containing hepatocytes. However, the effects of those drugs on cccDNA are unclear.

### **Immunotherapeutic strategies for HBV-DNA-integrated hepatocytes**

Three main mechanisms are responsible for hepatocarcinogenesis: (1) the oncogenic potential of the HBV-related proteins, HBsAg and HBx; (2) HBV-DNA integration into the host genome, dysregulating the cell cycle by the introduction of deletions, cis/trans-activations, and/or translocations, and/or inducing generalized genomic instability; and (3) persistent inflammation in the

liver causing rapid turnover of hepatocyte regeneration, enhancing the instability and/or mutagenesis of host genomes.

Therefore, if future advances in therapeutic modalities result in the complete elimination of cccDNA, hepatocarcinogenesis resulting from HBV-DNA integration into the host genome should be addressed. HBV-DNA integration into the hepatocyte genome has been observed in 86.4% of HBV-related HCCs and in 30.7% of adjacent liver tissue<sup>[87]</sup>. Integration of HBV-DNA into areas of the host genome encoding genes that regulate cellular proliferation, such as telomerase or proliferation signal transduction genes, may lead to cis-/trans-activation, inducing malignant transformation<sup>[88]</sup>. Furthermore, integration of HBV-DNA may induce genetic instability by altering the expression of oncogenes, tumor suppressor genes and microRNAs<sup>[87,89]</sup>. In addition, a viral-human chimeric transcript was reported to function as a noncoding RNA and promote hepatocarcinogenesis<sup>[90]</sup>. Integration of HBV-DNA into the host hepatocyte genome of transiently infected individuals has been reported to be a rare event, occurring in 0.01%-0.1% of hepatocytes<sup>[91]</sup>. Further investigations are needed to determine the mechanism by which HBV-DNA integration into the host genome induces carcinogenesis. The immune cytotoxicity of cells expressing HBV-related peptides may be the only strategy that effectively eliminates HBV-DNA-integrated hepatocytes. However, if non-immunogenic regions of HBV-DNA are integrated, elimination of those cells by immune attack would be impossible.

Taken together, these findings indicate that immunotherapy against HBV can control viral replication and reduce cccDNA, but may not be sufficient to completely eradicate HBV-infected or -integrated hepatocytes.

### **Inhibition of HBV replication**

Currently available NAs can efficiently reduce viremia but cannot eliminate intracellular cccDNA. However, complete suppression of HBV polymerase can result in the complete elimination of cccDNA through the death of cccDNA-containing hepatocytes after one natural lifespan of these cells<sup>[92]</sup>. Among the agents being tested are prodrugs of HBV polymerase inhibitors<sup>[93]</sup>. These include prodrugs of tenofovir, such as AGX1009 (Agenix) and TAF (GS-7340, Gilead Sciences), which have been evaluated in phase 3 trials<sup>[93,94]</sup>, and CMX157, a lipid conjugate of tenofovir, which has been evaluated in phase 1/2 trials<sup>[93,95]</sup>. RNase H inhibitors are also being tested, based on the specificity of HBV replication, which depends on the RNase H activity of HBV polymerase to degrade pgRNA<sup>[10]</sup>. Evaluations of selective inhibitors of HBV polymerase RNase H activity<sup>[96]</sup> suggest that they might be more effective when combined with NAs<sup>[93]</sup>.

### **Destruction of cccDNA**

Eradication of cccDNA in hepatocytes is essential to achieve radical cure of established HBV infection. Several trials have targeted cccDNA. For example, gene silencing techniques, such as small interfering RNAs (siRNAs) or

antisense oligonucleotides (ASOs), have been evaluated for their ability to reduce viremia and cccDNA. Although siRNAs may have promising activity, methods to effectively deliver them to hepatocytes have not been determined<sup>[97]</sup>. RNAi can inhibit all steps of HBV replication, and ARC-520 has been tested in a phase 2 trial in patients with chronic hepatitis B<sup>[95]</sup>. In contrast, a single injection of ASO, consisting of liver-targeted peptides, into a mouse model of chronic HBV infection was shown to reduce HBV-RNA, proteins and HBV-DNA for a long time, suggesting that ASO may become a promising treatment in patients with chronic HBV<sup>[98]</sup>. Furthermore, disubstituted sulfonamide was shown to selectively inhibit the formation of cccDNA<sup>[99]</sup>.

In addition, several genome editing technologies have been developed to silence sequence-specific cleavage of cccDNA. These include zinc finger nucleases (ZFNs)<sup>[100,101]</sup>, transcription activator-like effector nucleases (TALENs)<sup>[102,103]</sup>, and the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR associated system (Cas). These sequence-specific genome editing technologies could induce double-stranded breaks at certain DNA sites. ZFNs consist of a zinc finger domain, which contains a sequence-specific binding site, and a *Fok I* nuclease domain. ZFNs form heterodimers and induce double-stranded breaks at targeted sites. These breaks are subsequently repaired by homology-directed repair or non-homologous end joining. The specificity of ZFNs may be context-dependent, resulting from interactions between DNA binding domains and neighboring zinc fingers<sup>[104]</sup>. TALENs have transcription activator-like effector specific DNA binding activity, with DNA-binding sites more specific than those of ZFNs<sup>[105]</sup>. However, both ZFNs and TALENs require pairs of site-specific nucleases for each target to produce customized proteins<sup>[9]</sup>. In contrast, CRISPR/Cas technology is a novel genome-editing method, which is more useful than ZFNs or TALENs<sup>[106]</sup>. CRISPR/Cas loci encode RNA guided endonucleases, which are induced by immune responses against foreign genetic elements such as bacteriophages and plasmids<sup>[107]</sup>. The type 2 CRISPR/Cas system from *Streptococcus pyogenes* is a chimeric single-guide RNA with Cas9 protein<sup>[108]</sup>. The CRISPR/Cas9 system was shown to suppress HBV replication in cultured cells and in mouse models<sup>[109-117]</sup>, reducing both HBsAg<sup>[109,110,112-116]</sup> and cccDNA<sup>[110,113-115,117]</sup>. These findings suggest that genome editing technology, such as a CRISPR/Cas system, may be a potential therapeutic option for the complete eradication of HBV infection in future. However, cleavage of cccDNA and subsequent DNA repair may introduce mutations into the host genome. These mutations may be harmful to the host, resulting in the possible development of malignancy<sup>[9,118,119]</sup>, suggesting the need for further improvements in efficacy and safety prior to the therapeutic use of these systems.

### **Future perspectives on radical cure of chronic HBV infection**

Various trials have assessed agents that can terminate



**Table 2** Tgераaptic agents against hepatitis B virus currently in clinical development

Mode of actions	Target	Stage of development	Ref.
Entry inhibitions	NTCP	Myrcludex in phase 2	[14,123]
cccDNA			
Formation inhibitions	DSS	Preclinical	[99]
Transcription inhibitions	ASO	IONIS-HBVRx in phase 1	[98]
Destabilization/degradation	ZFN	Preclinical	[100,101]
	TALEN	Preclinical	[102,103]
	CRISPR/Cas9	Preclinical	[109-117]
SiRNA	PgRNA	ARC-520 in phase 2	[95]
Nucleocapsid assembly inhibitions	Capsid formation	BAY4109 in phase 1	[93,95]
		NV1221 in phase 1	[93,95]
Reverse transcription inhibitions	Polymerase	TAF in phase 3	[93,94]
		Cmx157 in phase 1/2	[93,95]
HBsAg release inhibitions	HBsAg secretion	Preclinical	[109]
	HBsAg secretion	Rep2139 in phase 1/2	[110]
Immune modulating	TLR-7 agonist	GS-9620 in phase 2	[67,68]
	HBV-specific	Preclinical	[84]
	cIAPS	Preclinical	[86]

cccDNA: Covalently-closed circular DNA; ZFN: Zinc finger nuclease; TALEN: Transcription activator-like effector nuclease; CRISPR: Clustered regularly interspaced short palindromic repeat; Cas: CRISPR associated system; pgRNA: Pregenomic RNAs; HBsAg: Hepatitis B virus surface antigen; TLR: Toll-like receptor; HBV: Hepatitis B virus; cIAPS: Cellular inhibitor of apoptosis proteins; DSS: Disubstituted sulfonamide; ASO: Antisense oligonucleotides; TAF: Tenofovir Alafenamide; NTCP: Na<sup>+</sup>/taurocholate cotransporting polypeptide.

the HBV life cycle in hepatocytes, including inhibitors of HBV-DNA polymerase, virus entry, core assembly and HBsAg secretion (Table 2)<sup>[93,95,120,121]</sup>. Especially Myrcludex B, a synthetic lipopeptide that targets NTCP, has been shown to efficiently prevent viral spread and has been applied in clinical trials<sup>[15,17,122,123]</sup>. These agents, including Myrcludex, are not themselves sufficient to eliminate HBV from chronically infected hepatocytes, as shown by the remaining cccDNA in the nuclei and HBV-DNA-integrated hepatocytes. Immunotherapy may potentially eliminate both cccDNA and HBV-DNA-integrated hepatocytes, but its effects would be limited. Although drugs targeting cccDNA in hepatocytes are theoretically ideal for complete eradication of HBV, no single drug or strategy, whether currently available or under development, has shown the ability to completely eliminate HBV with established safety and efficacy. Future trials, testing combination of different agents or strategies, will be necessary.

## REFERENCES

- Liaw YF, Chu CM. Hepatitis B virus infection. *Lancet* 2009; **373**: 582-592 [PMID: 19217993 DOI: 10.1016/S0140-6736(09)60207-5]
- Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine* 2012; **30**: 2212-2219 [PMID: 22273662 DOI: 10.1016/j.vaccine.2011.12.116]
- Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**: 2095-2128 [PMID: 23245604 DOI: 10.1016/S0140-6736(12)61728-0]
- Trépo C, Chan HL, Lok A. Hepatitis B virus infection. *Lancet* 2014; **384**: 2053-2063 [PMID: 24954675 DOI: 10.1016/S0140-6736(14)60220-8]
- Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005; **352**: 2682-2695 [PMID: 15987917 DOI: 10.1056/NEJMoa043470]
- Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Niesters

- HG, Zondervan P, Hansen B, Schalm SW. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. *Lancet* 2005; **365**: 123-129 [PMID: 15639293 DOI: 10.1016/S0140-6736(05)17701-0]
- 7 **Tuttleman JS**, Pourcel C, Summers J. Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. *Cell* 1986; **47**: 451-460 [PMID: 3768961]
  - 8 **Rehermann B**, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat Med* 1996; **2**: 1104-1108 [PMID: 8837608]
  - 9 **Ohno M**, Otsuka M, Kishikawa T, Yoshikawa T, Takata A, Koike K. Novel therapeutic approaches for hepatitis B virus covalently closed circular DNA. *World J Gastroenterol* 2015; **21**: 7084-7088 [PMID: 26109795 DOI: 10.3748/wjg.v21.i23.7084]
  - 10 **Seeger C**, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000; **64**: 51-68 [PMID: 10704474]
  - 11 **Lampertico P**, Maini M, Papatheodoridis G. Optimal management of hepatitis B virus infection - EASL Special Conference. *J Hepatol* 2015; **63**: 1238-1253 [PMID: 26150256 DOI: 10.1016/j.jhep.2015.06.026]
  - 12 **Lai CL**, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, Chen Y, Heathcote EJ, Rasenack J, Bzowej N, Naoumov NV, Di Bisceglie AM, Zeuzem S, Moon YM, Goodman Z, Chao G, Constance BF, Brown NA. Telbivudine versus lamivudine in patients with chronic hepatitis B. *N Engl J Med* 2007; **357**: 2576-2588 [PMID: 18094378 DOI: 10.1056/NEJMoa066422]
  - 13 **Chang TT**, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, Han KH, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R, Apelian D. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; **354**: 1001-1010 [PMID: 16525137 DOI: 10.1056/NEJMoa051285]
  - 14 **Summers J**, O'Connell A, Millman I. Genome of hepatitis B virus: restriction enzyme cleavage and structure of DNA extracted from Dane particles. *Proc Natl Acad Sci USA* 1975; **72**: 4597-4601 [PMID: 1060140]
  - 15 **Gripon P**, Canine I, Urban S. Efficient inhibition of hepatitis B virus infection by acylated peptides derived from the large viral surface protein. *J Virol* 2005; **79**: 1613-1622 [PMID: 15650187 DOI: 10.1128/JVI.79.3.1613-1622.2005]
  - 16 **Ni Y**, Lempp FA, Mehrle S, Nkongolo S, Kaufman C, Fälth M, Stindt J, Königer C, Nassal M, Kubitz R, Sülthmann H, Urban S. Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes. *Gastroenterology* 2014; **146**: 1070-1083 [PMID: 24361467 DOI: 10.1053/j.gastro.2013.12.024]
  - 17 **Brahmania M**, Feld J, Arif A, Janssen HL. New therapeutic agents for chronic hepatitis B. *Lancet Infect Dis* 2016; **16**: e10-e21 [PMID: 26795693 DOI: 10.1016/S1473-3099(15)00436-3]
  - 18 **Summers J**, Mason WS. Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. *Cell* 1982; **29**: 403-415 [PMID: 6180831]
  - 19 **Tang H**, McLachlan A. Transcriptional regulation of hepatitis B virus by nuclear hormone receptors is a critical determinant of viral tropism. *Proc Natl Acad Sci USA* 2001; **98**: 1841-1846 [PMID: 11172038 DOI: 10.1073/pnas.041479698]
  - 20 **Lucifora J**, Arzberger S, Durantal D, Belloni L, Strubin M, Levrero M, Zoulim F, Hantz O, Protzer U. Hepatitis B virus X protein is essential to initiate and maintain virus replication after infection. *J Hepatol* 2011; **55**: 996-1003 [PMID: 21376091 DOI: 10.1016/j.jhep.2011.02.015]
  - 21 **Nassal M**. HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. *Gut* 2015; **64**: 1972-1984 [PMID: 26048673 DOI: 10.1136/gutjnl-2015-309809]
  - 22 **Paterlini P**, Poussin K, Kew M, Franco D, Brechot C. Selective accumulation of the X transcript of hepatitis B virus in patients negative for hepatitis B surface antigen with hepatocellular carcinoma. *Hepatology* 1995; **21**: 313-321 [PMID: 7843699]
  - 23 **Zhang XD**, Wang Y, Ye LH. Hepatitis B virus X protein accelerates the development of hepatoma. *Cancer Biol Med* 2014; **11**: 182-190 [PMID: 25364579 DOI: 10.7497/j.issn.2095-3941.2014.03.004]
  - 24 **Kim CM**, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* 1991; **351**: 317-320 [PMID: 2034275 DOI: 10.1038/351317a0]
  - 25 **Yu DY**, Moon HB, Son JK, Jeong S, Yu SL, Yoon H, Han YM, Lee CS, Park JS, Lee CH, Hyun BH, Murakami S, Lee KK. Incidence of hepatocellular carcinoma in transgenic mice expressing the hepatitis B virus X-protein. *J Hepatol* 1999; **31**: 123-132 [PMID: 10424292]
  - 26 **Qiu X**, Dong S, Qiao F, Lu S, Song Y, Lao Y, Li Y, Zeng T, Hu J, Zhang L, Zhang L, Fan H. HBx-mediated miR-21 upregulation represses tumor-suppressor function of PDCD4 in hepatocellular carcinoma. *Oncogene* 2013; **32**: 3296-3305 [PMID: 23604124 DOI: 10.1038/ncr.2013.150]
  - 27 **Jung JK**, Arora P, Pagano JS, Jang KL. Expression of DNA methyltransferase 1 is activated by hepatitis B virus X protein via a regulatory circuit involving the p16INK4a-cyclin D1-CDK 4/6-pRb-E2F1 pathway. *Cancer Res* 2007; **67**: 5771-5778 [PMID: 17575144 DOI: 10.1158/0008-5472.CAN-07-0529]
  - 28 **Rivière L**, Gerossier L, Ducroux A, Dion S, Deng Q, Michel ML, Buendia MA, Hantz O, Neuveut C. HBx relieves chromatin-mediated transcriptional repression of hepatitis B viral cccDNA involving SETDB1 histone methyltransferase. *J Hepatol* 2015; **63**: 1093-1102 [PMID: 26143443 DOI: 10.1016/j.jhep.2015.06.023]
  - 29 **Arzumanyan A**, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer* 2013; **13**: 123-135 [PMID: 23344543 DOI: 10.1038/nrc3449]
  - 30 **Yuan K**, Lian Z, Sun B, Clayton MM, Ng IO, Feitelson MA. Role of miR-148a in hepatitis B associated hepatocellular carcinoma. *PLoS One* 2012; **7**: e35331 [PMID: 22496917 DOI: 10.1371/journal.pone.0035331]
  - 31 **Lee JO**, Kwun HJ, Jung JK, Choi KH, Min DS, Jang KL. Hepatitis B virus X protein represses E-cadherin expression via activation of DNA methyltransferase 1. *Oncogene* 2005; **24**: 6617-6625 [PMID: 16007161 DOI: 10.1038/sj.onc.1208827]
  - 32 **Arzumanyan A**, Friedman T, Kotei E, Ng IO, Lian Z, Feitelson MA. Epigenetic repression of E-cadherin expression by hepatitis B virus x antigen in liver cancer. *Oncogene* 2012; **31**: 563-572 [PMID: 21706058 DOI: 10.1038/ncr.2011.255]
  - 33 **Xie Y**, Liu S, Zhao Y, Guo Z, Xu J. X protein mutations in hepatitis B virus DNA predict postoperative survival in hepatocellular carcinoma. *Tumour Biol* 2014; **35**: 10325-10331 [PMID: 25034530 DOI: 10.1007/s13277-014-2331-0]
  - 34 **Su JJ**, Wang HC, Wu HC, Huang WY. Ground glass hepatocytes contain pre-S mutants and represent preneoplastic lesions in chronic hepatitis B virus infection. *J Gastroenterol Hepatol* 2008; **23**: 1169-1174 [PMID: 18505413 DOI: 10.1111/j.1440-1746.2008.05348.x]
  - 35 **Pollicino T**, Cacciola I, Saffioti F, Raimondo G. Hepatitis B virus PreS/S gene variants: pathobiology and clinical implications. *J Hepatol* 2014; **61**: 408-417 [PMID: 24801416 DOI: 10.1016/j.jhep.2014.04.041]
  - 36 **Wu HC**, Tsai HW, Teng CF, Hsieh WC, Lin YJ, Wang LH, Yuan Q, Su JJ. Ground-glass hepatocytes co-expressing hepatitis B virus X protein and surface antigens exhibit enhanced oncogenic effects and tumorigenesis. *Hum Pathol* 2014; **45**: 1294-1301 [PMID: 24767856 DOI: 10.1016/j.humpath.2013.10.039]
  - 37 **Na B**, Huang Z, Wang Q, Qi Z, Tian Y, Lu CC, Yu J, Hanes MA, Kakar S, Huang EJ, Ou JH, Liu L, Yen TS. Transgenic expression of entire hepatitis B virus in mice induces hepatocarcinogenesis independent of chronic liver injury. *PLoS One* 2011; **6**: e26240 [PMID: 22022578 DOI: 10.1371/journal.pone.0026240]
  - 38 **Hildt E**, Munz B, Saher G, Reifenberg K, Hofschneider PH. The PreS2 activator MHBs(t) of hepatitis B virus activates c-raf-1/Erk2 signaling in transgenic mice. *EMBO J* 2002; **21**: 525-535 [PMID: 11847101]
  - 39 **Kawanaka M**, Nishino K, Nakamura J, Oka T, Urata N, Goto D, Suehiro M, Kawamoto H, Kudo M, Yamada G. Quantitative Levels of Hepatitis B Virus DNA and Surface Antigen and the Risk of Hepatocellular Carcinoma in Patients with Hepatitis B Receiving

- Long-Term Nucleos(t)ide Analogue Therapy. *Liver Cancer* 2014; **3**: 41-52 [PMID: 24804176 DOI: 10.1159/000343857]
- 40 **Tseng TC**, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, Hsu CA, Kuo SF, Liu CH, Chen PJ, Chen DS, Kao JH. Serum hepatitis B surface antigen levels help predict disease progression in patients with low hepatitis B virus loads. *Hepatology* 2013; **57**: 441-450 [PMID: 22941922 DOI: 10.1002/hep.26041]
  - 41 **Liu J**, Yang HI, Lee MH, Lu SN, Jen CL, Batrla-Utermann R, Wang LY, You SL, Hsiao CK, Chen PJ, Chen CJ. Spontaneous seroclearance of hepatitis B seromarkers and subsequent risk of hepatocellular carcinoma. *Gut* 2014; **63**: 1648-1657 [PMID: 24225939 DOI: 10.1136/gutjnl-2013-305785]
  - 42 **Voehringer D**, Blaser C, Grawitz AB, Chisari FV, Buerki K, Pircher H. Break of T cell ignorance to a viral antigen in the liver induces hepatitis. *J Immunol* 2000; **165**: 2415-2422 [PMID: 10946266]
  - 43 **Chen WN**, Chen JY, Jiao BY, Lin WS, Wu YL, Liu LL, Lin X. Interaction of the hepatitis B spliced protein with cathepsin B promotes hepatoma cell migration and invasion. *J Virol* 2012; **86**: 13533-13541 [PMID: 23035214 DOI: 10.1128/JVI.02095-12]
  - 44 **Tan AT**, Koh S, Goh V, Bertolotti A. Understanding the immunopathogenesis of chronic hepatitis B virus: an Asian prospective. *J Gastroenterol Hepatol* 2008; **23**: 833-843 [PMID: 18565018 DOI: 10.1111/j.1440-1746.2008.05385.x]
  - 45 **Chisari FV**, Isogawa M, Wieland SF. Pathogenesis of hepatitis B virus infection. *Pathol Biol (Paris)* 2010; **58**: 258-266 [PMID: 20116937 DOI: 10.1016/j.patbio.2009.11.001]
  - 46 **Bertolotti A**, Wang FS. Overview of the special issue on HBV immunity. *Cell Mol Immunol* 2015; **12**: 253-254 [PMID: 25864914 DOI: 10.1038/cmi.2015.24]
  - 47 **Guidotti LG**, Isogawa M, Chisari FV. Host-virus interactions in hepatitis B virus infection. *Curr Opin Immunol* 2015; **36**: 61-66 [PMID: 26186123 DOI: 10.1016/j.coi.2015.06.016]
  - 48 **Moriyama T**, Guilhot S, Klopchin K, Moss B, Pinkert CA, Palmiter RD, Brinster RL, Kanagawa O, Chisari FV. Immunobiology and pathogenesis of hepatocellular injury in hepatitis B virus transgenic mice. *Science* 1990; **248**: 361-364 [PMID: 1691527]
  - 49 **Chisari FV**. Cytotoxic T cells and viral hepatitis. *J Clin Invest* 1997; **99**: 1472-1477 [PMID: 9119989 DOI: 10.1172/JCI119308]
  - 50 **Guidotti LG**, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity* 1996; **4**: 25-36 [PMID: 8574849]
  - 51 **Guidotti LG**, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 2001; **19**: 65-91 [PMID: 11244031 DOI: 10.1146/annurev.immunol.19.1.65]
  - 52 **Guidotti LG**, Matzke B, Schaller H, Chisari FV. High-level hepatitis B virus replication in transgenic mice. *J Virol* 1995; **69**: 6158-6169 [PMID: 7666518]
  - 53 **Xia Y**, Stadler D, Lucifora J, Reisinger F, Webb D, Hösel M, Wichter T, Wisskirchen K, Cheng X, Zhang K, Chou WM, Wettengel JM, Malo A, Bohne F, Hoffmann D, Eyer F, Thimme R, Falk CS, Thasler WE, Heikenwalder M, Protzer U. Interferon- $\gamma$  and Tumor Necrosis Factor- $\alpha$  Produced by T Cells Reduce the HBV Persistence Form, cccDNA, Without Cytolysis. *Gastroenterology* 2016; **150**: 194-205 [PMID: 26416327 DOI: 10.1053/j.gastro.2015.09.026]
  - 54 **Guidotti LG**, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. *Science* 1999; **284**: 825-829 [PMID: 10221919]
  - 55 **Rehermann B**, Fowler P, Sidney J, Person J, Redeker A, Brown M, Moss B, Sette A, Chisari FV. The cytotoxic T lymphocyte response to multiple hepatitis B virus polymerase epitopes during and after acute viral hepatitis. *J Exp Med* 1995; **181**: 1047-1058 [PMID: 7532675]
  - 56 **Webster GJ**, Reignat S, Maini MK, Whalley SA, Ogg GS, King A, Brown D, Amlot PL, Williams R, Vergani D, Dusheiko GM, Bertolotti A. Incubation phase of acute hepatitis B in man: dynamic of cellular immune mechanisms. *Hepatology* 2000; **32**: 1117-1124 [PMID: 11050064 DOI: 10.1053/jhep.2000.19324]
  - 57 **Wu JF**, Chang MH. Natural history of chronic hepatitis B virus infection from infancy to adult life - the mechanism of inflammation triggering and long-term impacts. *J Biomed Sci* 2015; **22**: 92 [PMID: 26487087 DOI: 10.1186/s12929-015-0199-y]
  - 58 **Mamun-Al-Mahtab SM**, Uddin H, Khan SI, Rahman S. Early termination of immune tolerance state of hepatitis B virus infection explains liver damage. *World J Hepatol* 2014; **6**: 621-625 [PMID: 25232455 DOI: 10.4254/wjh.v6.i8.621]
  - 59 **Shimizu Y**, Guidotti LG, Fowler P, Chisari FV. Dendritic cell immunization breaks cytotoxic T lymphocyte tolerance in hepatitis B virus transgenic mice. *J Immunol* 1998; **161**: 4520-4529 [PMID: 9794377]
  - 60 **Maini MK**, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, King AS, Herberg J, Gilson R, Alisa A, Williams R, Vergani D, Naoumov NV, Ferrari C, Bertolotti A. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med* 2000; **191**: 1269-1280 [PMID: 10770795]
  - 61 **Fiscaro P**, Valdatta C, Boni C, Massari M, Mori C, Zerbini A, Orlandini A, Sacchelli L, Missale G, Ferrari C. Early kinetics of innate and adaptive immune responses during hepatitis B virus infection. *Gut* 2009; **58**: 974-982 [PMID: 19201769 DOI: 10.1136/gut.2008.163600]
  - 62 **Busca A**, Kumar A. Innate immune responses in hepatitis B virus (HBV) infection. *Virol J* 2014; **11**: 22 [PMID: 24507433 DOI: 10.1186/1743-422X-11-22]
  - 63 **Xu L**, Yin W, Sun R, Wei H, Tian Z. Kupffer cell-derived IL-10 plays a key role in maintaining humoral immune tolerance in hepatitis B virus-persistent mice. *Hepatology* 2014; **59**: 443-452 [PMID: 23929689 DOI: 10.1002/hep.26668]
  - 64 **Zeisel MB**, Lucifora J, Mason WS, Sureau C, Beck J, Levrero M, Kann M, Knolle PA, Benkirane M, Durandel D, Michol ML, Autran B, Cosset FL, Strick-Marchand H, Trépo C, Kao JH, Carrat F, Lacombe K, Schinazi RF, Barré-Sinoussi F, Delfraissy JF, Zoulim F. Towards an HBV cure: state-of-the-art and unresolved questions-report of the ANRS workshop on HBV cure. *Gut* 2015; **64**: 1314-1326 [PMID: 25670809 DOI: 10.1136/gutjnl-2014-308943]
  - 65 **Lang T**, Lo C, Skinner N, Locarnini S, Visvanathan K, Mansell A. The hepatitis B e antigen (HBeAg) targets and suppresses activation of the toll-like receptor signaling pathway. *J Hepatol* 2011; **55**: 762-769 [PMID: 21334391 DOI: 10.1016/j.jhep.2010.12.042]
  - 66 **Visvanathan K**, Skinner NA, Thompson AJ, Riordan SM, Sozzi V, Edwards R, Rodgers S, Kurtovic J, Chang J, Lewin S, Desmond P, Locarnini S. Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. *Hepatology* 2007; **45**: 102-110 [PMID: 17187404 DOI: 10.1002/hep.21482]
  - 67 **Isorce N**, Lucifora J, Zoulim F, Durandel D. Immune-modulators to combat hepatitis B virus infection: From IFN- $\alpha$  to novel investigational immunotherapeutic strategies. *Antiviral Res* 2015; **122**: 69-81 [PMID: 26275801 DOI: 10.1016/j.antiviral.2015.08.008]
  - 68 **Gane EJ**, Lim YS, Gordon SC, Visvanathan K, Sicard E, Fedorak RN, Roberts S, Massetto B, Ye Z, Pflanz S, Garrison KL, Gaggar A, Mani Subramanian G, McHutchison JG, Kottitil S, Freilich B, Coffin CS, Cheng W, Kim YJ. The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection. *J Hepatol* 2015; **63**: 320-328 [PMID: 25733157 DOI: 10.1016/j.jhep.2015.02.037]
  - 69 **Perrillo R**. Benefits and risks of interferon therapy for hepatitis B. *Hepatology* 2009; **49**: S103-S111 [PMID: 19399806 DOI: 10.1002/hep.22956]
  - 70 **Lucifora J**, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, Sprinzl MF, Koppensteiner H, Makowska Z, Volz T, Remouchamps C, Chou WM, Thasler WE, Hüser N, Durandel D, Liang TJ, Münk C, Heim MH, Browning JL, Dejardin E, Dandri M, Schindler M, Heikenwalder M, Protzer U. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science* 2014; **343**: 1221-1228 [PMID: 24557838 DOI: 10.1126/science.1243462]
  - 71 **Stenglein MD**, Burns MB, Li M, Lengyel J, Harris RS. APOBEC3 proteins mediate the clearance of foreign DNA from human cells. *Nat Struct Mol Biol* 2010; **17**: 222-229 [PMID: 20062055 DOI: 10.1038/nsmb.1744]
  - 72 **Wang FS**, Xing LH, Liu MX, Zhu CL, Liu HG, Wang HF, Lei ZY.



- Dysfunction of peripheral blood dendritic cells from patients with chronic hepatitis B virus infection. *World J Gastroenterol* 2001; **7**: 537-541 [PMID: 11819824]
- 73 **Op den Brouw ML**, Binda RS, van Roosmalen MH, Protzer U, Janssen HL, van der Molen RG, Woltman AM. Hepatitis B virus surface antigen impairs myeloid dendritic cell function: a possible immune escape mechanism of hepatitis B virus. *Immunology* 2009; **126**: 280-289 [PMID: 18624732 DOI: 10.1111/j.1365-2567.2008.02896.x]
  - 74 **Webster G**, Bertoletti A. Quantity and quality of virus-specific CD8 cell response: relevance to the design of a therapeutic vaccine for chronic HBV infection. *Mol Immunol* 2001; **38**: 467-473 [PMID: 11741696]
  - 75 **Miyara M**, Sakaguchi S. Human FoxP3(+)CD4(+) regulatory T cells: their knowns and unknowns. *Immunol Cell Biol* 2011; **89**: 346-351 [PMID: 21301480 DOI: 10.1038/icb.2010.137]
  - 76 **Sakaguchi S**, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* 2008; **133**: 775-787 [PMID: 18510923 DOI: 10.1016/j.cell.2008.05.009]
  - 77 **Xu D**, Fu J, Jin L, Zhang H, Zhou C, Zou Z, Zhao JM, Zhang B, Shi M, Ding X, Tang Z, Fu YX, Wang FS. Circulating and liver resident CD4+CD25+ regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. *J Immunol* 2006; **177**: 739-747 [PMID: 16785573]
  - 78 **Francisco LM**, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* 2010; **236**: 219-242 [PMID: 20636820 DOI: 10.1111/j.1600-065X.2010.00923.x]
  - 79 **Fife BT**, Pauken KE. The role of the PD-1 pathway in autoimmunity and peripheral tolerance. *Ann N Y Acad Sci* 2011; **1217**: 45-59 [PMID: 21276005 DOI: 10.1111/j.1749-6632.2010.05919.x]
  - 80 **Ye B**, Liu X, Li X, Kong H, Tian L, Chen Y. T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. *Cell Death Dis* 2015; **6**: e1694 [PMID: 25789969 DOI: 10.1038/cddis.2015.42]
  - 81 **Chen CJ**, Yang HI, Iloeje UH. Hepatitis B virus DNA levels and outcomes in chronic hepatitis B. *Hepatology* 2009; **49**: S72-S84 [PMID: 19399801 DOI: 10.1002/hep.22884]
  - 82 **Invernizzi F**, Viganò M, Grossi G, Lampertico P. The prognosis and management of inactive HBV carriers. *Liver Int* 2016; **36** Suppl 1: 100-104 [PMID: 26725905 DOI: 10.1111/liv.13006]
  - 83 **Tong MJ**, Trieu J. Hepatitis B inactive carriers: clinical course and outcomes. *J Dig Dis* 2013; **14**: 311-317 [PMID: 23433008 DOI: 10.1111/1751-2980.12051]
  - 84 **Shimizu Y**. T cell immunopathogenesis and immunotherapeutic strategies for chronic hepatitis B virus infection. *World J Gastroenterol* 2012; **18**: 2443-2451 [PMID: 22654441 DOI: 10.3748/wjg.v18.i20.2443]
  - 85 **Ebert G**, Allison C, Preston S, Cooney J, Toe JG, Stutz MD, Ojaimi S, Baschuk N, Nachbur U, Torresi J, Silke J, Begley CG, Pellegrini M. Eliminating hepatitis B by antagonizing cellular inhibitors of apoptosis. *Proc Natl Acad Sci USA* 2015; **112**: 5803-5808 [PMID: 25902530 DOI: 10.1073/pnas.1502400112]
  - 86 **Ebert G**, Preston S, Allison C, Cooney J, Toe JG, Stutz MD, Ojaimi S, Scott HW, Baschuk N, Nachbur U, Torresi J, Chin R, Colledge D, Li X, Warner N, Revill P, Bowden S, Silke J, Begley CG, Pellegrini M. Cellular inhibitor of apoptosis proteins prevent clearance of hepatitis B virus. *Proc Natl Acad Sci USA* 2015; **112**: 5797-5802 [PMID: 25902529 DOI: 10.1073/pnas.1502390112]
  - 87 **Sung WK**, Zheng H, Li S, Chen R, Liu X, Li Y, Lee NP, Lee WH, Ariyaratne PN, Tennakoon C, Mulawadi FH, Wong KF, Liu AM, Poon RT, Fan ST, Chan KL, Gong Z, Hu Y, Lin Z, Wang G, Zhang Q, Barber TD, Chou WC, Aggarwal A, Hao K, Zhou W, Zhang C, Hardwick J, Buser C, Xu J, Kan Z, Dai H, Mao M, Reinhard C, Wang J, Luk JM. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. *Nat Genet* 2012; **44**: 765-769 [PMID: 22634754 DOI: 10.1038/ng.2295]
  - 88 **Paterlini-Bréchet P**, Saigo K, Murakami Y, Chami M, Gozuacik D, Mugnier C, Lagorce D, Bréchet C. Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene. *Oncogene* 2003; **22**: 3911-3916 [PMID: 12813464 DOI: 10.1038/sj.onc.1206492]
  - 89 **Feitelson MA**, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. *Cancer Lett* 2007; **252**: 157-170 [PMID: 17188425 DOI: 10.1016/j.canlet.2006.11.010]
  - 90 **Lau CC**, Sun T, Ching AK, He M, Li JW, Wong AM, Co NN, Chan AW, Li PS, Lung RW, Tong JH, Lai PB, Chan HL, To KF, Chan TF, Wong N. Viral-human chimeric transcript predisposes risk to liver cancer development and progression. *Cancer Cell* 2014; **25**: 335-349 [PMID: 24582836 DOI: 10.1016/j.ccr.2014.01.030]
  - 91 **Summers J**, Jilbert AR, Yang W, Aldrich CE, Saputelli J, Litwin S, Toll E, Mason WS. Hepatocyte turnover during resolution of a transient hepadnaviral infection. *Proc Natl Acad Sci USA* 2003; **100**: 11652-11659 [PMID: 14500915 DOI: 10.1073/pnas.1635109100]
  - 92 **Block TM**, Gish R, Guo H, Mehta A, Cuconati A, Thomas London W, Guo JT. Chronic hepatitis B: what should be the goal for new therapies? *Antiviral Res* 2013; **98**: 27-34 [PMID: 23391846 DOI: 10.1016/j.antiviral.2013.01.006]
  - 93 **Block TM**, Rawat S, Brosgart CL. Chronic hepatitis B: A wave of new therapies on the horizon. *Antiviral Res* 2015; **121**: 69-81 [PMID: 26112647 DOI: 10.1016/j.antiviral.2015.06.014]
  - 94 **Menéndez-Arias L**, Álvarez M, Pacheco B. Nucleoside/nucleotide analog inhibitors of hepatitis B virus polymerase: mechanism of action and resistance. *Curr Opin Virol* 2014; **8**: 1-9 [PMID: 24814823 DOI: 10.1016/j.coviro.2014.04.005]
  - 95 **Liang TJ**, Block TM, McMahon BJ, Ghany MG, Urban S, Guo JT, Locarnini S, Zoulim F, Chang KM, Lok AS. Present and future therapies of hepatitis B: From discovery to cure. *Hepatology* 2015; **62**: 1893-1908 [PMID: 26239691 DOI: 10.1002/hep.28025]
  - 96 **Tavis JE**, Lomonosova E. The hepatitis B virus ribonuclease H as a drug target. *Antiviral Res* 2015; **118**: 132-138 [PMID: 25862291 DOI: 10.1016/j.antiviral.2015.04.002]
  - 97 **Wooddell CI**, Rozema DB, Hossbach M, John M, Hamilton HL, Chu Q, Hegge JO, Klein JJ, Wakefield DH, Oropeza CE, Deckert J, Roehl I, Jahn-Hofmann K, Hadwiger P, Vormlocher HP, McLachlan A, Lewis DL. Hepatocyte-targeted RNAi therapeutics for the treatment of chronic hepatitis B virus infection. *Mol Ther* 2013; **21**: 973-985 [PMID: 23439496 DOI: 10.1038/mt.2013.31]
  - 98 **Billioud G**, Kruse RL, Carrillo M, Whitten-Bauer C, Gao D, Kim A, Chen L, McCaleb ML, Crosby JR, Hamatake R, Hong Z, Garaigorta U, Swayze E, Bissig KD, Wieland S. In vivo reduction of hepatitis B virus antigenemia and viremia by antisense oligonucleotides. *J Hepatol* 2016; **64**: 781-789 [PMID: 26658683 DOI: 10.1016/j.jhep.2015.11.032]
  - 99 **Cai D**, Mills C, Yu W, Yan R, Aldrich CE, Saputelli JR, Mason WS, Xu X, Guo JT, Block TM, Cuconati A, Guo H. Identification of disubstituted sulfonamide compounds as specific inhibitors of hepatitis B virus covalently closed circular DNA formation. *Antimicrob Agents Chemother* 2012; **56**: 4277-4288 [PMID: 22644022 DOI: 10.1128/AAC.00473-12]
  - 100 **Hoeksema KA**, Tyrrell DL. Inhibition of viral transcription using designed zinc finger proteins. *Methods Mol Biol* 2010; **649**: 97-116 [PMID: 20680830 DOI: 10.1007/978-1-60761-753-2\_6]
  - 101 **Zimmerman KA**, Fischer KP, Joyce MA, Tyrrell DL. Zinc finger proteins designed to specifically target duck hepatitis B virus covalently closed circular DNA inhibit viral transcription in tissue culture. *J Virol* 2008; **82**: 8013-8021 [PMID: 18524822 DOI: 10.1128/JVI.00366-08]
  - 102 **Bloom K**, Ely A, Mussolino C, Cathomen T, Arbuthnot P. Inactivation of hepatitis B virus replication in cultured cells and in vivo with engineered transcription activator-like effector nucleases. *Mol Ther* 2013; **21**: 1889-1897 [PMID: 23883864 DOI: 10.1038/mt.2013.170]
  - 103 **Chen J**, Zhang W, Lin J, Wang F, Wu M, Chen C, Zheng Y, Peng X, Li J, Yuan Z. An efficient antiviral strategy for targeting hepatitis B virus genome using transcription activator-like effector nucleases. *Mol Ther* 2014; **22**: 303-311 [PMID: 24025750 DOI: 10.1038/mt.2013.212]
  - 104 **Maeder ML**, Thibodeau-Beganny S, Osiaik A, Wright DA, Anthony RM, Eichinger M, Jiang T, Foley JE, Winfrey RJ, Townsend JA, Unger-Wallace E, Sander JD, Müller-Lerch F, Fu F, Pearlberg J,



- Göbel C, Dassie JP, Pruett-Miller SM, Porteus MH, Sgroi DC, Iafrate AJ, Dobbs D, McCray PB, Cathomen T, Voytas DF, Joung JK. Rapid “open-source” engineering of customized zinc-finger nucleases for highly efficient gene modification. *Mol Cell* 2008; **31**: 294-301 [PMID: 18657511 DOI: 10.1016/j.molcel.2008.06.016]
- 105 **Holkers M**, Maggio I, Liu J, Janssen JM, Miselli F, Mussolino C, Recchia A, Cathomen T, Gonçalves MA. Differential integrity of TALE nuclease genes following adenoviral and lentiviral vector gene transfer into human cells. *Nucleic Acids Res* 2013; **41**: e63 [PMID: 23275534 DOI: 10.1093/nar/gks1446]
- 106 **Sander JD**, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat Biotechnol* 2014; **32**: 347-355 [PMID: 24584096 DOI: 10.1038/nbt.2842]
- 107 **Barrangou R**, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero DA, Horvath P. CRISPR provides acquired resistance against viruses in prokaryotes. *Science* 2007; **315**: 1709-1712 [PMID: 17379808 DOI: 10.1126/science.1138140]
- 108 **Jinek M**, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012; **337**: 816-821 [PMID: 22745249 DOI: 10.1126/science.1225829]
- 109 **Lin SR**, Yang HC, Kuo YT, Liu CJ, Yang TY, Sung KC, Lin YY, Wang HY, Wang CC, Shen YC, Wu FY, Kao JH, Chen DS, Chen PJ. The CRISPR/Cas9 System Facilitates Clearance of the Intrahepatic HBV Templates In Vivo. *Mol Ther Nucleic Acids* 2014; **3**: e186 [PMID: 25137139 DOI: 10.1038/mtna.2014.38]
- 110 **Kennedy EM**, Bassit LC, Mueller H, Kornepati AV, Bogerd HP, Nie T, Chatterjee P, Javanbakht H, Schinazi RF, Cullen BR. Suppression of hepatitis B virus DNA accumulation in chronically infected cells using a bacterial CRISPR/Cas RNA-guided DNA endonuclease. *Virology* 2015; **476**: 196-205 [PMID: 25553515 DOI: 10.1016/j.virol.2014.12.001]
- 111 **Seeger C**, Sohn JA. Targeting Hepatitis B Virus With CRISPR/Cas9. *Mol Ther Nucleic Acids* 2014; **3**: e216 [PMID: 25514649 DOI: 10.1038/mtna.2014.68]
- 112 **Liu X**, Hao R, Chen S, Guo D, Chen Y. Inhibition of hepatitis B virus by the CRISPR/Cas9 system via targeting the conserved regions of the viral genome. *J Gen Virol* 2015; **96**: 2252-2261 [PMID: 25904148 DOI: 10.1099/vir.0.000159]
- 113 **Zhen S**, Hua L, Liu YH, Gao LC, Fu J, Wan DY, Dong LH, Song HF, Gao X. Harnessing the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated Cas9 system to disrupt the hepatitis B virus. *Gene Ther* 2015; **22**: 404-412 [PMID: 25652100 DOI: 10.1038/gt.2015.2]
- 114 **Dong C**, Qu L, Wang H, Wei L, Dong Y, Xiong S. Targeting hepatitis B virus cccDNA by CRISPR/Cas9 nuclease efficiently inhibits viral replication. *Antiviral Res* 2015; **118**: 110-117 [PMID: 25843425 DOI: 10.1016/j.antiviral.2015.03.015]
- 115 **Ramanan V**, Shlomai A, Cox DB, Schwartz RE, Michailidis E, Bhatta A, Scott DA, Zhang F, Rice CM, Bhatia SN. CRISPR/Cas9 cleavage of viral DNA efficiently suppresses hepatitis B virus. *Sci Rep* 2015; **5**: 10833 [PMID: 26035283 DOI: 10.1038/srep10833]
- 116 **Karimova M**, Beschoner N, Dammermann W, Chemnitz J, Indenbirken D, Bockmann JH, Grundhoff A, Lüth S, Buchholz F, Schulze zur Wiesch J, Hauber J. CRISPR/Cas9 nickase-mediated disruption of hepatitis B virus open reading frame S and X. *Sci Rep* 2015; **5**: 13734 [PMID: 26334116 DOI: 10.1038/srep13734]
- 117 **Wang J**, Xu ZW, Liu S, Zhang RY, Ding SL, Xie XM, Long L, Chen XM, Zhuang H, Lu FM. Dual gRNAs guided CRISPR/Cas9 system inhibits hepatitis B virus replication. *World J Gastroenterol* 2015; **21**: 9554-9565 [PMID: 26327763 DOI: 10.3748/wjg.v21.i32.9554]
- 118 **Liu S**, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; **101**: 1066-1082 [PMID: 19574418 DOI: 10.1093/jnci/djp180]
- 119 **Wu Y**, Gan Y, Gao F, Zhao Z, Jin Y, Zhu Y, Sun Z, Wu H, Chen T, Wang J, Sun Y, Fan C, Xiang Y, Qian G, Groopman JD, Gu J, Tu H. Novel natural mutations in the hepatitis B virus reverse transcriptase domain associated with hepatocellular carcinoma. *PLoS One* 2014; **9**: e94864 [PMID: 24788140 DOI: 10.1371/journal.pone.0094864]
- 120 **Yu W**, Goddard C, Clearfield E, Mills C, Xiao T, Guo H, Morrey JD, Motter NE, Zhao K, Block TM, Cuconati A, Xu X. Design, synthesis, and biological evaluation of triazolo-pyrimidine derivatives as novel inhibitors of hepatitis B virus surface antigen (HBsAg) secretion. *J Med Chem* 2011; **54**: 5660-5670 [PMID: 21786803 DOI: 10.1021/jm200696v]
- 121 **Noordeen F**, Vaillant A, Jilbert AR. Nucleic acid polymers inhibit duck hepatitis B virus infection in vitro. *Antimicrob Agents Chemother* 2013; **57**: 5291-5298 [PMID: 23939902 DOI: 10.1128/AAC.01003-13]
- 122 **Wang YJ**, Yang L, Zuo JP. Recent developments in antivirals against hepatitis B virus. *Virus Res* 2016; **213**: 205-213 [PMID: 26732483 DOI: 10.1016/j.virusres.2015.12.014]
- 123 **Urban S**, Bartenschlager R, Kubitz R, Zoulim F. Strategies to inhibit entry of HBV and HDV into hepatocytes. *Gastroenterology* 2014; **147**: 48-64 [PMID: 24768844 DOI: 10.1053/j.gastro.2014.04.030]

P- Reviewer: Bowden S, Gong ZJ, Jin DY

S- Editor: Qi Y L- Editor: A E- Editor: Li D



## Hepatocellular carcinoma beyond Milan criteria: Management and transplant selection criteria

Mohammed Elshamy, Federico Aucejo, KV Narayanan Menon, Bijan Egtesad

Mohammed Elshamy, Federico Aucejo, Bijan Egtesad, Hepato-biliary and Transplant Surgery, Digestive Disease and Surgery Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, United States

KV Narayanan Menon, Gastroenterology and Hepatology, Digestive Disease and Surgery Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, United States

**Author contributions:** This manuscript was written completely by the stated authors.

**Conflict-of-interest statement:** None.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Bijan Egtesad, MD, Hepato-biliary and Transplant Surgery, Digestive Disease and Surgery Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue/A100, Cleveland, OH 44195, United States. [egtesb@ccf.org](mailto:egtesb@ccf.org)  
 Telephone: +1-216-4445914  
 Fax: +1-216-4449375

Received: March 30, 2016  
 Peer-review started: April 6, 2016  
 First decision: June 7, 2016  
 Revised: June 17, 2016  
 Accepted: July 11, 2016  
 Article in press: July 13, 2016  
 Published online: July 28, 2016

### Abstract

Liver transplantation (LT) for hepatocellular carcinoma (HCC) has been established as a standard treatment in

selected patients for the last two and a half decades. After initially dismal outcomes, the Milan criteria (MC) (single HCC  $\leq 5$  cm or up to 3 HCCs  $\leq 3$  cm) have been adopted worldwide to select HCC patients for LT, however cumulative experience has shown that MC can be too strict. This has led to the development of numerous expanded criteria worldwide. Morphometric expansions on MC as well as various criteria which incorporate biomarkers as surrogates of tumor biology have been described. HCC that presents beyond MC initially can be downstaged with locoregional therapy (LRT). Post-LRT monitoring aims to identify candidates with favorable tumor behavior. Similarly, tumor marker levels as response to LRT has been utilized as surrogate of tumor biology. Molecular signatures of HCC have also been correlated to outcomes; these have yet to be incorporated into HCC-LT selection criteria formally. The ongoing discrepancy between organ demand and supply makes patient selection the most challenging element of organ allocation. Further validation of extended HCC-LT criteria models and pre-LT treatment strategies are required.

**Key words:** Hepatocellular carcinoma; Milan criteria; Liver transplantation; Expanded criteria; Locoregional therapy; Down staging

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Numerous expanded selection criteria for hepatocellular carcinoma (HCC)-liver transplantation (LT) have been proposed worldwide. Surrogates of favorable tumor biology such as Post-locoregional therapy strategies which observe tumor behavior, and the addition of HCC biomarkers to selection criteria have been explored. Further investigation is encouraged to identify patients beyond MC with the most favorable tumor biology for LT.

Elshamy M, Aucejo F, Menon KVN, Egtesad B. Hepatocellular carcinoma beyond Milan criteria: Management and transplant

selection criteria. *World J Hepatol* 2016; 8(21): 874-880 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i21/874.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i21.874>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver, with over 700000 new cases diagnosed yearly worldwide<sup>[1]</sup>. HCC continues to be a global health problem due to insufficient screening and surveillance and poorly controlled risk factors<sup>[2]</sup>. HCC arises most frequently in patients with chronic liver disease from diverse etiologies, and liver transplantation (LT) has been established as a standard treatment in selected patients for the last two and a half decades<sup>[3]</sup>. However, an ongoing conundrum is the discrepancy between organ demand and supply, making patient selection the most challenging piece of the puzzle to prevent organ misutilization<sup>[4]</sup>.

Poor patient selection (excessive tumor burden, unknown tumor biology) made initial results of LT for HCC quite dismal<sup>[5]</sup>. It wasn't until 1996, when Mazzaferro *et al*<sup>[6]</sup> defined tumor criteria for patient selection (single lesion  $\leq 5$  cm, or up to 3 lesions  $\leq 3$  cm each in the absence of tumor vascular invasion or evidence of extra-hepatic metastases) associated with comparable outcome to patients undergoing LT without HCC. The study revealed 4 year post-LT survival  $> 75\%$  and post-LT recurrence rate in the order of 8%. These criteria have since been known as the Milan criteria (MC), and have been adopted worldwide to select HCC patients for LT<sup>[7]</sup>.

Patients who present with HCC beyond MC can be down-staged *via* loco-regional therapy (LRT). LRT are trans-catheter, needle based or radiation treatments which target the tumor and induce selective tumor necrosis<sup>[8]</sup>. The efficacy of these treatments is gauged radiologically by the modified Response Evaluation Criteria in Solid Tumors<sup>[9]</sup>. Tumor response to LRT, post LRT observation before LT, and HCC biomarkers have been described for selecting the most favorable tumor biology in patients presenting with HCC beyond MC<sup>[9-11]</sup>.

Although strict adherence to MC can produce outcomes comparable to LT for non-HCC, cumulative experience over the last two decades have shown that MC can be too strict, and that select patients beyond MC may benefit from LT with adequate survival<sup>[12]</sup>. This has led to the development of numerous HCC expanded criteria worldwide, applied for both cadaveric and live donor liver transplantation.

Herein, we review various expanded HCC criteria and outcomes, impact of tumor response to LRT in post-LT outcome and emerging HCC molecular signatures that may be incorporated into patient selection criteria in the near future.

## EXTENDED LT-HCC CRITERIA

In 2001, Yao *et al*<sup>[13]</sup> published one of the most popular

expanded LT-HCC criteria. The University of California, San Francisco (UCSF) criteria considered a single lesion  $\leq 6.5$  cm, or 2-3 lesions  $\leq 4.5$  cm each, with total tumor diameter  $\leq 8$  cm.

Tumor recurrence was 11.4% and 5 years post-LT survival was in the order of 72.4%<sup>[13]</sup>. The original UCSF criteria were developed based on explant histopathological analysis, but subsequently have been validated utilizing pre-LT imaging. In 2007, Yao *et al*<sup>[14]</sup> published a prospective study utilizing the UCSF criteria revealing 80% 5 years post-LT recurrence free survival (RFS). Alongside MC, UCSF criteria have been the most widely recognized transplant criteria for HCC, and can expand 5%-20% the indication of LT for HCC patients<sup>[14]</sup>. Currently, some worldwide transplant centers utilize UCSF as the standard selection LT criteria for HCC<sup>[15]</sup>.

The Navarro extended criteria described by Herrero *et al*<sup>[16]</sup> in 2001 can expand the MC by considering LT for a single lesion  $\leq 6$  cm, or 2-3 lesions  $\leq 5$  cm each. In their analysis, 12.7% of the cohort experienced tumor recurrence. Post-LT 5 years overall survival and RFS was 79% and 70% respectively.

Silva *et al*<sup>[17]</sup> published the Valencia criteria in 2008. These would consider LT in HCC patients with 1-3 lesions  $\leq 5$  cm each, and total tumor  $\leq 10$  cm. Two hundred and fifty-seven patients undergoing LT for HCC were analyzed, however only 10% were beyond MC based on pre-LT imaging. Patients who fell within the Valencia criteria demonstrated post-LT 5 year survival comparable to patients within MC. The Valencia criteria expands LT to a higher maximum tumor burden compared to both MC and UCSF criteria, without detriment to patient survival, however similar to the Navarro criteria, due to the small number of patients in this cohort, these criteria require further validation.

Correlation of tumor size and number according to explant pathology and post-LT survival in 1206 patients from the International Registry of Hepatic Tumors, led to the recommendation of LT for a single lesion  $\leq 6$  cm, or 2-4 lesions  $\leq 5$  cm each by Onaca *et al*<sup>[18]</sup> in 2007. Survival in patients exceeding MC but meeting these criteria were not significantly lower than for patients meeting MC. Five years post-LT RFS with a single lesion 5.1-6.0 cm in diameter, or with 2-4 lesions (largest 3.1-5.0 cm) were 63.9%, and 64.6% respectively, compared to 5 years post-LT RFS of 61.8% if MC were met<sup>[18]</sup>.

Other proposed extended criteria do not put a limit to number of tumors recommended for LT. Roayaie *et al*<sup>[19]</sup> in 2002, demonstrated 55% 5 years post-LT RFS for patients with lesions 5-7 cm in diameter. In 2004, Kneteman *et al*<sup>[20]</sup> reported the outcomes of LT utilizing extended criteria described as a single lesion  $< 7.5$  cm, or multiple lesions  $< 5$  cm each. Four year post-LT survival was 82.9% vs 87.4% in the MC group.

One of the more recently proposed extended criteria is the Up-to-7 criteria proposed by Mazzaferro *et al*<sup>[21]</sup> in 2009. A cohort of 1556 patients undergoing cadaveric LT and LDLT for HCC from 36 transplant centers was analyzed, 71.5% of the cohort had HCC exceeding MC. The Up-to-7 criteria are defined as the sum of the

size of the largest tumor in cm and the total number of tumors in the absence of tumor microvascular invasion. Five years post-LT survival for patients within the Up-to-7 criteria compared to MC were 71.2% vs 73.3%<sup>[21]</sup>. The major limitation of these criteria is the lack of pre-LT information about microvascular invasion. Currently, this can only be partially projected *via* assessment of alpha-fetoprotein (AFP) level.

### Extended LT-HCC criteria using living donors

Outcomes in HCC patients undergoing living donor liver transplantation (LDLT) were shown to be equivalent to cadaveric liver transplantation<sup>[22]</sup>. Soejima *et al*<sup>[23]</sup> reported that tumor diameter > 5 cm was associated with worse prognosis; however the number of tumors was not. In the cohort of 60 patients who underwent LDLT for HCC, 67% were beyond MC based on pre-LT imaging. Three years post-LT survival of 68.6% was reported for patients beyond MC<sup>[23]</sup>.

Jonas *et al*<sup>[24]</sup> also described their extended criteria based on a cohort of 21 patients undergoing LDLT for HCC. Three year survival rates for patients not meeting MC or UCSF criteria were 62% and 53% respectively. Sugawara *et al*<sup>[25]</sup> proposed an expansion of selection criteria to include up to 5 HCC lesions,  $\leq 5$  cm each. In their cohort of 78 patients, post-LT RFS at 3 years was 94%.

Table 1 demonstrates an overview of proposed morphometric based expanded selection criteria.

## INCORPORATION OF SURROGATES OF TUMOR BIOLOGY TO SELECTION CRITERIA

### Tumor markers

Post-LT outcomes in patients with HCC are in part a consequence of tumor biology. As a result of the impossibility to unveil this feature solely through morphometric imaging characteristics, multiple studies have attempted to include other indicators of tumor behavior as selection criteria. AFP and des- $\gamma$ -carboxyprothrombin (DCP) both have established correlations with post treatment prognosis<sup>[26,27]</sup>. A pre-LT AFP level > 1000 ng/mL has been demonstrated as a significant predictor of HCC recurrence post-LT<sup>[26]</sup>. A large scale analysis of United Network for Organ Sharing (UNOS) data has demonstrated that patients transplanted beyond MC with an AFP level of 0 to 15 ng/mL (normal range) had improved survival<sup>[28]</sup>.

One of the most popular HCC-LT extended criteria including biomarkers as surrogates of tumor biology are the Hangzhou criteria (absence of macrovascular invasion and total tumor diameter  $\leq 8$  cm. If the tumor burden is > 8 cm, histopathology *via* tumor biopsy should be non-poorly differentiated HCC and AFP level should be  $\leq 400$  ng/mL<sup>[29]</sup>.

In the original cohort of 195 patients, fulfilling Hangzhou criteria led to a 5 year survival of 70.7% and DFS: 62.4%. On the other hand, patients beyond Hangzhou

criteria had a 5 year survival of 18.9% and DFS: 4.7%<sup>[29]</sup>. A large scale comparative study of multiple extended criteria confirmed post LT survival associated with LT beyond MC but meeting Hangzhou at 1-, 3-, 5- and 10-years was 89.5%, 70.8%, 62.4% and 52.9% respectively. Additionally, 1-, 3-, 5- and 10-year RFS was 81.6%, 64.3%, 56.5%, and 37.2% respectively. Compared to MC, expanded criteria expanded transplantable patients by 12.4% for Valencia, 16.3% for UCSF, 19.6% for Navarro, and 51.5% for Hangzhou. RFS rates were comparable to MC<sup>[30]</sup>.

In 2012, Lai *et al*<sup>[31]</sup> also suggested that the combination of total tumor diameter > 8 cm and an AFP level  $\leq 400$  ng/mL would result in favorable survival outcomes. The 5 year DFS rate was 74.4%. It was also noted that patients with increased AFP values in response to LRT had higher recurrence rates<sup>[31]</sup>. Duvoux *et al*<sup>[32]</sup> have suggested a predictive scoring model that combines the AFP level at listing with MC. In their model, an AFP level  $\leq 100$  ng/mL in the setting of patients beyond MC (1-3 lesions with a maximum tumor diameter of 6 cm) demonstrated 5- year survival near 70%<sup>[32]</sup>.

Similar criteria have been applied to LDLT as well. In a multicenter study from Japan, Todo *et al*<sup>[33]</sup> suggested that the combination of an AFP cut of level  $\geq 200$  ng/mL and protein induced by vitamin K absence or antagonism factor II (PIVKA II)  $\geq 100$  mAU/mL are significant predictors for poor post LT survival. These combined were described as the A-P level. Five year DFS for beyond MC HCC patients and within the A-P cutoff level was similar to those within MC at 78.7% and 90.4% respectively.

Kwon *et al*<sup>[34]</sup> demonstrated their outcomes incorporating an AFP level  $\leq 400$  ng/mL as a selection criteria along with any number of lesions  $\leq 5$  cm each. In a cohort of 139 patients, 5 year survival was noted at 79.9%, without a significant difference between patients within or beyond MC<sup>[34]</sup>. More recently in 2015, Toso *et al*<sup>[35]</sup> in a prospective study suggested extended LT criteria described as a combination of a total tumor volume  $\leq 115$  cm<sup>3</sup> and an AFP level  $\leq 400$  ng/mL. Four year post LT survival was similar between the extended criteria group and the MC group at 78.7% and 74.6% respectively<sup>[35]</sup>.

A lower AFP cut off rate of < 100 ng/mL as a criteria for HCC-LT was recommended by Grāt *et al*<sup>[36]</sup>. A retrospective analysis of a 121 patients demonstrated significant prediction of recurrence in patients transplanted within UCSF and Up-to-7 criteria who surpassed this limit. Five year RFS for patients meeting UCSF and within the AFP cut off was superior to those meeting USCF but beyond the cut off limit at 100% vs 69% respectively. Similarly, when applied to the Up-to-7 criteria, 5 year RFS for those meeting both the criteria and cut off limit was noted at 100% vs 71.9% for beyond the cut off limit<sup>[36]</sup>.

DCP, often utilized as a tumor marker for HCC in Japan, has been incorporated into the Kyoto criteria published by Fujiki *et al*<sup>[37]</sup> in 2009: A DCP level of  $\leq 400$  mAU/mL in addition to morphometric criteria of up



**Table 1** Expanded morphometric criteria for hepatocellular carcinoma-liver transplantation

Ref.	Year	Description	Donor type	n	Survival
Yao <i>et al</i> <sup>[13]</sup>	2001	1 lesion $\leq$ 6.5 cm, or 2-3 lesions $\leq$ 4.5 cm each. Total tumor diameter $\leq$ 8 cm	Cadaveric	70	5 yr OS: 72.4%
Herrero <i>et al</i> <sup>[16]</sup>	2001	1 lesion $\leq$ 6 cm, or 2-3 lesions $\leq$ 5 cm each	Cadaveric	47	5 yr OS: 79%
Roayaie <i>et al</i> <sup>[19]</sup>	2002	Any number of lesions, 5-7 cm each	Cadaveric	43	5 yr RFS: 55%
Keneteman <i>et al</i> <sup>[20]</sup>	2004	1 lesion $<$ 7.5 cm, or multiple lesions $<$ 5 cm each	Cadaveric	40	4 yr OS: 82.9% 4 yr RFS: 76.8%
Onaca <i>et al</i> <sup>[18]</sup>	2007	1 lesion $\leq$ 6 cm, or 2-4 lesions $\leq$ 5 cm each	Cadaveric	1206	5 yr RFS: 1 lesion $\leq$ 6 cm: 63.9%/or 2-4 lesions 3.1 cm-5 cm each: 64.6%
Soejima <i>et al</i> <sup>[23]</sup>	2007	Any number lesions $\leq$ 5 cm each	Living	67	3 yr OS: 68.6%
Jonas <i>et al</i> <sup>[24]</sup>	2007	Single lesion and diameter, or any number of lesions $\leq$ 6 cm each. Total tumor diameter $\leq$ 15 cm	Living	21	3 yr OS: 53%
Sugawara <i>et al</i> <sup>[25]</sup>	2007	Up to 5 lesions $\leq$ 5 cm each	Living	78	3 yr RFS: 94%
Silva <i>et al</i> <sup>[17]</sup>	2008	1-3 lesions $\leq$ 5 cm each. Total tumor diameter $\leq$ 10 cm	Cadaveric	257	5 yr OS: 67%
Mazzaferro <i>et al</i> <sup>[21]</sup>	2009	The sum of the size and number of tumors not exceeding 7 in the absence of microvascular invasion	Both	1556	5 yr OS: 71.2%

RFS: Recurrence free survival; OS: Overall survival.

to 10 nodules  $\leq$  5 cm each. Five year recurrence was similar for patients within MC, and patients beyond MC but meeting Kyoto criteria at 7% and 4% respectively. Five year survival for patients meeting Kyoto criteria was 89%<sup>[37]</sup>. Takada *et al*<sup>[38]</sup> also propose similar selection criteria. In their cohort of 136 patients, those who met the proposed selection criteria demonstrated a 5 year survival rate of 87%.

Lee *et al*<sup>[39]</sup> proposes the incorporation of 18F-Fluoro-deoxyglucose positron emission tomography (PET) to HCC-LT selection criteria. Retrospective analysis of 2806 patients demonstrated that patients with PET negative scans preoperatively in combination with a total tumor diameter  $\leq$  10 cm demonstrated 5 year overall survival and DFS rates of 73.4% and 80.4% respectively, which was not significantly different from those within MC<sup>[39]</sup>.

Table 2 demonstrates an overview of proposed expanded selection criteria which incorporate biomarkers to morphometric tumor measurements.

### Downstaging and response to LRT

LRT in HCC-LT candidates is considered an element of two approaches: For patients listed/to be listed within MC, LRT is applied neo-adjuvantly as bridging therapy to halt tumor progression<sup>[40]</sup>. Patients who present initially beyond MC are downstaged to reduce tumor size to meet MC<sup>[41]</sup>. Both strategies provide the opportunity to evaluate radiological and laboratory surrogates of tumor response, which could unveil more aggressive tumors with less favorable biology in order to be excluded from LT.

Since tumor behavior over time is a surrogate of tumor biology, LRT followed by a required waiting time before LT can help to unveil tumor biology and has been coined as the "ablate and wait" strategy<sup>[10]</sup>.

A systematic review and pooled analysis of 13 studies revealed the success rate of downstaging ranging between 11%-77%. There was no significant difference in utilizing Transarterial Chemoembolization or Transarterial Radioembolization. Post LT recurrence rates were noted to be as high as 16%, however survival outcomes could

not be calculated due to heterogeneity of the data which prevented adequate analysis. Further investigation is required to determine the effect of heterogeneous downstaging protocols in term of LRT modality, frequency, and waiting period pre- LT<sup>[42]</sup>.

The correlation between the AFP expression in response to LRT and post LT survival has also been investigated. A multicentric study which included 422 patients who underwent LRT before LT for HCC (306 within MC, 116 beyond MC) demonstrated an increased risk for HCC recurrence and death with an AFP slope  $>$  15 ng/mL per month<sup>[43]</sup>.

### Future directions: Molecular signatures

Genetic molecular signatures have been explored for their potential as biomarkers for HCC<sup>[44]</sup>. Dvorchik *et al*<sup>[45]</sup> assessed fractional allelic imbalance rates in a panel of 9 tumor suppressor genes. A higher rate of tumor suppressor gene mutation correlated with worse post-LT outcome independently of tumor vascular invasion or tumor burden<sup>[45]</sup>.

MicroRNA (miRNA) signatures detected in serum exosomes have also been described as potential biomarkers for HCC. In a cohort of 6 HCC patients miR-718 was described as significantly linked to HCC; and this was further validated in a cohort of 59 LDLT HCC cases. In the validation cohort, miR-718 expression levels were significantly lower in patients beyond MC, and with poorer histological differentiation. However, due to the small incidence of recurrence in this cohort, no direct association could be linked to miR-718<sup>[46]</sup>.

Another study analyzed paraffin embedded tissue from 69 HCC LT patients (which included 40 post LT recurrences) for miRNA expression. The biomarker proposed by this study consisted of 67 miRNAs, this biomarker had significantly identified the HCC recurrent cases, and it also displayed significance when applied to patients within and beyond MC<sup>[47]</sup>.

A predictive scoring system was recently published combining MC with miRNA markers to identify the risk of

**Table 2** Expanded criteria that incorporate tumor biomarkers for hepatocellular carcinoma-liver transplantation

Ref.	Year	Morphometric criteria	Biomarker criteria	Donor type	n	Survival
Kwon <i>et al</i> <sup>[34]</sup>	2007	Any number of lesions ≤ 5 cm each	AFP ≤ 400 ng/mL	Living	139	5 yr OS: 79.9%
Takada <i>et al</i> <sup>[38]</sup>	2007	Up to 10 lesions ≤ 5 cm each	PIVKA-II ≤ 400 mAU/mL	Living	136	5 yr OS: 87%
Zheng <i>et al</i> <sup>[29]</sup>	2008	Total tumor diameter ≤ 8 cm or total tumor diameter > 8 cm with histopathologic grade I or II	If total tumor diameter > 8 cm: AFP ≤ 400 ng/mL	Cadaveric	195	5 yr OS: 70.7%, 5 yr DFS: 62.4%
Fujiki <i>et al</i> <sup>[37]</sup>	2009	Up to 10 lesions ≤ 5 cm each	DCP ≤ 400 mAU/mL	Living	144	5 yr OS: 89%
Lai <i>et al</i> <sup>[31]</sup>	2012	Total tumor diameter ≤ 8 cm	AFP ≤ 400 ng/mL	Cadaveric	158	5 yr DFS: 74.4%
Grat <i>et al</i> <sup>[36]</sup>	2014	UCSF or Up-to-7 criteria	AFP < 100 ng/mL	Cadaveric	121	5 yr OS: 100%
Toso <i>et al</i> <sup>[35]</sup>	2015	Total tumor volume ≤ 115 cm <sup>3</sup>	AFP ≤ 400 ng/mL	Cadaveric	166	4 yr OS: 74.6%
Lee <i>et al</i> <sup>[39]</sup>	2015	Total tumor diameter ≤ 10 cm	PET/CT negative uptake	Living	280	5 yr OS: 73.4%, 5 yr DFS: 80.4%

AFP: Alpha fetal protein; UCSF: University of California, San Francisco; DFS: Disease free survival; PIVKA-II: Protein induced by vitamin K absence or antagonism factor II; OS: Overall survival.

HCC recurrence post- LT. Two miRNA markers significant of tumor recurrence (miR-214, miR-3187) were identified *via* microarray analysis of paraffin explant samples of 40 patients. In another validation cohort of 22 patients, high expression of miR-214 and low expression of miR-3187 were significantly associated with HCC recurrence. A predictive score including levels of these miRNAs and MC status was successful in identifying patients with a lower risk for tumor recurrence and death<sup>[48]</sup>.

## CONCLUSION

Although there remains a large discrepancy between cadaveric organ availability and demand, numerous selection criteria for HCC exceeding the well-established MC have been proposed worldwide. Only a few of these criteria have been validated by multiple independent studies. The current direction of incorporating biomarkers and other surrogates of tumor biology to morphometric criteria is highly encouraged, however this is not without challenge. The most commonly used HCC biomarker AFP, is not a reliable indicator for HCC. AFP levels are not elevated in up to 40% of cases<sup>[49,50]</sup>, furthermore AFP is challenged by its poor sensitivity and specificity<sup>[51]</sup>. Pre-LT tumor biopsy is somehow discouraged, due in part to tumor heterogeneity when multifocal HCC is present, as well as the risk of needle-tract seeding<sup>[52]</sup>.

In light of the current organ shortage, hepatic resection followed by salvage LT has also been suggested as a treatment strategy for HCC. A systematic review by Chan *et al*<sup>[53]</sup> demonstrated median overall survival at 1-, 3- and 5-years post LT was 89%, 80%, and 62% respectively. Additionally, tissue specimens obtained from a pre-LT resection can assist in selection of tumors with a favorable histopathological profile for LT<sup>[53]</sup>.

Monitoring radiologic and laboratory (tumor markers) tumor response post-LRT has been utilized to identify tumors with favorable biology; and in line with this current UNOS guidelines for organ allocation in the United States require listing HCC patients for 6 mo before qualification for HCC exception points<sup>[54]</sup>.

miRNAs are stable in blood and resistant to RNAases,

which makes them promising HCC biomarkers<sup>[46]</sup>. Further validation of extended HCC-LT criteria models that incorporate predictors of tumor biology are needed to optimize organ utilization in an ongoing era of organ shortage.

## REFERENCES

- 1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]
- 2 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]
- 3 **Freeman RB Jr**, Steffick DE, Guidinger MK, Farmer DG, Berg CL, Merion RM. Liver and intestine transplantation in the United States, 1997-2006. *Am J Transplant* 2008; **8**: 958-976 [PMID: 18336699 DOI: 10.1111/j.1600-6143.2008.02174.x]
- 4 **Martin P**, DiMartini A, Feng S, Brown R Jr, Fallon M. Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Hepatology* 2014; **59**: 1144-1165 [PMID: 24716201 DOI: 10.1002/hep.26972]
- 5 **Iwatsuki S**, Gordon RD, Shaw BW Jr, Starzl TE. Role of liver transplantation in cancer therapy. *Ann Surg* 1985; **202**: 401-407 [PMID: 2996449 DOI: 10.1097/00000658-198510000-00001]
- 6 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]
- 7 **Mazzaferro V**, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, Mariani L. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 2011; **17** Suppl 2: S44-S57 [PMID: 21695773 DOI: 10.1002/lt.22365]
- 8 **Cescon M**, Cucchetti A, Ravaioli M, Pinna AD. Hepatocellular carcinoma locoregional therapies for patients in the waiting list. Impact on transplantability and recurrence rate. *J Hepatol* 2013; **58**: 609-618 [PMID: 23041304 DOI: 10.1016/j.jhep.2012.09.021]
- 9 **Lencioni R**, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: 20175033 DOI: 10.1055/s-0030-1247132]
- 10 **Roberts JP**, Venook A, Kerlan R, Yao F. Hepatocellular carcinoma: Ablate and wait versus rapid transplantation. *Liver Transpl* 2010; **16**: 925-929 [PMID: 20658555 DOI: 10.1002/lt.22103]
- 11 **Merani S**, Majno P, Kneteman NM, Berney T, Morel P, Mentha G, Toso C. The impact of waiting list alpha-fetoprotein changes

- on the outcome of liver transplant for hepatocellular carcinoma. *J Hepatol* 2011; **55**: 814-819 [PMID: 21334400 DOI: 10.1016/j.jhep.2010.12.040]
- 12 **Yao FY**, Ferrell L, Bass NM, Bacchetti P, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: comparison of the proposed UCSF criteria with the Milan criteria and the Pittsburgh modified TNM criteria. *Liver Transpl* 2002; **8**: 765-774 [PMID: 12200775 DOI: 10.1053/jlts.2002.34892]
  - 13 **Yao FY**, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403 [PMID: 11391528 DOI: 10.1053/jhep.2001.24563]
  - 14 **Yao FY**, Xiao L, Bass NM, Kerlan R, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. *Am J Transplant* 2007; **7**: 2587-2596 [PMID: 17868066 DOI: 10.1111/j.1600-6143.2007.01965.x]
  - 15 The Transplantation Society Of Australia and New Zealand Organ Transplantation from Deceased Donors: Consensus Statement on Eligibility Criteria and Allocation Protocols. [updated 2015 Apr 15; accessed 2016 Mar 28]. Available from: URL: [http://www.tsanz.com.au/organallocationprotocols/documents/CSVs1.4\\_V4\\_Final.pdf](http://www.tsanz.com.au/organallocationprotocols/documents/CSVs1.4_V4_Final.pdf)
  - 16 **Herrero JI**, Sangro B, Quiroga J, Pardo F, Herraiz M, Cienfuegos JA, Prieto J. Influence of tumor characteristics on the outcome of liver transplantation among patients with liver cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2001; **7**: 631-636 [PMID: 11460231 DOI: 10.1053/jlts.2001.25458]
  - 17 **Silva M**, Moya A, Berenguer M, Sanjuan F, López-Andujar R, Pareja E, Torres-Quevedo R, Aguilera V, Montalva E, De Juan M, Mattos A, Prieto M, Mir J. Expanded criteria for liver transplantation in patients with cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2008; **14**: 1449-1460 [PMID: 18825681 DOI: 10.1002/lt.21576]
  - 18 **Onaca N**, Davis GL, Goldstein RM, Jennings LW, Klintmalm GB. Expanded criteria for liver transplantation in patients with hepatocellular carcinoma: a report from the International Registry of Hepatic Tumors in Liver Transplantation. *Liver Transpl* 2007; **13**: 391-399 [PMID: 17318865 DOI: 10.1002/lt.21095]
  - 19 **Roayaie S**, Frischer JS, Emre SH, Fishbein TM, Sheiner PA, Sung M, Miller CM, Schwartz ME. Long-term results with multimodal adjuvant therapy and liver transplantation for the treatment of hepatocellular carcinomas larger than 5 centimeters. *Ann Surg* 2002; **235**: 533-539 [PMID: 11923610 DOI: 10.1097/0000658-200204000-00012]
  - 20 **Kneteman NM**, Oberholzer J, Al Saghier M, Meeberg GA, Blitz M, Ma MM, Wong WW, Gutfreund K, Mason AL, Jewell LD, Shapiro AM, Bain VG, Bigam DL. Sirolimus-based immunosuppression for liver transplantation in the presence of extended criteria for hepatocellular carcinoma. *Liver Transpl* 2004; **10**: 1301-1311 [PMID: 15376305 DOI: 10.1002/lt.20237]
  - 21 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P; Metroticket Investigator Study Group. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045[08]70284-5]
  - 22 **Bhangui P**, Vibert E, Majno P, Salloum C, Andreani P, Zocrato J, Ichai P, Saliba F, Adam R, Castaing D, Azoulay D. Intention-to-treat analysis of liver transplantation for hepatocellular carcinoma: living versus deceased donor transplantation. *Hepatology* 2011; **53**: 1570-1579 [PMID: 21520172 DOI: 10.1002/hep.24231]
  - 23 **Soejima Y**, Taketomi A, Yoshizumi T, Uchiyama H, Aishima S, Terashi T, Shimada M, Maehara Y. Extended indication for living donor liver transplantation in patients with hepatocellular carcinoma. *Transplantation* 2007; **83**: 893-899 [PMID: 17460559 DOI: 10.1097/01.tp.0000259015.46798.ec]
  - 24 **Jonas S**, Mittler J, Pascher A, Schumacher G, Theruvath T, Benckert C, Rudolph B, Neuhaus P. Living donor liver transplantation of the right lobe for hepatocellular carcinoma in cirrhosis in a European center. *Liver Transpl* 2007; **13**: 896-903 [PMID: 17538994 DOI: 10.1002/lt.21189]
  - 25 **Sugawara Y**, Tamura S, Makuuchi M. Living donor liver transplantation for hepatocellular carcinoma: Tokyo University series. *Dig Dis* 2007; **25**: 310-312 [PMID: 17960065 DOI: 10.1159/000106910]
  - 26 **Hameed B**, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level & gt; 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. *Liver Transpl* 2014; **20**: 945-951 [PMID: 24797281 DOI: 10.1002/lt.23904]
  - 27 **Hakamada K**, Kimura N, Miura T, Morohashi H, Ishido K, Nara M, Toyoki Y, Narumi S, Sasaki M. Des-gamma-carboxy prothrombin as an important prognostic indicator in patients with small hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 1370-1377 [PMID: 18322950 DOI: 10.3748/wjg.14.1370]
  - 28 **Berry K**, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. *Liver Transpl* 2013; **19**: 634-645 [PMID: 23536495 DOI: 10.1002/lt.23652]
  - 29 **Zheng SS**, Xu X, Wu J, Chen J, Wang WL, Zhang M, Liang TB, Wu LM. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation* 2008; **85**: 1726-1732 [PMID: 18580463 DOI: 10.1097/TP.0b013e31816b67e4]
  - 30 **Xu X**, Lu D, Ling Q, Wei X, Wu J, Zhou L, Yan S, Wu L, Geng L, Ke Q, Gao F, Tu Z, Wang W, Zhang M, Shen Y, Xie H, Jiang W, Wang H, Zheng S. Liver transplantation for hepatocellular carcinoma beyond the Milan criteria. *Gut* 2016; **65**: 1035-1041 [PMID: 25804634 DOI: 10.1136/gutjnl-2014-308513]
  - 31 **Lai Q**, Avolio AW, Manzia TM, Sorge R, Agnes S, Tisone G, Berloco PB, Rossi M. Combination of biological and morphological parameters for the selection of patients with hepatocellular carcinoma waiting for liver transplantation. *Clin Transplant* 2012; **26**: E125-E131 [PMID: 22192083 DOI: 10.1111/j.1399-0012.2011.01572.x]
  - 32 **Duvoux C**, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, Francoz C, Compagnon P, Vanlemmens C, Dumortier J, Dharancy S, Gugenheim J, Bernard PH, Adam R, Radene S, Muscari F, Conti F, Hardwigsen J, Pageaux GP, Chazouillères O, Salame E, Hilleret MN, Lebray P, Abergel A, Debette-Gratien M, Kluger MD, Mallat A, Azoulay D, Cherqui D; Liver Transplantation French Study Group. Liver transplantation for hepatocellular carcinoma: a model including  $\alpha$ -fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012; **143**: 986-994.e3; quiz e14-e15 [PMID: 22750200 DOI: 10.1053/j.gastro.2012.05.052]
  - 33 **Todo S**, Furukawa H, Tada M; Japanese Liver Transplantation Study Group. Extending indication: role of living donor liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2007; **13**: S48-S54 [PMID: 17969069 DOI: 10.1002/lt.21334]
  - 34 **Kwon CH**, Kim DJ, Han YS, Park JB, Choi GS, Kim SJ, Joh JW, Lee SK. HCC in living donor liver transplantation: can we expand the Milan criteria? *Dig Dis* 2007; **25**: 313-319 [PMID: 17960066 DOI: 10.1159/000106911]
  - 35 **Toso C**, Meeberg G, Hernandez-Alejandro R, Dufour JF, Marotta P, Majno P, Kneteman NM. Total tumor volume and alpha-fetoprotein for selection of transplant candidates with hepatocellular carcinoma: A prospective validation. *Hepatology* 2015; **62**: 158-165 [PMID: 25777590 DOI: 10.1002/hep.27787]
  - 36 **Grąt M**, Kornasiewicz O, Lewandowski Z, Hołowko W, Grąt K, Kobryń K, Patkowski W, Zieniewicz K, Krawczyk M. Combination of morphologic criteria and  $\alpha$ -fetoprotein in selection of patients with hepatocellular carcinoma for liver transplantation minimizes the problem of posttransplant tumor recurrence. *World J Surg* 2014; **38**: 2698-2707 [PMID: 24858191 DOI: 10.1007/

- 00268-014-2647-3]
- 37 **Fujiki M**, Takada Y, Ogura Y, Oike F, Kaido T, Teramukai S, Uemoto S. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. *Am J Transplant* 2009; **9**: 2362-2371 [PMID: 19656125 DOI: 10.1111/j.1600-6143.2009.02783.x]
  - 38 **Takada Y**, Ito T, Ueda M, Sakamoto S, Haga H, Maetani Y, Ogawa K, Ogura Y, Oike F, Egawa H, Uemoto S. Living donor liver transplantation for patients with HCC exceeding the Milan criteria: a proposal of expanded criteria. *Dig Dis* 2007; **25**: 299-302 [PMID: 17960063]
  - 39 **Lee SD**, Kim SH, Kim SK, Kim YK, Park SJ. Clinical Impact of 18F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography in Living Donor Liver Transplantation for Advanced Hepatocellular Carcinoma. *Transplantation* 2015; **99**: 2142-2149 [PMID: 25905981 DOI: 10.1097/TP.0000000000000719]
  - 40 **Otto G**, Herber S, Heise M, Lohse AW, Mönch C, Bittinger F, Hoppe-Lotichius M, Schuchmann M, Victor A, Pitton M. Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. *Liver Transpl* 2006; **12**: 1260-1267 [PMID: 16826556 DOI: 10.1002/lt.20837]
  - 41 **Yao FY**, Kerlan RK, Hirose R, Davern TJ, Bass NM, Feng S, Peters M, Terrault N, Freise CE, Ascher NL, Roberts JP. Excellent outcome following down-staging of hepatocellular carcinoma prior to liver transplantation: an intention-to-treat analysis. *Hepatology* 2008; **48**: 819-827 [PMID: 18688876 DOI: 10.1002/hep.22412]
  - 42 **Parikh ND**, Waljee AK, Singal AG. Downstaging hepatocellular carcinoma: A systematic review and pooled analysis. *Liver Transpl* 2015; **21**: 1142-1152 [PMID: 25981135 DOI: 10.1002/lt.24169]
  - 43 **Lai Q**, Avolio AW, Graziadei I, Otto G, Rossi M, Tisone G, Goffette P, Vogel W, Pitton MB, Lerut J; European Hepatocellular Cancer Liver Transplant Study Group. Alpha-fetoprotein and modified response evaluation criteria in solid tumors progression after locoregional therapy as predictors of hepatocellular cancer recurrence and death after transplantation. *Liver Transpl* 2013; **19**: 1108-1118 [PMID: 23873764 DOI: 10.1002/lt.23706]
  - 44 **Woo HG**, Park ES, Thorgeirsson SS, Kim YJ. Exploring genomic profiles of hepatocellular carcinoma. *Mol Carcinog* 2011; **50**: 235-243 [PMID: 21465573 DOI: 10.1002/mc.20691]
  - 45 **Dvorchik I**, Schwartz M, Fiel MI, Finkelstein SD, Marsh JW. Fractional allelic imbalance could allow for the development of an equitable transplant selection policy for patients with hepatocellular carcinoma. *Liver Transpl* 2008; **14**: 443-450 [PMID: 18266211 DOI: 10.1002/lt.21393]
  - 46 **Sugimachi K**, Matsumura T, Hirata H, Uchi R, Ueda M, Ueo H, Shinden Y, Iguchi T, Eguchi H, Shirabe K, Ochiya T, Maehara Y, Mimori K. Identification of a bona fide microRNA biomarker in serum exosomes that predicts hepatocellular carcinoma recurrence after liver transplantation. *Br J Cancer* 2015; **112**: 532-538 [PMID: 25584485 DOI: 10.1038/bjc.2014.621]
  - 47 **Barry CT**, D'Souza M, McCall M, Safadjou S, Ryan C, Kashyap R, Marroquin C, Orloff M, Almudevar A, Godfrey TE. Micro RNA expression profiles as adjunctive data to assess the risk of hepatocellular carcinoma recurrence after liver transplantation. *Am J Transplant* 2012; **12**: 428-437 [PMID: 22008552 DOI: 10.1111/j.1600-6143.2011.03788.x]
  - 48 **Liese J**, Peveling-Oberhag J, Doering C, Schnitzbauer AA, Herrmann E, Zangos S, Hansmann ML, Moench C, Welker MW, Zeuzem S, Bechstein WO, Ulrich F. A possible role of microRNAs as predictive markers for the recurrence of hepatocellular carcinoma after liver transplantation. *Transpl Int* 2016; **29**: 369-380 [PMID: 26697811 DOI: 10.1111/tri.12733]
  - 49 **Chen DS**, Sung JL, Sheu JC, Lai MY, How SW, Hsu HC, Lee CS, Wei TC. Serum alpha-fetoprotein in the early stage of human hepatocellular carcinoma. *Gastroenterology* 1984; **86**: 1404-1409 [PMID: 6201411]
  - 50 **Sherman M**. Alpha-fetoprotein: an obituary. *J Hepatol* 2001; **34**: 603-605 [PMID: 11394662]
  - 51 **Waghay A**, Murali AR, Menon KN. Hepatocellular carcinoma: From diagnosis to treatment. *World J Hepatol* 2015; **7**: 1020-1029 [PMID: 26052391 DOI: 10.4254/wjh.v7.i8.1020]
  - 52 **Durand F**, Belghiti J, Paradis V. Liver transplantation for hepatocellular carcinoma: role of biopsy. *Liver Transpl* 2007; **13**: S17-S23 [PMID: 17969095 DOI: 10.1002/lt.21326]
  - 53 **Chan DL**, Alzahrani NA, Morris DL, Chua TC. Systematic review of efficacy and outcomes of salvage liver transplantation after primary hepatic resection for hepatocellular carcinoma. *J Gastroenterol Hepatol* 2014; **29**: 31-41 [PMID: 24117517 DOI: 10.1111/jgh.12399]
  - 54 **Wedd JP**, Nordstrom E, Nydam T, Durham J, Zimmerman M, Johnson T, Thomas Purcell W, Biggins SW. Hepatocellular carcinoma in patients listed for liver transplantation: Current and future allocation policy and management strategies for the individual patient. *Liver Transpl* 2015; **21**: 1543-1552 [PMID: 26457885 DOI: 10.1002/lt.24356]

**P- Reviewer:** Bouras AF, Dondossola D

**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Li D





2016 Hepatocellular Carcinoma: Global view

## Contribution of alpha-fetoprotein in liver transplantation for hepatocellular carcinoma

Bérénice Charrière, Charlotte Maulat, Bertrand Suc, Fabrice Muscari

Bérénice Charrière, Charlotte Maulat, Bertrand Suc, Fabrice Muscari, Department of Visceral Surgery, Toulouse-Rangueil University Hospital, 31059 Toulouse, France

Fabrice Muscari, Service de Chirurgie Digestive, CHU Toulouse Rangueil, 31059 Toulouse, France

**Author contributions:** Charrière B, Maulat C and Muscari F performed the research and wrote the paper; Suc B revised the manuscript for important intellectual content.

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Fabrice Muscari, Professor, Service de Chirurgie Digestive, CHU Toulouse Rangueil, 1 Avenue du Pr. Jean Poulhès, Cedex 9, 31059 Toulouse, France. [muscari.f@chu-toulouse.fr](mailto:muscari.f@chu-toulouse.fr)  
 Telephone: +33-561-322088  
 Fax: +33-561-322936

**Received:** March 27, 2016

**Peer-review started:** March 28, 2016

**First decision:** May 17, 2016

**Revised:** May 30, 2016

**Accepted:** June 27, 2016

**Article in press:** June 29, 2016

**Published online:** July 28, 2016

### Abstract

Alpha-fetoprotein (AFP) is the main tumor biomarker available for the management of hepatocellular carcinoma (HCC). Although it is neither a good screening test nor an accurate diagnostic tool for HCC, it seems to be a possible prognostic marker. However, its contribution in liver transplantation for HCC has not been fully determined, although its use to predict recurrence after liver transplantation has been underlined by international societies. In an era of organ shortages, it could also have a key role in the selection of patients eligible for liver transplantation. Yet unanswered questions remain. First, the cut-off value of serum AFP above which liver transplantation should not be performed is still a subject of debate. We show that a concentration of 1000 ng/mL could be an exclusion criterion, whereas values of < 15 ng/mL indicate patients with an excellent prognosis whatever the size and number of tumors. Monitoring the dynamics of AFP could also prove useful. However, evidence is lacking regarding the values that should be used. Today, the real input of AFP seems to be its integration into new criteria to select patients eligible for a liver transplantation. These recent tools have associated AFP values with morphological criteria, thus refining pre-existing criteria, such as Milan, University of California, San Francisco, or "up-to-seven". We provide a review of the different criteria submitted within the past years. Finally, AFP can be used to monitor recurrence after transplantation, although there is little evidence to support this claim. Future challenges will be to draft new international guidelines to implement the use of AFP as a selection tool, and to determine a clear cut-off value above which liver transplantation should not be performed.

**Key words:** Hepatocellular carcinoma; Downstaging; Alpha-fetoprotein; Liver transplantation; Selection criteria

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Alpha-fetoprotein (AFP) is the main biomarker available for the management of hepatocellular carcinoma (HCC). Yet, its contribution in liver transplantation for HCC has not been fully determined. We discuss the interest of AFP as a prognostic factor to predict tumor recurrence after liver transplantation, and as a selection tool to assess the best candidates to receive a graft. We also provide an overview of the different ways that AFP could be included in decisional algorithms before liver transplantation, through its static and dynamic values.

Charrière B, Maulat C, Suc B, Muscari F. Contribution of alpha-fetoprotein in liver transplantation for hepatocellular carcinoma. *World J Hepatol* 2016; 8(21): 881-890 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i21/881.htm> DOI: <http://dx.doi.org/10.4254/wjgh.v8.i21.881>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the second most common cause of death from cancer worldwide. It is estimated to have caused nearly 745000 deaths in 2012<sup>[1]</sup>. It represents a frequent indication for liver transplantation (LT). Good results are now achieved by accurate selection of patients. The Milan criteria (MC) are considered as the reference by health systems worldwide and are currently used by the United Network for Organ Sharing<sup>[2-4]</sup>. The overall survival rates after LT for HCC range from 65% to 80% at 5 years for patients fulfilling these criteria<sup>[5-7]</sup>. As the incidence of HCC is currently rising, several teams have attempted to extend the selection criteria in order to treat more patients: *i.e.*, University of California, San Francisco (UCSF), "up-to-seven", or "5/5" criteria<sup>[8-11]</sup>. These criteria are all based on the number and the size of nodules, but other features can influence recurrence rate after LT. Among these, histopathologic findings, poor differentiation, and microvascular invasion are negative prognostic factors<sup>[12-14]</sup>. However, data on these are difficult to obtain before transplantation. Therefore, we need preoperative prognostic elements to help improve the selection of patients eligible for LT. Today, alpha-fetoprotein (AFP) is the main tumor biomarker available to manage HCC<sup>[15]</sup>. It has many advantages, as it is simple to use, relatively inexpensive, and is widely available. In this article, we discuss the contribution of AFP in LT in HCC. First we assess its value as a screening and diagnosing tool, then we focus on its prognostic relevance, and finally we analyze its interest for the selection of the best candidates to receive a graft.

## AFP: WHAT IS IT?

AFP is a 67-kDa glycoprotein that is produced in early

fetal life by the liver and by a variety of tumors including HCC, hepatoblastoma, and non-seminomatous germ-cell tumors of the ovary and testis (*e.g.*, yolk sac and embryonal carcinoma). Tumor cells synthesize fetal proteins because of the "de-differentiation" of adult hepatocytes<sup>[16]</sup>. During fetal life, AFP is synthesized at first by the yolk sac, then by the liver. By the end of the first trimester, the fetal liver produces nearly all of the AFP. Although synthesis is reduced markedly shortly after birth, small amounts of AFP continue to be produced during adulthood<sup>[17]</sup>. Normal concentrations of AFP in adult serum are  $\leq 20$  ng/mL. AFP can increase temporarily in cases of liver injury or regeneration, particularly after liver resection, during fulminant viral hepatitis, or chronic viral hepatitis<sup>[18,19]</sup>. Patients with chronic hepatitis or cirrhosis and persistently elevated AFP levels are at higher risk of developing HCC<sup>[20-22]</sup>. More than the AFP rate at a given time, it is the increased expression of AFP that suggests the presence of HCC<sup>[23]</sup>.

Up to 20% of cases of HCC do not produce AFP<sup>[24]</sup>. For others, AFP can raise from normal to  $\geq 100000$   $\mu\text{g/L}$ <sup>[25]</sup>. AFP concentrations do not differ if HCC is developed on a cirrhotic liver or not. Serum AFP levels increase by 20%-80% in patients with HCC and are strongly related to tumor aggressiveness<sup>[26-28]</sup>. Its concentrations are correlated with tumor size, microvascular invasion and poorly differentiated HCC<sup>[15,20,29,30]</sup>. However, the utility of AFP is restricted by the existence of non-AFP-secreting tumors<sup>[24]</sup>.

## AFP: A POOR MARKER FOR SCREENING AND DIAGNOSING HCC AMONG PATIENTS ON A LT-WAITING LIST

### Use of AFP for HCC screening

Literature has shown that serum AFP ( $> 15$  or  $20$  ng/mL) as a screening test for HCC had a sensitivity of between 39% and 64%, and a specificity of between 76% and 91%. The positive predictive value is estimated at between 9% and 33%<sup>[20,31-33]</sup>.

The association of AFP with ultrasonography only improved the sensitivity by 6%-7% and the specificity by 2% compared to ultrasonography alone<sup>[31,34]</sup>, while also increasing the cost of HCC screening<sup>[35]</sup>.

These results clearly show that AFP is not a useful screening tool for HCC<sup>[36]</sup>. The first reason is that fluctuating levels of AFP in patients with cirrhosis can reflect flare-ups of HBV or HCV infection, or exacerbation of an underlying liver disease other than HCC development<sup>[7,37]</sup>. In addition, only a small proportion of tumors at an early stage (10%-20%) present with abnormal AFP serum levels<sup>[7]</sup>.

Current guidelines from the American Association for the Study of Liver Disease and the European Association for the Study of the Liver (EASL) have stopped recommending the use of AFP anymore to screen for HCC in cirrhotic patients. Only ultrasonography must be performed every 6 mo<sup>[7,38]</sup>.

### AFP for the diagnosis of HCC

In a case-control study of 340 cirrhotic patients, Trevisani *et al.*<sup>[39]</sup> have shown that AFP levels of > 20 ng/mL had a sensitivity of 60% and a specificity of 91% to diagnose HCC. At this threshold, 40% of all cases of HCC would be missed. An increase in this cut-off value would result in a lower rate of HCC detection whereas a lower cut-off value would increase the false-positive rate. These results demonstrate that AFP should not be used to diagnose HCC. Thus, AFP is no longer part of the diagnostic algorithm for HCC<sup>[7,38]</sup>.

## AFP: A PREDICTOR OF RECURRENCE AFTER LT

Although AFP is no longer used to diagnose HCC, several teams have shown that it could be a very interesting tool for prognosis<sup>[40,41]</sup>.

Thus, it could prove useful when discussing LT. Shetty *et al.*<sup>[42]</sup> in 2004, were among the first to suggest the potential prognostic usefulness of AFP when used specifically for patients who have received a liver graft. In their study, they have shown that elevated serum levels of AFP before LT were significantly associated with poorer recurrence-free survival and overall survival. In the following years, multiple studies have confirmed the prognostic role of AFP to predict outcomes after LT. Most of them are based on small cohorts of patients<sup>[28,43-47]</sup> and their main drawbacks are their retrospective designs. Yet all of them display the same tendency: Elevated AFP at the time of LT is associated with a worse prognosis after LT. Between 2008 and 2011, three large cohort studies that included thousands of patients, also showed the same pattern<sup>[48-50]</sup>. As a result, the EASL-EORTC advises on the prognostic relevance of AFP in their Clinical Practice Guidelines for the management of HCC<sup>[7]</sup>. Nevertheless, AFP alone is not sufficient to predict recurrence. Its interpretation must be associated with other demonstrated prognostic factors such as histopathologic findings, tumor differentiation, and microvascular invasion<sup>[12-14]</sup>.

## USE OF AFP TO SELECT LT CANDIDATES

Although the prognostic value of AFP seems well established today, one issue remains: How can we use AFP to improve the selection of LT candidates and ensure acceptable outcomes?

This question raises other issues: What cut-off value must we use to define an “elevated” level of AFP? Is it important to consider the evolution of AFP over time? Can AFP be included in an algorithm to help assess the best candidates for LT?

### Defining a cut-off value for AFP

To this day, there is no clear consensus regarding the level of AFP above which a patient should not be a

candidate for LT. The international consensus report regarding liver transplantation, published in 2012, mentions that “AFP concentration adds prognostic information in HCC patients and may be used for making decisions regarding transplantation”<sup>[4]</sup>, but with a weak level of evidence. According to these recommendations, whatever the level of AFP, LT can be considered as long as a patient fits within the Milan, UCSF, “up-to-seven” or “5/5” criteria<sup>[2,8,11,51]</sup>.

More than 20 studies have tried to define a cut-off value for pre-LT AFP, above which the prognosis would be too impaired to propose a LT. The main studies are reported Table 1. Several values have been studied, ranging from 15 ng/mL<sup>[52,53]</sup> to 1000 ng/mL<sup>[30,45,54-57]</sup>. Three reviews have also focused on the static values of AFP in an attempt to synthesize these various findings<sup>[58-60]</sup>, but none have been designed as a meta-analysis and thus no clear conclusion could be drawn.

However, three values appear repeatedly in the different studies: 15 ng/mL, 400 ng/mL and 1000 ng/mL.

The value of 15 ng/mL is interesting because it could indicate a population with a very good prognosis, even for patients with HCC graded beyond the MC. Lai *et al.*<sup>[52]</sup> and Berry *et al.*<sup>[53]</sup> report almost identical conclusions regarding this 15 ng/mL cut-off point: Patients outside the MC but with AFP < 15 ng/mL and no other adverse prognostic factors have excellent outcomes after a LT. This suggests that, in some cases, AFP could be used to select people with excellent outcomes and who would have been unfairly excluded from receiving a LT because they exceeded the MC.

The value of 1000 ng/mL appears as a value that should exclude patients from receiving a LT, at least in the absence of downstaging. Yao *et al.*<sup>[8]</sup>, when defining UCSF criteria in 2001, had already pointed out that an AFP of > 1000 ng/mL was related to a worse outcome, but only in univariate analyses. Later, the same team published a study concluding that AFP > 1000 ng/mL was an independent predictor of vascular invasion and should be an exclusion criterion for LT<sup>[30]</sup>. According to their study, using this cut-off value could have led to the exclusion of 4.7% of patients from receiving a LT, while decreasing tumor recurrence by 20%. Other publications observed that an AFP > 1000 ng/mL was a predictor of recurrence after a LT<sup>[45,55,61]</sup>. In 2012, Duvoux *et al.*<sup>[57]</sup> proposed a score that integrated AFP for the selection of patients eligible for LT. The value of 1000 ng/mL automatically led to the exclusion of these patients. In France, Duvoux’s algorithm is currently in use and an AFP value of 1000 ng/mL is recognized as a limit over which a LT should not be performed. The UCSF team now applies a similar policy<sup>[62]</sup>.

What about the values in between 15 and 1000 ng/mL? Several cut-off values have been studied over the last few years. The endpoints differ between studies: Some teams have studied the relationships between AFP and recurrence, whereas other have focused on the relationships between AFP and microvascular

**Table 1** Main studies suggesting a cut-off value for  $\alpha$ -fetoprotein when selecting candidates for liver transplantation

Ref.	Year	No. of patients	Country	Study design	AFP cut-off value	Endpoint
Yamashiki <i>et al</i> <sup>[43]</sup>	2004	93	United States	Prospective	100 ng/mL	Drop-out from list
Shetty <i>et al</i> <sup>[42]</sup>	2004	109	United States	Retrospective	300 ng/mL	Recurrence, death
Todo <i>et al</i> <sup>[54]</sup>	2007	653	Japan	Retrospective	200 ng/mL	Recurrence
Parfitt <i>et al</i> <sup>[61]</sup>	2007	75	Canada	Retrospective	1000 ng/mL	Recurrence
Pérez-Saborido <i>et al</i> <sup>[44]</sup>	2007	95	Spain	Retrospective	200 ng/mL	Recurrence
Onaca <i>et al</i> <sup>[10]</sup>	2007	902	United States	Retrospective	200 ng/mL	Recurrence
Adler <i>et al</i> <sup>[86]</sup>	2008	226	Belgium	Retrospective	100 ng/mL	Recurrence
Zou <i>et al</i> <sup>[45]</sup>	2008	303	China	Retrospective	1000 ng/mL	Fatal recurrence
Ioannou <i>et al</i> <sup>[50]</sup>	2008	5028	United States	Retrospective	455 ng/mL	Death
Xu <i>et al</i> <sup>[46]</sup>	2009	97	China	Retrospective	400 ng/mL	Recurrence
Toso <i>et al</i> <sup>[49]</sup>	2009	6478	Canada	Retrospective	400 ng/mL	Death
Lao <i>et al</i> <sup>[55]</sup>	2009	124	United States	Prospective	1000 ng/mL	Recurrence
Xiao <i>et al</i> <sup>[87]</sup>	2009	224	China	Retrospective	800 ng/mL	Death
McHugh <i>et al</i> <sup>[47]</sup>	2010	101	United States	Retrospective	100 ng/mL	Recurrence, death
Levi <i>et al</i> <sup>[88]</sup>	2010	244	United States	Retrospective	100 ng/mL	Recurrence
Merani <i>et al</i> <sup>[66]</sup>	2011	6817	United States	Retrospective	400 ng/mL	Death
Lai <i>et al</i> <sup>[89]</sup>	2011	153	Italy	Retrospective	210 ng/mL	Recurrence
Mailey <i>et al</i> <sup>[48]</sup>	2011	2253	United States	Retrospective	400 ng/mL	Death
Muscari <i>et al</i> <sup>[28]</sup>	2012	122	France	Retrospective	500 ng/mL	Recurrence, death
Ciccarelli <i>et al</i> <sup>[65]</sup>	2012	137	Belgium	Retrospective	400 ng/mL	Recurrence
Wong <i>et al</i> <sup>[59]</sup>	2013	211	United States	Retrospective	400 ng/mL	Recurrence
Harimoto <i>et al</i> <sup>[90]</sup>	2013	167	Japan	Retrospective	300 ng/mL	Recurrence
Abdel-Wahab <i>et al</i> <sup>[68]</sup>	2013	170	Egypt	Retrospective	200 ng/mL	Recurrence, death
Grąt <i>et al</i> <sup>[67]</sup>	2014	121	Poland	Retrospective	100 ng/mL	Recurrence
Hameed <i>et al</i> <sup>[30]</sup>	2014	211	United States	Retrospective	1000 ng/mL	Microvascular invasion
Lee <i>et al</i> <sup>[91]</sup>	2014	69	South Korea	Retrospective	200 ng/mL	Recurrence
Grąt <i>et al</i> <sup>[92]</sup>	2016	146	Poland	Retrospective	100 ng/mL	Recurrence

AFP: Alpha-fetoprotein.

invasion, or AFP and drop-out rates from waiting lists. The most frequent cut-off value reported in the literature is 400 ng/mL. This has been reported by authors from various countries in Asia<sup>[63]</sup>, Europe<sup>[64,65]</sup> and the United States<sup>[49,59,66]</sup>. It appears to be linked to recurrence but also to the risk of dropout while on a waiting list. However, it seems difficult to use the cut-off value of 400 ng/mL to directly exclude patients from a waiting list, because this value has been mostly studied as part of algorithms that include tumor volume, tumor size, the MC, and/or the UCSF-criteria. Moreover, many other cut-off values have been suggested, such as 100 ng/mL<sup>[47,57,67]</sup> and 200 ng/mL<sup>[10,68]</sup>. The level of evidence to define an optimal value is very weak and thus calls for further studies.

As to which AFP value should be considered, Merani *et al*<sup>[66]</sup> showed that only the last pre-transplant value of AFP independently predicted survival, unlike the AFP at the time of listing. Most of the studies cited above also used the last pre-transplant value of AFP to perform their analyses.

### Evolution of AFP over time: A critical marker

Studies have tried to assess the impact of the dynamic behavior of AFP. They are presented Table 2. The first team to address this issue was Han *et al*<sup>[69]</sup> in 2007. Although focusing on only 47 patients, this Canadian study found out that the preoperative AFP slope was an independent prognostic factor for recurrence, with a

cut-off at 50 ng per month. Later, Vibert *et al*<sup>[70]</sup> studied the outcomes of 153 patients in a monocentric French cohort, and concluded that a progression of AFP of > 15 ng per month was associated with decreased overall survival. Lai *et al*<sup>[52]</sup> in 2013, in a multicentric European study, obtained the same results. A fourth study proposed the cut-off value of 0.1 ng per day<sup>[71]</sup>. The main drawback of these four studies was the small number of data points used to determine the slope of AFP: Only two values were used by Vibert *et al*<sup>[70]</sup> (lowest and highest) and by Lai *et al*<sup>[52]</sup> (time of listing and time of LT). Han *et al*<sup>[69]</sup> used a median of 4 values (ranging from 2 to 11).

Other studies have focused on AFP dynamics, but with a different goal. They have evaluated the prognostic value of AFP evolution after loco-regional therapy. One of the first teams to address this question was Riaz *et al*<sup>[72]</sup> in 2009. They showed that a drop in AFP following loco-regional therapy was associated with better outcomes after LT. Bhat *et al*<sup>[73]</sup> used a logistic regression model to show that a decrease in AFP value after trans-arterial chemoembolization was significantly associated with better overall survival<sup>[73]</sup>. Wong *et al*<sup>[59]</sup> also obtained similar results. These studies enabled AFP to be part of the definition of a successful downstaging, along with radiological features. In fact, Yao *et al*<sup>[62]</sup> in California require that patients with an initial AFP > 1000 ng/mL have AFP decreased to < 500 ng/mL after loco-regional therapy, before undergoing LT. Similarly, in



**Table 2** Studies focusing on dynamic values of  $\alpha$ -fetoprotein before liver transplantation

Ref.	Year	No. of patients	AFP slope
Han <i>et al</i> <sup>[69]</sup>	2007	47	50 ng/mo
Vibert <i>et al</i> <sup>[70]</sup>	2010	153	15 ng/mo
Lai <i>et al</i> <sup>[52]</sup>	2013	422	15 ng/mo
Dumitra <i>et al</i> <sup>[71]</sup>	2013	92	0.1 ng/d

AFP: Alpha-fetoprotein.

France, the use of the Duvoux algorithm enables a patient with an AFP of > 1000 ng/mL to be back on the waiting list if AFP drops below this value<sup>[57]</sup>. Yet, to this day, the international recommendations only mention the number and size of viable tumors as criteria for successful downstaging<sup>[4]</sup>. The AFP concentrations before and after downstaging are just considered as giving “additional information” because evidence is not strong enough to enforce the wider use of AFP dynamics in the management of LT candidates. These recommendations date back from 2012 and they may evolve based on the recent studies mentioned above.

### Designing new scores that integrate AFP: The end of the MC?

If AFP can be used to obtain additional information to select LT candidates, then it appears logical to integrate it into an algorithm, along with other prognostic factors. Since Mazzaferro’s study in 1996<sup>[2]</sup>, attempts have been made to improve the MC. Including AFP to create a new selection tool could be a key.

This idea arose as early as 2007, when a Korean team designed a score based on tumor size, number of tumors, and value of AFP in order to select the best candidates for living donor LT<sup>[56]</sup>. For each feature, the patient was awarded between 1 and 4 points. In this small study ( $n = 63$ ), the different values of AFP used were < 20 ng/mL, 200 ng/mL, and 1000 ng/mL. According to the authors, this score allowed a slight expansion of the MC with comparable outcomes. Five years later, Duvoux *et al*<sup>[57]</sup> developed a very similar score. Their multicentric French study was based on a much larger cohort of patients ( $n = 492$ ), and used the same three characteristics for the selection of patients: *i.e.*, tumor size, number, and AFP. However, the number of points awarded for each feature was different; as were the cut-off values for AFP: *i.e.*, 100 ng/mL and 1000 ng/mL. It is interesting to note that in this latter score, an AFP > 1000 ng/mL provided enough points for patients to be excluded directly from LT, whatever the size and number of tumors. This means that, according to this score, AFP overpowers the MC. In France, Duvoux *et al*<sup>[51]</sup>’s study precluded to a radical change in the allocation policy for LT: This score is now used to select candidates for LT. Patients exceeding the criteria are classed as having a temporary contra-indication as long as a downstaging is not successfully performed. A recent study by Varona *et al*<sup>[74]</sup> has confirmed the accuracy of this model for the

prediction of recurrence and survival after a LT.

Other teams have come up with different scoring systems that include AFP when selecting LT candidates. The main ones are presented in Table 3. In 2008, a Chinese team designed the Hangzhou criteria<sup>[63]</sup>, based on total tumor diameter, AFP, and histopathologic grade. The main issue with this score was the necessity for histopathologic evaluation prior to LT, which is not easy to obtain and may be inaccurate as it is based on a biopsy. Nevertheless, this work raised the idea of total tumor size, rather than maximum size of tumor, or number of tumors. Lai’s team simplified the Hangzhou score and suggested using a score that featured only AFP and total tumor diameter (TTD), with a cut-off value at 400 ng/mL for AFP and 8 cm for TTD<sup>[64]</sup>. Various teams have developed slightly different scores, still using an AFP cut-off value of 400 ng/mL but replacing TTD with total tumor volume<sup>[49,75]</sup> or actual tumor volume<sup>[76]</sup>. More recently, a Korean team suggested that a combination of AFP and F-FDG PET data could be a very interesting selection tool<sup>[77]</sup>: A positive PET (cut-off at 1.10) and an AFP of > 200 ng/mL defined a group of patients with a high risk of recurrence and who should not be selected for LT. The main drawback of this study is the cost of F-FDG PET, but the authors point out the usefulness of PET to predict tumor aggressiveness, rather than sheer size and number.

Despite a few discrepancies, these studies share many common points: All of them agree that an AFP value of > 1000 ng/mL should lead to exclusion of these patients from receiving a LT; most suggest an association between AFP and morphological characteristics (size, number, and/or volume of tumors); and a few of these studies suggest the probable need for another marker for aggressiveness, such as histopathologic findings or PET.

## MONITORING AFP AFTER LIVER TRANSPLANTATION: A WISE POLICY OR A WASTE OF TIME (AND MONEY)?

In the absence of HCC recurrence, AFP levels decrease to < 20 ng/mL within 2 mo post-transplantation<sup>[78]</sup>. Hepatocellular carcinoma recurs in 10%-20% of transplant recipients, despite careful patient selection<sup>[2,7,78-80]</sup>. There is no evidence-based recommendation to be applied after transplantation in order to promptly detect and treat HCC recurrence.

Because few recurrences after LT can benefit from curative treatment, this raises questions about the usefulness of active surveillance after LT<sup>[81,82]</sup>. Roberts<sup>[82]</sup> suggest that screening all patients for HCC recurrence after transplantation, using both imaging and serum biomarkers, is probably not cost effective. However, AFP monitoring, in itself, is not very costly and may be appropriate at regular intervals<sup>[83]</sup>. Yamashiki *et al*<sup>[78]</sup> proposed to measure AFP at monthly periods for the first two years after LT, to detect any HCC recurrence. When a cut-off level of 20 ng/mL was used, the sensitivity and

**Table 3** Suggestions for new selection criteria for liver transplantation that integrate  $\alpha$ -fetoprotein

Ref.	Year	No. of patients	Study design	Criteria	AFP cut-off values
Yang <i>et al</i> <sup>[56]</sup>	2007	63	Retrospective	AFP Tumor size	20 ng/mL, 200 ng/mL, 1000 ng/mL
Zheng <i>et al</i> <sup>[63]</sup>	2008	195	Retrospective	Number of tumors AFP	400 ng/mL
Lai <i>et al</i> <sup>[64]</sup>	2012	158	Retrospective	Total tumor diameter Histopathologic grade AFP	400 ng/mL
Duvoux <i>et al</i> <sup>[57]</sup>	2012	435	Prospective	Total tumor diameter AFP Tumor size	100 ng/mL, 1000 ng/mL
Kashkoush <i>et al</i> <sup>[76]</sup>	2014	115	Retrospective	Number of tumors AFP	400 ng/mL
Toso <i>et al</i> <sup>[75]</sup>	2015	233	Prospective	Actual tumor volume AFP	400 ng/mL
Hong <i>et al</i> <sup>[77]</sup>	2015	123	Retrospective	Total tumor volume AFP F-FDG PET positivity	200 ng/mL

AFP: Alpha-fetoprotein; F-FDG PET: F-fluorodeoxyglucose positron emission tomography.

specificity of AFP to detect HCC recurrence after liver transplantation were 67% and 100%, respectively<sup>[78]</sup>. Several other studies suggest that active surveillance with AFP should be performed, but the optimal frequency is not clear<sup>[83-85]</sup>. Since 2010, international guidelines state that post-transplant monitoring may be performed every 6 to 12 mo, using contrast-enhanced computed tomography or magnetic resonance imaging in addition to AFP measurements<sup>[4]</sup>.

## CONCLUSION

Today, AFP is a key element to consider in the management of patients with HCC and who are eligible for LT. Although it does not contribute to screening or obtaining a diagnosis of HCC among patients on a LT waiting list, it can help predict the aggressiveness of the tumor and its risk of recurrence after LT.

The main usefulness of AFP regarding LT for HCC is its ability to assess the best LT candidates. It can be considered as an excellent selection criterion in association with the size and number of HCC nodules. This enables a reasonable enlargement of the MC while also guaranteeing satisfactory outcomes. Integrating an upper limit of 1000 ng/mL to the selection criteria would also allow exclusion of the few patients within the MC but who have a high risk of recurrence after LT. Furthermore, AFP can be used to monitor the evolution of HCC while on a waiting list, particularly in cases where there is downstaging.

Future challenges lie in the drafting of new international guidelines to implement the use of AFP as a selection tool, and to clarify the exact values that must be considered when using this biomarker in LT for HCC.

## REFERENCES

- 1 Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]
- 2 Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]
- 3 Duffy JP, Vardanian A, Benjamin E, Watson M, Farmer DG, Ghobrial RM, Lipshutz G, Yersiz H, Lu DS, Lassman C, Tong MJ, Hiatt JR, Busuttil RW. Liver transplantation criteria for hepatocellular carcinoma should be expanded: a 22-year experience with 467 patients at UCLA. *Ann Surg* 2007; **246**: 502-509; discussion 509-511 [PMID: 17717454 DOI: 10.1097/SLA.0b013e318148c704]
- 4 Clavien PA, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13**: e11-e22 [PMID: 22047762 DOI: 10.1016/S1473-2045(11)70175-9]
- 5 Mazzaferro V, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, Mariani L. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 2011; **17** Suppl 2: S44-S57 [PMID: 21695773 DOI: 10.1002/lt.22365]
- 6 Yoo HY, Patt CH, Geschwind JF, Thuluvath PJ. The outcome of liver transplantation in patients with hepatocellular carcinoma in the United States between 1988 and 2001: 5-year survival has improved significantly with time. *J Clin Oncol* 2003; **21**: 4329-4335 [PMID: 14581446 DOI: 10.1200/JCO.2003.11.137]
- 7 European Association for Study of Liver; European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *Eur J Cancer* 2012; **48**: 599-641 [PMID: 22424278 DOI: 10.1016/j.ejca.2011.12.021]
- 8 Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403 [PMID: 11391528 DOI: 10.1053/jhep.2001.24563]
- 9 Roayaie S, Frischer JS, Emre SH, Fishbein TM, Sheiner PA, Sung M, Miller CM, Schwartz ME. Long-term results with multimodal adjuvant therapy and liver transplantation for the treatment of hepatocellular carcinomas larger than 5 centimeters. *Ann Surg*

- 2002; **235**: 533-539 [PMID: 11923610]
- 10 **Onaca N**, Davis GL, Goldstein RM, Jennings LW, Klintmalm GB. Expanded criteria for liver transplantation in patients with hepatocellular carcinoma: a report from the International Registry of Hepatic Tumors in Liver Transplantation. *Liver Transpl* 2007; **13**: 391-399 [PMID: 17318865 DOI: 10.1002/lt.21095]
- 11 **Muscari F**, Foppa B, Kamar N, Peron JM, Selves J, Suc B. Liberal selection criteria for liver transplantation for hepatocellular carcinoma. *Br J Surg* 2009; **96**: 785-791 [PMID: 19526621 DOI: 10.1002/bjs.6619]
- 12 **Cha C**, Fong Y, Jarnagin WR, Blumgart LH, DeMatteo RP. Predictors and patterns of recurrence after resection of hepatocellular carcinoma. *J Am Coll Surg* 2003; **197**: 753-758 [PMID: 14585409 DOI: 10.1016/j.jamcollsurg.2003.07.003]
- 13 **Scotton O**, Zalinski S, Terris B, Lefevre JH, Casali A, Massault PP, Conti F, Calmus Y, Soubrane O. Hepatocellular carcinoma developed on compensated cirrhosis: resection as a selection tool for liver transplantation. *Liver Transpl* 2008; **14**: 779-788 [PMID: 18508370 DOI: 10.1002/lt.21431]
- 14 **Jonas S**, Bechstein WO, Steinmüller T, Herrmann M, Radke C, Berg T, Settmacher U, Neuhaus P. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. *Hepatology* 2001; **33**: 1080-1086 [PMID: 11343235 DOI: 10.1053/jhep.2001.23561]
- 15 **Liu C**, Xiao GQ, Yan LN, Li B, Jiang L, Wen TF, Wang WT, Xu MQ, Yang JY. Value of  $\alpha$ -fetoprotein in association with clinicopathological features of hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 1811-1819 [PMID: 23555170 DOI: 10.3748/wjg.v19.i11.1811]
- 16 **Sell S**. Alpha-fetoprotein, stem cells and cancer: how study of the production of alpha-fetoprotein during chemical hepatocarcinogenesis led to reaffirmation of the stem cell theory of cancer. *Tumour Biol* 2008; **29**: 161-180 [PMID: 18612221 DOI: 10.1159/000143402]
- 17 **Tomasi TB**. Structure and function of alpha-fetoprotein. *Annu Rev Med* 1977; **28**: 453-465 [PMID: 67821 DOI: 10.1146/annurev.me.28.020177.002321]
- 18 **Behne T**, Copur MS. Biomarkers for hepatocellular carcinoma. *Int J Hepatol* 2012; **2012**: 859076 [PMID: 22655201 DOI: 10.1155/2012/859076]
- 19 **Pastore G**, Dentico P, Angarano G, Zanetti AR, Ferroni P, Frappampina V, Schiraldi O, Roggendorf M, Frösner G. Hepatitis B virus markers, alpha-fetoprotein and survival in fulminant viral hepatitis. *J Med Virol* 1981; **7**: 97-103 [PMID: 6167671]
- 20 **Tsukuma H**, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328**: 1797-1801 [PMID: 7684822 DOI: 10.1056/NEJM199306243282501]
- 21 **Oka H**, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. *Hepatology* 1994; **19**: 61-66 [PMID: 7506227]
- 22 **Lok AS**, Everhart JE, Wright EC, Di Bisceglie AM, Kim HY, Sterling RK, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Morgan TR. Maintenance peginterferon therapy and other factors associated with hepatocellular carcinoma in patients with advanced hepatitis C. *Gastroenterology* 2011; **140**: 840-889; quiz e12 [PMID: 21129375 DOI: 10.1053/j.gastro.2010.11.050]
- 23 **Arrieta O**, Cacho B, Morales-Espinosa D, Ruelas-Villavicencio A, Flores-Estrada D, Hernández-Pedro N. The progressive elevation of alpha fetoprotein for the diagnosis of hepatocellular carcinoma in patients with liver cirrhosis. *BMC Cancer* 2007; **7**: 28 [PMID: 17288606 DOI: 10.1186/1471-2407-7-28]
- 24 **Ryder SD**. Guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. *Gut* 2003; **52** Suppl 3: iii1-iii8 [PMID: 12692148 DOI: 10.1136/gut.52.suppl\_3.iii1]
- 25 **Koteish A**, Thuluvath PJ. Screening for hepatocellular carcinoma. *J Vasc Interv Radiol* 2002; **13**: S185-S190 [PMID: 12354835]
- 26 **Fujioka M**, Nakashima Y, Nakashima O, Kojiro M. Immunohistologic study on the expressions of alpha-fetoprotein and protein induced by vitamin K absence or antagonist II in surgically resected small hepatocellular carcinoma. *Hepatology* 2001; **34**: 1128-1134 [PMID: 11732002 DOI: 10.1053/jhep.2001.29202]
- 27 **Sherman M**. The resurrection of alphafetoprotein. *J Hepatol* 2010; **52**: 939-940 [PMID: 20395007 DOI: 10.1016/j.jhep.2010.02.006]
- 28 **Muscari F**, Guinard JP, Kamar N, Peron JM, Ota P, Suc B. Impact of preoperative  $\alpha$ -fetoprotein level on disease-free survival after liver transplantation for hepatocellular carcinoma. *World J Surg* 2012; **36**: 1824-1831 [PMID: 22532309 DOI: 10.1007/s00268-012-1587-z]
- 29 **Toro A**, Arditi A, Mannino M, Arcerito MC, Mannino G, Palermo F, Bertino G, Di Carlo I. Effect of pre- and post-treatment  $\alpha$ -fetoprotein levels and tumor size on survival of patients with hepatocellular carcinoma treated by resection, transarterial chemoembolization or radiofrequency ablation: a retrospective study. *BMC Surg* 2014; **14**: 40 [PMID: 24993566 DOI: 10.1186/1471-2482-14-40]
- 30 **Hameed B**, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level > 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. *Liver Transpl* 2014; **20**: 945-951 [PMID: 24797281 DOI: 10.1002/lt.23904]
- 31 **Sherman M**, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology* 1995; **22**: 432-438 [PMID: 7543434]
- 32 **Chen JG**, Parkin DM, Chen QG, Lu JH, Shen QJ, Zhang BC, Zhu YR. Screening for liver cancer: results of a randomised controlled trial in Qidong, China. *J Med Screen* 2003; **10**: 204-209 [PMID: 14738659 DOI: 10.1258/096914103771773320]
- 33 **Pateron D**, Ganne N, Trinchet JC, Aourousseau MH, Mal F, Meicler C, Coderc E, Reboullet P, Beaugrand M. Prospective study of screening for hepatocellular carcinoma in Caucasian patients with cirrhosis. *J Hepatol* 1994; **20**: 65-71 [PMID: 7515408]
- 34 **Singal A**, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, Marrero JA. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. *Aliment Pharmacol Ther* 2009; **30**: 37-47 [PMID: 19392863 DOI: 10.1111/j.1365-2036.2009.04014.x]
- 35 **Zhang B**, Yang B. Combined alpha fetoprotein testing and ultrasonography as a screening test for primary liver cancer. *J Med Screen* 1999; **6**: 108-110 [PMID: 10444731]
- 36 **Gupta S**, Bent S, Kohlwe S. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med* 2003; **139**: 46-50 [PMID: 12834318]
- 37 **Di Bisceglie AM**, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, Wright EC, Everson GT, Lindsay KL, Lok AS, Lee WM, Morgan TR, Ghany MG, Gretch DR. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. *J Hepatol* 2005; **43**: 434-441 [PMID: 16136646]
- 38 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
- 39 **Trevisani F**, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, Domenicali M, De Notariis S, Roda E, Bernardi M. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol* 2001; **34**: 570-575 [PMID: 11394657]
- 40 **Vora SR**, Zheng H, Stadler ZK, Fuchs CS, Zhu AX. Serum alpha-fetoprotein response as a surrogate for clinical outcome in patients receiving systemic therapy for advanced hepatocellular carcinoma. *Oncologist* 2009; **14**: 717-725 [PMID: 19581525 DOI: 10.1634/

- theoncologist.2009-0038]
- 41 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794 DOI: 10.1053/jhep.2003.50047]
- 42 **Shetty K**, Timmins K, Brensinger C, Furth EE, Rattan S, Sun W, Rosen M, Soulen M, Shaked A, Reddy KR, Olthoff KM. Liver transplantation for hepatocellular carcinoma validation of present selection criteria in predicting outcome. *Liver Transpl* 2004; **10**: 911-918 [PMID: 15237377 DOI: 10.1002/lt.20140]
- 43 **Yamashiki N**, Gaynor JJ, Kato T, Reddy KR, Sobhonslidsuk A, Levi D, Nishida S, Madariaga J, Nery J, Schiff ER, Tzakis AG. Competing risks analysis of predictors of delisting owing to tumor progression in liver transplant candidates with hepatocellular carcinoma. *Am J Transplant* 2004; **4**: 774-781 [PMID: 15084174 DOI: 10.1111/j.1600-6143.2004.00412.x]
- 44 **Pérez-Saborido B**, de los Galanes SJ, Menéu-Díaz JC, Romero CJ, Elola-Olaso AM, Suárez YF, Valencia VB, Moreno-González E. Tumor recurrence after liver transplantation for hepatocellular carcinoma: recurrence pathway and prognostic factors. *Transplant Proc* 2007; **39**: 2304-2307 [PMID: 17889172 DOI: 10.1016/j.transproceed.2007.06.059]
- 45 **Zou WL**, Zang YJ, Chen XG, Shen ZY. Risk factors for fatal recurrence of hepatocellular carcinoma and their role in selecting candidates for liver transplantation. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 145-151 [PMID: 18397848]
- 46 **Xu X**, Ke QH, Shao ZX, Wu J, Chen J, Zhou L, Zheng SS. The value of serum alpha-fetoprotein in predicting tumor recurrence after liver transplantation for hepatocellular carcinoma. *Dig Dis Sci* 2009; **54**: 385-388 [PMID: 18563566 DOI: 10.1007/s10620-008-0349-0]
- 47 **McHugh PP**, Gilbert J, Vera S, Koch A, Ranjan D, Gedaly R. Alpha-fetoprotein and tumour size are associated with microvascular invasion in explanted livers of patients undergoing transplantation with hepatocellular carcinoma. *HPB (Oxford)* 2010; **12**: 56-61 [PMID: 20495646 DOI: 10.1111/j.1477-2574.2009.00128.x]
- 48 **Mailey B**, Artinyan A, Khalili J, Denitz J, Sanchez-Luege N, Sun CL, Bhatia S, Nissen N, Colquhoun SD, Kim J. Evaluation of absolute serum  $\alpha$ -fetoprotein levels in liver transplant for hepatocellular cancer. *Arch Surg* 2011; **146**: 26-33 [PMID: 21242442 DOI: 10.1001/archsurg.2010.295]
- 49 **Toso C**, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation for hepatocellular carcinoma utilizing the Scientific Registry of Transplant Recipients database. *Hepatology* 2009; **49**: 832-838 [PMID: 19152426 DOI: 10.1002/hep.22693]
- 50 **Ioannou GN**, Perkins JD, Carithers RL. Liver transplantation for hepatocellular carcinoma: impact of the MELD allocation system and predictors of survival. *Gastroenterology* 2008; **134**: 1342-1351 [PMID: 18471511 DOI: 10.1053/j.gastro.2008.02.013]
- 51 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]
- 52 **Lai Q**, Avolio AW, Graziadei I, Otto G, Rossi M, Tisone G, Goffette P, Vogel W, Pitzon MB, Lerut J. Alpha-fetoprotein and modified response evaluation criteria in solid tumors progression after locoregional therapy as predictors of hepatocellular cancer recurrence and death after transplantation. *Liver Transpl* 2013; **19**: 1108-1118 [PMID: 23873764 DOI: 10.1002/lt.23706]
- 53 **Berry K**, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. *Liver Transpl* 2013; **19**: 634-645 [PMID: 23536495 DOI: 10.1002/lt.23652]
- 54 **Todo S**, Furukawa H, Tada M. Extending indication: role of living donor liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2007; **13**: S48-S54 [PMID: 17969069 DOI: 10.1002/lt.21334]
- 55 **Lao OB**, Weissman J, Perkins JD. Pre-transplant therapy for hepatocellular carcinoma is associated with a lower recurrence after liver transplantation. *Clin Transplant* 2009; **23**: 874-881 [PMID: 19453644 DOI: 10.1111/j.1399-0012.2009.00993.x]
- 56 **Yang SH**, Suh KS, Lee HW, Cho EH, Cho JY, Cho YB, Kim IH, Yi NJ, Lee KU. A revised scoring system utilizing serum alphafetoprotein levels to expand candidates for living donor transplantation in hepatocellular carcinoma. *Surgery* 2007; **141**: 598-609 [PMID: 17462459 DOI: 10.1016/j.surg.2006.11.006]
- 57 **Duvoux C**, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, Francoz C, Compagnon P, Vanlemmens C, Dumortier J, Dharancy S, Gugenheim J, Bernard PH, Adam R, Radenne S, Muscari F, Conti F, Hardwigsen J, Pageaux GP, Chazouillères O, Salame E, Hilleret MN, Lebray P, Aberger A, Debbete-Gratien M, Kluger MD, Mallat A, Azoulay D, Cherqui D. Liver transplantation for hepatocellular carcinoma: a model including  $\alpha$ -fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012; **143**: 986-994.e3; quiz e14-15 [PMID: 22750200 DOI: 10.1053/j.gastro.2012.05.052]
- 58 **Lai Q**, Levi Sandri GB, Lerut J. Selection tool alpha-fetoprotein for patients waiting for liver transplantation: How to easily manage a fractal algorithm. *World J Hepatol* 2015; **7**: 1899-1904 [PMID: 26244064 DOI: 10.4254/wjh.v7.i15.1899]
- 59 **Wong LL**, Naugler WE, Schwartz J, Scott DL, Bhattacharya R, Reyes J, Orloff SL. Impact of locoregional therapy and alpha-fetoprotein on outcomes in transplantation for liver cancer: a UNOS Region 6 pooled analysis. *Clin Transplant* 2013; **27**: E72-E79 [PMID: 23278701 DOI: 10.1111/ctr.12056]
- 60 **Hakeem AR**, Young RS, Marangoni G, Lodge JP, Prasad KR. Systematic review: the prognostic role of alpha-fetoprotein following liver transplantation for hepatocellular carcinoma. *Aliment Pharmacol Ther* 2012; **35**: 987-999 [PMID: 22429190 DOI: 10.1111/j.1365-2036.2012.05060.x]
- 61 **Parfitt JR**, Marotta P, Alghamdi M, Wall W, Khakhar A, Suskin NG, Quan D, McAllister V, Ghent C, Levstik M, McLean C, Chakrabarti S, Garcia B, Driman DK. Recurrent hepatocellular carcinoma after transplantation: use of a pathological score on explanted livers to predict recurrence. *Liver Transpl* 2007; **13**: 543-551 [PMID: 17394152 DOI: 10.1002/lt.21078]
- 62 **Yao FY**, Hameed B, Mehta N, Roberts JP. Response to letter to the editors. *Liver Transpl* 2014; **20**: 1285 [PMID: 25155484 DOI: 10.1002/lt.23982]
- 63 **Zheng SS**, Xu X, Wu J, Chen J, Wang WL, Zhang M, Liang TB, Wu LM. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation* 2008; **85**: 1726-1732 [PMID: 18580463 DOI: 10.1097/TP.0b013e31816b67e4]
- 64 **Lai Q**, Avolio AW, Manzia TM, Sorge R, Agnes S, Tisone G, Berloco PB, Rossi M. Combination of biological and morphological parameters for the selection of patients with hepatocellular carcinoma waiting for liver transplantation. *Clin Transplant* 2012; **26**: E125-E131 [PMID: 22192083 DOI: 10.1111/j.1399-0012.2011.01572.x]
- 65 **Ciccarelli O**, Lai Q, Goffette P, Finet P, De Reyck C, Roggen F, Sempoux C, Doffagne E, Reding R, Lerut J. Liver transplantation for hepatocellular cancer: UCL experience in 137 adult cirrhotic patients. Alpha-fetoprotein level and locoregional treatment as refined selection criteria. *Transpl Int* 2012; **25**: 867-875 [PMID: 22716073 DOI: 10.1111/j.1432-2277.2012.01512.x]
- 66 **Merani S**, Majno P, Kneteman NM, Berney T, Morel P, Mentha G, Toso C. The impact of waiting list alpha-fetoprotein changes on the outcome of liver transplant for hepatocellular carcinoma. *J Hepatol* 2011; **55**: 814-819 [PMID: 21334400 DOI: 10.1016/j.jhep.2010.12.040]



- 67 **Grąt M**, Kornasiewicz O, Lewandowski Z, Hołówko W, Grąt K, Kobryń K, Patkowski W, Zieniewicz K, Krawczyk M. Combination of morphologic criteria and  $\alpha$ -fetoprotein in selection of patients with hepatocellular carcinoma for liver transplantation minimizes the problem of posttransplant tumor recurrence. *World J Surg* 2014; **38**: 2698-2707 [PMID: 24858191 DOI: 10.1007/s00268-014-2647-3]
- 68 **Abdel-Wahab M**, Sultan AM, Fathy OM, Salah T, Elshobary MM, Elghawalby NA, Yassen AM, Elsarraf WM, Elsaadany MF, Zalatah K. Factors affecting recurrence and survival after living donor liver transplantation for hepatocellular carcinoma. *Hepatogastroenterology* 2013; **60**: 1847-1853 [PMID: 24719918]
- 69 **Han K**, Tzimas GN, Barkun JS, Metrakos P, Tchervenkov JL, Hilzenrat N, Wong P, Deschênes M. Preoperative alpha-fetoprotein slope is predictive of hepatocellular carcinoma recurrence after liver transplantation. *Can J Gastroenterol* 2007; **21**: 39-45 [PMID: 17225881]
- 70 **Vibert E**, Azoulay D, Hoti E, Iacopinelli S, Samuel D, Salloum C, Lemoine A, Bismuth H, Castaing D, Adam R. Progression of alpha-fetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: a critical factor. *Am J Transplant* 2010; **10**: 129-137 [PMID: 20070666 DOI: 10.1111/j.1600-6143.2009.02750.x]
- 71 **Dumitra TC**, Dumitra S, Metrakos PP, Barkun JS, Chaudhury P, Deschênes M, Paraskevas S, Hassanain M, Tchervenkov JI. Pretransplantation  $\alpha$ -fetoprotein slope and milan criteria: strong predictors of hepatocellular carcinoma recurrence after transplantation. *Transplantation* 2013; **95**: 228-233 [PMID: 23222895 DOI: 10.1097/TP.0b013e31827743d7]
- 72 **Riaz A**, Ryu RK, Kulik LM, Mulcahy MF, Lewandowski RJ, Minocha J, Ibrahim SM, Sato KT, Baker T, Miller FH, Newman S, Omary R, Abecassis M, Benson AB, Salem R. Alpha-fetoprotein response after locoregional therapy for hepatocellular carcinoma: oncologic marker of radiologic response, progression, and survival. *J Clin Oncol* 2009; **27**: 5734-5742 [PMID: 19805671 DOI: 10.1200/JCO.2009.23.1282]
- 73 **Bhat M**, Hassanain M, Simoneau E, Tzimas GN, Chaudhury P, Deschenes M, Valenti D, Ghali P, Wong P, Cabrera T, Barkun J, Tchervenkov JI, Metrakos P. Magnitude of change in alpha-fetoprotein in response to transarterial chemoembolization predicts survival in patients undergoing liver transplantation for hepatocellular carcinoma. *Curr Oncol* 2013; **20**: 265-272 [PMID: 24155631 DOI: 10.3747/co.20.1270]
- 74 **Varona MA**, Soriano A, Aguirre-Jaime A, Garrido S, Oton E, Diaz D, Portero J, Bravo P, Barrera MA, Perera A. Risk factors of hepatocellular carcinoma recurrence after liver transplantation: accuracy of the alpha-fetoprotein model in a single-center experience. *Transplant Proc* 2015; **47**: 84-89 [PMID: 25645778 DOI: 10.1016/j.transproceed.2014.12.013]
- 75 **Toso C**, Meeberg G, Hernandez-Alejandro R, Dufour JF, Marotta P, Majno P, Kneteman NM. Total tumor volume and alpha-fetoprotein for selection of transplant candidates with hepatocellular carcinoma: A prospective validation. *Hepatology* 2015; **62**: 158-165 [PMID: 25777590 DOI: 10.1002/hep.27787]
- 76 **Kashkoush S**, El Moghazy W, Kawahara T, Gala-Lopez B, Toso C, Kneteman NM. Three-dimensional tumor volume and serum alpha-fetoprotein are predictors of hepatocellular carcinoma recurrence after liver transplantation: refined selection criteria. *Clin Transplant* 2014; **28**: 728-736 [PMID: 24708263 DOI: 10.1111/ctr.12373]
- 77 **Hong G**, Suh KS, Suh SW, Yoo T, Kim H, Park MS, Choi Y, Paeng JC, Yi NJ, Lee KW. Alpha-fetoprotein and (18)F-FDG positron emission tomography predict tumor recurrence better than Milan criteria in living donor liver transplantation. *J Hepatol* 2016; **64**: 852-859 [PMID: 26658686 DOI: 10.1016/j.jhep.2015.11.033]
- 78 **Yamashiki N**, Sugawara Y, Tamura S, Tateishi R, Yoshida H, Kaneko J, Matsui Y, Togashi J, Akahane M, Makuuchi M, Omata M, Kokudo N. Postoperative surveillance with monthly serum tumor markers after living-donor liver transplantation for hepatocellular carcinoma. *Hepatol Res* 2010; **40**: 278-286 [PMID: 20070400 DOI: 10.1111/j.1872-034X.2009.00591.x]
- 79 **Roayaie S**, Schwartz JD, Sung MW, Emre SH, Miller CM, Gondelesi GE, Krieger NR, Schwartz ME. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. *Liver Transpl* 2004; **10**: 534-540 [PMID: 15048797 DOI: 10.1002/lt.20128]
- 80 **Regalia E**, Fassati LR, Valente U, Pulvirenti A, Damilano I, Dardano G, Montalto F, Coppa J, Mazzaferro V. Pattern and management of recurrent hepatocellular carcinoma after liver transplantation. *J Hepatobiliary Pancreat Surg* 1998; **5**: 29-34 [PMID: 9683751]
- 81 **Kakodkar R**, Soin AS. Liver Transplantation for HCC: A Review. *Indian J Surg* 2012; **74**: 100-117 [PMID: 23372314 DOI: 10.1007/s12262-011-0387-2]
- 82 **Roberts JP**. Tumor surveillance-what can and should be done? Screening for recurrence of hepatocellular carcinoma after liver transplantation. *Liver Transpl* 2005; **11**: S45- S46 [PMID: 16237702 DOI: 10.1002/lt.20605]
- 83 **Kneteman N**, Livraghi T, Madoff D, de Santibañez E, Kew M. Tools for monitoring patients with hepatocellular carcinoma on the waiting list and after liver transplantation. *Liver Transpl* 2011; **17** Suppl 2: S117-S127 [PMID: 21584926 DOI: 10.1002/lt.22334]
- 84 **Sposito C**, Mariani L, Germini A, Flores Reyes M, Bongini M, Grossi G, Bhoori S, Mazzaferro V. Comparative efficacy of sorafenib versus best supportive care in recurrent hepatocellular carcinoma after liver transplantation: a case-control study. *J Hepatol* 2013; **59**: 59-66 [PMID: 23500153 DOI: 10.1016/j.jhep.2013.02.026]
- 85 **Ortiz J**, Dannel J, Chavez M, Davogusto G. Monitoring for post-transplant hepatocellular carcinoma recurrence. *HPB (Oxford)* 2012; **14**: 351; author reply 352 [PMID: 22487073 DOI: 10.1111/j.1477-2574.2012.00449.x]
- 86 **Adler M**, De Pauw F, Vereerstraeten P, Fancello A, Lerut J, Starkel P, Van Vlierberghe H, Troisi R, Donckier V, Detry O, Delwaide J, Michielsen P, Chapelle T, Pirenne J, Nevens F. Outcome of patients with hepatocellular carcinoma listed for liver transplantation within the Eurotransplant allocation system. *Liver Transpl* 2008; **14**: 526-533 [PMID: 18383082 DOI: 10.1002/lt.21399]
- 87 **Xiao L**, Fu ZR, Ding GS, Fu H, Ni ZJ, Wang ZX, Shi XM, Guo WY. Liver transplantation for hepatitis B virus-related hepatocellular carcinoma: one center's experience in China. *Transplant Proc* 2009; **41**: 1717-1721 [PMID: 19545714 DOI: 10.1016/j.transproceed.2009.03.058]
- 88 **Levi DM**, Tzakis AG, Martin P, Nishida S, Island E, Moon J, Selvaggi G, Tekin A, Madrazo BL, Narayanan G, Garcia MT, Feun LG, Tryphonopoulos P, Skartsis N, Livingstone AS. Liver transplantation for hepatocellular carcinoma in the model for end-stage liver disease era. *J Am Coll Surg* 2010; **210**: 727-734, 735-736 [PMID: 20421039 DOI: 10.1016/j.jamcollsurg.2010.01.007]
- 89 **Lai Q**, Avolio AW, Manzia TM, Agnes S, Tisone G, Berloco PB, Rossi M. Role of alpha-fetoprotein in selection of patients with hepatocellular carcinoma waiting for liver transplantation: must we reconsider it? *Int J Biol Markers* 2011; **26**: 153-159 [PMID: 21928243 DOI: 10.5301/IJBM.2011.8557]
- 90 **Harimoto N**, Shirabe K, Nakagawara H, Toshima T, Yamashita Y, Ikegami T, Yoshizumi T, Soejima Y, Ikeda T, Maehara Y. Prognostic factors affecting survival at recurrence of hepatocellular carcinoma after living-donor liver transplantation: with special reference to neutrophil/lymphocyte ratio. *Transplantation* 2013; **96**: 1008-1012 [PMID: 24113512 DOI: 10.1097/TP.0b013e3182a53f2b]
- 91 **Lee S**, Hyuck David Kwon C, Man Kim J, Joh JW, Woon Paik S, Kim BW, Wang HJ, Lee KW, Suh KS, Lee SK. Time of hepatocellular carcinoma recurrence after liver resection and alpha-fetoprotein are important prognostic factors for salvage liver transplantation. *Liver Transpl* 2014; **20**: 1057-1063 [PMID: 24862741 DOI: 10.1002/lt.23919]

- 92 **Grąt M**, Krasnodębski M, Patkowski W, Wronka KM, Masior Ł, Stypułkowski J, Grąt K, Krawczyk M. Relevance of Pre-Transplant

$\alpha$ -fetoprotein Dynamics in Liver Transplantation for Hepatocellular Cancer. *Ann Transplant* 2016; **21**: 115-124 [PMID: 26887339]

**P- Reviewer:** Guan YS, Miyoshi E **S- Editor:** Qi Y  
**L- Editor:** A **E- Editor:** Li D



## Acute renal injury after partial hepatectomy

Luis Alberto Batista Peres, Luis Cesar Bredt, Raphael Flavio Fachini Cipriani

Luis Alberto Batista Peres, Department of Nephrology, University Hospital of Western Paraná, State University of Western Paraná, Cascavel, Paraná 85819-110, Brazil

Luis Cesar Bredt, Department of Surgical Oncology and Hepatobiliary Surgery, University Hospital of Western Paraná, State University of Western Paraná, Cascavel, Paraná 85819-110, Brazil

Raphael Flavio Fachini Cipriani, Department of General Surgery, University Hospital of Western Paraná, State University of Western Paraná, Cascavel, Paraná 85819-110, Brazil

**Author contributions:** Peres LAB, Bredt LC and Cipriani RFF contributed equally to this review article; all authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

**Conflict-of-interest statement:** No potential conflicts of interest. No financial support.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Luis Cesar Bredt, Professor, Department of Surgical Oncology and Hepatobiliary Surgery, University Hospital of Western Paraná, State University of Western Paraná, Dom Pedro II Street, 2099, apto 701, Cascavel, Paraná 85819-110, Brazil. [lcbredt@gmail.com](mailto:lcbredt@gmail.com)  
Telephone: +55-45-99369943  
Fax: +55-45-33215151

Received: February 25, 2016  
Peer-review started: February 27, 2016  
First decision: May 13, 2016  
Revised: June 2, 2016  
Accepted: June 27, 2016  
Article in press: June 29, 2016

Published online: July 28, 2016

### Abstract

Currently, partial hepatectomy is the treatment of choice for a wide variety of liver and biliary conditions. Among the possible complications of partial hepatectomy, acute kidney injury (AKI) should be considered as an important cause of increased morbidity and postoperative mortality. Difficulties in the data analysis related to postoperative AKI after liver resections are mainly due to the multiplicity of factors to be considered in the surgical patients, moreover, there is no consensus of the exact definition of AKI after liver resection in the literature, which hampers comparison and analysis of the scarce data published on the subject. Despite this multiplicity of risk factors for postoperative AKI after partial hepatectomy, there are main factors that clearly contribute to its occurrence. First factor relates to large blood losses with renal hypoperfusion during the operation, second factor relates to the occurrence of post-hepatectomy liver failure with consequent distributive circulatory changes and hepatorenal syndrome. Eventually, patients can have more than one factor contributing to post-operative AKI, and frequently these combinations of acute insults can be aggravated by sepsis or exposure to nephrotoxic drugs.

**Key words:** Hepatectomy; Liver resection; Acute renal injury; Hepatorenal syndrome; Kidney

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In the specific scenario of liver resections, there are limited and heterogeneous data regarding the occurrence of acute kidney injury (AKI) in the post-operative period, and its clinical relevance (mortality, morbidity and hospital stay) were not conclusively explored and clarified. Difficulties in the data analysis related to postoperative AKI after liver resections are mainly due the scarce data published on the subject.

Peres LAB, Bredt LC, Cipriani RFF. Acute renal injury after partial hepatectomy. *World J Hepatol* 2016; 8(21): 891-901 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i21/891.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i21.891>

## INTRODUCTION

Currently, partial hepatectomy is the treatment of choice for a wide variety of primary liver tumors (benign or malignant), tumors of the bile ducts and secondary malignant liver tumors. The partial liver resections may also be necessary in the management of complex cystic liver diseases, benign biliary structures, some cases of hepatic trauma and more recently with living donor liver transplantation<sup>[1]</sup>. With the refinement of surgical techniques, improved selection of patients to procedure, advances in anesthetic support and perioperative care, this traditionally complex and feared operation has become a routine procedure in the past 20 years, with acceptable mortality rates ranging from 3.1% to 4.5%<sup>[2-4]</sup>.

Among the possible complications of major surgical procedures, including the partial hepatectomy, acute kidney injury (AKI) should be considered as an important cause of increased morbidity and postoperative mortality<sup>[5,6]</sup>, with an incidence ranging from 10% to 30% after major operations<sup>[7,8]</sup>. Literature data report an incidence of 1% of AKI in the postoperative major non-cardiac surgery without liver resection<sup>[6]</sup> about 20% after cardiac surgery<sup>[9-11]</sup> and 50% after liver transplantation<sup>[12-18]</sup>.

In the specific scenario of liver resections, there are limited and heterogeneous data regarding the occurrence of AKI in the postoperative period, with an incidence ranging from 0.9% to 15.1% of the patients<sup>[19-23]</sup>, and its clinical relevance (mortality, morbidity and hospital stay) were not conclusively explored and clarified.

Difficulties in the data analysis related to postoperative AKI after liver resections are mainly due to the multiplicity of factors to be considered in this surgical patients, such as general medical conditions and comorbidities, nutritional disorders, metastatic malignancy with low physiological reserve systems, immunological disorders, chemotherapy treatment, functional capacity and volume of liver parenchyma to be preserved, and the perioperative hemodynamic effects of the different modalities of partial hepatectomy. Moreover, there is no consensus of the exact definition of AKI after liver resection in the literature, which hampers comparison and analysis of the scarce data published on the subject<sup>[22]</sup>.

Despite this multiplicity of risk factors for postoperative AKI after partial hepatectomy, there are main factors that clearly contribute to its occurrence. First factor relates to large blood losses with renal hypoperfusion during the operation<sup>[20]</sup>, that very often can be associated by the deleterious renal effects of red blood cell transfusion<sup>[23]</sup>, and in some occasions this renal hypoperfusion occurs in patients with increased

renal susceptibility to ischemia, usually elderly patients with underlying cardiovascular or renal disorders, or eventually it may be drug-induced<sup>[21-24]</sup>. Second factor relates to the occurrence of post-hepatectomy liver failure (PLF) with consequent distributive circulatory changes and hepatorenal syndrome (HRS)<sup>[20]</sup>. Eventually, patients can have more than one factor contributing to post-operative AKI, and frequently these combinations of acute insults can be aggravated by sepsis<sup>[20-24]</sup> or exposure to nephrotoxic drugs, such as aminoglycosides<sup>[25]</sup>.

The aim of this review is to present the definition of postoperative AKI after partial hepatectomy, the different pathophysiological mechanisms for its occurrence and methods for preventing these events.

## DEFINITION OF POSTOPERATIVE AKI AFTER PARTIAL HEPATECTOMY

AKI is characterized by the deterioration of kidney function over a period of hours to days, resulting in the failure of the kidney to excrete nitrogenous waste products and to maintain fluid and electrolyte homeostasis<sup>[26]</sup>. In recent years, several criteria have been proposed for the diagnosis of AKI in general population, particularly the "Risk, Injury, Failure, Loss of Renal Function and End-Stage Renal Disease" (RIFLE) criteria<sup>[27]</sup>, the "Acute Kidney Injury Network" (AKIN) criteria<sup>[28]</sup> and more recently, the criteria suggested by a panel of experts, which combine the AKIN and the RIFLE criteria, thus proposing a new classification: The "Kidney Disease Improving Global Outcomes" criteria<sup>[29]</sup> (Table 1).

The first question regarding the definition of postoperative AKI after partial hepatectomy, would be determining which of these proposed AKI criteria is most appropriate for these patients undergoing liver resection. Whereas acute tubular necrosis (ATN), resulting from hypoxic damage to the renal medulla, is considered as a major cause of postoperative AKI<sup>[30]</sup>, different from general population, liver resections are often performed in the presence of functional deficit of the hepatic parenchyma, as in fibrosis, steatosis, cirrhosis, chemotherapy-induced injury and also in biliary obstruction<sup>[2]</sup>. Moreover, the recent technical improvements in liver surgery have resulted in an expansion and more liberal indications for major hepatectomies in patients with these underlying liver conditions<sup>[2,3,31-34]</sup>, however, the risk of postoperative complications, such as AKI, have remained important concerns<sup>[3,31,35]</sup>.

In the specific case of hepatocellular carcinoma, the tumor generally appears in a cirrhotic liver, which is a contributor to unfavorable postoperative results in large procedures<sup>[36]</sup>, regarding renal dysfunction, AKI is a common and potentially fatal event in patients with cirrhosis<sup>[37-39]</sup>, with a reported prevalence of 14%-50% in patients with cirrhosis<sup>[40-45]</sup>, this wide range in prevalence is likely due to different study populations and varying definitions of renal dysfunction. Studies evaluating survival predictors in cirrhosis, renal dysfunction was a



**Table 1** Current diagnostic criteria for acute kidney injury in general population

	RIFLE criteria <sup>[27]</sup>	AKIN criteria <sup>[28]</sup>	KDIGO criteria <sup>[29]</sup>
Diagnostic criteria	Increase in sCr to $\geq 1.5$ times baseline, within 7 d; or GFR decrease $> 25\%$ ; or urine volume $< 0.5$ mL/kg per hour for 6 h	Increase in sCr by $\geq 0.3$ mg/dL (26.5 mmol/L) within 48 h; or increase in sCr $\geq 1.5$ times baseline within 48 h; or urine volume $< 0.5$ mL/kg per hour for 6 h	Increase in sCr by $\geq 0.3$ mg/dL (26.5 mmol/L) within 48 h; or increase in sCr to $\geq 1.5$ times baseline, which is known or presumed to have occurred within the prior 7 d; or urine volume $< 0.5$ mL/kg per hour for 6 h
	Risk: sCr increase 1.5-1.9 times baseline; or GFR decrease 25%-50%; or urine output $< 0.5$ mL/kg per hour for 6 h	Stage 1: sCr increase 1.5-1.9 times baseline; or sCr increase $\geq 0.3$ mg/dL (26.5 mmol/L); or urine output $< 0.5$ mL/kg per hour for 6 h	Stage 1: sCr increase 1.5-1.9 times baseline; or sCr increase $\geq 0.3$ mg/dL (26.5 mmol/L); or urine output $< 0.5$ mL/kg per hour for 6-12 h
Staging	Injury: sCr increase 2.0-2.9 times baseline; or GFR decrease 50%-75%; or urine output $< 0.5$ mL/kg per hour for 12 h	Stage 2: sCr increase 2.0-2.9 times baseline; or urine output $< 0.5$ mL/kg per hour for 12 h	Stage 2: sCr increase 2.0-2.9 times baseline; or urine output $< 0.5$ mL/kg per hour for $\geq 12$ h
	Failure: sCr increase $\geq 3.0$ times baseline; or GFR decrease 50%-75%; or sCr increase $\geq 4.0$ mg/dL (353.6 mmol/L) with an acute increase of at least 0.5 mg/dL (44 mmol/L); or urine output $< 0.3$ mL/kg per hour for $\geq 24$ h; or anuria for $\geq 12$ h	Stage 3: sCr increase 3.0 times baseline; or sCr increase $\geq 4.0$ mg/dL (353.6 mmol/L) with an acute increase of at least 0.5 mg/dL (44 mmol/L); or urine output $< 0.3$ mL/kg per hour for $\geq 24$ h; or anuria for $\geq 12$ h	Stage 3: sCr increase 3.0 times baseline; or sCr increase to $\geq 4.0$ mg/dL (353.6 mmol/L); or initiation of renal replacement therapy; or urine output $< 0.3$ mL/kg per hour for $\geq 24$ h; or Anuria for $\geq 12$ h

AKIN: Acute Kidney Injury Network; GFR: Glomerular filtration rate; KDIGO: Kidney Disease Improving Global Outcome; RIFLE: Risk, Injury, Failure, Loss, End stage renal disease; sCr: Serum creatinine.

powerful predictor of death, as Child-Pugh score<sup>[46-48]</sup>.

Along with parenchymal dysfunction, the portal hypertension levels and its hemodynamic consequences are directly related to the degree of underlying liver injury<sup>[49-51]</sup>, as it is observed in cirrhosis and others conditions, such as severe steatosis and chemotherapy-induced injury<sup>[52]</sup>. The types of chemotherapy-induced liver toxicity include steatosis<sup>[53]</sup>, sinusoidal changes<sup>[54]</sup>, steatohepatitis<sup>[55]</sup>, and hemorrhagic central lobular necrosis<sup>[52]</sup>. Steatosis represents fatty changes in the liver, with the presence of fat droplets within the hepatocytes<sup>[56]</sup>, and it has been shown that steatosis may interfere with circulation through sinusoids and impair regeneration, and in addition the liver's protective mechanism against oxidative stress appear to be impaired<sup>[57,58]</sup>. The morbidity following liver resection associated with steatosis has been reported by Belghiti *et al*<sup>[2]</sup>, in this study with 747 patients, the mortality rate was higher in patients having steatosis than in those with no steatosis, 22% vs 8%, respectively ( $P = 0.003$ ). Likewise, according to Behrns *et al*<sup>[32]</sup> in 135 liver resections, morbidity was seen in 29% and 10% of the patients with steatosis and without steatosis, respectively.

Besides the fact that a significant portion of patients eligible for partial hepatectomy have underlying chronic liver disease or were exposed to systemic therapies with liver toxicity, the hemodynamic changes in patients after major liver resections may have similarities with those of patients with cirrhosis or acute liver failure, and depending on the remnant liver volume and functional quality of parenchyma (steatosis/cirrhosis) the clinical effects may be more evident<sup>[59]</sup>.

In 1953, Kowalski and Abelmann<sup>[60]</sup> reported the results of a study which have demonstrated that cardiac output in cirrhotic patients was significantly higher compared with healthy volunteers. The reason for this

so-called hyperdynamic state is that patients with cirrhosis develop portal hypertension with resultant splanchnic vasodilation and pooling of blood secondary to increased resistance to portal flow. This is due to (1) vasodilators such as nitric oxide, carbon monoxide, and endogenous cannabinoids<sup>[61,62]</sup>; and (2) vasodilation from inflammatory cytokines such as tumor necrosis factor- $\alpha$  and interleukin-6 induced by bacterial translocation from the gut<sup>[63]</sup>. As a result, the concentration of cyclic guanosine monophosphate cyclic is increased, resulting in splanchnic vasodilation, decrease in central and arterial blood volume, low capillary pressure, low central venous pressure (LCVP), low systemic vascular resistance, and reduction of mean arterial pressure<sup>[64]</sup>. This compensatory increase in cardiac output *via* activation of the sympathetic nervous system by carotid baroreceptors maintains sufficient renal perfusion, however, with decompensation of cirrhosis and increasing severity of portal hypertension, the compensatory increase in cardiac output is inadequate to maintain circulatory blood volume and adequate renal perfusion<sup>[65]</sup>. Therefore, it would be reasonable that diagnostic and staging AKI criteria that consider this circulatory impairment could be better applied in patients undergoing liver resections, particularly large resections and those with chronic liver disease.

It is extremely important to point out that in the case of patients with chronic liver disease, isolated dosages of serum creatinine (sCr) levels can not reveal the actual renal function of the patient, because: (1) there is decreased creatine formation in the secondary muscles loss of muscle mass<sup>[66]</sup>; (2) is increased renal tubular secretion of creatinine (Cr)<sup>[67]</sup>; (3) increasing the circulating volume of distribution in cirrhosis can dilute the sCr<sup>[68]</sup>; and (4) interference in the measurement of Cr due to elevated bilirubin<sup>[69]</sup>. As a result, the serum levels of Cr in patients

with cirrhosis overestimate glomerular filtration rate (GFR). Therefore, a dynamic definition referring to the elevation of serum Cr of  $\geq 50\%$  of preoperative levels to a final value  $\geq 1.5$  mg/dL (133  $\mu$ mol/L) could be more suitable for these patients, and clinical studies have shown that AKI according to these criteria was a strong predictor of hospital mortality in patients with liver disease<sup>[70-72]</sup>.

Another situation relates to the measurement of urine output of patients with chronic liver disease and ascites, since these patients can often present oliguria with high sodium retention, but they can still maintain a relatively normal GFR<sup>[73]</sup>. On the other hand, these patients can also have an increased diuresis because of diuretics therapy.

Thus, the current criteria suggested by the "International Ascites Club" for definition of AKI in cirrhotic patients do not include unreal measurements for these patients<sup>[68]</sup> (Table 2), and apparently would be the most appropriate criteria for the diagnosis and management of AKI after partial hepatectomy, especially in cases of large resections and underlying chronic liver disease.

## HEMODYNAMIC INSTABILITY AND RENAL HYPOPERFUSION

Although the extent of liver resection correlates with the magnitude of the procedure, and patients undergoing resection of more than three segments or an additional extrahepatic procedure have an increased risk of complications<sup>[74-76]</sup>, this is not a rigid rule. For example, an isolated resection of segment I is technically more demanding than a right hepatectomy, similarly, resection of segments IV, V, VIII or posterior right segments (segments VI, VII) may be technically more difficult than the left or right hepatectomy, although the transection area is larger. Therefore, a minor hepatectomy should not be considered as an operation of less magnitude, and most important, the prevention of intraoperative hemorrhage should not be neglected. If excessive blood loss persists and a reduction in oxygen delivery is not corrected, the renal medulla may be susceptible to ischemic ATN<sup>[77]</sup>, and as a result, patients may suffer from AKI. The results of two large studies<sup>[3,31]</sup> suggest that a blood loss of 1250 mL is the cutoff value for major complications after liver resections, such as AKI. Furthermore, red blood cell transfusion, that can be necessary in the case of haemorrhage, can be an additional risk factor for postoperative AKI<sup>[78]</sup>.

### **Increased susceptibility to renal hypoperfusion**

The kidneys are most vulnerable to moderate hypoperfusion when autoregulation is impaired. Factors increasing susceptibility to renal hypoperfusion may be seen in elderly patients or in patients with atherosclerosis, hypertension, or chronic renal failure, in whom hyaline and myointimal hyperplasia cause structural narrowing of the arterioles<sup>[79-81]</sup>. Increased susceptibility to renal

ischemia may also occur in malignant hypertension because of intimal thickening and fibrinoid necrosis of the small arteries and arterioles<sup>[82]</sup>. In addition, in chronic kidney disease, afferent arterioles in the functioning glomeruli become dilated with impairment of the kidney's ability to autoregulate the glomerular filtration rate in low-perfusion states<sup>[83]</sup>.

Impaired decreasing of afferent arteriolar resistance can occur when a patient is receiving nonsteroidal anti-inflammatory drugs or cyclooxygenase-2 inhibitors, which reduce the synthesis of prostaglandins in the kidneys, as consequence a decreasing in glomerular capillary pressure occurs in occasions of low-perfusion states<sup>[82,84-86]</sup>. In other situations, calcineurin inhibitors<sup>[87]</sup>, and radiocontrast agents<sup>[88]</sup> can act through various vasoconstrictor mediators to increase afferent arteriolar resistance, the later may have direct toxic effects on the tubules as well<sup>[81,82,88-92]</sup>. Decreased renal perfusion may also have an exaggerated drop in the GFR in low-perfusion states as a consequence of not raising efferent arteriolar resistance by angiotensin II in patients who are receiving angiotensin-receptor blockers or angiotensin-converting-enzyme inhibitors.

### **Red blood cell transfusion and postoperative AKI**

Despite the deleterious effect of hemodynamic instability in renal perfusion, red blood cell transfusion, that can be necessary in the case of haemorrhage, can be an additional risk factor for postoperative AKI<sup>[78]</sup>. Although the exact causal link between red blood cell transfusion and postoperative AKI is not fully elucidated, there are several mechanisms that may be implicated: Deficiency in 2,3-diphosphoglycerate with impaired oxygen unloading from hemoglobin, less deformability of stored red blood cells with obstruction of smaller capillaries<sup>[93]</sup> stored red blood cells hemolysis with an increase in circulating free iron<sup>[94]</sup>. Other mechanisms might include loss of the ability to generate nitric oxide, release of procoagulant phospholipids, increased adhesiveness to vascular endothelium, and accumulation of proinflammatory phospholipids<sup>[93,95-98]</sup>.

## POSTHEPATECTOMY LIVER FAILURE AND HEPATORENAL SYNDROME

Apart from blood loss, that can lead to ATN because of severe hemodynamic instability, others risk factors for postoperative AKI after partial hepatectomy would be those that favor PLF, characterized by jaundice, coagulopathy, encephalopathy, ascites, and renal and pulmonary failure, all of which may become apparent only 3 to 5 d after surgery<sup>[1]</sup>. These risk factors for PLF are well described, such as a small volume of remaining liver with marked volume reduction of organ parenchyma<sup>[35,99,100]</sup> associated to parenchymal cell injury due portal hyperperfusion<sup>[59,101]</sup>, liver cirrhosis or steatosis<sup>[102,103]</sup>, and liver toxicity induced by chemotherapy<sup>[104]</sup>. In patients with liver cirrhosis, the postoperative liver failure may occur

**Table 2** International Club of Ascites new definitions for the diagnosis and management of acute kidney injury in patients with cirrhosis<sup>[68]</sup>

Baseline sCr	A value of sCr obtained in the previous 3 mo, when available, can be used as baseline sCr. In patients with more than one value within the previous 3 mo, the value closest to the admission time to the hospital should be used. In patients without a previous sCr value, the sCr on admission should be used as baseline
Definition of AKI	Increase in sCr $\geq 0.3$ mg/dL ( $\geq 26.5$ mmol/L) within 48 h; or a percentage increase sCr $\geq 50\%$ from baseline which is known, or presumed, to have occurred within the prior 7 d
Staging of AKI	Stage 1: Increase in sCr $\geq 0.3$ mg/dL (26.5 mmol/L) or an increase in sCr $\geq 1.5$ -fold to twofold from baseline Stage 2: Increase in sCr > two to threefold from baseline Stage 3: Increase of sCr > threefold from baseline or sCr $\geq 4.0$ mg/dL (353.6 mmol/L) with an acute increase $\geq 0.3$ mg/dL (26.5 mmol/L) or initiation of renal replacement therapy
Progression of AKI	Progression: Progression of AKI to a higher stage and/or need for RRT Regression: Regression of AKI to a lower stage
Response to treatment	No response: No regression of AKI Partial response: Regression of AKI stage with a reduction of sCr to $\geq 0.3$ mg/dL (26.5 mmol/L) above the baseline value Full response: Return of sCr to a value within 0.3 mg/dL (26.5 mmol/L) of the baseline value

AKI: Acute kidney injury; RRT: Renal replacement therapy; sCr: Serum creatinine.

due the compromised liver microcirculation, with less resistance to ischemia-reperfusion injury<sup>[105]</sup> and impaired regeneration<sup>[106]</sup>, in addition, portal hypertension, if present, is associated with a poor outcome because of compromised portal flow and the risk of postoperative upper gastrointestinal bleeding<sup>[107]</sup>.

Liver steatosis is usually related to obesity, the presence of metabolic disorders, or the intake of alcohol or drugs, and this liver disorder increases the operative risk of partial hepatectomy<sup>[2,53,108]</sup>. The extent of liver resection in these patients with steatosis in order to avoid PLF is unclear, but the severity of fatty infiltration must be considered: Mild steatosis (up to 30% of hepatocytes containing fat) represents a minimal additional risk, in moderate steatosis (30% to 60% containing fat) caution is necessary, thus, a conservative resection should be favored, and patients with severe steatosis (more than 60% of hepatocytes containing fat) should undergo only limited resection<sup>[108]</sup>.

Regarding the chemotherapy-induced liver aggression, the rates of complications and death after major liver resection are likely to be increased<sup>[55,109]</sup>. Oxaliplatin can induce a veno-occlusive syndrome, occasionally associated with nodular regenerative hyperplasia, these vascular obstructions result in a bluish appearance of the liver (blue liver syndrome)<sup>[54,110,111]</sup>, and irinotecan can cause chemotherapy associated steatohepatitis<sup>[112]</sup>, and liver impairment can be amplified after partial hepatectomy in both situations, triggering PLF<sup>[113]</sup>.

A major concern regarding PLF is the onset of HRS. HRS is a reversible functional renal impairment that occurs in patients with advanced liver cirrhosis or hepatic failure. It is characterized by marked decrease in GFR and renal plasma flow in the absence of other cause of renal failure<sup>[114]</sup> (Table 3). The pathophysiological alterations of SHR consist of intravascular hypovolemia with activation of the renin-angiotensin-aldosterone system and vasoconstrictive sympathetic nervous system, leading to renal vasoconstriction of the afferent vessels and subsequent decrease in GFR<sup>[20]</sup>. Two subtypes of

HRS have been identified: SHR type 1 is characterized by a rapidly progressive renal insufficiency defined as a doubling of the initial serum creatinine to a level greater than 2.5 mg/dL or 220  $\mu$ mol/L in less than 2 wk, it is associated with very poor prognosis, and SHR Type 2 is characterized by a moderate renal insufficiency (Cr greater than 1.5 mg/dL or 133  $\mu$ mol/L), follows a steady course or slowly progressive, often associated with refractory ascites<sup>[114]</sup>.

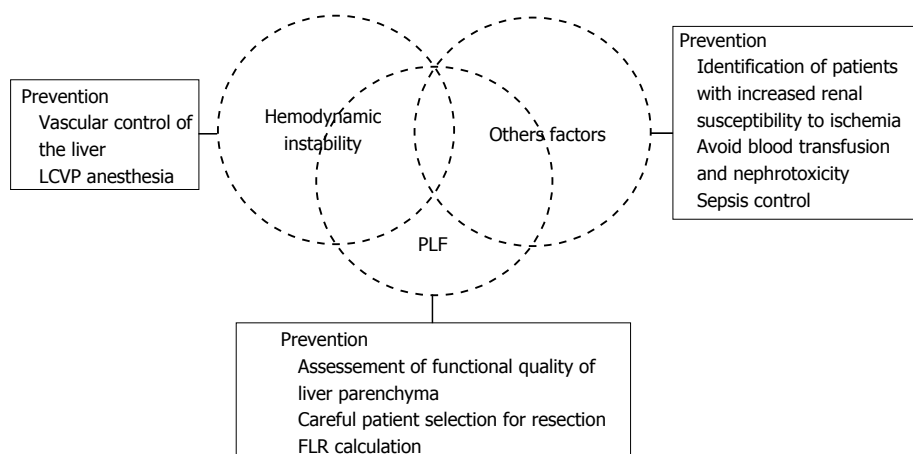
## KEYPOINTS FOR PREVENTION OF AKI AFTER PARTIAL HEPATECTOMY

Despite the fact that patients can have more than one factor contributing to post-operative AKI after partial hepatectomy, eventually aggravated by sepsis<sup>[20,21-24]</sup> or exposure to nephrotoxic drugs<sup>[25]</sup>, there are particular risk factors that must be controlled and specific operative and non-operative procedures that must be undertaken for prevention of post-operative renal injury in these patients (Figure 1).

### Vascular control of the liver

For prevention of intraoperative blood loss with consequent hemodynamic instability during the partial hepatectomy, there are intraoperative maneuvers that may be crucial in the moment of parenchymal transaction, such as vascular control of the liver<sup>[21]</sup>.

The vascular control of the liver is an effective method to reduce bleeding during the hepatectomy. While various techniques have been proposed, the two most widely used methods are the vascular inflow occlusion and complete vascular exclusion<sup>[115,116]</sup>. Occlusion of the hepatic vascular inflow<sup>[117]</sup> by the application of tourniquet in hepatoduodenal ligament<sup>[118]</sup> is the oldest and simplest way to reduce blood loss during hepatectomy. The "Pringle maneuver" can be used continuously to normal livers under normothermic conditions for a maximum of 60 min, and for 30 min in cirrhotic or steatotic livers, although longer periods have already been described<sup>[119-122]</sup>.



**Figure 1 Main risk factors and prevention of acute kidney injury after partial hepatectomy.** LCVP: Low central venous pressure; PLF: Posthepatectomy liver failure; FLR: Future liver remnant.

**Table 3 Diagnostic criteria of hepatorenal syndrome type of acute kidney injury in patients with cirrhosis<sup>[68]</sup>**

#### HRS-AKI

Diagnosis of cirrhosis and ascites  
Diagnosis of AKI according to ICA-AKI criteria (Table 2)  
No response after 2 consecutive days of diuretic withdrawal and plasma volume expansion with albumin 1 g/kg bodyweight  
Absence of shock  
No current or recent use of nephrotoxic drugs (NSAIDs, aminoglycosides, iodinated contrast media, etc.)  
No macroscopic signs of structural kidney injury, defined as  
Absence of proteinuria (> 500 mg/d)  
Absence of microhaematuria (> 50 RBCs per high power field)  
Normal findings on renal ultrasonography  
Patients who fulfil these criteria may still have structural damage such as tubular damage. Urine biomarkers will become an important element in making a more accurate differential diagnosis between HRS and acute tubular necrosis

HRS: Hepatorenal syndrome; AKI: Acute kidney injury; ICA: International club of ascites; NSAIDs: Non-steroidal anti-inflammatory drugs; RBCs: Red blood cells.

According Belghiti *et al.*<sup>[123]</sup> there is no significant difference in blood loss during surgery using the Pringle maneuver continuously or intermittently (15 min of ischemia for 5 min reperfusion). These concerns about longer periods of hepatic vascular inflow is mainly because that obstruction of the portal blood flow causes venous congestion of the bowel, and in combination with warm ischemic liver injury it results in a flush of anaerobic metabolites and cytokines back into the circulation on the clamp release<sup>[124]</sup>. In the total vascular exclusion<sup>[125]</sup>, the occlusion of the hepatic vascular inflow is combined to hepatic venous exclusion. The complete hepatic ischemia can be associated to hypothermic perfusion with cooled preservation solution<sup>[126]</sup> and extracorporeal venovenous bypass, with "ex situ" liver resection<sup>[127]</sup> or "in situ" liver resection<sup>[128]</sup>.

#### LCVP anesthesia

During the parenchymal transaction, a LCVP prevents

the back bleeding from hepaticveins<sup>[19,129,130]</sup>, and along with vascular control of the liver, these techniques test the patients cardiovascular reserve<sup>[21]</sup>. LCVP anesthesia is based on patients being maintained in hypovolaemic state until liver resection has been completed<sup>[19,129]</sup>, this is in contrast to most other major surgical procedures, where patients receive large volumes of crystalloid and colloid during the peri-operative period<sup>[21]</sup>. Moreover, vasodilators are often used to further reduce central venous pressure (CVP), leading to distributive changes in blood flow<sup>[129]</sup>, and whereas these techniques are applied for haemorrhage control and consequently promoting AKI prevention, a potential consequence of such circulatory changes is ATN, with subsequent renal impairment or failure<sup>[20]</sup>. The kidneys are at greater risk with abrupt fall in blood pressure, if the mean arterial pressure reaches values below 80 mmHg, there is a significant decrease in GFR<sup>[24]</sup>.

In the study of Wang *et al.*<sup>[131]</sup>, the maintenance of CVP  $\leq 4$  mmHg has reduced blood loss during partial hepatectomy, and has shortened the length of hospital stay, with no detrimental effects on hepatic or renal function. According to Melendez *et al.*<sup>[19]</sup>, in 496 liver resections with an anesthetic protocol of fluid restriction, with the use of nitroglycerin, furosemide, and with the maintenance of a systolic blood pressure of 90 mmHg, the median volume blood loss was 645 mL and the incidence of AKI was 3.1%. A study with 2116 LCVP-assisted hepatectomies reported an estimated mean blood loss of 300 mL (IQR: 200-600 mL), 90-d mortality of 2%, and postoperative AKI of 16% in the whole cohort (13% at risk, 2% at injury and 1% experienced failure)<sup>[132]</sup>. A study reported a low incidence of AKI requiring renal replacement therapy after liver resection (< 1%), confirming that the routine use of LCVP anaesthesia in combination with intermittent inflow occlusion is safe<sup>[21]</sup>.

Although there are strong evidences that LCVP during partial hepatectomy can minimize blood loss and mortality<sup>[19]</sup>, it is not clear whether it would play a role



in AKI prevention, as renal perfusion pressure can be decreased during relative hypovolemia, thus, further studies are required to prove this hypothesis.

### Prevention of post-hepatectomy liver failure

In order to reduce the incidence of PLF, a careful pre-operative planning and patient selection is mandatory. In the case of underlying cirrhosis, the best candidates for surgical resection are the exclusive Child-Pugh A patients with normal bilirubin values, the absence of clinical signs of portal hypertension (platelet count, splenomegaly and esophageal varices), only tumor diameter < 5 cm (without vascular invasion), asymptomatic and MELD < 8<sup>[107,133,134]</sup>. Hyperbilirubinemia, portal hypertension and clinical deterioration criteria are considered signs of poor postoperative course, despite the tumor resectability<sup>[135]</sup>.

Analyzing the issue of remnant liver volume after partial hepatectomy, the functional quality of parenchyma should not be ignored. In obtaining the computed tomography images, it enables the calculation of the future liver remnant (FLR), in patients with normal liver function, it must be greater than 25% of the liver total volume, corresponding to 0.5 of the patient weight. In patients with cirrhosis, prolonged exposure to chemotherapy and biliary obstruction, this value is 40%, corresponding to 0.7 of the patient weight<sup>[136]</sup>. The occlusion of a branch of the portal vein can be performed in order to minimize the occurrence of hepatic insufficiency after major resections. This procedure makes possible the treatment of tumors previously classified as unresectable, providing contralateral liver hypertrophy, thereby increasing the FLR<sup>[137,138]</sup>. In some situations resectability only occurs when performing two sequential hepatectomies associated with portal ligation for manipulation of the FLR, the two-stage hepatectomy<sup>[139]</sup>.

## FINAL CONSIDERATIONS

In the context of liver resections, the risk assessment of postoperative AKI requires the analysis of multiple variables involved in this complex universe, but probably there are main factors which significantly influence these patients for the occurrence of AKI: The massive blood loss during operation with or without an increased renal susceptibility to ischemia, and the occurrence of PLF. Certainly, the key interventions for preventing postoperative AKI after partial hepatectomy would be an appropriate preoperative work up, careful patient selection for surgery and rigorous perioperative control of the patient hemodynamic status by the surgical team.

## REFERENCES

- 1 **Clavien PA**, Petrowsky H, DeOliveira ML, Graf R. Strategies for safer liver surgery and partial liver transplantation. *N Engl J Med* 2007; **356**: 1545-1559 [PMID: 17429086 DOI: 10.1056/NEJMra065156]
- 2 **Belghiti J**, Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O. Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. *J Am Coll Surg* 2000; **191**: 38-46 [PMID: 10898182 DOI: 10.1016/S1072-7515(00)00261-1]
- 3 **Jarnagin WR**, Gonen M, Fong Y, DeMatteo RP, Ben-Porat L, Little S, Corvera C, Weber S, Blumgart LH. Improvement in perioperative outcome after hepatic resection: analysis of 1,803 consecutive cases over the past decade. *Ann Surg* 2002; **236**: 397-406; discussion 406-407 [PMID: 12368667 DOI: 10.1097/01.SLA.0000029003.66466.B3]
- 4 **Poon RT**, Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Improving perioperative outcome expands the role of hepatectomy in management of benign and malignant hepatobiliary diseases: analysis of 1222 consecutive patients from a prospective database. *Ann Surg* 2004; **240**: 698-708; discussion 708-710 [PMID: 15383797 DOI: 10.1097/01.sla.0000141195.66155.0c]
- 5 **Chertow GM**, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 2005; **16**: 3365-3370 [PMID: 16177006 DOI: 10.1681/asn.2004090740]
- 6 **Kheterpal S**, Tremper KK, Englesbe MJ, O'Reilly M, Shanks AM, Fetterman DM, Rosenberg AL, Swartz RD. Predictors of postoperative acute renal failure after noncardiac surgery in patients with previously normal renal function. *Anesthesiology* 2007; **107**: 892-902 [PMID: 18043057 DOI: 10.1097/01.anes.0000290588.29668.38]
- 7 **Bihorac A**, Yavas S, Subbiah S, Hobson CE, Schold JD, Gabrielli A, Layon AJ, Segal MS. Long-term risk of mortality and acute kidney injury during hospitalization after major surgery. *Ann Surg* 2009; **249**: 851-858 [PMID: 19387314 DOI: 10.1097/SLA.0b013e3181a40a0b]
- 8 **Abelha FJ**, Botelho M, Fernandes V, Barros H. Outcome and quality of life of patients with acute kidney injury after major surgery. *Nefrologia* 2009; **29**: 404-414 [PMID: 19820752 DOI: 10.3265/Nefrologia.2009.29.5.5456.en.full]
- 9 **Andersson LG**, Ekroth R, Bratteby LE, Hallhagen S, Wesslén O. Acute renal failure after coronary surgery—a study of incidence and risk factors in 2009 consecutive patients. *Thorac Cardiovasc Surg* 1993; **41**: 237-241 [PMID: 8211928 DOI: 10.1055/s-2007-1013861]
- 10 **Bove T**, Calabrò MG, Landoni G, Aletti G, Marino G, Crescenzi G, Rosica C, Zangrillo A. The incidence and risk of acute renal failure after cardiac surgery. *J Cardiothorac Vasc Anesth* 2004; **18**: 442-445 [PMID: 15365924 DOI: 10.1053/j.jvca.2004.05.021]
- 11 **Landoni G**, Bove T, Crivellari M, Poli D, Fochi O, Marchetti C, Romano A, Marino G, Zangrillo A. Acute renal failure after isolated CABG surgery: six years of experience. *Minerva Anestesiol* 2007; **73**: 559-565 [PMID: 17952028]
- 12 **Rimola A**, Gavaler JS, Schade RR, el-Lankany S, Starzl TE, Van Thiel DH. Effects of renal impairment on liver transplantation. *Gastroenterology* 1987; **93**: 148-156 [PMID: 3556303]
- 13 **McCauley J**, Van Thiel DH, Starzl TE, Puschett JB. Acute and chronic renal failure in liver transplantation. *Nephron* 1990; **55**: 121-128 [PMID: 2362625 DOI: 10.1159/000185938]
- 14 **Ishitani M**, Wilkowski M, Stevenson W, Pruett T. Outcome of patients requiring hemodialysis after liver transplantation. *Transplant Proc* 1993; **25**: 1762-1763 [PMID: 8470156]
- 15 **Nuño J**, Cuervas-Mons V, Vicente E, Turrión V, Pereira F, Mora NP, Barrios C, Millán I, Ardaiz J. Renal failure after liver transplantation: analysis of risk factors in 139 liver transplant recipients. *Transplant Proc* 1995; **27**: 2319-2320 [PMID: 7652826]
- 16 **Bilbao I**, Charco R, Balsells J, Lazaro JL, Hidalgo E, Llopart L, Murio E, Margarit C. Risk factors for acute renal failure requiring dialysis after liver transplantation. *Clin Transplant* 1998; **12**: 123-129 [PMID: 9575400]
- 17 **Cabezuelo JB**, Ramirez P, Ríos A, Acosta F, Torres D, Sansano T, Pons JA, Bru M, Montoya M, Bueno FS, Robles R, Parrilla P. Risk factors of acute renal failure after liver transplantation. *Kidney Int* 2006; **69**: 1073-1080 [PMID: 16528257 DOI: 10.1038/sj.ki.5000216]
- 18 **Yalavarthy R**, Edelstein CL, Teitelbaum I. Acute renal failure and chronic kidney disease following liver transplantation. *Hemodial Int* 2007; **11** Suppl 3: S7- S12 [PMID: 17897111 DOI: 10.1111/j.1542-4758.2007.00223.x]
- 19 **Melendez JA**, Arslan V, Fischer ME, Wuest D, Jarnagin WR,

- Fong Y, Blumgart LH. Perioperative outcomes of major hepatic resections under low central venous pressure anesthesia: blood loss, blood transfusion, and the risk of postoperative renal dysfunction. *J Am Coll Surg* 1998; **187**: 620-625 [PMID: 9849736 DOI: 10.1016/s1072-7515(98)00240-3]
- 20 **Saner F.** Kidney failure following liver resection. *Transplant Proc* 2008; **40**: 1221-1224 [PMID: 18555153 DOI: 10.1016/j.transproceed.2008.03.068]
- 21 **Armstrong T,** Welsh FK, Wells J, Chandrakumaran K, John TG, Rees M. The impact of pre-operative serum creatinine on short-term outcomes after liver resection. *HPB (Oxford)* 2009; **11**: 622-628 [PMID: 20495629 DOI: 10.1111/j.1477-2574.2009.00094.x]
- 22 **Slankamenac K,** Breitenstein S, Held U, Beck-Schimmer B, Puhan MA, Clavien PA. Development and validation of a prediction score for postoperative acute renal failure following liver resection. *Ann Surg* 2009; **250**: 720-728 [PMID: 19809295 DOI: 10.1097/sla.0b013e3181b8d840]
- 23 **Tomozawa A,** Ishikawa S, Shiota N, Cholvisudhi P, Makita K. Perioperative risk factors for acute kidney injury after liver resection surgery: an historical cohort study. *Can J Anaesth* 2015; **62**: 753-761 [PMID: 25925634 DOI: 10.1007/s12630-015-0397-9]
- 24 **Abuelo JG.** Normotensive ischemic acute renal failure. *N Engl J Med* 2007; **357**: 797-805 [PMID: 17715412 DOI: 10.1056/nejmra064398]
- 25 **Moore RD,** Smith CR, Lipsky JJ, Mellits ED, Lietman PS. Risk factors for nephrotoxicity in patients treated with aminoglycosides. *Ann Intern Med* 1984; **100**: 352-357 [PMID: 6364908 DOI: 10.7326/0003-4819-100-3-352]
- 26 **Thadhani R,** Pascual M, Bonventre JV. Acute renal failure. *N Engl J Med* 1996; **334**: 1448-1460 [PMID: 8618585 DOI: 10.1056/nejm199605303342207]
- 27 **Bellomo R,** Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004; **8**: R204-R212 [PMID: 15312219 DOI: 10.1186/cc2872]
- 28 **Mehta RL,** Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 2007; **11**: R31 [PMID: 17331245 DOI: 10.1186/cc5713]
- 29 **KDIGO Board Members.** KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Int Suppl* 2012; **2**: 1-138 [DOI: 10.1038/kisup.2012.3]
- 30 **Noor S,** Usmani A. Postoperative renal failure. *Clin Geriatr Med* 2008; **24**: 721-729, ix [PMID: 18984383 DOI: 10.1016/j.cger.2008.07.004]
- 31 **Imamura H,** Seyama Y, Kokudo N, Maema A, Sugawara Y, Sano K, Takayama T, Makuuchi M. One thousand fifty-six hepatectomies without mortality in 8 years. *Arch Surg* 2003; **138**: 1198-1206; discussion 1206 [PMID: 14609867 DOI: 10.1001/archsurg.138.11.1198]
- 32 **Behrns KE,** Tsiotos GG, DeSouza NF, Krishna MK, Ludwig J, Nagorney DM. Hepatic steatosis as a potential risk factor for major hepatic resection. *J Gastrointest Surg* 1998; **2**: 292-298 [PMID: 9841987 DOI: 10.1016/S1091-255X(98)80025-5]
- 33 **Cohnert TU,** Rau HG, Buttler E, Hernandez-Richter T, Sauter G, Reuter C, Schildberg FW. Preoperative risk assessment of hepatic resection for malignant disease. *World J Surg* 1997; **21**: 396-400; discussion 401 [PMID: 9143571 DOI: 10.1007/pl00012260]
- 34 **Choti MA,** Sitzmann JV, Tiburi MF, Sumetchotimetha W, Rangsiri R, Schulick RD, Lillemoe KD, Yeo CJ, Cameron JL. Trends in long-term survival following liver resection for hepatic colorectal metastases. *Ann Surg* 2002; **235**: 759-766 [PMID: 12035031]
- 35 **Schindl MJ,** Redhead DN, Fearon KC, Garden OJ, Wigmore SJ. The value of residual liver volume as a predictor of hepatic dysfunction and infection after major liver resection. *Gut* 2005; **54**: 289-296 [PMID: 15647196 DOI: 10.1136/gut.2004.046524]
- 36 **Simons JP,** Hill JS, Ng SC, Shah SA, Zhou Z, Whalen GF, Tseng JF. Perioperative mortality for management of hepatic neoplasm: a simple risk score. *Ann Surg* 2009; **250**: 929-934 [PMID: 19855257 DOI: 10.1097/sla.0b013e3181b9c2f]
- 37 **Moreau R,** Lebrech D. Acute renal failure in patients with cirrhosis: perspectives in the age of MELD. *Hepatology* 2003; **37**: 233-243 [PMID: 12540770 DOI: 10.1053/jhep.2003.50084]
- 38 **Mackelaite L,** Alsaukas ZC, Ranganna K. Renal failure in patients with cirrhosis. *Med Clin North Am* 2009; **93**: 855-869, viii [PMID: 19577118 DOI: 10.1016/j.mcna.2009.03.003]
- 39 **Salerno F,** Guevara M, Bernardi M, Moreau R, Wong F, Angeli P, Garcia-Tsao G, Lee SS. Refractory ascites: pathogenesis, definition and therapy of a severe complication in patients with cirrhosis. *Liver Int* 2010; **30**: 937-947 [PMID: 20492521 DOI: 10.1111/j.1478-3231.2010.02272.x]
- 40 **Hampel H,** Bynum GD, Zamora E, El-Serag HB. Risk factors for the development of renal dysfunction in hospitalized patients with cirrhosis. *Am J Gastroenterol* 2001; **96**: 2206-2210 [PMID: 11467654 DOI: 10.1111/j.1572-0241.2001.03958.x]
- 41 **Péron JM,** Bureau C, Gonzalez L, Garcia-Ricard F, de Soyres O, Dupuis E, Alric L, Pourrat J, Vinel JP. Treatment of hepatorenal syndrome as defined by the international ascites club by albumin and furosemide infusion according to the central venous pressure: a prospective pilot study. *Am J Gastroenterol* 2005; **100**: 2702-2707 [PMID: 16393223 DOI: 10.1111/j.1572-0241.2005.00271.x]
- 42 **Terra C,** Guevara M, Torre A, Gilabert R, Fernández J, Martín-Llahí M, Baccaro ME, Navasa M, Bru C, Arroyo V, Rodés J, Ginès P. Renal failure in patients with cirrhosis and sepsis unrelated to spontaneous bacterial peritonitis: value of MELD score. *Gastroenterology* 2005; **129**: 1944-1953 [PMID: 16344063 DOI: 10.1053/j.gastro.2005.09.024]
- 43 **du Cheyron D,** Bouchet B, Parienti JJ, Ramakers M, Charbonneau P. The attributable mortality of acute renal failure in critically ill patients with liver cirrhosis. *Intensive Care Med* 2005; **31**: 1693-1699 [PMID: 16244877 DOI: 10.1007/s00134-005-2842-7]
- 44 **Wu CC,** Yeung LK, Tsai WS, Tseng CF, Chu P, Huang TY, Lin YF, Lu KC. Incidence and factors predictive of acute renal failure in patients with advanced liver cirrhosis. *Clin Nephrol* 2006; **65**: 28-33 [PMID: 16429839 DOI: 10.5414/CNP65028]
- 45 **Montoliu S,** Ballesté B, Planas R, Alvarez MA, Rivera M, Miquel M, Masnou H, Cirera I, Morillas RM, Coll S, Sala M, García-Retortillo M, Cañete N, Solà R. Incidence and prognosis of different types of functional renal failure in cirrhotic patients with ascites. *Clin Gastroenterol Hepatol* 2010; **8**: 616-622; quiz e80 [PMID: 20399905 DOI: 10.1016/j.cgh.2010.03.029]
- 46 **Llach J,** Ginès P, Arroyo V, Rimola A, Titó L, Badalamenti S, Jiménez W, Gaya J, Rivera F, Rodés J. Prognostic value of arterial pressure, endogenous vasoactive systems, and renal function in cirrhotic patients admitted to the hospital for the treatment of ascites. *Gastroenterology* 1988; **94**: 482-487 [PMID: 3335320]
- 47 **Krag A,** Bendtsen F, Henriksen JH, Møller S. Low cardiac output predicts development of hepatorenal syndrome and survival in patients with cirrhosis and ascites. *Gut* 2010; **59**: 105-110 [PMID: 19837678 DOI: 10.1136/gut.2009.180570]
- 48 **Lim YS,** Larson TS, Benson JT, Kamath PS, Kremers WK, Therneau TM, Kim WR. Serum sodium, renal function, and survival of patients with end-stage liver disease. *J Hepatol* 2010; **52**: 523-528 [PMID: 20185195 DOI: 10.1016/j.jhep.2010.01.009]
- 49 **de Franchis R,** Dell'Era A, Primignani M. Diagnosis and monitoring of portal hypertension. *Dig Liver Dis* 2008; **40**: 312-317 [PMID: 18294933 DOI: 10.1016/j.dld.2007.12.007]
- 50 **Cucchetti A,** Ercolani G, Vivarelli M, Cescon M, Ravaioli M, Ramacciato G, Grazi GL, Pinna AD. Is portal hypertension a contraindication to hepatic resection? *Ann Surg* 2009; **250**: 922-928 [PMID: 19855258 DOI: 10.1097/SLA.0b013e3181b977a5]
- 51 **Santambrogio R,** Kluger MD, Costa M, Belli A, Barabino M, Laurent A, Opocher E, Azoulay D, Cherqui D. Hepatic resection for hepatocellular carcinoma in patients with Child-Pugh's A cirrhosis: is clinical evidence of portal hypertension a contraindication? *HPB (Oxford)* 2013; **15**: 78-84 [PMID: 23216782 DOI: 10.1111/j.1477-2574.2012.00594.x]
- 52 **Tisman G,** MacDonald D, Shindell N, Reece E, Patel P, Honda

- N, Nishimura EK, Garriss J, Shannahan W, Chisti N, McCarthy J, Nasser Moaddeli S, Sargent D, Plant A. Oxaliplatin toxicity masquerading as recurrent colon cancer. *J Clin Oncol* 2004; **22**: 3202-3204 [PMID: 15284280 DOI: 10.1200/JCO.2004.99.100]
- 53 **Kooby DA**, Fong Y, Suriawinata A, Gonen M, Allen PJ, Klimstra DS, DeMatteo RP, D'Angelica M, Blumgart LH, Jarnagin WR. Impact of steatosis on perioperative outcome following hepatic resection. *J Gastrointest Surg* 2003; **7**: 1034-1044 [PMID: 14675713 DOI: 10.1016/j.gassur.2003.09.012]
- 54 **Rubbia-Brandt L**, Audard V, Sartoretti P, Roth AD, Brezault C, Le Charpentier M, Dousset B, Morel P, Soubrane O, Chaussade S, Mentha G, Terris B. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. *Ann Oncol* 2004; **15**: 460-466 [PMID: 14998849 DOI: 10.1093/annonc/mdh095]
- 55 **Vauthey JN**, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM, Xiong HQ, Eng C, Lauwers GY, Mino-Kenudson M, Risio M, Muratore A, Capussotti L, Curley SA, Abdalla EK. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *J Clin Oncol* 2006; **24**: 2065-2072 [PMID: 16648507 DOI: 10.1200/JCO.2005.05.3074]
- 56 **Kemeny N**. Presurgical chemotherapy in patients being considered for liver resection. *Oncologist* 2007; **12**: 825-839 [PMID: 17673614 DOI: 10.1634/theoncologist.12-7-825]
- 57 **Maros T**, Seres-Sturm L, Lakatos O, Seres-Sturm M, Mody E, Blazsek V. Data regarding the restorative effects of the partial removal of the liver in advanced stages of toxic cirrhosis. *Morphol Embryol (Bucur)* 1975; **21**: 213-217 [PMID: 129696]
- 58 **Haney A**, Peacock EE, Madden JW. Liver regeneration and hepatic collagen deposition in rats with dimethylnitrosamine-induced cirrhosis. *Ann Surg* 1972; **175**: 863-869 [PMID: 5029843]
- 59 **Golriz M**, Majlesara A, El Sakka S, Ashrafi M, Arwin J, Fard N, Raisi H, Edalatpour A, Mehrabi A. Small for Size and Flow (SFSF) syndrome: An alternative description for posthepatectomy liver failure. *Clin Res Hepatol Gastroenterol* 2016; **40**: 267-275 [PMID: 26516057 DOI: 10.1016/J.Clinre.2015.06.024]
- 60 **Kowalski HJ**, Abelman WH. The cardiac output at rest in Laennec's cirrhosis. *J Clin Invest* 1953; **32**: 1025-1033 [PMID: 13096569 DOI: 10.1172/JCI102813]
- 61 **Martin PY**, Ginès P, Schrier RW. Nitric oxide as a mediator of hemodynamic abnormalities and sodium and water retention in cirrhosis. *N Engl J Med* 1998; **339**: 533-541 [PMID: 9709047 DOI: 10.1056/Nejm199808203390807]
- 62 **Ros J**, Clària J, To-Figueras J, Planagumà A, Cejudo-Martin P, Fernández-Varo G, Martín-Ruiz R, Arroyo V, Rivera F, Rodés J, Jiménez W. Endogenous cannabinoids: a new system involved in the homeostasis of arterial pressure in experimental cirrhosis in the rat. *Gastroenterology* 2002; **122**: 85-93 [PMID: 11781284 DOI: 10.1053/gast.2002.30305]
- 63 **Navasa M**, Follo A, Filella X, Jiménez W, Francitorra A, Planas R, Rimola A, Arroyo V, Rodés J. Tumor necrosis factor and interleukin-6 in spontaneous bacterial peritonitis in cirrhosis: relationship with the development of renal impairment and mortality. *Hepatology* 1998; **27**: 1227-1232 [PMID: 9581675 DOI: 10.1002/hep.510270507]
- 64 **Albillos A**, Rossi I, Cacho G, Martínez MV, Millán I, Abreu L, Barrios C, Escartín P. Enhanced endothelium-dependent vasodilation in patients with cirrhosis. *Am J Physiol* 1995; **268**: G459-G464 [PMID: 7900807]
- 65 **Ginès P**, Schrier RW. Renal failure in cirrhosis. *N Engl J Med* 2009; **361**: 1279-1290 [PMID: 19776409 DOI: 10.1056/nejmra0809139]
- 66 **Sherman DS**, Fish DN, Teitelbaum I. Assessing renal function in cirrhotic patients: problems and pitfalls. *Am J Kidney Dis* 2003; **41**: 269-278 [PMID: 12552488 DOI: 10.1053/ajkd.2003.50035]
- 67 **Caregaro L**, Menon F, Angeli P, Amodio P, Merkel C, Bortoluzzi A, Alberino F, Gatta A. Limitations of serum creatinine level and creatinine clearance as filtration markers in cirrhosis. *Arch Intern Med* 1994; **154**: 201-205 [PMID: 8285815 DOI: 10.1001/archinte.1994.00420020117013]
- 68 **Angeli P**, Gines P, Wong F, Bernardi M, Boyer TD, Gerbes A, Moreau R, Jalan R, Sarin SK, Piano S, Moore K, Lee SS, Durand F, Salerno F, Caraceni P, Kim WR, Arroyo V, Garcia-Tsao G. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. *Gut* 2015; **64**: 531-537 [PMID: 25631669 DOI: 10.1136/gutjnl-2014-308874]
- 69 **Spencer K**. Analytical reviews in clinical biochemistry: the estimation of creatinine. *Ann Clin Biochem* 1986; **23** (Pt 1): 1-25 [PMID: 3532908 DOI: 10.1177/000456328602300101]
- 70 **Ginès A**, Fernández-Esparrach G, Monescillo A, Vila C, Domènech E, Abecasis R, Angeli P, Ruiz-Del-Arbol L, Planas R, Solà R, Ginès P, Terg R, Inglada L, Vaqué P, Salerno F, Vargas V, Clemente G, Quer JC, Jiménez W, Arroyo V, Rodés J. Randomized trial comparing albumin, dextran 70, and polygeline in cirrhotic patients with ascites treated by paracentesis. *Gastroenterology* 1996; **111**: 1002-1010 [PMID: 8831595 DOI: 10.1016/S0016-5085(96)70068-9]
- 71 **Sort P**, Navasa M, Arroyo V, Aldeguez X, Planas R, Ruiz-del-Arbol L, Castells L, Vargas V, Soriano G, Guevara M, Ginès P, Rodés J. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med* 1999; **341**: 403-409 [PMID: 10432325 DOI: 10.1056/nejm199908053410603]
- 72 **Angeli P**, Fasolato S, Mazza E, Okolicsanyi L, Maresio G, Velo E, Galio A, Salinas F, D'Aquino M, Sticca A, Gatta A. Combined versus sequential diuretic treatment of ascites in non-azotaemic patients with cirrhosis: results of an open randomised clinical trial. *Gut* 2010; **59**: 98-104 [PMID: 19570764 DOI: 10.1136/gut.2008.176495]
- 73 **Angeli P**, Gatta A, Caregaro L, Menon F, Sacerdoti D, Merkel C, Rondana M, de Toni R, Ruol A. Tubular site of renal sodium retention in ascitic liver cirrhosis evaluated by lithium clearance. *Eur J Clin Invest* 1990; **20**: 111-117 [PMID: 2108033 DOI: 10.1111/j.1365-2362.1990.tb01800.x]
- 74 **Breitenstein S**, DeOliveira ML, Raptis DA, Slankamenac K, Kambakamba P, Nerl J, Clavien PA. Novel and simple preoperative score predicting complications after liver resection in noncirrhotic patients. *Ann Surg* 2010; **252**: 726-734 [PMID: 21037427 DOI: 10.1097/SLA.0b013e3181fb8c1a]
- 75 **Oussoultzoglou E**, Jaeck D, Addeo P, Fuchshuber P, Marzano E, Rosso E, Pessaux P, Bachellier P. Prediction of mortality rate after major hepatectomy in patients without cirrhosis. *Arch Surg* 2010; **145**: 1075-1081 [PMID: 21079096 DOI: 10.1001/archsurg.2010.225]
- 76 **Andres A**, Toso C, Moldovan B, Schiffer E, Rubbia-Brandt L, Terraz S, Klopfenstein CE, Morel P, Majno P, Mentha G. Complications of elective liver resections in a center with low mortality: a simple score to predict morbidity. *Arch Surg* 2011; **146**: 1246-1252 [PMID: 21768406 DOI: 10.1001/archsurg.2011.175]
- 77 **Jones DR**, Lee HT. Perioperative renal protection. *Best Pract Res Clin Anaesthesiol* 2008; **22**: 193-208 [PMID: 18494397 DOI: 10.1016/j.bpa.2007.08.005]
- 78 **Karkouti K**, Wijesundera DN, Yau TM, Callum JL, Cheng DC, Crowther M, Dupuis JY, Fremes SE, Kent B, Laflamme C, Lamy A, Legare JF, Mazer CD, McCluskey SA, Rubens FD, Sawchuk C, Beattie WS. Acute kidney injury after cardiac surgery: focus on modifiable risk factors. *Circulation* 2009; **119**: 495-502 [PMID: 19153273 DOI: 10.1161/circulationaha.108.786913]
- 79 **Badr KF**, Ichikawa I. Prerenal failure: a deleterious shift from renal compensation to decompensation. *N Engl J Med* 1988; **319**: 623-629 [PMID: 3045546 DOI: 10.1056/nejm198809083191007]
- 80 **Brady HR**, Clarkson MR, Lieberthal W. Acute Renal Failure. In: Brenner BM, Ed. Brenner and Rector's The Kidney. 7th ed. Philadelphia: Saunders, 2004: 1215-1292
- 81 **Palmer BF**. Renal dysfunction complicating the treatment of hypertension. *N Engl J Med* 2002; **347**: 1256-1261 [PMID: 12393824 DOI: 10.1056/NEJMra020676]
- 82 **Abuelo JG**. Diagnosing vascular causes of renal failure. *Ann Intern Med* 1995; **123**: 601-614 [PMID: 7677302]



- 83 **Taal MW**, Luyckx VA, Brenner BM. Adaptation to nephron loss. In: Brenner BM, Ed. Brenner And Rector's The Kidney. 7th ed. Philadelphia: Saunders, 2004: 1955-1997
- 84 **Schlondorff D**. Renal complications of nonsteroidal anti-inflammatory drugs. *Kidney Int* 1993; **44**: 643-653 [PMID: 8231040 DOI: 10.1038/ki.1993.293]
- 85 **Perazella MA**, Eras J. Are selective COX-2 inhibitors nephrotoxic? *Am J Kidney Dis* 2000; **35**: 937-940 [PMID: 10793030 DOI: 10.1016/S0272-6386(00)70266-6]
- 86 **Braden GL**, O'Shea MH, Mulhern JG, Germain MJ. Acute renal failure and hyperkalaemia associated with cyclooxygenase-2 inhibitors. *Nephrol Dial Transplant* 2004; **19**: 1149-1153 [PMID: 14993496 DOI: 10.1093/ndt/fgf622]
- 87 **Klein IH**, Abrahams A, van Ede T, Hené RJ, Koomans HA, Ligtenberg G. Different effects of tacrolimus and cyclosporine on renal hemodynamics and blood pressure in healthy subjects. *Transplantation* 2002; **73**: 732-736 [PMID: 11907418]
- 88 **Oudemans-van Straaten HM**. Contrast nephropathy, pathophysiology and prevention. *Int J Artif Organs* 2004; **27**: 1054-1065 [PMID: 15645616]
- 89 **Schor N**. Acute renal failure and the sepsis syndrome. *Kidney Int* 2002; **61**: 764-776 [PMID: 11849423 DOI: 10.1046/j.1523-1755.2002.00178.x]
- 90 **Lee HY**, Kim CH. Acute oliguric renal failure associated with angiotensin II receptor antagonists. *Am J Med* 2001; **111**: 162-163 [PMID: 11501548 DOI: 10.1016/S0002-9343(01)00784-7]
- 91 **Johansen TL**, Kjaer A. Reversible renal impairment induced by treatment with the angiotensin II receptor antagonist candesartan in a patient with bilateral renal artery stenosis. *BMC Nephrol* 2001; **2**: 1 [PMID: 11388887 DOI: 10.1186/1471-2369-2-1]
- 92 **Toto RD**. Renal insufficiency due to angiotensin-converting enzyme inhibitors. *Miner Electrolyte Metab* 1994; **20**: 193-200 [PMID: 7845322]
- 93 **Tinmouth A**, Fergusson D, Yee IC, Hébert PC. Clinical consequences of red cell storage in the critically ill. *Transfusion* 2006; **46**: 2014-2027 [PMID: 17076859 DOI: 10.1111/j.1537-2995.2006.01026.x]
- 94 **Karkouti K**, Wijeyesundera DN, Yau TM, McCluskey SA, Chan CT, Wong PY, Crowther MA, Hozhabri S, Beattie WS. Advance targeted transfusion in anemic cardiac surgical patients for kidney protection: an unblinded randomized pilot clinical trial. *Anesthesiology* 2012; **116**: 613-621 [PMID: 22354243 DOI: 10.1097/ALN.0b013e3182475e39]
- 95 **van de Watering L**. Red cell storage and prognosis. *Vox Sang* 2011; **100**: 36-45 [PMID: 21175654 DOI: 10.1111/j.1423-0410.2010.01441.x]
- 96 **Almac E**, Ince C. The impact of storage on red cell function in blood transfusion. *Best Pract Res Clin Anaesthesiol* 2007; **21**: 195-208 [PMID: 17650772 DOI: 10.1016/j.bpa.2007.01.004]
- 97 **Comporti M**, Signorini C, Buonocore G, Ciccoli L. Iron release, oxidative stress and erythrocyte ageing. *Free Radic Biol Med* 2002; **32**: 568-576 [PMID: 11909691 DOI: 10.1016/S0891-5849(02)00759-1]
- 98 **Bennett-Guerrero E**, Veldman TH, Doctor A, Telen MJ, Ortel TL, Reid TS, Mulherin MA, Zhu H, Buck RD, Califf RM, McMahon TJ. Evolution of adverse changes in stored RBCs. *Proc Natl Acad Sci USA* 2007; **104**: 17063-17068 [PMID: 17940021 DOI: 10.1073/pnas.0708160104]
- 99 **Lo CM**, Fan ST, Liu CL, Chan JK, Lam BK, Lau GK, Wei WJ, Wong J. Minimum graft size for successful living donor liver transplantation. *Transplantation* 1999; **68**: 1112-1116 [PMID: 10551638]
- 100 **Nishizaki T**, Ikegami T, Hiroshige S, Hashimoto K, Uchiyama H, Yoshizumi T, Kishikawa K, Shimada M, Sugimachi K. Small graft for living donor liver transplantation. *Ann Surg* 2001; **233**: 575-580 [PMID: 11303141]
- 101 **Wang F**, Pan KT, Chu SY, Chan KM, Chou HS, Wu TJ, Lee WC. Preoperative estimation of the liver graft weight in adult right lobe living donor liver transplantation using maximal portal vein diameters. *Liver Transpl* 2011; **17**: 373-380 [PMID: 21445920 DOI: 10.1002/lt.22274]
- 102 **Shirabe K**, Shimada M, Gion T, Hasegawa H, Takenaka K, Utsunomiya T, Sugimachi K. Postoperative liver failure after major hepatic resection for hepatocellular carcinoma in the modern era with special reference to remnant liver volume. *J Am Coll Surg* 1999; **188**: 304-309 [PMID: 10065820 DOI: 10.1016/S1072-7515(98)00301-9]
- 103 **Balzan S**, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D, Durand F. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**: 824-828, discussion 824-828 [PMID: 16327492 DOI: 10.1097/01.sla.0000189131.90876.9e]
- 104 **Fong Y**, Bentrem DJ. CASH (Chemotherapy-Associated Steatohepatitis) costs. *Ann Surg* 2006; **243**: 8-9 [PMID: 16371729 DOI: 10.1097/01.sla.0000193599.57858.9b]
- 105 **Seifalian AM**, Piasecki C, Agarwal A, Davidson BR. The effect of graded steatosis on flow in the hepatic parenchymal microcirculation. *Transplantation* 1999; **68**: 780-784 [PMID: 10515377]
- 106 **Yamanaka N**, Okamoto E, Kawamura E, Kato T, Oriyama T, Fujimoto J, Furukawa K, Tanaka T, Tomoda F, Tanaka W. Dynamics of normal and injured human liver regeneration after hepatectomy as assessed on the basis of computed tomography and liver function. *Hepatology* 1993; **18**: 79-85 [PMID: 8392029 DOI: 10.1002/hep.1840180114]
- 107 **Bruix J**, Castells A, Bosch J, Feu F, Fuster J, Garcia-Pagan JC, Visa J, Bru C, Rodés J. Surgical resection of hepatocellular carcinoma in cirrhotic patients: prognostic value of preoperative portal pressure. *Gastroenterology* 1996; **111**: 1018-1022 [PMID: 8831597 DOI: 10.1016/S0016-5085(96)70070-7]
- 108 **McCormack L**, Petrowsky H, Jochum W, Furrer K, Clavien PA. Hepatic steatosis is a risk factor for postoperative complications after major hepatectomy: a matched case-control study. *Ann Surg* 2007; **245**: 923-930 [PMID: 17522518 DOI: 10.1097/01.sla.0000251747.80025.b7]
- 109 **Fernandez FG**, Ritter J, Goodwin JW, Linehan DC, Hawkins WG, Strasberg SM. Effect of steatohepatitis associated with irinotecan or oxaliplatin pretreatment on resectability of hepatic colorectal metastases. *J Am Coll Surg* 2005; **200**: 845-853 [PMID: 15922194 DOI: 10.1016/j.jamcollsurg.2005.01.024]
- 110 **Bilchik AJ**, Poston G, Curley SA, Strasberg S, Saltz L, Adam R, Nordlinger B, Rougier P, Rosen LS. Neoadjuvant chemotherapy for metastatic colon cancer: a cautionary note. *J Clin Oncol* 2005; **23**: 9073-9078 [PMID: 16361615 DOI: 10.1200/jco.2005.03.2334]
- 111 **Aloia T**, Sebah M, Plasse M, Karam V, Lévi F, Giacchetti S, Azoulay D, Bismuth H, Castaing D, Adam R. Liver histology and surgical outcomes after preoperative chemotherapy with fluorouracil plus oxaliplatin in colorectal cancer liver metastases. *J Clin Oncol* 2006; **24**: 4983-4990 [PMID: 17075116 DOI: 10.1200/JCO.2006.05.8156]
- 112 **Morris-Stiff G**, Tan YM, Vauthey JN. Hepatic complications following preoperative chemotherapy with oxaliplatin or irinotecan for hepatic colorectal metastases. *Eur J Surg Oncol* 2008; **34**: 609-614 [PMID: 17764887 DOI: 10.1016/j.ejso.2007.07.007]
- 113 **Rahbari NN**, Garden OJ, Padbury R, Brooke-Smith M, Crawford M, Adam R, Koch M, Makuuchi M, Dematteo RP, Christophi C, Banting S, Usatoff V, Nagino M, Madden G, Hugh TJ, Vauthey JN, Greig P, Rees M, Yokoyama Y, Fan ST, Nimura Y, Figueras J, Capussotti L, Büchler MW, Weitz J. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). *Surgery* 2011; **149**: 713-724 [PMID: 21236455 DOI: 10.1016/j.surg.2010.10.001]
- 114 **Wadei HM**, Mai ML, Ahsan N, Gonwa TA. Hepatorenal syndrome: pathophysiology and management. *Clin J Am Soc Nephrol* 2006; **1**: 1066-1079 [PMID: 17699328 DOI: 10.2215/cjn.01340406]
- 115 **Abdalla EK**, Noun R, Belghiti J. Hepatic vascular occlusion: which technique? *Surg Clin North Am* 2004; **84**: 563-585 [PMID: 15062662 DOI: 10.1016/S0039-6109(03)00231-7]
- 116 **Smyrniotis V**, Farantos C, Kostopanagiotou G, Arkadopoulos N. Vascular control during hepatectomy: review of methods and results. *World J Surg* 2005; **29**: 1384-1396 [PMID: 16222453 DOI: 10.1002/lt.22274]



- 10.1007/s00268-005-0025-x]
- 117 **Pringle JH**. V. Notes on the Arrest of Hepatic Hemorrhage Due to Trauma. *Ann Surg* 1908; **48**: 541-549 [PMID: 17862242]
  - 118 **Li AK**, Mok SD. Simplified hepatectomy: the tourniquet method. *Aust N Z J Surg* 1989; **59**: 161-163 [PMID: 2920001 DOI: 10.1111/j.1445-2197.1989.tb01489.x]
  - 119 **Huguet C**, Gavelli A, Chieco PA, Bona S, Harb J, Joseph JM, Jobard J, Gramaglia M, Lasserre M. Liver ischemia for hepatic resection: where is the limit? *Surgery* 1992; **111**: 251-259 [PMID: 1311871]
  - 120 **Hannoun L**, Borie D, Delva E, Jones D, Vaillant JC, Nordlinger B, Parc R. Liver resection with normothermic ischaemia exceeding 1 h. *Br J Surg* 1993; **80**: 1161-1165 [PMID: 8402122 DOI: 10.1002/bjs.1800800933]
  - 121 **Midorikawa Y**, Kubota K, Takayama T, Toyoda H, Ijichi M, Torzilli G, Mori M, Makuuchi M. A comparative study of postoperative complications after hepatectomy in patients with and without chronic liver disease. *Surgery* 1999; **126**: 484-491 [PMID: 10486600 DOI: 10.1016/S0039-6060(99)70089-9]
  - 122 **Smyrniotis VE**, Kostopanagiotou GG, Contis JC, Farantos CI, Voros DC, Kannas DC, Koskinas JS. Selective hepatic vascular exclusion versus Pringle maneuver in major liver resections: prospective study. *World J Surg* 2003; **27**: 765-769 [PMID: 14509502 DOI: 10.1007/s00268-003-6978-8]
  - 123 **Belghiti J**, Noun R, Malafosse R, Jagot P, Sauvanet A, Pierangeli F, Marty J, Farges O. Continuous versus intermittent portal triad clamping for liver resection: a controlled study. *Ann Surg* 1999; **229**: 369-375 [PMID: 10077049]
  - 124 **Choukèr A**, Schachtner T, Schauer R, Dugas M, Løhe F, Martignoni A, Pollwein B, Niklas M, Rau HG, Jauch KW, Peter K, Thiel M. Effects of Pringle manoeuvre and ischaemic preconditioning on haemodynamic stability in patients undergoing elective hepatectomy: a randomized trial. *Br J Anaesth* 2004; **93**: 204-211 [PMID: 15194628 DOI: 10.1093/bja/ae195]
  - 125 **Delva E**, Nordlinger B, Parc R, Lienhart A, Hannoun L, Huguet C. Hepatic vascular exclusion (HVE) for major liver resections. *Int Surg* 1987; **72**: 78-81 [PMID: 3610538]
  - 126 **Belzer FO**, Southard JH. Principles of solid-organ preservation by cold storage. *Transplantation* 1988; **45**: 673-676 [PMID: 3282347]
  - 127 **Hannoun L**, Balladur P, Delva E, Panis Y, Camus Y, Honiger J, Levy E, Parc R. ["Ex situ-in vivo" surgery of the liver: a new technique in liver surgery. Principles and preliminary results]. *Gastroenterol Clin Biol* 1991; **15**: 758-761 [PMID: 1667768]
  - 128 **Delrivière L**, Hannoun L. In situ and ex situ in vivo procedures for complex major liver resections requiring prolonged hepatic vascular exclusion in normal and diseased livers. *J Am Coll Surg* 1995; **181**: 272-276 [PMID: 7670689]
  - 129 **Rees M**, Plant G, Wells J, Bygrave S. One hundred and fifty hepatic resections: evolution of technique towards bloodless surgery. *Br J Surg* 1996; **83**: 1526-1529 [PMID: 9014666 DOI: 10.1002/bjs.1800831110]
  - 130 **Smyrniotis V**, Kostopanagiotou G, Theodoraki K, Tsantoulas D, Contis JC. The role of central venous pressure and type of vascular control in blood loss during major liver resections. *Am J Surg* 2004; **187**: 398-402 [PMID: 15006570 DOI: 10.1016/j.amjsurg.2003.12.001]
  - 131 **Wang WD**, Liang LJ, Huang XQ, Yin XY. Low central venous pressure reduces blood loss in hepatectomy. *World J Gastroenterol* 2006; **12**: 935-939 [PMID: 16521223 DOI: 10.3748/wjg.v12.i6.935]
  - 132 **Correa-Gallego C**, Berman A, Denis SC, Langdon-Embry L, O'Connor D, Arslan-Carlson V, Kingham TP, D'Angelica MI, Allen PJ, Fong Y, DeMatteo RP, Jarnagin WR, Melendez J, Fischer M. Renal function after low central venous pressure-assisted liver resection: assessment of 2116 cases. *HPB (Oxford)* 2015; **17**: 258-264 [PMID: 25387727 DOI: 10.1111/hpb.12347]
  - 133 **Llovet JM**, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999; **30**: 1434-1440 [PMID: 10573522 DOI: 10.1002/hep.510300629]
  - 134 **Bellavance EC**, Lumpkins KM, Mentha G, Marques HP, Capussotti L, Pulitano C, Majno P, Mira P, Rubbia-Brandt L, Ferrero A, Aldrighetti L, Cunningham S, Russolillo N, Philosophe B, Barroso E, Pawlik TM. Surgical management of early-stage hepatocellular carcinoma: resection or transplantation? *J Gastrointest Surg* 2008; **12**: 1699-1708 [PMID: 18709418]
  - 135 **Emond JC**, Samstein B, Renz JF. A critical evaluation of hepatic resection in cirrhosis: optimizing patient selection and outcomes. *World J Surg* 2005; **29**: 124-130 [PMID: 15654659 DOI: 10.1007/s00268-004-7633-8]
  - 136 **Liau KH**, Blumgart LH, DeMatteo RP. Segment-oriented approach to liver resection. *Surg Clin North Am* 2004; **84**: 543-561 [PMID: 15062661 DOI: 10.1016/j.suc.2003.12.003]
  - 137 **Malinowski M**, Geisel D, Stary V, Denecke T, Seehofer D, Jara M, Baron A, Pratschke J, Gebauer B, Stockmann M. Portal vein embolization with plug/coils improves hepatectomy outcome. *J Surg Res* 2015; **194**: 202-211 [PMID: 25454977 DOI: 10.1016/j.jss.2014.10.028]
  - 138 **Broering DC**, Hillert C, Krupski G, Fischer L, Mueller L, Achilles EG, Schulte am Esch J, Rogiers X. Portal vein embolization vs. portal vein ligation for induction of hypertrophy of the future liver remnant. *J Gastrointest Surg* 2000; **6**: 905-913; discussion 913 [PMID: 12504230 DOI: 10.1016/S1091-255X(02)00122-1]
  - 139 **Adam R**, Laurent A, Azoulay D, Castaing D, Bismuth H. Two-stage hepatectomy: A planned strategy to treat irresectable liver tumors. *Ann Surg* 2000; **232**: 777-785 [PMID: 11088072]

**P- Reviewer:** He ST, Peltec A, Qin JM, Sirin G, Wang GY

**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Li D



Basic Study

## Anti-hepatitis C virus potency of a new autophagy inhibitor using human liver slices model

Sylvie Lagaye, Sonia Brun, Jesintha Gaston, Hong Shen, Ruzena Stranska, Claire Camus, Clarisse Dubray, Géraldine Rousseau, Pierre-Philippe Massault, Jérôme Courcambeck, Firas Bassisi, Philippe Halfon, Stanislas Pol

Sylvie Lagaye, Jesintha Gaston, Stanislas Pol, Institut Pasteur, INSERM U1223, 75015 Paris, France

Sylvie Lagaye, Jesintha Gaston, Hong Shen, Institut Cochin, INSERM U1016, CNRS UMR8104, Université Paris Descartes (UMR S1016), 75014 Paris, France

Sonia Brun, Clarisse Dubray, Jérôme Courcambeck, Firas Bassisi, Philippe Halfon, Genoscience Pharma, 13006 Marseille, France

Ruzena Stranska, KU Leuven, Rega Institute, 3000 Leuven, Belgium

Claire Camus, Philippe Halfon, Laboratoire Alphabio, 13006 Marseille, France

Géraldine Rousseau, APHP, Groupe Hospitalier La Pitié Salpêtrière, Service de Chirurgie digestive et Hépatobiliaire, 75013 Paris, France

Pierre-Philippe Massault, APHP, Groupe Hospitalier Cochin, Service de Chirurgie digestive, Hépatobiliaire et Endocrinienne, 75014 Paris, France

Stanislas Pol, Université Paris Descartes, 75014 Paris, France

Stanislas Pol, APHP, Groupe Hospitalier Cochin, Unité d'Hépatologie, 75014 Paris, France

Stanislas Pol, Institut Pasteur, Département de Recherche Translationnelle, INSERM UMS20, 75015 Paris, France

**Author contributions:** Lagaye S wrote the paper; Lagaye S, Brun S, Gaston J, Shen H, Stranska R, Camus C, Dubray C, Rousseau G, Massault PP, Courcambeck J and Bassisi F performed the experiments; Lagaye S, Brun S, Camus C, Halfon P and Pol S analyzed the data; Lagaye S, Halfon P and Pol S conceived and designed the experiments; Halfon P and Pol S contributed equally to the work.

Supported by The Institut National de la Santé et de la Recherche

Médicale (INSERM, France); and the personal support of Professor Jean-François Delfraissy as Director of the French Agency, Agence Nationale de Recherches sur le Sida et les hépatites virales (ANRS).

**Institutional review board statement:** Institutional review board statement is not required for manuscript submission in our Institution.

**Institutional animal care and use committee statement:** No animal use in the experiments.

**Conflict-of-interest statement:** The authors have no conflict of interest to declare.

**Data sharing statement:** No data sharing.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Sylvie Lagaye, PhD, DSc, Senior Scientist, Institut Pasteur, INSERM U1223, 25-28 rue du Dr Roux, 75015 Paris, France. [sylvie.lagaye@inserm.fr](mailto:sylvie.lagaye@inserm.fr)

**Telephone:** +33-1-40613424

**Fax:** +33-1-45688548

**Received:** March 24, 2016

**Peer-review started:** April 6, 2016

**First decision:** May 16, 2016

**Revised:** June 1, 2016

**Accepted:** June 27, 2016

**Article in press:** June 29, 2016

**Published online:** July 28, 2016

## Abstract

**AIM:** To evaluate the antiviral potency of a new anti-hepatitis C virus (HCV) antiviral agent targeting the cellular autophagy machinery.

**METHODS:** Non-infected liver slices, obtained from human liver resection and cut in 350  $\mu\text{m}$ -thick slices ( $2.7 \times 10^6$  cells per slice) were infected with cell culture-grown HCV Con1b/C3 supernatant (multiplicity of infection = 0.1) cultivated for up to ten days. HCV infected slices were treated at day 4 post-infection with GNS-396 for 6 d at different concentrations. HCV replication was evaluated by strand-specific real-time quantitative reverse transcription - polymerase chain reaction. The infectivity titers of supernatants were evaluated by foci formation upon inoculation into naive Huh-7.5.1 cells. The cytotoxic effect of the drugs was evaluated by lactate dehydrogenase leakage assays.

**RESULTS:** The antiviral efficacy of a new antiviral drug, GNS-396, an autophagy inhibitor, on HCV infection of adult human liver slices was evidenced in a dose-dependent manner. At day 6 post-treatment, GNS-396 EC<sub>50</sub> was 158 nmol/L without cytotoxic effect (compared to hydroxychloroquine EC<sub>50</sub> = 1.17  $\mu\text{mol/L}$ ).

**CONCLUSION:** Our results demonstrated that our *ex vivo* model is efficient for evaluation the potency of autophagy inhibitors, in particular a new quinoline derivative GNS-396 as antiviral could inhibit HCV infection in a dose-dependent manner without cytotoxic effect.

**Key words:** Host antiviral therapy; Hepatitis C virus; Tissue culture; Autophagy; Quinoline derivative

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatitis C virus (HCV) infection (or spread) is a serious public health challenge counting approximately 170 million people that are chronically infected worldwide. Efficient interferon-free treatments with new direct acting antivirals are expected to cure more than 90% of HCV-infected patients but they are not available in all the countries. Autophagy machinery is required to initiate HCV replication. Host antiviral therapy is an additional option for the treatment of HCV infection. The new autophagy inhibitor GNS-396 demonstrated significant efficacy and additive activity in inhibiting HCV replication and might be an additional option to treat HCV infected individuals.

Lagaye S, Brun S, Gaston J, Shen H, Stranska R, Camus C, Dubray C, Rousseau G, Massault PP, Courcambeck J, Bassisi F, Halfon P, Pol S. Anti-hepatitis C virus potency of a new autophagy inhibitor using human liver slices model. *World J Hepatol* 2016; 8(21): 902-914 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i21/902.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i21.902>

## INTRODUCTION

Approximately 170 million people worldwide are chronically infected with hepatitis C virus (HCV)<sup>[1]</sup>. Until recently, the most effective treatment against HCV infection was the combination of pegylated interferon- $\alpha$  2a or b and ribavirin (PR) which achieved sustained virological response (SVR) in about 45% of individuals infected by HCV genotype 1, 65% by HCV genotype 4, 70% by HCV genotype 3 and more than 85% by HCV genotype 2<sup>[2,3]</sup>. The frequent side effects associated with PR and the rates of non response to PR includes partial or null virologic response and breakthrough or relapse after PR discontinuation. Thus, development of novel and more effective antiviral treatments were essential<sup>[4]</sup>.

Two HCV NS3 protease inhibitors (PI), boceprevir (BOC) and telaprevir (TVR) have been approved and combined with PR, have increased the SVR to about 75% in therapy naïve HCV genotype 1 infected patients<sup>[5-9]</sup>. Over the past few years, other direct acting antivirals (DAAs) were developed<sup>[10-14]</sup> as second generation of PI with higher antiviral potency, HCV NS5A replication complex inhibitors and nucleotide analogue HCV NS5B polymerase inhibitors<sup>[13]</sup> as well as host-targeted indirect antivirals like cyclophilin inhibitors<sup>[15]</sup> and lambda interferon<sup>[15]</sup>. Interferon-free treatments with new DAAs are expected to cure more than 90% of HCV-infected patients<sup>[16]</sup>. But they are not available in all the countries<sup>[17]</sup>. At the present time, triple therapy combining PR with NS3 PI (TVR or BOC) is going to remain the main treatment for HCV patients<sup>[16-21]</sup>. That is why it appears important to continue research in limiting virus replication and the autophagy inhibition could be a new additional pathway because of recent evidences obtained regarding to an increased autophagic response in the liver of chronically HCV infected patients<sup>[22]</sup>.

Autophagy is a catabolic process which degrades a cellular own component through the lysosomal machinery<sup>[23]</sup>. It has been shown that autophagy is activated during virus and bacterial infection<sup>[24]</sup> and that some viruses can use the autophagy system to facilitate their own replication<sup>[25-29]</sup>. Previously, several studies evidenced that HCV infection resulted in endoplasmic reticulum stress and autophagy responses, that HCV regulated the autophagy pathway, that the autophagy machinery was required to initiate HCV replication, and finally, that the suppression of autophagy inhibited HCV replication<sup>[30-35]</sup>. Interestingly, it has been demonstrated that HCV induces autophagosomes *via* a Class III PI3K-independent pathway and uses autophagosomal membranes as sites for its RNA replication<sup>[36]</sup>.

The lysosomotropic anti-malarial drugs, chloroquine (CQ) and hydroxychloroquine (HCQ), belonging to the quinoline family, are among the autophagy inhibitors, which act by preventing the acidification of lysosomes, leading to the inhibition of both fusion of autophagosome with lysosome and lysosomal protein degradation<sup>[23]</sup>. In fact, CQ exerts an inhibitory effect for several RNA viruses including coronaviruses, flaviviruses and human

immunodeficiency virus<sup>[37-39]</sup>. Recently, it has been shown that a treatment with CQ of HCV infected cells suppressed the replication of the virus in a dose-dependent manner by preventing the autophagic proteolysis<sup>[40]</sup>.

In the present study, we used the established *ex vivo* model of primary human liver slices culture which allows to the *de novo* replication of primary viral isolates and production of high titer infectious HCV particles<sup>[41]</sup> to evaluate the potential antiviral potency GNS-396<sup>[42]</sup>, a new autophagy inhibitor in comparison with a well-known autophagy inhibitor, HCQ. Presented results might be additional options to treat HCV infected individuals.

## MATERIALS AND METHODS

### Human liver tissue specimens

Adult human primary liver tissue samples were obtained from HCV and also hepatitis B virus, and human immunodeficiency virus seronegative patients who underwent liver resection surgery, mainly for liver metastasis in the absence of underlying liver disease. Experimental procedures were carried out in accordance with French laws and Regulations.

### Liver slices preparation, culture and infection

Slices were prepared and cultured as described<sup>[41,43]</sup>. Briefly, uninfected human liver slices, obtained from human liver resection, were cut into 350  $\mu\text{m}$  thick slices of ( $2.7 \times 10^6$  cells per slice) with a vibratome (Leica, VTS1200) and transferred to 0.4  $\mu\text{m}$  organotypic culture inserts (Millipore) in 12-wells plates (1 slice/well) containing 2 mL of complete Dulbecco's modified eagles's medium (DMEM) culture media and maintained at 37 °C under a constant flow of humidified 95% O<sub>2</sub>/5% CO<sub>2</sub> for up to 24 h before viral infection. Cell number for tissue slices was estimated at approximately  $2.7 \times 10^6$  cells per slice based on a 14-cell thick slice (cell diameter approximately 25  $\mu\text{m}$ )<sup>[41]</sup>. The complete culture medium consisted of DMEM with 4.5 g/L D-glucose and glutamine (Life Technologies) supplemented with 10% fetal calf serum (Life Technologies, 16000-044), 5% penicillin-streptomycin (Life Technologies, 10378-016), 1% amphotericin (Sigma Aldrich), 5  $\mu\text{g/mL}$  insulin (Life Technologies, 51500-056), 0.4  $\mu\text{g/mL}$  dexamethasone (Sigma Aldrich, D4902), 10 mmol/L HEPES (Life Technologies, 15630080), non-essential amino acids (Life Technologies), 20 mmol/L sodium pyruvate (Life Technologies) and 50  $\mu\text{g/mL}$  ascorbic acid (Sigma Aldrich). One day post-culture in twelve-transwell plates, human primary liver slices were inoculated with HCV Con1/C3 at a multiplicity of infection equal to 0.1 at 37 °C in the same culture conditions as described above, for overnight. The infectious clone Con1/C3 (genotype 1b) (JFH1-derived chimeric viruses whose structural proteins are encoded by the genotype 1b-HCV sequence Con1)<sup>[44]</sup> could efficiently infect human liver slices which maintain their hepatocyte differentiation and retain normal physiological and biochemical parameters for at least 10 d. The inoculum was then removed; the slices were

washed three times with PBS and then supplemented with complete culture medium. Then, liver slices were cultured without medium replacements, as previously described<sup>[41]</sup>.

### HCV RNA transfection and virus production

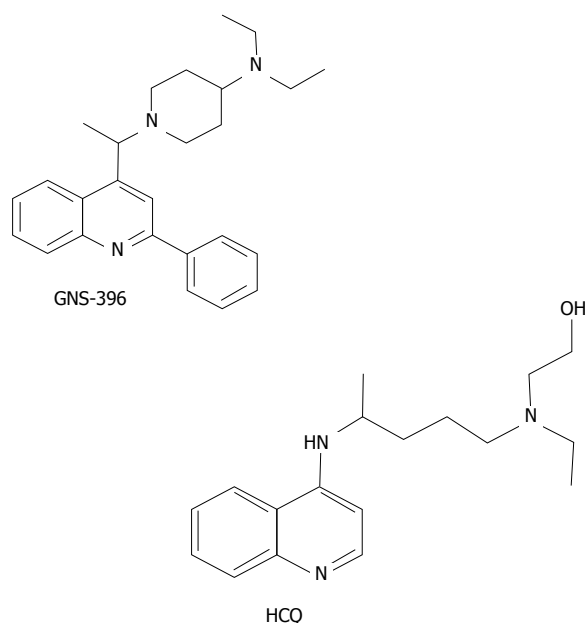
To produce HCVcc, viral RNAs were transcribed *in vitro* and electroporated into Huh-7.5.1 cell line (kindly provided by Professor Francis V Chisari, The Scripps Research Institute, La Jolla, CA), as described previously<sup>[45]</sup>. The infectious titer of cell culture supernatants was evaluated by classical titration assay<sup>[45]</sup>. In brief, the HCV infection of Huh-7.5.1 cells was performed with serial 10-fold dilution of viral supernatants. Seventy-two hours later, the formation of infected cells foci were detected by staining with human HCV positive sera or mouse monoclonal antibodies directed against HCV core (Ozyme) and non-structural (NS5A) (Virostat, clone1877) proteins. Titrations were performed in duplicate.

### Quantification of HCV strands RNA by real-time quantitative reverse transcription- polymerase chain reaction

A strand-specific real-time quantitative reverse transcription-polymerase chain reaction technique to quantify the intracellular levels of positive and negative strand HCV RNA was performed as previously described<sup>[46-49]</sup>. The quantification of 28S rRNA was used as an internal standard to quantify HCV in total liver RNA, as previously described<sup>[46]</sup>, (threshold of detection: 25 copies/reaction). Briefly, reverse transcription was carried out using oligo(dT) primer (Life Technologies) and Moloney murine leukemia virus reverse transcriptase (Promega) as recommended by the manufacturer. Real-time polymerase chain reactions were performed using the Light CyclerR (Roche Applied Science) and Fast Start DNA Master SYBR Green I kit (Life Science, Roche) according with the manufacturer's protocol.

Reverse transcription was performed using primers located in the 5' NCR region of HCV genome, tag-RC1 (5'-GGC CGT CAT GGT GGC GAA TAA GTC TAG CCA TGG CGT TAG TA-3')<sup>[47]</sup> and RC21 (5'-CTC CCG GGG CAC TCG CAA GC-3')<sup>[48]</sup> for the negative and positive strands, respectively, as described previously<sup>[46]</sup>. After a denaturation step performed at 70 °C for 8 min, the RNA template was incubated at 48 °C for 5 min in the presence of 200 ng of tag-RC1 primer and 1.25 mmol/L of each deoxynucleoside triphosphate (dNTP) (Promega) in a total volume of 12  $\mu\text{L}$ . Reverse transcription was carried out for 60 min at 60 °C in the presence of 20 U RNaseOutTM (Life Technologies) and 7.5 U ThermoscriptTM reverse transcriptase (Life Technologies), in the buffer recommended by the manufacturer. An additional treatment was applied by adding 1  $\mu\text{L}$  (2U) RNaseH (Life Technologies) for 20 min at 37 °C. The first round of nested PCR was performed with 2  $\mu\text{L}$  of the cDNA obtained in a total volume of 50  $\mu\text{L}$ , containing 3 U Taq polymerase (Promega), 0.5 mmol/L dNTP Mix (Promega), and 0.5  $\mu\text{mol/L}$  RC1 (5'-GTC TAG CCA TGG CGT TAG TA-3')





**Figure 1** GNS-396 and hydroxychloroquine structures. HCQ: Hydroxychloroquine.

and RC21 primers for positive-strand amplification, or tag (5'-GGC CGT CAT GGTGGC GAA TAA-3') and RC21 primers for negative strand amplification. The PCR protocol consisted of 18 cycles of denaturation (94 °C for 1 min), annealing (55 °C for 45 s), and extension (72 °C for 2 min). The cDNA obtained was purified using the Quick-clean kit (Qiagen), according to the manufacturer's instructions, and 2 µL of the purified product suspended in 10 µL nuclease free water (Promega) were then subjected to real-time PCR. The reaction was carried out using the DNA Fast Start SYBR Green Kit (Life Science, Roche), with Lightcycler<sup>TM</sup> instruments and technology (Roche Diagnostics). PCR amplifications were performed in a total volume of 20 µL, containing 3 mmol/L MgCl<sub>2</sub>, 2 µL DNA Master green (Life Science) and 50 ng of the 197 R (5'-CTTTCGCGACCCAACACTAC-3') and 104 (5'-AGAGCCATAGTGGTCTGCGG-3') primers<sup>[48,49]</sup>. The PCR protocol consisted of one step of initial denaturation for 10 min at 94 °C, followed by 40 cycles of denaturation (95 °C for 15 s), annealing (57 °C for 5 s), and extension (72 °C for 8 s). After amplification, the specificity of PCR products was checking by a melting curve analysis.

### Western blotting

Western blotting was performed as following. Each liver slice was washed 3 times in PBS, incubated in Laemmli buffer<sup>[50]</sup> at 100 °C for 10 min. The lysate was passed through a 26 G needle, 10 times and kept at -80 °C. Before electrophoresis in pre-cast sodium dodecyl sulfate polyacrylamide gel 4%-12% (Life Technologies), the samples were incubated at 95 °C for 5 min. After electrophoresis, proteins were transferred to a 0.22 µm Protran membrane BA83 (Schleicher and Schuell) and HCV proteins were detected by Western blotting using mouse monoclonal antibodies to core (C7-50, 1:10000) (Ozyme), to NS5A (1:2000) (Virostat, clone 1877), to

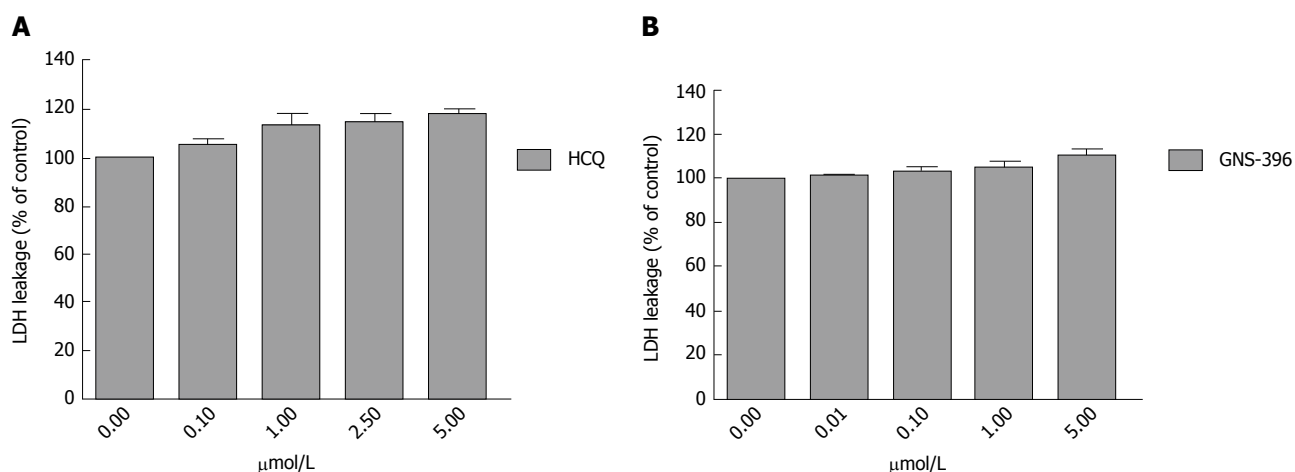
LC3 proteins (Sigma-Aldrich) and to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Abcam), and to β-actin (Pierce biotechnology). Horseradish peroxidase-conjugated anti-mouse IgG (GeHealthCare Life Sciences) at the dilution of 1:50000 were used as secondary antibodies. The reactions were developed using enhanced chemiluminescence detection reagents (GeHealthCare Life Sciences), followed by exposure to X-OMAT film (GeHealthCare Life Sciences). LC3-II protein expression analysis was performed with Image J software.

### Drugs inhibition of HCVcc Con1/C3 replication and cytotoxicity assays

The HCVcc Con1/C3 inhibition either by pegylated-interferon α-2a (peg-INF) (Roche, Pegasys) or/and ribavirin (RBV) (Schering Plough, Rebetol) or TVR (Janssen-Cilag, Incivo) or BOC (Schering-Plough, Boceprevir) or SOF (Gilead Sciences Intl Ltd, Sofosbuvir) or GNS-396 (Figure 1) (Genoscience Pharma, Marseille, France) or HCQ (Figure 1) (Genoscience Pharma, Marseille, France) or 0.5% dimethylsulfoxide (DMSO) (Sigma Aldrich) as a carrier control, and the cytotoxicity assays were performed as following. At day 4 post-infection with HCVcc Con1/C3 the human liver slices were treated by addition of different concentrations of the following drugs: peg-INF or/and RBV or TVR or BOC or SOF or HCQ (0.1, 1, 2.5, 5 µmol/L or a new quinoline derivative, GNS-396 (0.1, 1, 2.5, 5 µmol/L) alone or 0.5% DMSO as a carrier control, to culture medium, twice daily, up to day 10 post-infection. The infectivity (ffu/mL) was measured at day 2, day 4 or day 6 post-treatment depending on the experiment as described<sup>[41]</sup>. All the experiments were performed in triplicate. The cytoTox 96R Non-Radioactive Cytotoxicity Assay (Promega, G1780) was used to assess the potential cytotoxicity of the drugs. Results of lactate dehydrogenase (LDH) leakage were compared to the carrier control calculated (Figure 2) as described previously<sup>[51]</sup>.

### Evaluation of autophagy modulation and inhibition

Autophagy modulation was evaluated on HeLa cells treated with GNS-396, a new quinolone derivative. For tracking different stages of autophagy the tandem fusion of mRFP and EGFP fused to LC3 make a pH-sensitive sensor (mRFP-EGFP-LC3) that is used to monitor autophagy in live cells<sup>[52]</sup>. The EGFP tag is acid-sensitive while the mRFP tag is not. The double tagged LC3 can be used to label autophagosomes, amphisomes and autolysosomes. In autophagosomes, both tags emit yellow light. However, the fusion of autophagosomes to acidic lysosomes results in acidic autolysosomes where the green fluorescence from GFP is lost. Subsequently, the red fluorescence from mRFP is lost when the double tagged protein is degraded. The autophagic flux inhibition was shown using a SkBr3 mRFP1-EGFP-LC3 stable breast cancer cell line treated with 100 µmol/L GNS-396 or 100 µmol/L HCQ during 6 h. HCQ was used as a positive control of autophagy inhibition. Cell images were obtained using an epifluorescence microscope (Nikon,



**Figure 2** Cytotoxicity assays of the drugs used hydroxychloroquine (A) and GNS-396 (B). Percentages of lactate dehydrogenase (LDH) leakage are relative to DMSO control-treated liver slices. Drugs cytotoxicity is significant at % of control > 120. All experiments were performed on triplicate. Values are expressed as means  $\pm$  SE, comparisons were performed using the Mann-Whitney rank-sum test ( $P < 0.001$ ). HCQ: Hydroxychloroquine; DMSO: Dimethylsulfoxide.

Eclipse Ci).

Autophagy inhibition was evaluated on HeLa cells treated with GNS-396, at different drug concentrations (1, 10, 100  $\mu$ mol/L) during 4 h or 6 h in the presence or absence of bafilomycin A1 (100 nmol/L) (Sigma-Aldrich) added for the last 2 h. Bafilomycin A1 (BafA1) is an autophagy inhibitor as endosomal acidification inhibitor. It is a known inhibitor of the late phase of autophagy. Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes. Bafilomycin A1 acts by inhibiting vacuolar H<sup>+</sup> ATPase<sup>[53,54]</sup>. HCQ was used as a positive control of autophagy inhibition. The LC3-II protein expression in cell lysates was evaluated by Western-blot assay [anti-LC3 antibody (Sigma-Aldrich)] compared to either GAPDH protein expression [anti-GAPDH antibody (Abcam)] or  $\beta$ -actin protein expression [anti-actin antibody (Pierce biotechnology)]. LC3-II protein expression analysis was performed with Image J software.

### Statistical analysis

At different days of the kinetics, the results were obtained from the mean of the three slices culture. Statistical tests were performed using the GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA, United States). Values are expressed as means  $\pm$  standard errors of the mean. The data were compared using an unpaired two-tailed student's *t*-test or the Mann-Whitney rank-sum test. *P*-value < 0.05 was considered significant.

## RESULTS

### Validation of GNS-396, a new quinoline derivative, as inhibitor of autophagy

We evaluated the effect of GNS-396 (Figure 1)<sup>[42]</sup>, a new quinoline derivative, on autophagy by treatment of HeLa cells with various concentrations of GNS-396 during 6 h. HCQ was used as a positive control of autophagy inhibition. The microtubule-associated protein 1A/1B-

light chain 3 (LC3) is a soluble ubiquitin-like protein with a molecular mass of approximately 17 kDa that exists ubiquitously in mammalian tissues and cultured cells, as an unconjugated form (LC3-I) or conjugated to autophagosomes membranes (LC3-II: lipidated form). During autophagy, a cytosolic form of LC3 (LC3-I) is conjugated to phosphatidylethanolamine to form LC3-phosphatidylethanolamine conjugate (LC3-II), which is recruited to autophagosomal membranes allowing for the closure of the autophagic vacuole. Autophagosomes fuse with lysosomes to form autolysosomes, and intra-autophagosomal components are degraded by lysosomal hydrolases. At the same time, LC3-II in autolysosomal lumen is degraded. Thus, lysosomal turnover of the autophagosomal marker LC3-II reflects autophagic activity. Analysis of LC3 intracellular expression by Western blotting demonstrated an increase of normalized LC3-II protein expression when HeLa cells were treated with GNS-396, in a dose-dependent manner (Figure 3A), reflecting the accumulation of autophagosomes in cells, and therefore an effective modulation of the autophagy. Consequently, GNS-396 is a dose-dependent autophagy modulator with a magnitude of normalized LC3-II similar to which achieved with HCQ treatment, a well-known autophagic inhibitor (Figure 3B). Similar results were obtained on Huh7.5.1 cell line (data not shown).

To evaluate if the observed accumulation of autophagosomes after GNS-396 treatment was a consequence of either a stimulated production of new autophagosomes (in this case, GNS-396 would be an autophagy inducer) or a result of autophagosome clearance blockage (in this case, GNS-396 would be an autophagy inhibitor), HeLa cells were treated with different concentrations of GNS-396 in the absence or presence of a lysosomal protease inhibitor, Bafilomycin A1, that increases lysosomal pH and blocks autophagosome-lysosome fusion (Figure 3C) and LC3 protein levels were measured. HCQ was used as a positive control of autophagy inhibition (Figure 3D). After 4 h exposure of HeLa cells to GNS-396 (100  $\mu$ mol/L),

the accumulation of LC3-II was observed (Figure 3C) which was not enhanced in the presence of BafA1, supporting the idea that GNS-396 inhibits autophagic flux as HCQ (Figure 3D). To confirm that GNS-396 is an autophagy inhibitor, the autophagic flux was monitored by fluorescence microscopy, using the mRFP-EGFP-LC3 tandem-tagged fluorescent protein in SkBr3 mRFP-EGFP-LC3 stable cell line (Figure 4). In green/red merged images, yellow dots (*i.e.*, mRFP+EGFP+) indicate autophagosomes or non-acidic autolysosomes, while red dots (*i.e.*, mRFP+EGFP-) indicate autolysosomes. The autophagy flux is increased when both yellow and red puncta (dots) are increased in cells while the autophagic flux is blocked when only yellow puncta (dots) are increased without an accompanying increase of red puncta in cells. SkBr3 mRFP-EGFP-LC3 stable cell line was treated during 6 h with either GNS-396 (Figure 4C) or HCQ, a well-known autophagic inhibitor (Figure 4B) (100  $\mu\text{mol/L}$ ). An accumulation of yellow puncta (dots) corresponding to autophagosomes or non-acidic autolysosomes was noticeable (Figure 4B and C), indicating that GNS-396 blocks the autophagic flux, and may act as lysosomotropic agent as HCQ.

#### **Modulation of autophagy and inhibition of HCV infection in human liver slices model by GNS-396 treatment**

The level of LC3 and viral proteins expression were analysed by Western blotting after 1, 4, 6 and 10 d post-infection (Figure 5). HCV infection induced autophagy with an increase of protein LC3-II expression (Figure 5B) as compared to non-infected liver slices (Figure 5A), along with an increase of intracellular expression of the core and NS5A proteins consistent with the previous reports<sup>[22,36]</sup>. Intracellular expression of the viral proteins was decreased significantly at day 6 post-treatment with HCQ (1  $\mu\text{mol/L}$ ) or GNS-396 (1  $\mu\text{mol/L}$ ) (Figure 5D) in comparison with HCVcc infected liver slices not treated (Figure 5B). The HCQ- and GNS-396-treatment induced an accumulation of LC3-II protein in HCV infected liver slices treated with 1  $\mu\text{mol/L}$  HCQ or 1  $\mu\text{mol/L}$  GNS-396 (Figure 5D) in comparison either with not infected liver slices treated (Figure 5C) or not (Figure 5A), or with HCV infected liver slices without treatment (Figure 5B). At day 10, the normalized LC3-II protein expression increased when liver slices infected (Figure 5D) or not (Figure 5C) were treated either with GNS-396 (1  $\mu\text{mol/L}$ ) or HCQ (1  $\mu\text{mol/L}$ ). The GNS-396 and HCQ effects were tested on the *de novo* viral production of HCVcc Con1 infected liver slices (Figures 6 and 7). At day 4 post-infection, HCVcc Con1 infected liver slices were treated for 6 d with different concentrations either of GNS-396 or HCQ. From day 1 to day 6 post-treatment, the HCV RNA replication (Figure 6A and B) and the infectivity (Figure 7A and B) were inhibited in a dose-dependent manner. The addition of RBV with the new drug GNS-396 showed no significant difference in the viral inhibition (data not shown).

#### **EC50 analysis of HCV replication with GNS-396 treatment compared to that of validated antiviral drugs**

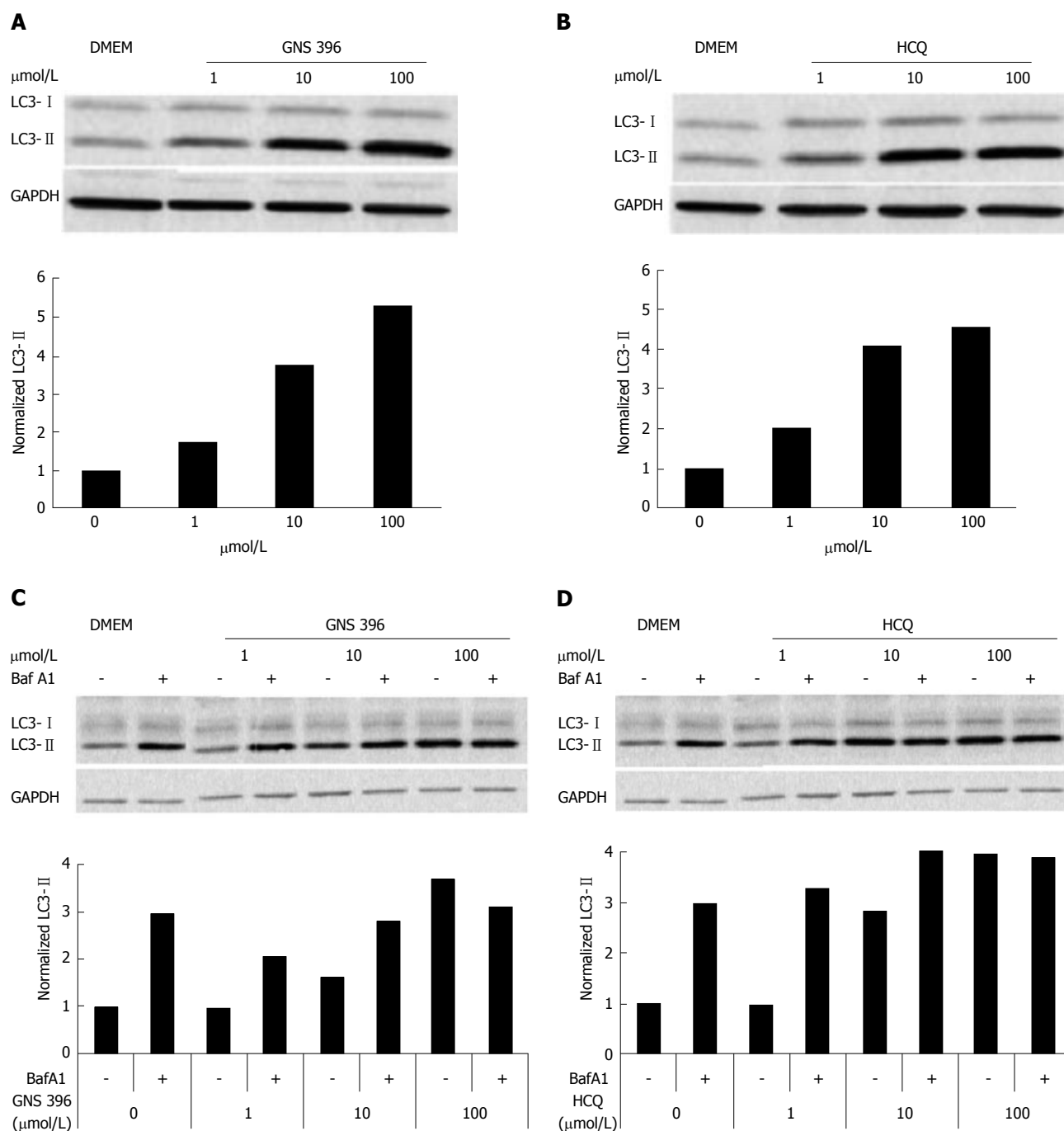
The ability of various concentrations of different antiviral

drugs to inhibit HCV replication was measured by detecting the replication of negative strands HCV RNA (Figure 6A and C) (Table 1). The calculated EC50 of different antiviral drugs is listed as Table 1 and compared to GNS-396. In summary, our model confirms that the antiviral activity of triple therapy was higher than that of the dual therapy by PR as extensively reported in clinical trials<sup>[5,6]</sup>. The new quinoline derivative GNS-396 has about 10-fold lower EC50 than HCQ (0.158  $\mu\text{mol/L}$  as compared to 1.17  $\mu\text{mol/L}$ ) (Figure 6B and D). No significant cytotoxic effects were observed when evaluated by the lactate dehydrogenase leakage (LDH) assays (Figure 2A and B). A 50% cytotoxic concentration (CC50 value) of 25  $\mu\text{mol/L}$  was obtained for GNS-396 in the human liver slices culture at day 6 post-treatment. Similar CC50 values were obtained in proliferating Huh-7-5-1 replicon cells (23  $\mu\text{mol/L}$ ).

## **DISCUSSION**

Our study evidenced that: (1) the *ex vivo* model of human liver slices HCVcc Con1 infection may be efficiently used for the assay of the antiviral potency of a new inhibitor (GNS-396 compared to HCQ) which interfered with autophagy; and (2) GNS-396 was a potent autophagy inhibitor, acting as "lysosomotropic agent" which was able to inhibit HCV replication in primary human adult HCVcc infected liver slices culture.

The establishment of the *ex vivo* model (feasibility, rapidity, specificity, potency) was already described in detail in 2012<sup>[41]</sup> with comparison between primary human hepatocytes, Huh-7.5.1 cell line. The Huh-7 cell system has several limitations that includes the inability to study the effects of pharmacologic inhibitors targeting the non-structural proteins of the most prevalent and problematic viral strains (*e.g.*, genotypes 1a and 1b). Moreover, the study of virus/host cell interactions is limited since the permissive cell lines are transformed and poorly differentiated. Firstly, the human liver slices culture maintains the original three-dimensional structure of the liver that allows cell crosstalk: The extra-cellular matrix and Kupffer cells essential for the normal function of the hepatocytes and the lobular structure. Secondly, the gene expression profiles in liver tissue slices were similar to that of the *in vivo* gene expression. Thirdly, primary hepatocytes preparations undergo treatment with collagenase (a treatment might have a negative effect on integrity of the proteins repertoire on the cell surface), but not the liver slices. Noteworthy, using established procedures, the tissue slices remained viable for, at least 10 d as it was shown by the secretion of albumin and urea. Moreover, the Huh-7 cell infection with primary isolates from patients are not very efficient. The infection of adult human liver slices culture allowed to achieve the robust replication of HCVcc genotype 2a, 1a and 1b genome and to obtain infectivity titers above 105 ffu/mL. In addition, we reported robust and productive infection using human primary isolates HCV genotype 1b. Stem cell-derived hepatocytes (hESC-Heps) displayed



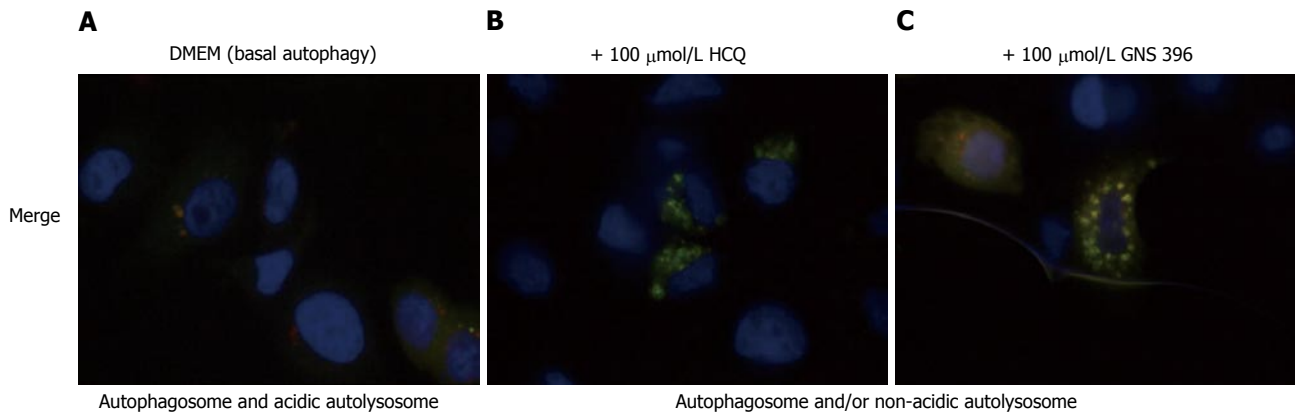
**Figure 3** Modulation and inhibition of autophagy by treatment with GNS-396 in HeLa cells. A and B: Autophagy modulation was evaluated using HeLa cells treated either with (A) GNS-396 or (B) HCQ at different concentrations (1, 10 and 100  $\mu\text{mol/L}$ ) during 6 h. HCQ was used as a positive control of autophagy modulation. Intracellular expression of proteins LC3 was evaluated by Western-blot assay and normalized for LC3- II; C and D: Autophagy inhibition by treatment with GNS-396 in HeLa cells. Autophagy inhibition was evaluated using HeLa cells treated either with (C) GNS-396 or (D) HCQ at different concentrations (1, 10 and 100  $\mu\text{mol/L}$ ) during 4 h in the presence or absence of 100 nmol/L bafilomycin A1. HCQ was used as a positive control of autophagy inhibition. LC3- II intracellular expression was evaluated by Western-blot assay and normalized. HCQ: Hydroxychloroquine; LC3: Microtubule-associated protein 1A/1B-light chain; DMEM: Dulbecco's modified eagles's medium; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; BafA1: Bafilomycin A1.

equivalence to primary adult hepatocytes. HESC-Heps were capable of supporting the full HCV life cycle (JFH1), including the release of infectious virions. Although supportive, hESC-Hep viral infection levels were not as great as those observed in Huh7 cells. Up to now, the hESC-Heps were not infected with primary isolates<sup>[55]</sup>. Currently, we are establishing a culture of liver slices for 21 d, which allows us to follow the variation of different

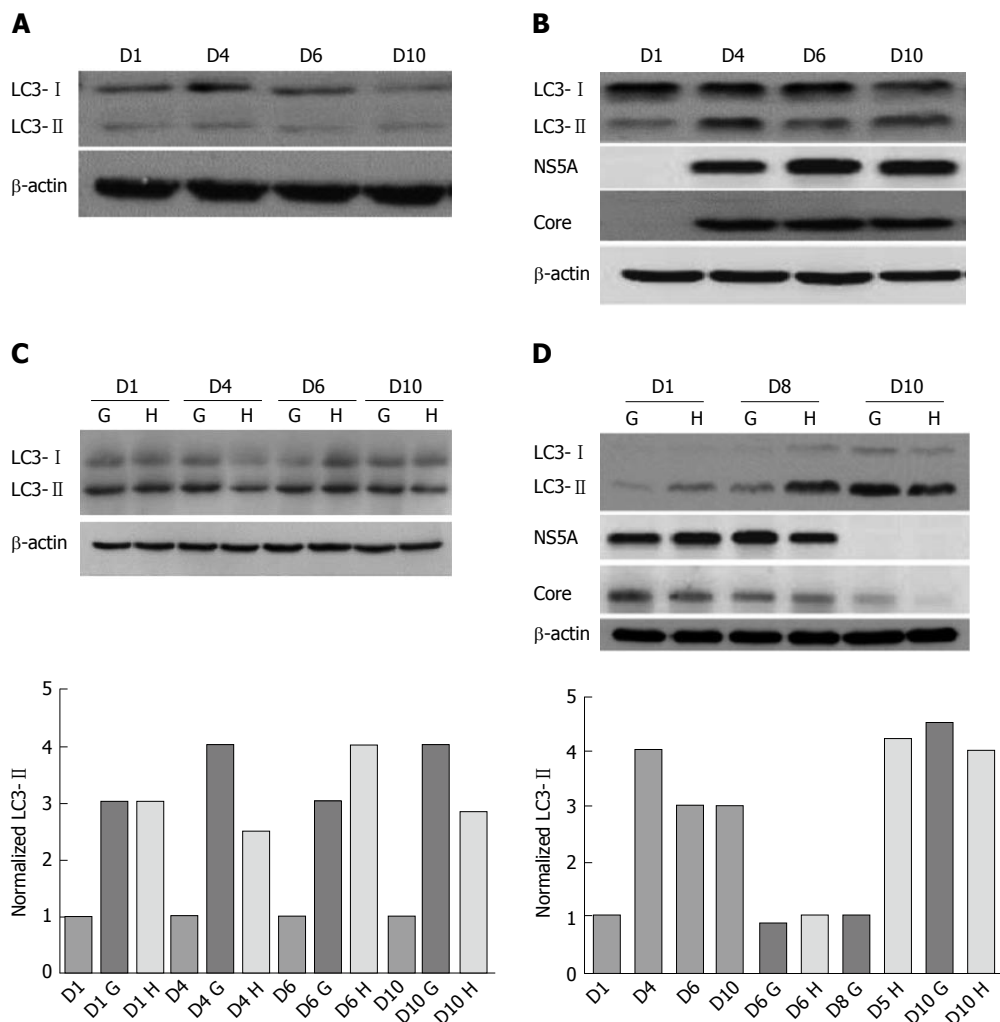
parameters and in particular, complete inhibition of viral production (data not shown).

Previous studies have reported that autophagy proteins are required to initiate HCV replication and translation<sup>[28,30-36]</sup>. Some data demonstrated that the suppression of LC3 protein lipidation, a necessary step for the formation of autophagosomes could also suppress HCV replication<sup>[30]</sup>. CQ is a well-known autophagic inhibitor

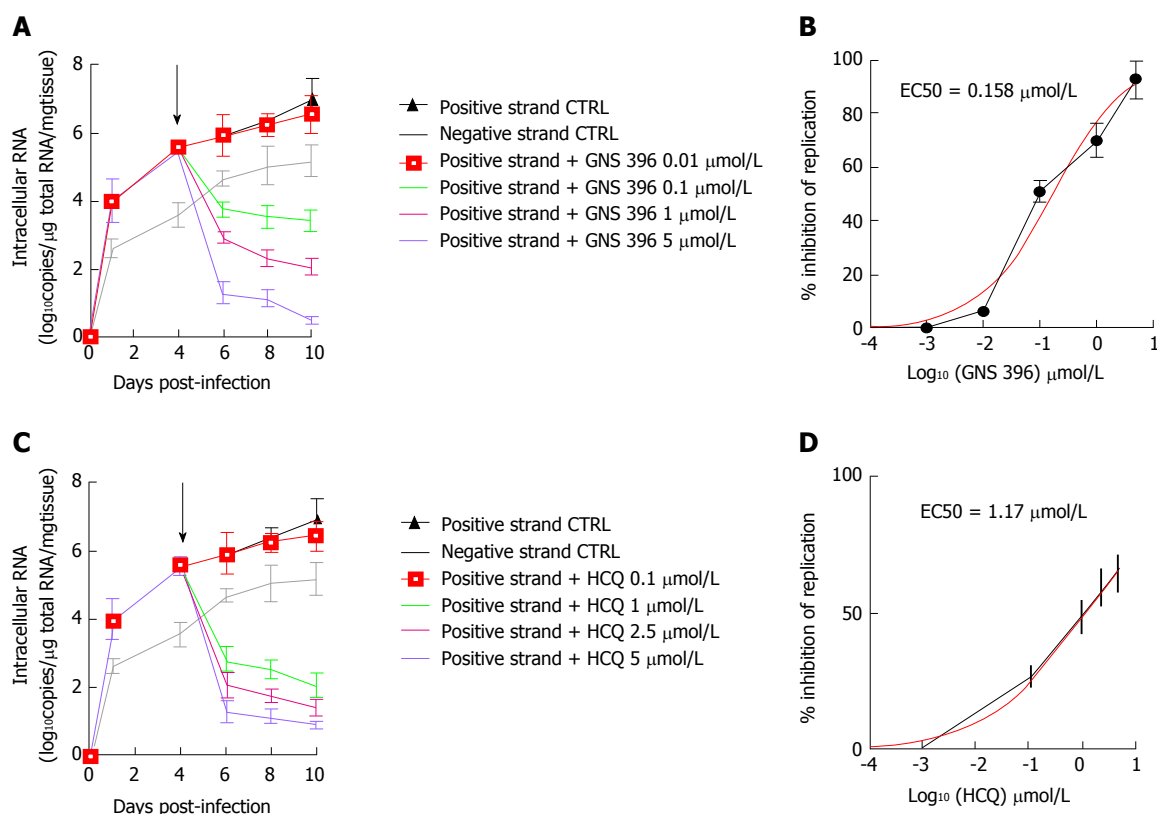




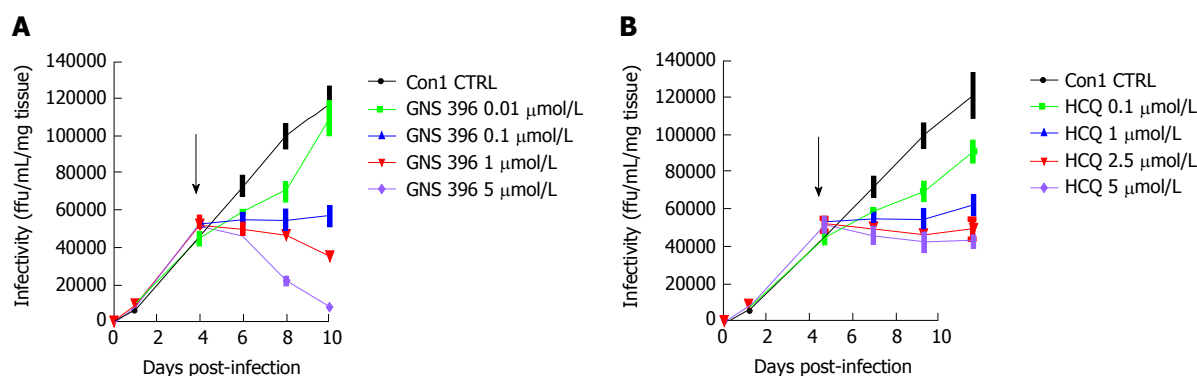
**Figure 4** Inhibition of autophagic flux by treatment with GNS-396 in SkBr3 mRFP-EGFP-LC3 stable cell line. Autophagic flux was monitored using the mRFP-EGFP-LC3 tandem-tagged fluorescent protein in SkBr3 mRFP-EGFP-LC3 stable cell line. A: SkBr3 mRFP-EGFP-LC3 stable cell line without any treatment is representative of basal autophagy; SkBr3 mRFP-EGFP-LC3 stable cell line was treated either with (B) 100  $\mu\text{mol/L}$  HCQ or (C) 100  $\mu\text{mol/L}$  GNS-396 during 6 h. In green/red merged images, yellow puncta (*i.e.*, mRFP+EGFP+) indicate autophagosomes or non-acidic autolysosomes, while red puncta (*i.e.*, mRFP+EGFP) indicate autolysosomes. HCQ is used as a positive control of autophagy inhibition. HCQ: Hydroxychloroquine; DMEM: Dulbecco's modified eagles's medium; LC3: Microtubule-associated protein 1A/1B-light chain.



**Figure 5** Inhibition of autophagy by treatment with GNS-396 (1  $\mu\text{mol/L}$ ) in primary adult human liver slices infected with cell culture-grown hepatitis C virus Con1 (multiplicity of infection = 1). A: Intracellular expression of LC3- I /LC3- II proteins in non-infected liver slices without treatment; B: Intracellular expression of LC3- I /LC3- II proteins and the normalization of intracellular protein LC3- II expression, for 10 d in non-infected liver slices with treatment either by GNS-396 (1  $\mu\text{mol/L}$ ) (G) or HCQ (1  $\mu\text{mol/L}$ ) (H) or without treatment (D: day); C: Expression of LC3- I /LC3- II proteins and HCV core and NS5A proteins in HCVcc Con1 infected liver slices either without treatment or (D) either with treatment by GNS-396 (1  $\mu\text{mol/L}$ ) or HCQ (1  $\mu\text{mol/L}$ ) and the normalization of Intracellular protein LC3- II expression for 10 d in HCVcc Con1 infected liver slices with treatment either by GNS-396 (1  $\mu\text{mol/L}$ ) (G) or HCQ (1  $\mu\text{mol/L}$ ) (H) or without treatment (D: day). LC3: Microtubule-associated protein 1A/1B-light chain; HCVcc: Cell culture-grown hepatitis C virus; NS5A: HCV nonstructural protein 5A.



**Figure 6** Inhibition of hepatitis C virus RNA replication by treatment either with GNS-396 or hydroxychloroquine in a dose-dependent manner in primary adult human cell culture-grown hepatitis C virus Con1 infected liver slices. Human liver slices were infected overnight with HCVcc Con1 (MOI = 0.1). The supernatant is then removed, the human liver slices washed and cultured. The liver slices and culture supernatants were collected different times post-infection. At day 4 post-infection, the liver slices were treated with increasing concentrations either of GNS-396 (0.01, 0.1, 1, 5 μmol/L) (A, B) or HCQ (C, D) for 6 d (black arrow: Start of the treatment either with GNS-396 or HCQ). Human HCVcc Con1 infected liver slices were lysed to evaluate intracellular levels of positive- and negative-strand HCV RNA by specific strand RT-qPCR at 1, 4, 6, 8, 10 d post-infection. The results were compared using the two-paired Student's test. Values are expressed as means ± standard errors: (A) HCV RNA replication by treatment with GNS-396: Positive strand (black line),  $P < 0.03$ ; negative strand (grey line),  $P < 0.013$ ; GNS-396 0.01 μmol/L (red line),  $P < 0.04$ ; GNS-396 0.1 μmol/L (green line),  $P < 0.05$ ; GNS-396 1 μmol/L (pink line),  $P < 0.05$ ; GNS-396 5 μmol/L (blue line),  $P < 0.05$ ; (C) HCV RNA replication by treatment with HCQ: Positive strand (black line),  $P < 0.03$ ; negative strand (grey line),  $P < 0.015$ ; HCQ 0.1 μmol/L (red line),  $P < 0.0001$ ; HCQ 1 μmol/L (green line),  $P < 0.0001$ ; HCQ 2.5 μmol/L (pink line),  $P < 0.01$ ; HCQ 5 μmol/L (blue line),  $P < 0.03$ . The detection of negative strand of HCV RNA evidences active replication as well as the increase overtime of both positive and negative strands of HCV RNA; B: Inhibition of HCV replication (%) with GNS-396 treatment  $P < 0.0038$ ; D: Inhibition of HCV replication (%) with HCQ treatment  $P < 0.0013$ . The replication was significantly inhibited in a dose-dependent manner in presence of increasing concentrations either of GNS-396 (B) or HCQ (D) for 6 d. HCVcc: Cell culture-grown hepatitis C virus; HCQ: Hydroxychloroquine; qRT-PCR: Quantitative technique consisting of reverse transcription followed by real-time polymerase chain reaction; MOI: Multiplicity of infection; CTRL: Control-treated liver slices.



**Figure 7** Dose-dependent inhibition of primary-culture-derived virus infectivity in primary adult human cell culture-grown hepatitis C virus Con1 infected liver slices by treatment either with GNS-396 (A) or hydroxychloroquine (B). Kinetics of infectivity of culture supernatants from human liver slices infected by HCV Con1 (MOI = 0.1) and treated either GNS-396 (A) or with HCQ (B) or at day 4 post-infection for 6 d. A: Con1 (black line),  $P < 0.0001$ ; GNS-396 0.01 μmol/L (green line),  $P < 0.0003$ ; GNS-396 0.1 μmol/L (blue line),  $P < 0.019$ ; GNS-396 1 μmol/L (red line),  $P < 0.05$ ; GNS-396 5 μmol/L (purple line),  $P < 0.05$ ; B: Con1 (black line),  $P < 0.0001$ ; HCQ 0.1 μmol/L (red line),  $P < 0.0001$ ; HCQ 1 μmol/L (green line),  $P < 0.0001$ ; HCQ 2.5 μmol/L (red line),  $P < 0.0001$ ; HCQ 5 μmol/L (purple line),  $P < 0.0003$ . Each curve represented the average of 2 independent infections performed in triplicate from 2 different donors. Values are expressed as means ± SE. The results were compared using the two-paired student's test. HCV: Hepatitis C virus; HCQ: Hydroxychloroquine; MOI: Multiplicity of infection; CTRL: Control-treated liver slices.

**Table 1** Inhibition of hepatitis C virus infectivity and 50% effective concentration of hepatitis C virus replication with direct active antivirals and autophagy inhibitors

Drugs <sup>1</sup>	Infectivity inhibition (%)	Average (SD) <sup>2</sup>	Replication inhibition (EC50) <sup>3</sup>	Average (SD) <sup>4</sup>	CC50 (SD) <sup>5</sup>
INF (2.6 to 260 nmol/L) <sup>6</sup>	Up to 95%	5	17 ng/mL	7.2	40 ng/mL (± 4)
RBV (1 to 100 µmol/L)	3% to 37%	3	146 µmol/L	13	400 µmol/L (± 21)
<sup>7</sup> TVR (0.01 to 50 µmol/L)	62% to 89%	4	0.395 µmol/L	0.038	40 µmol/L (± 3)
<sup>7</sup> BOC (0.01 to 50 µmol/L)	61% to 95%	5	0.417 µmol/L	0.024	41 µmol/L (± 5)
<sup>7</sup> SOF (0.01 to 50 µmol/L)	75% to 95%	4	0.147 µmol/L	0.017	23 µmol/L (± 2)
<sup>7</sup> HCQ (0.1 to 50 µmol/L)	25% to 94%	4	1.17 µmol/L	0.023	27 µmol/L (± 2)
<sup>7</sup> GNS-396 (0.01 to 5 µmol/L)	6% to 93%	3	0.158 µmol/L	0.014	25 µmol/L (± 2)
INF (2.6 to 260 nmol/L)/RBV 100 µmol/L	Up to 98%	6	10 ng/mL	3.1	43 ng/mL (± 4)
<sup>7</sup> HCQ (0.1 to 5 µmol/L)/RBV 50 µmol/L	27% to 85%	2	0.456 µmol/L	0.044	31 µmol/L (± 3)
<sup>7</sup> GNS-396 (0.01 to 5 µmol/L)/RBV 100 µmol/L	9% to 94%	2	0.157 µmol/L	0.012	26 µmol/L (± 2)
<sup>7</sup> TVR (0.01 to 50 µmol/L)/RBV 100 µmol/L	Up to 98%	3	0.310 µmol/L	0.029	49 µmol/L (± 3)
<sup>7</sup> BOC (0.01 to 50 µmol/L)/RBV 100 µmol/L	Up to 95%	2	0.370 µmol/L	0.035	48 µmol/L (± 4)
<sup>7</sup> SOF (0.01 to 50 µmol/L)/RBV 100 µmol/L	Up to 100%	2	0.080 µmol/L	0.028	17 µmol/L (± 5)
<sup>7</sup> TVR 1 µmol/L/ <sup>7</sup> BOC 1 µmol/L	Up to 89%	2	0.410 µmol/L	0.039	50 µmol/L (± 3)
<sup>7</sup> TVR 1 µmol/L/INF 26 nmol/L/RBV 100 µmol/L	Up to 99%	3	0.315 µmol/L	0.031	44 µmol/L (± 4)
<sup>7</sup> BOC 1 µmol/L/INF 26 nmol/L/RBV 100 µmol/L	Up to 97%	2	0.350 µmol/L	0.033	47 µmol/L (± 3)
<sup>7</sup> SOF 1 µmol/L/INF 26 nmol/L/RBV 100 µmol/L	Up to 100%	3	0.055 µmol/L	0.029	18 µmol/L (± 3)

<sup>1</sup>Drugs added at day 4 post-infection for 6 d; <sup>2</sup>Average (SD) of infectivity inhibition at day 6 post-treatment; <sup>3</sup>EC50 of the drugs written in bold at day 6 post-treatment; <sup>4</sup>Average (SD) of EC50 at day 6 post-treatment; <sup>5</sup>CC50 (SD): 50% cytotoxic concentration of the drugs written in bold at day 6 post-treatment (standard deviation); <sup>6</sup>INF 26 nmol/L: Peg-INF concentration corresponding to SOC; <sup>7</sup>DAAs and autophagy inhibitors in bold. EC50: 50% effective concentration; BOC: An inhibitor of the HCV-encoded NS3 protein; TVR: An inhibitor of the HCV-encoded NS3/4A hepatitis C protease; SOF: An uridine analogue inhibitor of the HCV NS5B polymerase; HCV: Hepatitis C virus; TVR: Telaprevir; BOC: Boceprevir; SOF: Sofosbuvir; RBV: Ribavirin; SD: Standard deviation; Peg-INF: Pegylated-interferon  $\alpha$ -2a; DAAs: Direct acting antivirals; HCQ: Hydroxychloroquine.

which is often used as an anti-malarial agent. HCQ is a "lysosomotropic" weak base that raises the lysosomal pH quickly<sup>[37]</sup>. Furthermore, many studies have reported the antiviral effect of CQ on other positive strand RNA viruses, such as polioviruses, coxsackieviruses, dengue viruses, coronaviruses (SARS-CoV virus)<sup>[24-29]</sup>, HIV-1<sup>[56]</sup>. In our study, we demonstrated the antiviral effect of HCQ and the new quinoline derivative GNS-396 on HCVcc replication in a dose - dependent manner. Compared to the treatment with HCQ alone, HCQ inhibition was more pronounced in combination with RBV or with other direct antivirals, suggesting a synergistic effect of the combined drugs on HCVcc infection in human liver slices. This result is consistent with a previous study which demonstrated the antiviral effect of CQ in combination with peg-IFN in HCV infected Huh-7 cell line<sup>[33]</sup>. Similarly, on Huh-7 cells infected with HCVpp (genotype 1a and 3a), it has been shown that CQ reduced by 50% virus infectivity at 50 µmol/L concentration, when the antiviral effect was tested<sup>[57]</sup>. Recently, ferroquine (FQ), an antimalarial ferrocenic analog of CQ, has been described as a novel inhibitor of HCV. FQ potently inhibited HCV infection of hepatoma cell lines<sup>[58]</sup>. Compared to these investigations, our study using the quinoline derivative GNS-396, revealed an inhibition of the virus infectivity up to 93% respectively at day 6 post-treatment with lower drug amounts (EC50 = 0.158 µmol/L). This demonstrates that GNS-396 is a stronger antiviral than HCQ (EC50 = 1.17 µmol/L). EC50 is a measure of the effectiveness of the drug in inhibiting the biochemical function. In our study, we evaluated the EC50 of HCV replication at day 6 post-treatment. The lower EC50 value indicates the greater potency of inhibiting HCV replication. As shown in Table

1, the infectivity inhibition, consistent with the inhibition of HCV replication, demonstrated that the new drug evaluated in the human HCV infected liver slices culture model, had a potent antiviral effect compared to the well-known established antivirals. In combination with the other well established drugs like DAA or inhibitors of other host targets (cyclophilin), quinoline derivatives could be additional therapeutic options for HCV infected patients.

In conclusion, this study demonstrated the relevance of the human HCV infected liver slices culture in preclinical studies of the new anti-viral drugs. New host-targeted therapies inhibiting autophagy (GNS-396, HCQ) have demonstrated significant efficiency and additive activity in inhibiting HCV replication. The *ex vivo* model of culture of human HCV infected liver slices might allow further evaluation of the efficacy of new antiviral drugs in single or in combined therapy and their potential toxicity in particular for patients "difficult to treat". Moreover, the infection of human liver slices culture with primary viral isolates from patients that we succeed to establish<sup>[41]</sup>, should allow highlighting the potential of early emergence of drug resistant viral variants during the anti-viral treatments.

## ACKNOWLEDGMENTS

The authors are grateful to Professor Francis V Chisari, for the kind gift of Huh-7.5.1 cells and to Professor Ralf Bartenschlager for kindly providing the chimeric pFK-Con1/C3 plasmid. We are also grateful to the members of the Departments of Digestive Surgery in the Groupe Hospitalier La Pitié Salpêtrière and Cochin-Hôtel Dieu

as to the members of the Department of Hepatology in Groupe Hospitalier Cochin-Hôtel Dieu, APHP, Paris, France, for the technical assistance. We are deeply indebted to Doctor Vladimir A. Morozov and Doctor Matthew Albert for the critical reading of the manuscript.

## COMMENTS

### Background

Hepatitis C virus (HCV) infection (or spread) is a serious public health challenge counting approximately 170 million people that are chronically infected worldwide. Host antiviral therapy is an additional option for the treatment of HCV infection.

### Research frontiers

Interferon-free treatments with new direct acting antivirals are expected to cure more than 90% of HCV-infected patients. But they are not available in all the countries. At the present time, triple therapy combining pegylated interferon- $\alpha$  2a or b and ribavirin with NS3 protease inhibitors (telaprevir or boceprevir) is going to remain the main treatment for HCV patients. That is why it appears important to continue research in limiting virus replication and the autophagy inhibition could be a new additional pathway because of recent evidences obtained regarding to an increased autophagic response in the liver of chronically HCV infected patients.

### Innovations and breakthroughs

This is the first study evaluating a new autophagy inhibitor as antiviral that could inhibit HCV infection in a dose-dependent manner without cytotoxic effect using the relevant ex vivo model of the human liver slices culture.

### Applications

This study highlight the relevance of the *ex vivo* model of the human HCV infected liver slices culture in preclinical studies of the new anti-viral drugs in single or in combined therapy and their potential toxicity in particular for patients "difficult to treat". Moreover, the infection of human liver slices culture with primary viral isolates from patients that the authors succeed to establish, should allow highlighting the potential of early emergence of drug resistant viral variants during the anti-viral treatments.

### Terminology

Autophagy is a catabolic process which degrades a cellular own component through the lysosomal machinery. It has been shown that autophagy is activated during virus and bacterial infection and that some viruses can use the autophagy system to facilitate their own replication.

### Peer-review

The manuscript is clear and comprehensive.

## REFERENCES

- 1 Szabó E, Lotz G, Páska C, Kiss A, Schaff Z. Viral hepatitis: new data on hepatitis C infection. *Pathol Oncol Res* 2003; **9**: 215-221 [PMID: 14688826 DOI: 10.1007/BF02893380]
- 2 Corouge M, Pol S. New treatments for chronic hepatitis C virus infection. *Med Mal Infect* 2011; **41**: 579-587 [PMID: 21764234 DOI: 10.1016/j.medmal.2011.04.003]
- 3 Mallet V, Gilgenkrantz H, Serpaggi J, Verkarre V, Vallet-Pichard A, Fontaine H, Pol S. Brief communication: the relationship of regression of cirrhosis to outcome in chronic hepatitis C. *Ann Intern Med* 2008; **149**: 399-403 [PMID: 18794559 DOI: 10.7326/0003-4819-149-6-200809160-00006]
- 4 Ashfaq UA, Javed T, Rehman S, Nawaz Z, Riazuddin S. An overview of HCV molecular biology, replication and immune responses. *Viral J* 2011; **8**: 161 [PMID: 21477382 DOI: 10.1186/1743-422X-8-161]
- 5 López-Labrador FX. Hepatitis C Virus NS3/4A Protease Inhibitors. *Recent Pat Antiinfect Drug Discov* 2008; **3**: 157-167 [PMID: 18991798]
- 6 Asselah T, Marcellin P. New direct-acting antivirals' combination for the treatment of chronic hepatitis C. *Liver Int* 2011; **31** Suppl 1: 68-77 [PMID: 21205141 DOI: 10.1111/j.1478-3231.2010.02411.x]
- 7 Poordad F, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]
- 8 Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]
- 9 Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]
- 10 Zeuzem S, Asselah T, Angus P, Zarski JP, Larrey D, Müllhaupt B, Gane E, Schuchmann M, Lohse A, Pol S, Bronowicki JP, Roberts S, Arasteh K, Zoulim F, Heim M, Stern JO, Kukulj G, Nehmiz G, Haefner C, Boecher WO. Efficacy of the protease inhibitor BI 201335, polymerase inhibitor BI 207127, and ribavirin in patients with chronic HCV infection. *Gastroenterology* 2011; **141**: 2047-2055; quiz e14 [PMID: 21925126 DOI: 10.1053/j.gastro.2011.08.051]
- 11 Jacobson IM, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]
- 12 Lawitz E, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
- 13 Sulkowski MS, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, McPhee F, Sherman D, Hindes R, Symonds W, Pasquinielli C, Grasela DM. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med* 2014; **370**: 211-221 [PMID: 24428467 DOI: 10.1056/NEJMoa1306218]
- 14 Poordad F, Schiff ER, Vierling JM, Landis C, Fontana RJ, Yang R, McPhee F, Hughes EA, Noviello S, Swenson ES. Daclatasvir with sofosbuvir and ribavirin for hepatitis C virus infection with advanced cirrhosis or post-liver transplantation recurrence. *Hepatology* 2016; **63**: 1493-1505 [PMID: 26754432 DOI: 10.1002/hep.28446]
- 15 Hopkins S, DiMassimo B, Rusnak P, Heuman D, Lalezari J, Sluder A, Scoreaux B, Mosier S, Kowalczyk P, Ribeill Y, Baugh J, Gallay P. The cyclophilin inhibitor SCY-635 suppresses viral replication and induces endogenous interferons in patients with chronic HCV genotype 1 infection. *J Hepatol* 2012; **57**: 47-54 [PMID: 22425702 DOI: 10.1016/j.jhep.2012.02.024]
- 16 Schaefer EA, Chung RT. Anti-hepatitis C virus drugs in development. *Gastroenterology* 2012; **142**: 1340-1350.e1 [PMID: 22537441 DOI: 10.1053/j.gastro.2012.02.015]



- 17 **Hill A**, Khoo S, Fortunak J, Simmons B, Ford N. Minimum costs for producing hepatitis C direct-acting antivirals for use in large-scale treatment access programs in developing countries. *Clin Infect Dis* 2014; **58**: 928-936 [PMID: 24399087 DOI: 10.1093/cid/ciu012]
- 18 **Deuffic-Burban S**, Schwarzingner M, Obach D, Mallet V, Pol S, Pageaux GP, Canva V, Deltenre P, Roudot-Thoraval F, Larrey D, Dhumeaux D, Mathurin P, Yazdanpanah Y. Should we await IFN-free regimens to treat HCV genotype 1 treatment-naïve patients? A cost-effectiveness analysis (ANRS 95141). *J Hepatol* 2014; **61**: 7-14 [PMID: 24650691 DOI: 10.1016/j.jhep.2014.03.011]
- 19 **European Association for the Study of the Liver**. EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol* 2015; **63**: 199-236 [PMID: 25911336 DOI: 10.1016/j.jhep.2015.03.025]
- 20 **AASLD/IDSA HCV Guidance Panel**. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology* 2015; **62**: 932-954 [PMID: 26111063 DOI: 10.1002/hep.27950]
- 21 **Zopf S**, Kremer AE, Neurath MF, Siebler J. Advances in hepatitis C therapy: What is the current state - what comes next? *World J Hepatol* 2016; **8**: 139-147 [PMID: 26839638 DOI: 10.4254/wjh.v8.i3.139]
- 22 **Rautou PE**, Cazals-Hatem D, Feldmann G, Mansouri A, Grodet A, Barge S, Martinot-Peignoux M, Duces A, Bièche I, Lebre C, Bedossa P, Paradis V, Marcellin P, Valla D, Asselah T, Moreau R. Changes in autophagic response in patients with chronic hepatitis C virus infection. *Am J Pathol* 2011; **178**: 2708-2715 [PMID: 21641393 DOI: 10.1016/j.ajpath.2011.02.021]
- 23 **Shintani T**, Klionsky DJ. Autophagy in health and disease: a double-edged sword. *Science* 2004; **306**: 990-995 [PMID: 15528435 DOI: 10.1126/science.1099993]
- 24 **Orvedahl A**, Levine B. Eating the enemy within: autophagy in infectious diseases. *Cell Death Differ* 2009; **16**: 57-69 [PMID: 18772897 DOI: 10.1038/cdd.2008.130]
- 25 **Jackson WT**, Giddings TH, Taylor MP, Mulinyawe S, Rabinovitch M, Kopito RR, Kirkegaard K. Subversion of cellular autophagosomal machinery by RNA viruses. *PLoS Biol* 2005; **3**: e156 [PMID: 15884975 DOI: 10.1371/journal.pbio.0030156]
- 26 **Lee YR**, Lei HY, Liu MT, Wang JR, Chen SH, Jiang-Shieh YF, Lin YS, Yeh TM, Liu CC, Liu HS. Autophagic machinery activated by dengue virus enhances virus replication. *Virology* 2008; **374**: 240-248 [PMID: 18353420 DOI: 10.1016/j.virol.2008.02.016]
- 27 **Wong J**, Zhang J, Si X, Gao G, Mao I, McManus BM, Luo H. Autophagosome supports coxsackievirus B3 replication in host cells. *J Virol* 2008; **82**: 9143-9153 [PMID: 18596087 DOI: 10.1128/JVI.00641-08]
- 28 **Dreux M**, Chisari FV. Viruses and the autophagy machinery. *Cell Cycle* 2010; **9**: 1295-1307 [PMID: 20305376 DOI: 10.4161/cc.9.7.11109]
- 29 **Silva LM**, Jung JU. Modulation of the autophagy pathway by human tumor viruses. *Semin Cancer Biol* 2013; **23**: 323-328 [PMID: 23727156 DOI: 10.1016/j.semcancer.2013.05.005]
- 30 **Sir D**, Chen WL, Choi J, Wakita T, Yen TS, Ou JH. Induction of incomplete autophagic response by hepatitis C virus via the unfolded protein response. *Hepatology* 2008; **48**: 1054-1061 [PMID: 18688877 DOI: 10.1002/hep.22464]
- 31 **Dreux M**, Gastaminza P, Wieland SF, Chisari FV. The autophagy machinery is required to initiate hepatitis C virus replication. *Proc Natl Acad Sci USA* 2009; **106**: 14046-14051 [PMID: 19666601 DOI: 10.1073/pnas.0907344106]
- 32 **Dreux M**, Chisari FV. Impact of the autophagy machinery on hepatitis C virus infection. *Viruses* 2011; **3**: 1342-1357 [PMID: 21994783 DOI: 10.3390/v3081342]
- 33 **Mohl BP**, Tedbury PR, Griffin S, Harris M. Hepatitis C virus-induced autophagy is independent of the unfolded protein response. *J Virol* 2012; **86**: 10724-10732 [PMID: 22837205 DOI: 10.1128/JVI.01667-12]
- 34 **Shrivastava S**, Bhanja Chowdhury J, Steele R, Ray R, Ray RB. Hepatitis C virus upregulates Beclin1 for induction of autophagy and activates mTOR signaling. *J Virol* 2012; **86**: 8705-8712 [PMID: 22674982 DOI: 10.1128/JVI.00616-12]
- 35 **Huang H**, Kang R, Wang J, Luo G, Yang W, Zhao Z. Hepatitis C virus inhibits AKT-tuberosclerosis complex (TSC), the mechanistic target of rapamycin (mTOR) pathway, through endoplasmic reticulum stress to induce autophagy. *Autophagy* 2013; **9**: 175-195 [PMID: 23169238 DOI: 10.4161/auto.22791]
- 36 **Sir D**, Kuo CF, Tian Y, Liu HM, Huang EJ, Jung JU, Machida K, Ou JH. Replication of hepatitis C virus RNA on autophagosomal membranes. *J Biol Chem* 2012; **287**: 18036-18043 [PMID: 22496373 DOI: 10.1074/jbc.M111.320085]
- 37 **Savarino A**, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis* 2003; **3**: 722-727 [PMID: 14592603 DOI: 10.1016/S1473-3099(03)00806-5]
- 38 **Vincent MJ**, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, Seidah NG, Nichol ST. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Viral J* 2005; **2**: 69 [PMID: 16115318 DOI: 10.1186/1743-422X-2-69]
- 39 **Rolain JM**, Colson P, Raoult D. Recycling of chloroquine and its hydroxyl analogue to face bacterial, fungal and viral infections in the 21st century. *Int J Antimicrob Agents* 2007; **30**: 297-308 [PMID: 17629679 DOI: 10.1016/j.ijantimicag.2007.05.015]
- 40 **Mizui T**, Yamashina S, Tanida I, Takei Y, Ueno T, Sakamoto N, Ikejima K, Kitamura T, Enomoto N, Sakai T, Kominami E, Watanabe S. Inhibition of hepatitis C virus replication by chloroquine targeting virus-associated autophagy. *J Gastroenterol* 2010; **45**: 195-203 [PMID: 19760134 DOI: 10.1007/s00535-009-0132-9]
- 41 **Lagaye S**, Shen H, Saunier B, Nascimbeni M, Gaston J, Bourdoncle P, Hannoun L, Massault PP, Vallet-Pichard A, Mallet V, Pol S. Efficient replication of primary or culture hepatitis C virus isolates in human liver slices: a relevant ex vivo model of liver infection. *Hepatology* 2012; **56**: 861-872 [PMID: 22454196 DOI: 10.1002/hep.25738]
- 42 **Halfon P**, Nallet J, Petit SJ, Bouzidi M, Joly F, Camus C, Benech P, Dubuisson, Courcambeck J, Wyckowski C. Chloroquine and related compounds are inhibitors of Hepatitis C Virus RNA by inhibiting autophagy proteolysis. *Hepatology* 2010; **52**: 807 [DOI: 10.1002/hep.23991]
- 43 **Olinga P**, Groothuis GM. Use of human tissue slices in drug targeting research. Drug targeting. Organ-specific strategies. Series: Methods and Principles in Medicinal Chemistry. G Molema, DKF Meijer, editors series, Mannhold R, Kubinyi H, Timmerman H, eds Weinheim, Germany: Wiley-VCH, 2001: 309-331 [DOI: 10.1002/352760006x.ch12]
- 44 **Pietschmann T**, Kaul A, Koutsoudakis G, Shavinskaya A, Kallis S, Steinmann E, Abid K, Negro F, Dreux M, Cosset FL, Bartenschlager R. Construction and characterization of infectious intragenotypic and intergenotypic hepatitis C virus chimeras. *Proc Natl Acad Sci USA* 2006; **103**: 7408-7413 [PMID: 16651538 DOI: 10.1073/pnas.0504877103]
- 45 **Zhong J**, Gastaminza P, Cheng G, Kapadia S, Kato T, Burton DR, Wieland SF, Uprichard SL, Wakita T, Chisari FV. Robust hepatitis C virus infection in vitro. *Proc Natl Acad Sci USA* 2005; **102**: 9294-9299 [PMID: 15939869 DOI: 10.1073/pnas.0503596102]
- 46 **Carrière M**, Pène V, Breiman A, Conti F, Chouzenoux S, Meurs E, Andrieu M, Jaffray P, Grira L, Soubrane O, Sogni P, Calmus Y, Chaussade S, Rosenberg AR, Pothelin P. A novel, sensitive, and specific RT-PCR technique for quantitation of hepatitis C virus replication. *J Med Virol* 2007; **79**: 155-160 [PMID: 17177304 DOI: 10.1002/jmv.20773]
- 47 **Komurian-Pradel F**, Perret M, Deiman B, Sodoyer M, Lotteau V, Paranhos-Baccalà G, André P. Strand specific quantitative real-time PCR to study replication of hepatitis C virus genome. *J Virol Methods* 2004; **116**: 103-106 [PMID: 14715313 DOI: 10.1016/j.jviromet.2003.10.004]
- 48 **Besnard NC**, Andre PM. Automated quantitative determination of hepatitis C virus viremia by reverse transcription-PCR. *J Clin Microbiol* 1994; **32**: 1887-1893 [PMID: 7989537]
- 49 **Nozaki A**, Kato N. Quantitative method of intracellular hepatitis C virus RNA using LightCycler PCR. *Acta Med Okayama* 2002; **56**:

- 107-110 [PMID: 12002616]
- 50 **Laemmli UK.** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680-685 [PMID: 5432063 DOI: 10.1038/227680a0]
  - 51 **Braydich-Stolle L,** Hussain S, Schlager JJ, Hofmann MC. In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. *Toxicol Sci* 2005; **88**: 412-419 [PMID: 16014736 DOI: 10.1093/toxsci/kfi256]
  - 52 **Kimura M,** Taniguchi M, Mikami Y, Masuda T, Yoshida T, Mishina M, Shimizu T. Identification and characterization of zebrafish semaphorin 6D. *Biochem Biophys Res Commun* 2007; **363**: 762-768 [PMID: 17897628 DOI: 10.1016/j.bbrc.2007.09.038]
  - 53 **Klionsky DJ,** Abeliovich H, Agostinis P, Agrawal DK, Aliev G, Askew DS, Baba M, Baehrecke EH, Bahr BA, Ballabio A, Bamber BA, Bassham DC, Bergamini E, Bi X, Biard-Piechaczyk M, Blum JS, Bredesen DE, Brodsky JL, Brumell JH, Brunk UT, Bursch W, Camougrand N, Cebollero E, Cecconi F, Chen Y, Chin LS, Choi A, Chu CT, Chung J, Clarke PG, Clark RS, Clarke SG, Clavé C, Cleveland JL, Codogno P, Colombo MI, Coto-Montes A, Cregg JM, Cuervo AM, Debnath J, Demarchi F, Dennis PB, Dennis PA, Deretic V, Devenish RJ, Di Sano F, Dice JF, Difiglia M, Dinesh-Kumar S, Distelhorst CW, Djavaheri-Mergny M, Dorsey FC, Dröge W, Dron M, Dunn WA, Duszenko M, Eissa NT, Elazar Z, Esclatine A, Eskelinen EL, Fésüs L, Finley KD, Fuentes JM, Fueyo J, Fujisaki K, Galliot B, Gao FB, Gewirtz DA, Gibson SB, Gohla A, Goldberg AL, Gonzalez R, González-Estévez C, Gorski S, Gottlieb RA, Häussinger D, He YW, Heidenreich K, Hill JA, Høyer-Hansen M, Hu X, Huang WP, Iwasaki A, Jäättelä M, Jackson WT, Jiang X, Jin S, Johansen T, Jung JU, Kadowaki M, Kang C, Kelekar A, Kessel DH, Kiel JA, Kim HP, Kimchi A, Kinsella TJ, Kiselyov K, Kitamoto K, Knecht E, Komatsu M, Kominami E, Kondo S, Kovács AL, Kroemer G, Kuan CY, Kumar R, Kundu M, Landry J, Laporte M, Le W, Lei HY, Lenardo MJ, Levine B, Lieberman A, Lim KL, Lin FC, Liou W, Liu LF, Lopez-Berestein G, López-Otín C, Lu B, Macleod KF, Malorni W, Martinet W, Matsuoka K, Mautner J, Meijer AJ, Meléndez A, Michels P, Miotto G, Mistiaen WP, Mizushima N, Mograbi B, Monastyrska I, Moore MN, Moreira PI, Moriyasu Y, Motyl T, Münz C, Murphy LO, Naqvi NI, Neufeld TP, Nishino I, Nixon RA, Noda T, Nürnberg B, Ogawa M, Oleinick NL, Olsen LJ, Ozpolat B, Paglin S, Palmer GE, Papassideri I, Parkes M, Perlmutter DH, Perry G, Piacentini M, Pinkas-Kramarski R, Prescott M, Proikas-Cezanne T, Raben N, Rami A, Reggiori F, Rohrer B, Rubinsztein DC, Ryan KM, Sadoshima J, Sakagami H, Sakai Y, Sandri M, Sasakawa C, Sass M, Schneider C, Seglen PO, Seleverstov O, Settleman J, Shacka JJ, Shapiro IM, Sibirny A, Silva-Zacarin EC, Simon HU, Simone C, Simonsen A, Smith MA, Spanel-Borowski K, Srinivas V, Steeves M, Stenmark H, Stromhaug PE, Subauste CS, Sugimoto S, Sulzer D, Suzuki T, Swanson MS, Tabas I, Takeshita F, Talbot NJ, Tallóczy Z, Tanaka K, Tanaka K, Tanida I, Taylor GS, Taylor JP, Terman A, Tettamanti G, Thompson CB, Thumm M, Tolkovsky AM, Tooze SA, Truant R, Tumanovska LV, Uchiyama Y, Ueno T, Uzcátegui NL, van der Klei I, Vaquero EC, Vellai T, Vogel MW, Wang HG, Webster P, Wiley JW, Xi Z, Xiao G, Yahalom J, Yang JM, Yap G, Yin XM, Yoshimori T, Yu L, Yue Z, Yuzaki M, Zabirnyk O, Zheng X, Zhu X, Deter RL. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. *Autophagy* 2008; **4**: 151-175 [PMID: 18188003 DOI: 10.4161/auto.5338]
  - 54 **Yamamoto A,** Tagawa Y, Yoshimori T, Moriyama Y, Masaki R, Tashiro Y. Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells. *Cell Struct Funct* 1998; **23**: 33-42 [PMID: 9639028 DOI: 10.1247/csf.23.33]
  - 55 **Zhou X,** Sun P, Lucendo-Villarin B, Angus AG, Szkolnicka D, Cameron K, Farnworth SL, Patel AH, Hay DC. Modulating innate immunity improves hepatitis C virus infection and replication in stem cell-derived hepatocytes. *Stem Cell Reports* 2014; **3**: 204-214 [PMID: 25068132 DOI: 10.1016/j.stemcr.2014.04.018]
  - 56 **Savarino A,** Gennero L, Chen HC, Serrano D, Malavasi F, Boelaert JR, Sperber K. Anti-HIV effects of chloroquine: mechanisms of inhibition and spectrum of activity. *AIDS* 2001; **15**: 2221-2229 [PMID: 11698694 DOI: 10.1097/00002030-200111230-00002]
  - 57 **Ashfaq UA,** Javed T, Rehman S, Nawaz Z, Riazuddin S. Lysosomotropic agents as HCV entry inhibitors. *Virol J* 2011; **8**: 163 [PMID: 21481279 DOI: 10.1186/1743-422X-8-163]
  - 58 **Vausselin T,** Calland N, Belouzard S, Descamps V, Douam F, Helle F, François C, Lavillette D, Duverlie G, Wahid A, Fénéant L, Cocquerel L, Guérardel Y, Wychowski C, Biot C, Dubuisson J. The antimalarial ferroquine is an inhibitor of hepatitis C virus. *Hepatology* 2013; **58**: 86-97 [PMID: 23348596 DOI: 10.1002/hep.26273]

P- Reviewer: Jin B, Irato P S- Editor: Ji FF  
L- Editor: A E- Editor: Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 August 8; 8(22): 915-956







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vysloulzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



**REVIEW**

- 915 Multimodal brain monitoring in fulminant hepatic failure  
*Paschoal Jr FM, Nogueira RC, Ronconi KDAL, de Lima Oliveira M, Teixeira MJ, Bor-Seng-Shu E*

**MINIREVIEWS**

- 924 Cholesterol metabolism in cholestatic liver disease and liver transplantation: From molecular mechanisms to clinical implications  
*Nemes K, Åberg F, Gylling H, Isoniemi H*

**ORIGINAL ARTICLE****Basic Study**

- 933 Antifibrotic effects of ambrisentan, an endothelin-A receptor antagonist, in a non-alcoholic steatohepatitis mouse model  
*Okamoto T, Koda M, Miyoshi K, Onoyama T, Kishina M, Matono T, Sugihara T, Hosho K, Okano J, Isomoto H, Murawaki Y*

**Retrospective Study**

- 942 Living donor liver transplantation for high model for end-stage liver disease score: What have we learned?  
*Dabbous H, Sakr M, Abdelhakam S, Montasser I, Bahaa M, Said H, El-Meteini M*

**Observational Study**

- 949 Boceprevir or telaprevir in hepatitis C virus chronic infection: The Italian real life experience  
*CLEO Study Group; Ascione A, Adinolfi LE, Amoroso P, Andriulli A, Armignacco O, Ascione T, Babudieri S, Barbarini G, Brogna M, Cesario F, Citro V, Claar E, Cozzolongo R, D'Adamo G, D'Amico E, Dattolo P, De Luca M, De Maria V, De Siena M, De Vita G, Di Giacomo A, De Marco R, De Stefano G, De Stefano G, Di Salvo S, Di Sarno R, Farella N, Felicioni L, Fimiani B, Fontanella L, Foti G, Furlan C, Giancotti F, Giolitto G, Gravina T, Guerrera B, Gulminetti R, Iacobellis A, Imperato M, Iodice A, Iovinella V, Izzi A, Liberti A, Leo P, Lettieri G, Luppino I, Marrone A, Mazzoni E, Messina V, Monarca R, Narciso V, Nosotti L, Pellicelli AM, Perrella A, Piai G, Picardi A, Pierri P, Pietromatera G, Resta F, Rinaldi L, Romano M, Rossini A, Russello M, Russo G, Sacco R, Sangiovanni V, Schiano A, Sciambra A, Scifo G, Simeone F, Sullo A, Tarquini P, Tundo P, Vallone A*

## Contents

*World Journal of Hepatology*  
Volume 8 Number 22 August 8, 2016

### ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Dr. Neil L Julie, MD, Department of Gastroenterology, Shady Grove Adventist Hospital, Bethesda, MD 20850, United States

### AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

### FLYLEAF

I-IV Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
August 8, 2016

#### COPYRIGHT

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

#### SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

#### INSTRUCTIONS TO AUTHORS

<http://www.wjgnet.com/bpg/gerinfo/204>

#### ONLINE SUBMISSION

<http://www.wjgnet.com/esps/>

## Multimodal brain monitoring in fulminant hepatic failure

Fernando Mendes Paschoal Jr, Ricardo Carvalho Nogueira, Karla De Almeida Lins Ronconi, Marcelo de Lima Oliveira, Manoel Jacobsen Teixeira, Edson Bor-Seng-Shu

Fernando Mendes Paschoal Jr, Ricardo Carvalho Nogueira, Karla De Almeida Lins Ronconi, Marcelo de Lima Oliveira, Edson Bor-Seng-Shu, Laboratory for Neurosonology and Cerebral Hemodynamics, Department of Neurology, Hospital das Clinicas, Sao Paulo University Medical School, São Paulo 04107-021, Brazil

Manoel Jacobsen Teixeira, Department of Neurology, Division of Neurosurgery, Hospital das Clinicas, Sao Paulo University Medical School, São Paulo 04107-021, Brazil

**Author contributions:** Paschoal Jr FM wrote the paper; Nogueira RC, Ronconi KDAL, de Lima Oliveira M, Teixeira MJ and Bor-Seng-Shu E collected the data.

**Conflict-of-interest statement:** The authors declare there is no conflict of interest regarding the publication of this paper.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Fernando Mendes Paschoal Jr, MD, Laboratory for Neurosonology and Cerebral Hemodynamics, Department of Neurology, Hospital das Clinicas, Sao Paulo University Medical School, Rua Paula Ney, 480, apt 42, São Paulo 04107-021, Brazil. [tenpaschoal@gmail.com](mailto:tenpaschoal@gmail.com)  
 Telephone: +55-11-30690435  
 Fax: +55-11-30633018

Received: February 20, 2016  
 Peer-review started: February 22, 2016  
 First decision: March 30, 2016  
 Revised: April 22, 2016  
 Accepted: June 14, 2016  
 Article in press: June 16, 2016  
 Published online: August 8, 2016

### Abstract

Acute liver failure, also known as fulminant hepatic failure (FHF), embraces a spectrum of clinical entities characterized by acute liver injury, severe hepatocellular dysfunction, and hepatic encephalopathy. Cerebral edema and intracranial hypertension are common causes of mortality in patients with FHF. The management of patients who present acute liver failure starts with determining the cause and an initial evaluation of prognosis. Regardless of whether or not patients are listed for liver transplantation, they should still be monitored for recovery, death, or transplantation. In the past, neuromonitoring was restricted to serial clinical neurologic examination and, in some cases, intracranial pressure monitoring. Over the years, this monitoring has proven insufficient, as brain abnormalities were detected at late and irreversible stages. The need for real-time monitoring of brain functions to favor prompt treatment and avert irreversible brain injuries led to the concepts of multimodal monitoring and neurophysiological decision support. New monitoring techniques, such as brain tissue oxygen tension, continuous electroencephalogram, transcranial Doppler, and cerebral microdialysis, have been developed. These techniques enable early diagnosis of brain hemodynamic, electrical, and biochemical changes, allow brain anatomical and physiological monitoring-guided therapy, and have improved patient survival rates. The purpose of this review is to discuss the multimodality methods available for monitoring patients with FHF in the neurocritical care setting.

**Key words:** Fulminant hepatic failure; Cerebral edema; Multimodality methods; Intracranial hypertension; Liver transplantation

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Cerebral edema and intracranial hypertension are common causes of mortality in patients with fulminant hepatic failure (FHF). The management of

patients who present acute liver failure starts with determining the cause and an initial evaluation of prognosis. Regardless of whether or not patients are listed for liver transplantation, they should still be monitored for recovery, death, or transplantation. The purpose of this review is to discuss the multimodality methods available for monitoring patients with FHF in the neurocritical care setting.

Paschoal Jr FM, Nogueira RC, Ronconi KDAL, de Lima Oliveira M, Teixeira MJ, Bor-Seng-Shu E. Multimodal brain monitoring in fulminant hepatic failure. *World J Hepatol* 2016; 8(22): 915-923 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i22/915.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i22.915>

## INTRODUCTION

Fulminant hepatic failure (FHF) is a complex clinical condition that is only partially understood and remains a major clinical challenge<sup>[1,2]</sup>. Hepatic encephalopathy (HE) associated with intracranial hypertension is a severe neurologic complication and the leading cause of death among patients with FHF<sup>[3]</sup>.

The management of patients who present acute liver failure starts with determining the cause and an initial evaluation of prognosis<sup>[3]</sup>. In the past, neuromonitoring was restricted to serial clinical neurologic examination and, in some cases, intracranial pressure (ICP) monitoring. Over the years, this monitoring has proven insufficient because brain abnormalities were detected at late and irreversible stages<sup>[4]</sup>. The need for real-time monitoring of brain functions to favor prompt treatment and avert irreversible brain injuries led to the concepts of multimodal monitoring and neurophysiological decision support. New monitoring techniques, such as brain tissue oxygen tension, continuous electroencephalogram (cEEG), transcranial Doppler (TCD), and cerebral microdialysis (MD), have been developed. These techniques enable early diagnosis of brain hemodynamic, electrical, and biochemical changes, allow brain anatomical and physiological monitoring-guided therapy, and have improved patient survival rates<sup>[4,5]</sup>.

The purpose of this review is to discuss the multimodality methods available for the monitoring of patients in the neurocritical care setting.

## MONITORING INTRACRANIAL PHYSIOLOGICAL VARIABLES

### Invasive ICP monitoring

ICP monitoring is indicated for brain swelling due to FHF and involves the use of catheters, which can be implanted into epidural, subdural-subarachnoid, or intraventricular spaces through a burr hole. The latest catheters allow real-time and continuous ICP data acquisition. The objective of ICP monitoring is to maintain ICP below 20 mmHg and have adequate cerebral perfusion pressure

(CPP) = arterial blood pressure (ABP) - ICP. The ideal management of CPP should take cerebral metabolic and hemodynamic data into account in order to avoid excessive cerebral hyperemia, as well as uncoupling of cerebral blood flow and metabolism<sup>[6,7]</sup>. Despite a lack of evidence that treatment of elevated ICP can improve survival rates of patients with FHF, it is generally accepted that Grade 3-4 HE patients, especially those awaiting liver transplantation, should undergo ICP monitoring<sup>[6,7]</sup>. ICP higher than 40 mmHg and prolonged low CPP < 50 mmHg are strongly associated with poor neurological recovery in FHF patients who are traditionally bad candidates for liver transplantation<sup>[8]</sup>.

Continuous perioperative measurement of ICP has been associated with a FHF survival rate of 54%-74%. Invasive ICP monitoring is especially risky in FHF patients with coagulopathy, for whom the incidence of intracranial bleeding due to catheter placement ranges from 5% to 22%<sup>[9,10]</sup>. Recombinant factor VII (rFVIIa) can be an alternative method for preventing intracranial hemorrhage associated with ICP placement. Acidosis can lead to low effectiveness of rFVIIa, therefore requiring its correction before use<sup>[5,11]</sup>.

Cerebral edema and intracranial hypertension (IH) are complications in approximately 75% to 80% of patients with FHF and grade III or IV encephalopathy, which remains a leading cause of death. The pathophysiology of these two complications still remains poorly understood, but may be related to vasogenic edema, cytotoxic edema, or cerebral hyperemia<sup>[8,12]</sup>. Vasogenic edema is the consequence of a breakdown of the blood brain barrier, while cytotoxic edema is related to the glutamine osmotic effects in the astrocytes that results in cerebral edema. On the other hand, hyperemia can be caused by failure of the sodium-potassium adenosine triphosphatase pump<sup>[8]</sup> and/or the accumulation of certain substances such as cytokines, products of the necrotic liver, or glutamine, which lead to vasodilatation of the microcirculation. Brain edema and hyperemia can lead to IH, with decreased cerebral perfusion pressure, cerebral ischemia, and herniation<sup>[8,12]</sup>.

## NON-INVASIVE ICP MONITORING

### Optic nerve ultrasound

The optic nerve has a sheath which is continuous with the dura mater of the brain. The subarachnoid space of the optic nerve sheath communicates with the brain and the subarachnoid space, meaning that optic nerve sheath diameter (ONSD) can be influenced by changes in the pressure of cerebrospinal fluid in the cranial cavity. ONSD has been increasingly used to monitor ICP in many different clinical settings, and is measured by an ultrasound probe placed on the eyes<sup>[13,14]</sup>. A linear correlation between ICP and ONSD measurements has been reported, and a significant reduction in ONSD occurs after draining the cerebrospinal fluid. The cut-off value of ONSD suggested to indicate ICP greater than 20 mmHg was 5.2 mm<sup>[15]</sup>. However, scant information



is available regarding the use of ONSD in patients undergoing liver transplantation. Kim *et al.*<sup>[13]</sup> concluded that patients undergoing liver transplantation are susceptible to severe bleeding disorders and elevated ICP during the procedure, reporting two cases of patients who underwent liver transplantation at different stages. In one case with severe hepatic encephalopathy, ONSD was measured before transplantation, yielding a value of 6.4 mm. Meanwhile, measurements made in the other case after reperfusion of the graft yielded a value of 5.7 mm. These data demonstrate that measurement of ONSD is a useful method for evaluating patients with FHF undergoing liver transplantation.

### **Transcranial color-coded duplex ultrasonography**

Midline shift (MLS) is a known prognostic factor for unfavorable outcome after the development of intracranial hemorrhage in patients with severe brain injury<sup>[16]</sup>. In clinical practice, the repetition of computed tomography is mostly used to monitor MLS. However, the examination leads to increased radiation exposure and requires the transport of critically-ill patients, which are associated with increased morbidity and mortality in these patients<sup>[17]</sup>. Transcranial color-coded duplex sonography (TCCD) represents a non-invasive bedside alternative to radiological methods. TCCD measurements are valid for the diagnosis and monitoring of various neurological diseases, including IH<sup>[18,19]</sup>. Furthermore, monitoring MLS *via* TCCD safely predicts early mortality and prognosis of conservative clinical treatment of hemispheric ischemic stroke<sup>[18]</sup>. Unlike ischemic stroke, intracranial hemorrhage MLS is caused by both the volume of hematoma and the formation of edema, which can make outcomes difficult to predict<sup>[20]</sup>. Patients with FHF who develop brain swelling and IH can benefit from this method, although it has not yet been described in the literature.

### **Brain computer tomography and magnetic resonance images**

Brain images have traditionally been used to diagnose strokes, but are also useful in ruling out other causes of changes in mental status<sup>[21]</sup>. Furthermore, a non-contrast computer tomography (CT) scan of the brain can disclose brain swelling, compressed basal cisterns, hydrocephalus, mass effect, and midline shift, which can be indicative of increased ICP. However, the absence of these findings does not exclude the presence of brain swelling<sup>[22]</sup>, which may be better visualized through magnetic resonance imaging (MRI) of the brain<sup>[21]</sup>. The imbalance in the homeostasis of cell volume consequent to elevation of cerebral ammonia concentration can be disclosed in MRI by the proton spectroscopy findings of decreased myo-inositol and choline signals<sup>[23]</sup>. Moreover, magnetization transfer ratio measurements of fast fluid-attenuated inversion recovery sequences and diffusion-weighted images can be used to detect abnormalities in white matter, thereby reflecting elevated ammonia concentrations in the central nervous system that

facilitate the diagnosis of brain swelling in patients with FHF<sup>[23,24]</sup>.

### **Cerebral blood flow monitoring**

Cerebral blood flow (CBF) can generally be maintained in the presence of varying CPP. However, this relationship is not linear in severe brain injury due to impaired cerebral autoregulation<sup>[25,26]</sup>. In such cases, assessment based on CPP alone can be inaccurate, as measurements assume that cerebral vascular resistance remains constant, which is not the case in serious brain injuries<sup>[24]</sup>. Therefore, direct monitoring of CBF can help in the management of patients with severe brain injury.

The gold standard method for CBF study is the Kety-Schmidt technique. This technique assesses the area between the curves of arterial and venous washout of a freely diffusible indicator such as nitrous oxide and calculates global CBF from the absorption rate of the indicator in brain tissue<sup>[26,27]</sup>. Radioisotopes such as krypton-85 and xenon-133 can also be used for CBF study in combination with compact scintillation detectors and microprocessors, as well as the indocyanine green dye dilution technique, which involves non-invasive near-infrared spectroscopy (NIRS) and the thermodilution method<sup>[28,29]</sup>. The principle of spectroscopy is based on the application of light in the near-infrared wavelength to assess, quantitatively and qualitatively, the molecular components related to tissue oxygenation. Based on deoxyhemoglobin and oxyhemoglobin concentrations in the tissue, NIRS is a non-invasive method which allows for the gathering of information for calculating tissue oxygenation<sup>[30]</sup>. Other techniques that evaluate CBF include: CT with xenon, CT by single photon emission tomography (SPECT), positron emission oxygen-15 tomography (PET), perfusion CT, and perfusion imaging by MRI<sup>[31,32]</sup>. SPECT studies the spatial distribution of the radioactive isomer technetium-99 (Tc-99) and its local metabolism in the brain. Since these radionuclides are unusual in the human body, Tc-99 metabolism or its connection may not be identical to the native molecule, and therefore difficulties in the interpretation of results may occur<sup>[33]</sup>. SPECT provides only a relative measurement of radioactivity and allows for the comparison of physiological parameters such as blood flow in different areas of the brain<sup>[34,35]</sup>.

Cerebrovascular resistance, according to Davies *et al.*<sup>[10]</sup>, tends to decrease during the course of FHF and can be influenced by the use of pharmacological agents (*i.e.*, sedatives and inotropes). Previous studies have shown increased blood flow in the basal ganglia of patients with minimal HE, which suggests an increased supply of ammonia to these areas, with resultant astrocyte dysfunction and cognitive impairment.

Nielsen *et al.*<sup>[36]</sup> evaluated CBF of FHF patients *via* the NIRS method. This method detects changes in cerebral perfusion pressure and constitutes a non-invasive method that, in conjunction with transcranial Doppler, may detect brain hyperperfusion before the manifestation of increased intracranial pressure.

TCD is a non-invasive method that measures cerebral blood flow velocity (CBFV). Access of ultrasound waves to the intracranial environment is possible through the "ultrasonic windows", namely the temporal, orbital, suboccipital, and submandibular windows. Thus, placing one transducer against these ultrasonic windows allows the obtention of the spectra of blood flow velocity vs time for some cerebral arteries<sup>[37]</sup>.

The previously mentioned arteries can be assessed every 1 mm to 2 mm along their lengths given the pulsed emission ultrasonic waves, which allow controlled modulation depth of the sampling area<sup>[38]</sup>. The examiner should acquire the most intense audible signal and best blood flow velocity spectra possible by adjusting the position and transducer angle so that the incidence angle between the emitted ultrasound beam and blood vessel is close to zero<sup>[39]</sup>; thus, more accurate measurements of blood flow velocity can be made.

TCD has proven a valuable method in studies of cerebral hemodynamics due to its high temporal resolution, non-invasiveness, portability, and ability to measure CBFV in real time. CBFV indirectly represents CBF if the cross-sectional area of the vessel is assumed to remain constant with fluctuations in arterial pressure. There is evidence that, despite variations in ABP, the caliber of the vessel does not change significantly<sup>[40,41]</sup>, thereby validating the method for clinical use.

TCD can provide indirect information on CBF and ICP in patients with FHF<sup>[22]</sup>. Changes in the shape of the spectral diastolic wave can be an early sign of IH and impaired cerebral perfusion pressure. In addition, the final stages of IH can lead to large attenuation of diastolic blood flow velocity (BFV)<sup>[42]</sup>.

ICP changes can influence cerebral blood circulation, which may be assessed with TCD. Currently, TCD publications are trying to predict ICP curves in a non-invasive manner. The pulsatility index (PI) is defined by the following formula: Systolic velocity - diastolic velocity/mean velocity, and is increased when cerebrovascular resistance is elevated. Increased ICP may lead to PI elevation, especially if there is an impairment of cerebral autoregulation. In this case, when diastolic blood pressure equals ICP, there is cessation of intracranial diastolic flow<sup>[43]</sup>; a further increase in ICP (oscillating flow) may appear during flow progress in the systole. During diastole, critically high ICP, CVR, and distended intracranial arteries eject the blood in a retrograde direction. When net forward flow is seriously reduced, severe ischemic brain damage or brain death may occur. In critical IH, the intracranial waveform degrades to become a small systolic spike and then disappears altogether<sup>[44]</sup>. The relationship between TCD-hemodynamic patterns and the different states of ICP reinforces the idea that TCD can be useful for determining the optimal range of arterial blood pressure for adequate cerebral blood flow dynamics in FHF patients<sup>[45,46]</sup>.

Cerebral autoregulation (CA) is impaired in patients with FHF, and CBF has been described as correlating with ICP in FHF<sup>[23]</sup>. CA is characterized by CBF remaining

relatively constant despite variations in CPP. This physiological response acts to protect the brain from the harmful effects (*i.e.*, ischemia or hyperemia) of large oscillations in perfusion pressure. Lassen *et al.*<sup>[47]</sup> use the term "autoregulation" to explain the relatively constant values of blood flow encountered during hypotension induction. However, autoregulation has been confused with other dynamic adjustment processes. Strictly speaking, autoregulation refers only to the brain's vascular response to changes in CPP, and is sometimes referred to specifically as pressure autoregulation. Brain vessels also dilate or contract as a physiological response to cellular metabolic activity, but should not strictly be called autoregulation. The influence of neuronal metabolism on CBF should be referred to as metabolic regulation of the flow-metabolism coupling<sup>[47,48]</sup>.

The methods used to estimate changes in cerebral perfusion are TCD ultrasound and clearance of xenon-133, while CT demonstrates stable CBF. Other techniques reflect tissue perfusion and estimate changes in CBF such as jugular arteriovenous difference in oxygen (AVDO<sub>2</sub>), electromagnetic flow meters, near-infrared spectroscopy, laser Doppler flowmetry, and venous occlusion plethysmography<sup>[49]</sup>.

With changes in technology, particularly the advent of TCD and high temporal resolution examination, it has become possible to calculate an index for static CA<sup>[50]</sup>, which relates cerebrovascular resistance to blood pressure, according to the following formula<sup>[51]</sup>:  $\Delta\text{CVR}\% / \Delta\text{CPP}\%$  (CVR - cerebrovascular resistance); where it is assumed that  $\text{CPP} = \text{ABP} - \text{ICP}$ , with the value of ICP being negligible and thus ABP replacing CPP<sup>[50]</sup>.

However, the nature of the estimates, the need for invasive procedures to change ABP, the inherent risk of exposing the patient to exhaustion of self-regulatory reserves, and the emergence of new dynamic CA study methods has reduced the use of the static method for evaluating CA in clinical studies<sup>[51,52]</sup>.

Abdo *et al.*<sup>[46]</sup> evaluated BFV by TCD in five patients with FHF and compared the results against a control group who had associated critical neurological conditions without FHF. Despite the limitations of the study, the authors concluded that patients with FHF may have a dominant pattern of brain hypoperfusion, with an average velocity below normal values and an increased pulsatility index, possibly due to an increase in ICP. The authors suggested that proper measurement by this method improves brain perfusion and prevents hypoxia in these patients. Another study that used TCD demonstrated that CA of CBF was re-established after the onset of HE improvement in patients with FHF<sup>[53]</sup>.

## NEUROPHYSIOLOGICAL MONITORING

### Electroencephalogram

Electroencephalogram (EEG) is a non-invasive method which analyzes spontaneous brain electrical activity and is performed by placing electrodes on the scalp with the aid of a conductive paste which, besides affixing the

electrodes, allows for the proper acquisition of the signals that constitute the brain's electrical activity<sup>[54]</sup>. Initially, a spontaneous recording of brain electrical activity is made while the patient is awake and conscious. If possible, this activity is also recorded during drowsiness and sleep. Recording during these different states increases the sensitivity of the method in detecting various defects, including patients with severe brain pathologies<sup>[21,54]</sup>.

Continuous video EEG (cEEG) provides long-term monitoring of brain electrical activity in critically-ill patients with altered mental status and in those at risk of secondary ischemia following acute brain injury. The main indications of cEEG are the detection of non-convulsive seizures or status epilepticus in order to investigate causes of impairment of consciousness, and to determine the prognosis of brain injury. EEG changes in hepatic encephalopathy may range from low alpha-rhythm frequency (8 Hz) mixed with bilateral theta activity, which can later develop into theta-delta with deceleration throughout both hemispheres, with or without three-phase curves. With increasing stupor, sleep activity disintegrates. In severe coma, arrhythmic delta activity diminishes, both in frequency and amplitude, and progresses to electrocerebral silence<sup>[54]</sup>.

The presence of subclinical seizure is often poorly recognized in patients with grade III and IV HE, emphasizing the importance of EEG monitoring in these patients. Cerebral ischemia has often been known to precede the onset of seizures in patients with FHF<sup>[54]</sup>. Seizures are susceptible to cerebral hypoxia and contribute to the development and perpetuation of brain swelling. During FHF, the increase in extracellular brain glutamate concentrations predisposes patients to epileptic activity<sup>[21]</sup>. Although no definitive recommendations can be made at the time of writing, the morbidity of untreated subclinical crisis should be considered concomitant with the prudent administration of anti-epileptic drugs until additional studies are established.

### Bispectral index

The bispectral index (BIS) is a neurophysiological monitoring system that continuously analyzes electroencephalograms to determine the level of consciousness of patients undergoing general anesthesia. The notion of "anesthetic depth" is usually associated with training experiences or memories during surgery, in which anesthesia does not prevent consciousness or even waking-up during general anesthesia. Although EEG is the gold standard used to determine electrical activity in comatose patients, standard EEG monitoring may not be feasible for all patients who require intensive care during pretransplant<sup>[55,56]</sup>.

Studies show that monitoring by BIS, which was developed in order to assist with the clinical evaluation of the degree of hypnosis with anesthesia, is useful for monitoring cases of FHF<sup>[55-57]</sup>. The BIS monitor uses the EEG signal derived from electrodes placed on the forehead that provide continuous monitoring. While monitoring for BIS has been developed to assess the

level of awareness during anesthesia, this method may also be useful to assess the degree of recovery of consciousness alongside improved liver function after liver transplantation. Hwang *et al*<sup>[9]</sup> showed that the BIS may be useful for evaluating state of consciousness during the peritransplant and intensive care periods for FHF patients who develop HE.

## BRAIN OXYGENATION MONITORING

Brain oxygenation monitoring after brain injury can lead to the detection or prevention of secondary ischemic episodes. The four methods used to measure cerebral oxygenation are: Jugular bulb oximetry, measurement of direct tissue oxygen tension, NIRS, and PET oxygen-15<sup>[32]</sup>.

### Jugular bulb oximetry

Catheterization of the jugular bulb and obtention of venous blood samples allow for an estimate of blood flow and cerebral metabolism. Monitoring blood oxygen saturation in veins that drain the brain provides an estimate of overall metabolic demand compared to oxygenation deprivation<sup>[32]</sup>. The parameter can be used as a measure of jugular venous oxygen content, as well as arteriovenous oxygen difference<sup>[57]</sup>.

Monitoring the oxygen saturation of the jugular vein provides an estimate of overall metabolic demand compared to oxygenation. The parameter used can be jugular venous oxygen content or arteriovenous oxygen difference ( $AVDO_2 = CMRO_2/CBF$ ;  $CMRO_2$  = cerebral metabolic rate of oxygen consumption). The extent of arteriovenous oxygen difference indicates the amount of oxygen extracted by the brain. Under normal conditions, this value is a 2.8  $\mu\text{mol/mL}$  (range 2.2-3.3  $\mu\text{mol/mL}$ ) or 6.3% volume (volume varies from 5%-7.5% oxygen) change in  $CMRO_2$  or cerebral blood flow extraction<sup>[24]</sup>. A reduction in cerebral blood flow, without changes in the energetic demands, increases oxygen extraction in the cerebral tissue. Thus, the jugular vein oxygen decreases and the difference between arterial and jugular venous oxygen increases. On the other hand, a disproportionate increase in cerebral blood flow or a decrease in energy consumption decreases  $AVDO_2$ <sup>[57]</sup>. The limitation of the method is the non-detection of oxygen consumption changes in small brain regions<sup>[58]</sup>.

### Brain tissue oxygen

Quantitation of tissue oxygen pressure ( $PtIO_2$ ) in the brain reflects the partial pressure of oxygen at the end of the capillary circuit. In ischemic situations, a fall in  $PtIO_2$  is accompanied by a decrease in pH (lactic acidosis) and an increase in tissue carbon dioxide pressure, with a lack of metabolic exchange between cells and the capillary circuit. Low values indicate  $PtIO_2$  tissue hypoxia and help guide therapy<sup>[59]</sup>. The patient should exhibit adequate hemoglobin content, balanced hemoglobin affinity for oxygen, and appropriate systemic arterial oxygen

content. Commonly-used sensors determine mean tissue oxygen pressure in an area of 17 mm<sup>3</sup>. The catheter is introduced into the cerebral white matter to a depth of 25 mm below the dura mater. The cathode comprises a gold and silver anode immersed in an electrolyte solution<sup>[58,59]</sup>. The oxygen molecules diffuse into the catheter, producing a reversible reaction at the cathode in which oxygen combines with water and forms ions (OH<sup>-</sup>). These reactions generate an electric current detected by a voltmeter, with the electrical signal subsequently digitized and transformed into a numeric value on the monitor display panel. Positioning the catheter in a circulatory border territory between the anterior and middle cerebral arteries allows for the early detection of changes in this area, which is more sensitive to flow variations<sup>[59]</sup>.

Based on previous studies, the cutoff point value for cerebral ischemia monitoring with PtIO<sub>2</sub> appears to lie within the 8 to 25 mmHg range. PtIO<sub>2</sub> monitoring can provide real-time information on the regulation of brain blood flow and has been shown to have a clear impact on the management of patients with severe brain injuries, such as traumatic brain injury and hemispheric infarcts<sup>[60]</sup>. Patients with FHF who develop brain swelling and IH can benefit from this method.

### Near infrared spectroscopy

As described above, this is a non-invasive technique for measuring regional cerebral oxygen saturation, as well as analyzing the difference in oxygenated hemoglobin and deoxygenated absorption spectra<sup>[61]</sup>.

Studies in patients with FHF demonstrate that the monitoring of brain oxygenation provides valuable data for the clinical management of this population<sup>[62]</sup>. Oxygen and cerebral glucose consumption have been observed before signs of brain swelling, suggesting that cerebral oxygen metabolism is intact at this stage<sup>[62]</sup>. In another study, CMRO<sub>2</sub> was found to be decreased in all patients with FHF<sup>[61]</sup>. There was also evidence of cerebral ischemia, as indicated by increased AVDO<sub>2</sub>. In the study, it was concluded that hyperemia alone was not related to the outcome, despite having occurred more frequently during elevated ICP. All patients with malignant intracranial hypertension previously had hyperemia<sup>[62,63]</sup>. Nielsen *et al.*<sup>[36]</sup> reported that both pressure and arterial oxygen saturation were maintained during infusion with norepinephrine. Additionally, hemoglobin concentration in blood flow was not compromised. Cerebral arterial oxygenation is capable of detecting brain perfusion changes during norepinephrine infusion in patients with acute liver failure. This suggests that NIRS can be valuable in monitoring critical changes in the cerebral oxygenation and blood volume of these patients.

## METABOLIC MONITORING

Brain metabolism can be evaluated by PET and MR spectroscopy, jugular oxygen saturation, monitoring of CBF, and MD. PET scans provide an estimate of the topographic view of glucose metabolism, while MRI

spectroscopy qualitatively shows the lactate content of a particular brain structure<sup>[58]</sup>.

MD techniques provide information on tissue metabolism, including the availability of substrates such as glucose and the production of local metabolites. This technique is based on the exchange of solutes through a semipermeable membrane that simulates the operation of a capillary and has the basic objective of monitoring the tissue availability of the different metabolites released by cells<sup>[64]</sup>.

The tip of the catheter contains a semipermeable membrane that separates a solution of known composition from the extracellular fluid space. MD fluid is then analyzed to quantify metabolites. This technique allows for the study of the release of excitatory neurotransmitters such as glutamate and aspartate, as well as other neuro-modulators, thereby indirectly analyzing the ischemic excitotoxicity phenomenon. It also allows for the analysis of the concentration of tissue degradation products such as glycerol. The catheter's semipermeable membrane used to study the cited substances only allows for the passage of ions of molecules with a molecular weight of less than 20000 daltons<sup>[64,65]</sup>.

Glucose is most frequently determined as the cellular energy substrate. In conditions where there is a decrease in both cerebral tissue glucose and PtIO<sub>2</sub>, a reduction of capillary blood flow may be inferred<sup>[63,64]</sup>. Lactate studies can indicate the intensity of anaerobic metabolism, while glycerol studies can evaluate tissue damage since glycerol is one of the structural components in the tissue lipid layer of cell membranes<sup>[66]</sup>. Glutamate is an important excitatory neurotransmitter in the mammalian nervous system, with aspartate following in importance. These amino acids are released in the synaptic cleft after neuronal depolarization. This depolarization can be associated with tissue ischemia in states of massive release<sup>[67]</sup>. In situations of excitotoxicity, massive release of glutamate into the synaptic cleft can be seen. Thus, large inputs of calcium into the cell are observed; as a consequence, there is production of oxygen free radicals in cell membranes and the release of more fatty acids and glycerol<sup>[66]</sup>. It is recommended that the MD catheter be placed in so-called "penumbra" areas adjacent to focal lesions in order to allow monitoring of potentially recoverable brain regions<sup>[68,69]</sup>. MD is currently considered one of the most important *in vitro* sampling methods in physiology and pharmacology. Applied in neurointensive care, it is the only tool that allows continuous measurement of chemicals in the brain extracellular space and elucidation of non-ischemic forms of cerebral hypoxia<sup>[67]</sup>.

The tissue volume evaluated by the MD catheter is a cylinder equivalent to the length of the dialysis membrane (10 mm) with a diameter of a few millimeters (0.6 mm). MD pumps perfuse the catheter with an artificial cerebrospinal fluid, which equilibrates with the interstice around the catheter. Balance occurs by diffusion through the dialysis membrane. Using a dialysis membrane with a 10 mm 0.3 perfusion flow L/min, the



concentration of dialyzed glucose, lactate, pyruvate, and glutamate is approximately 70% of the concentration of interstitial fluid. Samples are continuously collected and analyzed at the bedside every hour, or as needed, with the results being analyzed on trend curves<sup>[70]</sup>. When monitoring biochemical markers it is established that: Lactate/pyruvate ratio is the best marker of cerebral cortex state and early biomarkers in secondary ischemic injury glycerol and glutamate are additional markers of tissue hypoxia<sup>[70]</sup>.

Brain swelling predominantly involving glial cells is often reported as a serious complication of FHF. The swelling of astrocytes may result in elevated ICP and cerebral herniation syndrome in patients with FHF<sup>[70]</sup>. Tofteng *et al.*<sup>[71]</sup> found brain chemical changes in the MD of a young man with severe acute liver failure and brain swelling in the liver transplant, and found that both extracellular glutamate and glycerol levels were elevated before liver transplant, and tending to decrease after grafting. These results indicate changes in glutamate neurotransmission, arachidonic acid metabolism, lactate, and flow through the blood-brain barrier in patients with FHF.

In another study, Tofteng *et al.*<sup>[72]</sup> investigated whether an increased concentration of glutamate and brain extracellular lactate preceded episodes of elevated ICP in patients with FHF (7 women and 3 men; age range 20-55 years) by inserting MD and ICP catheters into the brain. A total of 352 MD samples were collected for a median of 3 d, allowing for the analysis of approximately 1760 dialyzed samples at the bedside. It has been shown that patients with FHF feature elevated concentrations of extracellular glutamate and cerebral lactate. However, high levels of glutamate are not correlated with increased intracranial pressure, while high levels of lactate precede episodes of elevated ICP. Hyperglycolysis to lactate accumulation is involved in brain microvascular vasodilation and ICP increase in patients with FHF. Therefore, it can be concluded that brain MD at the bedside can be a valuable tool for monitoring these patients.

## CONCLUSION

Patients with FHF are usually submitted for brain monitoring after undergoing liver transplantation or when they have a neurological decline. Brain monitoring in this critical phase is essential for maintaining hemodynamic, metabolic, and electrical parameters at acceptable levels. There are a myriad of methods for real time measuring of the aforementioned parameters, with each method having a particular contribution in the detection of "a brain at risk". The key point for proper patient management in order to prevent neurological complications is to combine the different methods in a multimodal approach.

The multimodal technique of extended neuro-monitoring offers an advanced option for further development and investigations in animal models of FHF. Furthermore, identification of patients at risk for neurologic complications before and after liver transplant

may allow for prompt neuroprotective interventions, including the optimal control of blood pressure.

## REFERENCES

- 1 **Kjaergard LL**, Liu J, Als-Nielsen B, Gluud C. Artificial and bioartificial support systems for acute and acute-on-chronic liver failure: a systematic review. *JAMA* 2003; **289**: 217-222 [PMID: 12517233 DOI: 10.1001/jama.289.2.217]
- 2 **O'Grady J**. Modern management of acute liver failure. *Clin Liver Dis* 2007; **11**: 291-303 [PMID: 17606208 DOI: 10.1016/j.cld.2007.04.011]
- 3 **Larsen FS**. Cerebral circulation in liver failure: Ohm's law in force. *Semin Liver Dis* 1996; **16**: 281-292 [PMID: 8989814 DOI: 10.1055/s-2007-1007241]
- 4 **Vespa PM**, Nenov V, Nuwer MR. Continuous EEG monitoring in the intensive care unit: early findings and clinical efficacy. *J Clin Neurophysiol* 1999; **16**: 1-13 [PMID: 10082088 DOI: 10.1097/0004691-199901000-00001]
- 5 **Shami VM**, Caldwell SH, Hespenheide EE, Arseneau KO, Bickston SJ, Macik BG. Recombinant activated factor VII for coagulopathy in fulminant hepatic failure compared with conventional therapy. *Liver Transpl* 2003; **9**: 138-143 [PMID: 12548507 DOI: 10.1053/jlts.2003.50017]
- 6 **Hanid MA**, Davies M, Mellon PJ, Silk DB, Strunin L, McCabe JJ, Williams R. Clinical monitoring of intracranial pressure in fulminant hepatic failure. *Gut* 1980; **21**: 866-869 [PMID: 6777264 DOI: 10.1136/gut.21.10.866]
- 7 **Frühaufl NR**, Radunz S, Grabellus F, Laube T, Uerschels AK, Kaiser GM. Neuromonitoring in a porcine model of acute hepatic failure. *Lab Anim* 2011; **45**: 174-178 [PMID: 21508115 DOI: 10.1258/la.2011.010083]
- 8 **Donovan JP**, Shaw BW, Langnas AN, Sorrell MF. Brain water and acute liver failure: the emerging role of intracranial pressure monitoring. *Hepatology* 1992; **16**: 267-268 [PMID: 1618475 DOI: 10.1002/hep.1840160138]
- 9 **Hwang S**, Lee SG, Park JI, Song GW, Ryu JH, Jung DH, Hwang GS, Jeong SM, Song JG, Hong SK, Lim YS, Kim KM. Continuous peritransplant assessment of consciousness using bispectral index monitoring for patients with fulminant hepatic failure undergoing urgent liver transplantation. *Clin Transplant* 2010; **24**: 91-97 [PMID: 19925461 DOI: 10.1111/j.1399-0012.2009.01148.x]
- 10 **Davies MH**, Mutimer D, Lowes J, Elias E, Neuberger J. Recovery despite impaired cerebral perfusion in fulminant hepatic failure. *Lancet* 1994; **343**: 1329-1330 [PMID: 7910328 DOI: 10.1016/S0140-6736(94)92471-6]
- 11 **Darlington DN**, Kheirabadi BS, Scherer MR, Martini WZ, Dubick MA. Acidosis and correction of acidosis does not affect rFVIIa function in swine. *Int J Burns Trauma* 2012; **2**: 145-157 [PMID: 23272296]
- 12 **Bingaman WE**, Frank JI. Malignant cerebral edema and intracranial hypertension. *Neurol Clin* 1995; **13**: 479-509 [PMID: 7476816]
- 13 **Kim YK**, Seo H, Yu J, Hwang GS. Noninvasive estimation of raised intracranial pressure using ocular ultrasonography in liver transplant recipients with acute liver failure - A report of two cases-. *Korean J Anesthesiol* 2013; **64**: 451-455 [PMID: 23741570 DOI: 10.4097/kjae.2013.64.5.451]
- 14 **Soldatos T**, Chatzimichail K, Papatheanasiou M, Gouliamos A. Optic nerve sonography: a new window for the non-invasive evaluation of intracranial pressure in brain injury. *Emerg Med J* 2009; **26**: 630-634 [PMID: 19700575 DOI: 10.1136/emj.2008.058453]
- 15 **Moretti R**, Pizzi B, Cassini F, Vivaldi N. Reliability of optic nerve ultrasound for the evaluation of patients with spontaneous intracranial hemorrhage. *Neurocrit Care* 2009; **11**: 406-410 [PMID: 19636971 DOI: 10.1007/s12028-009-9250-8]
- 16 **Hallevy C**, Ifergane G, Kordysh E, Herishanu Y. Spontaneous supratentorial intracerebral hemorrhage. Criteria for short-term functional outcome prediction. *J Neurol* 2002; **249**: 1704-1709

- [PMID: 12529793 DOI: 10.1007/s00415-002-0911-1]
- 17 Voigt LP, Pastores SM, Raouf ND, Thaler HT, Halpern NA. Review of a large clinical series: intrahospital transport of critically ill patients: outcomes, timing, and patterns. *J Intensive Care Med* 2009; **24**: 108-115 [PMID: 19188270 DOI: 10.1177/0885066608329946]
  - 18 Schlachetzki F, Herzberg M, Hölscher T, Ertl M, Zimmermann M, Ittner KP, Pels H, Bogdahn U, Boy S. Transcranial ultrasound from diagnosis to early stroke treatment: part 2: prehospital neurosonography in patients with acute stroke: the Regensburg stroke mobile project. *Cerebrovasc Dis* 2012; **33**: 262-271 [PMID: 22261817 DOI: 10.1159/000334667]
  - 19 Pérez ES, Delgado-Mederos R, Rubiera M, Delgado P, Ribó M, Maisterra O, Ortega G, Alvarez-Sabin J, Molina CA. Transcranial duplex sonography for monitoring hyperacute intracerebral hemorrhage. *Stroke* 2009; **40**: 987-990 [PMID: 19164795 DOI: 10.1016/s1073-5437(09)79362-3]
  - 20 Gerriets T, Stolz E, König S, Babacan S, Fiss I, Jauss M, Kaps M. Sonographic monitoring of midline shift in space-occupying stroke: an early outcome predictor. *Stroke* 2001; **32**: 442-447 [PMID: 11157180 DOI: 10.1161/01.str.32.2.442]
  - 21 Prakash R, Mullen KD. Mechanisms, diagnosis and management of hepatic encephalopathy. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 515-525 [PMID: 20703237 DOI: 10.1038/nrgastro.2010.116]
  - 22 Mohsenin V. Assessment and management of cerebral edema and intracranial hypertension in acute liver failure. *J Crit Care* 2013; **28**: 783-791 [PMID: 23683564 DOI: 10.1016/j.jccr.2013.04.002]
  - 23 Rovira A, Alonso J, Córdoba J. MR imaging findings in hepatic encephalopathy. *AJNR Am J Neuroradiol* 2008; **29**: 1612-1621 [PMID: 18583413 DOI: 10.1007/978-1-61779-836-8\_10]
  - 24 Robertson CS, Contant CF, Gokaslan ZL, Narayan RK, Grossman RG. Cerebral blood flow, arteriovenous oxygen difference, and outcome in head injured patients. *J Neurol Neurosurg Psychiatry* 1992; **55**: 594-603 [PMID: 1640238 DOI: 10.1136/jnnp.55.7.594]
  - 25 Panerai RB. The critical closing pressure of the cerebral circulation. *Med Eng Phys* 2003; **25**: 621-632 [PMID: 12900178 DOI: 10.1016/s1350-4533(03)00027-4]
  - 26 KETY SS, SCHMIDT CF. Measurement of cerebral blood flow and cerebral oxygen consumption in man. *Fed Proc* 1946; **5**: 264 [PMID: 21064908 DOI: 10.1007/978-3-7091-9101-9\_2]
  - 27 Cook DJ, Anderson RE, Michenfelder JD, Oliver WC, Orszulak TA, Daly RC, Bryce RD. Cerebral blood flow during cardiac operations: comparison of Kety-Schmidt and xenon-133 clearance methods. *Ann Thorac Surg* 1995; **59**: 614-620 [PMID: 7887699 DOI: 10.1016/0003-4975(94)00956-2]
  - 28 Obrist WD, Thompson HK, Wang HS, Wilkinson WE. Regional cerebral blood flow estimated by 133-xenon inhalation. *Stroke* 1975; **6**: 245-256 [PMID: 1154462 DOI: 10.1016/0304-3959(85)90215-5]
  - 29 Keller E, Nadler A, Alkadh H, Kollias SS, Yonekawa Y, Niederer P. Noninvasive measurement of regional cerebral blood flow and regional cerebral blood volume by near-infrared spectroscopy and indocyanine green dye dilution. *Neuroimage* 2003; **20**: 828-839 [PMID: 14568455 DOI: 10.1016/S1053-8119(03)00315-X]
  - 30 Mélot C, Berré J, Moraine JJ, Kahn RJ. Estimation of cerebral blood flow at bedside by continuous jugular thermodilution. *J Cereb Blood Flow Metab* 1996; **16**: 1263-1270 [PMID: 8898700 DOI: 10.1097/00004647-199611000-00022]
  - 31 Yonas H, Jungreis C. Xenon CT cerebral blood flow: past, present, and future. *AJNR Am J Neuroradiol* 1995; **16**: 219-220 [PMID: 7900599 DOI: 10.5005/jp/books/11824\_25]
  - 32 Latchaw RE. Cerebral perfusion imaging in acute stroke. *J Vasc Interv Radiol* 2004; **15**: S29-S46 [PMID: 15101514 DOI: 10.1097/01.RVI.0000112976.88422.86]
  - 33 Lammertsma AA. PET/SPECT: functional imaging beyond flow. *Vision Res* 2001; **41**: 1277-1281 [PMID: 11322972 DOI: 10.1016/s0042-6989(00)00262-5]
  - 34 O'Carroll RE, Hayes PC, Ebmeier KP, Dougall N, Murray C, Best JJ, Bouchier IA, Goodwin GM. Regional cerebral blood flow and cognitive function in patients with chronic liver disease. *Lancet* 1991; **337**: 1250-1253 [PMID: 1674063 DOI: 10.1016/0140-6736(91)92920-w]
  - 35 Catafau AM, Kulisevsky J, Bernà L, Pujol J, Martin JC, Otermin P, Balanzó J, Carrió I. Relationship between cerebral perfusion in frontal-limbic-basal ganglia circuits and neuropsychologic impairment in patients with subclinical hepatic encephalopathy. *J Nucl Med* 2000; **41**: 405-410 [PMID: 10716310 DOI: 10.1016/s0002-9270(01)04132-6]
  - 36 Nielsen HB, Tofteng F, Wang LP, Larsen FS. Cerebral oxygenation determined by near-infrared spectrophotometry in patients with fulminant hepatic failure. *J Hepatol* 2003; **38**: 188-192 [PMID: 12547407 DOI: 10.1016/s0168-8278(02)00377-x]
  - 37 Ringelstein E. A practical guide to transcranial Doppler sonography, 1989
  - 38 McCartney JT, Gomez CR. Handbook of transcranial Doppler. New York: Springer-Verlag, 1997
  - 39 Torbey MT, Hauser TK, Bhardwaj A, Williams MA, Ulatowski JA, Mirski MA, Razumovsky AY. Effect of age on cerebral blood flow velocity and incidence of vasospasm after aneurysmal subarachnoid hemorrhage. *Stroke* 2001; **32**: 2005-2011 [PMID: 11546889 DOI: 10.1161/hs0901.094622]
  - 40 Newell DW, Aaslid R, Lam A, Mayberg TS, Winn HR. Comparison of flow and velocity during dynamic autoregulation testing in humans. *Stroke* 1994; **25**: 793-797 [PMID: 7909175 DOI: 10.1161/01.str.25.4.793]
  - 41 Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 2000; **31**: 1672-1678 [PMID: 10884472 DOI: 10.1161/01.str.31.7.1672]
  - 42 Kudo M. Cerebral vascular resistance in hepatic insufficiency. *J Gastroenterol Hepatol* 2001; **16**: 845-847 [PMID: 11555094 DOI: 10.1046/j.1440-1746.2001.02552.x]
  - 43 Kawakami M, Koda M, Murawaki Y. Cerebral pulsatility index by transcranial Doppler sonography predicts the prognosis of patients with fulminant hepatic failure. *Clin Imaging* 2010; **34**: 327-331 [PMID: 20813293 DOI: 10.1016/j.clinimag.2009.09.006]
  - 44 Paschoal FM, Bor-Seng-Shu E, Teixeira MJ. Transcranial Doppler ultrasonography with jugular vein compression can detect impairment of intracranial compliance. *Clin Neurol Neurosurg* 2013; **115**: 1196-1198 [PMID: 23128012 DOI: 10.1016/j.clineuro.2012.09.028]
  - 45 Bor-Seng-Shu E, Teixeira MJ, Hirsch R, Andrade AF, Marino R Jr. Transcranial Doppler sonography in two patients who underwent decompressive craniectomy for traumatic brain swelling: report of two cases. *Arq Neuropsiquiatr* 2004; **62**: 715-721 [PMID: 15334237 DOI: 10.1590/S0004-282X2004000400028]
  - 46 Abdo A, López O, Fernández A, Santos J, Castillo J, Castellanos R, González L, Gómez F, Limonta D. Transcranial Doppler sonography in fulminant hepatic failure. *Transplant Proc* 2003; **35**: 1859-1860 [PMID: 12962825 DOI: 10.1016/s0041-1345(03)00592-x]
  - 47 Lassen NA. Cerebral blood flow and oxygen consumption in man. *Physiol Rev* 1959; **39**: 183-238 [PMID: 13645234]
  - 48 MacKenzie ET, Strandgaard S, Graham DI, Jones JV, Harper AM, Farrar JK. Effects of acutely induced hypertension in cats on pial arteriolar caliber, local cerebral blood flow, and the blood-brain barrier. *Circ Res* 1976; **39**: 33-41 [PMID: 1277403 DOI: 10.1161/01.res.39.1.33]
  - 49 Rangel-Castilla L, Gasco J, Nauta HJ, Okonkwo DO, Robertson CS. Cerebral pressure autoregulation in traumatic brain injury. *Neurosurg Focus* 2008; **25**: E7 [PMID: 18828705 DOI: 10.3171/foc.2008.25.10.e7]
  - 50 Panerai RB. Cerebral autoregulation: from models to clinical applications. *Cardiovasc Eng* 2008; **8**: 42-59 [PMID: 18041584 DOI: 10.1007/s10558-007-9044-6]
  - 51 Tiecks FP, Lam AM, Aaslid R, Newell DW. Comparison of static and dynamic cerebral autoregulation measurements. *Stroke* 1995; **26**: 1014-1019 [PMID: 7762016 DOI: 10.1161/01.str.26.6.1014]
  - 52 Aaslid R. Cerebral autoregulation and vasomotor reactivity. *Front Neurol Neurosci* 2006; **21**: 216-228 [PMID: 17290140 DOI: 10.1159/000092434]

- 53 **Strauss G**, Hansen BA, Kirkegaard P, Rasmussen A, Hjortrup A, Larsen FS. Liver function, cerebral blood flow autoregulation, and hepatic encephalopathy in fulminant hepatic failure. *Hepatology* 1997; **25**: 837-839 [PMID: 9096585 DOI: 10.1002/hep.510250409]
- 54 **Ellis AJ**, Wendon JA, Williams R. Subclinical seizure activity and prophylactic phenytoin infusion in acute liver failure: a controlled clinical trial. *Hepatology* 2000; **32**: 536-541 [PMID: 10960446 DOI: 10.1053/jhep.2000.9775]
- 55 **Vivien B**, Paqueron X, Le Cosquer P, Langeron O, Coriat P, Riou B. Detection of brain death onset using the bispectral index in severely comatose patients. *Intensive Care Med* 2002; **28**: 419-425 [PMID: 11967595 DOI: 10.1007/s00134-002-1219-4]
- 56 **Dahaba AA**, Worm HC, Zhu SM, Bao FP, Salah A, Zakaria S, Bornemann H, Stadlbauer V, Rehak PH, Metzler H, Stauber RE. Sensitivity and specificity of bispectral index for classification of overt hepatic encephalopathy: a multicentre, observer blinded, validation study. *Gut* 2008; **57**: 77-83 [PMID: 17698861 DOI: 10.1136/gut.2007.129130]
- 57 **Lewis SB**, Myburgh JA, Reilly PL. Detection of cerebral venous desaturation by continuous jugular bulb oximetry following acute neurotrauma. *Anaesth Intensive Care* 1995; **23**: 307-314 [PMID: 7573917]
- 58 **Feldman Z**, Robertson CS. Monitoring of cerebral hemodynamics with jugular bulb catheters. *Crit Care Clin* 1997; **13**: 51-77 [PMID: 9012576 DOI: 10.1016/s0749-0704(05)70296-7]
- 59 **Sarratzadeh AS**, Kiening KL, Unterberg AW. Neuromonitoring: brain oxygenation and microdialysis. *Curr Neurol Neurosci Rep* 2003; **3**: 517-523 [PMID: 14565908 DOI: 10.1007/s11910-003-0057-2]
- 60 **Tolias CM**, Reinert M, Seiler R, Gilman C, Scharf A, Bullock MR. Normobaric hyperoxia--induced improvement in cerebral metabolism and reduction in intracranial pressure in patients with severe head injury: a prospective historical cohort-matched study. *J Neurosurg* 2004; **101**: 435-444 [PMID: 15352601 DOI: 10.3171/jns.2004.101.3.0435]
- 61 **Jöbsis FF**. Non-invasive, infra-red monitoring of cerebral O<sub>2</sub> sufficiency, blood volume, HbO<sub>2</sub>-Hb shifts and blood flow. *Acta Neurol Scand Suppl* 1977; **64**: 452-453 [PMID: 268870]
- 62 **Strauss GI**, Möller K, Larsen FS, Kondrup J, Knudsen GM. Cerebral glucose and oxygen metabolism in patients with fulminant hepatic failure. *Liver Transpl* 2003; **9**: 1244-1252 [PMID: 14625823 DOI: 10.1016/j.lts.2003.09.020]
- 63 **Aggarwal S**, Obrist W, Yonas H, Kramer D, Kang Y, Scott V, Planinsic R. Cerebral hemodynamic and metabolic profiles in fulminant hepatic failure: relationship to outcome. *Liver Transpl* 2005; **11**: 1353-1360 [PMID: 16237715 DOI: 10.1002/lt.20479]
- 64 **Johnston AJ**, Gupta AK. Advanced monitoring in the neurology intensive care unit: microdialysis. *Curr Opin Crit Care* 2002; **8**: 121-127 [PMID: 12386512 DOI: 10.1097/00075198-200204000-00006]
- 65 **Ungerstedt U**, Rostami E. Microdialysis in neurointensive care. *Curr Pharm Des* 2004; **10**: 2145-2152 [PMID: 15281890 DOI: 10.2174/1381612043384105]
- 66 **Rosenbloom AJ**, Sipe DM, Weedn VW. Microdialysis of proteins: performance of the CMA/20 probe. *J Neurosci Methods* 2005; **148**: 147-153 [PMID: 16043227 DOI: 10.1016/j.jneumeth.2005.04.018]
- 67 **Bor-Seng-Shu E**, Figueiredo EG, Fonoff ET, Fujimoto Y, Panerai RB, Teixeira MJ. Decompressive craniectomy and head injury: brain morphometry, ICP, cerebral hemodynamics, cerebral microvascular reactivity, and neurochemistry. *Neurosurg Rev* 2013; **36**: 361-370 [PMID: 23385739 DOI: 10.1007/s10143-013-0453-2]
- 68 **de Lima Oliveira M**, Kairalla AC, Fonoff ET, Martinez RC, Teixeira MJ, Bor-Seng-Shu E. Cerebral microdialysis in traumatic brain injury and subarachnoid hemorrhage: state of the art. *Neurocrit Care* 2014; **21**: 152-162 [PMID: 24072457 DOI: 10.1007/s12028-013-9884-4]
- 69 **de Lima Oliveira M**, Paiva W, Teixeira MJ, Bor-Seng-Shu E. Brain metabolic crisis in traumatic brain injury: what does it mean? *J Neurotrauma* 2014; **31**: 1750-1751 [PMID: 24915159 DOI: 10.1089/neu.2014.3386]
- 70 **Bellander BM**, Cantais E, Enblad P, Hutchinson P, Nordström CH, Robertson C, Sahuquillo J, Smith M, Stocchetti N, Ungerstedt U, Unterberg A, Olsen NV. Consensus meeting on microdialysis in neurointensive care. *Intensive Care Med* 2004; **30**: 2166-2169 [PMID: 15549254 DOI: 10.1007/s00134-004-2461-8]
- 71 **Tofteng F**, Jorgensen L, Hansen BA, Ott P, Kondrup J, Larsen FS. Cerebral microdialysis in patients with fulminant hepatic failure. *Hepatology* 2002; **36**: 1333-1340 [PMID: 12447856 DOI: 10.1002/hep.1840360607]
- 72 **Tofteng F**, Larsen FS. Monitoring extracellular concentrations of lactate, glutamate, and glycerol by in vivo microdialysis in the brain during liver transplantation in acute liver failure. *Liver Transpl* 2002; **8**: 302-305 [PMID: 11910577 DOI: 10.1053/jlts.2002.32283]

**P- Reviewer:** Hashimoto N **S- Editor:** Gong ZM

**L- Editor:** Rutherford A **E- Editor:** Li D



# Cholesterol metabolism in cholestatic liver disease and liver transplantation: From molecular mechanisms to clinical implications

Katriina Nemes, Fredrik Åberg, Helena Gylling, Helena Isoniemi

Katriina Nemes, Fredrik Åberg, Helena Isoniemi, University of Helsinki and Helsinki University Central Hospital, Transplantation and Liver Surgery Clinic, Meilahti Hospital, P.O. BOX 340, FI-00029 HUS, Finland

Helena Gylling, University of Helsinki and Helsinki University Central Hospital, Internal Medicine, Biomedicum Helsinki C 4 22, P.O. BOX 700, FI-00029 HUS, Finland

**Author contributions:** Nemes K wrote the first draft; Åberg F made critical revisions and constructed the figures; Gylling H and Isoniemi H further revised the manuscript; all authors have approved the final version of the manuscript and its submission.

**Conflict-of-interest statement:** The authors declare no conflict of interests for this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Katriina Nemes, MD, PhD, University of Helsinki and Helsinki University Central Hospital, Transplantation and Liver Surgery Clinic, Meilahti Hospital, P.O. BOX 340, FI-00029 HUS, Finland. [katriina.nemes@outlook.com](mailto:katriina.nemes@outlook.com)  
 Telephone: +358-40-5002151  
 Fax: +358-9-174975

Received: March 31, 2016

Peer-review started: April 6, 2016

First decision: May 17, 2016

Revised: June 7, 2016

Accepted: July 11, 2016

Article in press: July 13, 2016

Published online: August 8, 2016

## Abstract

The aim of this review is to enlighten the critical roles that the liver plays in cholesterol metabolism. Liver transplantation can serve as gene therapy or a source of gene transmission in certain conditions that affect cholesterol metabolism, such as low-density-lipoprotein (LDL) receptor gene mutations that are associated with familial hypercholesterolemia. On the other hand, cholestatic liver disease often alters cholesterol metabolism. Cholestasis can lead to formation of lipoprotein X (Lp-X), which is frequently mistaken for LDL on routine clinical tests. In contrast to LDL, Lp-X is non-atherogenic, and failure to differentiate between the two can interfere with cardiovascular risk assessment, potentially leading to prescription of futile lipid-lowering therapy. Statins do not effectively lower Lp-X levels, and cholestasis may lead to accumulation of toxic levels of statins. Moreover, severe cholestasis results in poor micellar formation, which reduces cholesterol absorption, potentially impairing the cholesterol-lowering effect of ezetimibe. Apolipoprotein B-100 measurement can help distinguish between atherogenic and non-atherogenic hypercholesterolemia. Furthermore, routine serum cholesterol measurements alone cannot reflect cholesterol absorption and synthesis. Measurements of serum non-cholesterol sterol biomarkers - such as cholesterol precursor sterols, plant sterols, and cholestanol - may help with the comprehensive assessment of cholesterol metabolism. An adequate cholesterol supply is essential for liver-regenerative capacity. Low preoperative and perioperative serum cholesterol levels seem to predict mortality in liver cirrhosis and after liver transplantation. Thus, accurate lipid profile evaluation is highly important in liver disease and after liver transplantation.



**Key words:** Cholesterol metabolism; Cholestasis; Liver transplantation; Non-cholesterol sterols; Cholestanol; Donor; Low density lipoprotein receptor mutation; Apolipoprotein B-100; Lipoprotein-X

© **The Author(s)** 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The liver plays key roles in cholesterol metabolism. Cholestatic liver disease leads to alterations of cholesterol metabolism: Cholesterol homeostasis is disturbed and cholesterol synthesis and especially cholesterol absorption are reduced, and lipoprotein X may develop. The latter can interfere with cardiovascular risk assessment. Apolipoprotein B-100 measurement may be useful in such cases. Cholesterol metabolism in cholestasis could be better described using cholesterol precursor sterols, diet-derived plant sterols, and cholestanol (the liver-synthesized derivative of cholesterol). Accurate lipid profile evaluation is particularly important after liver transplantation, when both atherogenic and non-atherogenic hypercholesterolemia may co-exist.

Nemes K, Åberg F, Gylling H, Isoniemi H. Cholesterol metabolism in cholestatic liver disease and liver transplantation: From molecular mechanisms to clinical implications. *World J Hepatol* 2016; 8(22): 924-932 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i22/924.htm> DOI: <http://dx.doi.org/10.4254/wjgh.v8.i22.924>

## INTRODUCTION

Abundant in the bloodstream and in cell membranes, cholesterol is a critical component of vertebrate cell-membrane structure and function, allowing cells to maintain the permeability and fluidity that is fundamental for all animal life<sup>[1,2]</sup>. Cholesterol biosynthesis defects, such as Smith-Lemli-Opitz syndrome and lathosterolosis, reveal cholesterol's importance in normal embryonic development. Lathosterolosis is a defect of postsqualene cholesterol biosynthesis that results in deficient transformation of lathosterol into 7-dehydrocholesterol by sterol-C5-desaturase/dehydrogenase. This disorder is characterized by high serum levels of the cholesterol precursor lathosterol, and low cholesterol levels in cells, plasma, and tissues-which causes multiple congenital anomalies, including microcephaly and progressive cholestasis leading to liver failure<sup>[3]</sup>.

The tightly inter-regulated whole-body cholesterol homeostasis includes the following main components: Intestinal cholesterol absorption, hepatic *de-novo* cholesterol synthesis, and cholesterol excretion from the body. Recent advances in the field have further clarified the mechanisms of intestinal transporters and regulatory pathways<sup>[4-11]</sup>. The brain is home to about 23% of total body cholesterol, which is mainly synthesized *in situ* following blood-brain barrier establishment since dietary cholesterol does not cross this boundary<sup>[3]</sup>. In contrast

to other species, humans exhibit a high cholesterol synthesis rate in the brain only after birth<sup>[3]</sup>.

Under normal circumstances, the liver is the primary site of cholesterol biosynthesis and storage<sup>[12]</sup>. The liver is also the principal site of cholesterol excretion, converting cholesterol to bile acids and removing free cholesterol as neutral sterols *via* biliary excretion<sup>[4,5,13,14]</sup>. Since the liver plays a central role in cholesterol metabolism, liver disease can impact cholesterol metabolism, depending on the type of liver injury (parenchymal, cholestatic, or mixed)<sup>[15]</sup>. In one case of lathosterolosis, liver transplantation (LT) removed the liver disease, reversing the cholesterol metabolism defect and somewhat improving the postnatal neurological symptoms<sup>[3]</sup>. Conversely, various changes in cholesterol metabolism can be indicators of hepatic and biliary dysfunction.

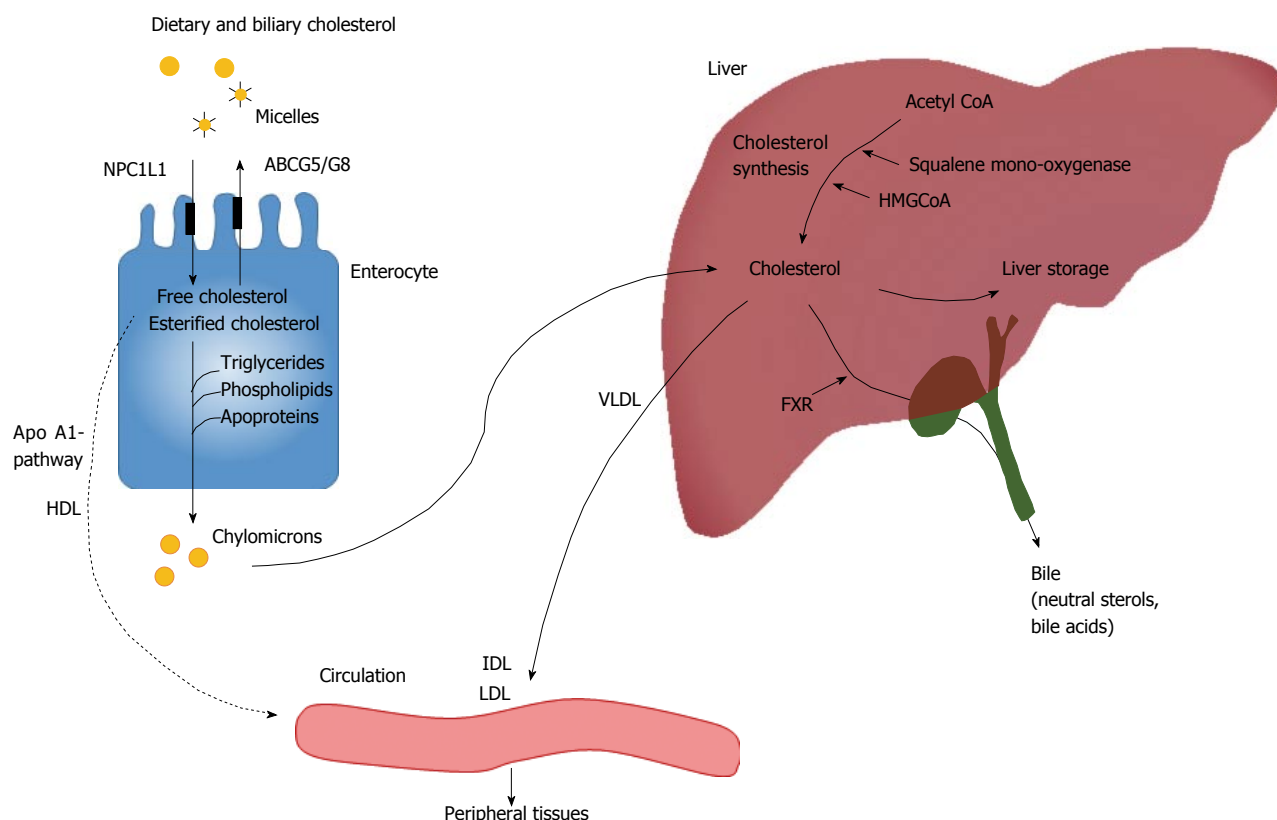
In the present review, we aimed to summarize current concepts regarding the regulation of cholesterol metabolism in health and in cholestatic liver disease. We discuss difficulties in assessing cholesterol metabolism, and summarize the cholesterol metabolism disturbances seen in cholestatic liver disease and before and after LT. Cholesterol metabolism in the setting of non-alcoholic steatohepatitis was recently reviewed<sup>[16]</sup>, and is not discussed here.

## OVERVIEW OF CHOLESTEROL METABOLISM

The following are the main components involved in liver-related cholesterol metabolism and the control of plasma cholesterol levels: (1) intestinal absorption of dietary and biliary cholesterol; (2) bile acid synthesis; (3) endogenous cholesterol synthesis; (4) biliary excretion of cholesterol; (5) low-density lipoprotein (LDL) receptor activity; (6) very-low-density lipoprotein (VLDL) particle synthesis and transport into circulation; and (7) reverse cholesterol transport from peripheral tissues for biliary or non-biliary excretion [trans-intestinal cholesterol efflux (TICE)], the latter of which has been demonstrated only in animal models<sup>[4-11,13,17-19]</sup>.

## ABSORPTION OF DIETARY AND BILIARY CHOLESTEROL IN THE SMALL INTESTINE

Intestine-driven pathways are an important component of cholesterol homeostasis, through which cholesterol is both taken up from and pumped back to the intestinal lumen. Intestinal cholesterol absorption is a selective multistep process that is regulated by multiple sterol-transporter genes at the enterocyte level<sup>[17,18]</sup>. Uptake of free cholesterol from mixed micelles in the intestinal lumen to enterocytes occurs *via* the specific transporter protein Niemann-Pick C1 Like 1 (NPC1L1), which is highly expressed in the brush-border membrane of small-intestinal enterocytes<sup>[10,11,18,19]</sup> (Figure 1). These enterocytes then selectively efflux about half of the free cholesterol and about 90% of plant sterols back to the



**Figure 1 From the gut to the circulation: An overview of the pathways involved in cholesterol absorption and synthesis.** IDL: Intermediate density lipoprotein; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; NPC1L1: Niemann-Pick C1 like 1; ABCG5: Adenosine triphosphate-binding cassette transporter G5 heterodimer; HMGCoA: 3-hydroxy-3-methyl-glutaryl CoA; FXR: Farnesoid X receptor.

intestinal lumen *via* the adenosine triphosphate (ATP)-binding cassette (ABC) G5/G8 transporters<sup>[20]</sup>.

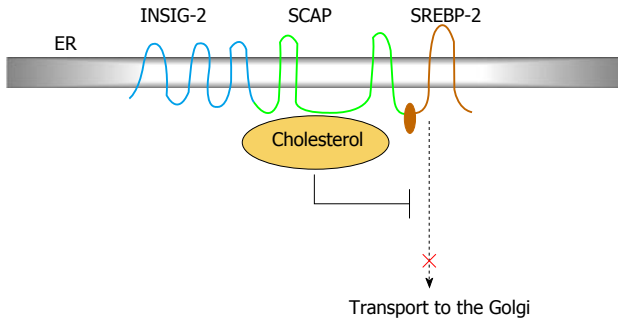
Lipoproteins synthesized in the liver and intestine play central roles in mediating the cholesterol transport to and from tissues through the bloodstream. Within enterocytes, free cholesterol is esterified and assembled - together with triglycerides, phospholipids, and apolipoproteins - to form chylomicrons (lipoproteins). Chylomicrons next enter the lymphatic system and the blood circulation at the thoracic duct, such that chylomicron remnants can be transported to the liver<sup>[21]</sup> (Figure 1). Some of the cholesterol in enterocytes is generated by endogenous synthesis<sup>[4,22]</sup>. Cholesterol is also reportedly secreted *via* an apolipoprotein A1-dependent pathway to form high-density lipoproteins (HDL) in the extracellular milieu, which then enter circulation<sup>[21]</sup>.

## CHOLESTEROL SYNTHESIS IN THE LIVER AND PERIPHERAL TISSUE

Cholesterol primarily enters blood circulation from two sources: From intestinal cholesterol absorption, and from the *de novo* cholesterol synthesis that is ubiquitous in all nucleated cells<sup>[1]</sup>. The majority of the body's endogenous cholesterol is produced by the liver<sup>[4]</sup>. Through a complex 37-step process, cholesterol is synthesized from simpler precursor molecules, starting with acetyl CoA<sup>[12]</sup>. The rate-limiting factors in the cholesterol synthesis pathway

include two enzymes: The target of statins 3-hydroxy-3-methyl-glutaryl CoA reductase and squalene mono-oxygenase, which oxidizes the precursor squalene to lanosterol<sup>[1,23]</sup> (Figure 1).

The membrane of the endoplasmic reticulum (ER) contains an intracellular feedback system-a tightly controlled protein network that modulates the transcription of genes that mediate cholesterol synthesis and uptake (Figure 2). Sterol regulatory element-binding protein isoform 2 (SREBP-2) is an ER membrane-bound transcription factor that activates genes encoding the enzymes required for cholesterol synthesis<sup>[1,6-8,24]</sup>. A key event in cholesterol synthesis is the gated movement of SREBP-2 from the ER to the Golgi complex. A crucial ER membrane component, the cleavage-activating protein (SCAP), acts as both an escort for SREBP-2 and a sterol sensor. Immediately after SREBP-2 synthesis in the ER, its COOH-terminal regulatory domain binds to the COOH-terminal domain of SCAP. When cells become cholesterol depleted, SCAP escorts SREBP-2 from the ER to the Golgi apparatus, where SREBP-2 is cleaved by two proteases and then trans-located to the nucleus, where it activates transcription of multiple target genes for cholesterol synthesis. Upon accumulation of excess cellular cholesterol, the SCAP-SREBP complex binds to the resident ER protein INSIG-2, remaining in the ER in a sterol-regulated manner and thereby blocking cholesterol synthesis<sup>[25]</sup> (Figure 2). Interactions between cholesterol, SCAP, and the SCAP-binding protein INSIG-2



**Figure 2** One key event in cholesterol homeostasis is the gated movement of sterol regulatory element-binding protein isoform 2 from the endoplasmic reticulum to the Golgi complex, involving the cleavage-activating protein, and INSIG-2. SREBP-2: Sterol regulatory element-binding protein isoform 2; ER: Endoplasmic reticulum; SCAP: Cleavage-activating protein.

create a sensitive switch that can respond to minor alterations of intracellular cholesterol levels, thus exerting precise control over the cholesterol composition of cell membranes.

In liver cells, free cholesterol can be excreted as neutral sterols into bile or transformed into bile acids, or it can be esterified and either stored in the liver as cholesterol esters or assembled into VLDL and secreted into circulation. The microsomal transfer protein assembles VLDL from cholesterol esters, triglycerides, phospholipids, free cholesterol, and apolipoprotein B-100 (apo B-100) as its structural protein. Triglycerides in VLDL are subsequently broken down by the enzymes lipoprotein lipase and hepatic lipase, producing intermediate-density lipoproteins (IDLs), followed by LDLs that transport cholesterol to peripheral tissues<sup>[21]</sup>.

## CHOLESTEROL ELIMINATION

Free cholesterol is toxic and mammalian somatic cells cannot catabolize it; thus, the removal of excess intracellular cholesterol by a distinct regulatory system is crucial (Figure 3). Liver X receptors (LXRs) act as whole-body cholesterol sensors. Under physiological conditions, cholesterol pool expansion and high intracellular cholesterol levels raise the intracellular concentration of oxygenated cholesterol metabolites termed oxysterols, which are important intermediate or end products in cholesterol excretion pathways. Oxysterols trigger liver-specific LXR activation, generating a transcriptional response that results in net elimination of cholesterol from the body *via* mobilization of cholesterol from peripheral tissues and promotion of hepatic excretion<sup>[4]</sup>. Quantitatively, the most important oxygenation reactions are those involved in the early steps of converting cholesterol into bile acids, a metabolically strictly controlled process. Cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) is the initial and rate-limiting enzyme of bile-acid synthesis<sup>[7,26]</sup>. CYP7A1 gene transcription is inhibited by the farnesoid X nuclear receptor, thereby producing negative feedback that reduces the bile acid synthesis from cholesterol. The farnesoid X receptor-agonist obeticholic acid is currently

under investigation for possible use in therapy for primary biliary cholangitis (PBC)<sup>[27]</sup>.

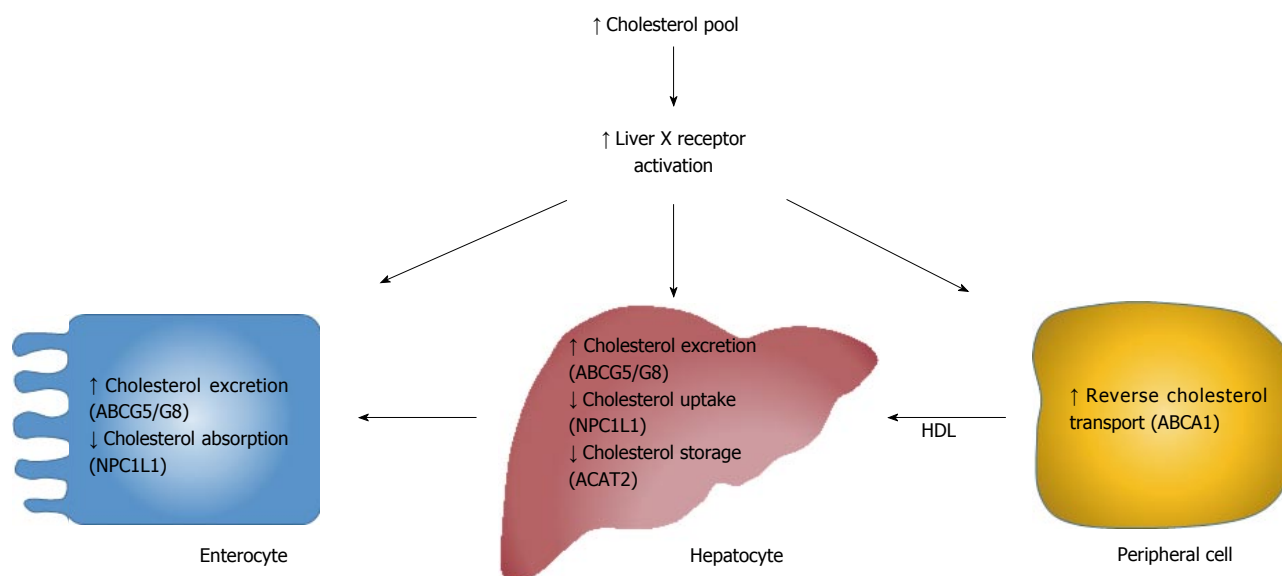
The most important target genes of LXRs include ABCG5/G8, NPC1L1, acetyl-CoA cholesterol acyltransferase 2 (ACAT-2), and ATP-binding cassette transporter sub-family member A 1 (ABCA1; also known as the cholesterol efflux regulatory protein)<sup>[4]</sup>. Activation of these genes can increase intestinal and hepatic cholesterol excretion (ABCG5/G8), reduce cholesterol absorption (NPC1L1), and reduce cholesterol storage (ACAT-2). ABCA1 is involved in reverse cholesterol transport, in which surplus free cholesterol from peripheral tissues is eliminated from the body *via* biliary excretion or through the non-biliary TICE pathway<sup>[4,5]</sup> (Figure 3).

HDL mediates the transfer of cholesterol from peripheral tissues to the liver. Nascent cholesterol-poor pre- $\beta$  HDL particles take up free cholesterol from peripheral tissues *via* ABCA1, after which this free cholesterol is esterified by lecithin-cholesterol acyltransferase. The esterified cholesterol is moved to the HDL particle's hydrophobic core, and progressive lipidation of the HDL particle causes it to mature, enlarge, and become more spherical. The cholesterol esters in mature HDL particles can be removed from circulation by hepatic scavenger receptor B1, or *via* transfer to apo B-100-containing lipoproteins (VLDL, IDL, and LDL) in a manner mediated by the cholesterol-ester transfer protein. By means of the LDL receptor and the LDL receptor-related protein, the liver can take up the apo B-100-containing lipoprotein particles from circulation<sup>[21]</sup>.

## DIFFICULTIES OF ASSESSING CHOLESTEROL METABOLISM IN CHOLESTASIS

Changes in cholesterol metabolism are not mirrored by routine serum cholesterol and lipoprotein measurements<sup>[28]</sup>. Moreover, the direct methods available to evaluate cholesterol metabolism are complex and laborious, and require labeling techniques, feces collection, and dietary recalls over several days.

In clinical research under steady state conditions, several non-cholesterol sterols that are measurable in serum can serve as valid biomarkers of cholesterol metabolism, especially when expressed as ratios to cholesterol<sup>[13,29-31]</sup>. Cholesterol precursor sterols, such as desmosterol and lathosterol, are markers of cholesterol synthesis<sup>[29]</sup>. On the other hand, diet-derived plant sterols (e.g., campesterol and sitosterol) and the liver-synthesized cholesterol metabolite cholestanol are markers of cholesterol absorption efficiency<sup>[30]</sup>. These markers have been investigated in PBC before and after LT<sup>[15,28,32-34]</sup>. Compared to that in healthy controls, intestinal cholesterol absorption is reportedly reduced by 2/3 in cases of prolonged severe intrahepatic cholestasis leading to cirrhosis and end-stage liver failure, as seen in PBC<sup>[35]</sup>. Cholestasis impairs the intestinal absorption of all types of sterols due to poor micellar formation secondary



**Figure 3 Cholesterol elimination- mechanisms and key transporters.** Expansion of the cholesterol pool activates liver X receptor, thereby generating transcriptional responses resulting in cholesterol excretion and catabolism. NPC1L1: Niemann-Pick C1 like 1; ABCA1: ATP-binding cassette transporter sub-family member A 1, also known as the cholesterol efflux regulatory protein; ACAT2: Acetyl-CoA acyltransferase 2; ABCG5: Adenosine triphosphate-binding cassette transporter G5 heterodimer; HDL: High density lipoprotein.

to reduced bile formation and excretion. However, striking increases of serum and hepatic plant sterol and cholestanol levels are also observed, indicating that the serum levels of plant sterols and cholestanol do not correctly mirror cholesterol absorption in cholestasis<sup>[15,28]</sup>. These changes can be used as biomarkers of the degree of cholestasis, with serum cholestanol/cholesterol being an even more sensitive marker of cholestasis among early-stage PBC patients than serum bilirubin<sup>[33]</sup>. Moreover, in end-stage cholestasis, serum cholestanol levels increase to levels that are otherwise only seen in the rare genetic disorder cerebrotendinous xanthomatosis<sup>[36]</sup>. This genetic disorder manifests with extremely high cholesterol deposits in tissues, including nerve tissues, resulting in severe neurologic symptoms<sup>[36]</sup>.

The liver is almost solely responsible for the secretion of sterols (*e.g.*, cholesterol, plant sterols, and cholestanol) from the human body *via* bile, which is regulated by hepatic proteins, including ABCG5/G8, NPC1L1, and LXRs<sup>[4-11]</sup>. ABCG5/G8 is active in cholesterol and sterol excretion across the canalicular membrane into bile. To our knowledge, no human studies have been performed to clarify how these sterol transporters function on the biliary canalicular level in intrahepatic cholestasis. Mutations in the genes of these transporters cause phytosterolemia, characterized by increased intestinal absorption and reduced biliary secretion of plant sterols, cholesterol, and cholestanol. Interestingly, Miettinen *et al.*<sup>[37]</sup> reported the case of a patient with phytosterolemia who presented with cholestatic liver disease necessitating LT. Following LT, the grossly elevated pre-transplant serum levels of plant sterols decreased to values only slightly above normal. This case highlights that the liver apparently plays a predominant role in maintaining sterol balance, since the intestinal ABCG5/G8 defect was not

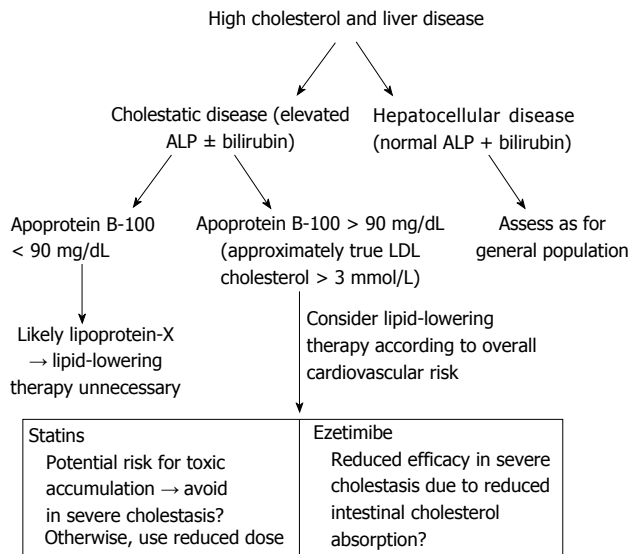
altered by LT<sup>[37]</sup>.

## LIPOPROTEIN X

Despite reduced cholesterol synthesis, high serum total and LDL cholesterol concentrations and even xanthomata are common features in PBC and other forms of cholestatic liver disease<sup>[35,38-47]</sup>. In PBC, serum total cholesterol varies widely, ranging from 2.9 to 46.1 mmol/L (112-1779 mg/dL), and even up to 83 mmol/L (3204 mg/dL)<sup>[43]</sup>.

High serum LDL cholesterol concentration is associated with atherosclerosis. Apo B-100 is present in all liver-derived atherogenic lipoproteins-including VLDL, IDL, LDL, and lipoprotein (a). However, in chronic cholestasis, LDL cholesterol measured using standard hospital laboratory methods is frequently elevated due to abnormal lipoprotein X (Lp-X), which is distinct from apo B-100-containing lipoproteins. Lp-X is characterized by a vesicular structure comprising a 30- to 70-nm lipid bilayer enclosing an aqueous compartment. Lp-X possesses strikingly high contents of unesterified cholesterol and phospholipids; low contents of cholesterol esters and triglycerides; small amounts of albumin and apolipoproteins C, E, and A-1; and no or a low concentration of apo B-100. Lp-X and LDL have the same density and are thus indistinguishable by standard lipoprotein ultracentrifugation. On the other hand, the physical size of Lp-X is in the range of VLDL or larger. Routine clinical laboratory methods currently used to measure LDL cholesterol are markedly affected by the presence of Lp-X, leading to false interpretations of elevated LDL cholesterol levels. Nuclear magnetic resonance spectroscopy measurements of lipoproteins reveal that Lp-X exist in PBC patients more commonly than currently recognized<sup>[48]</sup>. This phenomenon explains





**Figure 4** Algorithm for assessing hyperlipidemia in the setting of chronic liver disease. LDL: Low density lipoprotein; ALP: Alkaline phosphatase.

why high LDL cholesterol concentrations within the context of PBC, when actually caused by Lp-X, show no association with atherosclerotic events<sup>[38-41]</sup>.

Lp-X formation is typically associated with a low apo B-100 concentration together with a high total cholesterol concentration<sup>[44]</sup>. The usual target level of apo B-100 is below 90 mg/dL, corresponding to a true LDL cholesterol concentration of below 3.0 mmol/L (116 mg/dL)<sup>[49-51]</sup>. The ratio of apo B-100 to total cholesterol is normally around 1:2, but may be 1:10 in cases of severe Lp-X formation<sup>[45]</sup>. Since an elevated apo B-100 concentration is a risk factor for atherosclerosis<sup>[49-51]</sup>, apo B-100 concentrations should be measured when considering lipid-lowering treatment in PBC and other cholestatic conditions (Figure 4). Even when LDL cholesterol levels are high, cholesterol-lowering medication is unnecessary in cases where apo B-100 is below 90 mg/dL, since this suggests prevalence of non-atherogenic Lp-X. Lp-X resolves after successful cholestasis treatment<sup>[52]</sup>.

Importantly, most statins are excreted into bile and, thus, cholestatic liver disease may lead to toxic levels of drug accumulation<sup>[41]</sup>. Furthermore, in Lp-X-related hypercholesterolemia, statin therapy does not effectively lower cholesterol levels because Lp-X does not undergo LDL receptor-mediated hepatic clearance<sup>[43,48]</sup>. Therefore, statins must be used cautiously in cholestatic conditions. Moreover, cholesterol absorption is low in severe cholestasis due to poor micellar formation, potentially diminishing the effect of ezetimibe, which lowers cholesterol levels by decreasing intestinal cholesterol absorption. In severe cholestasis, a lipid phenotype suggesting high cardiovascular risk necessitates accurate evaluation with consultation of a lipidologist. An additional caveat is that elevated Lp-X may affect various laboratory tests - for instance, potentially leading to pseudohyponatremia<sup>[45]</sup>. Although hypercholesterolemia is well-acknowledged in

PBC, Lp-X formation is often neglected<sup>[53]</sup>.

## CHOLESTEROL AND LIVER REGENERATION

An ample cholesterol supply is critical for liver regeneration and for hepatocyte, stellate cell, and Kupffer cell function<sup>[54]</sup>. The importance of a circulating cholesterol supply for liver regeneration is exemplified following liver resection, where declining serum cholesterol coincides with intrahepatic cholesterol accumulation. In parallel, a serum total cholesterol concentration of below 2.8 mmol/L (108 mg/dL) in decompensated liver cirrhosis is associated with reduced transplant-free survival<sup>[55]</sup>. Additionally, among patients with non-cholestatic cirrhosis who underwent LT, a recipient serum total cholesterol level of below 1.8 mmol/L (69 mg/dL) at LT was associated with reduced post-LT graft outcome, independent of relevant donor, graft, and pre-operative recipient variables<sup>[56]</sup>. Both recipient cholesterol levels and the expressions of cholesterol metabolism genes in the liver graft could conceivably influence liver graft cholesterol availability and graft regeneration<sup>[56]</sup>.

## DONOR-DERIVED HYPERCHOLESTEROLEMIA

The LDL receptor is critical in mediating the catabolism of cholesterol-enriched particles and is abundant in the liver, with hepatocytes expressing up to 70%-80% of all LDL receptors in humans<sup>[57]</sup>. Pathogenic mutations in the LDL receptor gene cause familial hypercholesterolemia (FH) characterized by markedly elevated serum total and LDL cholesterol levels, tendon xanthomas, and early atherosclerosis. LT presents an effective therapy for homozygous FH.

On the other hand, we recently reported a case in which an LDL receptor mutation was unintentionally transmitted from a donor to an LT recipient, causing severe hypercholesterolemia in the recipient<sup>[58]</sup>. Prior to LT, the patient had hepatic epithelioid hemangioendothelioma without cirrhosis or cholestasis and exhibited no dyslipidemia. Following LT, the recipient's lipid levels were similar to those observed in FH, but her genomic DNA was normal in this regard. DNA was extracted from biopsy specimens of the liver allograft, and subjected to sequencing of the LDL receptor coding region, revealing a heterozygous splicing mutation in intron 9 that was previously reported as an FH-associated pathogenic mutation<sup>[58]</sup>. This finding essentially represents a transgenic model, consistent with previous evidence suggesting that most LDL cholesterol uptake in the body occurs in the liver and is mediated by LDL receptors. Since heterozygous FH is not extremely rare (prevalence 1/200 to 1/500<sup>[59]</sup>), our report raises concern of LT recipients acquiring unidentified FH from LT donors, especially

since FH manifestations are extrahepatic and thus easily overseen during donor evaluation<sup>[60]</sup>.

## POST-TRANSPLANT FOLLOW-UP

Hyperlipidemia reportedly occurs in 40%-66% of patients following LT<sup>[61]</sup>. Many mechanisms contribute to post-LT hypercholesterolemia and hypertriglyceridemia, including genetic susceptibility, diet, obesity, metabolic syndrome, diabetes, cholestatic problems, and immunosuppressive medication. The immunosuppressive drug cyclosporine induces hypercholesterolemia by inhibiting sterol 27-hydroxylase, a key enzyme in the bile synthesis pathway. Corticosteroids are usually tapered in the early post-LT period, and thus have minimal long-term influence on serum lipids<sup>[61]</sup>. Post-transplant cholestasis is also relatively common, and often secondary to anastomotic or non-anastomotic biliary stricturing<sup>[62]</sup>. Prolonged cholestasis may lead to Lp-X formation, but very few post-LT cases are reported<sup>[52,63,64]</sup>.

Compared to the general population, LT recipients more commonly experience cardiovascular events, especially LT recipients with metabolic syndrome and/or diabetes<sup>[65]</sup>. The overall lipoprotein profile in LT recipients is generally proatherogenic, but variation exists<sup>[66]</sup>, warranting an individualized detailed assessment of cardiovascular risk. Hepatic steatosis is considered a manifestation of metabolic syndrome and/or diabetes and is associated with a proatherogenic profile. Importantly, liver graft steatosis is increasingly detected. Thus, lipid profile assessment should include apo B-100 quantification in addition to the routine measurements of total, LDL, and HDL cholesterol, and total triglycerides. It is assumed that reducing intrahepatic lipids reduces the risks of hepatic and cardiovascular complications. Recent data suggest that treatment with a combination of dietary intervention, weight loss, and ezetimibe (which is well tolerated and can be combined with a statin) can reduce LDL cholesterol and apo B-100 concentrations in these patients<sup>[22,67-70]</sup>.

## CONCLUSION

Various liver disorders, particularly cholestasis, affect cholesterol metabolism and can cause variable hypercholesterolemia, including Lp-X appearance. Mistaking Lp-X for LDL cholesterol may interfere with cardiovascular risk assessment, leading to the prescription of futile lipid-lowering therapy. Lipid panel assessment should be regularly performed in all LT recipients, and at LT evaluation. Apo B-100 measurement can help in distinguishing between atherogenic and non-atherogenic hypercholesterolemia. Therefore, the measurement of apo B-100 can help in evaluating overall cardiovascular risk, as well as the effects of therapy during follow-up. This is particularly important after LT, when cholestasis and Lp-X may coexist with true atherogenic hypercholesterolemia and increased cardiovascular risk.

## ACKNOWLEDGMENTS

I recently changed my name from Katriina Nikkilä to Katriina Nemes. My previous publication history has been released by using the name Katriina Nikkilä (e.g., Nikkilä K) partly shown also in the actual references.

## REFERENCES

- 1 **Ikonen E.** Cellular cholesterol trafficking and compartmentalization. *Nat Rev Mol Cell Biol* 2008; **9**: 125-138 [PMID: 18216769 DOI: 10.1038/nrm2336]
- 2 **Maxfield FR, van Meer G.** Cholesterol, the central lipid of mammalian cells. *Curr Opin Cell Biol* 2010; **22**: 422-429 [PMID: 20627678 DOI: 10.1016/j.ceb.2010.05.004]
- 3 **Calvo PL, Brunati A, Spada M, Romagnoli R, Corso G, Parenti G, Rossi M, Baldi M, Carbonaro G, David E, Pucci A, Amoroso A, Salizzoni M.** Liver transplantation in defects of cholesterol biosynthesis: the case of lathosterolosis. *Am J Transplant* 2014; **14**: 960-965 [PMID: 24621408 DOI: 10.1111/ajt.12645]
- 4 **Bonamassa B, Moschetta A.** Arteriosclerosis: lessons from LXR and the intestine. *Trends Endocrinol Metab* 2013; **24**: 120-128 [PMID: 23158108 DOI: 10.1016/j.tem.2012.10.004]
- 5 **Degirrolamo C, Sabbà C, Moschetta A.** Intestinal nuclear receptors in HDL cholesterol metabolism. *J Lipid Res* 2015; **56**: 1262-1270 [PMID: 25070952 DOI: 10.1194/jlr.R052704]
- 6 **Horton JD, Goldstein JL, Brown MS.** SREBs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; **109**: 1125-1131 [PMID: 11994399 DOI: 10.1172/JCI15593]
- 7 **Weber LW, Boll M, Stampfl A.** Maintaining cholesterol homeostasis: Sterol regulatory element-binding proteins. *World J Gastroenterol* 2004; **10**: 3081-3087 [PMID: 15457548 DOI: 10.3748/wjg.v10.i21.3081]
- 8 **Eberlé D, Hegarty B, Bossard P, Ferré P, Foufelle F.** SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie* 2004; **86**: 839-848 [PMID: 15589694 DOI: 10.1016/j.biochi.2004.09.018]
- 9 **Moschetta A.** Nuclear receptors and cholesterol metabolism in the intestine. *Atheroscler Suppl* 2015; **17**: 9-11 [PMID: 25659870 DOI: 10.1016/S1567-5688(15)50003-2]
- 10 **Davis HR Jr, Altmann SW, Niemann-Pick C1 Like 1 (NPC1L1) an intestinal sterol transporter.** *Biochim Biophys Acta* 2009; **1791**: 679-683 [PMID: 19272334 DOI: 10.1016/j.bbalip.2009.01.002]
- 11 **Betters JL, Yu L.** NPC1L1 and cholesterol transport. *FEBS Lett* 2010; **584**: 2740-2747 [PIMD: 20307540 DOI: 10.1016/j.febslet.2010.03.030]
- 12 **Goldstein JL, Brown MS.** Regulation of the mevalonate pathway. *Nature* 1990; **343**: 425-430 [PMID: 1967820 DOI: 10.1038/343425a0]
- 13 **Gylling H.** Clinical utility of serum markers of cholesterol absorption and synthesis. *Curr Opin Lipidol* 2014; **25**: 207-212 [PMID: 24811297 DOI: 10.1097/MOL.0000000000000069]
- 14 **Turner S, Voogt J, Davidson M, Glass A, Killion S, Decaris J, Mohammed H, Minehira K, Boban D, Murphy E, Luchoomun J, Awada M, Neese R, Hellerstein M.** Measurement of reverse cholesterol transport pathways in humans: in vivo rates of free cholesterol efflux, esterification, and excretion. *J Am Heart Assoc* 2012; **1**: e001826 [PMID: 23130164 DOI: 10.1161/JAHA.112.001826]
- 15 **Nikkilä K, Höckerstedt K, Miettinen TA.** High cholestanol and low campesterol-to-sitosterol ratio in serum of patients with primary biliary cirrhosis before liver transplantation. *Hepatology* 1991; **13**: 663-669 [PMID: 2010161 DOI: 10.1002/hep.1840130409]
- 16 **Arguello G, Balboa E, Arrese M, Zanlungo S.** Recent insights on the role of cholesterol in non-alcoholic fatty liver disease. *Biochim Biophys Acta* 2015; **1852**: 1765-1778 [PMID: 26027904 DOI: 10.1016/j.bbdis.2015.05.015]
- 17 **Sudhop T, Lütjohann D, von Bergmann K.** Sterol transporters:

- target of natural sterols and new lipid lowering drugs. *Pharmacol Ther* 2005; **105**: 333-341 [PMID: 15737409 DOI: 10.1016/j.pharmthera.2004.10.011]
- 18 **Hui DY**, Labonté ED, Howles PN. Development and physiological regulation of intestinal lipid absorption. III. Intestinal transporters and cholesterol absorption. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G839-G843 [PMID: 18276831 DOI: 10.1152/ajpgi.00061.2008]
  - 19 **Phan BA**, Dayspring TD, Toth PP. Ezetimibe therapy: mechanism of action and clinical update. *Vasc Health Risk Manag* 2012; **8**: 415-427 [PMID: 22910633 DOI: 10.2147/VHRM.S33664]
  - 20 **Ostlund RE Jr**, McGill JB, Zeng CM, Covey DF, Stearns J, Stenson WF, Spilburg CA. Gastrointestinal absorption and plasma kinetics of soy Delta(5)-phytosterols in humans. *Am J Physiol Endocrinol Metabol* 2002; **282**: E911-E 916 [PMID: 11882512 DOI: 10.1152/ajpendo.00328.2001]
  - 21 **Hussain MM**. Intestinal lipid absorption and lipoprotein formation. *Curr Opin Lipidol* 2014; **25**: 200-206 [PMID: 24751933 DOI: 10.1097/MOL.0000000000000084]
  - 22 **Arca M**. Alterations of intestinal lipoprotein metabolism in diabetes mellitus and metabolic syndrome. *Atheroscler Supp* 2015; **17**: 12-16 [PMID: 25659871 DOI: 10.1016/S1567-5688(15)50004-4]
  - 23 **Buhaescu I**, Izzedine H. Mevalonate pathway: a review of clinical and therapeutical implications. *Clin Biochem* 2007; **40**: 575-584 [PMID: 17467679 DOI: 10.1016/j.clinbiochem.2007.03.016]
  - 24 **Radhakrishnan A**, Goldstein JL, McDonald JG, Brown MS. Switch-like control of SREBP-2 transport triggered by small changes in ER cholesterol: a delicate balance. *Cell Metab* 2008; **8**: 512-521 [PMID: 19041766 DOI: 10.1016/j.cmet.2008.10.008]
  - 25 **Yabe D**, Brown MS, Goldstein JL. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. *Proc Natl Acad Sci USA* 2002; **99**: 12753-12758 [PMID: 12242332 DOI: 10.1073/pnas.162488899]
  - 26 **Repa JJ**, Berge KE, Pomajzl C, Richardson JA, Hobbs H, Mangelsdorf DJ. Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by liver X receptors  $\alpha$  and  $\beta$ . *J Biol Chem* 2002; **277**: 18793-18800 [PMID: 11901146 DOI: 10.1074/jbc.M109927200]
  - 27 **Corpechot C**. Primary biliary cirrhosis beyond ursodeoxycholic acid. *Semin Liver Dis* 2016; **36**: 15-26 [PMID: 26870929 DOI: 10.1055/s-0035-1571273]
  - 28 **Nikkilä K**, Höckerstedt K, Miettinen TA. Serum and hepatic cholestanol, squalene and noncholesterol sterols in man: a study on liver transplantation. *Hepatology* 1992; **15**: 863-870 [PMID: 1568728 DOI: 10.1002/hep.1840150519]
  - 29 **Miettinen TA**, Tilvis RS, Kesäniemi YA. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am J Epidemiol* 1990; **131**: 20-31 [PMID: 2293749]
  - 30 **Miettinen TA**, Tilvis RS, Kesäniemi YA. Serum cholestanol and plant sterol levels in relation to cholesterol metabolism in middle-aged men. *Metabolism* 1989; **38**: 136-140 [PMID: 2913464 DOI: 10.1016/0026-0495(89)90252-7]
  - 31 **Miettinen TA**, Gylling H, Nissinen MJ. The role of serum non-cholesterol sterols as surrogate markers of absolute cholesterol synthesis and absorption. *Nutr Metab Cardiovasc Dis* 2011; **21**: 765-769 [PMID: 21899991 DOI: 10.1016/j.numecd.2011.05.005]
  - 32 **Nikkilä K**, Miettinen TA, Höckerstedt KV, Isoniemi H. Sterol parameters as markers of liver function in primary biliary cirrhosis before and after liver transplantation. *Transpl Int* 2005; **18**: 221-225 [PMID: 15691276 DOI: 10.1111/j.1432-2277.2004.00002.x]
  - 33 **Nikkilä K**, Nissinen MJ, Gylling H, Isoniemi H, Miettinen TA. Serum sterols in patients with primary biliary cirrhosis and acute liver failure before and after liver transplantation. *J Hepatol* 2008; **49**: 936-945 [PMID: 18926587 DOI: 10.1016/j.jhep.2008.07.026]
  - 34 **Nikkilä K**, Höckerstedt K, Miettinen TA. Liver transplantation modifies serum cholestanol, cholesterol precursor and plant sterol levels. *Clin Chim Acta* 1992; **208**: 205-218 [PMID: 1499139]
  - 35 **Gylling H**, Färkkilä M, Vuoristo M, Miettinen TA. Metabolism of cholesterol and low- and high-density lipoproteins in primary biliary cirrhosis: cholesterol absorption and synthesis related to lipoproteins levels and their kinetics. *Hepatology* 1995; **21**: 89-95 [PMID: 7806174]
  - 36 **Bhattacharyya AK**, Lin DS, Connor WE. Cholestanol metabolism in patients with cerebrotendinous xanthomatosis: absorption, turnover, and tissue deposition. *J Lipid Res* 2007; **48**: 185-192 [PMID: 17012751 DOI: 10.1194/jlr.M600113-JLR200]
  - 37 **Miettinen TA**, Klett EL, Gylling H, Isoniemi H, Patel SB. Liver transplantation in a patient with sitosterolemia and cirrhosis. *Gastroenterology* 2006; **130**: 542-547 [PMID: 16472606 DOI: 10.1053/j.gastro.2005.10.022]
  - 38 **Crippin JS**, Lindor KD, Jorgensen R, Kottke BA, Harrison JM, Murtaugh PA, Dickson ER. Hypercholesterolemia and atherosclerosis in primary biliary cirrhosis: what is the risk? *Hepatology* 1992; **15**: 858-862 [PMID: 1568727 DOI: 10.1002/hep.1840150518]
  - 39 **Longo M**, Crosignani A, Battezzati PM, Squarcia Giussani C, Iernizzi P, Zuin M, Podda M. Hyperlipidaemic state and cardiovascular risk in primary biliary cirrhosis. *Gut* 2002; **51**: 265-269 [PMID: 12117892 DOI: 10.1136/gut.51.2.265]
  - 40 **Chang PY**, Lu SC, Su TC, Chou SF, Huang WH, Morrisett JD, Chen CH, Liau CS, Lee YT. Lipoprotein-X reduces LDL atherogenicity in primary biliary cirrhosis by preventing LDL oxidation. *J Lipid Res* 2004; **45**: 2116-2122 [PMID: 15314101 DOI: 10.1194/jlr.M400229-JLR200]
  - 41 **Sorokin A**, Brown JL, Thompson PD. Primary biliary cirrhosis, hyperlipidemia, and atherosclerotic risk: a systematic review. *Atherosclerosis* 2007; **194**: 293-299 [PMID: 17240380 DOI: 10.1016/j.atherosclerosis.2006.11.036]
  - 42 **Citkowitz E**. Was the low-density lipoprotein cholesterol level really elevated? *Liver Transpl* 2011; **17**: 1234, author reply 1235 [PMID: 21506250 DOI: 10.1002/lt.22317]
  - 43 **Wong ML**, Raghavan RP, Hedger NA, Ellis RD, Meeking DR, Albon L. The use of plasmapheresis in managing primary biliary cirrhosis presenting with profound hypercholesterolaemia. *The British Journal of Diabetes and Vascular Diseases* 2012; **12**: 156-158 [DOI: 10.1177/1474651412442410]
  - 44 **Ooi YK**, Mietus-Snyder M, Torres C, Mohan P, Harahsheh A. Lipoprotein-X-accumulation: a mimic of familial hypercholesterolemia. *Consult Pediatr* 2013; **12**: 63-65
  - 45 **Hussain I**, Ahmad Z, Garg A. Extreme hypercholesterolemia presenting with pseudohyponatremia - a case report and review of the literature. *J Clin Lipidol* 2015; **9**: 260-264 [PMID: 25911084 DOI: 10.1016/j.jacl.2014.11.007]
  - 46 **Joukhadar R**, Chiu K. Severe hypercholesterolemia in patients with graft-vs-host disease affecting the liver after stem cell transplantation. *Endocr Pract* 2012; **18**: 90-97 [PMID: 21940276 DOI: 10.4158/EP11212.RA]
  - 47 **Chow A**, Rifci VA, Schneider SH. Lipoprotein-X in a patient with lymphoplasmacytic sclerosing cholangitis: an unusual cause of secondary hypercholesterolemia. *AACE Clinical Case Reports* 2016; **2**: e20- e 24 [DOI: 10.4158/EP14249.CR]
  - 48 **Foley KF**, Silveira MG, Hornseth JM, Lindor KD, McConnell JP. A patient with primary biliary cirrhosis and elevated LDL cholesterol. *Clin Chem* 2009; **55**: 187-191, discussion 191-192 [PMID: 19106186 DOI: 10.1373/clinchem.2008.108720]
  - 49 **Assessment by the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices**. Cole TG, Contois JH, Csako G, McConnell JP, Remaley AT, Devaraj S, Hoefner DM, Mallory T, Sethi AA, Warnick GR. Association of apolipoprotein B and nuclear magnetic resonance spectroscopy-derived LDL particle number with outcomes in 25 clinical studies. *Clin Chem* 2013; **59**: 752-770 [PMID: 23386699 DOI: 10.1373/clinchem.2012.196733]
  - 50 **Sniderman AD**, Williams K, Contois JH, Monroe HM, McQueen MJ, de Graaf J, Furberg CD. A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. *Circ Cardiovasc Qual Outcomes* 2011; **4**: 337-345 [PMID: 21487090 DOI: 10.1161/CIRCOUTCOMES.110.959247]

- 51 **Varvel SA**, Dayspring TD, Edmons Y, Thiselton DL, Ghaedi L, Voros S, McConnel JP, Sasinowski M, Dall T, Warnick GR. Discordance between apolipoprotein B and low-density lipoprotein particle number is associated with insulin resistance in clinical practice. *J Clin Lipidol* 2015; **9**: 247-255 [PMID: 25911082 DOI: 10.1016/j.jacl.2014.11.005]
- 52 **Jankowski K**, Wyzgal A, Wierzbicka A, Tronina O, Durlak M, Pruszczyk P. Rapid normalization of severe hypercholesterolemia mediated by lipoprotein X after liver transplantation in a patient with cholestasis - a case report. *Acta Biochim Pol* 2015; **62**: 621-623 [PMID: 26317127 DOI: 10.18388/abp.2015.971]
- 53 **Purohit T**, Cappell MS. Primary biliary cirrhosis: Pathophysiology, clinical presentation and therapy. *World J Hepatol* 2015; **7**: 926-941 [PMID: 25954476 DOI: 10.4254/wjh.v7.i7.926]
- 54 **Delgado-Coello B**, Briones-Orta MA, Macias-Silva M, Mas-Oliva J. Cholesterol: recapitulation of its active role during liver regeneration. *Liver Int* 2011; **31**: 1271-1284 [PMID: 21745289 DOI: 10.1111/j.1478-3231.2011.02542.x]
- 55 **Jiang M**, Liu F, Xiong WJ, Zhong L, Xu W, Xu F, Liu YB. Combined MELD and blood lipid level in evaluating the prognosis of decompensated cirrhosis. *World J Gastroenterol* 2010; **16**: 1397-1401 [PMID: 20238407 DOI: 10.3748/wjg.v16.i11.1397]
- 56 **Ginanni Corradini S**, Siciliano M, Parlanti L, Molinaro A, Cantafora A, Poli E, Mennini G, Melandro F, Vestri AR, Merli M, Bianco P, Corsi A, Toniutto P, Bitetto D, Falletti E, Attili AF, Berloco P, Rossi M. Recipient perioperative cholesterolaemia and graft cholesterol metabolism gene expression predict liver transplant outcome. *Liver Int* 2014; **34**: e290-e301 [PMID: 24256518 DOI: 10.1111/liv.12351]
- 57 **Goldstein JL**, Brown MS. The LDL receptor. *Arterioscler Thromb Vasc Biol* 2009; **29**: 431-438 [PMID: 19299327 DOI: 10.1161/ATVBAHA.108.179564]
- 58 **Nikkilä K**, Åberg F, Isoniemi H. Transmission of LDLR mutation from donor through liver transplantation resulting in hypercholesterolemia in the recipient. *Am J Transplant* 2014; **14**: 2898-2902 [PMID: 25231171 DOI: 10.1111/ajt.12961]
- 59 **Nordestgaard BG**, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, Wiklund O, Hegele RA, Raal FJ, Defesche JC, Wiegman A, Santos RD, Watts GF, Parhofer KG, Hovingh GK, Kovanen PT, Boileau C, Averna M, Borén J, Bruckert E, Catapano AL, Kuivenhoven JA, Pajukanta P, Ray K, Stalenhoef AF, Stroes E, Taskinen MR, Tybjaerg-Hansen A; European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J* 2013; **34**: 3478-3490a [PMID: 23956253 DOI: 10.1093/eurheartj.eht273]
- 60 **Najam O**, Ray KK. Familial hypercholesterolemia: a review of the natural history, diagnosis, and management. *Cardiol Ther* 2015; **4**: 25-38 [PMID: 25769531 DOI: 10.1007/s40119-015-0037-z]
- 61 **Sethi A**, Stravitz RT. Review article: medical management of the liver transplant recipient - a primer for non-transplant doctors. *Aliment Pharmacol Ther* 2007; **25**: 229-245 [PMID: 17217455 DOI: 10.1111/j.1365-2036.2006.03166.x]
- 62 **Ben-Ari Z**, Pappo O, Mor E. Intrahepatic cholestasis after liver transplantation. *Liver Transpl* 2003; **9**: 1005-1018 [PMID: 14526393 DOI: 10.1053/jlts.2003.50212]
- 63 **Tejera P**, Karalis D, Xiao G, Simon B, Amori R. Severe hypercholesterolemia and cholestatic liver disease after liver transplant: a case of lipoprotein X. The ICE/ENDO 2014 International Congress of Endocrinology and the 96<sup>th</sup> annual meeting of Endocrine Society in Chicago, June 21-24, 2014 Chicago, SUN-0851
- 64 **Yeh H**, Kitchens WH, Elias N, Kelsey PB, Markmann JF, Hertl M. Hyperlipidemia due to biliary stricture after living-donor liver transplantation. *Transplantation* 2011; **92**: e29-e30 [PMID: 21909017 DOI: 10.1097/TP.0b013e31822d095d]
- 65 **Madhwal S**, Atreja A, Albeldawi M, Lopez R, Post A, Costa MA. Is liver transplantation a risk for cardiovascular disease? A meta-analysis of observational studies. *Liver Transpl* 2012; **18**: 1140-1146 [PMID: 22821899 DOI: 10.1002/lt.23508]
- 66 **Chhatrala R**, Siddiqui MB, Stravitz RT, Driscoll C, Sanyal A, Sargeant C, Luketic V, Sharma A, Sterling R, Matherly S, Puri P, Siddiqui MS. Evolution of serum atherogenic risk in liver transplant recipients: Role of lipoproteins and metabolic and inflammatory markers. *Liver Transpl* 2015; **21**: 623-630 [PMID: 25762084 DOI: 10.1002/lt.24100]
- 67 **Chan DC**, Watts GF, Gan SK, Ooi EM, Barrett PH. Effect of ezetimibe on hepatic fat, inflammatory markers, and apolipoprotein B-100 kinetics in insulin-resistant obese subjects on a weight loss diet. *Diabetes Care* 2010; **33**: 1134-1139 [PMID: 20185740 DOI: 10.2337/dc09-1765]
- 68 **Åberg F**, Koljonen V, Nikkilä K, Boyd S, Arola J, Isoniemi H. Thiazolidinedione therapy versus lifestyle recommendation in the treatment of post-liver transplant graft steatosis. *Ann Transplant* 2014; **19**: 389-396 [PMID: 25105443 DOI: 10.12659/AOT.890664]
- 69 **Farnier M**. Ezetimibe/statin combination therapy to treat patients with type 2 diabetes. *Atheroscler Suppl* 2015; **17**: 2-8 [PMID: 25659869 DOI: 10.1016/S1567-5688(15)50002-0]
- 70 **Averna M**. The effect of ezetimibe on NALFD. *Atheroscler Suppl* 2015; **17**: 27-34 [PMID: 25659874 DOI: 10.1016/S1567-5688(15)50007-X]

**P- Reviewer:** Stieger B, Tiao MM, Zhao YL

**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Li D





## Basic Study

# Antifibrotic effects of ambrisentan, an endothelin-A receptor antagonist, in a non-alcoholic steatohepatitis mouse model

Toshiaki Okamoto, Masahiko Koda, Kennichi Miyoshi, Takumi Onoyama, Manabu Kishina, Tomomitsu Matono, Takaaki Sugihara, Keiko Hosho, Junichi Okano, Hajime Isomoto, Yoshikazu Murawaki

Toshiaki Okamoto, Masahiko Koda, Kennichi Miyoshi, Takumi Onoyama, Manabu Kishina, Tomomitsu Matono, Takaaki Sugihara, Keiko Hosho, Junichi Okano, Hajime Isomoto, Yoshikazu Murawaki, Division of Medicine and Clinical Science, Department of Multidisciplinary Internal Medicine, Tottori University, Yonago, Tottori 683-8504, Japan

Masahiko Koda, Second Department of Internal Medicine, Tottori University, Yonago, Tottori 683-8504, Japan

**Author contributions:** Okamoto T and Koda M contributed equally to this work; Koda M designed the research; Okamoto T, Koda M, Miyoshi K, Onoyama T, Kishina M, Matono T, Sugihara T, Hosho K and Okano J performed the research; Okamoto T and Koda M analyzed the data; Okamoto T, Koda M, Isomoto H and Murawaki Y wrote the paper.

**Institutional review board statement:** All experiments were performed in accordance with the Animal Experimentation Guidelines of Tottori University (Yonago, Japan). The study was reviewed and approved by the ethics committee of Tottori University. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Tottori University (approval number; 14-Y-8).

**Conflict-of-interest statement:** All authors declare that they have no conflict of interest. All authors certify that this article is not under consideration for publication elsewhere. Publication is approved by all authors and by the responsible authorities where the work was carried out.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

[licenses/by-nc/4.0/](http://creativecommons.org/licenses/by-nc/4.0/)

Manuscript source: Invited manuscript

Correspondence to: Masahiko Koda, MD, PhD, Associate Professor, Second Department of Internal Medicine, Tottori University, Nishi-cho 36-1, Yonago, Tottori 683-8504, Japan. [masakoda@grape.med.tottori-u.ac.jp](mailto:masakoda@grape.med.tottori-u.ac.jp)  
Telephone: +81-859-386527  
Fax: +81-859-386529

Received: February 24, 2016  
Peer-review started: March 4, 2016  
First decision: April 15, 2016  
Revised: June 22, 2016  
Accepted: July 11, 2016  
Article in press: July 13, 2016  
Published online: August 8, 2016

## Abstract

**AIM:** To examine the effects of the endothelin type A receptor antagonist ambrisentan on hepatic steatosis and fibrosis in a steatohepatitis mouse model.

**METHODS:** Fatty liver shionogi (FLS) FLS-*ob/ob* mice (male, 12 wk old) received ambrisentan (2.5 mg/kg orally per day;  $n = 8$ ) or water as a control ( $n = 5$ ) for 4 wk. Factors were compared between the two groups, including steatosis, fibrosis, inflammation, and endothelin-related gene expression in the liver.

**RESULTS:** In the ambrisentan group, hepatic hydroxyproline content was significantly lower than in the control group ( $18.0 \mu\text{g/g} \pm 6.1 \mu\text{g/g}$  vs  $33.9 \mu\text{g/g} \pm 13.5 \mu\text{g/g}$  liver, respectively,  $P = 0.014$ ). Hepatic fibrosis estimated by Sirius red staining and areas positive

for  $\alpha$ -smooth muscle actin, indicative of activated hepatic stellate cells, were also significantly lower in the ambrisentan group ( $0.46\% \pm 0.18\%$  vs  $1.11\% \pm 0.28\%$ , respectively,  $P = 0.0003$ ; and  $0.12\% \pm 0.08\%$  vs  $0.25\% \pm 0.11\%$ , respectively,  $P = 0.047$ ). Moreover, hepatic RNA expression levels of procollagen-1 and tissue inhibitor of metalloproteinase-1 (TIMP-1) were significantly lower by 60% and 45%, respectively, in the ambrisentan group. Inflammation, steatosis, and endothelin-related mRNA expression in the liver were not significantly different between the groups.

**CONCLUSION:** Ambrisentan attenuated the progression of hepatic fibrosis by inhibiting hepatic stellate cell activation and reducing procollagen-1 and *TIMP-1* gene expression. Ambrisentan did not affect inflammation or steatosis.

**Key words:** Endothelin; Ambrisentan; Steatohepatitis; Hepatic stellate cell; Hepatic fibrosis; Oxidative stress; Hepatic hydroxyproline

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Endothelin (ET) can activate hepatic stellate cells, leading to the progression of hepatic fibrosis. Furthermore, ET-1 may increase the inflow of free fatty acids from the fat tissue into the liver and exacerbate hepatic steatosis. Therefore, ET-1 antagonism may be a novel target for steatohepatitis. The present study showed that ambrisentan, an ET type A receptor antagonist, attenuated hepatic fibrosis by inhibiting hepatic stellate cell activation, without affecting hepatic steatosis, in a non-alcoholic steatohepatitis mouse model.

Okamoto T, Koda M, Miyoshi K, Onoyama T, Kishina M, Matono T, Sugihara T, Hosho K, Okano J, Isomoto H, Murawaki Y. Antifibrotic effects of ambrisentan, an endothelin-A receptor antagonist, in a non-alcoholic steatohepatitis mouse model. *World J Hepatol* 2016; 8(22): 933-941 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i22/933.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i22.933>

## INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is characterized by hepatic fat deposition, inflammation, and differing degrees of fibrosis<sup>[1]</sup>. In the pathophysiology of NASH, the deposition of fat in liver cells, which occurs in association with obesity and insulin resistance, is a benign process in most patients but is followed by inflammation and fibrosis in the liver in response to multiple insults, such as oxidative stress and various adipokines or cytokines acting in parallel<sup>[2]</sup>. In NASH, the serum endothelin-1 (ET-1) level is elevated and is correlated with hepatic fibrosis severity<sup>[3]</sup>. The development of hepatic fibrosis is mediated to a large extent by the activation of hepatic

stellate cells (HSCs). ET-1 is released from sinusoidal endothelial cells and HSCs, which serves to activate the HSCs and accelerate collagen fiber synthesis in them<sup>[4]</sup>. Furthermore, ET-1 acts as a mediator and is elevated in conditions such as insulin resistance, hyperglycemia, oxidative stress, and endothelial cell dysfunction<sup>[5,6]</sup>. ET-1 also increases vascular superoxide production and promotes cell proliferation by inducing reactive oxygen species<sup>[7]</sup>.

Ambrisentan is a selective ET type A receptor (ETAR) antagonist approved for the treatment of patients with pulmonary arterial hypertension<sup>[8]</sup>. ETAR antagonists improve liver fibrosis in cirrhotic rats<sup>[9]</sup>, but their effects on NASH are unknown. Fatty liver shionogi (FLS)-*ob/ob* mice are characterized by hyperphagia, obesity, hyperlipidemia, and diabetes mellitus<sup>[10]</sup>. As described in our previous study using these mice<sup>[11]</sup>, FLS-*ob/ob* mice are generated by transferring the *Lep<sup>ob</sup>* gene into the FLS mouse genome, causing FLS mice to spontaneously develop chronic hepatic steatosis but not obesity. The resultant FLS-*ob/ob* mice show severe steatosis, hepatocellular ballooning, and advanced hepatic fibrosis histologically. They also display increased oxidative stress, elevated production of inflammatory and profibrotic cytokines, and increased apoptosis of hepatocytes, and eventually develop cirrhosis and liver tumors<sup>[12,13]</sup>. For these reasons, FLS-*ob/ob* are considered to be animal model the most closely represents human metabolic syndrome-related NASH. Against this background, this study investigated the therapeutic effects of ambrisentan on hepatic steatosis and fibrosis in NASH using FLS-*ob/ob* male mice.

## MATERIALS AND METHODS

### Animals

A total of 13 male FLS-*ob/ob* mice (age, 8 wk; body weight,  $42.88 \pm 1.74$  g) were obtained from Shionogi Research Laboratories (Shiga, Japan) and housed in a controlled environment ( $24^\circ\text{C} \pm 2^\circ\text{C}$ ; 12:12-h light:Dark cycle). Mice were provided *ad libitum* water and a standard powdered diet (CE-2, 4.6% fat; CLEA Japan, Tokyo, Japan). To maintain dietary intake in both groups at an equal level, food consumption and body weight were monitored throughout observation. All experiments were performed in accordance with the Animal Experimentation Guidelines of Tottori University (Yonago, Japan). The study was reviewed and approved by the ethics committee of Tottori University. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Tottori University (approval number, 14-Y-8) and the animal protocol was designed to minimize pain and discomfort to the animals.

### Administration of ambrisentan

At the age of 12 wk, male FLS-*ob/ob* mice were randomly assigned to the ambrisentan ( $n = 8$ ) or control ( $n = 5$ ) group. Intragastric gavage administration was carried out in conscious animals with an appropriately

sized gastric tube. Ambrisentan (2.5 mg/kg per day; ADooQ BioScience, Irvine, CA) was orally administered every afternoon for 4 wk as a bolus through a gastric tube. Water was administered to the control group. At week 4, animals were fasted for 4 h and tail vein blood was drawn and subjected to blood glucose determination. Animals were killed by pentobarbital anesthesia injection (Dainippon Sumitomo Pharma, Osaka, Japan) after 4 wk and blood was collected from the right ventricle. Plasma samples were frozen and stored at -80 °C. Liver and visceral fat were then weighed, snap-frozen in liquid nitrogen, and stored at -80 °C. Additional liver specimens were fixed in 10% buffered formalin (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and embedded in paraffin (Wako Pure Chemical Industries, Ltd.) for histological analysis.

#### **Analysis of hepatic cholesterol and triglycerides**

Snap-frozen liver samples (50 mg) were homogenized and extracted using chloroform-methanol (2:1 v/v; Wako Pure Chemical Industries, Ltd.). The organic phase was then dried and resuspended in 2-propanol containing 10% Triton X-100. Total cholesterol and triglyceride contents were measured with the Cholesterol E-test (Wako Pure Chemical Industries, Ltd.) and Triglyceride E-test (Wako Pure Chemical Industries, Ltd.), respectively.

#### **Biochemical analysis**

Blood samples were immediately separated by centrifugation at 2000 *g* for 15 min at 4 °C and stored at -80 °C until further use. Serum samples were analyzed to determine the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

#### **Measurement of hepatic hydroxyproline content**

Hepatic tissue (400 mg wet weight) was hydrolyzed in 4 mL of 6 mol/L HCl at 105 °C overnight. The hydrolysate was then thoroughly evaporated under vacuum. The sediment was resuspended in distilled water, decolorized with activated charcoal, and filtered; the filtrate was then acidified to pH 5.0 and evaporated under vacuum. The sediment was resuspended in distilled water, mixed with 2 mL of isopropanol, and then incubated with 1 mL of 7% chloramine-T for 5 min at room temperature. After addition of Ehrlich's solution (2 mL; 1.76 g p-dimethylaminobenzaldehyde dissolved in 4.08 mL 60% perchloric acid and 95.5 mL of isopropanol), the mixture was incubated at 60 °C for 10 min. The absorbance of the cooled mixture was measured at 562 nm.

#### **Measurement of hepatic fibrosis area**

As in our previous study<sup>[11]</sup>, formalin-fixed, paraffin-embedded liver sections (4- $\mu$ m-thick) were stained with picrosirius red (Chroma-Gesellschaft Schmid GmbH and Co., Munster, Germany) and counterstained with fast green (Chroma-Gesellschaft Schmid GmbH and Co.). The areas of hepatic fibrosis were subsequently measured in 10 randomly selected fields in each specimen (magnification,  $\times$  400) using WinROOF ver.5.71 software and the Olympus BX51N-34 microscope.

fication,  $\times$  400) using WinROOF ver.5.71 software and the Olympus BX51N-34 microscope.

#### **Measurement of hepatic steatosis area**

Following the staining of neutral lipids in frozen-fixed, cryostat-embedded liver sections (4-mm-thick) with oil red O (Sigma-Aldrich, St. Louis, MO), areas of hepatic steatosis were measured using WinROOF version 5.71 software (Mitani Corporation, Tokyo, Japan) in 10 randomly selected fields (magnification,  $\times$  400; Olympus BX51N-34; Olympus Corporation, Tokyo, Japan) per specimen<sup>[11]</sup>.

#### **Immunostaining for $\alpha$ -smooth muscle actin**

Immunostaining for  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) was used for the detection and counting of activated HSCs. As described previously<sup>[11]</sup>,  $\alpha$ -SMA was detected by staining with mouse monoclonal anti- $\alpha$ -SMA antibody (cat. No. MS-113-R7; Thermo Fisher Scientific, Fremont, CA) without dilution. Goat anti-mouse Ig from the Histofine Mouse Stain kit (cat. No. 414322; Nichirei Biosciences, Inc., Tokyo, Japan) was used without dilution as the secondary antibody. HSCs activation was assessed by using WinROOF ver.5.71 software to measure the areas of  $\alpha$ -SMA staining in 10 randomly selected fields (magnification  $\times$  400; Olympus BX51N-34) per specimen.

#### **Analysis of inflammatory cell infiltration of hepatic tissue**

F4/80, a mature mouse cell surface glycoprotein expressed at high levels on Kupffer cells, was immunohistochemically stained using a rat monoclonal anti-mouse F4/80 antibody (cat. No. ab6640; Abcam, Tokyo, Japan) diluted to 1:100 with 0.01 mol/L PBS according to the manufacturer's instructions. Goat anti-rat secondary antibody from the Histofine Simple Stain Mouse MAX-PO (Rat) kit (cat. No. 414311; Nichirei Biosciences, Inc.) was used without dilution. Immunopositive cells were analyzed in 10 intralobular ocular fields (magnification,  $\times$  400; Olympus BX41N-34) per specimen<sup>[11]</sup>.

#### **Analysis of oxidative stress**

Immunohistochemical staining for 8-hydroxy-2-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, was used to assess oxidative stress<sup>[11]</sup>. A monoclonal mouse anti-8-OHdG antibody (cat. No. MOG-020P; Nikken SEIL, Shizuoka, Japan) diluted in 200  $\mu$ L distilled water was used, following the manufacturer's instructions. Goat anti-mouse Ig from the Histofine Mouse Stain kit served as the secondary antibody without dilution. WinROOF ver.5.71 software was used to analyze immunopositive cells using 10 intralobular ocular fields (magnification  $\times$  400; Olympus BX41N-34) per specimen, and values are expressed as the ratios (%) of fields. Also, 4-hydroxynonenal (4-HNE) was semi-quantified *via* immunohistochemical staining using a monoclonal mouse anti-4-HNE antibody (cat. no. MHN-020P; Nikken SEIL) diluted in 200  $\mu$ L distilled water following the

manufacturer's instructions. Goat anti-mouse Ig from the Histofine Mouse Stain kit was used as the secondary antibody without dilution. Ten randomly selected fields (magnification,  $\times 400$ ) in each 4-HNE-stained specimen were classified into immunopositive grades 1, 2, 3 and 4 (0%-10%, 11%-20%, 21%-30%, and  $> 30\%$ , respectively) and the mean values of 10 fields were calculated.

#### RNA extraction and reverse transcription-PCR analysis

As described previously<sup>[11]</sup>, total RNA was extracted from homogenized hepatic tissue samples using the RNeasy Lipid Tissue Mini kit (Qiagen, Hilden, Germany). Absorbance at 260 nm was measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific), to determine RNA concentrations and RNA quality was confirmed by electrophoresis on ethidium bromide-stained 1% agarose gels. Total RNA (2  $\mu\text{g}$ ) was reverse transcribed in a final volume of 11.5  $\mu\text{L}$  containing 4  $\mu\text{L}$  of 5  $\times$  standard buffer, 2  $\mu\text{L}$  of 0.1 mol/L dithiothreitol, 1  $\mu\text{L}$  of SuperScript II RNase H reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA), 2  $\mu\text{L}$  of 10 mol/L MdNTP (Promega, Madison, WI), 1  $\mu\text{L}$  of 50 pmol/ $\mu\text{L}$  Random Primer (Promega), 0.5  $\mu\text{L}$  of 100 pmol/ $\mu\text{L}$  Oligo (dT)15 Primer (Promega), and 1  $\mu\text{L}$  of 40 U/ $\mu\text{L}$  ribonuclease inhibitor (Wako Pure Chemical Industries, Ltd.). Mixtures were incubated at 37  $^{\circ}\text{C}$  for 60 min and 95  $^{\circ}\text{C}$  for 5 min, and were then cooled to 4  $^{\circ}\text{C}$  for 5 min using a MyCycler Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA).

#### Real-time PCR

Quantitative real-time PCR assays (7900HT Fast Real-time PCR system; Applied Biosystems, Carlsbad, CA) proceeded as described previously<sup>[11]</sup>. The assays were used a final volume of 10  $\mu\text{L}$  containing 250 nmol/L Universal ProbeLibrary probe (Roche, Basel, Switzerland), 900 nmol/L forward primer, 900 nmol/L reverse primer, 5  $\mu\text{L}$  EXPRESS qPCR Supermix with Premixed Rox (Invitrogen), and 2  $\mu\text{L}$  cDNA. mRNA level of transforming growth factor- $\beta 1$  (TGF- $\beta 1$ ; GenBank: NM\_011577), procollagen-type I (GenBank: U08020), connective tissue growth factor (CTGF; GenBank: NM\_010217), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ; GenBank: NM\_013693), monocyte chemoattractant protein-1 (MCP-1; GenBank: NM\_100127112), tissue inhibitor of metalloproteinases-1 (TIMP-1; GenBank: NM\_011593), peroxisome proliferator-activated receptor (PPAR- $\alpha$ ; GenBank: NM\_007988.3), sterol regulatory element-binding protein 1c (SREBP1c; GenBank: NM\_011480), microsomal triglyceride transfer protein (MTP; GenBank: NM\_008642), endothelin-1 (ET-1; GenBank: NM\_010204), endothelin-converting enzyme (ECE; GenBank: NM\_199307), endothelin-1 type A receptor (ET-1A; GenBank: NM\_010332), and endothelin-1 type B receptor (ET-1B; GenBank: U32329) were assessed using the 7900HT Fast Real-Time PCR System with SDS2.3 software (Applied Biosystems) and with  $\beta$ -actin (GenBank: NM\_007393) as an internal standard.

Thermal cycle conditions were 95  $^{\circ}\text{C}$  for 20 s, followed by 45 cycles of 1 s at 95  $^{\circ}\text{C}$  and 20 s at 60  $^{\circ}\text{C}$ . The relative mRNA expression levels were calculated using the  $2^{-\Delta\Delta\text{CT}}$  method.

#### Statistical analysis

Differences between groups were statistically analyzed using unpaired Student's *t*-tests. All statistical analysis was performed using StatFlex ver.6.0 for Windows software (Artech Co. Ltd., Osaka, Japan). All data are expressed as means  $\pm$  SD, with *P* values less than 0.05 considered to indicate significant differences.

## RESULTS

#### Characteristics of FLS-ob/ob mice

As shown in Table 1, the two groups of mice did not differ in terms of food consumption, bodyweight, liver weight, liver-to-bodyweight ratio, visceral fat weight, or levels of serum AST and ALT. There was no difference in hepatic histology with hematoxylin-eosin staining between the two groups (Figure 1A and B).

#### Effects of ambrisentan on hepatic steatosis

To assess the effects of ambrisentan on lipid metabolism, we determined the hepatic steatosis area, hepatic lipid contents, and gene expression of hepatic lipogenesis, lipolysis, and lipid transporter genes. Oil red O staining showed no differences in area of hepatic steatosis between the groups (ambrisentan vs control; 15.0%  $\pm$  6.0% vs 17.0%  $\pm$  7.7%; *P* = 0.614; Figure 1C-E). Steatosis-related mRNA expression levels (PPAR- $\alpha$ , SREBP-1c, FAS, and MTP) were not different between the two groups (Table 2). Hepatic total cholesterol and triglyceride contents also revealed no differences between the two groups (Table 1). These findings suggested that ambrisentan did not affect lipid metabolism and accumulation in the liver of FLS-ob/ob mice.

#### Effects of ambrisentan on hepatic fibrosis

To assess whether ambrisentan attenuated hepatic fibrosis, we determined the antifibrotic effects of ambrisentan in the FLS-ob/ob mice. Sirius red staining showed that the area of fibrosis was decreased by ambrisentan compared with the control (0.46%  $\pm$  0.18% vs 1.11%  $\pm$  0.28%, respectively, *P* = 0.0003; Figure 1F-H). Hepatic hydroxyproline (Hyp) content was significantly reduced by ambrisentan compared with the control (18.0  $\mu\text{g/g}$   $\pm$  6.1  $\mu\text{g/g}$  liver vs 33.9  $\mu\text{g/g}$   $\pm$  13.5  $\mu\text{g/g}$  liver, respectively, *P* = 0.014; Figure 1I). Moreover, the area of positive  $\alpha$ -SMA immunostaining was significantly reduced by ambrisentan (0.12%  $\pm$  0.08% vs 0.25%  $\pm$  0.11%, respectively *P* = 0.047; Figure 1J-M).

In relation to extracellular matrix metabolism in the liver, as shown in Table 2, ambrisentan reduced the mRNA expression levels of procollagen-1 by 60% and TIMP-1 by 45% but the mRNA expression of TGF- $\beta 1$  and CTGF did not differ between the two groups.



**Table 1** Effects of ambrisentan administration on various parameters in fatty liver shionogi-*ob/ob* mice

Parameters	Control group ( <i>n</i> = 5)	Ambrisentan group ( <i>n</i> = 8)	<i>P</i> value
Body weight (g)	47.3 ± 3.6	47.0 ± 4.6	0.27
Liver weight (g)	5.4 ± 1.2	5.1 ± 1.1	0.75
Liver/body weight ratio	0.11 ± 0.02	0.11 ± 0.01	0.68
Visceral fat weight (g)	2.5 ± 0.3	2.7 ± 0.3	0.32
Weekly dietary intake (g)	31.7 ± 9.3	29.4 ± 9.0	0.66
Serum AST (U/L)	143 ± 20	155 ± 43	0.59
Serum ALT (U/L)	120 ± 52	151 ± 65	0.38
Hepatic cholesterol (mg/dL)	24.5 ± 1.56	27.2 ± 2.58	0.06
Hepatic triglyceride (mg/dL)	1152 ± 500	929 ± 210	0.28

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

### Effects of ambrisentan on the inflammatory reaction in the liver

The process of hepatic fibrosis is driven primarily by inflammation in response to liver damage. There were fewer F4/80-positive cells in the ambrisentan group than in the control group, but not significantly so ( $6.5 \pm 3.9$  vs  $15.2 \pm 11.5$ , respectively,  $P = 0.055$ ; Figure 2A-C). Levels of inflammation-related mRNA (TNF- $\alpha$  and MCP-1) did not differ between the two groups (Table 2).

### Effects of ambrisentan on oxidative stress

Oxidative stress is involved in the development of NASH. We determined oxidative stress by two methods: 8-OHdG as an index of DNA damage and 4-HNE as an index of lipid peroxidation. Ambrisentan did not affect the ratio of 8-OHdG-positive cells in the liver compared with the control ( $73.8\% \pm 12.4\%$  vs  $78.2\% \pm 11.5\%$ , respectively,  $P = 0.538$ ; Figure 2D-F) and did not alter the immunostaining grade for liver 4-HNE ( $2.36 \pm 0.37$  vs  $2.35 \pm 0.41$ , respectively,  $P = 0.958$ ; Figure 2G-I).

### Effects of ambrisentan on ET-related mRNA in the liver

Finally, we measured ET-related gene expression in FLS-*ob/ob* mice. The levels of ET-related mRNAs (ET-1, ECE, ETAR, and ETBR) were not different between the two groups (Table 2).

## DISCUSSION

This study had two important findings. First, ambrisentan did not affect lipid metabolism. Second, it significantly attenuated the progression of hepatic fibrosis. Thus, ET-1 antagonism reduced hepatic fibrosis without improving hepatic steatosis. Ambrisentan did not reduce body weight, blood glucose levels, or hepatic steatosis compared with the control group. ET-1 is reported to increase lipolysis in human and bovine adipocytes<sup>[14]</sup>. Therefore, ET-1 may increase the inflow of free fatty acids from the fat tissue into the liver and exacerbate hepatic steatosis. ET-1 reduced the cholesterol efflux in macrophages, resulting in exacerbation of lipid accumulation in macrophages<sup>[15]</sup>. However, the present study showed that ambrisentan did not affect lipid accumulation

**Table 2** Hepatic mRNA expression levels of various genes in the control and ambrisentan groups

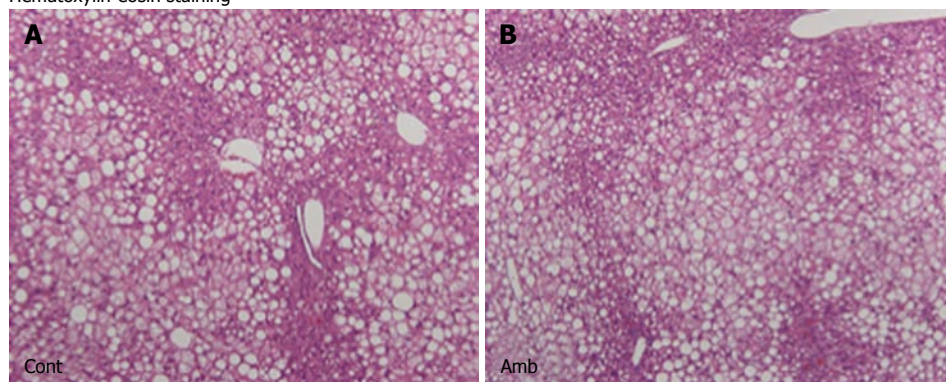
mRNA	Control group ( <i>n</i> = 5)	Ambrisentan group ( <i>n</i> = 8)	<i>P</i> value
Procollagen-1	1.76 ± 0.58	1.06 ± 0.43	0.024
TGF- $\beta$ 1	1.60 ± 0.80	1.14 ± 0.17	0.13
CTGF	1.43 ± 0.49	1.52 ± 0.40	0.36
TIMP-1	2.98 ± 1.58	1.34 ± 0.61	0.02
TNF- $\alpha$	2.37 ± 2.65	2.37 ± 3.02	1
MCP-1	10.20 ± 10.06	8.14 ± 8.90	0.39
SREBP1c	0.69 ± 0.19	0.80 ± 0.17	0.29
FAS	0.76 ± 0.34	0.87 ± 0.46	0.67
PPAR- $\alpha$	0.81 ± 0.16	0.98 ± 0.27	0.24
MTP	0.95 ± 0.09	0.99 ± 0.09	0.45
ET-1	1.40 ± 0.57	1.47 ± 0.50	0.82
ECE	1.02 ± 0.13	1.23 ± 0.23	0.09
ETAR	3.74 ± 3.35	2.55 ± 1.56	0.4
ETBR	2.07 ± 0.76	1.87 ± 0.49	0.59

TGF: Transforming growth factor; CTGF: Connective tissue growth factor; TIMP: Tissue inhibitor of metalloproteinase; TNF: Tumor necrosis factor; MCP: Monocyte chemoattractant protein; SREBP: Sterol regulatory element-binding protein; FAS: Fatty acid synthase; PPAR: Peroxisome proliferator-activated receptor; MTP: Microsomal triglyceride transfer protein; ET: Endothelin; ECE: Endothelin-converting enzyme; ETAR: Endothelin type A receptor; ETBR: Endothelin type B receptor.

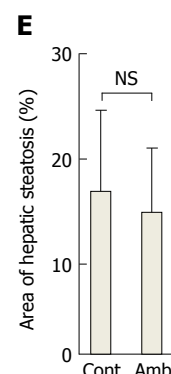
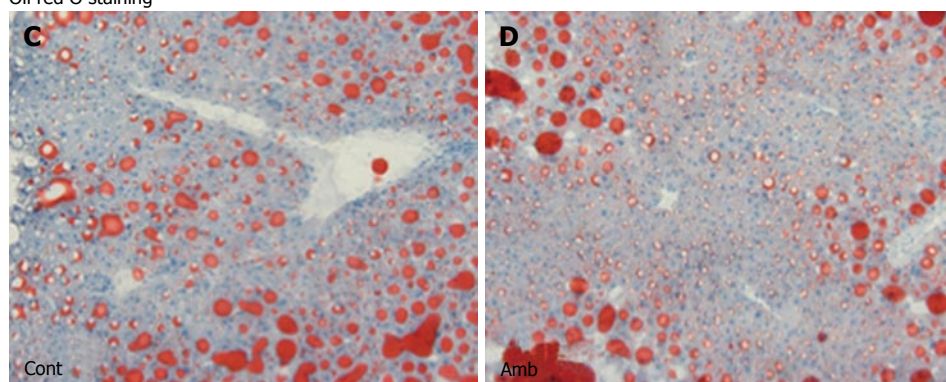
in hepatocytes or the contents of hepatic cholesterol and triglyceride. Furthermore, the expression levels of lipid metabolism-related genes such as SREBP-1c and FAS, which are involved in hepatic lipogenesis<sup>[16]</sup>, PPAR- $\alpha$ , which is involved in  $\beta$ -oxidation of fatty acids, and MTP, which transports triglyceride to very low-density lipoprotein-were not affected by ambrisentan. From these findings, our *in vivo* experiments using FLS-*ob/ob* mice indicated that ETAR antagonism was not involved in hepatic lipid metabolism. Hyperleptinemia is reported to regulate the sensitivity of ET-1 for steatosis in NASH cirrhotic rats<sup>[16]</sup>. Because the FLS-*ob/ob* mice used in our study are leptin deficient<sup>[12]</sup>, FLS-*ob/ob* mice may have low sensitivity for ET-1 in steatosis, and ET-1 may be less involved in hepatic steatosis in these mice.

Second, we investigated the effect of ambrisentan on hepatic fibrosis. The present study showed that ETAR antagonism reduced the hepatic Hyp content and the area of hepatic fibrosis through the inhibition of HSC activation. Several studies have implicated ET-1 in fibrogenesis of the kidney, cardiovascular system, and liver<sup>[2,9,17,18]</sup>. HSCs express ETAR and ET type B receptors. ET-1 is secreted from HSCs and acts in HSCs and other cells in an autocrine and paracrine manner. Our previous *in vitro* experiments showed that ET-1 increased fibrogenic gene expression *via* ETAR<sup>[17]</sup>. Furthermore, Cho *et al.*<sup>[19]</sup> reported that an oral ETAR antagonist attenuated collagen synthesis in rat liver fibrosis due to cholestasis. The present study confirmed that the ETAR antagonist also inhibited hepatic fibrosis in a mouse NASH model. HSCs are activated by several factors and stimulants and produce extracellular matrix proteins. Rocky *et al.*<sup>[9]</sup> and Pinzani *et al.*<sup>[20]</sup> showed that ET-1 increased DNA synthesis and cell growth *via* ETAR in cultured HSCs.

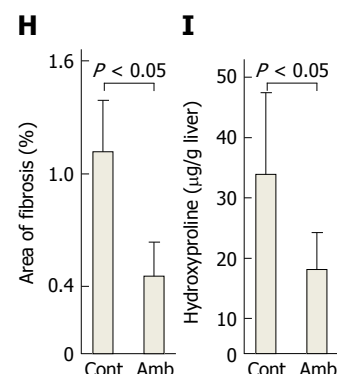
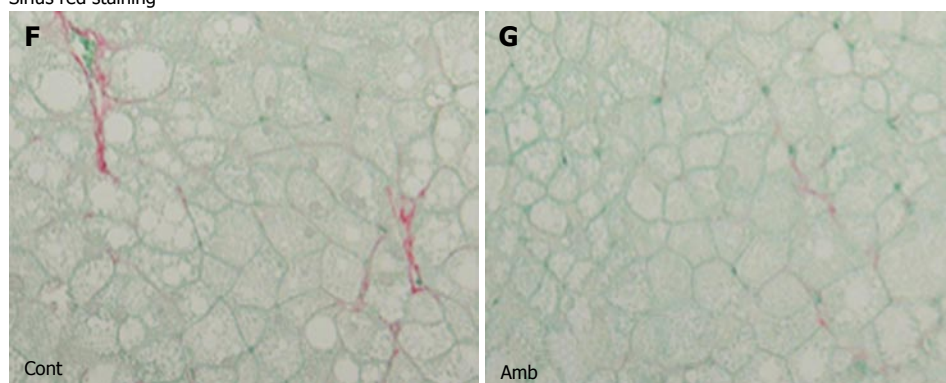
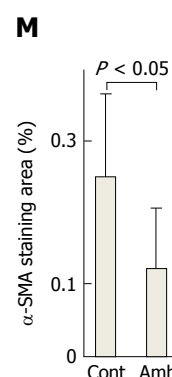
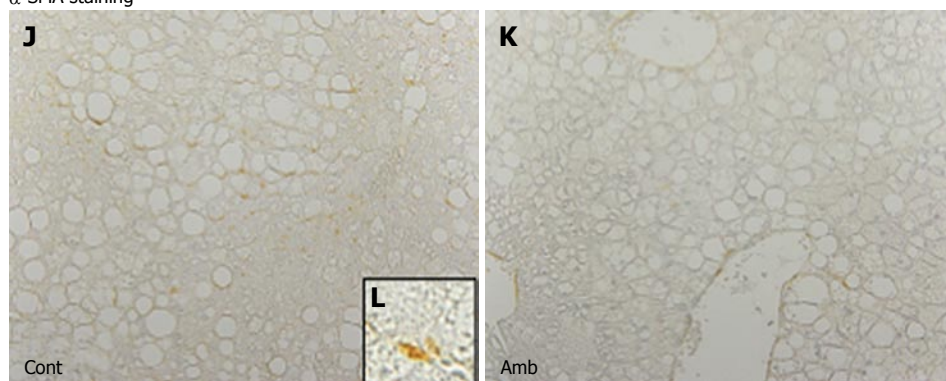
## Hematoxylin-eosin staining



## Oil red O staining



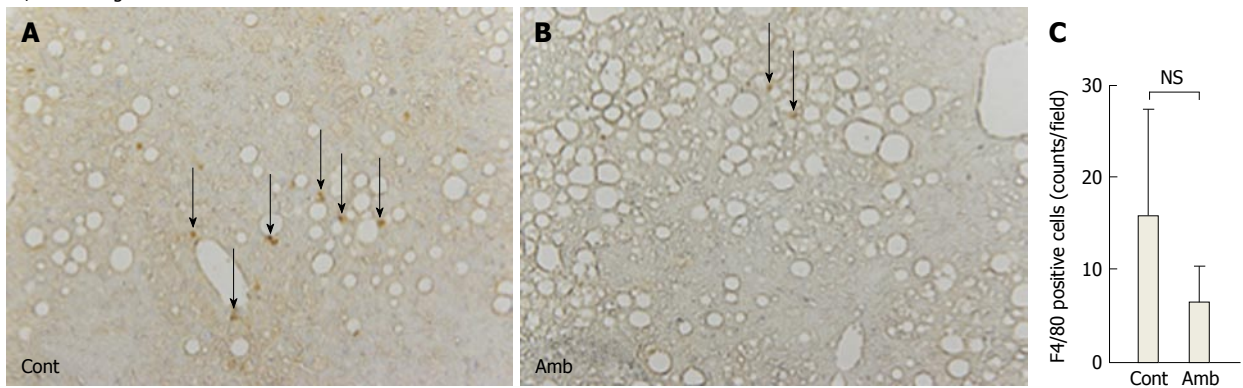
## Sirius red staining

 $\alpha$ -SMA staining

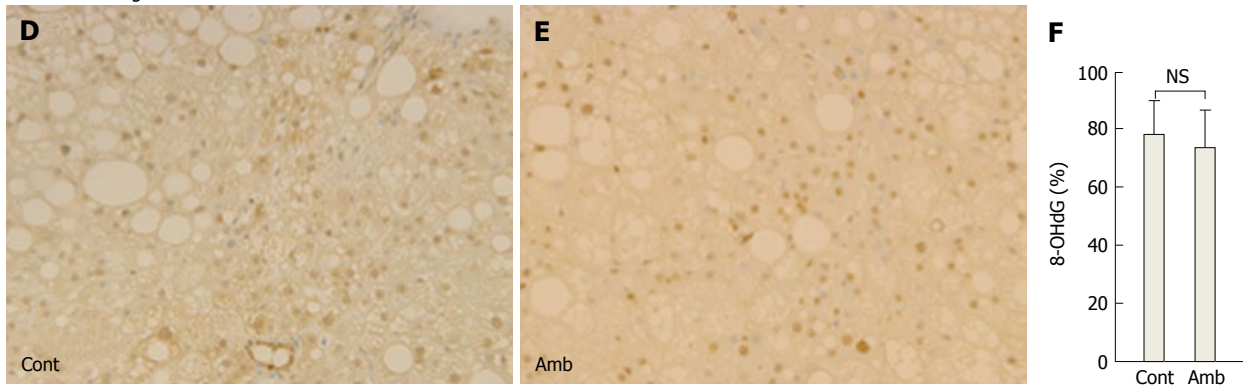
**Figure 1** Histological analyses of liver tissues. Representative images of hematoxylin-eosin staining (magnification,  $\times 100$ ) in the (A) control and (B) ambrisentan groups; representative images of oil red O staining (magnification,  $\times 100$ ) in the (C) control and (D) ambrisentan groups; E: The proportion (%) of the hepatic steatosis area stained with oil red O was measured using image analysis. Hepatic fibrosis was determined by Sirius red staining. Representative images of Sirius red staining (magnification,  $\times 400$ ) of the (F) control and (G) ambrisentan groups; the proportion (%) of the hepatic fibrosis area stained with Sirius red was measured using image analysis ( $P < 0.01$ ); H: The area of fibrosis was significantly decreased in the ambrisentan group compared with the control group; I: Comparison of hepatic hydroxyproline content between groups; representative images of  $\alpha$ -SMA immunostaining (magnification,  $\times 400$ ) in the (J) control and (K) ambrisentan groups; L: Shows a higher magnification ( $\times 1000$ ) of an  $\alpha$ -SMA-positive cell (arrow); M: Quantitation of an area of  $\alpha$ -SMA immunostaining measured by image analysis ( $P < 0.05$ ). The area of  $\alpha$ -SMA immunostaining was significantly reduced in the ambrisentan group compared with the control.  $\alpha$ -SMA:  $\alpha$ -smooth muscle actin.



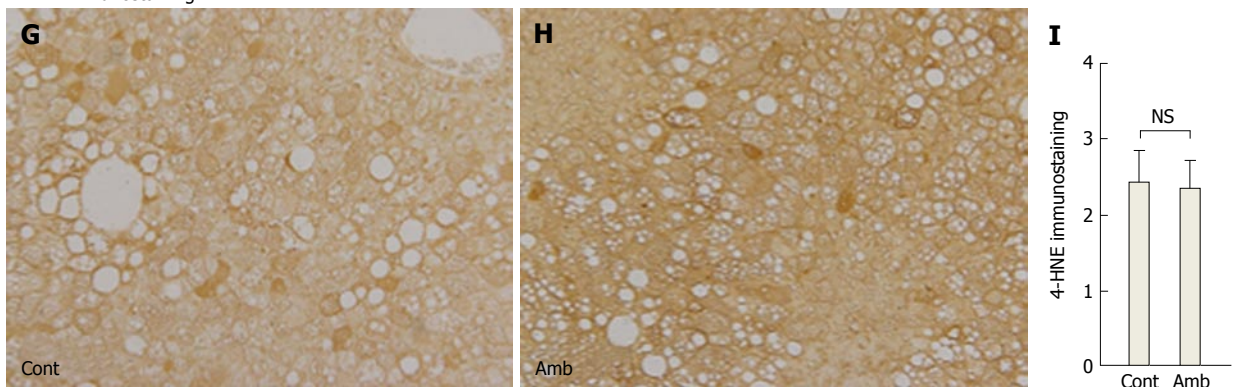
## F4/80 staining



## 8-OHdG staining



## 4-HNE immunostaining



**Figure 2 F4/80 immunostaining.** Representative images of F4/80 immunostaining (magnification,  $\times 400$ ) of Kupffer cells in the (A) control and (B) ambrisentan groups; C: Numbers of immunopositive F4/80 cells (arrows) in both groups; representative immunostaining for 8-OHdG (magnification,  $\times 400$ ) in the (D) control and (E) ambrisentan groups; F: Comparison of 8-OHdG-immunopositive cells between the groups; immunostaining for 4-HNE (magnification,  $\times 400$ ) in the (G) control and (H) ambrisentan groups; I: Comparison of 4-HNE-immunopositive cells between the groups. 4-HNE: 4-hydroxynonenal.

Our study showed that ETAR antagonism reduced HSC activation. Therefore, in the NASH model, ET-1 is involved in the activation of HSCs *via* ETAR. HSCs are activated by cytokines, oxidative stress, and inflammation. However, ambrisentan did not affect oxidative stress, as assessed by 8-OHdG and 4-HNE, or the inflammatory reaction, as assessed by *TNF- $\alpha$*  and *MCP-1* gene expression or F4/80-positive cells. Therefore, ET-1 may directly activate HSCs.

ET-1 stimulates extracellular matrix protein production by HSCs. In an HSC culture study, ET-1 increased the production of procollagen-1 and TGF- $\beta$ 1 *via* ETAR<sup>[17]</sup>. However, although the present study indicated that ETAR antagonism attenuated the gene expression of procollagen-1, it did not influence the gene expression of

TGF- $\beta$ 1 and CTGF, which is downstream of TGF- $\beta$ 1. This discrepancy may be attributable to the model of liver injury. A previous report<sup>[9]</sup> showed that ET antagonism reduced TGF- $\beta$ 1 mRNA levels in the carbon tetrachloride model, but its levels were not altered in cholestatic-induced liver injury. Such data showed that the effects of ET-1 antagonism in TGF- $\beta$ 1 may depend on the liver injury model. Therefore, ET-1 might not play a major role in TGF- $\beta$ 1 expression in mild liver injury models such as cholestasis or steatohepatitis.

The present study showed that ETAR antagonism reduced TIMP-1 gene expression. TIMP-1 is a high-affinity inhibitor of many matrix metalloproteinases and suppresses matrix degradation, resulting in the progression of liver

fibrosis. ET-1 is reported to increase TIMP-1 mRNA in fibroblasts<sup>[21]</sup>. In our study, ETAR antagonism attenuated TIMP-1 expression and might improve hepatic fibrosis by increasing fibrolysis. From these results, it appears that ambrisentan improved hepatic fibrosis by inhibiting HSC activation and suppressing procollagen-1 and *TIMP-1* gene expression.

The present study has some limitations. First, it involved a small number of mice and a relatively short duration of ambrisentan treatment. We included only 8 ambrisentan-treated mice and 5 controls and the study duration was only 4 wk. Therefore, examination of a larger number of mice and a longer administration period is required to validate these results. Second, our experiments did not include non-NASH mice arms because we could not obtain DS mice, the original wild-type of FLS-*ob/ob* mice. Therefore, further study is needed using another NASH mouse model.

In conclusion, ambrisentan attenuated the progression of hepatic fibrosis by suppressing the activation of HSCs and reducing procollagen-1 and TIMP-1 expression.

## COMMENTS

### Background

In non-alcoholic steatohepatitis (NASH), the serum endothelin-1 (ET-1) level is elevated and is correlated with hepatic fibrosis severity. The development of hepatic fibrosis is mediated to a large extent by the activation of hepatic stellate cells (HSCs). ET-1 serves to activate the HSCs and accelerates collagen fiber synthesis in them. Furthermore, ET-1 acts as a mediator and is elevated in conditions such as insulin resistance, hyperglycemia, oxidative stress, and endothelial cell dysfunction.

### Research frontiers

Ambrisentan, a selective ET type A receptor (ETAR) antagonist improves liver fibrosis in cirrhotic rats, but their effects on NASH are unknown. ET-1 may become a novel target for the treatment of NASH.

### Applications

The present study has shown ambrisentan improved hepatic fibrosis by inhibiting HSC activation and suppressing procollagen-1 and tissue inhibitor of metalloproteinase-1 (*TIMP-1*) gene expression, but did not affect hepatic steatosis. The combination therapy of ambrisentan with other drugs for lipid accumulation may be more effective for NASH.

### Terminology

NASH: Nonalcoholic steatohepatitis is characterized by hepatic fat deposition, inflammation, and differing degrees of fibrosis.

### Peer-review

This is an interesting study. The authors report that "ambrisentan" attenuates the progression of hepatic fibrosis by inhibiting the activation of HSCs and reducing procollagen-1 and *TIMP-1* gene expression. According to them it did not affect inflammation and steatosis. No doubt these results are interesting.

## REFERENCES

- 1 **Pascale A**, Pais R, Ratzliff V. An overview of nonalcoholic steatohepatitis: past, present and future directions. *J Gastrointest Liver Dis* 2010; **19**: 415-423 [PMID: 21188334]
- 2 **Tilg H**, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 2010; **52**: 1836-1846 [PMID: 21038418 DOI: 10.1002/hep.24001]
- 3 **Degertekin B**, Ozenirler S, Elbeg S, Akyol G. The serum endothelin-1 level in steatosis and NASH, and its relation with severity of liver fibrosis. *Dig Dis Sci* 2007; **52**: 2622-2628 [PMID: 17429733 DOI: 10.1007/s10620-006-9147-8]
- 4 **Mallat A**, Préaux AM, Serradeil-Le Gal C, Raufaste D, Gallois C, Brenner DA, Bradham C, Maclouf J, Iourgenko V, Fouassier L, Dhumeaux D, Mavrier P, Lotersztajn S. Growth inhibitory properties of endothelin-1 in activated human hepatic stellate cells: a cyclic adenosine monophosphate-mediated pathway. Inhibition of both extracellular signal-regulated kinase and c-Jun kinase and upregulation of endothelin B receptors. *J Clin Invest* 1996; **98**: 2771-2778 [PMID: 8981923 DOI: 10.1172/JCI119103]
- 5 **Ottosson-Seeberger A**, Lundberg JM, Alvestrand A, Ahlborg G. Exogenous endothelin-1 causes peripheral insulin resistance in healthy humans. *Acta Physiol Scand* 1997; **161**: 211-220 [PMID: 9366964 DOI: 10.1046/j.1365-201X.1997.00212.x]
- 6 **Shaw SG**, Boden PJ. Insulin resistance, obesity and the metabolic syndrome. Is there a therapeutic role for endothelin-1 antagonists? *Curr Vasc Pharmacol* 2005; **3**: 359-363 [PMID: 16248779 DOI: 10.2174/157016105774329471]
- 7 **Wedgwood S**, Dettman RW, Black SM. ET-1 stimulates pulmonary arterial smooth muscle cell proliferation via induction of reactive oxygen species. *Am J Physiol Lung Cell Mol Physiol* 2001; **281**: L1058-L1067 [PMID: 11597896 DOI: 10.1161/CIRCULATIONAHA.107.742510]
- 8 **Galiè N**, Olschewski H, Oudiz RJ, Torres F, Frost A, Ghofrani HA, Badesch DB, McGoon MD, McLaughlin VV, Roecker EB, Gerber MJ, Dufton C, Wiens BL, Rubin LJ. Ambrisentan for the treatment of pulmonary arterial hypertension: results of the ambrisentan in pulmonary arterial hypertension, randomized, double-blind, placebo-controlled, multicenter, efficacy (ARIES) study 1 and 2. *Circulation* 2008; **117**: 3010-3019 [PMID: 18506008]
- 9 **Rockey DC**, Chung JJ. Endothelin antagonism in experimental hepatic fibrosis. Implications for endothelin in the pathogenesis of wound healing. *J Clin Invest* 1996; **98**: 1381-1388 [PMID: 8823303 DOI: 10.1172/JCI118925]
- 10 **Soga M**, Kishimoto Y, Kawaguchi J, Nakai Y, Kawamura Y, Inagaki S, Katoh K, Oohara T, Makino S, Oshima I. The FLS mouse: a new inbred strain with spontaneous fatty liver. *Lab Anim Sci* 1999; **49**: 269-275 [PMID: 10403441]
- 11 **Kishina M**, Koda M, Kato J, Tokunaga S, Matono T, Sugihara T, Ueki M, Murawaki Y. Therapeutic effects of the direct renin inhibitor, aliskiren, on non-alcoholic steatohepatitis in fatty liver Shionogi ob/ob male mice. *Hepatol Res* 2014; **44**: 888-896 [PMID: 23777387 DOI: 10.1111/hepr.12186]
- 12 **Soga M**, Hashimoto S, Kishimoto Y, Hirasawa T, Makino S, Inagaki S. Insulin resistance, steatohepatitis, and hepatocellular carcinoma in a new congenic strain of Fatty Liver Shionogi (FLS) mice with the *Lep(ob)* gene. *Exp Anim* 2010; **59**: 407-419 [PMID: 20660987 DOI: 10.1538/expanim.59.407]
- 13 **Sugihara T**, Koda M, Kishina M, Kato J, Tokunaga S, Matono T, Ueki M, Murawaki Y. Fatty liver Shionogi-ob/ob mouse: A new candidate for a non-alcoholic steatohepatitis model. *Hepatol Res* 2013; **43**: 547-556 [PMID: 23057725 DOI: 10.1111/j.1872-034X.2012.01101.x]
- 14 **Eriksson AK**, van Harmelen V, Stenson BM, Aström G, Wåhlén K, Laurencikiene J, Rydén M. Endothelin-1 stimulates human adipocyte lipolysis through the ET A receptor. *Int J Obes (Lond)* 2009; **33**: 67-74 [PMID: 18982011 DOI: 10.1038/ijo.2008.212]
- 15 **Lin CY**, Lee TS, Chen CC, Chang CA, Lin YJ, Hsu YP, Ho LT. Endothelin-1 exacerbates lipid accumulation by increasing the protein degradation of the ATP-binding cassette transporter G1 in macrophages. *J Cell Physiol* 2011; **226**: 2198-2205 [PMID: 21520072 DOI: 10.1002/jcp.22556]
- 16 **Yang YY**, Tsai TH, Huang YT, Lee TY, Chan CC, Lee KC, Lin HC. Hepatic endothelin-1 and endocannabinoids-dependent effects of hyperleptinemia in nonalcoholic steatohepatitis-cirrhotic rats. *Hepatology* 2012; **55**: 1540-1550 [PMID: 22183953 DOI: 10.1002/hep.25534]
- 17 **Koda M**, Bauer M, Krebs A, Hahn EG, Schuppan D, Murawaki Y. Endothelin-1 enhances fibrogenic gene expression, but does not



- promote DNA synthesis or apoptosis in hepatic stellate cells. *Comp Hepatol* 2006; **5**: 5 [DOI: 10.1186/1476-5926-5-5]
- 18 **Arthur MJ**, Mann DA, Iredale JP. Tissue inhibitors of metalloproteinases, hepatic stellate cells and liver fibrosis. *J Gastroenterol Hepatol* 1998; **13** Suppl: S33-S38 [PMID: 9792032]
  - 19 **Cho JJ**, Hoche B, Herbst H, Jia JD, Ruehl M, Hahn EG, Riecken EO, Schuppan D. An oral endothelin-A receptor antagonist blocks collagen synthesis and deposition in advanced rat liver fibrosis. *Gastroenterology* 2000; **118**: 1169-1178 [PMID: 10833492 DOI: 10.1016/S0016-5085(00)70370-2]
  - 20 **Pinzani M**, Milani S, De Franco R, Grappone C, Caligiuri A, Gentilini A, Tosti-Guerra C, Maggi M, Failli P, Ruocco C, Gentilini P. Endothelin 1 is overexpressed in human cirrhotic liver and exerts multiple effects on activated hepatic stellate cells. *Gastroenterology* 1996; **110**: 534-548 [PMID: 8566602 DOI: 10.1053/gast.1996.v110.pm8566602]
  - 21 **Knowles JP**, Shi-Wen X, Haque SU, Bhalla A, Dashwood MR, Yang S, Taylor I, Winslet MC, Abraham DJ, Loizidou M. Endothelin-1 stimulates colon cancer adjacent fibroblasts. *Int J Cancer* 2012; **130**: 1264-1272 [PMID: 21445967]

**P- Reviewer:** Murdaca G, Ohkoshi S, Sanal MG, Tasci I

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D



Retrospective Study

## Living donor liver transplantation for high model for end-stage liver disease score: What have we learned?

Hany Dabbous, Mohammad Sakr, Sara Abdelhakam, Iman Montasser, Mohamed Bahaa, Hany Said, Mahmoud El-Meteini

Hany Dabbous, Mohammad Sakr, Sara Abdelhakam, Iman Montasser, Department of Tropical Medicine, Ain Shams Center for Organ Transplant, Faculty of Medicine, Ain Shams University, Cairo 11341, Egypt

Mohamed Bahaa, Hany Said, Mahmoud El-Meteini, Department of Hepatobiliary Surgery, Ain Shams Center for Organ Transplant, Faculty of Medicine, Ain Shams University, Cairo 11341, Egypt

**Author contributions:** Dabbous H, Sakr M, Abdelhakam S, Montasser I and El-Meteini M designed the research; Dabbous H and El-Meteini M contributed equally to the work; Dabbous H, Bahaa M, Said H and El-Meteini M performed the research; Dabbous H, Abdelhakam S, Montasser I and El-Meteini M contributed analytic tools; Dabbous H, Sakr M, Abdelhakam S, Montasser I, Bahaa M and El-Meteini M analyzed the data; Dabbous H, Abdelhakam S, Montasser I, Said H and El-Meteini M wrote the paper.

**Institutional review board statement:** This study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University Institutional Review Board.

**Informed consent statement:** Patients were not required to give informed consent to the study because the analysis used anonymous data that were obtained after each patient agreed to management by written consent.

**Conflict-of-interest statement:** None of the authors have any conflicts of interests and no financial disclosure.

**Data sharing statement:** The technical appendix, statistical code, and dataset are available from the corresponding author at [saratropical@yahoo.com](mailto:saratropical@yahoo.com). The participants gave informed consent for the data sharing.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this

work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Sara Abdelhakam, MD, Assistant Professor of Tropical Medicine, Department of Tropical Medicine, Ain Shams Center for Organ Transplant, Faculty of Medicine, Ain Shams University, Khalifa El-Maamon St., Abbassia, Cairo 11341, Egypt. [saratropical@yahoo.com](mailto:saratropical@yahoo.com)  
**Telephone:** +20-100-1601548  
**Fax:** +20-222-598751

**Received:** March 15, 2016  
**Peer-review started:** March 18, 2016  
**First decision:** April 19, 2016  
**Revised:** May 12, 2016  
**Accepted:** July 11, 2016  
**Article in press:** July 13, 2016  
**Published online:** August 8, 2016

### Abstract

**AIM:** To assess the impact of model for end-stage liver disease (MELD) score on patient survival and morbidity post living donor liver transplantation (LDLT).

**METHODS:** A retrospective study was performed on 80 adult patients who had LDLT from 2011-2013. Nine patients were excluded and 71 patients were divided into two groups; Group 1 included 38 patients with a MELD score < 20, and Group 2 included 33 patients with a MELD score > 20. Comparison between both groups was done regarding operative time, intra-operative blood requirement, intensive care unit (ICU) and hospital stay, infection, and patient survival.

**RESULTS:** Eleven patients died (15.5%); 3/38 (7.9%)

patients in Group 1 and 8/33 (24.2%) in Group 2 with significant difference ( $P = 0.02$ ). Mean operative time, duration of hospital stay, and ICU stay were similar in both groups. Mean volume of blood transfusion and cell saver re-transfusion were  $8 \pm 4$  units and  $1668 \pm 202$  mL, respectively, in Group 1 in comparison to  $10 \pm 6$  units and  $1910 \pm 679$  mL, respectively, in Group 2 with no significant difference ( $P = 0.09$  and  $0.167$ , respectively). The rates of infection and systemic complications (renal, respiratory, cardiovascular and neurological complications) were similar in both groups.

**CONCLUSION:** A MELD score  $> 20$  may predict mortality after LDLT.

**Key words:** Living donor liver transplantation; Model for end-stage liver disease score; Morbidity; Mortality; Infection

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We assessed the impact of model for end-stage liver disease (MELD) score on patient survival and morbidity after living donor liver transplantation (LDLT). A total of 71 patients were included and divided into two groups: Group 1 had 38 patients with a MELD score  $< 20$  and Group 2 had 33 patients with a MELD score  $> 20$ . We compared between both groups regarding operative time, intra-operative blood requirement, duration of intensive care unit and hospital stay, infection, and patient survival. We found that a MELD score  $> 20$  could predict mortality after LDLT.

Dabbous H, Sakr M, Abdelhakam S, Montasser I, Bahaa M, Said H, El-Meteini M. Living donor liver transplantation for high model for end-stage liver disease score: What have we learned? *World J Hepatol* 2016; 8(22): 942-948 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i22/942.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i22.942>

## INTRODUCTION

Orthotopic liver transplantation (OLT) is now considered an established treatment option for patients with end-stage liver diseases (ESLD). However, the increasing scarcity of grafts in comparison to the number of waiting patients, as well as the high procedure cost, lead to difficult decisions about how to distribute such scarce organs<sup>[1,2]</sup>. This highlights the need to identify patients who are likely to have good outcome following liver transplantation<sup>[3,4]</sup>. The Child-Turcotte-Pugh (CTP) score was originally developed for assessing the outcome of patients with liver cirrhosis and portal hypertension and was extended to stratify patients on the waiting list for liver transplantation<sup>[5]</sup>. The use of CTP in prioritizing potential liver transplant recipients is limited by several factors. Ascites and hepatic encephalopathy

are subjective variables and are affected by medical treatment; also CTP score lacks renal function assessment which strongly affects prognosis in cirrhotic patients<sup>[6]</sup>. The model for end-stage liver disease (MELD) was first described by Malinchoc *et al.*<sup>[7]</sup> as a mathematical model for predicting postoperative three-month survival for patients who underwent transjugular intrahepatic porto-systemic shunt. The MELD score was then validated as a predictor of mortality for a wide variety of liver diseases<sup>[8]</sup>, including cirrhotic patients awaiting liver transplantation<sup>[9]</sup>. Afterwards, MELD score was incorporated as a clear and objective system based on easily measurable laboratory parameters to reduce mortality among patients on the waiting list<sup>[10,11]</sup>. The ideal allocation system should allocate livers to candidates who are most likely to die without transplantation, and also to those who have a high probability of survival after OLT<sup>[12]</sup>. In February 2002, the United Network for Organ Sharing introduced a new allocation policy for cadaveric liver transplants based on the MELD score<sup>[13]</sup>. This new policy stratified patients according to the risk of death while they are on the waiting list<sup>[14]</sup>. The impact of the MELD score on postoperative mortality is still elusive.

The aim of this retrospective study was to assess the impact of the MELD score on patient survival and morbidity post living donor liver transplantation (LDLT).

## MATERIALS AND METHODS

Between January 2011 and January 2013, 80 adult patients with ESLD had received LDLT at the Ain Shams Center for Organ Transplant, Cairo, Egypt. Nine patients were excluded: Three had small-for-size grafts; one recipient had a combined organ (liver and kidney) transplant and 5 recipients had incomplete follow-up records. The remaining 71 transplants were included in this retrospective study. Seventy patients had LDLT with a right liver graft, and one patient had a left liver graft. The graft recipient weight ratio was between 0.8 and 1.7. The immunosuppressive regimen included cyclosporine or tacrolimus, mycophenolate mofetil (MMF), and corticosteroids in all patients except those transplanted for hepatocellular carcinoma (HCC). In patients transplanted for HCC, the regimen included calcineurin inhibitor and steroids only. Trough levels of cyclosporine were maintained between 200 and 300 ng/mL. Trough levels of tacrolimus were maintained between 8 and 12 ng/mL. Rapid withdrawal of corticosteroids within three months was routine in all patients (all transplanted for hepatitis C virus). In cases of acute rejection, the first-line therapy consisted of optimization of the maintenance level of immunosuppression. If there was no response, then MMF or rapamycin were added to the patient's regimen, if not already being taken. In some cases, a shift from cyclosporine to tacrolimus was beneficial. A small dose of steroids was used if all other measures failed.

The seventy one patients included in this study were

**Table 1** Demographic data, Child classification, and cold and warm ischemia time among the studied groups *n* (%)

Variable	MELD < 20 ( <i>n</i> = 38)	MELD > 20 ( <i>n</i> = 33)
Age (yr) (mean ± SD)	47.8 ± 7.8	46.2 ± 7.9
Sex		
Male	34 (89.5)	32 (97)
Female	4 (10.5)	1 (3)
Diagnosis		
ESLD	27 (71.1)	26 (78.8)
HCC	3 (7.9)	0
ESLD + HCC	8 (21)	7 (21.2)
Child-Turcotte-Pugh		
A	0	0
B	3 (7.9)	0
C	35 (92.1)	33 (100)
Cold ischemia time (min)	47 ± 23	42 ± 30
Warm ischemia time (min)	54.4 ± 20.2	53.7 ± 16.9

ESLD: End-stage liver disease; HCC: Hepatocellular carcinoma; MELD: Model for end-stage liver disease.

divided into two groups. Group 1 included 38 patients with a MELD score less than 20, and Group 2 included 33 patients with a MELD score more than 20.

The MELD score was calculated using laboratory results collected immediately before liver transplantation with no adjustments for malignancy. We calculated the MELD score using the following formula: MELD =  $[0.957 \times \ln(\text{creatinine mg/dL}) + 0.378 \times \ln(\text{bilirubin mg/dL}) + 1.12 \times \ln(\text{INR}) + 0.643 \times 10^8]$ . We reported the age, sex of the recipient, diagnosis, indication for liver transplantation, modified CTP score as well as cold and warm ischemia time. The diagnosis of chronic liver disease was confirmed by histopathology of the explanted liver. The modified CTP score was calculated and each patient was categorized as A, B, or C. Operative data (including operative time and intra-operative blood transfusion) and early post-operative outcomes [including intensive care unit (ICU) stay, hospital stay, incidence of infection and other morbidities including renal impairment, cardiovascular, respiratory and neurological complications] were compared between the two groups. Overall patient survival was also compared between the two groups. Survival was calculated using the date of transplant to either 5 years post-transplant or to the end-point of this study in January 2016.

### Statistical analysis

Categorical data were presented as numbers and percentages. Quantitative data were presented as the mean, standard deviations, ranges, median and interquartile ranges. For qualitative data, the comparison between the two groups was performed by using the  $\chi^2$  test and Fisher exact test. For quantitative data, the comparison between the two groups was performed using an independent *t*-test for parametric data and a Mann-Whitney test for non-parametric data. The Kaplan-Meier survival analysis was used to assess the overall survival of both groups. The confidence interval was set to 95%,

and the margin of error that was accepted was set to 5%. All data were analyzed using SPSS version 17. A *P*-value more than 0.05 was considered to indicate a non-significant (NS) difference between the two groups; a *P*-value less than 0.05 was considered to be statistically significant (S).

The statistical methods of this study were reviewed by Ahmed Mukhtar, Department of Anesthesia and Critical Care, Diploma of Medical Biostatistics, Faculty of Medicine, Cairo University, Cairo, Egypt.

## RESULTS

This retrospective study included 71 patients classified into two groups according to their preoperative MELD score. Demographic data, Child classification, and cold and warm ischemia time were comparable between both groups (Table 1).

### MELD score and survival

Overall patient survival was compared between both groups from the date of transplant to 5 years post-transplant or to the end-point of this study in January 2016. Eleven patients (15.5%) died during this study: Three patients out of 38 (7.9%) in Group 1 with a MELD less than 20 and 8 patients out of 33 (24.2%) in Group 2 with a MELD more than 20.

The 1, 3 and 5-year survival rates in Group 1 were 94.7%, 94.7% and 92.1% respectively, in comparison to 81.8%, 81.8% and 75.8% respectively in Group 2 with statistically significant difference between both groups (*P* = 0.02). Mortality occurred mainly in the early postoperative period in ICU because of respiratory failure due to weak respiratory muscles with poor weaning capability from mechanical ventilation (two patients in Group 1 and six patients in Group 2).

Figure 1 shows the Kaplan-Meier curve for overall survival of both groups, where Group 1 patients had a statistically significant higher overall survival rate compared to Group 2 patients.

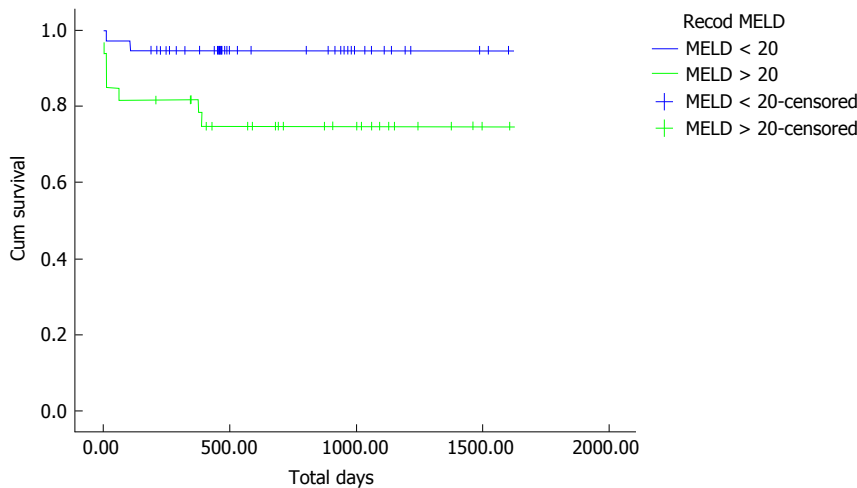
### MELD score and hospital stay

In this study, there was no statistically significant difference observed among the two groups with regard to mean hospital and ICU stay. In Group 1, the mean hospital stay was 30 ± 14 d in comparison to 29 ± 18 d in Group 2 (*P* = 0.937). The mean ICU stay in Group 1 was 7 ± 3 d, while in Group 2, it was 9 ± 4 d (*P* = 0.315).

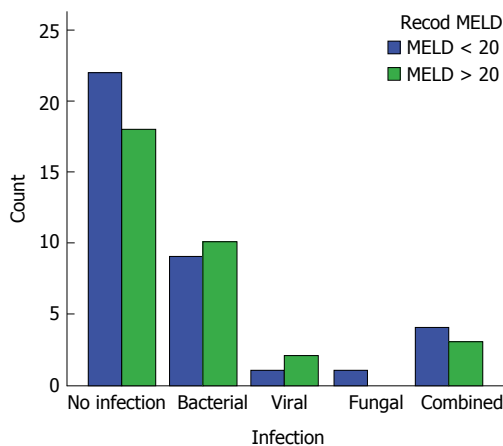
### MELD score and operative data

There was no statistically significant difference between the groups with respect to operative time, blood loss, and intra-operative blood transfusion (cell saver, blood product). The mean operative time in Group 1 was 11.1 ± 2 h (with a range of 7-15 h), and in Group 2, it was 10.6 ± 1.4 h (with a range of 9-14 h), (*P* = 0.292). The mean volume of blood transfusion and cell saver re-transfusion were 8 ± 4 units and 1668 ± 202 mL, respectively, in Group 1 in comparison to 10 ± 6 units and





**Figure 1** Kaplan-Meier curve for overall survival of both groups. The Group 1 patients that had a MELD score < 20 had higher overall survival rates than the Group 2 patients that had a MELD score > 20. MELD: Model for end-stage liver disease; Cum survival: Cumulative survival.



**Figure 2** Infection rates in both groups. MELD: Model for end-stage liver disease.

1910 ± 679 mL, respectively, in Group 2 ( $P = 0.09$  and  $0.167$ ).

#### MELD score and postoperative complications

**Infection:** The overall incidence of infection in this study was 42.3% (30 out of 71 patients). In Group 1, the incidence of infection was 39.5% (15/38 patients). Bacterial infection was the most common representing 23.6% of the patients, while viral infection [cytomegalovirus (CMV)] was detected in 2.6%, fungal in 2.6% and combined infection in 10.5%. In Group 2, the incidence of infection was 45.5% (15/33 patients). Bacterial infection was the most common type of infection, representing 30.3%, while viral infection (CMV) was detected in 6%, fungal in 0% and combined infection in 9.1%. No statistically significant difference was detected between the groups regarding infection rates ( $P = 0.79$ ) (Figure 2).

**Systemic complications:** There were no significant differences observed between groups with regard to the incidence of systemic complications including renal,

respiratory, cardiovascular, and neurological complications (34.2% and 45.5% in Groups 1 and 2, respectively,  $P = 0.869$ ).

Renal impairment was the most common complication in both group (10.5% in Group 1 and 15.2% in Group 2), followed by cardiovascular complications (13.2% in Group 1 and 12.1% in Group 2) consisting of mainly hypertension in most patients and arrhythmia in 2 patients. Neurological complications occurred in 2.6% and in 3% of the patients in Groups 1 and 2, respectively. Respiratory complications (basal atelectasis, pleural effusion, adult respiratory distress syndrome and respiratory infection) occurred in 7.9% of the patients in Group 1 compared to 15.2% in Group 2. Two patients in Group 1 (5.3%) and 2 patients in Group 2 (6.1%) had a combined respiratory and other system complications (Figure 3).

## DISCUSSION

The large imbalance between patient demand and donated organs is a pressing problem in LDLT. The best solution to this problem is still a matter of debate. Unfortunately, prioritizing extremely sick patients makes it likely that patients who are not as sick will be forced to wait until getting worse and their chances for success become also diminished<sup>[15]</sup>. Patients who are very sick may have worse post-transplant outcomes than healthier patients<sup>[16]</sup>. Thus, the optimal system would offer grafts to those who are sufficiently sick to justify the transplantation but not too sick to benefit from it<sup>[17]</sup>. The urgency of need should be optimized with the likelihood of satisfactory postoperative outcomes so as to avoid "ineffective transplantation"<sup>[18]</sup>.

An accurate prognostic model could also help potential transplant recipients and their families make decisions by providing them with information on the patient's survival probability post-transplantation<sup>[19,20]</sup>. The MELD score was achieved to help prioritizing prospective liver allograft recipients. Its accuracy to predict short-term mortality

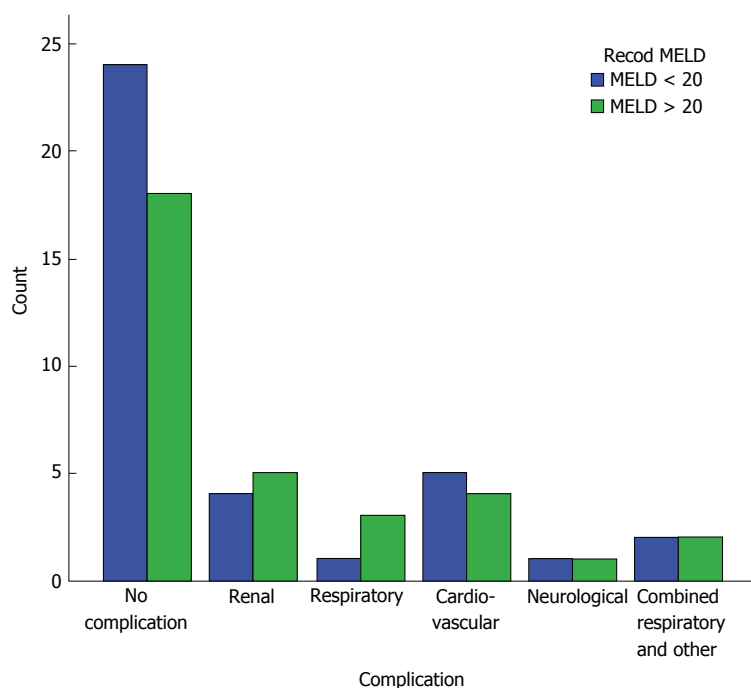


Figure 3 Incidence of systemic complications in both groups. MELD: Model for end-stage liver disease.

among patients with end-stage liver disease has been largely established<sup>[21]</sup>. However, an ideal selection system should incorporate predictions for survival while the patient is on the waiting list as well as following transplantation. The development of a model that may predict post-transplant outcomes based on pre-transplant variables is difficult because of variation in surgical skills and chance events that occur in the perioperative period. In addition to other factors such as graft rejection, biliary and vascular complications which are independent of pre-transplant events. Although it seems reasonable that pre-transplant variables which constitute the MELD score may influence the immediate post-transplant phase, their ability to predict long term outcome appears less likely. Recently, several investigators examined the predictive value of MELD for post-transplantation outcome, but with conflicting results and limited follow-up period; thus, a clear consensus has not emerged yet<sup>[22,23]</sup>.

In a systematic review about the performance of MELD score in the setting of liver transplantation, Cholongitas *et al*<sup>[9]</sup> concluded that the MELD is not a good predictor for short-term mortality following liver transplantation, and further studies were needed to assess its long term performance. Additionally, Batista *et al*<sup>[24]</sup> demonstrated that the preoperative MELD score showed low overall accuracy for predicting survival after liver transplantation; similar to what was described in other Brazilian studies. On the other hand, worse survival rates in recipients with higher MELD scores has been reported by some authors<sup>[25-27]</sup>. The current study confirms the relation between MELD score and post liver transplantation survival. The incidence of mortality was 7.9% in patients with a MELD score less than 20 compared to 24.2% in patients with a MELD greater than 20, with significant difference

between both groups ( $P = 0.02$ ).

Our study shows no significant impact of MELD score on the duration of hospital and ICU stay; these findings are comparable with those of Poon *et al*<sup>[28]</sup>, while many studies such as Foxton *et al*<sup>[29]</sup>, demonstrated that liver transplantation of patients with higher MELD scores resulted in an increased ICU and hospital stay as well as increased need for renal replacement therapy. Additionally, Buchanan *et al*<sup>[30]</sup> showed that patients in the highest MELD group had a longer ICU stay than those in the lower MELD group ( $P = 0.008$ ). Lee *et al*<sup>[31]</sup> and Massicotte *et al*<sup>[32]</sup> concluded that the MELD score did not predict blood loss or blood product requirements during liver transplantation, while others such as Feng *et al*<sup>[33]</sup> demonstrated that massive blood transfusion during liver transplantation can be predicted by preoperative MELD score. In our study, no definite relation was detected between MELD score and intra-operative blood loss or requirements of blood transfusion.

In the current study, the incidence of infection was comparable between both groups with no significant difference between a MELD score that was less or more than 20. This conclusion is the same finding of Li *et al*<sup>[34]</sup> in which a univariate analysis of risk factors for post-operative bacterial and fungal infections showed no statistically significant difference in regards to the MELD score. However, in the study of Selzner *et al*<sup>[35]</sup>, high MELD score recipients had more frequent postoperative pneumonia in comparison to those with low MELD ( $P = 0.003$ ), while no differences were observed in rates of biliary complications or overall infections.

In conclusion, a MELD score more than 20 can predict poor overall survival post LDLT. No significant relation was found between MELD score and intra-operative blood

loss or blood requirement, hospital and ICU stay, or post LDLT morbidity.

## COMMENTS

### Background

Orthotopic liver transplantation has become an established treatment approach for patients with end-stage liver disease, but the growing scarcity of grafts compared to numbers of waiting patients, and the high cost of this procedure, make it difficult to make decisions about how to distribute such scarce organs. The impact of the model for end-stage liver disease (MELD) score on postoperative mortality and morbidity following liver transplantation is not well-established yet.

### Research frontiers

The authors assessed the impact of MELD score on patient survival and morbidity post living donor liver transplantation (LDLT) in the current retrospective study that was performed on 71 adult patients who had LDLT from 2011-2013. They were divided into two groups: Group 1 included 38 patients with a MELD score < 20, and Group 2 included 33 patients with a MELD score > 20. They found that MELD score > 20 can predict poor overall survival post LDLT. No significant relation was found between MELD score and intra-operative blood loss or blood requirement, hospital and intensive care unit stay, or post LDLT morbidity.

### Innovations and breakthroughs

This is the first Egyptian study that addresses the impact of MELD score on patient survival and morbidity post living donor liver transplantation.

### Applications

The findings of this study may represent a future strategy that may help prioritize prospective liver allograft recipients and predict post-transplantation outcome.

### Terminology

The MELD score is a mathematical model based on easily measurable laboratory tests. It is calculated immediately prior to liver transplantation through the following formula:  $MELD = [0.957 \times \ln(\text{creatinine mg/dL}) + 0.378 \times \ln(\text{bilirubin mg/dL}) + 1.12 \times \ln(INR) + 0.643 \times 10^3]$ .

### Peer-review

The authors have done a retrospective study on impact of MELD score on patient survival and morbidity after living donor liver transplantation. The data may be useful for liver transplantation.

## REFERENCES

- Merion RM, Schaubel DE, Dykstra DM, Freeman RB, Port FK, Wolfe RA. The survival benefit of liver transplantation. *Am J Transplant* 2005; **5**: 307-313 [PMID: 15643990 DOI: 10.1111/j.1600-6143.2004.00703.x]
- Biggins SW. Beyond the numbers: rational and ethical application of outcome models for organ allocation in liver transplantation. *Liver Transpl* 2007; **13**: 1080-1083 [PMID: 17663407 DOI: 10.1002/lt.21210]
- Schaubel DE, Sima CS, Goodrich NP, Feng S, Merion RM. The survival benefit of deceased donor liver transplantation as a function of candidate disease severity and donor quality. *Am J Transplant* 2008; **8**: 419-425 [PMID: 18190658 DOI: 10.1111/j.1600-6143.2007.02086.x]
- Schaubel DE, Guidinger MK, Biggins SW, Kalbfleisch JD, Pomfret EA, Sharma P, Merion RM. Survival benefit-based deceased-donor liver allocation. *Am J Transplant* 2009; **9**: 970-981 [PMID: 19341419 DOI: 10.1111/j.1600-6143.2009.02571.x]
- Christensen E. Prognostic models including the Child-Pugh, MELD and Mayo risk scores--where are we and where should we go? *J Hepatol* 2004; **41**: 344-350 [PMID: 15288486 DOI: 10.1016/j.jhep.2004.06.005]
- Durand F, Valla D. Assessment of the prognosis of cirrhosis: Child-Pugh versus MELD. *J Hepatol* 2005; **42** Suppl: S100-S107 [PMID: 15777564 DOI: 10.1016/j.jhep.2004.11.015]
- Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871 [PMID: 10733541 DOI: 10.1053/he.2000.5852]
- Boursier J, Cesbron E, Tropet AL, Pilette C. Comparison and improvement of MELD and Child-Pugh score accuracies for the prediction of 6-month mortality in cirrhotic patients. *J Clin Gastroenterol* 2009; **43**: 580-585 [PMID: 19197195 DOI: 10.1097/MCG.0b013e3181889468]
- Cholongitas E, Marelli L, Shusang V, Senzolo M, Rolles K, Patch D, Burroughs AK. A systematic review of the performance of the model for end-stage liver disease (MELD) in the setting of liver transplantation. *Liver Transpl* 2006; **12**: 1049-1061 [PMID: 16799946 DOI: 10.1002/lt.20824]
- Tenório AL, Macedo FI, Miranda LE, Fernandes JL, da Silva CM, Neto OL, Lacerda CM. Survival on waiting list for liver transplantation before and after introduction of the model for end-stage liver disease score. *Transplant Proc* 2010; **42**: 407-411 [PMID: 20304152 DOI: 10.1016/j.transproceed.2010.01.005]
- Freeman RB, Wiesner RH, Edwards E, Harper A, Merion R, Wolfe R. Results of the first year of the new liver allocation plan. *Liver Transpl* 2004; **10**: 7-15 [PMID: 14755772 DOI: 10.1002/lt.20024]
- Ghobrial RM, Gornbein J, Steadman R, Danino N, Markmann JF, Holt C, Anselmo D, Amersi F, Chen P, Farmer DG, Han S, Derazo F, Saab S, Goldstein LI, McDiarmid SV, Busuttil RW. Pretransplant model to predict posttransplant survival in liver transplant patients. *Ann Surg* 2002; **236**: 315-322; discussion 322-323 [PMID: 12192318]
- Martin AP, Bartels M, Hauss J, Fangmann J. Overview of the MELD score and the UNOS adult liver allocation system. *Transplant Proc* 2007; **39**: 3169-3174 [PMID: 18089345 DOI: 10.1016/j.transproceed.2007.04.025]
- Ravaioli M, Grazi GL, Ballardini G, Cavrini G, Ercolani G, Cescon M, Zanella M, Cucchetti A, Tuci F, Del Gaudio M, Varotti G, Vetrone G, Trevisani F, Bolondi L, Pinna AD. Liver transplantation with the Meld system: a prospective study from a single European center. *Am J Transplant* 2006; **6**: 1572-1577 [PMID: 16827857 DOI: 10.1111/j.1600-6143.2006.01354.x]
- UNOS. Rationale for Objectives of Equitable Organ Allocation. [accessed 2011 Aug 15]. Available from: URL: <http://www.unos.org/resources/bioethics.asp?index=10>
- Neuberger J, Gimson A, Davies M, Akyol M, O'Grady J, Burroughs A, Hudson M. Selection of patients for liver transplantation and allocation of donated livers in the UK. *Gut* 2008; **57**: 252-257 [PMID: 17895356]
- Dawwas MF, Gimson AE. Candidate selection and organ allocation in liver transplantation. *Semin Liver Dis* 2009; **29**: 40-52 [PMID: 19235658 DOI: 10.1055/s-0029-1192054]
- Zhang M, Yin F, Chen B, Li YP, Yan LN, Wen TF, Li B. Pretransplant prediction of posttransplant survival for liver recipients with benign end-stage liver diseases: a nonlinear model. *PLoS One* 2012; **7**: e31256 [PMID: 22396731 DOI: 10.1371/journal.pone.0031256]
- Jacob M, Lewsey JD, Sharpin C, Gimson A, Rela M, van der Meulen JH. Systematic review and validation of prognostic models in liver transplantation. *Liver Transpl* 2005; **11**: 814-825 [PMID: 15973726 DOI: 10.1002/lt.20456]
- Lewsey JD, Dawwas M, Copley LP, Gimson A, Van der Meulen JH. Developing a prognostic model for 90-day mortality after liver transplantation based on pretransplant recipient factors. *Transplantation* 2006; **82**: 898-907 [PMID: 17038904]
- Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: 11172350 DOI: 10.1053/jhep.2001.22172]
- Brown RS, Kumar KS, Russo MW, Kinkhabwala M, Rudow DL, Harren P, Lobritto S, Emond JC. Model for end-stage liver disease

- and Child-Turcotte-Pugh score as predictors of pretransplantation disease severity, posttransplantation outcome, and resource utilization in United Network for Organ Sharing status 2A patients. *Liver Transpl* 2002; **8**: 278-284 [PMID: 11910574 DOI: 10.1053/jlts.2002.31340]
- 23 **Jacob M**, Copley LP, Lewsey JD, Gimson A, Toogood GJ, Rela M, van der Meulen JH. Pretransplant MELD score and post liver transplantation survival in the UK and Ireland. *Liver Transpl* 2004; **10**: 903-907 [PMID: 15237375 DOI: 10.1002/lt.20169]
  - 24 **Batista TP**, Sabat BD, Melo PS, Miranda LE, Fonseca-Neto OC, Amorim AG, Lacerda CM. Employment of MELD score for the prediction of survival after liver transplantation. *Rev Col Bras Cir* 2012; **39**: 105-111 [PMID: 22664516 DOI: 10.1590/S0100-69912012000200005]
  - 25 **Brandão A**, Fuchs SC, Gleisner AL, Marroni C, Zanotelli ML, Cantisani G. MELD and other predictors of survival after liver transplantation. *Clin Transplant* 2009; **23**: 220-227 [PMID: 19210688 DOI: 10.1111/j.1399-0012.2008.00943.x]
  - 26 **Monteiro F**, Coria SA, Boni R, Pereira LA. Model for end-stage liver disease: impact of the new deceased donor liver allocation policy in São Paulo, Brazil. *Transplant Proc* 2009; **41**: 226-228 [PMID: 19249520 DOI: 10.1016/j.transproceed.2008.09.059]
  - 27 **Yoo HY**, Thuluvath PJ. Short-term postliver transplant survival after the introduction of MELD scores for organ allocation in the United States. *Liver Int* 2005; **25**: 536-541 [PMID: 15910490 DOI: 10.1111/j.1478-3231.2005.01011.x]
  - 28 **Poon KS**, Chen TH, Jeng LB, Yang HR, Li PC, Lee CC, Yeh CC, Lai HC, Su WP, Peng CY, Chen YF, Ho YJ, Tsai PP. A high model for end-stage liver disease score should not be considered a contraindication to living donor liver transplantation. *Transplant Proc* 2012; **44**: 316-319 [PMID: 22410005 DOI: 10.1016/j.transproceed.2012.02.006]
  - 29 **Foxton MR**, Al-Freah MA, Portal AJ, Sizer E, Bernal W, Auzinger G, Rela M, Wendon JA, Heaton ND, O'Grady JG, Heneghan MA. Increased model for end-stage liver disease score at the time of liver transplant results in prolonged hospitalization and overall intensive care unit costs. *Liver Transpl* 2010; **16**: 668-677 [PMID: 20440776 DOI: 10.1002/lt.22027]
  - 30 **Buchanan P**, Dzebisashvili N, Lentine KL, Axelrod DA, Schnitzler MA, Salvalaggio PR. Liver transplantation cost in the model for end-stage liver disease era: looking beyond the transplant admission. *Liver Transpl* 2009; **15**: 1270-1277 [PMID: 19790155 DOI: 10.1002/lt.21802]
  - 31 **Lee J**, Chung MY. Does the model for end-stage liver disease score predict transfusion amount, acid-base imbalance, haemodynamic and oxidative abnormalities during living donor liver transplantation? *J Int Med Res* 2011; **39**: 1773-1782 [PMID: 22117978]
  - 32 **Massicotte L**, Beaulieu D, Roy JD, Marleau D, Vandenbroucke F, Dagenais M, Lapointe R, Roy A. MELD score and blood product requirements during liver transplantation: no link. *Transplantation* 2009; **87**: 1689-1694 [PMID: 19502961 DOI: 10.1097/TP.0b013e3181a5e5f1]
  - 33 **Feng ZY**, Jin XD, Chen YZ. [Predictors of massive blood transfusion in liver transplantation for patients with benign end-stage liver disease]. *Zhonghua Yi Xue Za Zhi* 2008; **88**: 3040-3044 [PMID: 19192401]
  - 34 **Li C**, Wen TF, Mi K, Wang C, Yan LN, Li B. Analysis of infections in the first 3-month after living donor liver transplantation. *World J Gastroenterol* 2012; **18**: 1975-1980 [PMID: 22563180 DOI: 10.3748/wjg.v18.i16.1975]
  - 35 **Selzner M**, Kashfi A, Cattral MS, Selzner N, McGilvray ID, Greig PD, Levy GA, Renner EL, Grant DR. Live donor liver transplantation in high MELD score recipients. *Ann Surg* 2010; **251**: 153-157 [PMID: 19858705 DOI: 10.1097/SLA.0b013e3181bc9c6a]

**P- Reviewer:** He JY, Julie NL, Srivastava M  
**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Li D





Observational Study

## Boceprevir or telaprevir in hepatitis C virus chronic infection: The Italian real life experience

CLEO Study Group; Antonio Ascione, Luigi Elio Adinolfi, Pietro Amoroso, Angelo Andriulli, Orlando Armignacco, Tiziana Ascione, Sergio Babudieri, Giorgio Barbarini, Michele Brogna, Francesco Cesario, Vincenzo Citro, Ernesto Claar, Raffaele Cozzolongo, Giuseppe D'Adamo, Emilio D'Amico, Pellegrino Dattolo, Massimo De Luca, Vincenzo De Maria, Massimo De Siena, Giuseppe De Vita, Antonio Di Giacomo, Rosanna De Marco, Giorgio De Stefano, Giulio De Stefano, Sebastiano Di Salvo, Raffaele Di Sarno, Nunzia Farella, Laura Felicioni, Basilio Fimiani, Luca Fontanella, Giuseppe Foti, Caterina Furlan, Francesca Giancotti, Giancarlo Giolitto, Tiziana Gravina, Barbara Guerrera, Roberto Gulminetti, Angelo Iacobellis, Michele Imperato, Angelo Iodice, Vincenzo Iovinella, Antonio Izzi, Alfonso Liberti, Pietro Leo, Gennaro Lettieri, Ileana Luppino, Aldo Marrone, Ettore Mazzoni, Vincenzo Messina, Roberto Monarca, Vincenzo Narciso, Lorenzo Nosotti, Adriano Maria Pellicelli, Alessandro Perrella, Guido Piai, Antonio Picardi, Paola Pierri, Grazia Pietromatera, Francesco Resta, Luca Rinaldi, Mario Romano, Angelo Rossini, Maurizio Russello, Grazia Russo, Rodolfo Sacco, Vincenzo Sangiovanni, Antonio Schiano, Antonio Sciambra, Gaetano Scifo, Filomena Simeone, Annarita Sullo, Pierluigi Tarquini, Paolo Tundo, Alfredo Vallone

Antonio Ascione, Luca Fontanella, Michele Imperato, Department of Medicine, Center for Liver Diseases, "Buon Consiglio" - Fatebenefratelli Hospital, 80126 Naples, Italy

Luigi Elio Adinolfi, Barbara Guerrera, Internal Medicine Unit, Second University of Naples, 80125 Marcianise, Italy

Pietro Amoroso, Gennaro Lettieri, Paola Pierri, VI Division of Infectious Diseases, Cotugno Hospital, AORN "Ospedali dei Colli", 80135 Naples, Italy

Angelo Andriulli, Angelo Iacobellis, Division of Gastroenterology, Casa Sollievo Sofferenza Hospital, IRCCS, 71013 San Giovanni Rotondo, Italy

Orlando Armignacco, Division of Infectious Diseases, Belcolle Hospital, 01100 Viterbo, Italy

Tiziana Ascione, Giorgio De Stefano, Nunzia Farella, IX Division of Infectious Diseases, Cotugno Hospital, AORN "Ospedali dei Colli", 80135 Naples, Italy

Sergio Babudieri, Clinical of Infectious Disease, University of Sassari, 07100 Sassari, Italy

Giorgio Barbarini, Roberto Gulminetti, Infectious Disease IRCCS San Matteo, 27100 Pavia, Italy

Michele Brogna, Alfredo Vallone, Division of Infectious Diseases and Liver Unit, "G. Iazzolino" Hospital, 89900 Vibo Valentia, Italy

Francesco Cesario, Division of Infectious Diseases, "Annunziata" Hospital, 87100 Cosenza, Italy

Vincenzo Citro, Giuseppe D'Adamo, Basilio Fimiani, Department of Internal Medicine, Umberto I Hospital, 84014 Nocera Inferiore, Italy

Ernesto Claar, Antonio Sciambra, Internal Medicine, Ospedale Evangelico Villa Betania, 80147 Naples, Italy

Raffaele Cozzolongo, Division of Gastroenterology, IRCCS "S. de Bellis" Hospital, 70013 Castellana Grotte, Italy

Emilio D'Amico, Laura Felicioni, Internal Medicine Unit, Pescara-Penne Hospital, 65121 Pescara, Italy

Pellegrino Dattolo, Gastroenterology Unit, Marcianise Hospital, 81025 Marcianise, Italy

Massimo De Luca, Liver Unit, AORN Cardarelli, 80131 Napoli, Italy

Vincenzo De Maria, Massimo De Siena, Sebastiano Di Salvo,

Francesca Giacotti, Tiziana Gravina, Liver Unit, Policlinico “Mater Domini”, 80020 Catanzaro, Italy

Giuseppe De Vita, Service of Medical Day Hospital, “Rummo” Hospital, 82100 Benevento, Italy

Antonio Di Giacomo, Internal Medicine, Regina Margherita Hospital, 97013 Comiso, Ragusa, Italy

Rosanna De Marco, Pietro Leo, Ileana Luppino, Division of Gastroenterology, “Annunziata” Hospital, 87100 Cosenza, Italy

Giulio De Stefano, Grazia Pietromatera, Infectious Disease, Matera Hospital, 75100 Matera, Italy

Raffaele Di Sarno, Antonio Izzi, First Division of Infectious Diseases, Cotugno Hospital, AORN “Ospedali dei Colli”, 80135 Naples, Italy

Giuseppe Foti, Division of Infectious Diseases, AO Melacrino-Bianchi-Morelli, 89121 Reggio Calabria, Italy

Caterina Furlan, Infectious and Tropical Disease, Policlinico Umberto I, 00185 Rome, Italy

Giancarlo Giolitto, Grazia Russo, Division of Infectious Disease, Maria SS Addolorata Hospital, 84025 Eboli, Salerno, Italy

Angelo Iodice, Vincenzo Messina, Filomena Simeone, Division of Infectious Diseases, S. Anna and S. Sebastiano Hospital, 81100 Caserta, Italy

Vincenzo Iovinella, Outpatients Service for Liver Diseases, “Loreto Crispi” Hospital, 80121 Naples, Italy

Alfonso Liberti, V Division of Infectious Diseases, Cotugno Hospital, AORN “Ospedali dei Colli”, 80135 Naples, Italy

Aldo Marrone, Luca Rinaldi, Department of Medical Surgical, Neurological, Geriatric, and Metabolic Sciences, Second University of Naples, 80131 Naples, Italy

Ettore Mazzone, Liver Unit, Policlinico Casilino, 80132 Roma, Italy

Roberto Monarca, Medicine and Health Unit for Prisoners, Belcolle Hospital, 01100 Viterbo, Italy

Vincenzo Narciso, Internal Medicine Unit, “Ascalesi” Hospital, 80100 Naples, Italy

Lorenzo Nosotti, Gastrointestinal and Liver Department, National Institute for Health, Migration and Poverty, 80199 Rome, Italy

Adriano Maria Pellicelli, Liver Unit, Azienda Ospedaliera San Camillo Forlanini, 00151 Rome, Italy

Alessandro Perrella, VII Division of Infectious Diseases, Cotugno Hospital, AORN “Ospedali dei Colli”, 80135 Naples, Italy

Guido Piai, Division of Gastroenterology, S. Anna and S. Sebastiano Hospital, 8100 Caserta, Italy

Antonio Picardi, Liver Unit, University “Campus Biomedico”, 80199 Rome, Italy

Francesco Resta, Division of Infectious Disease, Taranto Hospital, 74121 Taranto, Italy

Mario Romano, Liver Unit, “Sandro Pertini” Hospital, 80132 Rome, Italy

Angelo Rossini, Liver Unit, Service “Spedali Civili” Hospital, 24121 Brescia, Italy

Maurizio Russello, Liver Unit, Garibaldi-Nesima Hospital, 95121 Catania, Italy

Rodolfo Sacco, Gastroenterology and Metabolism Diseases Unit, AO Pisana, 56121 Pisa, Italy

Vincenzo Sangiovanni, III Division of Infectious Diseases, Cotugno Hospital, AORN “Ospedali dei Colli”, 80135 Naples, Italy

Antonio Schiano, Hepatology Unit, S. Giovanni di Dio Hospital, 80027 Frattamaggiore, Italy

Gaetano Scifo, Infectious Diseases Unit, P.O. Umberto I, 96100 Siracusa, Italy

Annarita Sullo, Infectious Diseases Unit, “Umberto 1°” Hospital, 84014 Nocera Inferiore, Italy

Pierluigi Tarquini, Infectious Diseases Unit, Giuseppe Mazzini Hospital, 64100 Teramo, Italy

Paolo Tundo, Division of Infectious Diseases, S. Caterina Novella Hospital, 73013 Galatina, Italy

**Author contributions:** All authors of CLEO study group equally contributed to conception and design of the study, acquisition of data, review the draft, and approved the final version.

**Institutional review board statement:** The study was reviewed and approved by the CLEO Governing Board.

**Informed consent statement:** All study participants, or their legal representative, provided verbal informed consent prior to study enrolment as decided by the CLEO Governing Board and according to the local rules.

**Conflict-of-interest statement:** The authors declare that they have no conflicts of interest regarding this study.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Antonio Ascione, MD, Consultant Hepatologist, Department of Medicine, Center for Liver Diseases, “Buon Consiglio” - Fatebenefratelli Hospital, Via A. Manzoni 220, 80126 Naples, Italy. [antonio.ascione@paginemediche.it](mailto:antonio.ascione@paginemediche.it)

Telephone: +39-081-5981877

Received: February 23, 2016

Peer-review started: February 24, 2016

First decision: April 15, 2016

Revised: June 23, 2016

Accepted: July 20, 2016

Article in press: July 22, 2016

Published online: August 8, 2016

## Abstract

**AIM:** To check the safety and efficacy of boceprevir/telaprevir with peginterferon/ribavirin for hepatitis C virus (HCV) genotype 1 in the real-world settings.

**METHODS:** This study was a non-randomized, observational, prospective, multicenter. This study involved 47 centers in Italy. A database was prepared for the homogenous collection of the data, was used by all of the centers for data collection, and was updated continuously. All of the patients enrolled in this study were older than 18 years of age and were diagnosed with chronic infection due to HCV genotype 1. The HCV RNA testing was performed using COBAS-TaqMan2.0 (Roche, LLQ 25 IU/mL).

**RESULTS:** All consecutively treated patients were included. Forty-seven centers enrolled 834 patients as follows: Male 64%; median age 57 (range 18-78), of whom 18.3% were over 65; mean body mass index 25.6 (range 16-39); genotype 1b (79.4%); diagnosis of cirrhosis (38.2%); and fibrosis F3/4 (71.2%). The following drugs were used: Telaprevir (66.2%) and PEG-IFN-alpha2a (67.6%). Patients were naïve (24.4%), relapsers (30.5%), partial responders (14.8%) and null responders (30.3%). Overall, adverse events (AEs) occurred in 617 patients (73.9%) during the treatment. Anemia was the most frequent AE (52.9% of cases), especially in cirrhotic. The therapy was stopped for 14.6% of the patients because of adverse events or virological failure (15%). Sustained virological response was achieved in 62.7% of the cases, but was 43.8% in cirrhotic patients over 65 years of age.

**CONCLUSION:** In everyday practice, triple therapy is safe but has moderate efficacy, especially for patients over 65 years of age, with advanced fibrosis, non-responders to peginterferon + ribavirin.

**Key words:** Boceprevir; Telaprevir; Chronic hepatitis; Antiviral therapy; Peg-interferon; Ribavirin

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This study describes the role of antiviral therapy for chronic hepatitis C virus infections in everyday practice. Boceprevir or telaprevir, in combination with pegylated interferon and ribavirin, were

used in this multicenter study organized by the Italian Association of Hospital Hepatologists (CLEO). A total of 834 patients were enrolled with this first available combination of direct-acting antiviral drugs. The data on the efficacies were quite similar to those produced by the registration studies; however, in the real world experience, patients were older and had more advanced liver disease. In this category of patients, the sustained virological response was less than 50%.

CLEO Study Group; Ascione A, Adinolfi LE, Amoroso P, Andriulli A, Armignacco O, Ascione T, Babudieri S, Barbarini G, Brogna M, Cesario F, Citro V, Claar E, Cozzolongo R, D'Adamo G, D'Amico E, Dattolo P, De Luca M, De Maria V, De Siena M, De Vita G, Di Giacomo A, De Marco R, De Stefano G, De Stefano G, Di Salvo S, Di Sarno R, Farella N, Felicioni L, Fimiani B, Fontanella L, Foti G, Furlan C, Giancotti F, Giolitto G, Gravina T, Guerrero B, Gulminetti R, Iacobellis A, Imperato M, Iodice A, Iovinella V, Izzi A, Liberti A, Leo P, Lettieri G, Luppino I, Marrone A, Mazzoni E, Messina V, Monarca R, Narciso V, Nosotti L, Pellicelli AM, Perrella A, Piai G, Picardi A, Pierri P, Pietromatera G, Resta F, Rinaldi L, Romano M, Rossini A, Russello M, Russo G, Sacco R, Sangiovanni V, Schiano A, Sciambra A, Scifo G, Simeone F, Sullo A, Tarquini P, Tundo P, Vallone A. Boceprevir or telaprevir in hepatitis C virus chronic infection: The Italian real life experience. *World J Hepatol* 2016; 8(22): 949-956 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i22/949.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i22.949>

## INTRODUCTION

Chronic hepatitis C virus (HCV) infection is one of the main causes of liver cirrhosis, end-stage liver disease, hepatocellular carcinoma (HCC) and liver transplantation worldwide. Pegylated interferon-alpha (P) and ribavirin (R) have been the backbone of HCV treatment for more than a decade. In 2011, the approval of telaprevir (TVR) and boceprevir (BOC), two protease inhibitors (PI), opened the first generation of direct antiviral agents (DAAs) for the treatment of genotype 1 HCV infection.

In many randomized studies, triple therapy (the combination of P plus R with PI, such as TVR or BOC) is demonstrated to be more effective than P plus R alone, with an increased likelihood of sustained virological response (SVR) of more than 30%, when compared with the dual therapy (P + R), reaching 68%-75% of naïve patients and 29%-83% of the experienced patients depending on the previous response to P + R<sup>[1-4]</sup>. The increase in SVR is associated with more side effects, and some of them, such as anemia and rash, were frequently causes of the withdrawal from treatment. However, as is well known, in the registered trials, the number of difficult-to-treat patients is rather small (cirrhotic, elderly, null responders to previous treatments and patients with comorbidities). However, even with restricted criteria for enrollment in phase 3 studies, a number of patients had

to stop the triple therapy due to either viral failure or adverse events (12%-15%).

TVR/BOC, approved for reimbursement in Italy in December 2012, have been used since January 2013. Since then, the group of the Association of Hospital Hepatologists (CLEO DAAs Study Group) was deeply involved in using these drugs, and the Governing Board of the Association decided to collect data from the Hospital centers belonging to the CLEO. The aim of our study was to determine what happens in everyday practice in terms of safety and efficacy using the triple therapy.

## MATERIALS AND METHODS

### Study design

This study was a non-randomized, observational, prospective, multicenter. This study involved 47 centers in Italy. A database was prepared for the homogenous collection of the data, was used by all of the centers for data collection, and was updated continuously.

### Subjects

All of the patients enrolled in this study were older than 18 years of age, were diagnosed with chronic infection due to HCV genotype 1, and were consecutively seen in at least one of the centers between January 2013 and June 2014. No distinction was made between naive and previously treated patients. With regard to age, patients were divided into the following three groups: (1) less than 50; (2) between 50 and 65; and (3) over the age of 65. In this manner, we tried to avoid the division into only two categories (under 65 and over 65), which is presented in many papers and flattens the differences. Hepatitis B virus/human immunodeficiency virus positive patients or patients suffering from chronic liver disease due to other etiologies were excluded.

### Treatment

Each center made the choice between TVR or BOC and Peg-IFN- $\alpha$ 2a or Peg-IFN- $\alpha$ 2b; patients were also treated with ribavirin (dose depending on the type of P chosen). The drugs were administered according to the manufacturer's instructions. TVR was administered with P + R for 12 wk followed by 36 wk of P + R; while patients treated with BOC received 4 wk of P + R (lead-in phase) followed by 44 wk of BOC + P + R. Patients treated with BOC or TVR had to respect the stopping rule concerning the kinetics of the viral load as follows: BOC patients with an HCV-RNA at week 12 greater than or equal to 100 IU/mL or detectable at 24 wk had to stop the therapy, while TVR patients with an HCV-RNA greater than 1000 IU/mL at week 4 or 12 or detectable at week 24 had to stop the treatment. They were classified as non-responders because of the virological failure.

### Methods

Fibrosis was evaluated by a liver biopsy or by measuring the liver stiffness according to the manufacturer's in-

structions (Fibroscan<sup>®</sup>, Echosens, Paris, France). The results were expressed in kilopascal (kPa), and the cut-off values according to the literature were as follows: F1 was defined by a liver stiffness < 7.0 kPa; F2 was defined by a liver stiffness between 7.1-9.5; F3 was defined by a liver stiffness between 9.6-12.4; F4 (cirrhotic patients) was defined by liver stiffness values of up to 12.5 kPa<sup>[5]</sup>. Patients, according to their response to the previous treatment, were categorized as naive (never treated with antiviral drugs); relapsers (patients who were HCV RNA negative at the end of treatment and HCV RNA positive during the follow-up); partial responders (those with a reduction of HCV RNA during the treatment, but never become HCV RNA negative); and null responders (patients without any change in HCV RNA during the treatment and thereafter)<sup>[6]</sup>.

AEs were graded by the investigators, according to the NIH grading system (CTCAE version 4.0). Hematological disorders, mainly anemia, were managed by reducing the ribavirin dose, giving erythropoietin, and/or with a blood transfusion, at the discretion of the physicians of each center. Hepatic decompensation during the therapy was defined by the new onset of one of the following clinical manifestations: Ascites, variceal hemorrhage, hepatic encephalopathy and onset of HCC.

A quantification of the HCV-RNA level was performed at baseline, 4 wk, 8 wk, 12 wk, the end of treatment, and 12 wk after the end of treatment. The HCV-RNA level was detected using real-time polymerase chain reaction (COBAS<sup>®</sup> TaqMan<sup>®</sup> HCV Test v2.0, Roche Diagnostics, Basel, Switzerland) with a lower limit of detection of 25 IU/mL. SVR was defined as HCV-RNA below the level of quantification 12 wk after the end of treatment.

### Statistical analysis

All consecutively treated patients were included; data were analyzed according to the intention-to-treat principle. A preliminary descriptive analysis of the main demographic, virological and clinical baseline variables [gender, age, body mass index (BMI), HCV genotype, HCV RNA level, fibrosis grade, IL-28B, type of response to previous antiviral therapy, biochemical laboratory tests, concomitant diseases, side effects, and virological response during, at the end, and 12 wk after the end of therapy] of the entire population under investigation was carried out. Statistics measurements were as follows: Mean and standard deviation, mean standard error and 95%CI, median and range (when appropriate). At a later stage, univariate analysis and one-way ANOVA were conducted to verify the relationships between each independent variable and the dependent variable (SVR12). A  $\chi^2$  test for categorical variables and a *t*-test or Mann-Whitney test (when appropriate) for quantitative variables was used. A two-tailed *P*-value < 0.05 was considered to indicate statistical significance. Then, we looked for multicollinearity between those independent variables that statistically associated with SVR12. Finally, a multivariable logistic-regression analysis (step-



**Table 1 Baseline characteristics of 834 patients enrolled**

Age	Median 57 (range 18-78); age > 65: 18.3%
Sex	Male 64%, female 36%
BMI	Mean 25.6 ( $\pm$ SD) = 3.2 (range 16-39)
Genotype (%)	
1a	19.2
1b	79.4
1	1.4
HCV-RNA	
HCV-RNA $\leq 10^6$	42%
HCV-RNA $> 10^6$	58%
IL 28B (%) <sup>1</sup>	
TT	21.1
CT	65.4
CC	13.5
Fibrosis (%)	
F1	7.7
F2	21.1
F3	33
F4	38.2
Cirrhosis (CTP%)	
A5	70.8
A6	23.1
B7	4.5
B8	1.6
Previous treatment (%)	
Naive	24.4
Relapser	30.5
Partial responder	14.8
Null responder	30.3
Comorbidity (%)	
Diabetes mellitus	11.5
Alcohol	12.1

<sup>1</sup>Available on 513 patients (61.5%). BMI: Body mass index; HCV: Hepatitis C virus; IL: Interleukin; CTP: Child-Turcotte-Pugh classification; SD: Standard deviation.

wise selection procedure) was conducted to assess the relationship between the SVR and the pre-specified demographic and baseline clinical characteristics.

We have not carried out a statistical analysis comparing the two treatments. The reasons are as follows: (1) as already mentioned, this comparison was not one of the purposes of the study; and (2) each center not only chose BOC or TVR in its absolute discretion but also the type of pegylated interferon. This aspect would determine the division into the four groups with a very different dimension and would not provide acceptable results. Moreover, other studies similar to ours did not make any comparative analysis between the two treatments because of the same reasons<sup>[7,8]</sup>.

All statistical analyses were performed using the software package SPSS for Windows (Rel SPSS 15.0; SPSS Chicago, IL, United States).

## RESULTS

Eight hundred and thirty-four Caucasian patients observed in the 47 participating centers from January 2013 to June 2014 were enrolled, of whom 12.1% were also alcohol abusers, and 11.5% were affected by type 2 diabetes.

The two treatments (BOC/TVR) were analyzed to-

gether. The characteristics of the patients are reported in Table 1.

The majority of our patients were affected by genotype 1b (79.4%) and cirrhosis (38.2%). Among these 319 cirrhotic patients, 70.8% had a Child-Turcotte-Pugh Score of A5, 23.1% had A6; while 4.5% were B7 and 1.6% were B8. According to the response to previous treatments, 24.4% were naive, 30.5% were relapsers, 14.8% were partial responders and 30.3% were null-responders. According to the fibrosis grade, 7.7% of patients were F1, 21.1% were F2, 33.0% were F3 and 38.2% were F4.

HCV genotype 1b (79.4%) infections were more frequent than HCV 1a (19.2%), but the HCV genotype was not defined as 1b or 1a in 1.4% of the cases. As expected, in this population of relapsers and non-responders to prior antiviral therapy, only 13.5% of the patients had an IL-28B genotype CC. However, not all of the centers had this test available, but it was carried out on 61.5% of treated patients. Each center decided the choice of therapy, with the following percentage: TVR 66.2%, BOC 33.8%, Peg-IFN alpha2a 67.6% and Peg-IFN alpha2b 32.4%.

Overall, 70.4% of the patients completed a full course of therapy, while the treatment was stopped due to virological failure in 15% of the cases and for adverse events in 14.6%.

The overall SVR rate was 62.7% (95%CI: 59.1-66.3), while 70.1% of the patients had undetectable HCV-RNA levels at the end of triple therapy with a rate of relapse of 7.3% (Table 2). According to age, SVR was observed in 67.4% of patients < 50 years, 63.1% of the patients whose ages ranged from 50 to 65, and 55.3% of patients > 65 years ( $P = 0.037$ ). SVR was observed in 65.7% of the naive patients, 73.7% of relapsers, 67.2% of partial responders and 55.1% of the null responders ( $P = 0.012$ ). Only 53.4% of the cirrhotic patients had an SVR vs the 72.7% of patients with fibrosis F1 ( $P = 0.003$ ), 73.4% with F2 ( $P = 0.0001$ ), and 63.3% with F3 ( $P = 0.013$ ); the lower rate of SVR of 43.8% was observed in cirrhotic patients over 65 years of age ( $P = 0.0001$ ). When we compared the SVR observed in the categories F0/1/2 and 3 (68.1%) vs F4 (53.4%), there was a statistically significant difference ( $P = 0.0001$ ). As for the relationship between SVR and the IL28B, the CC (70%), CT (57.5%), and TT (45.7%) groups, there was a statistically significant difference ( $P = 0.029$ ) in favor of the CC group. Alcohol did not affect the percentage of SVR, while type 2 diabetes was statistically associated with SVR (OR = 0.55; 95%CI: 0.34-0.87,  $P = 0.006$ ). The univariate analysis showed that six factors were independently associated with SVR. These factors were as follows: (1) a relapse after P + R treatment; (2) the stage of fibrosis; (3) age; (4) gender; (5) diabetes; and (6) the IL-28B status; while BMI, HCV-RNA at baseline, biochemistry at baseline and genotype subtype were not associated with SVR. The multivariate analysis with logistical regression revealed that only fibrosis F0/F1/F2 stages, IL-28B-CC and the absence of diabetes are independently associated

**Table 2** Percentage of sustained virological response according to demographics and clinical characteristics

RVR <sup>1</sup>	66.5%
HCV-RNA negative at EOT	70.1%
Relapse <sup>2</sup>	7.3%
SVR 12 <sup>3</sup>	62.7%
Age	
< 50 yr	67.4%
50-65 yr	63.1%
> 65 yr	55.3%
Previous treatment	
Naive	65.7%
Relapser	73.7%
Partial responder	67.2%
Null responder	55.1%
Fibrosis (%)	
F1	72.7%
F2	73.4%
F3	63.3%
F4	53.4%
F4 > 65 yr	43.8%

<sup>1</sup>HCV-RNA negative at week 4; <sup>2</sup>Those who achieved EOT but had HCV-RNA positive at week 12; <sup>3</sup>HCV-RNA negative 12 wk after the EOT. RVR: Rapid virological response; EOT: End of treatment; SVR: Sustained virological response; HCV: Hepatitis C virus.

with SVR ( $P < 0.05$ ). The odds ratios for fibrosis stages F0/F1/F2 and F3 vs F4 (the reference category) were 2.3 (95%CI: 1.3-3.8;  $P = 0.002$ ) and 1.5 (95%CI: 0.9-2.3;  $P = 0.096$ ), respectively. The OR for IL28B-CC and IL-28B-CT vs IL-28B-TT (the reference category) were 3.2 (95%CI: 1.5-6.7;  $P = 0.003$ ) and 1.5 (95%CI: 0.9-2.4;  $P = 0.11$ ), respectively. As for diabetes, the odds ratio was 1.8 (95%CI: 0.9-3.5;  $P = 0.075$ ).

### Safety

Overall, AEs occurred in 617 patients (73.9%) during the treatment (Table 3). A total of 122 (14.6%) of the patients suspended the therapy due to AEs. In general, females stopped the treatment more often than males (16% vs 11%;  $P = 0.043$ ). With increasing age, there was a statistically significant increase in AEs (9.4% vs 12.6% vs 18.4%;  $P = 0.040$ ). There was no statistically significant difference in relation to subtype (1b 13.7% vs 9.3% 1a;  $P = 0.18$ ); nor was there a statistically significant difference in relation to the histological diagnosis ( $P = 0.58$ ) even if the F4 class showed the highest percentage (13.8%) of AEs compared to the other classes as follows: F3 (12.9%), F2 (9.8%), F1 (11.7%) and F0 (0.6%, four patients only in this group).

Anemia was the most frequent AE (52.9% of cases), especially in cirrhotic as already described<sup>[9]</sup>, followed by asthenia (39.6%), neutro-thrombocytopenia (29.6%), rash/itching (23.2%), dysgeusia (8.6%), psychiatric disorders (6.7%), anorectal discomfort (5.9%) and others (14.9%). Among this last group, we recorded the following: Gastrointestinal disorders (23 cases), pulmonary infections (9), ascites (3), pancreatitis (2), thrombosis of retina (2), and new onset of cancer as follows: Hepatocellular carcinoma (1), breast (1), and

**Table 3** Adverse events (%) and treatment discontinuation

Adverse events (73.9%)	
Anemia	52.9
Asthenia	39.6
Neutro/thrombopenia	29.6
Dysgeusia	8.6
Psychiatric disorders	6.7
Anorectal symptoms	5.9
Others (see text)	14.9
Treatment discontinuation (122 cases; 14.6%)	Number of cases
Rash/Itch	36 (29.5%)
Anemia	28 (22.9%)
Asthenia	18 (14.7%)
Psychiatric disorders	6 (5%)
Pancytopenia	3 (2.5%)
Neutro/thrombopenia	3 (2.5%)
Others (see text)	28 (22.9%)

kidney (1). Anemia was observed regardless of the DAA used, while rash was more frequently observed in the TVR treated patients. The main AEs that led to treatment discontinuation were rash (29.8%) and anemia (23.4%). There were no fatalities as the included patients had cirrhosis, but not as advanced as in the French study<sup>[8]</sup> where the 2.2% of the patients died.

### DISCUSSION

This study, conducted in 47 hospital centers in Italy, enrolled 834 patients consecutively seen in clinical settings. Because there was no selection of the cases, all of the patients seen and judged to be treatable by each center were included. For this reason, we can safely assume that this study mirrors what happens in real life. This is the main reason of the need for studies that monitor the safety after registration of the authorization of the prescription of new drugs. It is at this stage that many older patients with morbidity, concurrently taking other medications, are enrolled. Observational studies, such as those already published and our own, serve not only to validate the results of pivotal trials but also to provide information on safety and predictors of response that helps to more appropriately use the new drugs. Some aspects should be underlined, such as the age of the patients (18.3% more than 65), the percentage of advanced liver disease (Fibrosis score F3 plus F4 = 70.9%) and the high percentage (75.6%) of patients previously treated with P + R. It is quite remarkable that the percentage of patients with compensated cirrhosis was 37.1%; while in the registration studies, this group of difficult-to-treat patients did not exceed 15%.

When we analyzed the differences between the major registration studies conducted using TVR/BOC and our findings, the first observation was that the AEs causing discontinuation of drugs were different from those reported in the phase 3 trials, where these percentages ranged between 8%-15%. The true strength of "real life" studies is the inclusion of patients who visit the clinics in every day practice and represent HCV-related disease at every stage. The only weakness is that they are not

randomized, and specialized centers in different parts of the country are involved, which favors a certain degree of heterogeneity. However, this aspect is also present in the pivotal studies in which many centers participate, often scattered in different countries. Analyzing other studies similar to ours, the percentages of drug discontinuation varies from a minimum of 8% to a maximum of 38%<sup>[7-10]</sup>. However, it is difficult to entirely blame DDAs for some AEs, as in addition to BOC and TVR, there were two drugs, including P and R, with AEs well known for many years, especially anemia, itching, and nervousness.

In this study, among the AEs causing withdrawal from treatment, rash (29.5%) was the most frequent, although we did not observe DRESS syndrome or toxic epidermal necrolysis.

Rash was detected in both treatment groups, although it was more frequent in patients treated with TVR. Anemia was the second most important AE leading to discontinuation of therapy. In 11% of the patients, it was necessary to perform blood transfusions, while in 25%, epoetin was administered. Other cases were simply treated with a dose reduction of ribavirin. As for the AEs not causing withdrawal from therapy, we did not find remarkable differences with the pivotal trials (Table 3).

The SVR at 12 wk after the end of treatment was achieved by 62.7%, more than that achieved by the other similar studies. The high number of patients with cirrhosis and the presence of older patients explain the results, such as SVR, which was a percentage lower than that obtained from the pivotal studies. In naive patients, the results were similar to those previously obtained by partial responders, while those who had the best performance (SVR = 73.7%) were those who had a relapse at the end of the previous treatments. Similar data for this category of patients were achieved by the other studies<sup>[9,11,12]</sup> for experienced patients. Null responder patients to previous treatments had an SVR of 55.1%, better than that reported in other similar studies, whereas in one study<sup>[10]</sup>, the SVR was less than 20%. The most relevant finding of this study was the negative correlation between the SVR and fibrosis grade. This result has been recently confirmed<sup>[13]</sup>. In fact, as reported in Table 3, the worst result (SVR = 43.8%) was achieved in patients with cirrhosis, who were older than 65 years of age. Indeed, these categories of patients (elderly, with cirrhosis and with many failures to previous treatments) represent the majority of patients requiring treatment today. Multivariate analyses showed that the most important factors linked to SVR were the grade of fibrosis, IL-28B-CC and not being diabetic.

In conclusion, the treatment with first generation PI (BOC/TVR) plus P + R is quite safe, but its efficacy is limited, especially for elderly cirrhotic patients. This information is very useful as DDA IFN-free drugs may change the antiviral therapy options for HCV, and there is no doubt that in many countries, these drugs will only be selectively available due to cost. Therefore, real life studies on "old" less expensive DDAs could be very

useful for establishing drug delivery policies in relation to the resources available in each country.

## ACKNOWLEDGMENTS

The CLEO Study Group thanks Michele Imparato, MD, for the data management, Massimo De Luca, MD, for the statistical analysis and Antonio Ascione, MD, for writing the draft.

## COMMENTS

### Background

Protease inhibitors (boceprevir or telaprevir) in combination with pegylated interferons and ribavirin are the first direct antiviral therapy for chronic infections with hepatitis C virus (HCV) genotype 1. They were introduced in 2011 and since then have been a step forward in the development of this therapy. In Italy, these therapies were introduced in 2013 and the Italian Association of Hospital hepatologists (CLEO) has begun, among the members of the association, the data collection.

### Research frontiers

This study represents one of the few real-life studies with high number of cases, published in the international field and the only one regarding the Italian patients. Compared to the registration studies, the collection of data from patients who are treated every day provides valuable data to validate in clinical practice this treatment.

### Innovations and breakthroughs

Therefore, the present study tested in practice the first two innovative drugs in chronic infections with HCV therapy that were expected at least for ten years. With their arrival in the therapeutic baggage of hepatologists, the authors have obtained results certainly better than the performance of conventional therapy with interferon and ribavirin alone, which has represented the standard of care for about fifteen years.

### Applications

The data generated from this study show that these drugs have an acceptable safety profile but their effectiveness, especially in cirrhotic patients and with over 65 years of age, is quite modest. Their greater efficacy is obtained in patients with non-advanced liver damage. The new drugs, which are currently on the market for hepatitis C, are more active than the triple therapy, but their cost is extremely high. Therefore, these studies are of great social importance because, in countries that do not have an economy that allows the purchase of these drugs, the triple therapy can be offered with excellent results, choosing carefully the categories of patients to be treated.

### Terminology

The letter "F" expresses the degree of fibrosis in the liver. In this study this aspect was defined by liver biopsy or by the Fibroscan tool, which, in a non-invasive way, is able to define the degree of rigidity and, therefore, the actual degree of fibrosis in the liver. The physical principle is that the higher the number in kilopascals, the higher the degree of fibrosis.

### Peer-review

This topic of study is very topical and important. The authors' concept and ideas for this investigation is very note worthwhile and studies of real life experiences are most useful for the field.

## REFERENCES

- 1 Poordad F, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl*

- J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]
- 2 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]
- 3 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]
- 4 **Zeuzem S**, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; **364**: 2417-2428 [PMID: 21696308 DOI: 10.1056/NEJMoa1013086]
- 5 **Ziol M**, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Ledinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48-54 [PMID: 15690481]
- 6 **Wedemeyer H**, Jensen DM, Godofsky E, Mani N, Pawlowsky JM, Miller V. Recommendations for standardized nomenclature and definitions of viral response in trials of hepatitis C virus investigational agents. *Hepatology* 2012; **56**: 2398-2403 [PMID: 22707382 DOI: 10.1002/hep.25888]
- 7 **Gordon SC**, Muir AJ, Lim JK, Pearlman B, Argo CK, Ramani A, Maliakkal B, Alam I, Stewart TG, Vainorius M, Peter J, Nelson DR, Fried MW, Reddy KR. Safety profile of boceprevir and telaprevir in chronic hepatitis C: real world experience from HCV-TARGET. *J Hepatol* 2015; **62**: 286-293 [PMID: 25218788 DOI: 10.1016/j.jhep.2014.08.052]
- 8 **Hézode C**, Fontaine H, Dorival C, Zoulim F, Larrey D, Canva V, De Ledinghen V, Poynard T, Samuel D, Bourliere M, Alric L, Raabe JJ, Zarski JP, Marcellin P, Riachi G, Bernard PH, Loustaud-Ratti V, Chazouilleres O, Abergel A, Guyader D, Metivier S, Tran A, Di Martino V, Causse X, Dao T, Lucidarme D, Portal I, Cacoub P, Gournay J, Grando-Lemaire V, Hillon P, Attali P, Fontanges T, Rosa I, Petrov-Sanchez V, Barthe Y, Pawlowsky JM, Pol S, Carrat F, Bronowicki JP. Effectiveness of telaprevir or boceprevir in treatment-experienced patients with HCV genotype 1 infection and cirrhosis. *Gastroenterology* 2014; **147**: 132-142.e4 [PMID: 24704719 DOI: 10.1053/j.gastro.2014.03.051]
- 9 **Bruno S**, Vierling JM, Esteban R, Nyberg LM, Tanno H, Goodman Z, Poordad F, Bacon B, Gottesdiener K, Pedicone LD, Albrecht JK, Brass CA, Thompson S, Burroughs MH. Efficacy and safety of boceprevir plus peginterferon-ribavirin in patients with HCV G1 infection and advanced fibrosis/cirrhosis. *J Hepatol* 2013; **58**: 479-487 [PMID: 23183529 DOI: 10.1016/j.jhep.2012.11.020]
- 10 **Colombo M**, Strasser S, Moreno C, Abrao Ferreira P, Urbanek P, Fernández I, Abdurakmonov D, Streinu-Cercel A, Verheyen A, Iraqi W, DeMasi R, Hill A, Lonjon-Domanec I, Wedemeyer H. Sustained virological response with telaprevir in 1,078 patients with advanced hepatitis C: the international telaprevir access program. *J Hepatol* 2014; **61**: 976-983 [PMID: 24946280 DOI: 10.1016/j.jhep.2014.06.005]
- 11 **Bonnet D**, Guivarch M, Bérard E, Combis JM, Remy AJ, Glibert A, Payen JL, Metivier S, Barange K, Desmorat H, Palacin A, Nicot F, Abravanel F, Alric L. Telaprevir- and boceprevir-based tritherapies in real practice for F3-F4 pretreated hepatitis C virus patients. *World J Hepatol* 2014; **6**: 660-669 [PMID: 25276282 DOI: 10.4254/wjh.v6.i9.660]
- 12 **Perry CM**. Telaprevir: a review of its use in the management of genotype 1 chronic hepatitis C. *Drugs* 2012; **72**: 619-641 [PMID: 22439668 DOI: 10.2165/11208370-000000000-00000]
- 13 **Ferenci P**, Caruntu FA, Lengyel G, Messinger D, Bakalos G, Flisiak R. Boceprevir Plus Peginterferon Alfa-2a/Ribavirin in Treatment-Naïve Hepatitis C Virus Genotype 1 Patients: International Phase IIIb/IV TriCo Trial. *Infect Dis Ther* 2016; **5**: 113-124 [PMID: 27228998 DOI: 10.1007/s40121-016-0110-5]

**P- Reviewer:** Abd El-Wahab EW, Li J, Lawless MW, Tang ZH

**S- Editor:** Gong XM **L- Editor:** A **E- Editor:** Li D







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 August 18; 8(23): 957-998





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verducci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**EDITORIAL**

- 957 Adjuvant sorafenib in hepatocellular carcinoma: A cautionary comment of STORM trial  
*Zhong JH, Du XK, Xiang BD, Li LQ*

**REVIEW**

- 961 Dynamics of hepatic and intestinal cholesterol and bile acid pathways: The impact of the animal model of estrogen deficiency and exercise training  
*Lavoie JM*

**ORIGINAL ARTICLE****Basic Study**

- 976 Interplay between microRNA-17-5p, insulin-like growth factor- II through binding protein-3 in hepatocellular carcinoma  
*Habashy DA, El Tayebi HM, Fawzy IO, Hosny KA, Esmat G, Abdelaziz AI*
- 985 Reversal of multidrug resistance of hepatocellular carcinoma cells by metformin through inhibiting *NF- $\kappa$ B* gene transcription  
*Wu W, Yang JL, Wang YL, Wang H, Yao M, Wang L, Gu JJ, Cai Y, Shi Y, Yao DF*

**CASE REPORT**

- 994 Metastatic recurrence to a solitary lymph node four years after hepatic lobectomy for primary hepatocellular carcinoma  
*Caparelli ML, Roberts NJ, Braverman TS, Stevens RM, Broun ER, Allamaneni S*

## Contents

*World Journal of Hepatology*  
Volume 8 Number 23 August 18, 2016

### ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Oliver Ebert, MD, Attending Doctor, II. Medizinische Klinik und Poliklinik, Klinikum rechts der Isar, Technical University of Munich, D-81675 Muenchen, Germany

### AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

### FLYLEAF

I-IV Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
August 18, 2016

#### COPYRIGHT

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

#### SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

#### INSTRUCTIONS TO AUTHORS

<http://www.wjgnet.com/bpg/gerinfo/204>

#### ONLINE SUBMISSION

<http://www.wjgnet.com/esps/>



## Adjuvant sorafenib in hepatocellular carcinoma: A cautionary comment of STORM trial

Jian-Hong Zhong, Xue-Ke Du, Bang-De Xiang, Le-Qun Li

Jian-Hong Zhong, Bang-De Xiang, Le-Qun Li, Department of Hepatobiliary Surgery, Affiliated Tumor Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China

Xue-Ke Du, Department of Anesthesia, Affiliated Tumor Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China

**Author contributions:** Zhong JH, Du XK and Xiang BD contributed equally to this work; Zhong JH and Du XK designed the study and wrote the manuscript; Zhong JH, Xiang BD and Li LQ analyzed the data from the included studies; all authors reviewed the manuscript and approved publication.

**Supported by** Guangxi Science and Technology Development Projects, No. 14124003-4; Guangxi University of Science and Technology Research Projects, No. KY2015LX056; the Self-Raised Scientific Research Fund of the Ministry of Health of Guangxi Province, Nos. Z2015621, Z2015601, GZZC15-34 and Z2014241; and the Innovation Project of Guangxi Graduate Education, No. YCBZ2015030.

**Conflict-of-interest statement:** The authors declare no conflicts of interest regarding this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Le-Qun Li, MD, Department of Hepatobiliary Surgery, Affiliated Tumor Hospital of Guangxi Medical University, He Di Rd #71, Nanning 530021, Guangxi Zhuang Autonomous Region, China. [xitongpingjia@163.com](mailto:xitongpingjia@163.com)  
**Telephone:** +86-771-5330855  
**Fax:** +86-771-5312000

**Received:** April 29, 2016

Peer-review started: May 4, 2016

First decision: July 4, 2016

Revised: July 6, 2016

Accepted: July 29, 2016

Article in press: August 1, 2016

Published online: August 18, 2016

### Abstract

Recurrence rate of hepatocellular carcinoma (HCC) is very high even after curative surgery, and no postoperative therapies have been definitively shown to prevent HCC recurrence. Sorafenib is proved to be effective for advanced HCC by two large randomized controlled trials in 2008 and 2009. Therefore it stands to reason to expect that adjuvant sorafenib may improve post-surgery outcomes of patients with HCC. However, many questions still exist about the value of sorafenib for patients with HCC after surgery or transarterial chemoembolization. In this editorial, we comprehensively reviewed the safety and efficacy of adjuvant sorafenib for patients with hepatocellular carcinoma after surgery or transarterial chemoembolization. We emphasized the positive and negative role of sorafenib.

**Key words:** Adjuvant; Hepatocellular carcinoma; Tumor recurrence; Sorafenib

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Sorafenib is effective for advanced hepatocellular carcinoma (HCC). However, its positive role as adjuvant therapy for HCC after surgery or transarterial chemoembolization is controversy.

Zhong JH, Du XK, Xiang BD, Li LQ. Adjuvant sorafenib in hepatocellular carcinoma: A cautionary comment of STORM trial. *World J Hepatol* 2016; 8(23): 957-960 Available from:

## INTRODUCTION

Large randomized controlled trials have shown transarterial chemoembolization (TACE)<sup>[1,2]</sup> and sorafenib<sup>[3,4]</sup> monotherapy to extend median overall survival by approximately 3 mo over best supportive care in patients with hepatocellular carcinoma (HCC) in Barcelona Clinic Liver Cancer (BCLC) stage B or C. Though hepatic resection is the mainstay treatment for HCC, tumor recurrence is very high after surgery<sup>[5]</sup>. Therefore it stands to reason to expect that sorafenib may improve post-resection outcomes of patients with multinodular HCC or patients at high risk of HCC recurrence.

## STUDY ANALYSIS

In the recent issue of the *World J Gastroenterol*, Li *et al*<sup>[6]</sup> reported a small retrospective study which enrolled 36 male patients with BCLC stage C HCC after hepatic resection. Twelve patients received resection plus sorafenib while other 24 patients received resection alone. The authors found patients in the resection plus sorafenib group had a significantly longer time-to-tumor progression (TTP) and median overall survival compared to patients in the resection alone group.

However, the phase III placebo-controlled study STORM trial<sup>[7]</sup>, which included 1602 patients from 28 countries with early-stage HCC following surgical resection or local ablation, found that adjuvant sorafenib did not significantly affect recurrence-free survival, time to recurrence or overall survival. The authors concluded that no evidence of clinical benefit exists for adjuvant sorafenib therapy in such patients.

Also, the phase II SPACE trial comparing the efficacy and safety of TACE with or without sorafenib failed to meet its endpoint of prolonging TTP<sup>[8]</sup>. This raises important questions about the use of adjuvant sorafenib in the clinic.

The SPACE trial<sup>[8]</sup>, which involved 307 Asian and non-Asian patients with multinodular HCC in BCLC stage B, showed that the combination of TACE and sorafenib did not significantly increase TTP or overall survival over TACE alone. This negative result adds to another previous study calling into question the clinical benefits of adjuvant sorafenib. A phase III trial involving 458 Asian patients with HCC in stage B or C found that sorafenib did not significantly prolong TTP or overall survival in patients who responded to TACE<sup>[9]</sup>. In addition to non-efficacy, sorafenib add the incidence of adverse events or may worsen outcomes in certain patients<sup>[3,7,10]</sup>.

## REASONS OF NEGATIVE RESULTS

These negative results (Table 1) call for caution in the

adjuvant use of sorafenib. Why the results would be negative when our therapeutic aim shifts from control of established tumor cells to the eradication of occult micrometastases? One reason for caution lies in the mechanism of sorafenib, which inhibits tumor angiogenesis. Preclinical studies suggest that anti-angiogenic therapy can, in principle, increase the likelihood of tumor invasion and spread<sup>[11]</sup>, and that tumor angiogenesis can rapidly recover when anti-angiogenic therapy is halted<sup>[12]</sup>. Another reason for caution is that sorafenib may not be effective against recurrent or metastatic tumors, even if it is effective against primary tumors. The two types of tumors behave differently, and it is possible that recurrent or metastatic tumors are more malignant because they were not eliminated by initial therapy (TACE, resection, ablation). In fact, studies suggest that sorafenib has poor efficacy against intrahepatic metastases (derived from the primary tumor) as well as multicentric tumors arising spontaneously in the residual liver<sup>[7]</sup>.

While previous works strengthens the arguments for re-assessing adjuvant use of sorafenib, some of their results should be interpreted with caution. For example, the findings of Li *et al*<sup>[6]</sup> were based on a very small retrospective study; Lencioni *et al*<sup>[8]</sup> reported that the combination of TACE and sorafenib showed greater benefit in Asian patients than in non-Asian ones, yet median TTP was nearly the same (24 mo) in Asian and non-Asian subgroups as well as the total study population<sup>[8]</sup>. This TTP is substantially longer than the 5.4 mo reported in another phase III trial involving only Asian patients<sup>[9]</sup>.

Lack of efficacy with sorafenib has been attributed to insufficient duration of therapy<sup>[8]</sup>, such as because of delays in starting sorafenib after TACE, as well as to insufficient daily sorafenib doses<sup>[9]</sup>. These explanations seem less likely given that all published phase II or III multicenter randomized controlled trials concur that adjuvant anti-angiogenic agents, including sorafenib, are associated with negative TTP, overall survival, or recurrence-free survival for solid cancers<sup>[7-9,13]</sup>. In fact, a large dosing study involving 1943 patients with non-metastatic renal-cell carcinoma supports the notion that disease-free survival does not depend on treatment duration<sup>[13]</sup>.

## PERSPECTIVE

The growing evidence for lack of adjuvant sorafenib efficacy against HCC<sup>[7-9]</sup>, and substantial evidence against adjuvant anti-angiogenic therapy against solid cancers in general<sup>[13-16]</sup>, should lead clinicians to re-assess their treatment approaches. In this sense, some ongoing trials of adjuvant anti-angiogenic agents for solid cancers (*e.g.*, NCT00908752, NCT01009801) are already terminated.

Nowadays, more and more trials revealed the definite efficacy of postoperative antiviral treatment with nucleot(s)ide analogs for hepatitis B virus-related HCC<sup>[17-19]</sup>. Adjuvant adoptive immunotherapy may also improve recurrence-free and overall survival<sup>[20]</sup>. But more rando-

**Table 1** Adjuvant sorafenib for hepatocellular carcinoma

Ref.	Recruited period	Sample size (T/C)	HCC characteristics	First therapy	Adjuvant therapy	Outcomes
Li <i>et al</i> <sup>[6]</sup> , 2016	2009-2013	12/24	With portal vein thrombus	Hepatic resection	Sorafenib (200-800 mg/d)	TTP, <i>P</i> = 0.041 OS, <i>P</i> = 0.01
Bruix <i>et al</i> <sup>[7]</sup> , 2015	2008-2010	556/558	Early stage HCC	Hepatic resection or ablation	Sorafenib (400 mg) twice a day	RFS, <i>P</i> = 0.26 OS, <i>P</i> = 0.48
Lencioni <i>et al</i> <sup>[8]</sup> , 2016	-	154/153	Intermediate stage multinodular HCC	TACE with doxorubicin-eluting beads	Sorafenib (400 mg) twice a day	TTP, <i>P</i> = 0.07 OS, <i>P</i> = 0.29
Kudo <i>et al</i> <sup>[9]</sup> , 2011	2006-2009	229/227	Unresectable HCC who responded to TACE	Conventional TACE	Sorafenib (400 mg) twice a day	TTP, <i>P</i> = 0.25 OS, <i>P</i> = 0.79

C: Control group; HCC: Hepatocellular carcinoma; OS: Overall survival; RFS: Recurrence-free survival; T: Adjuvant treated group; TACE: Transarterial chemoembolization; TTP: Time-to-tumor progression.

mized trials are warranted because of inconsistent findings from new randomized trials<sup>[21,22]</sup>. For HCC patients with high risk of recurrence, adjuvant TACE has positive effect in terms of improving overall survival<sup>[23]</sup>. However, each postoperative or adjuvant therapy has its own indication, revealing that not all patients with HCC after surgery should receive specific postoperative or adjuvant therapy. New drugs may help further define therapeutic directions for the future.

## REFERENCES

- Llovet JM, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J; Barcelona Liver Cancer Group. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862 DOI: 10.1016/S0140-6736(02)08649-X]
- Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, Fan ST, Wong J. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**: 1164-1171 [PMID: 11981766 DOI: 10.1053/jhep.2002.33156]
- Bruix J, Raoul JL, Sherman M, Mazzaferro V, Bolondi L, Craxi A, Galle PR, Santoro A, Beaugrand M, Sangiovanni A, Porta C, Gerken G, Marrero JA, Nadel A, Shan M, Moscovici M, Voliotis D, Llovet JM. Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma: subanalyses of a phase III trial. *J Hepatol* 2012; **57**: 821-829 [PMID: 22727733 DOI: 10.1016/j.jhep.2012.06.014]
- Cheng AL, Guan Z, Chen Z, Tsao CJ, Qin S, Kim JS, Yang TS, Tak WY, Pan H, Yu S, Xu J, Fang F, Zou J, Lentini G, Voliotis D, Kang YK. Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma according to baseline status: subset analyses of the phase III Sorafenib Asia-Pacific trial. *Eur J Cancer* 2012; **48**: 1452-1465 [PMID: 22240282 DOI: 10.1016/j.ejca.2011.12.006]
- Zhong JH, Ma L, Li LQ. Postoperative therapy options for hepatocellular carcinoma. *Scand J Gastroenterol* 2014; **49**: 649-661 [PMID: 24716523 DOI: 10.3109/00365521.2014.905626]
- Li J, Hou Y, Cai XB, Liu B. Sorafenib after resection improves the outcome of BCLC stage C hepatocellular carcinoma. *World J Gastroenterol* 2016; **22**: 4034-4040 [PMID: 27099447 DOI: 10.3748/wjg.v22.i15.4034]
- Bruix J, Takayama T, Mazzaferro V, Chau GY, Yang J, Kudo M, Cai J, Poon RT, Han KH, Tak WY, Lee HC, Song T, Roayaie S, Bolondi L, Lee KS, Makuuchi M, Souza F, Berre MA, Meinhardt G, Llovet JM; STORM investigators. Adjuvant sorafenib for hepatocellular carcinoma after resection or ablation (STORM): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2015; **16**: 1344-1354 [PMID: 26361969 DOI: 10.1016/S1470-2045(15)00198-9]
- Lencioni R, Llovet JM, Han G, Tak WY, Yang J, Guglielmi A, Paik SW, Reig M, Kim do Y, Chau GY, Luca A, Del Arbol LR, Leberre MA, Niu W, Nicholson K, Meinhardt G, Bruix J. Sorafenib or placebo plus TACE with doxorubicin-eluting beads for intermediate stage HCC: The SPACE trial. *J Hepatol* 2016; **64**: 1090-1098 [PMID: 26809111 DOI: 10.1016/j.jhep.2016.01.012]
- Kudo M, Imanaka K, Chida N, Nakachi K, Tak WY, Takayama T, Yoon JH, Hori T, Kumada H, Hayashi N, Kaneko S, Tsubouchi H, Suh DJ, Furuse J, Okusaka T, Tanaka K, Matsui O, Wada M, Yamaguchi I, Ohya T, Meinhardt G, Okita K. Phase III study of sorafenib after transarterial chemoembolisation in Japanese and Korean patients with unresectable hepatocellular carcinoma. *Eur J Cancer* 2011; **47**: 2117-2127 [PMID: 21664811 DOI: 10.1016/j.ejca.2011.05.007]
- Zhong JH. The STORM trial and beyond: narrowing the horizon of adjuvant sorafenib for postoperative hepatocellular carcinoma. *Tumour Biol* 2015; **36**: 8271-8272 [PMID: 26499777 DOI: 10.1007/s13277-015-4279-0]
- Pàez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Viñals F, Inoue M, Bergers G, Hanahan D, Casanovas O. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell* 2009; **15**: 220-231 [PMID: 19249680 DOI: 10.1016/j.ccr.2009.01.027]
- Mancuso MR, Davis R, Norberg SM, O'Brien S, Sennino B, Nakahara T, Yao VJ, Inai T, Brooks P, Freemark B, Shalinsky DR, Hu-Lowe DD, McDonald DM. Rapid vascular regrowth in tumors after reversal of VEGF inhibition. *J Clin Invest* 2006; **116**: 2610-2621 [PMID: 17016557 DOI: 10.1172/JCI24612]
- Haas NB, Manola J, Uzzo RG, Flaherty KT, Wood CG, Kane C, Jewett M, Dutcher JP, Atkins MB, Pins M, Wilding G, Cella D, Wagner L, Matin S, Kuzel TM, Sexton WJ, Wong YN, Choueiri TK, Pili R, Puzanov I, Kohli M, Stadler W, Carducci M, Combes R, DiPaola RS. Adjuvant sunitinib or sorafenib for high-risk, non-metastatic renal-cell carcinoma (ECOG-ACRIN E2805): a double-blind, placebo-controlled, randomised, phase 3 trial. *Lancet* 2016; **387**: 2008-2016 [PMID: 26969090 DOI: 10.1016/S0140-6736(16)00559-6]
- Kudo M, Han G, Finn RS, Poon RT, Blanc JF, Yan L, Yang J, Lu L, Tak WY, Yu X, Lee JH, Lin SM, Wu C, Tanwandee T, Shao G, Walters IB, Dela Cruz C, Poulart V, Wang JH. Brivanib as adjuvant therapy to transarterial chemoembolization in patients with hepatocellular carcinoma: A randomized phase III trial. *Hepatology* 2014; **60**: 1697-1707 [PMID: 24996197 DOI: 10.1002/hep.27290]
- Cameron D, Brown J, Dent R, Jackisch C, Mackey J, Pivov X, Steger GG, Suter TM, Toi M, Parmar M, Laeufle R, Im YH, Romieu G, Harvey V, Lipatov O, Pienkowski T, Cottu P, Chan A, Im SA, Hall PS, Bubuteishvili-Pacaud L, Henschel V, Deurloo RJ, Pallaud C, Bell R. Adjuvant bevacizumab-containing therapy in triple-negative breast cancer (BEATRICE): primary results of a randomised, phase 3 trial. *Lancet Oncol* 2013; **14**: 933-942 [PMID: 23611111 DOI: 10.1016/S1473-3099(13)70750-9]

- 23932548 DOI: 10.1016/S1470-2045(13)70335-8]
- 16 **de Gramont A**, Van Cutsem E, Schmoll HJ, Tabernero J, Clarke S, Moore MJ, Cunningham D, Cartwright TH, Hecht JR, Rivera F, Im SA, Bodoky G, Salazar R, Maindrault-Goebel F, Shacham-Shmueli E, Bajetta E, Makrutzki M, Shang A, André T, Hoff PM. Bevacizumab plus oxaliplatin-based chemotherapy as adjuvant treatment for colon cancer (AVANT): a phase 3 randomised controlled trial. *Lancet Oncol* 2012; **13**: 1225-1233 [PMID: 23168362 DOI: 10.1016/S1470-2045(12)70509-0]
- 17 **Zhong JH**, Ma L, Li LQ. Postoperative Antiviral Therapy With Nucleos(t)ide Analogs in Patients With Hepatitis B Virus-related Hepatocellular Carcinoma. *Ann Surg* 2015; Epub ahead of print [PMID: 25822679 DOI: 10.1097/SLA.0000000000001224]
- 18 **Huang G**, Lau WY, Wang ZG, Pan ZY, Yuan SX, Shen F, Zhou WP, Wu MC. Antiviral therapy improves postoperative survival in patients with hepatocellular carcinoma: a randomized controlled trial. *Ann Surg* 2015; **261**: 56-66 [PMID: 25072444 DOI: 10.1097/SLA.0000000000000858]
- 19 **Yin J**, Li N, Han Y, Xue J, Deng Y, Shi J, Guo W, Zhang H, Wang H, Cheng S, Cao G. Effect of antiviral treatment with nucleotide/nucleoside analogs on postoperative prognosis of hepatitis B virus-related hepatocellular carcinoma: a two-stage longitudinal clinical study. *J Clin Oncol* 2013; **31**: 3647-3655 [PMID: 24002499 DOI: 10.1200/JCO.2012.48.5896]
- 20 **Zhong JH**, Ma L, Wu LC, Zhao W, Yuan WP, Wu FX, Zhang ZM, Huang S, You XM, Li LQ. Adoptive immunotherapy for postoperative hepatocellular carcinoma: a systematic review. *Int J Clin Pract* 2012; **66**: 21-27 [PMID: 22171902 DOI: 10.1111/j.1742-1241.2011.02814.x]
- 21 **Xu L**, Wang J, Kim Y, Shuang ZY, Zhang YJ, Lao XM, Li YQ, Chen MS, Pawlik TM, Xia JC, Li SP, Lau WY. A randomized controlled trial on patients with or without adjuvant autologous cytokine-induced killer cells after curative resection for hepatocellular carcinoma. *Oncoimmunology* 2016; **5**: e1083671 [PMID: 27141337 DOI: 10.1080/2162402X.2015.1083671]
- 22 **Lee JH**, Lee JH, Lim YS, Yeon JE, Song TJ, Yu SJ, Gwak GY, Kim KM, Kim YJ, Lee JW, Yoon JH. Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. *Gastroenterology* 2015; **148**: 1383-91.e6 [PMID: 25747273 DOI: 10.1053/j.gastro.2015.02.055]
- 23 **Zhong JH**, Li LQ. Postoperative adjuvant transarterial chemembolization for participants with hepatocellular carcinoma: A meta-analysis. *Hepatol Res* 2010; **40**: 943-953 [PMID: 20887328 DOI: 10.1111/j.1872-034X.2010.00710.x]

**P- Reviewer:** Hernanda PY, Takeda H, Tarazov PG  
**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Li D





## Dynamics of hepatic and intestinal cholesterol and bile acid pathways: The impact of the animal model of estrogen deficiency and exercise training

Jean-Marc Lavoie

Jean-Marc Lavoie, Department of Kinesiology, University of Montreal, Montreal, Québec H3C 3J7, Canada

**Author contributions:** Lavoie JM performed all the work of this manuscript.

**Supported by** The Natural Sciences and Engineering Research Council of Canada, No. NSERC 7594.

**Conflict-of-interest statement:** The author declares that he has no competing interests.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Jean-Marc Lavoie, PhD, Professor, Department of Kinesiology, University of Montreal, CP 6128, Succ "Centre-ville", Montreal, Québec H3C 3J7, Canada. [jean-marc.lavoie@umontreal.ca](mailto:jean-marc.lavoie@umontreal.ca)  
 Telephone: +1-514-3437044

**Received:** March 20, 2016

**Peer-review started:** March 22, 2016

**First decision:** May 19, 2016

**Revised:** May 25, 2016

**Accepted:** July 14, 2016

**Article in press:** July 18, 2016

**Published online:** August 18, 2016

dynamics that involves transport lipoproteins which levels are tightly dependent on how the liver and the intestine regulate cholesterol and biliary acid metabolism. Regulation of cholesterol and biliary acids by the liver and the intestine is in turn coupled to a large array of enzymes and transporters that largely influence the inflow and the outflow of cholesterol and biliary acids through these organs. The activity of the key regulators of cholesterol and biliary acids may be influenced by several external factors such as pharmacological drugs and the nutritional status. In recent years, more information has been gathered about the impact of estrogens on regulation of cholesterol in the body. Exposure to high levels of estrogens has been reported to promote cholesterol gallstone formation and women are twice as likely as men to develop cholesterol gallstones. The impact of estrogen withdrawal, such as experienced by menopausal women, is therefore of importance and more information on how the absence of estrogens influence cholesterol regulation is started to come out, especially through the use of animal models. An interesting alternative to metabolic deterioration due to estrogen deficiency is exercise training. The present review is intended to summarize the present information that links key regulators of cholesterol and biliary acid pathways in liver and intestine to the absence of estrogens in an animal model and to discuss the potential role of exercise training as an alternative.

**Key words:** PSCK9; Low-density lipoprotein receptor; Very low-density lipoprotein; Sterol regulatory element binding proteins; Ovariectomy; High-density lipoprotein; Lipoproteins

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

### Abstract

Plasma cholesterol level is determined by a complex

**Core tip:** The liver is considered the master piece in regulation of plasma cholesterol levels. Together with

the intestine they control the influx and the efflux of cholesterol and biliary acids in the body. Cholesterol and its conversion into biliary acids are regulated by an extended network of enzymes and transporters that largely influence plasma cholesterol levels. The key regulators of cholesterol and biliary acids in liver and intestine are in turn affected by several factors including estrogens levels and more recently exercise training. Low estrogenic levels, such as seen in post-menopausal women, are associated with higher plasma cholesterol levels. In recent years more information has been accumulated on the extent to which low estrogenic levels, such as seen in an ovariectomized animal model, influence cholesterol and biliary metabolism at the molecular level. As an alternative to a deficiency in estrogens, exercise training has been reported to exert a beneficial effect on these key regulators of cholesterol and biliary acids.

Lavoie JM. Dynamics of hepatic and intestinal cholesterol and bile acid pathways: The impact of the animal model of estrogen deficiency and exercise training. *World J Hepatol* 2016; 8(23): 961-975 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i23/961.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i23.961>

## INTRODUCTION

The importance of estrogens in regulating cholesterol and biliary acid metabolism in liver is enlightened by clinical studies confirming that women are twice as likely as men to develop cholesterol gallstones<sup>[1,2]</sup>. Oversaturation of biliary cholesterol is the requisite defect for the formation of gallstones<sup>[1]</sup>. This pathophysiological state is induced by either hypersecretion of biliary cholesterol or decreased secretion of bile acids. Therefore, both the cholesterol secreted into bile and the bile acids synthesized from cholesterol in liver are involved in the disease<sup>[3]</sup>. Exposure to high levels of estrogens has been reported to promote cholesterol gallstone formation<sup>[4]</sup>. Similarly, the estrogen receptor  $\alpha$ -selective agonist propylpyrazole and tamoxifen treatment, that have estrogen-like activity, augment biliary cholesterol secretion in mice<sup>[4]</sup> and increase gallstone prevalence in women<sup>[5]</sup>. On the whole, these findings indicate that there is a close relationship between estrogens, cholesterol and biliary acid metabolism in liver. This in turn raises the question of the extent to which a deficiency in estrogens, as happens with menopause, affects cholesterol and biliary acid regulation in liver. The first element to take into consideration is the fact that estrogen withdrawal in animals decreases gene expression of HMGCoA-reductase (-r), the rate-limiting enzyme in hepatic cholesterol biosynthesis<sup>[6]</sup>.

Estrogen-deficient state in Ovx animals has been repeatedly reported to result in substantial liver fat accumulation indicating that fat metabolism is perturbed

by the absence of estrogens<sup>[7,8]</sup>. The information on the impact of the absence of estrogens on cholesterol metabolism, however, is scarce. An increase in plasma cholesterol levels in Ovx rats has been reported 30 years ago<sup>[9,10]</sup>. This has been confirmed in more recent studies in Ovx animals<sup>[6,11]</sup> as well as in post-menopausal women<sup>[12]</sup>. The situation of liver cholesterol levels in Ovx animals is more controversial. Liver total cholesterol level was reported not to be affected by estrogen withdrawal in some studies<sup>[11,13]</sup> while it has been found to be increased in rats ovariectomized for 5-8 wk<sup>[14,15]</sup>. Large cholesterol accumulation has also been found in liver of Ovx rats fed with a high-fat diet, that was not observed in liver of Ovx rats fed a standard diet and in Sham rats fed a high-fat diet<sup>[6]</sup>. The authors suggested a vulnerability to cholesterol accumulation in liver of Ovx animals fed a high fat diet. These findings have, at least, the merit of raising questions on the impact of the lack of estrogens on regulatory pathways involved in liver cholesterol metabolism. Cholesterol homeostasis in liver depends on cholesterol synthesis, uptake, and clearance. One of the aims of the present review is to summarize our present knowledge of the extent to which the lack of estrogens in an ovariectomized animal model affects the regulation of molecular pathways of cholesterol and bile acids in liver and intestine.

One of the best non-pharmacological strategies for the treatment of metabolic disturbances leading to coronary artery disease is exercise training<sup>[16,17]</sup>. In recent years, there has been a fair amount of studies indicating that exercise training is also beneficial in circumventing the detrimental effects of estrogen removal on metabolic pathways involved in liver fat accumulation<sup>[18]</sup>. Treadmill exercise for 12 wk has also been reported to reduce plasma low density lipoprotein (LDL)-cholesterol (-C) and total cholesterol in Ovx rats<sup>[19]</sup> while plasma LDL-C was decreased in 6-wk trained Ovx rats fed a high fat diet for 10 mo<sup>[20]</sup>. Although limited, there is recent information on the impact of exercise training on regulation of cholesterol pathways in liver and intestine in response to metabolic disturbances. For instance there are reports indicating an increased fecal cholesterol excretion in exercising animals<sup>[21]</sup>. There is also a recent report of changes with exercise training in gene expression of intestinal nuclear receptors involved in the defense system against endobiotic and xenobiotic insults suggesting that regular exercise contributes to the intestinal maintenance of cholesterol and bile acid homeostasis<sup>[22]</sup>. In the present review, a consideration will be given to the effects of exercise training on cholesterol and bile acids pathways, especially in the context of estrogens deficiency.

The present review is divided in two large sections related respectively to the pathways involved into hepatic cholesterol influx and efflux and how estrogen deficiency affects key regulators of these pathways. This will be followed by a discussion of the known effects of exercise training on these pathways.

## HEPATIC CHOLESTEROL INFLUX

Western-type diets provide approximately 400 mg of cholesterol per day while our body synthesizes approximately 1 g *de novo*<sup>[23,24]</sup>. Hence, blood cholesterol levels reflect both dietary and endogenously synthesized cholesterol. The liver is a central component in regulation of cholesterol metabolism. This organ is able to acquire cholesterol through *de novo* synthesis and from all classes of circulating lipoproteins<sup>[25]</sup>.

### Cholesterol biosynthesis

The total body content of cholesterol is approximately 100 g, of which approximately 90% are found at the cellular levels and 10% in circulation<sup>[26]</sup>. Cholesterol is synthesised virtually in all nucleated cells<sup>[27]</sup>. For instance, the central nervous system contains approximately 25% of the unesterified cholesterol present in the body and it comes almost entirely for *in situ* synthesis<sup>[28]</sup>. It is assumed that approximately 24% of cholesterol synthesis occurs in small intestine of rats and a significant fraction of it is transported to liver where nearly 50% of total cholesterol synthesis occurs<sup>[29]</sup>. Cholesterol synthesis starts, similarly to *de novo* lipogenesis, by the transfer of acetyl CoA from mitochondria to cytosol. The further condensation of three units of acetyl CoA forms an HMG-CoA that is transported to the endoplasmic reticulum (ER) where it is reduced to mevalonate by the enzyme HMGCoA-r follows by several steps leading to the formation of isoprene, squalene, lanosterol, and finally cholesterol. The action of the enzyme HMGCoA-r is the rate-limited step in endogenous cholesterol synthesis.

**Regulation of cholesterol biosynthesis:** The view that cholesterol is randomly distributed within cell membrane no longer holds. For instance the distribution of lipids and cholesterol in the outer leaflet is organized into domains so-called rafts and caveolae playing intricate roles to maintain cellular homeostasis<sup>[30,31]</sup>. On the other hand, membranes of the endoplasmic reticulum and the Golgi apparatus contain comparatively little cholesterol, an important factor in cholesterol homeostasis<sup>[32]</sup>. Maintenance of cholesterol homeostasis is orchestrated mainly by a feedback regulatory system that senses the level of cholesterol in cell membranes and modulates cholesterol biosynthesis and uptake from plasma lipoproteins<sup>[33]</sup>. The molecular mechanism of how hepatocytes maintain cholesterol homeostasis has become more precise with the discovery of the transcription factors sterol regulatory element binding proteins (SREBPs)<sup>[32]</sup>.

Short-term regulation of the enzyme HMGCoA-r is operated by mechanisms such as phosphorylation/dephosphorylation of the catalytic domain (serine 871) by specific kinases (AMPK) and phosphatases (protein phosphatase 2A)<sup>[34,35]</sup>. HMGCoA-r is physiologically present in the cell in unphosphorylated active form (30%) and phosphorylated inactive form (70%)<sup>[36]</sup>.

Long-term regulation of HMGCoA-r relies on synthesis and degradation rate of the enzyme. The cholesterol system is unique in that the regulated end-product, cholesterol, is sequestered entirely within cell membranes. Sterol regulatory elements (SREs) are nucleotidic sequences in the gene promoters, encoding proteins involved in cholesterol homeostasis such as HMGCoA-r and LDL receptor (LDL-R). These sequences are recognized by a family of transcription factors called SREBP<sup>[37]</sup>. The SREBP family members, SREBP-1 (a and c) and SREBP-2, are synthesized as membrane protein in the endoplasmic reticulum.

SREBP-2 is considered to be largely involved in the regulation of cholesterol metabolism. In ER, SREBP interacts with a cargo protein called SREBP cleavage-activated protein (SCAP), which acts as a transporter and cholesterol sensor<sup>[37,38]</sup>. The complex formation is essential for the exit of SREBPs from the ER and subsequent proteolytic activation<sup>[39]</sup>. The SREBP/SCAP containing vesicles from the ER also contain a membrane anchored serine protease of the subtilisin family called Site-1 protease (SIP-1). Sip becomes activated only during its transport to the Golgi<sup>[40]</sup>. SCAP escorts SREBP from the ER to the Golgi apparatus where the SREBPs are proteolytically processed by SIP-1 to yield active fragments that migrate to the nucleus encoding its target genes<sup>[33]</sup>. To release active SREBP, another enzyme is required, Site-2 protease. Interestingly, the nuclear action of SREBP induces new SREBP mRNA through SREs located in the promoter regions of their own genes<sup>[41]</sup>. When cholesterol builds up in the ER membrane, a conformational change in SCAP occurs through the direct cholesterol binding to the sterol domain and triggers SCAP to bind to Insig, another ER membrane protein<sup>[42]</sup>. This association hampers the transport of the SREBP/SCAP complex to the Golgi apparatus, resulting in a reduced proteolytic activation of precursor SREBP. For instance, high dietary cholesterol prevents maturation of SREBPs and cuts off cholesterol and LDL receptor synthesis.

**Estrogen deficiency and HMGCoA-r regulation:** Since plasma cholesterol level is increased in Ovx animals<sup>[6,11]</sup> one might expect an increase in cholesterol synthesis. However, HMGCoA-r mRNA secondary to Ovx was found to be decreased in several studies in rats<sup>[6,11,43,44]</sup> and in mice fed a high-fat high-cholesterol diet<sup>[13]</sup>. Along with HMGCoA-r, gene expression of SREBP-2, the transcription factor involved in the regulation of HMGCoA-r, was also decreased in Ovx animals<sup>[14,43]</sup>. On the opposite, an increase in HMGCoA-r protein content has been reported in frog and rat after 5 d of estrogen administration<sup>[45,46]</sup>. On the whole these results strongly suggest that an increased cholesterol biosynthesis is not responsible for the increased higher plasma cholesterol found with estrogen deficiency in animals and in post-menopausal women. They also suggest an accumulation of cholesterol in the ER membrane.

### Receptors involved in hepatic uptake of cholesterol from lipoproteins

**Lipoprotein remnant receptors:** Upon completion of hydrolysis (approximately 50% of TG removal) chylomicrons and VLDL lose affinity for lipoprotein lipase (LPL) and dissociate<sup>[47]</sup>. The apoproteins A1 and C are then transferred to high-density lipoprotein (HDL) in exchange for apo E upon what they are then called chylomicrons and VLDL remnants<sup>[48,49]</sup>. The acquisition of apo E is crucial since it will serve eventually as ligands for receptor mediated clearance. Intermediate density lipoproteins (IDL) which are VLDLs that interact for prolonged period with LPL are also remnants particles. The remnant lipoproteins are then small enough to enter the space of Disse. Once into the space of Disse, remnant lipoproteins small enough to fit between the endothelial cells are sequestered by high-molecular-weight heparin proteoglycan (HSPG) molecules. Within the space of Disse the particles are remodeled by hepatic lipase. Final uptake by the hepatocytes is receptor mediated that include LDL-R, LDL related protein (LRP), a complex LRP-HSPG or HSPG alone<sup>[25,50]</sup>. These mechanisms are efficient so that half-life of remnants in plasma is 30 min. The apoB-48 containing chylomicron remnants are completely cleared from the plasma. However the presence of apoB-100 in VLDL alters their metabolism so that only 50% of VLDL remnants are cleared by lipoproteins remnant receptors.

**Receptors involved in hepatic uptake of LDL-cholesterol:** VLDL remnants that are not taken up by the remnant receptors are metabolized to a greater extent by LPL, become increasing smaller, relatively deficient in TG and enriched in cholesterol esters. These particles are called IDL. Because IDL contains apoE a fraction of these particles may be taken up by the liver through the remnant receptors<sup>[51]</sup>. However, the remainder will be changed to LDL following further hydrolysis of the TG by the hepatic lipase. The apoE and apoC- II molecules will then transfer to HDL and leave apoB as their only apolipoprotein<sup>[52]</sup>. The LDL-R is the only receptor able to clear up LDL from the circulation. Because of the lack of apoE, the LDL particle is a relatively weak ligand for the LDL receptor<sup>[53]</sup>. As a result, the half-life of the LDL particle is relatively long (two to four days) thus accounting for 65%-75% of total plasma cholesterol. Interaction of apoB with the LDL-R facilitates the internalisation and the further degradation of LDL<sup>[53]</sup>. Inside the cell, the LDL particle is hydrolysed to release unesterified cholesterol. The LDL-R is expressed on the cell surface of several tissues including liver, macrophages, lymphocytes, adrenal cortex, gonads, and smooth muscle<sup>[25]</sup>.

**Metabolism of the LDL-R:** The LDL-R is a cell surface receptor that mediates specific uptake and catabolism of plasma lipoproteins containing apoB or apoE<sup>[53]</sup>. The primary function of this receptor is the removal of highly atherogenic LDL particles from circulation<sup>[53]</sup>. Since the liver contains approximately 70% of total

LDL-R found in the body<sup>[54]</sup>, hepatic LDL-R activity is an important contributor to regulation of plasma cholesterol LDL levels. The LDL-R activity is downregulated post-transcriptionally by a protease, proprotein convertase subtilisin kexin type 9 (PCSK9)<sup>[55]</sup>. PCSK9 is highly expressed in liver and intestine<sup>[56]</sup>. However, circulating PCSK9 originates exclusively from hepatocytes<sup>[57]</sup>. The gene expressions of LDL-R and PCSK9 as well as HMGCoA-r are regulated by a transcription factor, SREBP-2<sup>[58]</sup>. Within the endoplasmic reticulum, PCSK9 undergoes an auto catalytic cleavage<sup>[56]</sup> that results in a tightly bound secretable heterodimeric complex<sup>[59]</sup>. PCSK9 is, therefore, readily measured in plasma. PCSK9 binds to the LDL-R at the surface of the hepatocytes and/or within the cell<sup>[60]</sup>. LDL-R is then directed from the cell surface recycling toward degradation in the endosome/lysosome pathway<sup>[61]</sup>. Mutations leading to a loss of function or genetic invalidation of PCSK9 largely reduce circulating LDL-C levels and reduce cardiovascular events (88%) in humans (for a review see<sup>[60]</sup>).

The co-regulation of PCSK9 and HMGCoA-r by the same transcription factor has consequences. As discussed by Poirier *et al*<sup>[60]</sup>, statins that lower LDL-C by inhibiting HMGCoA-r also increase the expression of PCSK9<sup>[62]</sup> which decreases their capacity at increasing LDL-R. This may explain why LDL-C levels do not reach therapeutic goals in many patients with statins therapy. Hepatocyte nuclear factor 1 alpha, a key mediator of the effects of bile acids on gene expression, also regulates PCSK9<sup>[63]</sup>.

**Estrogen deficiency and LDL-R:** In line with the reduction in HMGCoA-r, gene expression of hepatic LDL-R has been repeatedly reported to be reduced in Ovx animals<sup>[11,13,14,43,64]</sup>. Along with LDL-R, PCSK9 transcripts in liver and PCSK9 plasma levels have also been shown to be reduced in Ovx rats<sup>[14]</sup>. These results concord with the reports that estrogens administration upregulates *LDL-R* gene expression in rat liver<sup>[46,65]</sup>. In a recent study, Roubtsova *et al*<sup>[66]</sup> showed, using PCSK9 KO mice, that the interaction between PCSK9 and LDL-R was sex-specific, thus depending on estrogens. The similar decrease in PCSK9 and LDL-R in Ovx animals is, however, puzzling considering that a decrease in PCSK9 should lead to an increase in LDL-R. It has been proposed that the rate of cycling of hepatic LDL-R on cell surface might be an explanation. When hepatic cholesterol increases, as it is observed in Ovx animals<sup>[11,14]</sup>, the transcriptional regulation of PCSK9 and LDL-R both mediated by SREBP-2 would be inhibited, and the rate of cycling of the hepatic LDL-R slowed down leading to higher levels in circulating LDL-cholesterol. The transcriptional regulation of the LDL-R is, however, paradoxical since SREBP-2 also regulates the transcription of PCSK9, thus leading to two opposing effects initiated by the same signal. In a recent publication, Starr *et al*<sup>[67]</sup> proposed a more dynamic role for PCSK9, suggesting that phosphorylated PCSK9 promotes degradation of LDL-R, whereas nonphosphorylated PCSK9 is in an LDL-R-protective state. Taken together, these results emphasize



the need to a better understanding of the sex specific interaction between LDL-R and PCSK9, especially in view of a new class of cholesterol lowering drugs, the PCSK9 inhibitors<sup>[68]</sup>.

**Metabolism of the LRP1 receptor:** LRP1 is a member of the *LDL-R* gene family which also includes receptors such as LRP2 (megalin), LRP8 (apoE receptor 2), and the VLDL receptor (VLDLR)<sup>[69]</sup>. LRP1 is expressed in several types of cells including hepatocytes, fibroblasts, smooth muscle cells, and neurons<sup>[70]</sup>. This transmembrane protein displays both scavenging and signaling functions. LRP1 mediates removal of at least 30 different ligands, including VLDL remnants or IDL and chylomicron remnants from the circulation<sup>[71]</sup>, but also several molecules unrelated to lipid homeostasis including proteases, protease inhibitor complexes, extracellular matrix proteins, growth factors, toxins, and viral proteins<sup>[72]</sup>. LRP1 also acts as an endocytic receptor for several intracellular proteins released by necrotic cells, which failure to be efficiently cleared may be associated with the onset of autoimmune disease<sup>[73,74]</sup>. Interestingly, LRP1, by regulating cell signaling through several mechanisms, may change the activity of other receptors by controlling the abundance of these receptors in the plasma membrane<sup>[75]</sup>. For instance, disruption of the LRP gene in adult normal mice resulted in a compensatory upregulation of the LDL-R in the liver<sup>[76]</sup>.

The gene expression of LRP1 is complex and appears to be regulated by hormones and growth factors<sup>[77]</sup>. LRP1, as well as other members of the LDL-R family, are bound by a molecule called receptor-associated protein (RAP) that blocks the bindings of ligands to these receptors<sup>[78]</sup>. RAP functions as a molecular chaperone that assists in the trafficking of the LRP1 to the cell surface<sup>[79]</sup>. In different tissues, LRP1 gene expression has been reported to be affected by factors such as hypercholesterolemia, lipopolysaccharides, growth factors, and hypoxia (for a review see<sup>[80]</sup>). Hepatic LRP1 expression has been reported to be negatively associated with intracellular cholesterol level and positively associated with expression of SREBP-2<sup>[81]</sup>. On the whole, LRP1 may be seen as a complex biosensor allowing the cells to answer to micro-environmental variations<sup>[80]</sup>.

**Estrogen deficiency and LRP1 receptor:** A reduction in gene expression of LRP1 in Ovx rats was first reported by Ngo Sock *et al*<sup>[14]</sup> and confirmed in recent studies at the protein levels<sup>[15]</sup>. This decrease in LRP1 in Ovx animals may be associated with the decrease in the SREBP-2 transcription factor<sup>[81]</sup>. Interestingly, it has been recently reported that LRP1 is also a target for PCSK9 in HepG2 cells<sup>[82]</sup>. These authors postulated that LDL-R can effectively compete with LRP1 for PCSK9 activity. A reduction in *LRP1* gene expression could contribute to the increase in plasma cholesterol in Ovx rats by reducing the uptake of circulating lipoprotein remnants. Finally, inducible degrader of the low-density lipoprotein receptor an ubiquitin ligase that also me-

diates the degradation of the LDL-R was found not to be affected by an ovariectomy<sup>[66]</sup>.

### VLDLR

In addition to LRP, the LDL-R gene family includes a further member that functions as receptor for VLDL<sup>[83]</sup>. The VLDLR is expressed in several tissues including heart, muscle, adipose tissue, and macrophages but barely detectable in liver under normal conditions<sup>[83,84]</sup>. This receptor has been suggested to be important for the metabolism of apoE-containing triacylglycerol-rich lipoproteins, such as VLDL and IDL.

Interestingly, circulating PCSK9 originating from liver can regulate VLDLR in adipose tissue, which tissue does not express PCSK9<sup>[57]</sup>. In that manner, the absence of circulating PCSK9 resulted in an increase in the level of surface of VLDLR in the perigonadal tissue<sup>[57]</sup>. Interestingly, the increase was 10 times higher in female than in male mice<sup>[57]</sup>. This response was in line with the typical female pattern in mice that implies a high surface VLDLR levels in perigonadal fat and low surface LDLR levels in hepatocytes<sup>[66]</sup>.

### Hepatic cholesterol uptake from HDL

HDL is a class of lipoproteins that is able to remove excess cholesterol from cells and transport it through plasma to the liver. The apoA1 is the major structural determinant of HDL. It is involved in the formation as well as in the interaction with its receptor, scavenger receptor class B, type 1 (SR-B1)<sup>[85]</sup>. HDL formation occurs mainly in the liver and to a lesser extent in the intestine<sup>[85]</sup>. The events start when lipid-poor apoA1 is secreted by the liver or the intestine<sup>[86]</sup> or dissociates from lipoprotein particles in the plasma<sup>[87]</sup>. ApoA1 interacts with the membrane-embedded ATP binding cassette A1 (ABCA1) and incorporates small amount of phospholipids and unesterified cholesterol into the apoA1 molecule<sup>[88]</sup>. Maturation of these pre $\beta$ HDL in the plasma occurs due to two enzymes, lecithin: Cholesterol acyl transferase (LCAT) that esterifies cholesterol and phospholipid transfer protein (PLTP) that transfers phospholipids from remnant particles to HDL.

HDLs have the ability of removing excess cholesterol from cells. The first mechanism involved the action of pre $\beta$ HDL interacting with ABCA1 that in addition of forming a new HDL by the liver is used to remove excess cholesterol from macrophages<sup>[89]</sup>. Spherical mature HDL may remove cholesterol from cells using several mechanisms. The particle may interact with SR-B1 on the plasma membrane. Macrophages also express ABCG1 transporters that mediate transfer of excess cholesterol to HDL. Finally excess cholesterol from cells may also efflux in absence of binding to transport protein, travels short distance through plasma and be taken up by HDL<sup>[25]</sup>. The activity of LCAT and PLTP prevents the HDL from being saturated with cholesterol. The enzyme cholesterol ester transfer protein (CETP) that transfer cholesteryl ester molecules from HDL to remnant particles in exchange for TG also increases the capacity of HDL to accept

unesterified cholesterol from cells.

HDLs circulating to the liver interact with SR-B1 the main HDL receptor<sup>[90]</sup>. SR-B1 in the liver facilitates the uptake of cholesterol and cholesterol esters from the HDL particle without the apoA1<sup>[86]</sup>. ApoA1 may then be recycled to form a new pre $\beta$ HDL. The action of SR-B1 is facilitated by the hydrolysis of TG by the hepatic lipase. The adrenal gland and gonads also highly express SR-B1 most likely due to their requirement in cholesterol<sup>[86]</sup>.

HDLs are considered limiting for the reverse cholesterol transport because it is assumed that they deliver peripheral cholesterol to the liver for biliary secretion and eventually fecal excretion<sup>[91,92]</sup>. As discussed by Temel and Brown<sup>[93]</sup>, however, there is evidence that HDL-driven cholesterol efflux does not correlate with how much is lost in bile or in the feces. Mice genetically lacking ApoA1 or ABCA1 and, therefore having very low circulating levels of HDL, or showing different steady-state concentrations of HDL-C have normal biliary and fecal cholesterol loss<sup>[94,95]</sup>. Some authors argue that apoB-containing lipoproteins and particularly the activity of CETP play a substantial role in reverse cholesterol transport<sup>[96]</sup>.

#### Estrogen deficiency and hepatic HDL receptor:

SR-B1 mRNA in liver that allows the return of cholesterol to liver *via* HDL was reported to be higher in Ovx compared to Sham rats<sup>[14]</sup>. Interestingly, *ABCA1* gene expression, involved in biosynthesis of nascent HDL was also found to be increased in Ovx rats<sup>[14]</sup>. An increase in gene expression of *ABCA1* was also found in jejunum of Ovx rats<sup>[14]</sup>. Although limited, these findings point to the direction as if the hepatic contribution to HDL metabolism was increased with estrogen withdrawal.

## HEPATIC CHOLESTEROL EFFLUX

There are essentially two ways by which liver can excrete cholesterol: (1) secretion of unmodified cholesterol or after its transformation in bile salts into bile caniculi; and (2) through VLDL secretion.

#### Hepatic cholesterol-bile acid metabolism

The liver is the only organ that has ability to eliminate cholesterol through its secretion into bile or its transformation into bile salts. Bile acids synthesis from cholesterol is stimulated by the nuclear factor liver X receptor (LXR) through its target gene cytochrome P450, family 7, subfamily a, polypeptide 1 (CYP7A1), the main enzyme in the conversion of cholesterol into bile acids<sup>[97]</sup>. The synthesis of a full complement of bile acids requires 17 enzymes<sup>[98]</sup>. The bile acid pool size is reduced by 75% in mice deficient in CYP7A1<sup>[99]</sup>. An alternative biosynthetic pathway is initiated by the enzyme cholesterol 27 $\alpha$ -hydroxylase (Cyp27 $\alpha$ 1<sup>[99]</sup>). Bile salts are highly soluble in water. They form aggregate with phospholipids derived from hepatocyte membranes and solubilize cholesterol in bile for transport from liver to intestine<sup>[100]</sup>. Nuclear factor farnesoid X receptor (FXR) activated by bile acids, stimulates bile and cholesterol

efflux from liver. Opposite to LXR, FXR suppresses bile acids synthesis by inhibiting Cyp7A1. At the canalicular membrane of the hepatocytes, bile salts are pump into bile by a membrane transporter, ABCB11, also referred to as bile salt export pump (BSEP) and to a lesser extent by the multidrug resistance-associated protein 2 (MDR2; ABCC2<sup>[101]</sup>), which activates two other transporters, ABCB4 involved in the transport of phospholipids and ABCG5/G8 a heterodimer involved in the secretion of cholesterol<sup>[102-104]</sup>. Alternative mechanisms to ABCG5/G8 cholesterol secretion involve ATP8B1 and diffusion<sup>[105]</sup>. Altogether bile salts and phospholipids form micelles which are stored in the gall bladder during fasting. In addition, bile salts may be exported to the blood at the sinusoidal membrane mediated by MRP3 (ABCC3) and MRP4 (ABCC4), as well as the organic solute transporter OST  $\alpha/\beta$ <sup>[106]</sup>. Conversion of cholesterol to bile salts accounts for about 50% of daily cholesterol excretion<sup>[107]</sup>.

#### Estrogen deficiency and hepatic cholesterol-bile acid metabolism:

Cyp7A1 and Cyp8b1 transcripts have been reported to be decreased in Ovx rats and mice<sup>[6,11,13,43]</sup> suggesting a reduction in cholesterol elimination *via* bile acid formation. This decrease has been found in Ovx rats fed a standard diet and even more so when Ovx rats were fed a high-fat (42%) diet<sup>[6]</sup>. On the opposite, estrogen treatment has been reported to result in an increase in biliary cholesterol hypersecretion in mice<sup>[4]</sup>.

Estrogen deficiency was associated with lower transcript levels of BSEP and MDR2 suggesting that, in addition to synthesis, excretion of bile acids from hepatocytes to caniculi was decreased in Ovx rats<sup>[15,43]</sup>. Furthermore, the gene expression of nuclear receptors FXR and LXR was found to be lower in Ovx compared to Sham animals<sup>[43]</sup>. The decrease in gene expression of FXR suggests that bile acids did not accumulate in liver of Ovx rats. FXR mRNA levels are controlled by bile acids<sup>[108]</sup>. The specific role of hepatic FXR is to prevent bile acid hepato-toxicity by initiating the expression of a gene network involved in the synthesis and excretion of bile acids. Accordingly, FXR-null mice show massive accumulation of cholesterol in hepatocytes<sup>[109]</sup>. The indication that bile acid metabolism is disrupted in Ovx rats may in turn favours cholesterol accumulation in liver since bile acid secretion exerts a driven force for biliary cholesterol excretion<sup>[110]</sup>. Supporting the hypothesis that biliary metabolic pathways are indeed disrupted in Ovx animals is the finding of a decrease in total bile production in Ovx rats<sup>[111]</sup>.

Gene expression of ABCG5/G8 transporters involved in exportation of cholesterol from the liver to the bile ducts was unchanged in Ovx compared to Sham rats<sup>[6,15,43]</sup> and in aromatase knockout mice<sup>[112]</sup> suggesting that these transporters are not regulated by estrogens.

#### Hepatic excretion of cholesterol through VLDL

VLDL assembly in liver is initiated by the entry of apoB100 in the lumen of the endoplasmic reticulum<sup>[113]</sup>.

The apoB protein is lipidated by the action of microsomal transfer protein (MTP) accumulating TG as well as cholesterol esters molecules. Besides MTP and apoB100, other molecular markers of VLDL assembly include diacylglycerol acyltransferase 2 (DGAT2), involved in the reesterification of TG<sup>[114]</sup>, and acyl-CoA: Cholesterol acyltransferase 2 (ACAT2) that converts free cholesterol into cholesterol esters<sup>[115]</sup>. Further lipidation of the VLDL particles after they exit the endoplasmic reticulum compartment is carried on by a lipid droplet-associated protein, cell death-inducing DNA fragmentation factor alpha-like-effector B (Cideb)<sup>[116]</sup>. The importance of Cideb has been enlightened by the finding of a reduction in plasma LDL levels in Cideb-null mice<sup>[117]</sup>. However, hepatic cholesterol storage was increased in liver of these animals due to its increased LDL-R and ACAT expression. Finally, small GTP binding protein (Sar1a), an intracellular vesicular trafficking protein, facilitates the movements of VLDL particles between the endoplasmic reticulum and the Golgi apparatus where they are secreted in the plasma.

#### **Estrogen deficiency and hepatic VLDL metabolism:**

The observation that plasma cholesterol level is increased in OvX animals<sup>[6,11]</sup> might suggest an increased cholesterol excretion through VLDL. On the opposite, a decrease in VLDL-TG production has been reported in estrogen-deficient animals<sup>[118,119]</sup>. Supporting such a decrease in VLDL production at the molecular level is the repeatedly reported decrease in gene expression of MTP, the rate-limiting molecule for VLDL assembly and secretion, in OvX animals<sup>[15,43,118]</sup>. Transcripts of other genes involved in VLDL synthesis, including apoB, DGAT2, ACAT2, Cideb, and Sar1a have also been reported to be decreased in OvX rats fed a standard diet<sup>[15,43]</sup> and even more so for some genes (MTP and apoB100) in OvX rats fed an enriched-cholesterol diet<sup>[15]</sup>. The additive effect of estrogen withdrawal and high-cholesterol diet on reducing markers of VLDL production was corroborated by an accumulation of total cholesterol and TG in liver and lower levels of these two forms of lipids in plasma<sup>[15]</sup>. In search of an explanation for the postulated reduced VLDL production in OvX rats fed the cholesterol diet, it has been suggested that cholesterol may induce ER stress through cholesterol accumulation<sup>[120]</sup> and that ER stress limits VLDL assembly and secretion through apoB degradation<sup>[121]</sup>. Collectively, these results points toward the interpretation that VLDL assembly is disrupted upon ovariectomy leading to reduced excretion of TG and cholesterol from the liver, thus contributing to exacerbate liver fat and cholesterol accumulation<sup>[14,15]</sup>.

Molecular mechanisms by which estrogens regulate transcription of target genes involved in VLDL pathway are not well known. The classical genomic mechanism of estrogen action involves activation of its nuclear receptor (ER $\alpha$  and  $\beta$ ) and subsequent binding to estrogen response elements located in the promoters of target genes<sup>[122,123]</sup>. Estrogens have also been shown to have non-genomic actions mediated through a subpopulation

of ER $\alpha$  and  $\beta$  located at the plasma membrane<sup>[124]</sup>. It is thus possible that estrogens affect expression of target genes involved in different metabolic pathways through interaction in the nucleus and/or activation of signal transduction pathways at the plasma membrane.

#### **Intestinal excretion of biliary cholesterol**

As mentioned above, hepatic cholesterol is secreted into bile unmodified or after its conversion into bile salts. These bile salts participate in cholesterol transport and eventually in fat digestion in the intestine. However, rather than being lost in the feces, most of the bile salts are recycled when they are taken up by transport proteins in the distal ileum. FXR controls the absorption of bile acids in the intestine through the regulation of bile acid transporters from the intestine to the portal system<sup>[125]</sup>. These include apical sodium-dependent bile acid transporter, the ileal bile acid binding protein, and at the basolateral membrane of enterocytes the heterodimeric organic solute transporters  $\alpha$  and  $\beta$  (OST $\alpha$ , OST $\beta$ )<sup>[126,127]</sup>. Bile salts picked up by these transporters enter the portal circulation and are transported back to the liver where they are eventually re-secreted into bile. This process of recycling back the bile salts between the intestine and the liver is called the enterohepatic circulation<sup>[128]</sup>. The Na<sup>+</sup>-taurocholate cotransporting polypeptide (NTCP) is the major uptake system to transport bile salts from the blood into parenchymal cells<sup>[129]</sup>. Together with several organic anions transporting polypeptide, it controls bile salt uptake at the sinusoidal membrane<sup>[130]</sup>. Bile salt accumulation down-regulates NTCP at the transcriptional level mediated by FXR and the short heterodimer partner 1<sup>[131]</sup>.

Less than 10% of transported bile salts are lost in the feces (0.4 g/d)<sup>[132]</sup>. Therefore, dietary cholesterol (0.4 g/d) constitutes only 25% compared to endogenous cholesterol (1.2 g/d) that passes through intestine in one day<sup>[133]</sup>. Coordination between intestinal bile acids levels and hepatic bile acids biosynthesis is assured through the intestinal secretion of fibroblast growth factor 15/19 that inhibits Cyp7 $\alpha$ 1 in liver under FXR activation<sup>[134]</sup>.

**Excretion of intestinal absorbed cholesterol:** The cellular mechanisms by which chylomicrons in the intestine and VLDL in the liver are assembly are very similar. Their assembly depends of the availability of apoB, triglycerides, and the TG transfer protein MTP. However, opposite to liver, enterocytes express a protein called apoB editing complex-1<sup>[135]</sup>. As a result of the action of this enzyme, translation of apoB comes to a premature stop making intestinal apoB in the intestine 48% as long as the protein expressed in the liver (apoB100). Cholesteryl esters added to the core molecule of chylomicrons come from biliary acids (75%) and from dietary sources. During digestion, cholesteryl esters in food are hydrolyzed to form unesterified cholesterol<sup>[136]</sup>. Dietary and biliary cholesterol from micelles enter the enterocytes mainly (80%) *via* a protein channel, Neimann-Pick C-1 like 1 protein (NPC1L1)<sup>[137]</sup>. Some of

this cholesterol is immediately pumped back into the lumen by the heterodimer transporter ABCG5/G8<sup>[138]</sup>. A portion of cholesterol is also transferred to apoA1 by the ABCA1 transporter to form a nascent HDL. The fraction of cholesterol remaining is esterified to a long-chain fatty acid by ACAT2<sup>[139]</sup>.

**Estrogen deficiency and intestinal bile acid-cholesterol metabolism:** The information is rather limited in regard to biliary cholesterol metabolism in the intestine. A greater faecal excretion of bile acids has been reported in Ovx rats<sup>[11]</sup>. The authors explain this response by suggesting a decreased reabsorption of bile acids from the ileum through a decrease in bile acid transporters. Gene expression of Ntcp, the major uptake system to transport bile salts from the blood into parenchymal cells, was found to be unchanged in Ovx compared to Sham rats<sup>[15]</sup>. On the other hand, gene expression of ABCA1 was reported to be increased in jejunum of Ovx rats, suggesting an increased efflux of intestinal cholesterol through HDL synthesis in Ovx animals<sup>[14]</sup>.

#### **Transintestinal cholesterol excretion**

The hepatobiliary pathway also referred to as the reverse cholesterol transport pathway is considered the major elimination cholesterol route. Nevertheless, fecal cholesterol excretion was observed in several states of disturbances in cholesterol biliary excretion supporting the existence of a new route for cholesterol excretion<sup>[140-142]</sup>. In other words, a large part of the cholesterol found in the feces originates from a source other than bile and diet. The non-biliary alternative called the transintestinal cholesterol excretion pathway implies the direct secretion of plasma lipoprotein-derived cholesterol by the small intestine<sup>[94,143,144]</sup>. Among the numerous studies on transintestinal cholesterol excretion (TICE), there is some agreement that under normal conditions TICE contributes to less than 30% of cholesterol found in the feces (for a review see<sup>[93]</sup>). However, the TICE pathway may be stimulated under pathophysiological or pharmacological conditions. For instance, intestinal cholesterol excretion is inducible by a high-fat diet<sup>[145]</sup> or pharmacologically by ligands of LXR<sup>[146]</sup>. The importance of the role of TICE has been recently highlighted by the demonstration that TICE is essential to macrophage reverse cholesterol transport in mice<sup>[142]</sup>.

It seems that the liver initiates the activation of the TICE<sup>[93]</sup>. Findings in mice with impaired hepatobiliary cholesterol excretion indicate that cholesterol is first transported to the liver before being delivered to the intestine<sup>[93]</sup>. Temel and Brown<sup>[93]</sup> summarized evidence that indicate that it is the subsequent steps within the liver that determine the amount of cholesterol eliminated through the biliary and non-biliary excretory mechanism. The excess cholesterol is most likely repacked into apoB rich lipoproteins secreted by the liver. These liver-derived apoB-containing lipoproteins are recognized by the proximal small intestine through LDL-R and probably

other mechanisms<sup>[147]</sup>. Le May *et al*<sup>[147]</sup> provided data suggesting that PCSK9 is a repressor of TICE dependent on the LDL-R. They also demonstrated that both LDL and HDL (possibly through SR-B1 transporter) provided cholesterol to TICE. Once the free cholesterol is liberated from the TICE lipoproteins, it may efflux from the apical side of the enterocyte through the ABCG5/G8 transporters or the multidrug transporter ABCG1a/b<sup>[93]</sup>.

## **EFFECTS OF EXERCISE TRAINING ON LIVER AND INTESTINAL CHOLESTEROL METABOLISM**

The main finding supporting the contention that exercise training improves lipid and cholesterol metabolism is the reported increase in plasma HDL levels and the concomitant decrease in LDL-cholesterol and triglycerides in human studies<sup>[148,149]</sup>. In animals, positive effects of exercise training on the outcome of disturbances in lipid and cholesterol metabolism has been demonstrated by Ramachandran *et al*<sup>[150]</sup> who reported a 50% reduction in pre-existing atherosclerotic lesions in LDL-R KO mice. Similarly, Matsumoto *et al*<sup>[151]</sup> reported that exercise training in LDL-R KO mice prevented aortic valve sclerosis. These authors specified that exercise exerted several numerous favourable effects that include preservation of valvular endothelial integrity, reduced recruitment of inflammatory cells, and oxidative stress. A decrease in aortic lesion size was also reported by Meissner *et al*<sup>[21]</sup> after 12 wk of voluntary running wheel in LDL-R deficient mice.

However, as mentioned by Meissner *et al*<sup>[152]</sup>, the molecular pathways behind such exercise-induced improvements in plasma lipids are not well defined. In addition, the analysis of the effects of exercise training on the molecular components of cholesterol metabolism in liver is complicated by the variety of animal models used.

#### **HMGCoA-r and exercise training**

There is a paucity of information on the effects of exercise training on cholesterol biosynthesis. Ngo Sock *et al*<sup>[14]</sup> reported that training (8 wk) did not appear to have any effect on HMGCoA-r as well as on SREBP-2 transcripts whether in Sham or in Ovx rats. Previously, Meissner *et al*<sup>[152]</sup> reported an increase in lanosterol/cholesterol ratio in mice submitted to two weeks of voluntary exercise suggesting an increase in cholesterol biosynthesis. However, the same group of authors reported a decrease in HMGCoA-r after 12 wk of voluntary wheel running in LDL-R deficient mice<sup>[21]</sup>. On the whole, there is no clear indication that hepatic cholesterol biosynthesis is changed with exercise training.

#### **LDL-R and exercise training**

Using CETP transgenic mice, an animal model that simulates reverse cholesterol transport (RCT) in human, Rocco *et al*<sup>[153]</sup> found an increase in hepatic LDL-R protein



levels following 6 wk of treadmill exercise. Using this animal model they also found that exercise training improved macrophage RCT. An increase in *LDL-R* gene expression in liver of normal mice exercised for two weeks had been previously found<sup>[154]</sup>. At the same time, Wilund *et al*<sup>[155]</sup> reported an increase in *LDL-R* gene expression and a reduction in gallstone development in gallstone-sensitive mice fed a lithogenic diet after 12 wk of exercise training.

In a recent study, Wen *et al*<sup>[156]</sup> found that treadmill exercise for 8 wk resulted in an increase in PCSK9, *LDL-R*, and SREBP-2 mRNA in high-fat fed mice. On the other hand, they found a reduction in plasma PCSK9 levels and no difference in *LDL-R* protein abundance. They attributed these latter responses to the lower levels of circulating *LDL-C* in trained animals.

In other respects, exercise training (8 wk) did not alter *LDL-R*, *PCSK9*, and *LRP1* gene expression in Sham rats as well as being ineffective in correcting reductions in these molecular markers in Ovx rats<sup>[14]</sup>. On the opposite, Pinto *et al*<sup>[157]</sup> recently reported an increase in *LDL-R* protein levels in male mice trained for 6 wk. Taken together, there is indication that exercise training may favour liver cholesterol uptake from circulation through *LDL-R* thus, supporting the general finding of a reduction in circulating *LDL-C* in human<sup>[149]</sup>.

#### **HDL metabolism and exercise training**

Exercise training (8 wk) did not influence SR-B1 and ABCA1 responses in Sham as well as in Ovx rats<sup>[14]</sup>. On the other hand, an increase in ABCA1 mRNA had previously been reported following 6 wk of treadmill exercise in rats accompanied by an increase in plasma HDL-C concentration<sup>[158]</sup>.

Two weeks of exercise training resulted in an increase in SR-B1 in livers of exercised mice<sup>[154]</sup>. Wilund *et al*<sup>[155]</sup> also reported an increase in *SR-B1* gene expression and a reduction in gallstone development in gallstone-sensitive mice fed a lithogenic diet after 12 wk of exercise training. An increase in SR-B1 protein level in liver has also been reported in male mice trained for 6 wk along with the demonstration of an increased macrophage cholesterol flux to the liver<sup>[157]</sup>.

In CETP transgenic mice, Rocco *et al*<sup>[153]</sup> found an increase in hepatic ABCA1 protein levels following 6 wk of treadmill exercise but no effects on SR-B1. On the whole, it appears that exercise training stimulates positive adaptations of molecular markers of HDL metabolism that would tend to support the finding of an increase circulating HDL levels with exercise training in human<sup>[149]</sup>.

#### **Bile acids and exercise training**

Wilund *et al*<sup>[155]</sup> reported an increase in gene expression of Cyp27A1 in mice fed a lithogenic diet after 12 wk of exercise training. On the opposite, Meissner *et al*<sup>[21,152]</sup> did not observe any effects of exercise on key genes expression involved in bile acid synthesis (*CYP7A1*, *CYP8B1*, and *CYP27A1*) in mice despite an increased fecal bile acid and cholesterol excretion, leading the authors to assume

a posttranscriptional regulation of these genes. The authors hypothesized that physical activity might increase bile acid synthesis to increase the capacity for micelle formation, thus increasing fatty acid absorption<sup>[21]</sup>. More recently, Pinto *et al*<sup>[157]</sup> reported an increase in *CYP7A1* gene expression in male mice trained for 6 wk. On the whole the existing molecular data would tend to support the physiological finding of an increase in fecal bile acid and cholesterol excretion in exercise trained animals.

#### **VLDL and exercise training**

There is a report that VLDL-TG secretion rate is reduced in human following exercise training<sup>[159]</sup>. A decrease in VLDL-TG accumulation and apoB mRNA after exercise training has also been reported in male Wistar rats<sup>[160]</sup>. Accordingly, liver MTP protein content has been found to be decreased with exercise training in mice<sup>[21]</sup> and in standard and high-fat fed female Sprague-Dawley strain rats<sup>[161]</sup>. Since liver fat accumulation is reduced with exercise training<sup>[162]</sup>, the latter authors argue that the reduced liver VLDL production induced by regular exercise is a consequence of an increased lipid disposal through oxidation<sup>[163]</sup>. It is also possible that an increased hepatic insulin sensitivity following exercise training may have resulted in a decrease in VLDL-TG synthesis and secretion. It is well documented that insulin suppresses the secretion of VLDL particles by the liver<sup>[164]</sup> and *MTP* gene expression has been reported to be reduced by insulin in culture liver cells<sup>[165]</sup>.

Plasma VLDL-TG levels have also been reported to be reduced following exercise training in Ovx rats for which VLDL-TG levels were already reduced<sup>[118]</sup>. This suggests that the effects of exercise training and estrogen withdrawal on VLDL-TG synthesis and/or secretion are additive and most likely take place through different pathways. On the other hand, the reduction in VLDL-TG production with exercise training in Ovx rats did not result in an accumulation of liver TG<sup>[118]</sup>. This was explained by the fact that exercise training increases the use of lipids, therefore, reducing fat delivery to the liver.

#### **Intestinal markers and exercise training**

Gene expression of ABCA1 was reported to be increased in jejunum of Ovx rats but unchanged by exercise training (8 wk)<sup>[14]</sup>. On the other hand, the same group of authors found an increase in ABCA1 in ileum of 8-wk trained rats<sup>[22]</sup>. An increase in ABCA1 mRNA in the upper part of the small intestine in Wistar rats trained for 6 wk had been previously reported<sup>[166]</sup>. Although limited, these findings concord with what has been found in liver and suggest that HDL synthesis from the intestine is increased following exercise training.

Wilund *et al*<sup>[155]</sup> found a decrease in *NPC1L1* and *ABCG5/G8* gene expression in duodenum of mice after 12 wk of exercise training. The authors explain that the reduction in *ABCG5/G8* might have been the consequence of the reduction in *NPC1L1* and less cholesterol transported into the enterocytes. A decrease in *NPC1L1* and *ABCG5/G8* was also recently reported in the ileum of

8-wk trained rats<sup>[22]</sup>.

On the other hand, Meissner *et al*<sup>[152]</sup> reported an increase in fecal bile and cholesterol loss and a decrease in jejunal NPC1L1, suggesting a decrease intestinal cholesterol absorption, in male mice submitted to voluntary exercise for two weeks. Running mice also displayed lower ileal OST $\alpha$ , OST $\beta$ , and NTCP transporters, all involved in the enterohepatic circulation of bile acids. However, running did not affect mRNA levels of cholesterol efflux ABCG5/G8 in jejunum. On the whole these authors<sup>[152]</sup> reached the conclusion of an increase cholesterol turnover with regular exercise. In a subsequent study, Meissner *et al*<sup>[21]</sup> found a massive fecal bile acid loss in hypercholesterolemic LDL-R deficient mice trained for 12 wk. Decreases in ileal OST $\alpha$  and OST $\beta$  mRNA have also been reported in 8-wk trained rats along with a decrease in FXR transcription factor indicating that the need to protect the intestine against bile acid overload is reduced in trained animals<sup>[22]</sup>. Finally, Ngo Sock *et al*<sup>[22]</sup> found a decrease in pregnane X receptor (PXR) mRNA in ileum of trained rats. Since PXR receptors protect organisms from exogenous chemical insults, and several endobiotics such as lipids, steroids, and bile acids<sup>[167]</sup>, the authors advocate that exercise training contributes to the maintenance of cholesterol and bile acid homeostasis<sup>[22]</sup>.

On the whole it appears that, at the molecular level, exercise training would contribute to the maintenance of normal circulating cholesterol levels by increasing hepatic LDL-R and HDL metabolism and by favouring adaptations to bile acid metabolism that stimulate fecal bile and cholesterol excretion. When discussing the effects of exercise training on cholesterol metabolism one has to consider that on contrary of fatty acids and glucose or glycogen, cholesterol is not metabolized during exercise. Therefore, it might be an interesting avenue to look at the impact of exercise training on cholesterol metabolism through its link with lipid and glucose metabolism such as intestinal lipid absorption or hepatic *de novo* lipogenesis.

## IN SUMMARY (ESTROGEN DEFICIENCY EFFECTS)

*HMGCoA-r* gene expression in liver along with its transcription factor SREBP-2 is decreased in Ovx animals suggesting a decrease in cholesterol synthesis. There are also indications that bile acid synthesis (*i.e.*, CYP7A1) and transporters of bile acid excretion into caniculi (*i.e.*, BSEP) are also decreased with estrogen deficiency. The reduction in hepatic bile acid metabolism would support the finding that total bile production is reduced in Ovx rats<sup>[111]</sup>.

Although it has been shown that hepatic PCSK9 as well as SREBP-2 and LDL-R mRNA levels are reduced in estrogen deficient animals, there is on the whole data supporting the contention that LDL-R protein levels are increased in Ovx animals most likely associated with a reduction in *PCSK9* gene expression. Although it is

difficult at the present time to reconcile clearly the impact of the absence of estrogens on the dynamics of hepatic PCSK9 and LDL-R and its consequence on plasma LDL-cholesterol, it is evident that estrogen levels play a critical role. The sex specific interaction between LDL-R and PCSK9 would be particularly relevant to post-menopausal women, especially in view of a new class of cholesterol lowering drugs, the PCSK9 inhibitors<sup>[68]</sup>.

There are also data supporting the finding that VLDL and HDL metabolism are changed with the absence of estrogens. VLDL production and its main regulatory factor (MTP) have been repeatedly reported to be decreased in Ovx animals. On the other hand, increases in SR-B1 and ABCA1 mRNA in liver of Ovx animals support the contention that HDL metabolism is increased in these animals. An increase in ABCA1 in intestine suggesting an increase in HDL biosynthesis has also been reported<sup>[14]</sup>.

Although it is obvious that more work has to be done to clearly understand the changes in cholesterol and bile acid metabolism in liver and intestine with the absence of estrogens, the data actually available in Ovx models tend to indicate an increase in cholesterol influx into the liver and a decrease in cholesterol efflux.

## REFERENCES

- 1 Wang DQ, Afdhal NH. Genetic analysis of cholesterol gallstone formation: searching for Lith (gallstone) genes. *Curr Gastroenterol Rep* 2004; **6**: 140-150 [PMID: 15191694]
- 2 Wang HH, Afdhal NH, Wang DQ. Overexpression of estrogen receptor alpha increases hepatic cholestero-genesis, leading to biliary hypersecretion in mice. *J Lipid Res* 2006; **47**: 778-786 [PMID: 16380638 DOI: 10.1194/jlr.M500454-JLR200]
- 3 Cai Q, Wang ZQ, Cai Q, Li C, Chen EZ, Jiang ZY. Relationship between CYP7A1 -204A& gt; C polymorphism with gallbladder stone disease and serum lipid levels: a meta-analysis. *Lipids Health Dis* 2014; **13**: 126 [PMID: 25103562 DOI: 10.1186/1476-511X-13-126]
- 4 Wang HH, Afdhal NH, Wang DQ. Estrogen receptor alpha, but not beta, plays a major role in 17beta-estradiol-induced murine cholesterol gallstones. *Gastroenterology* 2004; **127**: 239-249 [PMID: 15236189 DOI: 10.1053/j.gastro.2004.03.059]
- 5 Akin ML, Uluutku H, Erenoglu C, Karadag A, Gulluoglu BM, Sakar B, Celenk T. Tamoxifen and gallstone formation in postmenopausal breast cancer patients: retrospective cohort study. *World J Surg* 2003; **27**: 395-399 [PMID: 12658480]
- 6 Ngo Sock ET, Côté I, Mentor JS, Prud'homme D, Bergeron R, Lavoie JM. Ovariectomy stimulates hepatic fat and cholesterol accumulation in high-fat diet-fed rats. *Horm Metab Res* 2013; **45**: 283-290 [PMID: 23225241 DOI: 10.1055/s-0032-1329964]
- 7 Paquette A, Shinoda M, Rabasa Lhoret R, Prud'homme D, Lavoie JM. Time course of liver lipid infiltration in ovariectomized rats: impact of a high-fat diet. *Maturitas* 2007; **58**: 182-190 [PMID: 17889461 DOI: 10.1016/j.maturitas.2007.08.002]
- 8 Picard F, Deshaies Y, Lalonde J, Samson P, Labrie C, Bélanger A, Labrie F, Richard D. Effects of the estrogen antagonist EM-652. HCl on energy balance and lipid metabolism in ovariectomized rats. *Int J Obes Relat Metab Disord* 2000; **24**: 830-840 [PMID: 10918529]
- 9 van Lenten BJ, Melchior GW, Roheim PS. Lipoprotein metabolism in the ovariectomized rat. *J Lipid Res* 1983; **24**: 1475-1484 [PMID: 6655365]
- 10 Van Lenten BJ, Roheim PS. The apolipoprotein profile of the ovariectomized rat. Implications of estrogen in receptor-mediated uptake of lipoproteins. *Life Sci* 1981; **28**: 273-278 [PMID: 7219047]
- 11 Kato M, Ogawa H, Kishida T, Ebihara K. The mechanism of the cholesterol-lowering effect of water-insoluble fish protein in

- ovariectomised rats. *Br J Nutr* 2009; **102**: 816-824 [PMID: 19335928 DOI: 10.1017/S0007114509316153]
- 12 **Kimura T**, Matsumoto T, Akiyoshi M, Owa Y, Miyasaka N, Aso T, Moritani T. Body fat and blood lipids in postmenopausal women are related to resting autonomic nervous system activity. *Eur J Appl Physiol* 2006; **97**: 542-547 [PMID: 16779552]
  - 13 **Kamada Y**, Kiso S, Yoshida Y, Chatani N, Kizu T, Hamano M, Tsubakio M, Takemura T, Ezaki H, Hayashi N, Takehara T. Estrogen deficiency worsens steatohepatitis in mice fed high-fat and high-cholesterol diet. *Am J Physiol Gastrointest Liver Physiol* 2011; **301**: G1031-G1043 [PMID: 21885686]
  - 14 **Ngo Sock ET**, Chapados NA, Lavoie JM. LDL receptor and Pcsk9 transcripts are decreased in liver of ovariectomized rats: effects of exercise training. *Horm Metab Res* 2014; **46**: 550-555 [PMID: 24619822]
  - 15 **Farahnak Z**, Côté I, Ngo Sock ET, Lavoie JM. High dietary cholesterol and ovariectomy in rats repress gene expression of key markers of VLDL and bile acid metabolism in liver. *Lipids Health Dis* 2015; **14**: 125 [PMID: 26453540 DOI: 10.1186/s12944-015-0128-9]
  - 16 **Berlin JA**, Colditz GA. A meta-analysis of physical activity in the prevention of coronary heart disease. *Am J Epidemiol* 1990; **132**: 612-628 [PMID: 2144946]
  - 17 **Powell KE**, Thompson PD, Caspersen CJ, Kendrick JS. Physical activity and the incidence of coronary heart disease. *Annu Rev Public Health* 1987; **8**: 253-287 [PMID: 3555525 DOI: 10.1146/annurev.pu.08.050187.001345]
  - 18 **Lavoie JM**, Pighon A. NAFLD, Estrogens, and Physical Exercise: The Animal Model. *J Nutr Metab* 2012; **2012**: 914938 [PMID: 21845221 DOI: 10.1155/2012/014938]
  - 19 **Oh HY**, Lim S, Lee JM, Kim DY, Ann ES, Yoon S. A combination of soy isoflavone supplementation and exercise improves lipid profiles and protects antioxidant defense-systems against exercise-induced oxidative stress in ovariectomized rats. *Biofactors* 2007; **29**: 175-185 [PMID: 18057549 DOI: 10.1002/biof.5520290402]
  - 20 **Zoth N**, Weigt C, Zengin S, Selder O, Selke N, Kalicinski M, Piechotta M, Diel P. Metabolic effects of estrogen substitution in combination with targeted exercise training on the therapy of obesity in ovariectomized Wistar rats. *J Steroid Biochem Mol Biol* 2012; **130**: 64-72 [PMID: 22330197 DOI: 10.1016/j.jsbmb.2012.01.004]
  - 21 **Meissner M**, Lombardo E, Havinga R, Tietge UJ, Kuipers F, Groen AK. Voluntary wheel running increases bile acid as well as cholesterol excretion and decreases atherosclerosis in hypercholesterolemic mice. *Atherosclerosis* 2011; **218**: 323-329 [PMID: 21802084 DOI: 10.1016/j.atherosclerosis.2011.06.040]
  - 22 **Ngo Sock ET**, Farahnak Z, Lavoie JM. Exercise training decreases gene expression of endo- and xeno-sensors in rat small intestine. *Appl Physiol Nutr Metab* 2014; **39**: 1098-1103 [PMID: 24933213 DOI: 10.1139/apnm-2013-0573]
  - 23 **Jones PJ**, Lichtenstein AH, Schaefer EJ. Interaction of dietary fat saturation and cholesterol level on cholesterol synthesis measured using deuterium incorporation. *J Lipid Res* 1994; **35**: 1093-1101 [PMID: 8077848]
  - 24 **Haggarty P**, Shetty P, Thangam S, Kumar S, Kurpad A, Ashton J, Milne E, Earl C. Free and esterified fatty acid and cholesterol synthesis in adult males and its effect on the doubly-labelled water method. *Br J Nutr* 2000; **83**: 227-234 [PMID: 10884710 DOI: 10.1017/S0007114500000295]
  - 25 **Cohen DE**. Lipoprotein metabolism and cholesterol balance. In: IM Arias, DA Cohen, N Fausto, DA Shafritz, and AW Wolkoff (Eds), *The liver: Biology and Pathology* 5th ed. Oxford, 2009: 271-285
  - 26 **Iglesias P**, Díez JJ. New drugs for the treatment of hypercholesterolaemia. *Expert Opin Investig Drugs* 2003; **12**: 1777-1789 [PMID: 14585054 DOI: 10.1517/13543784.12.11.1777]
  - 27 **Dietschy JM**, Turley SD, Spady DK. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J Lipid Res* 1993; **34**: 1637-1659 [PMID: 8245716]
  - 28 **Dietschy JM**, Turley SD. Cholesterol metabolism in the brain. *Curr Opin Lipidol* 2001; **12**: 105-112 [PMID: 11264981]
  - 29 **Turley SD**, Andersen JM, Dietschy JM. Rates of sterol synthesis and uptake in the major organs of the rat in vivo. *J Lipid Res* 1981; **22**: 551-569 [PMID: 7276735]
  - 30 **Simons K**, Ikonen E. Functional rafts in cell membranes. *Nature* 1997; **387**: 569-572 [PMID: 9177342 DOI: 10.1038/42408]
  - 31 **Anderson RG**. The caveolae membrane system. *Annu Rev Biochem* 1998; **67**: 199-225 [PMID: 9759488 DOI: 10.1146/annurev.biochem.67.1.199]
  - 32 **Weber LW**, Boll M, Stampfl A. Maintaining cholesterol homeostasis: sterol regulatory element-binding proteins. *World J Gastroenterol* 2004; **10**: 3081-3087 [PMID: 15457548 DOI: 10.3748/wjg.v10.i21.3081]
  - 33 **Brown MS**, Goldstein JL. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc Natl Acad Sci USA* 1999; **96**: 11041-11048 [PMID: 10500120 DOI: 10.1073/pnas.96.20.11041]
  - 34 **Ching YP**, Davies SP, Hardie DG. Analysis of the specificity of the AMP-activated protein kinase by site-directed mutagenesis of bacterially expressed 3-hydroxy 3-methylglutaryl-CoA reductase, using a single primer variant of the unique-site-elimination method. *Eur J Biochem* 1996; **237**: 800-808 [PMID: 8647128 DOI: 10.1111/j.1432-1033.1996.0800p.x]
  - 35 **Gaussin V**, Skarlas P, Ching YP, Hardie DG, Hue L. Distinct type-2A protein phosphatases activate HMGCoA reductase and acetyl-CoA carboxylase in liver. *FEBS Lett* 1997; **413**: 115-118 [PMID: 9287127 DOI: 10.1016/S0014-5793(97)00890-9]
  - 36 **Pallottini V**, Montanari L, Cavallini G, Bergamini E, Gori Z, Trentalance A. Mechanisms underlying the impaired regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in aged rat liver. *Mech Ageing Dev* 2004; **125**: 633-639 [PMID: 15491682 DOI: 10.1016/j.mad.2004.08.001]
  - 37 **Goldstein JL**, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. *Cell* 2006; **124**: 35-46 [PMID: 16413480 DOI: 10.1016/j.cell.2005.12.022]
  - 38 **Edwards PA**, Tabor D, Kast HR, Venkateswaran A. Regulation of gene expression by SREBP and SCAP. *Biochim Biophys Acta* 2000; **1529**: 103-113 [PMID: 11111080 DOI: 10.1016/S1388-1981(00)00140-2]
  - 39 **Sato R**. Sterol metabolism and SREBP activation. *Arch Biochem Biophys* 2010; **501**: 177-181 [PMID: 20541520 DOI: 10.1016/j.jabb.2010.06.004]
  - 40 **Espenshade PJ**, Cheng D, Goldstein JL, Brown MS. Autocatalytic processing of site-1 protease removes propeptide and permits cleavage of sterol regulatory element-binding proteins. *J Biol Chem* 1999; **274**: 22795-22804 [PMID: 10428864 DOI: 10.1074/jbc.274.32.22795]
  - 41 **Sato R**, Inoue J, Kawabe Y, Kodama T, Takano T, Maeda M. Sterol-dependent transcriptional regulation of sterol regulatory element-binding protein-2. *J Biol Chem* 1996; **271**: 26461-26464 [PMID: 8900111]
  - 42 **Yang T**, Espenshade PJ, Wright ME, Yabe D, Gong Y, Aebersold R, Goldstein JL, Brown MS. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* 2002; **110**: 489-500 [PMID: 12202038 DOI: 10.1016/S0092-8674(02)00872-3]
  - 43 **Côté I**, Chapados NA, Lavoie JM. Impaired VLDL assembly: a novel mechanism contributing to hepatic lipid accumulation following ovariectomy and high-fat/high-cholesterol diets? *Br J Nutr* 2014; **112**: 1592-1600 [PMID: 25263431 DOI: 10.1017/S0007114514002517]
  - 44 **De Marinis E**, Martini C, Trentalance A, Pallottini V. Sex differences in hepatic regulation of cholesterol homeostasis. *J Endocrinol* 2008; **198**: 635-643 [PMID: 18603607 DOI: 10.1677/JOE-08-0242]
  - 45 **Di Croce L**, Bruscalupi G, Trentalance A. Independent behavior of rat liver LDL receptor and HMGCoA reductase under estrogen treatment. *Biochem Biophys Res Commun* 1996; **224**: 345-350 [PMID: 8702393 DOI: 10.1006/bbrc.1996.1031]
  - 46 **Di Croce L**, Bruscalupi G, Trentalance A. Independent responsiveness of frog liver low-density lipoprotein receptor and HMGCoA reductase to estrogen treatment. *Pflugers Arch* 1997; **435**: 107-111 [PMID: 9359909]



- 47 **Fielding PE**, Fielding CJ. Dynamics of lipoprotein transport in the human circulatory system. In: DE Vance and JR Vance (Eds), *Biochemistry of Lipids, Lipoproteins and Membranes*. Amsterdam: Elsevier, 2002: 527-552
- 48 **Cooper AD**. Hepatic uptake of chylomicron remnants. *J Lipid Res* 1997; **38**: 2173-2192 [PMID: 9392416]
- 49 **Mahley RW**, Ji ZS. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J Lipid Res* 1999; **40**: 1-16 [PMID: 9869645]
- 50 **Scappa MC**, Kanno K, Cohen DE. Lipoprotein metabolism. In: J Rodes, JP Benhamou, MA Rizzetto, J Reichen, and A Blei. *The Textbook of Hepatology From Basic Science to Clinical Practice*, 3rd Edition. Blackwell, Oxford, 2007
- 51 **Packard CJ**, Shepherd J. Lipoprotein heterogeneity and apolipoprotein B metabolism. *Arterioscler Thromb Vasc Biol* 1997; **17**: 3542-3556 [PMID: 9437204 DOI: 10.1161/01.ATV.17.12.3542]
- 52 **Cohen DE**, Armstrong EJ. Pharmacology of Cholesterol and Lipoprotein Metabolism, In: Golan DE, Tashjian AH, Armstrong EJ, Galanter JM, Armstrong AW, Armaout RA and Rose HS (eds), 2nd ed. *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*. Philadelphia: Lippincott Williams and Wilkins, 2007
- 53 **Brown MS**, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 1986; **232**: 34-47 [PMID: 3513311 DOI: 10.1126/science.3513311]
- 54 **Niesen M**, Bedi M, Lopez D. Diabetes alters LDL receptor and PCSK9 expression in rat liver. *Arch Biochem Biophys* 2008; **470**: 111-115 [PMID: 18054320 DOI: 10.1016/j.abb.2007.11.009]
- 55 **Abifadel M**, Varret M, Rabès JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, Derré A, Villéger L, Farnier M, Beaucier I, Bruckert E, Chambaz J, Chanu B, Lecerf JM, Luc G, Moulin P, Weissenbach J, Prat A, Krempf M, Junien C, Seidah NG, Boileau C. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003; **34**: 154-156 [PMID: 12730697 DOI: 10.1038/ng1161]
- 56 **Seidah NG**, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, Basak A, Prat A, Chrétien M. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci USA* 2003; **100**: 928-933 [PMID: 12552133 DOI: 10.1073/pnas.0335507100]
- 57 **Roubtsova A**, Munkonda MN, Awan Z, Marcinkiewicz J, Chamberland A, Lazure C, Cianflone K, Seidah NG, Prat A. Circulating proprotein convertase subtilisin/kexin 9 (PCSK9) regulates VLDL protein and triglyceride accumulation in visceral adipose tissue. *Arterioscler Thromb Vasc Biol* 2011; **31**: 785-791 [PMID: 21273557 DOI: 10.1161/ATVBAHA.110.220988]
- 58 **Smith JR**, Osborne TF, Goldstein JL, Brown MS. Identification of nucleotides responsible for enhancer activity of sterol regulatory element in low density lipoprotein receptor gene. *J Biol Chem* 1990; **265**: 2306-2310 [PMID: 2298751]
- 59 **Cunningham D**, Danley DE, Geoghegan KF, Griffor MC, Hawkins JL, Subashi TA, Varghese AH, Ammirati MJ, Culp JS, Hoth LR, Mansour MN, McGrath KM, Seddon AP, Shenolikar S, Stutzman-Engwall KJ, Warren LC, Xia D, Qiu X. Structural and biophysical studies of PCSK9 and its mutants linked to familial hypercholesterolemia. *Nat Struct Mol Biol* 2007; **14**: 413-419 [PMID: 17435765 DOI: 10.1038/nsmb1235]
- 60 **Poirier S**, Mamarbachi M, Chen WT, Lee AS, Mayer G. GRP94 Regulates Circulating Cholesterol Levels through Blockade of PCSK9-Induced LDLR Degradation. *Cell Rep* 2015; **13**: 2064-2071 [PMID: 26628375 DOI: 10.1016/j.celrep.2015.11.006]
- 61 **Poirier S**, Mayer G. The biology of PCSK9 from the endoplasmic reticulum to lysosomes: new and emerging therapeutics to control low-density lipoprotein cholesterol. *Drug Des Devel Ther* 2013; **7**: 1135-1148 [PMID: 24115837 DOI: 10.2147/DDDT.S36984]
- 62 **Dubuc G**, Chamberland A, Wassef H, Davignon J, Seidah NG, Bernier L, Prat A. Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 2004; **24**: 1454-1459 [PMID: 15178557 DOI: 10.1161/01.ATV.0000134621.14315.43]
- 63 **Jung D**, Kullak-Ublick GA. Hepatocyte nuclear factor 1 alpha: a key mediator of the effect of bile acids on gene expression. *Hepatology* 2003; **37**: 622-631 [PMID: 12601360 DOI: 10.1053/jhep.2003.50100]
- 64 **Ge XZ**, Tian PF, Lin Q, Huo Q. [The influence of soybean isoflavone on expression of low density lipoprotein receptor (LDLR) mRNA in ovariectomized rats]. *Zhong Yao Cai* 2006; **29**: 349-351 [PMID: 16913490]
- 65 **Parini P**, Angelin B, Stavrèus-Evers A, Freyschuss B, Eriksson H, Rudling M. Biphasic effects of the natural estrogen 17beta-estradiol on hepatic cholesterol metabolism in intact female rats. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1817-1823 [PMID: 10894823 DOI: 10.1161/01.ATV.20.7.1817]
- 66 **Roubtsova A**, Chamberland A, Marcinkiewicz J, Essalmani R, Fazel A, Bergeron JJ, Seidah NG, Prat A. PCSK9 deficiency unmasks a sex- and tissue-specific subcellular distribution of the LDL and VLDL receptors in mice. *J Lipid Res* 2015; **56**: 2133-2142 [PMID: 26323289 DOI: 10.1194/jlr.M061952]
- 67 **Starr AE**, Lemieux V, Noad J, Moore JJ, Dewpura T, Raymond A, Chrétien M, Figeys D, Mayne J.  $\beta$ -Estradiol results in a proprotein convertase subtilisin/kexin type 9-dependent increase in low-density lipoprotein receptor levels in human hepatic HuH7 cells. *FEBS J* 2015; **282**: 2682-2696 [PMID: 25913303 DOI: 10.1111/febs.13309]
- 68 **Raal FJ**, Stein EA, Dufour R, Turner T, Civeira F, Burgess L, Langslet G, Scott R, Olsson AG, Sullivan D, Hovingh GK, Cariou B, Gouni-Berthold I, Somaratne R, Bridges I, Scott R, Wasserman SM, Gaudet D. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. *Lancet* 2015; **385**: 331-340 [PMID: 25282519 DOI: 10.1016/S0140-6736(14)61399-4]
- 69 **Gonias SL**, Campana WM. LDL receptor-related protein-1: a regulator of inflammation in atherosclerosis, cancer, and injury to the nervous system. *Am J Pathol* 2014; **184**: 18-27 [PMID: 24128688 DOI: 10.1016/j.ajpath.2013.08.029]
- 70 **Moestrup SK**, Gliemann J, Pallesen G. Distribution of the alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein in human tissues. *Cell Tissue Res* 1992; **269**: 375-382 [PMID: 1423505]
- 71 **Kim C**, Vaziri ND. Down-regulation of hepatic LDL receptor-related protein (LRP) in chronic renal failure. *Kidney Int* 2005; **67**: 1028-1032 [PMID: 15698441 DOI: 10.1111/j.1523-1755.2005.00166.x]
- 72 **Strickland DK**, Gonias SL, Argraves WS. Diverse roles for the LDL receptor family. *Trends Endocrinol Metab* 2002; **13**: 66-74 [PMID: 11854021 DOI: 10.1016/S1043-2760(01)00526-4]
- 73 **Fernandez-Castaneda A**, Arandjelovic S, Stiles TL, Schlobach RK, Mowen KA, Gonias SL, Gaultier A. Identification of the low density lipoprotein (LDL) receptor-related protein-1 interactome in central nervous system myelin suggests a role in the clearance of necrotic cell debris. *J Biol Chem* 2013; **288**: 4538-4548 [PMID: 23264627 DOI: 10.1074/jbc.M112.384693]
- 74 **Lauber K**, Blumenthal SG, Waibel M, Wesselborg S. Clearance of apoptotic cells: getting rid of the corpses. *Mol Cell* 2004; **14**: 277-287 [PMID: 15125832 DOI: 10.1016/S1097-2765(04)00237-0]
- 75 **Gonias SL**, Wu L, Salicioni AM. Low density lipoprotein receptor-related protein: regulation of the plasma membrane proteome. *Thromb Haemost* 2004; **91**: 1056-1064 [PMID: 15175790 DOI: 10.1160/TH04-01-0023]
- 76 **Rohlmann A**, Gotthardt M, Hammer RE, Herz J. Inducible inactivation of hepatic LRP gene by cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. *J Clin Invest* 1998; **101**: 689-695 [PMID: 9449704]
- 77 **Kütt H**, Herz J, Stanley KK. Structure of the low-density lipoprotein receptor-related protein (LRP) promoter. *Biochim Biophys Acta* 1989; **1009**: 229-236 [PMID: 2597675]
- 78 **Williams SE**, Ashcom JD, Argraves WS, Strickland DK. A novel mechanism for controlling the activity of alpha 2-macroglobulin



- receptor/low density lipoprotein receptor-related protein. Multiple regulatory sites for 39-kDa receptor-associated protein. *J Biol Chem* 1992; **267**: 9035-9040 [PMID: 1374383]
- 79 **Willnow TE**, Armstrong SA, Hammer RE, Herz J. Functional expression of low density lipoprotein receptor-related protein is controlled by receptor-associated protein in vivo. *Proc Natl Acad Sci USA* 1995; **92**: 4537-4541 [PMID: 7538675]
  - 80 **Emonard H**, Th  ret L, Bennisroune AH, Dedieu S. Regulation of LRP-1 expression: make the point. *Pathol Biol (Paris)* 2014; **62**: 84-90 [PMID: 24661974 DOI: 10.1016/j.patbio.2014.02.002]
  - 81 **Moon JH**, Kang SB, Park JS, Lee BW, Kang ES, Ahn CW, Lee HC, Cha BS. Up-regulation of hepatic low-density lipoprotein receptor-related protein 1: a possible novel mechanism of antiatherogenic activity of hydroxymethylglutaryl-coenzyme A reductase inhibitor Atorvastatin and hepatic LRP1 expression. *Metabolism* 2011; **60**: 930-940 [PMID: 20951395 DOI: 10.1016/j.metabol.2010.08.013]
  - 82 **Canuel M**, Sun X, Asselin MC, Paramithiotis E, Prat A, Seidah NG. Proprotein convertase subtilisin/kexin type 9 (PCSK9) can mediate degradation of the low density lipoprotein receptor-related protein 1 (LRP-1). *PLoS One* 2013; **8**: e64145 [PMID: 23675525 DOI: 10.1371/journal.pone.0064145]
  - 83 **Webb JC**, Patel DD, Jones MD, Knight BL, Soutar AK. Characterization and tissue-specific expression of the human 'very low density lipoprotein (VLDL) receptor' mRNA. *Hum Mol Genet* 1994; **3**: 531-537 [PMID: 8069294 DOI: 10.1093/hmg/3.4.531]
  - 84 **Oka K**, Ishimura-Oka K, Chu MJ, Sullivan M, Krushkal J, Li WH, Chan L. Mouse very-low-density-lipoprotein receptor (VLDLR) cDNA cloning, tissue-specific expression and evolutionary relationship with the low-density-lipoprotein receptor. *Eur J Biochem* 1994; **224**: 975-982 [PMID: 7925422 DOI: 10.1111/j.1432-1033.1994.00975.x]
  - 85 **Silver DL**, Jiang XC, Arai T, Bruce C, Tall AR. Receptors and lipid transfer proteins in HDL metabolism. *Ann N Y Acad Sci* 2000; **902**: 103-111; discussion 111-112 [PMID: 10865830 DOI: 10.1111/j.1749-6632.2000.tb06305.x]
  - 86 **Timmins JM**, Lee JY, Boudyguina E, Kluckman KD, Brunham LR, Mulya A, Gebre AK, Coutinho JM, Colvin PL, Smith TL, Hayden MR, Maeda N, Parks JS. Targeted inactivation of hepatic Abca1 causes profound hypoalphalipoproteinemia and kidney hypercatabolism of apoA-I. *J Clin Invest* 2005; **115**: 1333-1342 [PMID: 15841208 DOI: 10.1172/JCI23915]
  - 87 **Kiss RS**, McManus DC, Franklin V, Tan WL, McKenzie A, Chimini G, Marcel YL. The lipidation by hepatocytes of human apolipoprotein A-I occurs by both ABCA1-dependent and -independent pathways. *J Biol Chem* 2003; **278**: 10119-10127 [PMID: 12547832 DOI: 10.1074/jbc.M300137200]
  - 88 **Zannis VI**, Chroni A, Krieger M. Role of apoA-I, ABCA1, LCAT, and SR-BI in the biogenesis of HDL. *J Mol Med (Berl)* 2006; **84**: 276-294 [PMID: 16501936]
  - 89 **Rothblat GH**, de la Llera-Moya M, Atger V, Kellner-Weibel G, Williams DL, Phillips MC. Cell cholesterol efflux: integration of old and new observations provides new insights. *J Lipid Res* 1999; **40**: 781-796 [PMID: 10224147]
  - 90 **Acton S**, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* 1996; **271**: 518-520 [PMID: 8560269 DOI: 10.1126/science.271.5248.518]
  - 91 **Rader DJ**, Alexander ET, Weibel GL, Billheimer J, Rothblat GH. The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis. *J Lipid Res* 2009; **50** Suppl: S189-S194 [PMID: 19064999 DOI: 10.1194/jlr.R800088-JLR200]
  - 92 **Rosenson RS**, Brewer HB, Davidson WS, Fayad ZA, Fuster V, Goldstein J, Hellerstein M, Jiang XC, Phillips MC, Rader DJ, Remaley AT, Rothblat GH, Tall AR, Yvan-Charvet L. Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. *Circulation* 2012; **125**: 1905-1919 [PMID: 22508840 DOI: 10.1161/CIRCULATIONAHA.111.066589]
  - 93 **Temel RE**, Brown JM. A new model of reverse cholesterol transport: enTICEing strategies to stimulate intestinal cholesterol excretion. *Trends Pharmacol Sci* 2015; **36**: 440-451 [PMID: 25930707 DOI: 10.1016/j.tips.2015.04.002]
  - 94 **Osono Y**, Woollett LA, Marotti KR, Melchior GW, Dietschy JM. Centripetal cholesterol flux from extrahepatic organs to the liver is independent of the concentration of high density lipoprotein-cholesterol in plasma. *Proc Natl Acad Sci USA* 1996; **93**: 4114-4119 [PMID: 8633025]
  - 95 **Groen AK**, Bloks VW, Bandsma RH, Ottenhoff R, Chimini G, Kuipers F. Hepatobiliary cholesterol transport is not impaired in Abca1-null mice lacking HDL. *J Clin Invest* 2001; **108**: 843-850 [PMID: 11560953 DOI: 10.1172/JCI12473]
  - 96 **Hellerstein M**, Turner S. Reverse cholesterol transport fluxes. *Curr Opin Lipidol* 2014; **25**: 40-47 [PMID: 24362356 DOI: 10.1097/MOL.0000000000000050]
  - 97 **Redinger RN**. Nuclear receptors in cholesterol catabolism: molecular biology of the enterohepatic circulation of bile salts and its role in cholesterol homeostasis. *J Lab Clin Med* 2003; **142**: 7-20 [PMID: 12878981 DOI: 10.1016/S0022-2143(03)00088-X]
  - 98 **Russell DW**. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem* 2003; **72**: 137-174 [PMID: 12543708 DOI: 10.1146/annurev.biochem.72.121801.161712]
  - 99 **Schwarz M**, Russell DW, Dietschy JM, Turley SD. Marked reduction in bile acid synthesis in cholesterol 7alpha-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. *J Lipid Res* 1998; **39**: 1833-1843 [PMID: 9741696]
  - 100 **Wang DQ**, Cohen DE, Carey MC. Biliary lipids and cholesterol gallstone disease. *J Lipid Res* 2009; **50** Suppl: S406-S411 [PMID: 19017613 DOI: 10.1194/jlr.R800075-JLR200]
  - 101 **Akita H**, Suzuki H, Ito K, Kinoshita S, Sato N, Takikawa H, Sugiyama Y. Characterization of bile acid transport mediated by multidrug resistance associated protein 2 and bile salt export pump. *Biochim Biophys Acta* 2001; **1511**: 7-16 [PMID: 11248200 DOI: 10.1016/S0005-2736(00)00355-2]
  - 102 **Stieger B**, Meier Y, Meier PJ. The bile salt export pump. *Pflugs Arch* 2007; **453**: 611-620 [PMID: 17051391 DOI: 10.1007/s00424-006-0152-8]
  - 103 **Oude Elferink RP**, Paulusma CC. Function and pathophysiological importance of ABCB4 (MDR3 P-glycoprotein). *Pflugs Arch* 2007; **453**: 601-610 [PMID: 16622704 DOI: 10.1007/s00424-006-0062-9]
  - 104 **Hazard SE**, Patel SB. Sterolins ABCG5 and ABCG8: regulators of whole body dietary sterols. *Pflugs Arch* 2007; **453**: 745-752 [PMID: 16440216 DOI: 10.1007/s00424-005-0040-7]
  - 105 **Naik J**, de Waart DR, Utsunomiya K, Duijst S, Mok KH, Oude Elferink RP, Bosma PJ, Paulusma CC. ATP8B1 and ATP11C: Two Lipid Flippases Important for Hepatocyte Function. *Dig Dis* 2015; **33**: 314-318 [PMID: 26045263 DOI: 10.1159/000371665]
  - 106 **M  hlfeld S**, Domanova O, Berlage T, Stross C, Helmer A, Keitel V, H  ussinger D, Kubitz R. Short-term feedback regulation of bile salt uptake by bile salts in rodent liver. *Hepatology* 2012; **56**: 2387-2397 [PMID: 22806967 DOI: 10.1002/hep.25955]
  - 107 **Kuwabara T**, Han KH, Hashimoto N, Yamauchi H, Shimada K, Sekikawa M, Fukushima M. Tartary buckwheat sprout powder lowers plasma cholesterol level in rats. *J Nutr Sci Vitaminol (Tokyo)* 2007; **53**: 501-507 [PMID: 18202538 DOI: 10.3177/jnsv.53.501]
  - 108 **Xu G**, Pan LX, Li H, Forman BM, Erickson SK, Shefer S, Bollineni J, Batta AK, Christie J, Wang TH, Michel J, Yang S, Tsai R, Lai L, Shimada K, Tint GS, Salen G. Regulation of the farnesoid X receptor (FXR) by bile acid flux in rabbits. *J Biol Chem* 2002; **277**: 50491-50496 [PMID: 12401785 DOI: 10.1074/jbc.M209176200]
  - 109 **Sinal CJ**, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* 2000; **102**: 731-744 [PMID: 11030617 DOI: 10.1016/S0092-8674(00)00062-3]
  - 110 **Oude Elferink RP**, Paulusma CC, Groen AK. Hepatocanalicular transport defects: pathophysiologic mechanisms of rare diseases. *Gastroenterology* 2006; **130**: 908-925 [PMID: 16530529 DOI: 10.1053/j.gastro.2005.08.052]
  - 111 **Czerny B**, Teister M, Juzyszyn Z, Teister L, Pawlik A, Gazda P,

- Kaminski A, Chalas A. The effect of retinoic acid receptor agonist acitretin on the production of bile and concentrations of some serum components in ovariectomized rats. *Menopause* 2011; **18**: 213-218 [PMID: 20861754 DOI: 10.1097/gme.0b013e3181ef22b8]
- 112 **Hewitt KN**, Boon WC, Murata Y, Jones ME, Simpson ER. The aromatase knockout mouse presents with a sexually dimorphic disruption to cholesterol homeostasis. *Endocrinology* 2003; **144**: 3895-3903 [PMID: 12933663 DOI: 10.1210/en.2003-0244#sthash.QVBR65mz.dpuf]
- 113 **Hussain MM**, Shi J, Dreizen P. Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. *J Lipid Res* 2003; **44**: 22-32 [PMID: 12518019 DOI: 10.1194/jlr.R200014-JLR200]
- 114 **Gibbons GF**, Wiggins D, Brown AM, Hebbachi AM. Synthesis and function of hepatic very-low-density lipoprotein. *Biochem Soc Trans* 2004; **32**: 59-64 [PMID: 14748713 DOI: 10.1042/bst0320059]
- 115 **Cianflone KM**, Yasrael Z, Rodriguez MA, Vas D, Sniderman AD. Regulation of apoB secretion from HepG2 cells: evidence for a critical role for cholesteryl ester synthesis in the response to a fatty acid challenge. *J Lipid Res* 1990; **31**: 2045-2055 [PMID: 1964953]
- 116 **Ye J**, Li JZ, Liu Y, Li X, Yang T, Ma X, Li Q, Yao Z, Li P. Cideb, an ER- and lipid droplet-associated protein, mediates VLDL lipitation and maturation by interacting with apolipoprotein B. *Cell Metab* 2009; **9**: 177-190 [PMID: 19187774 DOI: 10.1016/j.cmet.2008.12.013]
- 117 **Li JZ**, Lei Y, Wang Y, Zhang Y, Ye J, Xia X, Pan X, Li P. Control of cholesterol biosynthesis, uptake and storage in hepatocytes by Cideb. *Biochim Biophys Acta* 2010; **1801**: 577-586 [PMID: 20123130 DOI: 10.1016/j.bbalip.2010.01.012]
- 118 **Barsalani R**, Chapados NA, Lavoie JM. Hepatic VLDL-TG production and MTP gene expression are decreased in ovariectomized rats: effects of exercise training. *Horm Metab Res* 2010; **42**: 860-867 [PMID: 20938890 DOI: 10.1055/s-0030-1267173]
- 119 **Lemieux C**, Gélinas Y, Lalonde J, Labrie F, Cianflone K, Deshaies Y. Hypolipidemic action of the SERM acolbifene is associated with decreased liver MTP and increased SR-BI and LDL receptors. *J Lipid Res* 2005; **46**: 1285-1294 [PMID: 15741653 DOI: 10.1194/jlr.M400448-JLR200]
- 120 **Hager L**, Li L, Pun H, Liu L, Hossain MA, Maguire GF, Naples M, Baker C, Magomedova L, Tam J, Adeli K, Cummins CL, Connelly PW, Ng DS. Lecithin: cholesterol acyltransferase deficiency protects against cholesterol-induced hepatic endoplasmic reticulum stress in mice. *J Biol Chem* 2012; **287**: 20755-20768 [PMID: 22500017 DOI: 10.1074/jbc.M112.340919]
- 121 **Ota T**, Gayet C, Ginsberg HN. Inhibition of apolipoprotein B100 secretion by lipid-induced hepatic endoplasmic reticulum stress in rodents. *J Clin Invest* 2008; **118**: 316-332 [PMID: 18060040 DOI: 10.1172/JCI32752]
- 122 **D'Eon TM**, Souza SC, Aronovitz M, Obin MS, Fried SK, Greenberg AS. Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J Biol Chem* 2005; **280**: 35983-35991 [PMID: 16109719 DOI: 10.1074/jbc.M507339200]
- 123 **Nilsson S**, Mäkelä S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson JA. Mechanisms of estrogen action. *Physiol Rev* 2001; **81**: 1535-1565 [PMID: 11581496]
- 124 **Pappas TC**, Gametchu B, Watson CS. Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. *FASEB J* 1995; **9**: 404-410 [PMID: 7896011]
- 125 **Matsubara T**, Li F, Gonzalez FJ. FXR signaling in the enterohepatic system. *Mol Cell Endocrinol* 2013; **368**: 17-29 [PMID: 22609541 DOI: 10.1016/j.mce.2012.05.004]
- 126 **Shneider BL**. Intestinal bile acid transport: biology, physiology, and pathophysiology. *J Pediatr Gastroenterol Nutr* 2001; **32**: 407-417 [PMID: 11396803]
- 127 **Dawson PA**, Hubbert M, Haywood J, Craddock AL, Zerangue N, Christian WV, Ballatori N. The heteromeric organic solute transporter alpha-beta, Ostalpha-Ostbeta, is an ileal basolateral bile acid transporter. *J Biol Chem* 2005; **280**: 6960-6968 [PMID: 15563450 DOI: 10.1074/jbc.M412752200]
- 128 **Carey MC**, Duane WC. Enterohepatic circulation. In: Arias IM, Boyer JL, Fausto N, Jakoby WB, Schachter DA, and Shafritz DA (eds), Raven Press New York, 1994: 719-767
- 129 **Stieger B**, Hagenbuch B, Landmann L, Höchli M, Schroeder A, Meier PJ. In situ localization of the hepatocytic Na<sup>+</sup>/Taurocholate cotransporting polypeptide in rat liver. *Gastroenterology* 1994; **107**: 1781-1787 [PMID: 7958692]
- 130 **Hagenbuch B**, Meier PJ. The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta* 2003; **1609**: 1-18 [PMID: 12507753 DOI: 10.1016/S0005-2736(02)00633-8]
- 131 **Denson LA**, Sturm E, Echevarria W, Zimmerman TL, Makishima M, Mangelsdorf DJ, Karpen SJ. The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. *Gastroenterology* 2001; **121**: 140-147 [PMID: 11438503 DOI: 10.1053/gast.2001.25503]
- 132 **Dawson PA**, Lan T, Rao A. Bile acid transporters. *J Lipid Res* 2009; **50**: 2340-2357 [PMID: 19498215 DOI: 10.1194/jlr.R900012-JLR200]
- 133 **Cohen DE**. Balancing cholesterol synthesis and absorption in the gastrointestinal tract. *J Clin Lipidol* 2008; **2**: S1-S3 [PMID: 19343078 DOI: 10.1016/j.jacl.2008.01.004]
- 134 **Jones S**. Mini-review: endocrine actions of fibroblast growth factor 19. *Mol Pharm* 2008; **5**: 42-48 [PMID: 18179175 DOI: 10.1021/mp700105z]
- 135 **Anant S**, Davidson NO. Molecular mechanisms of apolipoprotein B mRNA editing. *Curr Opin Lipidol* 2001; **12**: 159-165 [PMID: 11264987]
- 136 **Wang DQ**. Regulation of intestinal cholesterol absorption. *Annu Rev Physiol* 2007; **69**: 221-248 [PMID: 17002594 DOI: 10.1146/annurev.physiol.69.031905.160725]
- 137 **Altmann SW**, Davis HR, Zhu LJ, Yao X, Hoos LM, Tetzloff G, Iyer SP, Maguire M, Golovko A, Zeng M, Wang L, Murgolo N, Graziano MP. Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science* 2004; **303**: 1201-1204 [PMID: 14976318 DOI: 10.1126/science.1093131]
- 138 **Yu L**, Li-Hawkins J, Hammer RE, Berge KE, Horton JD, Cohen JC, Hobbs HH. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. *J Clin Invest* 2002; **110**: 671-680 [PMID: 12208868 DOI: 10.1172/JCI16001]
- 139 **Joyce C**, Skinner K, Anderson RA, Rudel LL. Acyl-coenzyme A: cholesteryl acyltransferase 2. *Curr Opin Lipidol* 1999; **10**: 89-95 [PMID: 10327276]
- 140 **Brown JM**, Bell TA, Alger HM, Sawyer JK, Smith TL, Kelley K, Shah R, Wilson MD, Davis MA, Lee RG, Graham MJ, Crooke RM, Rudel LL. Targeted depletion of hepatic ACAT2-driven cholesterol esterification reveals a non-biliary route for fecal neutral sterol loss. *J Biol Chem* 2008; **283**: 10522-10534 [PMID: 18281279 DOI: 10.1074/jbc.M707659200]
- 141 **Kruit JK**, Plösch T, Havinga R, Boverhof R, Groot PH, Groen AK, Kuipers F. Increased fecal neutral sterol loss upon liver X receptor activation is independent of biliary sterol secretion in mice. *Gastroenterology* 2005; **128**: 147-156 [PMID: 15633131 DOI: 10.1053/j.gastro.2004.10.006]
- 142 **Temel RE**, Sawyer JK, Yu L, Lord C, Degirolamo C, McDaniel A, Marshall S, Wang N, Shah R, Rudel LL, Brown JM. Biliary sterol secretion is not required for macrophage reverse cholesterol transport. *Cell Metab* 2010; **12**: 96-102 [PMID: 20620999 DOI: 10.1016/j.cmet.2010.05.011]
- 143 **Temel RE**, Brown JM. Biliary and nonbiliary contributions to reverse cholesterol transport. *Curr Opin Lipidol* 2012; **23**: 85-90 [PMID: 22262055 DOI: 10.1097/MOL.0b013e3283508c21]
- 144 **van der Velde AE**, Vries CL, van den Oever K, Kunne C, Oude Elferink RP, Kuipers F, Groen AK. Direct intestinal cholesterol secretion contributes significantly to total fecal neutral sterol excretion in mice. *Gastroenterology* 2007; **133**: 967-975 [PMID: 17854600 DOI: 10.1053/j.gastro.2007.06.019]
- 145 **van der Velde AE**, Vries CL, van den Oever K, Seemann I, Oude Elferink RP, van Eck M, Kuipers F, Groen AK. Regulation of

- direct transintestinal cholesterol excretion in mice. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G203-G208 [PMID: 18511744 DOI: 10.1152/ajpgi.90231.2008]
- 146 **van der Veen JN**, van Dijk TH, Vrans CL, van Meer H, Havinga R, Bijsterveld K, Tietge UJ, Groen AK, Kuipers F. Activation of the liver X receptor stimulates trans-intestinal excretion of plasma cholesterol. *J Biol Chem* 2009; **284**: 19211-19219 [PMID: 19416968 DOI: 10.1074/jbc.M109.014860]
  - 147 **Le May C**, Berger JM, Lespine A, Pillot B, Prieur X, Letessier E, Hussain MM, Collet X, Cariou B, Costet P. Transintestinal cholesterol excretion is an active metabolic process modulated by PCSK9 and statin involving ABCB1. *Arterioscler Thromb Vasc Biol* 2013; **33**: 1484-1493 [PMID: 23559630 DOI: 10.1161/ATVBAHA.112.300263]
  - 148 **Durstine JL**, Grandjean PW, Cox CA, Thompson PD. Lipids, lipoproteins, and exercise. *J Cardiopulm Rehabil* 2002; **22**: 385-398 [PMID: 12464825]
  - 149 **Halverstadt A**, Phares DA, Wilund KR, Goldberg AP, Hagberg JM. Endurance exercise training raises high-density lipoprotein cholesterol and lowers small low-density lipoprotein and very low-density lipoprotein independent of body fat phenotypes in older men and women. *Metabolism* 2007; **56**: 444-450 [PMID: 17378998 DOI: 10.1016/j.metabol.2006.10.019]
  - 150 **Ramachandran S**, Penumetcha M, Merchant NK, Santanam N, Rong R, Parthasarathy S. Exercise reduces preexisting atherosclerotic lesions in LDL receptor knock out mice. *Atherosclerosis* 2005; **178**: 33-38 [PMID: 15585198 DOI: 10.1016/j.atherosclerosis.2004.08.010]
  - 151 **Matsumoto Y**, Adams V, Jacob S, Mangner N, Schuler G, Linke A. Regular exercise training prevents aortic valve disease in low-density lipoprotein-receptor-deficient mice. *Circulation* 2010; **121**: 759-767 [PMID: 20124122 DOI: 10.1161/CIRCULATIONAHA.109.892224]
  - 152 **Meissner M**, Havinga R, Boverhof R, Kema I, Groen AK, Kuipers F. Exercise enhances whole-body cholesterol turnover in mice. *Med Sci Sports Exerc* 2010; **42**: 1460-1468 [PMID: 20139791 DOI: 10.1249/MSS.0b013e3181cfc02]
  - 153 **Rocco DD**, Okuda LS, Pinto RS, Ferreira FD, Kubo SK, Nakandakare ER, Quintão EC, Catanozi S, Passarelli M. Aerobic exercise improves reverse cholesterol transport in cholesteryl ester transfer protein transgenic mice. *Lipids* 2011; **46**: 617-625 [PMID: 21479674 DOI: 10.1007/s11745-011-3555-z]
  - 154 **Wei C**, Penumetcha M, Santanam N, Liu YG, Garelnabi M, Parthasarathy S. Exercise might favor reverse cholesterol transport and lipoprotein clearance: potential mechanism for its anti-atherosclerotic effects. *Biochim Biophys Acta* 2005; **1723**: 124-127 [PMID: 15820521 DOI: 10.1016/j.bbagen.2005.03.005]
  - 155 **Wilund KR**, Feeney LA, Tomayko EJ, Chung HR, Kim K. Endurance exercise training reduces gallstone development in mice. *J Appl Physiol* (1985) 2008; **104**: 761-765 [PMID: 18187606 DOI: 10.1152/japplphysiol.01292.2007]
  - 156 **Wen S**, Jadhav KS, Williamson DL, Rideout TC. Treadmill Exercise Training Modulates Hepatic Cholesterol Metabolism and Circulating PCSK9 Concentration in High-Fat-Fed Mice. *J Lipids* 2013; **2013**: 908048 [PMID: 23862065 DOI: 10.1155/2013/908048]
  - 157 **Pinto PR**, Rocco DD, Okuda LS, Machado-Lima A, Castilho G, da Silva KS, Gomes DJ, Pinto Rde S, Iborra RT, Ferreira Gda S, Nakandakare ER, Machado UF, Correa-Giannella ML, Catanozi S, Passarelli M. Aerobic exercise training enhances the in vivo cholesterol trafficking from macrophages to the liver independently of changes in the expression of genes involved in lipid flux in macrophages and aorta. *Lipids Health Dis* 2015; **14**: 109 [PMID: 26377330 DOI: 10.1186/s12944-015-0093-3]
  - 158 **Ghanbari-Niaki A**, Khabazian BM, Hossaini-Kakhak SA, Rahbarizadeh F, Hedayati M. Treadmill exercise enhances ABCA1 expression in rat liver. *Biochem Biophys Res Commun* 2007; **361**: 841-846 [PMID: 17689492 DOI: 10.1016/j.bbrc.2007.07.100]
  - 159 **Tsekouras YE**, Magkos F, Kellas Y, Basioukas KN, Kavouras SA, Sidossis LS. High-intensity interval aerobic training reduces hepatic very low-density lipoprotein-triglyceride secretion rate in men. *Am J Physiol Endocrinol Metab* 2008; **295**: E851-E858 [PMID: 18664593 DOI: 10.1152/ajpendo.90545.2008]
  - 160 **Lira FS**, Tavares FL, Yamashita AS, Koyama CH, Alves MJ, Caperuto EC, Batista ML, Seelaender M. Effect of endurance training upon lipid metabolism in the liver of cachectic tumour-bearing rats. *Cell Biochem Funct* 2008; **26**: 701-708 [PMID: 18636434 DOI: 10.1002/cbf.1495]
  - 161 **Chapados NA**, Seelaender M, Levy E, Lavoie JM. Effects of exercise training on hepatic microsomal triglyceride transfer protein content in rats. *Horm Metab Res* 2009; **41**: 287-293 [PMID: 19023847 DOI: 10.1055/s-0028-1102937]
  - 162 **Gauthier MS**, Couturier K, Latour JG, Lavoie JM. Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis. *J Appl Physiol* (1985) 2003; **94**: 2127-2134 [PMID: 12547845 DOI: 10.1152/japplphysiol.01164.2002]
  - 163 **Rector RS**, Thyfault JP, Morris RT, Laye MJ, Borengasser SJ, Booth FW, Ibdah JA. Daily exercise increases hepatic fatty acid oxidation and prevents steatosis in Otsuka Long-Evans Tokushima Fatty rats. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G619-G626 [PMID: 18174272 DOI: 10.1152/ajpgi.00428.2007]
  - 164 **Sparks JD**, Sparks CE. Insulin modulation of hepatic synthesis and secretion of apolipoprotein B by rat hepatocytes. *J Biol Chem* 1990; **265**: 8854-8862 [PMID: 2187873]
  - 165 **Lin MC**, Gordon D, Wetterau JR. Microsomal triglyceride transfer protein (MTP) regulation in HepG2 cells: insulin negatively regulates MTP gene expression. *J Lipid Res* 1995; **36**: 1073-1081 [PMID: 7658155]
  - 166 **Khabazian BM**, Ghanbari-Niaki A, Safarzadeh-Golpordesar Ar, Ebrahimi M, Rahbarizadeh F, Abednazari H. Endurance training enhances ABCA1 expression in rat small intestine. *Eur J Appl Physiol* 2009; **107**: 351-358 [PMID: 19629515 DOI: 10.1007/s00421-009-1133-3]
  - 167 **Ihunnah CA**, Jiang M, Xie W. Nuclear receptor PXR, transcriptional circuits and metabolic relevance. *Biochim Biophys Acta* 2011; **1812**: 956-963 [PMID: 21295138 DOI: 10.1016/j.bbadis.2011.01.014]

**P-Reviewer:** Chiang TA, Morales-Gonzalez J, Skrypnik IN

**S-Editor:** Qiu S **L-Editor:** A **E-Editor:** Li D



Basic Study

## Interplay between microRNA-17-5p, insulin-like growth factor- II through binding protein-3 in hepatocellular carcinoma

Danira Ashraf Habashy, Hend Mohamed El Tayebi, Injie Omar Fawzy, Karim Adel Hosny, Gamal Esmat, Ahmed Ihab Abdelaziz

Danira Ashraf Habashy, Hend Mohamed El Tayebi, Injie Omar Fawzy, Department of Pharmacology and Toxicology, German University in Cairo, Main Entrance Al Tagamoa Al Khames, Cairo 11835, Egypt

Karim Adel Hosny, Department of General Surgery, Faculty of Medicine, Cairo University, Cairo 11562, Egypt

Gamal Esmat, Department of Endemic Medicine and Hepatology, Cairo University, Cairo 11562, Egypt

Ahmed Ihab Abdelaziz, Department of Biology, American University in Cairo, New Cairo City, Cairo 11835, Egypt

**Author contributions:** Habashy DA planned and executed all the experiments, acquired the data, performed the statistical analysis, interpretation of data, and wrote the manuscript; Habashy DA, El Tayebi HM and Abdelaziz AI contributed in study concept and design; El Tayebi HM contributed in study co-supervision and revision of the manuscript; Fawzy IO assisted in the binding confirmation experiment and revision of the manuscript; Hosny KA and Esmat G provided tissue samples and clinical data; Abdelaziz AI contributed in study supervision, and critical revision of the manuscript for important intellectual content; all authors approved the final version of the article to be published.

**Institutional review board statement:** The study was approved by the ethical review committees of the German University in Cairo and Cairo University.

**Informed consent statement:** All liver biopsy specimens from patients and healthy donors were taken after informed consent and ethical permission was obtained for participation in the study.

**Conflict-of-interest statement:** To the best of our knowledge, no conflict of interest exists.

**Data sharing statement:** All liver biopsy specimens from patients and healthy donors were taken after informed consent was obtained for participation in the study. Any clinical data stated in the manuscript is anonymous.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Ahmed Ihab Abdelaziz, MD, PhD, Associate Professor of Molecular Medicine, Department of Biology, American University in Cairo, New Cairo City, AUC Avenue, Cairo 11835, Egypt. [ahmed.ihab.abdelaziz@gmail.com](mailto:ahmed.ihab.abdelaziz@gmail.com)  
**Telephone:** +20-2-26151000  
**Fax:** +20-2-27957565

**Received:** March 6, 2016  
**Peer-review started:** March 7, 2016  
**First decision:** May 17, 2016  
**Revised:** June 1, 2016  
**Accepted:** July 11, 2016  
**Article in press:** July 13, 2016  
**Published online:** August 18, 2016

### Abstract

**AIM:** To investigate the effect of microRNA on insulin-like growth factor binding protein-3 (IGFBP-3) and hence on insulin-like growth factor- II (IGF- II) bioavailability in hepatocellular carcinoma (HCC).

**METHODS:** Bioinformatic analysis was performed using microrna.org, DIANA lab and Segal lab softwares. Total RNA was extracted from 23 HCC and 10 healthy liver tissues using mirVana miRNA Isolation Kit. microRNA-17-5p (miR-17-5p) expression was mimicked and antagonized in HuH-7 cell lines using HiPerFect



Transfection Reagent, then total RNA was extracted using Biozol reagent then reverse transcribed into cDNA followed by quantification of miR-17-5p and IGFBP-3 expression using TaqMan real-time quantitative PCR. Luciferase reporter assay was performed to validate the binding of miR-17-5p to the 3'UTR of IGFBP-3. Free IGF- II protein was measured in transfected HuH-7 cells using IGF- II ELISA kit.

**RESULTS:** Bioinformatic analysis revealed IGFBP-3 as a potential target for miR-17-5p. Screening of miR-17-5p and IGFBP-3 revealed a moderate negative correlation in HCC patients, where miR-17-5p was extensively underexpressed in HCC tissues ( $P = 0.0012$ ), while IGFBP-3 showed significant upregulation in the same set of patients ( $P = 0.0041$ ) compared to healthy donors. Forcing miR-17-5p expression in HuH-7 cell lines showed a significant downregulation of IGFBP-3 mRNA expression ( $P = 0.0267$ ) and a significant increase in free IGF- II protein ( $P = 0.0339$ ) compared to mock untransfected cells using unpaired *t*-test. Luciferase assay validated IGFBP-3 as a direct target of miR-17-5p; luciferase activity was inhibited by 27.5% in cells co-transfected with miR-17-5p mimics and the construct harboring the wild-type binding region 2 of IGFBP-3 compared to cells transfected with this construct alone ( $P = 0.0474$ ).

**CONCLUSION:** These data suggest that regulating IGF- II bioavailability and hence HCC progression can be achieved through targeting IGFBP-3 *via* manipulating the expression of miRNAs.

**Key words:** Insulin-like growth factor binding protein-3; Insulin-like growth factor signaling pathway; MicroRNA; Insulin-like growth factor- II ; Hepatocellular carcinoma

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** microRNA-17-5p (miR-17-5p) was extensively underexpressed in hepatocellular carcinoma tissues, while insulin-like growth factor binding protein-3 (IGFBP-3) mRNA showed significant upregulation in the same set of patients. In HuH-7 cell line, miR-17-5p directly targets and downregulates IGFBP-3, consequently elevating the level of free insulin-like growth factor- II (IGF- II). Thus, manipulation of microRNAs can potentially control the activation of the oncogenic IGF axis.

Habashy DA, El Tayebi HM, Fawzy IO, Hosny KA, Esmat G, Abdelaziz AI. Interplay between microRNA-17-5p, insulin-like growth factor- II through binding protein-3 in hepatocellular carcinoma. *World J Hepatol* 2016; 8(23): 976-984 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i23/976.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i23.976>

## INTRODUCTION

The insulin-like growth factor (IGF) signaling pathway

is composed of IGF ligands, IGF receptors and insulin-like growth factor binding proteins (IGFBPs) which work in unison to regulate cell growth, differentiation, proliferation, and apoptosis. This axis is activated when the IGFs, IGF- I and IGF- II , bind to the insulin-like growth factor-1 receptor (IGF-1R) and activate a series of downstream signaling pathways controlling the cell cycle<sup>[1,2]</sup>. IGFBPs are transport proteins which bind to IGF- II with high affinity thereby prolonging their half-life and circulation turnover, and negatively regulate the activity of IGFs by controlling their binding to IGF receptors<sup>[3]</sup>. The levels of IGFBPs are modulated by various IGFBP proteases, such as matrix metalloproteinases (MMPs), which regulate the bioavailability and activity of IGFBPs, by mediating their proteolytic cleavage<sup>[4]</sup>.

Multiple IGF axis members were found to play an important role in hepatocellular carcinoma (HCC) pathogenesis. IGF- II was found to be overexpressed in HCC and to promote tumor cell migration, proliferation and extra-hepatic metastasis<sup>[5-8]</sup>. Moreover, our research group has shown IGF- II to be overexpressed in peripheral blood monocytes of HCC patients, and this aberrant expression was directly correlated with elevated serum levels of alfa-fetoprotein and poor prognosis<sup>[9]</sup>. IGF-1R was reported to be upregulated in 59% of HCC tissues in which it was associated with poor prognosis and tumors exceeding the Milan criteria<sup>[10]</sup>. The tumorigenic effect of IGF-1R was reversed through its efficient blockage by combination of two IGF-1R antibodies which dramatically reduced liver tumor growth<sup>[11]</sup>. On the other hand, IGFBP-3 expression was found to be inversely correlated to HCC metastasis and proliferation<sup>[12,13]</sup>.

The potential regulation of IGF axis members by microRNAs is an appealing subject of investigation. We have previously shown that miR-615-5p downregulates IGF- II expression and forcing its expression reduces tumorigenesis in HCC<sup>[14]</sup>. miR-122 was found to suppress IGF-1R expression thus inhibiting HCC progression<sup>[15,16]</sup>. Conversely, we have demonstrated that forcing the expression of the oncomiR miR-96 leads to the up-regulation of IGF-1R and IGFBP-3 expression, while forcing the expression of the oncomiR-182 leads to the downregulation of IGF-1R and the upregulation of IGFBP-3 expression<sup>[17]</sup>. On the other hand, our research group reported that miR-155 induces the expression of IGF- II and IGF-1R and downregulates IGFBP-3 expression<sup>[18]</sup>. Nevertheless, the regulation of IGF-axis members by microRNAs still needs further investigation, particularly for the IGFBP-3. *In silico* analysis revealed IGFBP-3 as a potential downstream target for several microRNAs, one of which is microRNA-17-5p (miR-17-5p). This microRNA is an oncomiR that belongs to miR-17-92 cluster<sup>[19]</sup>. We have previously shown miR-17-5p to be significantly downregulated in non-metastatic HCC tissues compared to healthy tissues, where forcing its expression in HuH-7 cells resulted in enhancement of tumor cell growth, proliferation, migration, and colony-formation<sup>[20]</sup>. Therefore, this study aimed at identifying the impact of this important microRNA on IGFBP-3 expression, and

**Table 1** Characteristic features of non-metastatic hepatocellular carcinoma patients and healthy controls

	Average $\pm$ SD
HCC and cirrhotic patient parameters	
Mean age	49 $\pm$ 13.5
Sex: Male/female	22/1
Ethanol abuse	None
AST (U/L)	100.5 $\pm$ 65.8
ALT (U/L)	85.6 $\pm$ 95.6
Alkaline phosphatase (U/L)	110.2 $\pm$ 60.7
Serum albumin (g/dL)	4.6 $\pm$ 1.5
Serum AFP (ng/mL)	155.7 $\pm$ 22.3
HCV Ab	100% (23/23 HCC patients)
HBV Ab	17.3% (4/23 HCC patients)
Healthy control (liver donor) parameters	
Mean age	31 $\pm$ 10.5
Sex: Male/female	7/3
Ethanol abuse	None
HCV Ab	None
HBV Ab	None

HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; AFP: Alpha fetal protein; HBV: Hepatitis B virus; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; SD: Standard deviation.

consequently on the IGF- II bioavailability, and hence on HCC tumorigenesis.

## MATERIALS AND METHODS

### Bioinformatics

Bioinformatics algorithms microrna.org, DIANA Lab, and Segal lab were used to predict microRNAs that may target IGFBP-3.

### Study subjects

This study included 23 HCC patients who underwent liver transplantation surgery in the Kasr Al Aini Hospital, Cairo University, Egypt. Ten healthy liver tissues were obtained from the healthy liver donors. Healthy donors were non-diabetic, non-hypertensive and negative for hepatitis B and C viruses (Table 1). The study was approved by the ethical review committees of the German University in Cairo and Cairo University, and is in accordance with the standards set by the Declaration of Helsinki. All participants gave their written informed consent. All patients were non-metastatic with no extrahepatic manifestations and no vascular invasion. Most of the patients (65.5%) had more than one focal lesion as indicated in the pathology report and were subjected to clinical assessment as shown in (Table 2).

### Cell cultures and transfection of microRNA oligonucleotides

HuH-7 cells were maintained in Dulbecco's modified Eagle's medium (Lonza, Switzerland) supplemented with 4.5 g/L glucose, 4 mmol/L L-glutamine, 10% fetal bovine serum and Mycozap (1:500, Lonza, Switzerland) at 37 °C in 5% CO<sub>2</sub> atmosphere. HuH-7 cells were transfected with mimics and inhibitors of miR-17-5p (Qiagen, Ger-

**Table 2** Number/sizes of focal lesions according to Milan criteria

Patients	No. of focal lesions	Size of focal lesions (cm)
Patient 1	3 focal lesions	1.5, 1 and 1
Patient 2	Unifocal	2.5
Patient 3	3 focal lesions	2, 2.5 and 3
Patient 4	3 focal lesions	2, 2 and 3.5
Patient 5	Unifocal	1.5-2
Patient 6	3 focal lesions	3-4, 1 and 1
Patient 7	Unifocal	4
Patient 8	3 focal lesions	4, 1 and 1
Patient 9	3 focal lesions	1, 1 and 1.5
Patient 10	Unifocal	2.5
Patient 11	2 focal lesions	1 and 1.7
Patient 12	3 focal lesions	1, 1 and 1
Patient 13	Unifocal	3
Patient 14	3 focal lesions	3, 1.5 and 2
Patient 15	3 focal lesions	1, 1 and 4
Patient 16	2 focal lesions	3 and 1.5
Patient 17	2 focal lesions	1.5 and 3
Patient 18	3 focal lesions	2.5, 2.5 and 1.5
Patient 19	3 focal lesions	1.5, 1 and 1
Patient 20	Unifocal	2
Patient 21	Unifocal	1.5
Patient 22	3 focal lesion	3, 2.5 and 1
Patient 23	Unifocal	3

many) (Qiagen ID: MSY0000070 and MIN0000070, respectively). All transfection experiments were carried out in triplicates using HiPerFect Transfection Reagent (Qiagen, Germany), according to the manufacturer's protocol; the experiments were repeated three times. Cells that were only exposed to transfection reagent are designated as mock. Cells transfected with miR-17-5p mimics are designated as miR-17-5p, whereas cells transfected with miR-17-5p inhibitor are designated as anti-miR-17-5p.

### mRNA and microRNA isolation from liver tissues and HCC cell lines

mRNAs and microRNAs were extracted from liver tissues and HCC cell lines. Fresh liver samples (HCC and healthy tissues) were collected during surgery and were immediately snapfrozen in liquid nitrogen. The specimens were manually pulverized in liquid nitrogen, and about 100 mg of tissues powder were used for large and small RNA extraction using mirVana miRNA Isolation Kit (Ambion, United States), according to the manufacturer's protocol. HCC cell lines were harvested 48 h after transfection according to HiPerFect Transfection Reagent protocol and total RNA was extracted using Biozol Reagent (Bioer Technology, China).

### miRNA and mRNA quantification

The extracted microRNAs were reverse transcribed into single stranded complementary DNA (cDNA) using TaqMan MicroRNA Reverse Transcription Kit (ABI, United States) and specific primers for has-miR-17-5p and RNU6B. mRNA was reverse transcribed into cDNA using the high-capacity cDNA reverse transcription kit (ABI, United States)

**Table 3** The forward and reverse primer sequences used in the wild type 1 and 2, and the mutant type 1 and 2 insulin-like growth factor binding protein-3 3'UTR constructs

Primer name	Primer sequence
WT1 forward	5'-CAATGGTAAACTTGAGCATCTTTTCACTTTCCAGTAGT-3'
WT1 reverse	5'-CTAGACTACTGGAAAAGTGAAAAGATGCTCAAGTTTACCATTGAGCT-3'
WT2 forward	5'-CGTCGAAGCGGCCGACCACTGACTTTGTGACTTT-3'
WT2 reverse	5'-CTAGAAAAGTCACAAAGTCAGTGGTCGGCCGCTTCGACGAGCT-3'
MUT1 forward	5'-CAATGGTAAACTTGAGCATCTTTTCACTCCAGTAGT3'
MUT1 reverse	5'-CTAGACTACTGGATGAAAAGATGCTCAAGTTTACCATTGAGCT-3'
MUT2 forward	5'-CGTCGAAGCGGCCGACCACTGACGTGACTTT-3'
MUT2 reverse	5'-CTAGAAAAGTCACGTCAAGTGGTCGGCCGCTTCGACGAGCT-3'

WT: Wild type; MUT: Mutant type.

according to the manufacturer's instructions. Relative expression of miR-17-5p and RNU6B (for normalization) as well as IGFBP-3 and beta-2 microglobulin (B2M; as housekeeping gene for normalization) was quantified using TaqMan Real-Time quantitative PCR (ABI Assay IDs: 002308, 001093, Hs00365742\_g1 and Hs00984230\_m1, respectively) using StepOne™ Systems (ABI, United States). Relative expression was calculated using the  $2^{-\Delta\Delta CT}$  method. All PCR reactions including controls were run in duplicate reactions.

#### IGFBP-3 3'UTR construct and luciferase assay

The two predicted target sites for miR-17-5p on IGFBP-3 3'UTR were each designed as sticky ended oligonucleotides flanked by *Sac* I and *Xba* I restriction sites, and ligated into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Germany) to form the two wild-type (WT) constructs. Also, two mutant constructs (MUT) were designed where 3 nucleotides from the binding region had been deleted from each site. The first target site is denoted as WT1 and its mutant form as MUT1; the second target site is WT2 and its mutant form is MUT2. The forward and reverse primer sequences for each construct are as shown in (Table 3). HuH-7 cells were seeded in 24-well plates and either WT or MUT constructs were transfected by lipofection technique using SuperFect Transfection Reagent (Qiagen, Germany). The following day, the cells were co-transfected with miR-17-5p mimics using HiPerFect according to the protocol (Qiagen). After 48 h, luciferase assay was performed using Steady-GLO Luciferase Reporter System (Promega, Germany) according to the manufacturer's protocol. After 5 min, luminescence was measured at 545 nm. Luciferase experiments were done in triplicates.

#### Quantitative detection of free IGF-II protein

Free IGF- II protein was measured in the cell culture supernatant from miR-17-5p mimicked, miR-17-5p antagonized, and mock untransfected HuH-7 cells, using the human IGF- II ELISA kit (CUSABIO, China), according to the manufacturer's instructions. Absorbance was measured at 450 nm in a microplate reader.

#### Statistical analysis

miRNA and gene expression data analysis was performed

according to the  $2^{-\Delta\Delta CT}$  method. An assessment of the normality of data was done as a prerequisite for all the statistical tests to identify the correct statistical methods to analyze our data with. We used Shapiro Wilks test since the size of the sample is less than 50. The normality test for miR-17-5p and IGFBP-3 screening experiments of "Healthy controls" and "HCC patients" showed that the dependent variable, "RQ", isn't normally distributed since the significant value of the Shapiro Wilks test is less than 0.05, so the data significantly deviate from a normal distribution, with an exception in the data obtained from IGFBP-3 expression in the healthy controls were found to be normally distributed. In view of this fact the statistical significance of the data was analyzed by performing the non-parametric Mann-Whitney test. The degree of the relationship between linear related variables was measured by the Pearson *r* correlation test. The normality test for the transfection and binding confirmation experiments showed that the data are normally distributed; therefore the parametric unpaired *t*-test was used. The specific types of tests, when applicable, are indicated in the figure legends. All data are presented as mean  $\pm$  standard error of the mean (SEM). All tests were 2-tailed and a two-tailed *P* value  $< 0.05$  was required for statistical significance. All the data were statistically analyzed using GraphPad Prism 5 software.

The statistical methods of this study were reviewed by Dr. Nihal Aly Etman, Department of Statistics, Mathematics and Insurance, Faculty of Commerce, Ain Shams University.

## RESULTS

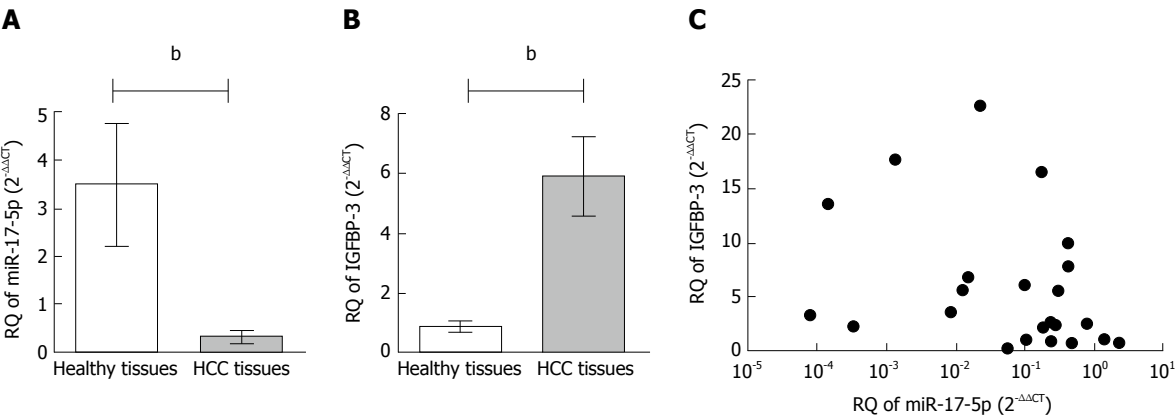
#### Bioinformatics

miR-17-5p accession number and mature sequence were retrieved using miRBase database (<http://www.mirbase.org/>). *In silico* predictions were carried out using three different softwares, and results showed IGFBP-3 to be a potential downstream target to miR-17-5p, where the microRNA was predicted to bind to the 3'UTR of IGFBP-3 at two different regions. The interactions between miR-17-5p seed sequence and its target sequence on the 3'UTR of IGFBP-3 are as shown in (Table 4). Where, the seed sequence of miR-17-5p

**Table 4** Predicted target region-seed sequence binding for miR-17-5p on the 3'UTR of insulin-like growth factor binding protein-3

Target region	hsa-miR-17-5p (seed sequence) binding to IGFBP-3 (target sequence)	Target sequence position on 3'UTR of IGFBP-3	6mer/7mer/8mer
Region 1	miR-17-5p 3'gaUGGACGUG-ACAUUCGUGAAAc 5'     :     :                     IGFBP-3 5'aaACUUGAGCAUCUUUU <u>CACUUU</u> c 3'	196-204	6mer
Region 2	miR-17-5p 3'GAUGGAC- GUGACAUUCGUGAAAC 5'                                 IGFBP-3 5' CGGCCGACCACUG----- <u>ACUUUG</u> 3'	335-343	6mer

IGFBP-3: Insulin-like growth factor binding protein-3; miR-17-5p: MicroRNA-17-5p.



**Figure 1** Expression profile of microRNA-17-5p and insulin-like growth factor binding protein-3 and their correlation in liver tissues. The expression of miR-17-5p and IGFBP-3 were investigated in 10 healthy and 23 HCC liver tissues using TaqMan qRT-PCR and normalized in each sample to RNU6B endogenous control for miR-17-5p and B2M for IGFBP-3. A: miR-17-5p expression was down-regulated in non-metastatic HCC patients compared to healthy liver tissues ( $P = 0.0012$ ); B: Regarding IGFBP-3, its mRNA expression showed a significant higher expression in HCC tissues compared to healthy tissues ( $P = 0.0041$ ). Statistical analysis was performed using the Mann-Whitney test; C: Relative quantitation (RQ) values of miR-17-5p and IGFBP-3 mRNA in HCC tissues were analyzed using Pearson's method of correlation. A non-significant inverse correlation was found with Pearson's  $r = -0.3244$  ( $P = 0.1310$ ). <sup>b</sup> $P < 0.01$ . HCC: Hepatocellular carcinoma; IGFBP-3: Insulin-like growth factor binding protein-3; miR-17-5p: MicroRNA-17-5p; qRT-PCR: Real-time quantitative PCR.

is shown in bold and italic, while the target sequence of the 3'UTR of IGFBP-3 is underlined. The lines indicate complementarity between the binding region of the mRNA and the seed sequence of the microRNA, while the dots indicate mismatches or GU wobbles.

**Expression profile of miR-17-5p and IGFBP-3 in non-metastatic HCC liver tissues**

Expression of miR-17-5p in non-metastatic HCC tissues ( $n = 23$ ) ( $0.318 \pm 0.109$ ) was significantly lower compared to healthy tissues ( $n = 10$ ) ( $3.488 \pm 1.267$ ,  $P = 0.0012$ ; Figure 1A). On the other hand, the expression of IGFBP-3 in the same non-metastatic HCC tissues ( $5.913 \pm 1.294$ ) was significantly higher compared to healthy tissues ( $1.352 \pm 0.272$ ,  $P = 0.0041$ ; Figure 1B).

**Correlation analysis between miR-17-5p and IGFBP-3 mRNA expression in HCC tissues**

IGFBP-3 mRNA was quantified in all HCC tissues and correlated to miR-17-5p expression in the same patients. Using Pearson's statistical method of correlation, miR-17-5p expression was found to be moderately inversely correlated but not statistically significant with IGFBP-3 transcript levels in all HCC tissues studied ( $r = -0.3244$ ,  $P$

$= 0.1310$ ; Figure 1C).

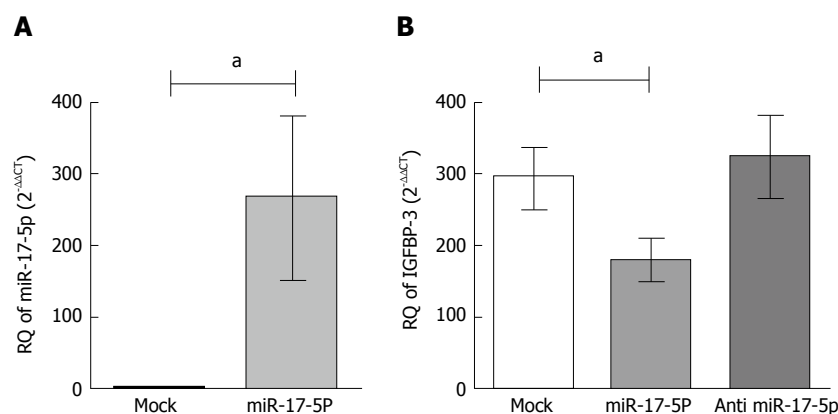
**Impact of miR-17-5p on IGFBP-3 mRNA in HuH-7 cells**

HuH-7 cells were transfected with miR-17-5p mimics and transfection efficiency was achieved with an observed 250 fold increase ( $P = 0.0470$ ) in miR-17-5p levels in transfected cells ( $266.6 \pm 113.2$ ) compared to their respective untransfected mock cells ( $1.069 \pm 0.1927$ ) (Figure 2A). Mimicking of miR-17-5p in HuH-7 resulted in a significant downregulation of IGFBP-3 mRNA levels ( $0.6527 \pm 0.1021$ ) compared to mock untransfected cells ( $1.069 \pm 0.1502$ ,  $P = 0.0267$ ). Conversely, inhibitors of miR-17-5p in HuH-7 cells showed a tendency of increase compared to mock untransfected HuH-7 cell lines (Figure 2B).

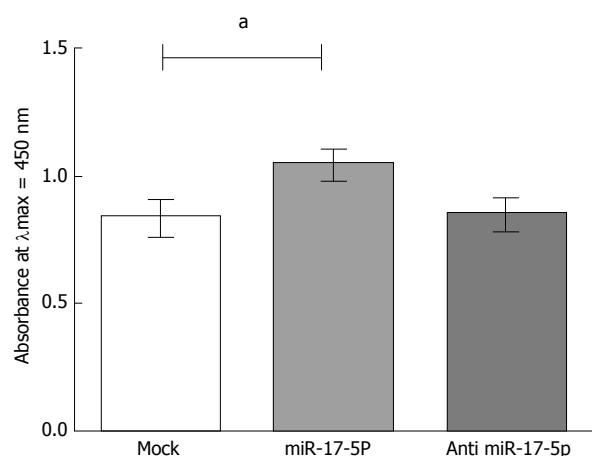
**Impact of miR-17-5p on free IGF-II protein in HuH-7 cells**

In miR-17-5p mimicked HuH-7 cells, there was a significant upregulation in the amount of the free IGF- II protein ( $1.045 \pm 0.05255$ ) compared to mock untransfected HuH-7 cells ( $0.8344 \pm 0.06783$ ,  $P = 0.0339$ ). Antagonizing the expression of miR-17-5p had no effect on the amount of the free IGF- II protein compared to the mock HuH-7 cells (Figure 3).





**Figure 2** Impact of microRNA-17-5p on insulin-like growth factor binding protein-3 mRNA expression in HuH-7 cell line. A: The expression of miR-17-5p was determined by TaqMan qRT-PCR in HuH-7 cells transfected with oligonucleotide mimics of miR-17-5p, 48 h post-transfection, relative to their expression in mock untransfected HuH-7 cells. The expression of miR-17-5p was normalized to RNU6B endogenous control. A: Transfection of miR-17-5p mimics increased miR-17-5p levels in HuH-7 by 250 fold compared to mock cells ( $P = 0.0470$ ). Unpaired *t*-test was performed; B: HuH-7 cells were transfected with miR-17-5p mimics or inhibitors, and the relative expression of IGFBP-3 was determined using TaqMan qRT-PCR, relative to mock untransfected cells, and gene expression was normalized to endogenous control B2M. IGFBP-3 mRNA expression was dramatically suppressed upon mimicking of miR-17-5p compared to mock cells ( $P = 0.0267$ ), while inhibitors of miR-17-5p showed a tendency of increase compared to mock cells. Unpaired *t*-test was performed. <sup>a</sup> $P < 0.05$ . IGFBP-3: Insulin-like growth factor binding protein-3; miR-17-5p: MicroRNA-17-5p; qRT-PCR: Real-time quantitative PCR.



**Figure 3** Impact of microRNA-17-5p on free insulin-like growth factor-II protein in HuH-7 cells. HuH-7 cells were transfected with miR-17-5p mimics or inhibitors. The free IGF- II protein was measured in media of mimicked and antagonized HuH-7 cells using an IGF- II ELISA Kit. Free IGF- II protein, measured at  $\lambda_{max} = 450$ , was found to be significantly increased upon mimicking of miR-17-5p expression compared to mock untransfected cells ( $P = 0.0339$ ), while inhibitors of miR-17-5p showed no effect on the levels of free IGF- II protein levels compared to mock cells. Unpaired *t*-test was performed. <sup>a</sup> $P < 0.05$ . IGF- II: Insulin-like growth factor- II; miR-17-5p: MicroRNA-17-5p.

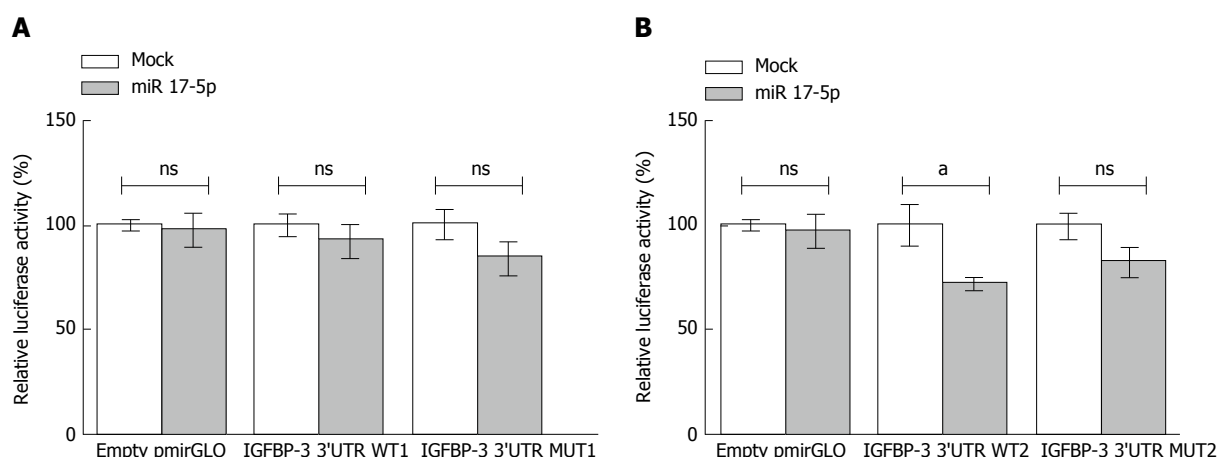
### Confirming IGFBP-3 as a direct target of miR-17-5p

To confirm that miR-17-5p directly targets the 3'UTR of IGFBP-3, wild-type constructs (WT1 and WT2) were designed where each of the two predicted 3'UTR target regions were inserted downstream to a luciferase reporter gene in pmiRGLO vector. To assess that the effects were due to specific binding to these binding regions, a mutant construct for each binding site was also prepared in which 3 base pairs were deleted from the predicted binding sequence in the 3'UTR of IGFBP-3, to form mutant constructs MUT1 and MUT2, respectively. Also, in a set of cells, empty pmiRGLO vector was transfected as a control to ensure that miR-17-5p mimics have no effect

on the vector itself. For each binding region, experiments were performed by transfecting HuH-7 cells with either the construct containing the WT 3'UTR binding region of IGFBP-3, or the construct containing the MUT 3'UTR binding region. Then miR-17-5p mimics were co-transfected with the vectors or constructs and luciferase reporter activity was assessed. In cells transfected with WT1 construct, luciferase activity was not affected upon co-transfection with miR-17-5p mimics (Figure 4A). On the other hand, luciferase activity was inhibited by 27.5% in cells co-transfected with miR-17-5p mimics and WT2 construct ( $72.48 \pm 2.383$ ) compared to cells transfected with the WT2 construct alone ( $100.0 \pm 9.432$ ,  $P = 0.0474$ ) (Figure 4B). In contrast, in cells transfected with either MUT1 or MUT2, no change in luciferase activity was observed upon mimicking with miR-17-5p (Figure 4). The inhibition in the luciferase activity observed only in the WT2 construct indicates direct targeting and transcriptional inhibition of IGFBP-3 by miR-17-5p mimics through only one of the two predicted target regions.

## DISCUSSION

The regulation of IGFBP-3 by microRNAs has not been extensively studied but recently our research group showed that the oncomiR miR-155 represses IGFBP-3 expression in HCC cell lines<sup>[18]</sup>. In addition, we showed an increased expression of IGFBP-3 upon forcing the expression of miR-96 and miR-182<sup>[17]</sup>. To the best of our knowledge the IGF- II bioavailability has never been investigated after targeting IGFBPs with microRNAs, therefore in this study, we aimed at identifying a new microRNA which could regulate the IGFBP-3 and consequently the IGF- II bioavailability, and hence influence HCC tumorigenesis. *In silico* analysis revealed IGFBP-3 as a potential downstream target for miR-17-5p (Table 4), a microRNA which we have previously shown to have



**Figure 4** Insulin-like growth factor binding protein-3 is a direct target of miR-17-5p. For each target sequence, experiments were performed by transfecting HuH-7 cells with either empty pmirGLO vector, or the construct with the wild-type (WT) miR-17-5p target region insert, or the construct with the mutant (MUT) miR-17-5p target region insert. Then miR-17-5p mimics were co-transfected with the vectors or constructs. A: Luciferase activity was not affected in cells co-transfected with miR-17-5p mimics and WT1 construct compared to cells transfected with the WT1 construct alone; B: On the other hand, luciferase activity was inhibited by 27.5%, in cells co-transfected with miR-17-5p mimics and WT2 construct compared to cells transfected with the WT2 construct alone ( $P = 0.0474$ ). The cells transfected with either of the mutant constructs (MUT1 or MUT2) show no change in the luciferase activity upon mimicking with miR-17-5p. Unpaired *t*-test was performed. <sup>a</sup> $P < 0.05$ . NS: Not significant; IGFBP-3: Insulin-like growth factor binding protein-3; miR 17-5p: MicroRNA-17-5p.

oncogenic properties in HCC<sup>[20]</sup>.

No correlation analysis was previously done between miR-17-5p and IGFBP-3 expression in HCC patients, therefore non-metastatic liver tissues of HCC patients were screened for that purpose. miR-17-5p was markedly downregulated (Figure 1A) while IGFBP-3 was significantly upregulated (Figure 1B) in the non-metastatic liver tissues of HCC patients compared to healthy controls. This goes in line with previous studies showing IGFBP-3 to be highly expressed in breast and esophageal cancer<sup>[21,22]</sup>. But on the other hand, it contradicts other studies that reported reduced IGFBP-3 mRNA expression and protein levels in metastatic HCC patients<sup>[12,13]</sup>. Moreover, the repression of miR-17-5p in HCC tissues (Figure 1A) corroborates our previous results that showed a significant downregulation of miR-17-5p expression in non-metastatic HCC patients<sup>[20]</sup>, but nonetheless it contradicts other studies in metastatic HCC tissues<sup>[23]</sup>. These disparities can, however, be attributed to differences in the cohorts of patients included in the various studies, with regards to stage and etiology of the disease as well as other factors such as ethnicity, gender and age. Of note, the results of the correlation analysis revealed a moderate negative correlation between miR-17-5p and IGFBP-3 expression in HCC patients (Figure 1C), suggesting that IGFBP-3, as predicted by *in silico* analysis, may in fact be under the posttranscriptional regulation of miR-17-5p.

In order to investigate the effect of miR-17-5p on IGFBP-3, transfection experiments were performed by forcing miR-17-5p expression in HuH-7 cell lines and the expression of IGFBP-3 mRNA was assessed, where it was found that upon forcing miR-17-5p expression in HuH-7 cells, there was a significant downregulation in IGFBP-3 expression (Figure 2B). This finding further implies that miR-17-5p may target and regulate IGFBP-3

expression. As revealed by *in silico* analysis, the 3'UTR of the IGFBP-3 transcript contains two exclusive putative binding sites for miR-17-5p. In order to validate IGFBP-3 as a direct downstream target of miR-17-5p, a WT and a MUT luciferase reporter gene construct was prepared for each binding region on the 3'UTR of IGFBP-3. Using these microRNA-target expression constructs, it was demonstrated that forcing the expression of miR-17-5p significantly decreased luciferase activity only in the construct harboring the WT2 binding region of the 3' UTR of IGFBP-3 target gene (Figure 4). This interesting finding indicates that only one of the two putative binding sites is in fact functionally active and that miR-17-5p effectively targets and inhibits the transcription of IGFBP-3 by directly associating with this specific target region. This unusual observation has also been found in colon cancer where bioinformatic tools predicted two target sites on the oncogene Friend leukemia virus integration 1 (Fli-1) for the tumor suppressor miR-145; however, upon measuring the luciferase activity only the construct harboring one of these two predicted target sites of Fli-1 showed a decrease in luciferase activity by more than 50% upon miR-145 mimicking, while the other construct harboring the second target site did not respond to miR-145<sup>[24]</sup>.

Since IGFBP-3 is a crucial negative regulator of the bioavailability of IGF- II, therefore the levels of free IGF- II protein were quantified in the media of miR-17-5p mimicked and mock untransfected HuH-7 cells. The results showed a significant increase in unbound IGF- II in miR-17-5p mimicked HuH-7 cells compared to mock untransfected cells (Figure 3). This in turn confirms that miR-17-5p regulates IGF- II bioavailability through direct targeting of IGFBP-3. In this regard, the biological function of miR-17-5p appears to simulate the effect of another regulator of the IGF pathway, the MMPs, whose

overexpression leads to the decrease in IGFBP-3 and subsequent increase in IGF- II bioavailability<sup>[25]</sup>.

In conclusion, the findings of this study shed light on the important role of the oncogenic miR-17-5p in hepatocarcinogenesis, where this microRNA was found to increase IGF- II bioavailability by directly targeting and repressing IGFBP-3 expression. Hence, manipulating microRNA expression might be a compelling potential therapeutic approach in preventing HCC progression.

## ACKNOWLEDGMENTS

The authors would like to thank the English instructor, Ms. Gilan Hamdi, and the English Department at the German University in Cairo for revising the manuscript. We would like to thank Ms. Nihal Etman, from the Department of Statistics, Mathematics and Insurance at Ain Shams University, Cairo, Egypt for reviewing the statistics of the manuscript.

## COMMENTS

### Background

Insulin-like growth factor-II (IGF-II) is a major activator of the oncogenic IGF axis, often overexpressed in hepatocellular carcinoma leading to the promotion of tumor cell migration, proliferation and metastasis. IGF-II protein bioavailability is controlled by a class of insulin-like growth factor binding proteins (IGFBPs) 1-6 which regulate the binding of IGF-II to its receptor, IGF-1 receptor. Very few studies have investigated the regulation of IGFBPs by microRNAs.

### Research frontiers

Recently, microRNAs have entered the first clinical trials investigating their therapeutic potential in primary liver cancer.

### Innovations and breakthroughs

This is the first study to investigate the effect of a microRNA on an IGFBP and consequently on the IGF- II bioavailability.

### Applications

microRNA-17-5p affected IGFBP-3 and consequently the level of free IGF- II which could allow for the activation of the oncogenic IGF axis. This suggests that microRNAs can be manipulated to regulate the activation of this axis.

### Terminology

microRNAs are approximately 22 nucleotide long single stranded, small, non-coding RNA sequences that post-transcriptionally regulate gene expression by binding to the 3'UTR of their target mRNA, suppressing its translation or causing its degradation.

### Peer-review

The study is well planned involves proving of a concept by bioinformatics tools and then confirming *in vitro* and patient's tissues.

## REFERENCES

- 1 **Zha J**, Lackner MR. Targeting the insulin-like growth factor receptor-1R pathway for cancer therapy. *Clin Cancer Res* 2010; **16**: 2512-2517 [PMID: 20388853 DOI: 10.1158/1078-0432.CCR-09-2232]
- 2 **Blundell TL**, Bedarkar S, Rinderknecht E, Humbel RE. Insulin-like growth factor: a model for tertiary structure accounting for immunoreactivity and receptor binding. *Proc Natl Acad Sci USA* 1978; **75**: 180-184 [PMID: 272633 DOI: 10.1073/pnas.75.1.180]
- 3 **Firth SM**, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. *Endocr Rev* 2002; **23**: 824-854 [PMID: 12466191 DOI: 10.1210/er.2001-0033]
- 4 **Rajah R**, Katz L, Nunn S, Solberg P, Beers T, Cohen P. Insulin-like growth factor binding protein (IGFBP) proteases: functional regulators of cell growth. *Prog Growth Factor Res* 1995; **6**: 273-284 [PMID: 8817670 DOI: 10.1016/0955-2235(95)00012-7]
- 5 **Nussbaum T**, Samarin J, Ehemann V, Bissinger M, Ryschich E, Khamidjanov A, Yu X, Gretz N, Schirmacher P, Breuhahn K. Autocrine insulin-like growth factor-II stimulation of tumor cell migration is a progression step in human hepatocarcinogenesis. *Hepatology* 2008; **48**: 146-156 [PMID: 18537183 DOI: 10.1002/hep.22297]
- 6 **Qian J**, Yao D, Dong Z, Wu W, Qiu L, Yao N, Li S, Bian Y, Wang Z, Shi G. Characteristics of hepatic igf-ii expression and monitored levels of circulating igf-ii mRNA in metastasis of hepatocellular carcinoma. *Am J Clin Pathol* 2010; **134**: 799-806 [PMID: 20959664 DOI: 10.1309/AJCPTFDSE2V3LCZP]
- 7 **Breuhahn K**, Longerich T, Schirmacher P. Dysregulation of growth factor signaling in human hepatocellular carcinoma. *Oncogene* 2006; **25**: 3787-3800 [PMID: 16799620 DOI: 10.1038/sj.onc.1209556]
- 8 **Couvert P**, Carrié A, Pariès J, Vaysse J, Miroglia A, Kerjean A, Nahon P, Chelly J, Trinchet JC, Beaugrand M, Ganne-Carrié N. Liver insulin-like growth factor 2 methylation in hepatitis C virus cirrhosis and further occurrence of hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 5419-5427 [PMID: 18803353 DOI: 10.3748/wjg.14.5419]
- 9 **El Tayebi HM**, Salah W, El Sayed IH, Salam EM, Zekri AR, Zayed N, Salem ES, Esmat G, Abdelaziz AI. Expression of insulin-like growth factor-II, matrix metalloproteinases, and their tissue inhibitors as predictive markers in the peripheral blood of HCC patients. *Biomarkers* 2011; **16**: 346-354 [PMID: 21506705 DOI: 10.3109/1354750X.2011.573095]
- 10 **Liu X**, Jiang W, Aucejo F, Kim R, Miller C, Byrne M, Lopez R, Yerian L. Insulin-like growth factor I receptor  $\beta$  expression in hepatocellular carcinoma. *Hum Pathol* 2011; **42**: 882-891 [PMID: 21292299 DOI: 10.1016/j.humpath.2010.10.007]
- 11 **Dong J**, Demarest SJ, Sereno A, Tamraz S, Langley E, Doern A, Snipas T, Perron K, Joseph I, Glaser SM, Ho SN, Reff ME, Hariharan K. Combination of two insulin-like growth factor-I receptor inhibitory antibodies targeting distinct epitopes leads to an enhanced antitumor response. *Mol Cancer Ther* 2010; **9**: 2593-2604 [PMID: 20716637 DOI: 10.1158/1535-7163.MCT-09-1018]
- 12 **Luo SM**, Tan WM, Deng WX, Zhuang SM, Luo JW. Expression of albumin, IGF-1, IGFBP-3 in tumor tissues and adjacent non-tumor tissues of hepatocellular carcinoma patients with cirrhosis. *World J Gastroenterol* 2005; **11**: 4272-4276 [PMID: 16015705 DOI: 10.3748/wjg.v11.i27.4272]
- 13 **Huynh H**, Chow PK, Ooi LL, Soo KC. A possible role for insulin-like growth factor-binding protein-3 autocrine/paracrine loops in controlling hepatocellular carcinoma cell proliferation. *Cell Growth Differ* 2002; **13**: 115-122 [PMID: 11959812]
- 14 **El Tayebi HM**, Hosny KA, Esmat G, Breuhahn K, Abdelaziz AI. miR-615-5p is restrictedly expressed in cirrhotic and cancerous liver tissues and its overexpression alleviates the tumorigenic effects in hepatocellular carcinoma. *FEBS Lett* 2012; **586**: 3309-3316 [PMID: 22819824 DOI: 10.1016/j.febslet.2012.06.054]
- 15 **Girard M**, Jacquemin E, Munnich A, Lyonnet S, Henrion-Caude A. miR-122, a paradigm for the role of microRNAs in the liver. *J Hepatol* 2008; **48**: 648-656 [PMID: 18291553 DOI: 10.1016/j.jhep.2008.01.019]
- 16 **Zeng C**, Wang R, Li D, Lin XJ, Wei QK, Yuan Y, Wang Q, Chen W, Zhuang SM. A novel GSK-3  $\beta$ -C/EBP  $\alpha$ -miR-122-insulin-like growth factor 1 receptor regulatory circuitry in human hepatocellular carcinoma. *Hepatology* 2010; **52**: 1702-1712 [PMID: 21038412 DOI: 10.1002/hep.23875]
- 17 **Assal RA**, El Tayebi HM, Hosny KA, Esmat G, Abdelaziz AI. A pleiotropic effect of the single clustered hepatic metastamirs miR-96-5p and miR-182-5p on insulin-like growth factor II, insulin-like growth factor-1 receptor and insulin-like growth factor-binding

- protein-3 in hepatocellular carcinoma. *Mol Med Rep* 2015; **12**: 645-650 [PMID: 25739014 DOI: 10.3892/mmr.2015.3382]
- 18 **El Tayebi HM**, Waly AA, Assal RA, Hosny KA, Esmat G, Abdelaziz AI. Transcriptional activation of the IGF-II/IGF-1R axis and inhibition of IGFBP-3 by miR-155 in hepatocellular carcinoma. *Oncol Lett* 2015; **10**: 3206-3212 [PMID: 26722313 DOI: 10.3892/ol.2015.3725]
  - 19 **He L**, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM. A microRNA polycistron as a potential human oncogene. *Nature* 2005; **435**: 828-833 [PMID: 15944707 DOI: 10.1038/nature03552]
  - 20 **El Tayebi HM**, Omar K, Hegy S, El Maghrabi M, El Brolosy M, Hosny KA, Esmat G, Abdelaziz AI. Repression of miR-17-5p with elevated expression of E2F-1 and c-MYC in non-metastatic hepatocellular carcinoma and enhancement of cell growth upon reversing this expression pattern. *Biochem Biophys Res Commun* 2013; **434**: 421-427 [PMID: 23583198 DOI: 10.1016/j.bbrc.2013.04.003]
  - 21 **Figueroa JA**, Jackson JG, McGuire WL, Krywicki RF, Yee D. Expression of insulin-like growth factor binding proteins in human breast cancer correlates with estrogen receptor status. *J Cell Biochem* 1993; **52**: 196-205 [PMID: 7690042 DOI: 10.1002/jcb.240520211]
  - 22 **Takaoka M**, Harada H, Andl CD, Oyama K, Naomoto Y, Dempsey KL, Klein-Szanto AJ, El-Deiry WS, Grimberg A, Nakagawa H. Epidermal growth factor receptor regulates aberrant expression of insulin-like growth factor-binding protein 3. *Cancer Res* 2004; **64**: 7711-7723 [PMID: 15520175 DOI: 10.1158/0008-5472.CAN-04-0715]
  - 23 **Chen L**, Jiang M, Yuan W, Tang H. miR-17-5p as a novel prognostic marker for hepatocellular carcinoma. *J Invest Surg* 2012; **25**: 156-161 [PMID: 22583011 DOI: 10.3109/08941939.2011.618523]
  - 24 **Zhang J**, Guo H, Zhang H, Wang H, Qian G, Fan X, Hoffman AR, Hu JF, Ge S. Putative tumor suppressor miR-145 inhibits colon cancer cell growth by targeting oncogene Friend leukemia virus integration 1 gene. *Cancer* 2011; **117**: 86-95 [PMID: 20737575 DOI: 10.1002/cncr.25522]
  - 25 **Lee YM**, Bae MH, Lee OH, Moon EJ, Moon CK, Kim WH, Kim KW. Synergistic induction of in vivo angiogenesis by the combination of insulin-like growth factor-II and epidermal growth factor. *Oncol Rep* 2004; **12**: 843-848 [PMID: 15375510 DOI: 10.3892/or.12.4.843]

**P- Reviewer:** Patial V **S- Editor:** Gong ZM

**L- Editor:** A **E- Editor:** Li D





Basic Study

## Reversal of multidrug resistance of hepatocellular carcinoma cells by metformin through inhibiting *NF-κB* gene transcription

Wei Wu, Jun-Ling Yang, Yi-Lang Wang, Han Wang, Min Yao, Li Wang, Juan-Juan Gu, Yin Cai, Yun Shi, Deng-Fu Yao

Wei Wu, Jun-Ling Yang, Deng-Fu Yao, Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu Province, China

Yi-Lang Wang, Department of Oncology, the 2<sup>nd</sup> Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu Province, China

Han Wang, Department of Liver Surgery, Zhongshan Hospital of Fudan University, Shanghai 200032, China

Min Yao, Departments of Immunology, Medical College of Nantong University, Nantong 226001, Jiangsu Province, China

Li Wang, Department of Medical Informatics, Medical College of Nantong University, Nantong 226001, Jiangsu Province, China

Juan-Juan Gu, Yin Cai, Yu Shi, Department of Oncology, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu Province, China

**Author contributions:** Wu W, Yang JL, Wang YL and Wang H contributed equally to this work; Wu W, Yao M, Shi Y and Wang L designed and performed the research; Yang JL, Gu JJ and Cai Y contributed new reagents/analytic tools; Yao M and Wang L analyzed the data; Wu W, Shi Y and Yao DF wrote the paper; Yao DF is the guarantor; all authors have read and approved the final version to be published.

**Supported by** Projects of Jiangsu Elitist Peak in Six Fields, Nos. 2013-WSN-078, 2013-WSW-011, and 2014-YY-028; the QingLan Program of Jiangsu Higher Education, the Youth Science Foundation of Nantong Health Department, No. WQ2014005; and the International Science and Technology Cooperation Program, No. 2013DFA32150.

**Institutional review board statement:** The study was reviewed and approved by the Affiliated Hospital of Nantong University.

**Conflict-of-interest statement:** The authors declare no potential conflict of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Deng-Fu Yao, MD, PhD, Professor, Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, No. 20 West Temple Road, Nantong 226001, Jiangsu Province, China. [yaodf@ahnmc.com](mailto:yaodf@ahnmc.com)  
**Telephone:** +86-513-85052297  
**Fax:** +86-513-85052523

**Received:** April 7, 2016

**Peer-review started:** April 8, 2016

**First decision:** May 23, 2016

**Revised:** May 25, 2016

**Accepted:** June 14, 2016

**Article in press:** June 16, 2016

**Published online:** August 18, 2016

### Abstract

**AIM:** To interfere with the activation of nuclear factor-κB (NF-κB) with metformin and explore its effect in reversing multidrug resistance (MDR) of hepatocellular carcinoma (HCC) cells.

**METHODS:** Expression of P-glycoprotein (P-gp) and NF-κB in human HepG2 or HepG2/adriamycin (ADM) cells

treated with pCMV-NF- $\kappa$ B-small interference RNA (siRNA) with or without metformin, was analyzed by Western blot or fluorescence quantitative PCR. Cell viability was tested by CCK-8 assay. Cell cycle and apoptosis were measured by flow cytometry and Annexin-V-PE/7-AnnexinV apoptosis detection double staining assay, respectively.

**RESULTS:** P-gp overexpression in HepG2 and HepG2/ADM cells was closely related to *mdr1* mRNA ( $3.310 \pm 0.154$ ) and NF- $\kappa$ B mRNA ( $2.580 \pm 0.040$ ) expression. NF- $\kappa$ B gene transcription was inhibited by specific siRNA with significant down-regulation of P-gp and enhanced HCC cell chemosensitivity to doxorubicin. After pretreatment with metformin, HepG2/ADM cells were sensitized to doxorubicin and P-gp was decreased through the NF- $\kappa$ B signaling pathway. The synergistic effect of metformin and NF- $\kappa$ B siRNA were found in HepG2/ADM cells with regard to proliferation inhibition, cell cycle arrest and inducing cell apoptosis.

**CONCLUSION:** Metformin *via* silencing NF- $\kappa$ B signaling could effectively reverse MDR of HCC cells by down-regulating MDR1/P-gp expression.

**Key words:** Metformin; Reversal; Multidrug resistance; Hepatocellular carcinoma

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Metformin might target AMP-activated protein kinase mammalian target of rapamycin pathway, suppress hypoxia-inducible factor-1 $\alpha$  and transcriptionally down-regulate P-glycoprotein (P-gp) and multidrug resistance (MDR)-associated protein 1, suggesting that metformin may reverse MDR by targeting the AMP-activated protein kinase/mammalian target of rapamycin/hypoxia-inducible factor-1 $\alpha$ /P-gp and MDR-associated protein 1 pathways. In the present study, HepG2/ADM cells pretreated with metformin were sensitized to doxorubicin and P-gp was decreased through the nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling pathway. The synergistic effects were found in the cells with regard to proliferation inhibition, cell cycle arrest and inducing apoptosis, and inhibiting P-gp expression *via* the NF- $\kappa$ B signaling pathway effectively reversed MDR by down-regulating MDR1/P-gp expression.

Wu W, Yang JL, Wang YL, Wang H, Yao M, Wang L, Gu JJ, Cai Y, Shi Y, Yao DF. Reversal of multidrug resistance of hepatocellular carcinoma cells by metformin through inhibiting NF- $\kappa$ B gene transcription. *World J Hepatol* 2016; 8(23): 985-993 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i23/985.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i23.985>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers and causes of cancer-related mortality

worldwide<sup>[1-3]</sup>. Due to the lack of specific symptoms, the vast majority of HCCs are diagnosed at late and/or advanced stages<sup>[4,5]</sup>. Although recent advances in surgical techniques and interventional therapy have improved survival, the emergence of multidrug resistance (MDR) to a series of clinical chemotherapeutics with different structures or different target sites severely blocks the successful management of HCC<sup>[6,7]</sup>. The well recognized mechanism of classical MDR is the significant overexpression of human *MDR1* gene encoding MDR1/P-glycoprotein (P-gp) that acts as an efflux pump on cell surface<sup>[8,9]</sup>. Intracellular anti-cancer drugs increasingly flow from cells through the efflux pump, thus drug concentrations become lower and cancer cells become resistant to chemotherapeutic drugs such as doxorubicin<sup>[10,11]</sup>.

Recently, some studies have found diverse anticancer effects of metformin in the cells of lung, gastric, endometrial, breast, and other types of cancer<sup>[12,13]</sup>. Metformin exhibits anti-proliferative effects in tumor cells *in vitro* and *in vivo*<sup>[14,15]</sup>. Metformin might target the AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) pathway<sup>[16,17]</sup>, suppress the hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ )<sup>[18,19]</sup> and transcriptionally down-regulate P-gp and MDR-associated protein 1 (MRP1), suggesting that metformin may reverse MDR by targeting the AMPK/mTOR/HIF-1 $\alpha$ /P-gp and MRP1 pathways<sup>[20,21]</sup>. In addition, the activation of nuclear factor-kappa B (NF- $\kappa$ B) pathway plays an important role in the development of HCC<sup>[22-24]</sup>, but whether it is related to MDR and the underlying molecular mechanisms remain to be explored<sup>[25,26]</sup>. In this study, we silenced NF- $\kappa$ B gene transcription with specific small interference RNA (siRNA) in human resistant HepG2/adriamycin (HepG2/ADM) cells, and explored the impact of metformin and NF- $\kappa$ B silencing, alone or in combination, on MDR1 regulation and MDR in HCC cells.

## MATERIALS AND METHODS

### Cell culture

Human hepatoma cell line HepG2, HepG2/ADM cell line and hepatocyte cell line LO2 were purchased from Aibio Biotech Company (Shanghai, China). LO2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, KeyGen Biotech Co., Ltd, Nanjing, China) containing 10% fetal bovine serum (FBS, Invitrogen, United States), penicillin (100 U/mL)/streptomycin (100 U/mL), at 37 °C with 5% CO<sub>2</sub>. HepG2 and HepG2/ADM cells were cultured in RPMI 1640 (KeyGen Biotech Co., Ltd, Nanjing, China) complete medium supplemented with 10% FBS, penicillin (100 U/mL)/streptomycin (100 U/mL) at 37 °C in a humidified incubator containing 5% CO<sub>2</sub>.

### Western blot

The cultured cells were washed with phosphate buffered saline (PBS) twice and lysed in phenylmethane sulfonyl fluoride (PMSF, Beyotime, Nantong, China) cell lysis buffer (1:1000), and the protein concentrations were determined with the bicinchoninic acid (BCA, Beyotime,

Nantong, China) protein assay kit. The protein samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride (PVDF, Millipore, United States) membranes. After blocking with 5% skim milk in Tris-buffered saline with tween (TBST) at room temperature for 3 h, the membranes were incubated with the primary antibody overnight at 4 °C. The primary antibodies were diluted as follows: p65 and P-p65 (rabbit anti-human, 1:1000, Cell Signaling, United States), MDR1 (rabbit anti-human, 1:500, Abcam, United States) and  $\beta$ -actin (mouse anti-human, 1:2000, internal reference, Proteintech, United States). Then the membranes were washed three times with TBST and incubated with a horseradish peroxidase-conjugated secondary antibody (mouse or rabbit anti-human, 1:1000, Univ-bio, Nanjing, China) for 2.5 h at room temperature. Finally, the samples were detected with Quantity One software using the electrochemiluminescence kit (Millipore, United States). All Western blot experiments were repeated three times.

### Real-time quantitative PCR

The cultured cells were digested with trypsin. Total RNA was extracted with TRIzol (Invitrogen, United States) reagent according to the protocol of the manufacturer. The quantity of total RNA was determined based on absorbance at 260 nm, and the purity of total RNA was analyzed based on the absorbance ratio at 260 and 280 nm ( $A_{260}/A_{280}$ ). Reverse transcription of total RNA to complementary DNA (cDNA) was performed with RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, CA, United States). PCR was carried with an SYBR Premix Ex Taq™ II kit (TaKaRa, Dalian, China), and GAPDH was used as an internal reference. The sequences of the primers used<sup>[27]</sup> were: NF- $\kappa$ B/p65 (forward: 5'-CTATCAGTCAGCGCATCCAG-3' and reverse: 5'-GCCAGAGTTTCGGTTCCTC-3'); mdr1 (forward: 5'-CCGGTT TGGAGCCTACTTG-3' and reverse: 5'-TCCAA TGTGTTCCGGCATTAG-3'); and GAPDH (forward: 5'-CAAGTTCATCCATGACAAC TTTG-3' and reverse: 5'-GTCCACCACCCTGTTGCTGTAG-3'). Real-time PCR cycling parameters consisted of initial denaturation at 94 °C for 2 min and 40 cycles of 95 °C for 10 s, 55 °C for 30 s, and 70 °C for 45 s. The amplification specificity was confirmed by the melting curves. Ct values were calculated based on duplicates and normalized to GAPDH. The relative expression was calculated using the  $2^{-\Delta\Delta Ct}$  method. All PCR experiments were repeated three times.

### Cell viability assay

Cell viability was evaluated with CCK-8 kit (Dojindo, Japan). Cells were divided into blank, negative control and experimental groups. Briefly, cells in logarithmic growth phase were digested with trypsin, and the cell suspension liquid (100  $\mu$ L) was seeded in 96-well plates. Toxicity tests were performed with different concentrations of ADM added to 96-well plates in the experimental group. The micro-plates were pre-cultured

at 37 °C in a humidified incubator containing 5% CO<sub>2</sub>, and liquid was changed at a fixed time interval. Then 10  $\mu$ L/well CCK-8 solution was added and incubated at 37 °C for 4 h. The absorbance (A) was measured with a microplate reader at a wavelength of 450 nm. Cell survival rate was calculated as  $A_{exp}/A_{con} \times 100\%$ . Values of IC<sub>50</sub> were evaluated with the Graphpad Prism5 software. Each individual experiment was performed at least three times.

### Metformin treatment

HepG2/ADM cells were divided into three groups: Blank, control and experiment. The experimental group was treated with 1  $\mu$ mol/L metformin for 24 h, and then continued to be cultured for 48 h with 1.5  $\mu$ mol/L doxorubicin. The control group was only treated with doxorubicin, and the blank group did not undergo any treatment.

### Analysis of cell apoptosis

HepG2/ADM cells were treated with drugs for 48 h, and then continued to be cultured for 24 h with another culture solution. Cells were harvested using trypsin without EDTA and washed with cold PBS twice. Cell cycle and apoptosis ( $n = 3$ ) were measured by flow cytometry and Annexin-V-PE/7-AnnexinV apoptosis detection double staining assay (BD, United States), respectively.

### Plasmid construction and cell transfection

NF- $\kappa$ B-siRNAs were designed according to the previously reported sequences<sup>[28]</sup> and synthesized by the Biomix Company (Nantong, China) according to Rel A sequence obtained from Gene ID 5970. The sequences of siRNAs were: NF- $\kappa$ B/p65 siRNA (forward, 5'-TGCTGTTCATCTCCTG AAAGGAGGCCGTTTTGGCCACTGACTGACGGCCTCCT CAGGAGATGAA-3' and reverse, 5'-CCTGTTTCATCTCCT GAGGAGGCCGTGCTAGTCAGTGGCCAAAACGGCCTCC TTTCAGGAGATGAAC-3'; and negative-siRNA (forward, 5'-TGCTGAAATGTACTGCGCGTGGAGACGTTTTGGCCA CTGACTGACGTCTCCACGCAGTACATTT-3' and reverse, 5'-CCTGAAATGTACTGCGTGGAGACGTGCTAGTCAGTGGCC AAAACGTCTCCACGCAGTACATTT-3'). Each siRNA was inserted to a pcDNA™ 6.2-GW/EmGFPmiR vector (Invitrogen, United States). HepG2/ADM cells were divided into blank control, negative siRNA control and NF- $\kappa$ B/p65 siRNA transfection groups. After cells were planted into microwell plates at a density of 70%, the plasmids were transfected into cells for incubation for 24 h according to the manufacturer's instructions. The medium was removed on another day and replaced with the fresh one, and the transfection efficiency was observed with a fluorescence microscope. These experiments were performed in triplicate.

### Statistical analysis

Data are expressed as the mean  $\pm$  SD. Statistical analyses were done using the SPSS21.0 software package. Differences between groups were assessed using analysis of variance or *t*-test.  $P \leq 0.05$  was regarded as

**Table 1** Absorbance values ( $n = 3$ , mean  $\pm$  SD) of HepG2/adriamycin cells treated with different concentrations of metformin

Time (h)	0 (blank)	0.1 mmol/L	0.3 mmol/L	1 mmol/L	3 mmol/L	10 mmol/L
24	1.242 $\pm$ 0.03	1.233 $\pm$ 0.04	1.221 $\pm$ 0.02	1.195 $\pm$ 0.00	1.189 $\pm$ 0.02	1.101 $\pm$ 0.02 <sup>a</sup>
48	1.744 $\pm$ 0.01	1.734 $\pm$ 0.02	1.718 $\pm$ 0.04	1.703 $\pm$ 0.03	1.583 $\pm$ 0.03 <sup>a</sup>	1.483 $\pm$ 0.01 <sup>a</sup>
72	1.692 $\pm$ 0.04	1.677 $\pm$ 0.01	1.650 $\pm$ 0.06	1.583 $\pm$ 0.06	1.420 $\pm$ 0.06 <sup>a</sup>	1.300 $\pm$ 0.04 <sup>a</sup>

<sup>a</sup> $P < 0.05$  vs the blank group.**Table 2** Effect of adriamycin combined with metformin on the proliferation of HepG2/adriamycin cells ( $n = 3$ , mean  $\pm$  SD)

Adriamycin ( $\mu$ mol/L)	24 h		48 h		72 h	
	Control	Metformin	Control	Metformin	Control	Metformin
0	1.434 $\pm$ 0.03	1.327 $\pm$ 0.04 <sup>a</sup>	1.477 $\pm$ 0.08	1.357 $\pm$ 0.01	1.695 $\pm$ 0.08	1.507 $\pm$ 0.05 <sup>a</sup>
0.01	1.280 $\pm$ 0.06	1.160 $\pm$ 0.01 <sup>a</sup>	1.489 $\pm$ 0.03	1.314 $\pm$ 0.03 <sup>a</sup>	1.505 $\pm$ 0.01	1.378 $\pm$ 0.07 <sup>a</sup>
0.1	1.194 $\pm$ 0.10	1.111 $\pm$ 0.09	1.418 $\pm$ 0.01	1.213 $\pm$ 0.02 <sup>a</sup>	1.453 $\pm$ 0.02	1.249 $\pm$ 0.04 <sup>a</sup>
1	0.847 $\pm$ 0.02	0.662 $\pm$ 0.02 <sup>a</sup>	0.661 $\pm$ 0.01	0.661 $\pm$ 0.06	0.753 $\pm$ 0.04	0.508 $\pm$ 0.04 <sup>a</sup>
5	0.628 $\pm$ 0.08	0.458 $\pm$ 0.02 <sup>a</sup>	0.358 $\pm$ 0.02	0.208 $\pm$ 0.03 <sup>a</sup>	0.347 $\pm$ 0.03	0.194 $\pm$ 0.03 <sup>a</sup>
10	0.531 $\pm$ 0.00	0.399 $\pm$ 0.01 <sup>a</sup>	0.162 $\pm$ 0.01	0.062 $\pm$ 0.01 <sup>a</sup>	0.122 $\pm$ 0.01	0.049 $\pm$ 0.01 <sup>a</sup>
20	0.284 $\pm$ 0.01	0.162 $\pm$ 0.01 <sup>a</sup>	0.143 $\pm$ 0.01	0.051 $\pm$ 0.00 <sup>a</sup>	0.084 $\pm$ 0.01	0.027 $\pm$ 0.00 <sup>a</sup>

<sup>a</sup> $P < 0.05$  vs the control group. The proliferation of HepG2/adriamycin cells calculated with SPSS21.0 is presented as mean  $\pm$  SD from CCK-8 assay in triplicate.

statistically significant.

## RESULTS

### Expression of P-gp, *mdr1*, and NF- $\kappa$ B in different liver cell lines

The levels of P-gp, *mdr1*, and NF- $\kappa$ B expression in different liver cell lines are shown in Figure 1. The proliferation of HepG2 and HepG2/ADM cells was decreased along with the increase of the concentration of doxorubicin, and the ability of proliferation was higher in HepG2/ADM cells than in HepG2 cells. At 24, 48 and 72 h, the IC<sub>50</sub> values of doxorubicin against HepG2 cells were 0.489, 0.221 and 0.224  $\mu$ mol/L, respectively, and the IC<sub>50</sub> values of doxorubicin against HepG2/ADM cells were 4.166, 1.522 and 1.380  $\mu$ mol/L, respectively. The resistance index (RI,  $\mu$ mol/L) of HepG2/ADM cells was 8.519 at 24 h, 6.874 at 48 h and 6.166 at 72 h. There was almost no P-gp expression in LO2 cells. Different degrees of expression of P-gp protein were observed in HepG2 and HepG2/ADM cells, but the P-gp expression in HepG2/ADM cells was significantly higher than that in HepG2 cells (Figure 1A and B). The p-p65 expression was significantly increased, while the expression of p65 was significantly decreased in HepG2/ADM cells (Figure 1C and D). The levels of *mdr1* mRNA and NF- $\kappa$ B mRNA were  $3.310 \pm 0.154$  and  $2.580 \pm 0.040$ , respectively, in HepG2/ADM cells, and  $0.084 \pm 0.038$  and  $0.607 \pm 0.032$ , respectively, in HepG2 cells; the former was significantly higher than the latter ( $P < 0.01$ ). Relative transcript levels ( $2^{-\Delta\Delta Ct}$ ) of *mdr1* mRNA and NF- $\kappa$ B mRNA were  $9.381 \pm 0.750$  and  $3.927 \pm 0.069$ , respectively (Figure 1E).

### Effect of metformin on HepG2/ADM cells

The effect of metformin on the proliferation of HepG2/ADM

cells was concentration- and time-dependent (Table 1). Metformin showed no significant effect on HepG2/ADM cells when its concentration was less than 3 mmol/L, but had different degrees of inhibition on the proliferation of HepG2/ADM cells when its concentration was between 3–10 mmol/L ( $P < 0.05$ ). The HepG2/ADM cells were divided into experimental and control groups. After pretreatment with metformin, the experimental group cells were treated with different concentrations of doxorubicin. The effect of adriamycin combined with metformin on the proliferation of HepG2/ADM cells is shown in Table 2. After treatment with metformin, HepG2/ADM cells were more sensitive to adriamycin.

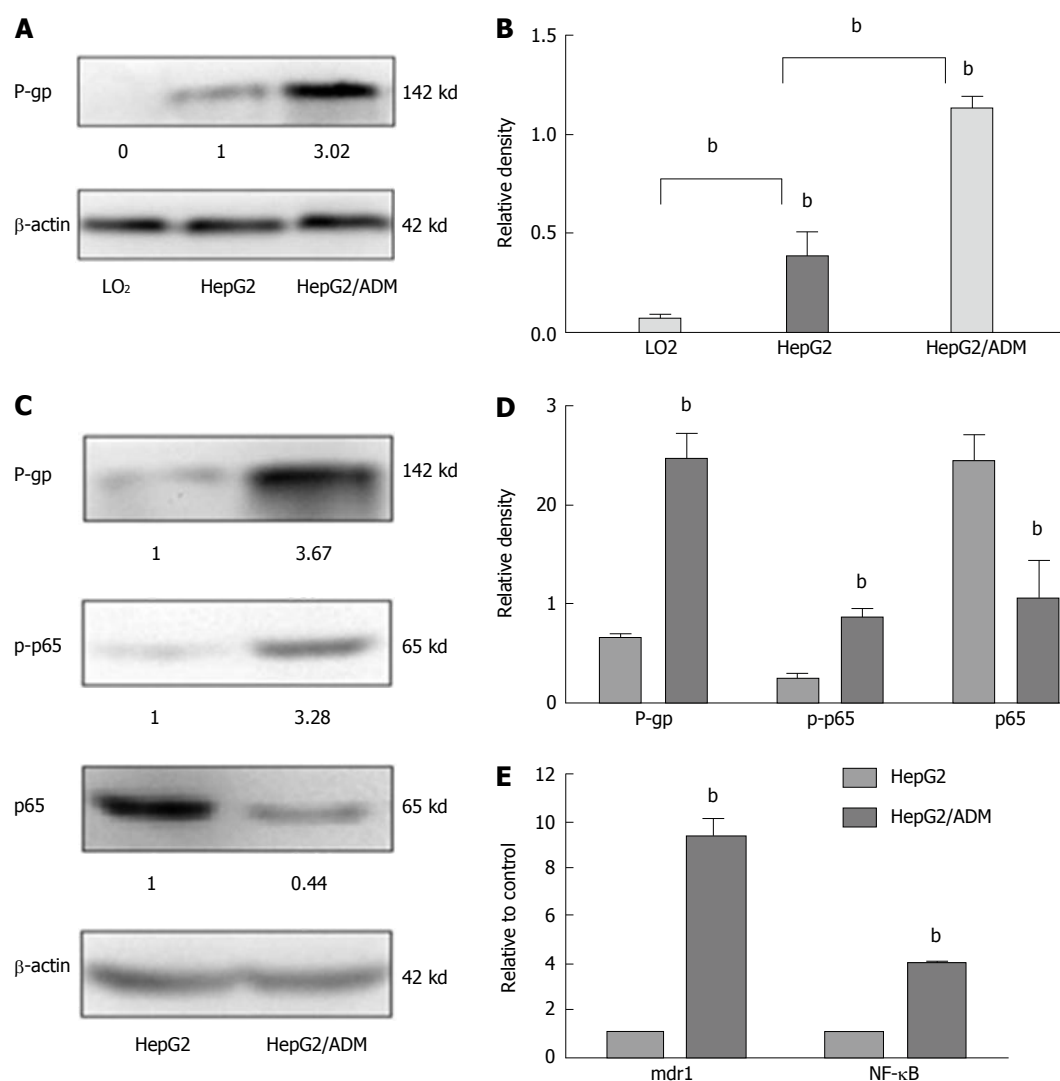
### Metformin promotes HepG2/ADM cell apoptosis

The levels of HepG2/ADM cell apoptosis in the experimental (treated with metformin plus adriamycin), control (only treated with adriamycin) and blank (without adriamycin or metformin) groups are shown in Figure 2. After the cells were pretreated with 1 mmol/L metformin for 24 h, adriamycin was added. MDR1 in HepG2/ADM cells was down-regulated, the cell cycle was blocked at G<sub>0</sub>/G<sub>1</sub> phase, and apoptosis was enhanced. Significant differences in the apoptosis rates were found among different groups ( $F = 3726.97$ ,  $P < 0.001$ ), and the apoptosis rate was significantly higher in the experimental group ( $22.17\% \pm 0.37\%$ ) than in the control group ( $14.86\% \pm 0.21\%$ ) or the blank group ( $4.17\% \pm 0.13\%$ ).

### Metformin reverses MDR via the NF- $\kappa$ B signaling pathway

Metformin reversed the MDR of HCC cells via the NF- $\kappa$ B signaling pathway (Figure 3). The levels of P-gp expression in the HepG2/ADM cells were decreased with





**Figure 1** The levels of P-glycoprotein, mdr1 and nuclear factor- $\kappa$ B expression in different cell lines. A and C: The levels of P-gp and NF- $\kappa$ B expression in HepG2 or HepG2/ADM cells were determined by Western blot. The number indicates the ratio of HepG2/ADM cells to HepG2 cells ( $n = 3$ , mean  $\pm$  SD); B and D: The gray intensity images of Figure 1A and Figure 1C, respectively; E: The levels of mdr1 and NF- $\kappa$ B mRNA expression were determined by qRT-PCR. <sup>b</sup> $P < 0.01$  ( $n = 3$ , mean  $\pm$  SD), compared with hepG2 or LO<sub>2</sub> cell line. P-gp: P-glycoprotein; NF- $\kappa$ B: Nuclear factor- $\kappa$ B; ADM: Adriamycin.

the increasing dose of metformin, and the phosphorylated p65 expression in the nucleus was also decreased. Metformin could down-regulate P-gp expression by inhibiting NF- $\kappa$ B activation in a dose- and time-dependent manner.

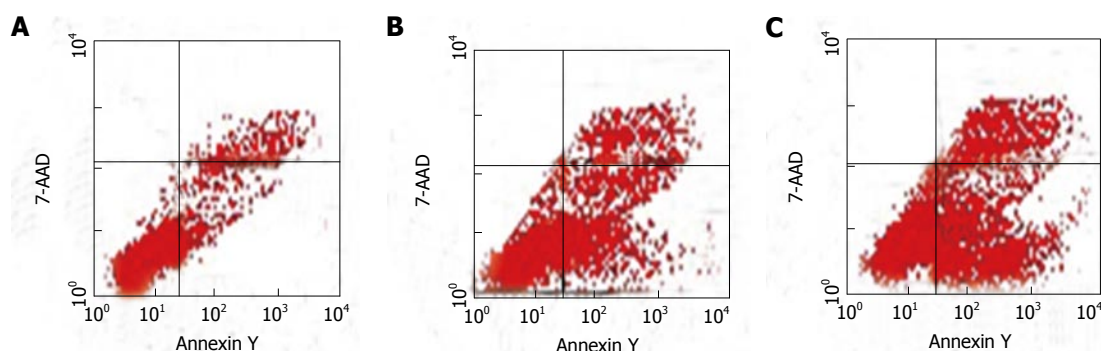
#### Synergistic effect of metformin plus NF- $\kappa$ B-siRNA

The synergistic effects of metformin combined with NF- $\kappa$ B siRNA in reversing MDR are shown in Figure 4. HepG2/ADM cells were divided into three groups: Untreated cells, cells treated with metformin alone and those treated with metformin combined with NF- $\kappa$ B-siRNA. In the NF- $\kappa$ B-siRNA group, NF- $\kappa$ B-siRNA was transfected into HepG2/ADM cells for 24 h, and then cells were treated with 1 mmol/L metformin for 48 h. The levels of P-gp expression were  $0.91 \pm 0.24$ ,  $0.63 \pm 0.13$  and  $0.22 \pm 0.02$  ( $F = 14.47$ ,  $P = 0.005$ ) in untreated, metformin and the metformin combined with NF- $\kappa$ B-siRNA groups, respectively. The expression of P-gp was significantly

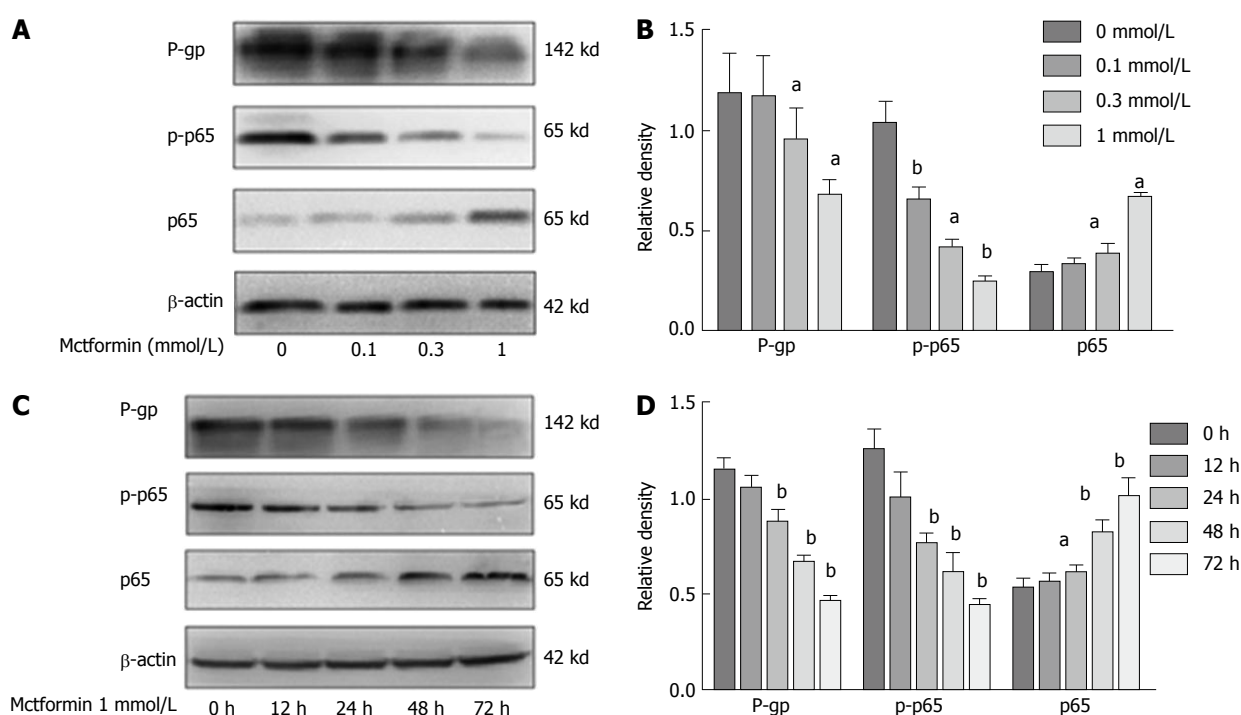
reduced in cells treated with metformin plus NF- $\kappa$ B-siRNA compared with that in cells only treated with metformin ( $t = 5.39$ ,  $P = 0.006$ ).

## DISCUSSION

Recent advances in surgical techniques and interventional therapy have improved survival of HCC patients<sup>[6,7,29]</sup>. However, the emergence of MDR to a series of clinical chemotherapeutics with different structures or target sites severely blocks the successful management of HCC and still is a difficult problem to be solved in clinical practice<sup>[30,31]</sup>. MDR in HCC could result from several biochemical mechanisms including decreased drug influx, increased drug efflux, altered cell cycle checkpoints, altered drug targets, increased drug metabolism and/or resistance to drug-induced apoptosis. Therefore, it is very important to find safe and effective MDR reversal agents for HCC<sup>[32]</sup>. In the present study, metformin with



**Figure 2** Metformin enhances adriamycin-induced apoptosis of HepG2/adriamycin cells. Cell early apoptosis was measured by Annexin-V-PE/7-AAD double staining assay in triplicate. A: The blank group (untreated); B: The control group (only treated with adriamycin); C: The experiment group (treated with metformin plus adriamycin). AAD: AnnexinV apoptosis detection.



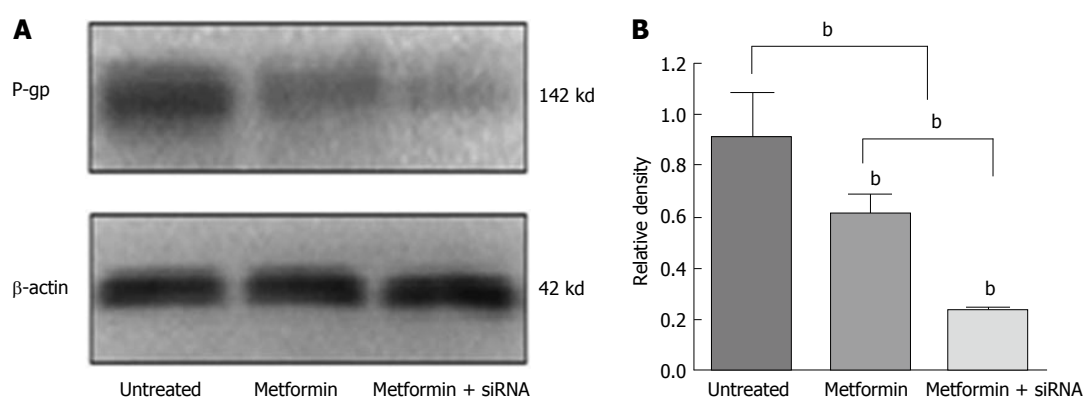
**Figure 3** Metformin down-regulates P-glycoprotein expression via the nuclear factor- $\kappa$ B signaling pathway. A: After HepG2/ADM cells were treated with different doses of metformin for 24 h, the levels of P-gp and p-p65 expression analyzed by Western blot were decreased in a dose-dependent manner, and the cytoplasmic p65 increased in a dose-dependent manner; B: The gray intensity images of Figure 3A. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs the blank group ( $n = 3$ , mean  $\pm$  SD); C: After HepG2/ADM cells were treated with 1 mmol/L metformin for different time periods, the levels of P-gp and p-p65 expression analyzed by Western blot were decreased in a time-dependent manner, and the cytoplasmic p65 increased in a time-dependent manner; D: The gray intensity images of Figure 3C. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs the blank group ( $n = 3$ , mean  $\pm$  SD). P-gp: P-glycoprotein; NF- $\kappa$ B: Nuclear factor- $\kappa$ B; ADM: Adriamycin.

silencing NF- $\kappa$ B gene transcription was used to reverse MDR of HepG2/ADM cells with high NF- $\kappa$ B expression.

Anti-cancer drug efflux is one of the most common mechanisms of MDR of HCC cells, and it is mediated by ATP-binding cassette transporters<sup>[33,34]</sup>, such as P-gp encoded by *MDR1* gene, which is located downstream of the NF- $\kappa$ B signaling pathway. P-gp expression regulated by *MDR1* is the most important and common cause of MDR, and weakened the apoptosis of cancer cells induced by chemotherapeutic drugs. Both P-gp expression and NF- $\kappa$ B activation are linked closely with HCC progression<sup>[35]</sup>. Usually NF- $\kappa$ B takes part in gene transcription by means of homodimers or heterodimers,

such as p50/p65, p65/p65, and p65/Rel. In quiescent cells, they are predominantly cytoplasmic, associating with members of inhibitory I $\kappa$ B family and forming NF- $\kappa$ B/I $\kappa$ B complexes without activity. Both P-gp and NF- $\kappa$ B at the protein or transcriptional level were significantly higher (Figure 1), with p65 expression decreasing in HepG2/ADM cells, indicating that abnormal P-gp and NF- $\kappa$ B expression could associate with the MDR formation of HCC cells<sup>[20]</sup>.

Metformin is a safe, low-cost drug, and therefore remains one of the most commonly prescribed drugs worldwide<sup>[16,36]</sup>. The anticancer effects of metformin indicate the possibility that certain diabetes-associated types of



**Figure 4** Alteration of P-glycoprotein expression in cells treated with metformin plus nuclear factor- $\kappa$ B-small interference RNA. A: Alteration of P-gp expression in different groups of cells. A significant decrease of P-gp expression analyzed by Western blot was found in HepG2/ADM cells treated with the metformin plus NF- $\kappa$ B-siRNA; B: The gray intensity images of Figure 4A. <sup>b</sup> $P < 0.01$  vs the blank group ( $n = 3$ , mean  $\pm$  SD). P-gp: P-glycoprotein; NF- $\kappa$ B: Nuclear factor- $\kappa$ B; ADM: Adriamycin; siRNA: Small interference RNA.

cancer<sup>[37,38]</sup> may be circumvented, and metformin has anti-proliferative potential against cancer cells or reversing MDR *in vitro* and *in vivo*<sup>[39,40]</sup>. However, the precise molecular mechanisms whereby metformin works in cancer prevention remain multi-factorial and ill-defined. Metformin affected HepG2/ADM cell proliferation in a dose- and time-dependent manner (Table 1). Metformin at  $< 3$  mmol/L had no significant impact on HepG2/ADM cells, but the cells treated with metformin between 3-10 mmol/L were more sensitive to adriamycin with regard to promoting cell apoptosis (Figure 2) and inhibiting cell proliferation (Table 2), suggesting that metformin could increase the sensitivity of HepG2/ADM cells to anti-cancer drugs.

There are few studies on the effect of metformin on MDR of HCC cells. siRNA strategy is a powerful technique to inhibit specific gene expression, which has highlighted the potential use of siRNA molecules to study gene function or explore new HCC therapeutic agents<sup>[41,42]</sup>. The expression of NF- $\kappa$ B gene transcription was inhibited by specific siRNA, which significantly down-regulated P-gp and enhanced the chemosensitivity of HCC cells to doxorubicin, confirming the mechanism of decreasing P-gp *via* the NF- $\kappa$ B signaling pathway. The synergistic effects of metformin and NF- $\kappa$ B siRNA were found in HepG2/ADM cells with regard to cell proliferation inhibition, cell cycle arrest, and inducing cell apoptosis. These data confirm that the metformin could enhance the HepG2/ADM cells sensitivity to adriamycin and reverse MDR *via* the NF- $\kappa$ B signaling pathway (Figure 4).

In conclusion, the development of MDR still is one of major causes of HCC chemotherapy failure<sup>[43,44]</sup>. Although specific NF- $\kappa$ B siRNA is a powerful small molecule reagent designed to silence expression of NF- $\kappa$ B and MDR1/P-gp related to MDR to increase tumor cell sensitivity to anti-cancer drugs, how to apply metformin plus interfering NF- $\kappa$ B activation for effective reversal of MDR of HCC cells still needs to be further explored.

## ACKNOWLEDGMENTS

The authors thank Dr. FitzGibbon T for comments on

earlier drafts of the manuscript.

## COMMENTS

### Background

Hepatocellular carcinoma (HCC) multidrug resistance (MDR) to a series of clinical chemotherapeutics with different structures or different target sites severely blocks the successful management of HCC. The mechanism of classical MDR is the significant overexpression of MDR1/P-glycoprotein (P-gp) that acts as an efflux pump on cell surface. Intracellular anti-cancer drugs increasingly flow from cells through the efflux pump, thus drug concentrations become lower and cancer cells become resistant to chemotherapeutic drugs such as doxorubicin.

### Research frontiers

Metformin could target AMP-activated protein kinase mammalian target of rapamycin pathway, suppress hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) and transcriptionally down-regulate P-gp and MDR-associated protein 1, suggesting that metformin may reverse MDR by targeting the AMP-activated protein kinase/mammalian target of rapamycin/HIF-1 $\alpha$ /P-gp and MDR-associated protein 1 pathways. However, whether metformin plus nuclear factor- $\kappa$ B (NF- $\kappa$ B) inhibition might effectively reverse MDR of HCC cells remains to be explored.

### Innovations and breakthroughs

Recently, there are few studies on the effects of metformin on MDR of HCC cells. In this study, the data suggested that the abnormal expression of MDR1/P-gp and NF- $\kappa$ B activation during HCC development were related to MDR formation, which might be down-regulated through inhibiting activation of the NF- $\kappa$ B signaling pathway with specific small interference RNA (siRNA). The combination of metformin with interfering NF- $\kappa$ B gene transcription could effectively reverse the MDR of HCC cells.

### Applications

The abnormal expression of MDR1/P-gp in HCC was related to MDR formation, which could be down-regulated through inhibiting activation of the NF- $\kappa$ B signaling pathway with specific siRNA and increasing sensitivity of HCC cells to chemotherapy drugs. Interfering NF- $\kappa$ B activation with metformin is effective to reverse MDR of HCC cells. However, how to apply metformin plus interfering NF- $\kappa$ B activation for effective reversal of MDR of HCC cells still needs to be explored.

### Terminology

Metformin is a safe, low-cost drug. The anticancer effects of metformin indicate the possibility that certain diabetes-associated types of cancer may be circumvented. Indeed, many retrospective meta-analyses have shown that metformin possesses anti-cancer activities and decreases the incidence of primary cancer development in those taking metformin routinely, and a multitude of clinical cancer trials are actively assessing its benefits in non-diabetic population who have already developed cancer. However, the precise molecular

mechanisms whereby metformin works in cancer prevention remain multi-factorial and ill-defined.

### Peer-review

Authors have done excellent work in this present study. They have explored the effect of metformin and interfering *NF-κB* gene transcription with specific siRNA, alone or in combination, on *MDR1* gene regulation. The application of interfering *NF-κB* activation with metformin was more effective to reverse MDR of HCC cells.

## REFERENCES

- 1 Singal AG, El-Serag HB. Hepatocellular Carcinoma From Epidemiology to Prevention: Translating Knowledge into Practice. *Clin Gastroenterol Hepatol* 2015; **13**: 2140-2151 [PMID: 26284591 DOI: 10.1016/j.cgh.2015.08.014]
- 2 de Martel C, Maucourt-Boulch D, Plummer M, Franceschi S. World-wide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma. *Hepatology* 2015; **62**: 1190-1200 [PMID: 26146815 DOI: 10.1002/hep.27969]
- 3 Ashtari S, Pourhoseingholi MA, Sharifian A, Zali MR. Hepatocellular carcinoma in Asia: Prevention strategy and planning. *World J Hepatol* 2015; **7**: 1708-1717 [PMID: 26140091 DOI: 10.4254/wjh.v7.i12.1708]
- 4 Yao M, Wang L, Yao Y, Gu HB, Yao DF. Biomarker-based Micro-RNA Therapeutic Strategies for Hepatocellular Carcinoma. *J Clin Transl Hepatol* 2014; **2**: 253-258 [PMID: 26355266 DOI: 10.14218/JCTH.2014.00020 26355266]
- 5 Zhang H, Yao M, Wu W, Qiu L, Sai W, Yang J, Zheng W, Huang J, Yao D. Up-regulation of annexin A2 expression predicates advanced clinicopathological features and poor prognosis in hepatocellular carcinoma. *Tumour Biol* 2015; **36**: 9373-9383 [PMID: 26109000 DOI: 10.1007/s13277-015-3678-6]
- 6 Bruix J, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 2014; **63**: 844-855 [PMID: 24531850]
- 7 Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. *Hepatology* 2008; **48**: 1312-1327 [PMID: 18821591]
- 8 Fang P, Zhang X, Gao Y, Ding CR, Cui F, Jiao SC. Reversal effect of melanoma differentiation associated gene-7/interleukin-24 on multidrug resistance in human hepatocellular carcinoma cells. *Anat Rec (Hoboken)* 2012; **295**: 1639-1646 [PMID: 22899557 DOI: 10.1002/ar.22551]
- 9 Liu Y, Lou G, Wu W, Zheng M, Shi Y, Zhao D, Chen Z. Involvement of the NF-κB pathway in multidrug resistance induced by HBx in a hepatoma cell line. *J Viral Hepat* 2011; **18**: e439-e446 [PMID: 21914061 DOI: 10.1111/j.1365-2893.2011.01463.x]
- 10 Zheng W, Sai W, Yao M, Gu H, Yao Y, Qian Q, Yao D. Silencing clusterin gene transcription on effects of multidrug resistance reversing of human hepatoma HepG2/ADM cells. *Tumour Biol* 2015; **36**: 3995-4003 [PMID: 25600802 DOI: 10.1007/s13277-015]
- 11 Zhao X, Chen Q, Li Y, Tang H, Liu W, Yang X. Doxorubicin and curcumin co-delivery by lipid nanoparticles for enhanced treatment of diethylnitrosamine-induced hepatocellular carcinoma in mice. *Eur J Pharm Biopharm* 2015; **93**: 27-36 [PMID: 25770771 DOI: 10.1016/j.ejpb.2015.03.003]
- 12 Cimai A, Ichigo S, Matsunami K, Takagi H, Yasuda K. Clinical benefits of metformin in gynecologic oncology. *Oncol Lett* 2015; **10**: 577-582 [PMID: 26622536]
- 13 Cazzaniga M, Bonanni B. Breast Cancer Metabolism and Mitochondrial Activity: The Possibility of Chemoprevention with Metformin. *Biomed Res Int* 2015; **2015**: 972193 [PMID: 26605341 DOI: 10.1155/2015/972193]
- 14 Hall C, Stone RL, Gehlot A, Zorn KK, Burnett AF. Use of Metformin in Obese Women With Type I Endometrial Cancer Is Associated With a Reduced Incidence of Cancer Recurrence. *Int J Gynecol Cancer* 2016; **26**: 313-317 [PMID: 26588235]
- 15 Cazzaniga M, Bonanni B. Relationship Between Metabolic Reprogramming and Mitochondrial Activity in Cancer Cells. Understanding The Anticancer Effect of Metformin and Its Clinical Implications. *Anticancer Res* 2015; **35**: 5789-5796 [PMID: 26503999]
- 16 Kim HG, Hien TT, Han EH, Hwang YP, Choi JH, Kang KW, Kwon KI, Kim BH, Kim SK, Song GY, Jeong TC, Jeong HG. Metformin inhibits P-glycoprotein expression via the NF-κB pathway and CRE transcriptional activity through AMPK activation. *Br J Pharmacol* 2011; **162**: 1096-1108 [PMID: 21054339 DOI: 10.1111/j.1476-5381.2010.01101.x]
- 17 Chen S, Wang Y, Ruan W, Wang X, Pan C. Reversing multidrug resistance in hepatocellular carcinoma cells by inhibiting extracellular signal-regulated kinase/mitogen-activated protein kinase signaling pathway activity. *Oncol Lett* 2014; **8**: 2333-2339 [PMID: 25295120]
- 18 Li S, Yao D, Wang L, Wu W, Qiu L, Yao M, Yao N, Zhang H, Yu D, Ni Q. Expression characteristics of hypoxia-inducible factor-1α and its clinical values in diagnosis and prognosis of hepatocellular carcinoma. *Hepat Mon* 2011; **11**: 821-828 [PMID: 22224081 DOI: 10.5812/kowsar.1735143X.771]
- 19 Dong ZZ, Yao DF, Li SS, Yao M, Yu DD, Yao NH, Qian YJ, Qiu LW. Inhibitory effect of miRNA silencing hypoxia-inducible factor alpha subunit gene on the proliferation of HepG2 cells. *Zhonghua Gan Zang Bing Za Zhi* 2011; **19**: 281-285 [PMID: 21586227 DOI: 10.3760/cma.j.issn.1007-3418.2011.04.012]
- 20 Xiang QF, Zhang DM, Wang JN, Zhang HW, Zheng ZY, Yu DC, Li YJ, Xu J, Chen YJ, Shang CZ. Cabozantinib reverses multidrug resistance of human hepatoma HepG2/adr cells by modulating the function of P-glycoprotein. *Liver Int* 2015; **35**: 1010-1023 [PMID: 24621440 DOI: 10.1111/liv.12524]
- 21 Fantappiè O, Sassoli C, Tani A, Nosi D, Marchetti S, Formigli L, Mazzanti R. Mitochondria of a human multidrug-resistant hepatocellular carcinoma cell line constitutively express inducible nitric oxide synthase in the inner membrane. *J Cell Mol Med* 2015; **19**: 1410-1417 [PMID: 25691007 DOI: 10.1111/jcmm.12528]
- 22 Wu W, Yao DF, Dong ZZ, Bian YZ, Yao NH, Qiu LW, Yang JL, Sai WL. [Abnormality of NF-κB expression and the clinical implications in patients with HBV-related hepatocellular carcinoma]. *Zhonghua Gan Zang Bing Za Zhi* 2011; **19**: 466-468 [PMID: 22053381]
- 23 Dong ZZ, Yao DF, Wu W, Yao M, Yu HB, Shen JJ, Qiu LW, Yao NH, Sai WL, Yang JL. Delayed hepatocarcinogenesis through antiangiogenic intervention in the nuclear factor-kappa B activation pathway in rats. *Hepatobiliary Pancreat Dis Int* 2010; **9**: 169-174 [PMID: 20382589]
- 24 Yao DF, Yu HB, Shen JJ, Wang YL, Wu XH, Qiu LW, Wu W. [The effect of thalidomine-induced NF-kappa B activation on malignant transformation of hepatocytes]. *Zhonghua Gan Zang Bing Za Zhi* 2009; **17**: 312-314 [PMID: 19403036]
- 25 Shi Y, Wang SY, Yao M, Sai WL, Wu W, Yang JL, Cai Y, Zheng WJ, Yao DF. Chemosensitization of HepG2 cells by suppression of NF-κB/p65 gene transcription with specific-siRNA. *World J Gastroenterol* 2015; **21**: 12814-12821 [PMID: 26668505 DOI: 10.3748/wjg.v21.i45.12814 26668505]
- 26 Liu Y, Lou G, Wu W, Shi Y, Zheng M, Chen Z. Interferon-α sensitizes HBx-expressing hepatocarcinoma cells to chemotherapeutic drugs through inhibition of HBx-mediated NF-κB activation. *Viral J* 2013; **10**: 168 [PMID: 23718853 DOI: 10.1186/1743]
- 27 Wu W, Yao D, Wang Y, Qiu L, Sai W, Yang J, Yao N, Li S, Bian Y, Wang Z, Yao D. Suppression of human hepatoma (HepG2) cell growth by nuclear factor-kappaB/p65 specific siRNA. *Tumour Biol* 2010; **31**: 605-611 [PMID: 20628843 DOI: 10.1007/s13277]
- 28 Wang YL, Yao DF, Wu W, Sai WL, Qiu LW, Yang JL, Zhu JW. [Effect of siRNA-mediated inhibition of nuclear transcription factor-kappa B on apoptosis of hepatocarcinoma cells]. *Zhonghua Gan Zang Bing Za Zhi* 2010; **18**: 609-613 [PMID: 20825717 DOI: 10.3760/cma.j.issn.1007-3418.2010.08.014 20825717]
- 29 Gillet JP, Andersen JB, Madigan JP, Varma S, Bagni RK, Powell K, Burgan WE, Wu CP, Calcagno AM, Ambudkar SV, Thorgeirsson SS, Gottesman MM. A Gene Expression Signature Associated with Overall Survival in Patients with Hepatocellular Carcinoma Suggests a New Treatment Strategy. *Mol Pharmacol* 2016; **89**: 263-272 [PMID: 26668215 DOI: 10.1124/mol.115.101360]



- 30 **Ho CT**, Shang HS, Chang JB, Liu JJ, Liu TZ. Folate deficiency-triggered redox pathways confer drug resistance in hepatocellular carcinoma. *Oncotarget* 2015; **6**: 26104-26118 [PMID: 26327128 DOI: 10.18632/oncotarget.4422]
- 31 **Xiang Y**, Liu Y, Yang Y, Hu H, Hu P, Ren H, Zhang D. A secretomic study on human hepatocellular carcinoma multiple drug-resistant cell lines. *Oncol Rep* 2015; **34**: 1249-1260 [PMID: 26151126 DOI: 10.3892/or.2015.4106]
- 32 **Yan J**, Zhou Y, Chen D, Li L, Yang X, You Y, Ling X. Effects of mitochondrial translocation of telomerase on drug resistance in hepatocellular carcinoma cells. *J Cancer* 2015; **6**: 151-159 [PMID: 25561980 DOI: 10.7150/jca.10419]
- 33 **Colombo F**, Trombetta E, Cetrangolo P, Maggioni M, Razini P, De Santis F, Torrente Y, Prati D, Torresani E, Porretti L. Giant Lysosomes as a Chemotherapy Resistance Mechanism in Hepatocellular Carcinoma Cells. *PLoS One* 2014; **9**: e114787 [PMID: 25493932 DOI: 10.1371/journal.pone.0114787]
- 34 **Shibasaki Y**, Sakaguchi T, Hiraide T, Morita Y, Suzuki A, Baba S, Setou M, Konno H. Expression of indocyanine green-related transporters in hepatocellular carcinoma. *J Surg Res* 2015; **193**: 567-576 [PMID: 25173835 DOI: 10.1016/j.jss.2014.07.055]
- 35 **Wu W**, Yao DF, Qiu LW, Sai WL, Shen JJ, Yu HB, Wu XH, Li YM, Wang YL, Gu WJ. Characteristics of hepatic nuclear-transcription factor-kappa B expression and quantitative analysis in rat hepatocarcinogenesis. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 504-509 [PMID: 19822494]
- 36 **Pryor R**, Cabreiro F. Repurposing metformin: an old drug with new tricks in its binding pockets. *Biochem J* 2015; **471**: 307-322 [PMID: 26475449 DOI: 10.1042/BJ20150497]
- 37 **Bhat A**, Sebastiani G, Bhat M. Systematic review: Preventive and therapeutic applications of metformin in liver disease. *World J Hepatol* 2015; **7**: 1652-1659 [PMID: 26140084 DOI: 10.4254/wjh.v7.i12.1652]
- 38 **Ling S**, Tian Y, Zhang H, Jia K, Feng T, Sun D, Gao Z, Xu F, Hou Z, Li Y, Wang L. Metformin reverses multidrug resistance in human hepatocellular carcinoma Bel-7402/5-fluorouracil cells. *Mol Med Rep* 2014; **10**: 2891-2897 [PMID: 25310259 DOI: 10.3892/mmr.2014.2614]
- 39 **Yan F**, Bai LP, Gao H, Zhu CM, Lin L, Kang XP. EGF reverses multi-drug resistance via the p-ERK pathway in HepG2/ADM and SMMC7721/ADM hepatocellular carcinoma models. *Asian Pac J Cancer Prev* 2014; **15**: 2619-2623 [PMID: 24761873]
- 40 **Qin Y**, Bao H, Pan Y, Yin M, Liu Y, Wu S, Li H. SUMOylation alterations are associated with multidrug resistance in hepatocellular carcinoma. *Mol Med Rep* 2014; **9**: 877-881 [PMID: 24399357 DOI: 10.3892/mmr.2014.1882]
- 41 **Shen J**, Sun H, Meng Q, Yin Q, Zhang Z, Yu H, Li Y. Simultaneous inhibition of tumor growth and angiogenesis for resistant hepatocellular carcinoma by co-delivery of sorafenib and survivin small hairpin RNA. *Mol Pharm* 2014; **11**: 3342-3351 [PMID: 24495194 DOI: 10.1021/mp4006408 24495194]
- 42 **Yao M**, Wang L, Qiu L, Qian Q, Yao D. Encouraging microRNA-based Therapeutic Strategies for Hepatocellular Carcinoma. *Anti-cancer Agents Med Chem* 2015; **15**: 453-460 [PMID: 25511513]
- 43 **Cheng L**, Luo S, Jin C, Ma H, Zhou H, Jia L. FUT family mediates the multidrug resistance of human hepatocellular carcinoma via the PI3K/Akt signaling pathway. *Cell Death Dis* 2013; **4**: e923 [PMID: 24232099 DOI: 10.1038/cddis.2013.450]
- 44 **Gu W**, Liu L, Fang FF, Huang F, Cheng BB, Li B. Reversal effect of bufalin on multidrug resistance in human hepatocellular carcinoma BEL-7402/5-FU cells. *Oncol Rep* 2014; **31**: 216-222 [PMID: 24173654 DOI: 10.3892/or.2013.2817]

**P- Reviewer:** Chiang TA, Jamall IS **S- Editor:** Qi Y

**L- Editor:** Wang TQ **E- Editor:** Li D



## Metastatic recurrence to a solitary lymph node four years after hepatic lobectomy for primary hepatocellular carcinoma

Michael L Caparelli, Nathan J Roberts, Timothy S Braverman, Robert M Stevens, Edward R Broun, Shyam Allamaneni

Michael L Caparelli, Nathan J Roberts, Shyam Allamaneni, Department of Surgery, the Jewish Hospital, Cincinnati, OH 45236, United States

Timothy S Braverman, Department of Pathology, the Jewish Hospital, Cincinnati, OH 45236, United States

Robert M Stevens, Department of Radiology, the Jewish Hospital, Cincinnati, OH 45236, United States

Edward R Broun, Department of Oncology, Hematology Care, Inc., Cincinnati, OH 45236, United States

**Author contributions:** Caparelli ML wrote the paper; Caparelli ML, Roberts NJ and Allamaneni S designed the report and edited the content; Roberts NJ and Allamaneni S performed the operation; Braverman TS provided pathology report and micrographs; Stevens RM provided critical findings for imaging studies; Broun ER contributed intellectual discussion of the patient case; all members edited the report.

**Institutional review board statement:** This case report was exempt from the Institutional Review Board standards at the Jewish Hospital in Cincinnati.

**Informed consent statement:** The patient involved in this study gave her verbal consent authorizing use and disclosure of her protected health information.

**Conflict-of-interest statement:** All the authors have no conflicts of interest to declare.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Shyam Allamaneni, MD, Surgical Oncologist, Department of Surgery, the Jewish Hospital, 4777 E Galbraith Road, Cincinnati, OH 45236, United States. [drallamaneni@gmail.com](mailto:drallamaneni@gmail.com)  
 Telephone: +1-513-6865392  
 Fax: +1-513-6865394

Received: March 14, 2016

Peer-review started: March 14, 2016

First decision: April 20, 2016

Revised: May 9, 2016

Accepted: July 14, 2016

Article in press: July 18, 2016

Published online: August 18, 2016

### Abstract

This report describes a patient that developed recurrent metastatic hepatocellular carcinoma (HCC) to a supra-pancreatic lymph node four years after being treated for primary HCC *via* complete left hepatectomy. Metastatic HCC was proven by pathologic confirmation. The report addresses the role of surgical resection as a treatment modality for recurrent HCC to solitary lymph nodes. The role of biological chemotherapy as adjuvant treatment is also addressed.

**Key words:** Hepatocellular carcinoma; Lymph node; Recurrence; Metastatic; Extrahepatic

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Recurrence of primary hepatocellular carcinoma to a solitary extracellular site is a rare occurrence, especially after complete hepatic lobectomy for the

primary tumor. In this report we describe a case of recurrence to a solitary suprapancreatic lymph node four years after initial resection. This is the only report to describe such a recurrence this long after the primary resection.

Caparelli ML, Roberts NJ, Braverman TS, Stevens RM, Broun ER, Allamaneni S. Metastatic recurrence to a solitary lymph node four years after hepatic lobectomy for primary hepatocellular carcinoma. *World J Hepatol* 2016; 8(23): 994-998 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i23/994.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i23.994>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, with the highest prevalence rates occurring in the eastern hemisphere. However, there has been a rise in prevalence in the Western hemisphere. It has been postulated that this pattern is due to higher incidence of hepatitis B and C virus seen outside of the United States<sup>[1]</sup>. Tumor staging and strategies for treatment of HCC have been well described with current guidelines following the recommendations of the 2010 AHPBA/SSO/SSAT consensus conference on HCC<sup>[2]</sup>. Current guidelines are primarily geared toward patients with primary resectable and non-resectable HCC. However, data is lacking with regard to the treatment of recurrent extrahepatic HCC. Systemic chemotherapy has proven to be of minimal benefit for patients with advanced, and recurrent extrahepatic HCC. There are current studies being conducted that support the use of multikinase inhibitors, including Sorafenib, as a viable option for patients with advanced and extrahepatic HCC<sup>[3]</sup>.

It is well known that the most common type of recurrence of HCC is intrahepatic. The most common sites for hematogenous spread are the lung, followed by the adrenal gland, and bone<sup>[4]</sup>. Metastases of HCC to lymph nodes (LN) are quite rare. In one report that included a subset of Japanese patients who underwent hepatic resection, the prevalence of lymphatic involvement was as low as 2.2%<sup>[5]</sup>. Another study showed that the 5-year survival rate for patients with lymph node metastasis is approximately 20%<sup>[6]</sup>. There have been few reports describing metastasis to LN that have been treated with surgical resection, and their results have been varied<sup>[7-11]</sup>. With this in mind, the importance of surgical resection of extrahepatic HCC recurrent to lymph nodes cannot be understated as a viable treatment modality. Interestingly, this is the first reported case where isolated lymph node metastasis has occurred greater than 3 years after initial hepatic resection. We describe a case of HCC recurrent to a solitary suprapancreatic lymph node treated by complete surgical resection.

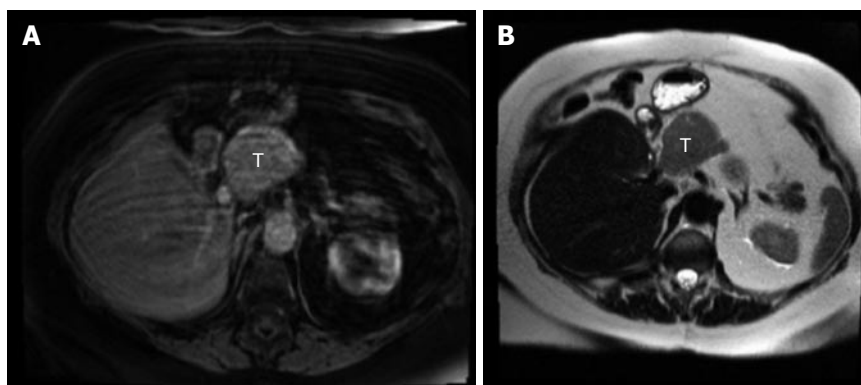
## CASE REPORT

The patient is a 67-year-old woman who presented with a suprapancreatic mass on magnetic resonance imaging (MRI). She initially presented 4 years prior with HCC of the left lobe of the liver measuring 10.8 cm × 7.4 cm × 9.5 cm. She was asymptomatic at the time of the discovery and the tumor was found due to imaging studies prior to a recent thoracic aortic aneurysm repair. Interestingly she did not have known risk factors for developing HCC such as cirrhosis, chronic hepatitis, tobacco use, diabetes, nonalcoholic fatty liver disease, hemochromatosis, or alpha-1 antitrypsin deficiency. Laboratory findings at that time showed a alpha fetoprotein (AFP) level of 119000 ng/mL. She subsequently underwent complete left hepatic lobectomy and had no complications post procedure. The patient was in remission for almost 4 years, but had a steady increase in AFP, 177-883 ng/mL, from year 3 to 4. Serial computed tomography (CT) imaging showed no evidence of recurrence over that time period. Subsequent MRI showed a soft tissue mass medial to the right hepatic lobe/porta hepatis measuring 4.6 cm × 5.6 cm (Figure 1). CT guided biopsy of the mass revealed a poorly differentiated malignant neoplasm, favoring HCC. The patient had no history of viral hepatitis, alcoholic liver disease, jaundice, abdominal pain, weight loss, chronic cough, bloody stools, bone pain, or any other signs to suggest metastatic disease. She was subsequently taken to the operating room for *en bloc* resection of a large suprapancreatic retroperitoneal mass, celiac and portal lymphadenectomy. Pathology showed the suprapancreatic mass to be consistent with HCC, high grade within a lymph node structure. Portal and celiac axis lymph nodes were negative for metastasis. Interestingly, immunohistochemical stains for the recurrent carcinoma showed not only tumor markers that confirm hepatocellular origin, but might suggest a more aggressive tumor - staining positive for cytokeratin 19 (CK19), glypican 3 (G3) and hepatocyte paraffin 1 (HP1). Microscopic pathologic figures are shown in Figures 2 and 3. The patient's post-operative course has been uncomplicated and at eight months post op she is disease free. Current AFP level is 2.2 ng/mL.

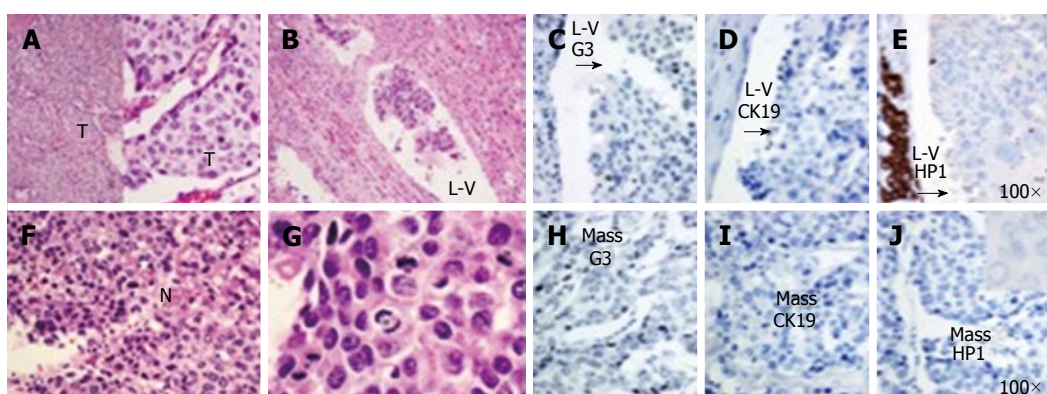
## DISCUSSION

The recurrence of HCC can be classified as early or late phase<sup>[12]</sup>. Early phase recurrence typically occurs within the first two years post-resection, and is related to aggressive features of the primary tumor such as high tumor grade, local invasion, and multifocal tumors. Late recurrence occurs more than two years after resection and is related to *de novo* tumor formation, typically in patients with cirrhotic liver disease. The fact that our patient recurred to an extrahepatic LN nearly four years post-surgery is remarkable, and of the first to be reported this late, post-resection. The initial tumor was without aggressive characteristics, as it was moderately

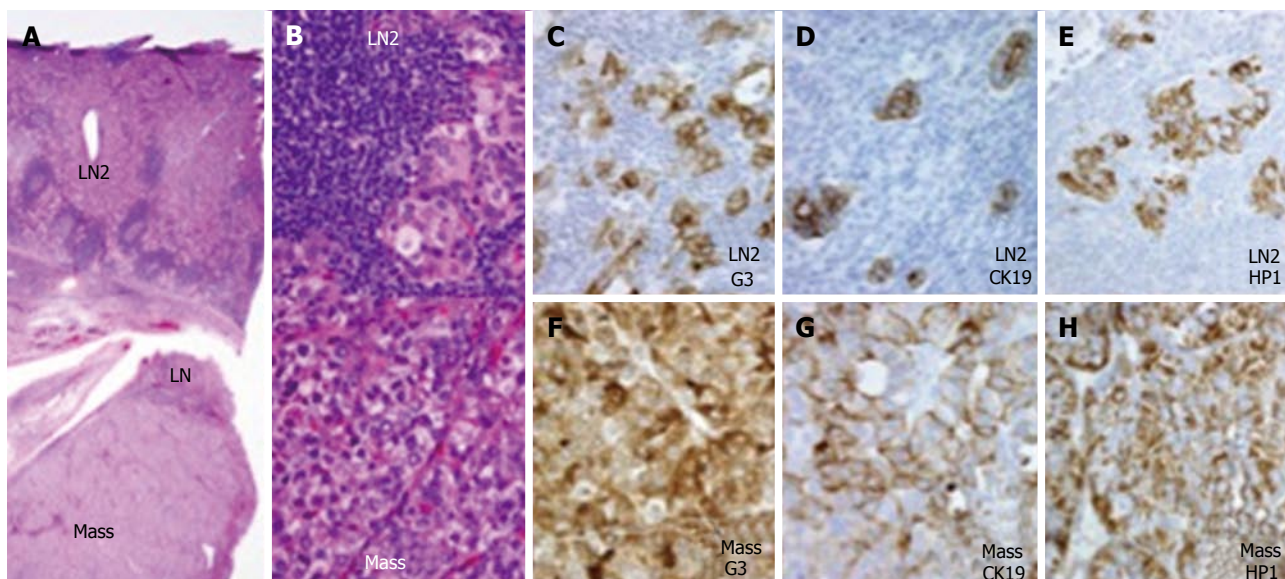




**Figure 1 Suprapancreatic mass.** The left panel (A) shows an axial post contrast T1 fat suppressed sequence that demonstrates an arterial phase enhancing mass medial to the liver; the right panel (B) shows an axial T2 HASTE sequence demonstrating a mass with increased T2 signal medial to the liver. T: Tumor.



**Figure 2 Initial hepatic lobectomy.** On the left-hand set of H and E-stained quarter panel images, the upper left two-image combination quarter panel (A) shows the trabecular architecture, the trabeculae as "T"; the upper right H and E quarter panel (B) shows L-V; the lower H and E quarter panels show high power views of solid pattern tumor with focal geographic necrosis [lower left (F) as "N"] and numerous mitoses [lower right (G) with "M" times 4]; on the right-hand set of IHC-stained split full-height images, the mass, along the bottom (H-J) show negative G3 (H), CK19 (I), HP1 (J); the L-V, along the top (C-E) with a similar pattern, but with weak but convincing HP1 positivity. Both HP1 stains are inset with 100 × high power images (100 ×). Arrows on the L-V IHC (C and D) stains indicate the tumor (opposite vessel wall). L-V: Lymph-vascular tumor; Mass: Main mass; LN: Lymph node; G3: Glypican 3; CK19: Cytokeratin 19; HP1: Hepatocyte paraffin 1; T: Tumor.



**Figure 3 Resected recurrence.** A: The H and E-stained shows a tiny area of likely LN at one end; B: Shows obvious tumor cells nests; On the right-hand set of IHC stained split full-height images, both the main mass (F-H); and obvious nodal tumor (LN2) (C-E), are strongly positive on G3 (C), CK19 (D), and HP1 (E) stains. Mass: Main mass; LN: Lymph node; G3: Glypican 3; CK19: Cytokeratin 19; HP1: Hepatocyte paraffin 1.



differentiated and without local invasion. Additionally, the initial tumor stained negative for CK19, G3 and was only weakly positive for HP1 in the lympho-vascular invasive sample as seen in Figure 1. Interestingly, the recurrent tumor was positive for these three biomarkers, suggesting hepatocellular origin and a more aggressive tumor<sup>[13]</sup>. Clonal selection, therapeutic selection, or possibly both may explain this finding.

LN status is essential to the staging of cancers, including HCC. The presence of LN metastasis is associated with poorer survival and higher risk of tumor recurrence<sup>[4]</sup>. Although the most common intra- and extra-hepatic recurrence is to liver and lung respectively, metastases to LNs are not that uncommon. There have been two reports that showed LN metastases in 28% and 25% of autopsied cases of HCC, respectively<sup>[14,15]</sup>. However, a more recent study of surgical patients in Japan showed only 2.2% LN involvement in patients that underwent hepatic resection<sup>[5]</sup>. This discrepancy may be due to the fact that more advanced HCC cases that are more likely to have extrahepatic metastases are less likely to undergo resection. This finding illuminates the importance LN dissection in hepatic surgery. LN dissection is not the current standard when performing hepatic resection for HCC. In a study by Ercolani *et al.*<sup>[16]</sup> the role of lymphadenectomy was addressed. In 40 patients with HCC the incidence of LN metastases was 7.5%. It was also found that the most common site of LN metastases from HCC is the hepatic pedicle node, followed by the retropancreatic space, and common hepatic artery station. The authors concluded that regional lymphadenectomy is a safe procedure after liver resection; however, this is yet to become common practice.

Several case reports have been published on the findings of metastatic HCC to LNs<sup>[7-11]</sup>. Patients in these reports often had cirrhosis, and all but one of these patients underwent resection with varied short-term survival results. One report described a patient with a solitary suprapancreatic LN metastasis that underwent pancreaticoduodenectomy and had reported disease free survival for 27 mo. Another patient with LN metastases to two paraaortic mediastinal LNs underwent complete resection, but had recurrence and died 13 mo later<sup>[10]</sup>. It is reasonable to argue liver disease, and multiple LN involvement may be factors for worse prognosis post LN resection.

Our patient appears to be an excellent candidate for resection, as she had a solitary LN, and is without cirrhotic, viral or alcoholic liver disease. In addition, adjuvant treatment with sorafenib - an oral multikinase inhibitor that has been shown to suppress tumor growth and angiogenesis by inhibiting the Raf/MEK/ERK signaling pathway and receptor kinases, such as VEGFR-1, VEGFR-2, VEGFR-3, and PDGFR $\beta$  - should be considered<sup>[3]</sup>. Sorafenib was shown to increase survival in patients with advanced HCC in the SHARP (Sorafenib HCC Assessment Randomized Protocol) trial. However, data is lacking on whether this multikinase inhibitor is useful in the

treatment of recurrent extrahepatic HCC. One recent study showed that the therapeutic effect of sorafenib was comparable in advanced HCC with or without extra-hepatic metastasis<sup>[3]</sup>. It may be beneficial to initiate adjuvant treatment in patients with recurrent LN involvement, but further studies need to be performed prior to this becoming standard.

## COMMENTS

### Case characteristic

A 67-year-old woman who presented with a suprapancreatic mass on magnetic resonance imaging (MRI). The patient was asymptomatic at the time of presentation. Imaging studies were performed because of increased serum alpha fetoprotein levels led to increase suspicion for recurrence of primary hepatocellular carcinoma (HCC) resected four years prior.

### Clinical diagnosis

The patient was asymptomatic at the time of presentation.

### Differential diagnosis

Recurrent primary HCC, metastatic cancer, reactive lymphadenopathy, primary tumor of unknown origin, lymphoma.

### Laboratory diagnosis

Elevated alpha fetoprotein level of 883 ng/mL.

### Imaging diagnosis

MRI showed a soft tissue mass medial to the right hepatic lobe/porta hepatis measuring 4.6 cm  $\times$  5.6 cm.

### Pathological diagnosis

HCC, high grade within a lymph node structure.

### Treatment

Surgical resection of lesion.

### Related reports

HCC is a primary liver cancer. HCC typically does not recur to an extrahepatic solitary lymph node after primary resection.

### Term explanation

HCC is a primary liver cancer. It is the fifth most common human cancer worldwide.

### Experiences and lessons

Surgical resection of HCC recurrence to a solitary lymph node is a viable option and may also be curative. Long term follow-up of this patient will further illuminate the possibility of cure.

### Peer-review

An interesting case presentation with a long period disease-free up to 4 years. It should be benefit to the knowledge of the hepatologists and keep in mind for the importance of clinical follow-up after extensive hepatectomy.

## REFERENCES

1. Chen KW, Ou TM, Hsu CW, Horng CT, Lee CC, Tsai YY, Tsai CC, Liou YS, Yang CC, Hsueh CW, Kuo WH. Current systemic treatment of hepatocellular carcinoma: A review of the literature. *World J Hepatol* 2015; 7: 1412-1420 [PMID: 26052386 DOI: 10.4254/wjh.v7.i10.1412]
2. Munene G, Vauthey JN, Dixon E. Summary of the 2010 AHPBA/

- SSO/SSAT Consensus Conference on HCC. *Int J Hepatol* 2011; **2011**: 565060 [PMID: 21994863 DOI: 10.4061/2011/565060]
- 3 **Nakano M**, Tanaka M, Kuromatsu R, Nagamatsu H, Tajiri N, Satani M, Niizeki T, Aino H, Okamura S, Iwamoto H, Shimose S, Shirono T, Koga H, Torimura T. Sorafenib for the treatment of advanced hepatocellular carcinoma with extrahepatic metastasis: a prospective multicenter cohort study. *Cancer Med* 2015; **4**: 1836-1843 [PMID: 26471348 DOI: 10.1002/cam4.548]
- 4 **Katyal S**, Oliver JH, Peterson MS, Ferris JV, Carr BS, Baron RL. Extrahepatic metastases of hepatocellular carcinoma. *Radiology* 2000; **216**: 698-703 [PMID: 10966697 DOI: 10.1148/radiology.216.3.r00se24698]
- 5 **Liver Cancer Study Group of Japan**. Primary liver cancer in Japan. Clinicopathologic features and results of surgical treatment. *Ann Surg* 1990; **211**: 277-287 [PMID: 2155591]
- 6 **Xiaohong S**, Huikai L, Feng W, Ti Z, Yunlong C, Qiang L. Clinical significance of lymph node metastasis in patients undergoing partial hepatectomy for hepatocellular carcinoma. *World J Surg* 2010; **34**: 1028-1033 [PMID: 20174806 DOI: 10.1007/s00268-010-0400-0]
- 7 **Shoji F**, Shirabe K, Yano T, Maehara Y. Surgical resection of solitary cardiophrenic lymph node metastasis by video-assisted thoracic surgery after complete resection of hepatocellular carcinoma. *Interact Cardiovasc Thorac Surg* 2010; **10**: 446-447 [PMID: 20022882 DOI: 10.1510/icvts.2009.225284]
- 8 **Taniai N**, Yoshida H, Mamada Y, Mizuguchi Y, Fujihira T, Akimaru K, Tajiri T. A case of recurring hepatocellular carcinoma with a solitary Virchow's lymph node metastasis. *J Nippon Med Sch* 2005; **72**: 245-249 [PMID: 16113497 DOI: 10.1272/jnms.72.245]
- 9 **Kurokawa T**, Yamazaki S, Moriguchi M, Aoki M, Watanabe Y, Higaki T, Takayama T. Resection of solitary metachronous lymph node metastasis from hepatocellular carcinoma following transarterial chemotherapy with cisplatin: a case report. *Anticancer Res* 2011; **31**: 3991-3993 [PMID: 22110232]
- 10 **Utsumi M**, Matsuda H, Sadamori H, Shinoura S, Umeda Y, Yoshida R, Satoh D, Hashimoto M, Yagi T, Fujiwara T. Resection of metachronous lymph node metastases from hepatocellular carcinoma after hepatectomy: report of four cases. *Acta Med Okayama* 2012; **66**: 177-182 [PMID: 22525476]
- 11 **Ueda J**, Yoshida H, Mamada Y, Taniai N, Mineta S, Yoshioka M, Kawano Y, Shimizu T, Hara E, Kawamoto C, Kaneko K, Uchida E. Surgical resection of a solitary para-aortic lymph node metastasis from hepatocellular carcinoma. *World J Gastroenterol* 2012; **18**: 3027-3031 [PMID: 22736929 DOI: 10.3748/wjg.v18.i23.3027]
- 12 **Colecchia A**, Schiumerini R, Cucchetti A, Cescon M, Taddia M, Marasco G, Festi D. Prognostic factors for hepatocellular carcinoma recurrence. *World J Gastroenterol* 2014; **20**: 5935-5950 [PMID: 24876717 DOI: 10.3748/wjg.v20.i20.5935]
- 13 **Feng J**, Zhu R, Chang C, Yu L, Cao F, Zhu G, Chen F, Xia H, Lv F, Zhang S, Sun L. CK19 and Glypican 3 Expression Profiling in the Prognostic Indication for Patients with HCC after Surgical Resection. *PLoS One* 2016; **11**: e0151501 [PMID: 26977595 DOI: 10.1371/journal.pone.0151501]
- 14 **Edmondson HA**, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer* 1954; **7**: 462-503 [PMID: 13160935]
- 15 **Anthony PP**. Hepatocellular carcinoma: an overview. *Histopathology* 2001; **39**: 109-118 [PMID: 11493326]
- 16 **Ercolani G**, Grazi GL, Ravaioli M, Grigioni WF, Cescon M, Gardini A, Del Gaudio M, Cavallari A. The role of lymphadenectomy for liver tumors: further considerations on the appropriateness of treatment strategy. *Ann Surg* 2004; **239**: 202-209 [PMID: 14745328 DOI: 10.1097/01.sla.0000109154.00020.e0]

**P- Reviewer:** Chetty R, Chiu KW, Delladetsima IK, Mihaila RG, Pan JJ, Zhu X **S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 August 28; 8(24): 999-1046







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verducci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*





## Contents

Three issues per month Volume 8 Number 24 August 28, 2016

### REVIEW

- 999 Outcomes of liver transplantation in patients with hepatorenal syndrome  
*Modi RM, Patel N, Metwally SN, Mumtaz K*

### MINIREVIEWS

- 1012 Rethinking the role of non-selective beta blockers in patients with cirrhosis and portal hypertension  
*Ferrarese A, Zanetto A, Germani G, Burra P, Senzolo M*

### ORIGINAL ARTICLE

#### Observational Study

- 1019 Hypolactasia is associated with insulin resistance in nonalcoholic steatohepatitis  
*de Campos Mazo DF, Mattar R, Stefano JT, da Silva-Elto JMK, Diniz MA, Duarte SMB, Rabelo F, Lima RVC, de Campos PB, Carrilho FJ, Oliveira CP*
- 1028 Diagnostic non-invasive model of large risky esophageal varices in cirrhotic hepatitis C virus patients  
*Elalfy H, Elsherbiny W, Abdel Rahman A, Elhammady D, Shaltout SW, Elsamanoudy AZ, El Deek B*

#### Prospective Study

- 1038 Liver resections can be performed safely without Pringle maneuver: A prospective study  
*Maurer CA, Walensi M, Käser SA, Künzli BM, Lötscher R, Zuse A*

## Contents

*World Journal of Hepatology*  
Volume 8 Number 24 August 28, 2016

### ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Christoph A Maurer, MD, FACS, FRCS(Hon), FEBS, Professor of Surgery, Department of Surgery, Hirslanden-Clinic Beau-Site, 3013 Bern, Switzerland

### AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

### FLYLEAF

I-IV Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL OFFICE  
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director  
*World Journal of Hepatology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
August 28, 2016

#### COPYRIGHT

© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

#### SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

#### INSTRUCTIONS TO AUTHORS

<http://www.wjgnet.com/bpg/gerinfo/204>

#### ONLINE SUBMISSION

<http://www.wjgnet.com/esps/>

## Outcomes of liver transplantation in patients with hepatorenal syndrome

Rohan M Modi, Nishi Patel, Sherif N Metwally, Khalid Mumtaz

Rohan M Modi, Nishi Patel, Sherif N Metwally, Department of Internal Medicine, the Ohio State University Wexner Medical Center, Columbus, OH 43210, United States

Khalid Mumtaz, Department of Internal Medicine, Division of Gastroenterology, Hepatology and Nutrition, the Ohio State University Medical Center, Columbus, OH 43210, United States

**Author contributions:** Modi RM and Mumtaz K decided upon the aims of the article, wrote the manuscript, and made necessary revisions; Patel N and Metwally SN helped in writing and reviewing the manuscript.

**Conflict-of-interest statement:** The authors do not have any disclosures to report.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Khalid Mumtaz, MD, MSc, Assistant Professor, Department of Internal Medicine, Division of Gastroenterology, Hepatology and Nutrition, the Ohio State University Medical Center, 410 West 10<sup>th</sup> Ave, Columbus, OH 43210, United States. [drkmumtaz@yahoo.com](mailto:drkmumtaz@yahoo.com)  
 Telephone: +1-614-6858657  
 Fax: +1-614-2938518

Received: April 28, 2016  
 Peer-review started: April 28, 2016  
 First decision: June 16, 2016  
 Revised: June 20, 2016  
 Accepted: July 14, 2016  
 Article in press: July 18, 2016  
 Published online: August 28, 2016

### Abstract

Hepatorenal syndrome (HRS) plays an important role in patients with liver cirrhosis on the wait list for liver transplantation (LT). The 1 and 5-year probability of developing HRS in cirrhotic with ascites is 20% and 40%, respectively. In this article, we reviewed current concepts in HRS pathophysiology, guidelines for HRS diagnosis, effective treatment options presently available, and controversies surrounding liver alone *vs* simultaneous liver kidney transplant (SLKT) in transplant candidates. Many treatment options including albumin, vasoconstrictors, renal replacement therapy, and eventual LT have remained a mainstay in the treatment of HRS. Unfortunately, even after aggressive measures such as terlipressin use, the rate of recovery is less than 50% of patients. Moreover, current SLKT guidelines include: (1) estimation of glomerular filtration rate of 30 mL/min or less for 4-8 wk; (2) proteinuria > 2 g/d; or (3) biopsy proven interstitial fibrosis or glomerulosclerosis. Even with these updated criteria there is a lack of consistency regarding long-term benefits for SLKT *vs* LT alone. Finally, in regards to kidney dysfunction in the post-transplant setting, an estimation of glomerular filtration rate < 60 mL/min per 1.73 m<sup>2</sup> may be associated with an increased risk of patients having long-term end stage renal disease. HRS is common in patients with cirrhosis and those on liver transplant waitlist. Prompt identification and therapy initiation in transplant candidates with HRS may improve post-transplantation outcomes. Future studies identifying optimal vasoconstrictor regimens, alternative therapies, and factors predictive of response to therapy are needed. The appropriate use of SLKT in patients with HRS remains controversial and requires further evidence by the transplant community.

**Key words:** Liver transplantation; Simultaneous liver kidney transplantation; Vasopressors; Dialysis; Post-transplant outcomes; Hepatorenal syndrome

© The Author(s) 2016. Published by Baishideng Publishing

Group Inc. All rights reserved.

**Core tip:** We aim to review the literature on hepatorenal syndrome (HRS) in the setting of liver transplantation (LT) and address critical issues that are barriers to improved outcomes. Many consistencies have remained as treatment options including albumin, vasoconstrictors, renal replacement therapy, and eventual LT. Moreover, the utility of simultaneous liver kidney transplantation in HRS patients still requires further evidence by the transplant community.

Modi RM, Patel N, Metwally SN, Mumtaz K. Outcomes of liver transplantation in patients with hepatorenal syndrome. *World J Hepatol* 2016; 8(24): 999-1011 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i24/999.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i24.999>

## INTRODUCTION

Prior to diagnosing a patient with hepatorenal syndrome (HRS) in the setting of liver transplantation (LT), it is important to rule out other etiologies of renal dysfunction. A broad differential should include reversible causes such as acute kidney injury (AKI) or acute tubular necrosis (ATN) and irreversible cause like chronic kidney disease (CKD) or parenchymal kidney disease.

Traditionally there are three types of AKI (pre-renal azotemia, intrinsic kidney disease, and post-obstructive causes) that are still common in patients with liver disease in addition to HRS<sup>[1]</sup>. Common causes of pre-renal injury independent of HRS include infection, intravascular fluid depletion, GI fluid losses, surgery or bleeding, and renal artery occlusion<sup>[2]</sup>, all of which should appropriately respond to volume expansion with albumin within 48 h. If there is any recent contrast media or nephrotoxic agent with granular casts and proteinuria > 500 mg, it is important to consider ATN as a likely diagnosis<sup>[3]</sup>. A recent study evaluating patients with AKI [pre-renal azotemia ( $n = 35$ ), HRS ( $n = 35$ ), ATN ( $n = 36$ )] revealed that pre-renal azotemia has a lower mortality when compared to both HRS ( $P = 0.05$ ) and ATN ( $P = 0.04$ )<sup>[4]</sup>.

Intrinsic kidney disease is more common than previously believed in the cirrhotic population, and is thought to be related to the underlying etiology of cirrhosis<sup>[2]</sup>. Kidney biopsy is most useful in intrinsic kidney disease with hematuria (50 red blood cells per high power field), proteinuria (> 500 mg/d), renal insufficiency of unknown origin, or HRS for a prolonged period of time. Histologically, IgA nephropathy, membranoproliferative glomerulonephritis, focal global glomerulosclerosis, and diabetic nephropathy<sup>[5]</sup> are the most common biopsy findings. The importance of diagnosing parenchymal disease is especially important if a patient is being considered for combined liver-kidney transplantation<sup>[6]</sup>. Additionally, obstructive causes of renal dysfunction

including nephrolithiasis, bladder outlet obstruction and other intra-abdominal etiologies should be assessed.

If the aforementioned workup returns negative, HRS should be considered as a potential cause of renal dysfunction. The 1-year and 5-year probability of developing HRS in patients with ascites is 20% and 40%, respectively<sup>[7]</sup>. The most recent diagnostic criteria for HRS from the International Ascites Club (IAC) in 2007 include creatinine (Cr) > 1.5 mg/dL, no improvement of Cr after volume expansion with albumin after 48 h, no current or recent exposure to nephrotoxic drugs, absence of parenchymal disease (proteinuria > 500 mg/d), microscopic hematuria (50 red blood cells per high power field), and abnormal renal ultrasonography<sup>[8]</sup>.

In this review, we will focus on various aspects of HRS and its impact on various phases of LT. Literature was searched for this review from various search engines including PubMed, Cochrane, and Scopus. Each of the citations for the papers originally pulled was then reviewed for additional articles for inclusion.

## ROLE OF CR AND OTHER MARKERS OF RENAL IMPAIRMENT IN CIRRHOTICS

There is concern that serum Cr may not reflect accurate kidney function in the setting of HRS with significant liver dysfunction<sup>[8,9]</sup>. Cr is an indirect measure of renal function as it is derived from non-enzymatic conversion of creatine, which is stored in muscle and being produced within the liver. As patients develop cirrhosis there is increased muscle wasting, decreased protein intake, and diminished creatine synthesis resulting in overestimation of renal function<sup>[9,10]</sup>. Moreover, two individuals with similar glomerular filtration rates may have varying Cr levels due to variation associated with age, sex, race, body mass index, and bilirubin concentrations<sup>[11]</sup>. For example, women generally have lower serum Cr levels compared to men resulting in lower median MELD scores (14 vs 15,  $P < 0.001$ ) and a higher likelihood to die on the transplant list when compared to the pre-MELD era<sup>[12]</sup>.

Multiple mathematical formulas have been developed to utilize serum Cr to calculate an estimation of glomerular filtration rate (eGFR). These include Cockcroft-Gault (C-G) and Modification of Diet in Renal Disease (MDRD) which incorporate different variables. C-G requires age, gender, weight, and serum Cr, while MDRD-4 utilizes age, gender, ethnicity, and serum Cr and MDRD-6 also involves albumin and urea<sup>[13,14]</sup>. In our cirrhotic population, MDRD-6 is used more widely when compared to C-G given inclusion of albumin and urea. Moreover, exogenous markers such as inulin have been previously documented to improve accuracy when determining renal function. Unfortunately the "gold standard" inulin infusion technique is time consuming, expensive, and potentially invasive making it a less viable option<sup>[15]</sup>.

Multiple AKI biomarkers including NGAL, Cystatin C, IL-18, NAG, and KIM-1 have been well characterized



and may delineate patients who have the risk of progression of disease and will require renal replacement therapy (RRT)<sup>[2,16,17]</sup>. For example, Aberg *et al.*<sup>[18]</sup> looked specifically at the urinary marker neutrophil gelatinase-associated lipocalin (NGAL) in 203 LT patients and demonstrated that raised urinary levels of NGAL independently predicted pre-LT kidney dysfunction in the setting of HRS and could have the potential to help decide the need to performed combined liver-kidney transplantation. Additionally, urinary NGAL levels to be a strong predictor for short-term mortality, with HRS patients having intermediate levels between prerenal azotemia and intrinsic AKI<sup>[19]</sup>. Furthermore, certain studies have also shown cystatin C level may be an important marker for predicting mortality in HRS<sup>[20,21]</sup>. However, it is important to note that at this time current IAC or Acute Dialysis Quality Initiative do not recommend evaluating for these biomarkers.

## BRIEF PATHOPHYSIOLOGY AND TYPES OF HRS

The pathophysiology of HRS has been well documented previously with portal hypertension leading to splanchnic artery dilatation<sup>[22,23]</sup>. This phenomenon results in a number of downstream effects including arterial under-filling, increased cardiac output, and vasoconstriction of renal arteries<sup>[8]</sup>. Ultimately the kidneys respond with increased activity of renin-angiotensin-aldosterone system as well as non-osmotic release of vasopressin, both of which result in worsening GFR, ascites, and hemodynamic instability<sup>[24,25]</sup>.

HRS is typically divided into two subtypes, type 1 and type 2, based on the rate of progression of renal disease and prognosis. Diagnostic criteria for type 1 HRS (in addition to criteria for HRS according to IAC mentioned above) include serum Cr > 2.5 mg/dL, doubling of serum Cr in less than 2 wk, no history of diuretic resistant ascites, and generally a precipitating event. On the other hand, type 2 HRS is a gradually progressive renal impairment without any precipitating events and usually associated with diuretic resistant ascites. Additionally, patient outcomes in terms of survival were reported to be better with type 2 HRS vs type 1<sup>[26]</sup>.

## CLASSIFICATIONS OF RENAL DYSFUNCTION IN PATIENTS WITH CIRRHOSIS

Various criteria are used for classification of renal dysfunction in patients with liver cirrhosis. Two of the most commonly used criteria include the Risk, Injury, Failure, Loss, and End-Stage Kidney Disease (RIFLE) and AKI network (AKIN). The RIFLE criteria utilize both serum Cr level and urine output to assess what stage of renal injury has occurred. For example, acute renal injury is Cr doubled from baseline and urine output <

0.5 mL/kg per hour over 12 h while acute renal failure is Cr tripled from baseline and urine output < 0.3 mL/kg per hour over 24 h. A major limitation of the RIFLE classification is that per these criteria a large number of cirrhotic patients would already present with some degree of AKI. In 2007 the AKIN has proposed a new definition of AKI that condenses RIFLE into 3 stages to increase sensitivity and specificity of diagnosing AKI. Moreover, the Kidney Disease Improving Global Outcomes recently defined AKI as diminished kidney function resulting in 0.3 mg/dL increase in serum Cr in 48 h, or a 50% increase in baseline Cr (within 7 d), or a urine volume of < 0.5 mL/kg per hour for 6 h<sup>[8,27]</sup>. It has been well documented that approximately 20% of patients hospitalized for decompensated cirrhosis present with a concomitant AKI<sup>[28]</sup>. This phenomenon is related to the progressive vasodilatory state of cirrhosis causing a decrease in arterial volume and resultant vasoconstriction of renal vessels. Interestingly, two prospective studies assessing AKI criteria in patients with cirrhosis found that AKI with serum Cr values < 1.5 mg/dL is a relatively benign and potentially reversible condition, while significant increase in Cr (> 1.5 mg/dL) is associated with a worse prognosis<sup>[29,30]</sup>.

A retrospective study utilized the RIFLE classification to look at 283 patients who underwent LT and stratified them into three cohorts: Risk, injury, and failure. Moreover, the failure group was further subdivided by etiology (HRS vs ATN) and the clinical course was followed for 5 years. Comparing these groups, the ATN group had significantly worse 1- and 5-year survival and renal outcomes, with an increased incidence of stage 4 and 5 CKD<sup>[31]</sup>. While only a single-center retrospective study, it is instrumental in demonstrating that the etiology of AKI may be more important than initially thought in predicting renal recovery<sup>[32]</sup>.

Prerenal injury, ATN, and HRS encompass close to 80% of AKI etiology in the in the pre-transplantation setting<sup>[33]</sup>. A United Network for Organ Sharing (UNOS) based study in 2002 found that 40% of LT candidates have kidney dysfunction, best defined as a GFR < 60 cm<sup>3</sup>/min per square meter<sup>[34]</sup>. More recently, a prospective study following 463 patients classified renal failure into four main categories: Infections (*n* = 213, 46%), hypovolemia associated renal failure (*n* = 149, 32%), HRS (*n* = 60, 13%), parenchymal nephropathy (*n* = 41, 9%)<sup>[35]</sup>. While this is a simple classification, it is useful to assess prognosis and decisions regarding LT.

## PREVALENCE AND PRECIPITANTS OF HRS IN WAIT-LIST AND TRANSPLANT PATIENTS

The prevalence of HRS has been reported to increase with severity and duration of cirrhosis. Ginès *et al.*<sup>[36]</sup> studied 229 patients with cirrhosis and found an 18% incidence of HRS at one year, with an increase to 39% within five years. Additionally, Wong *et al.*<sup>[37]</sup> reported

HRS in 48% of patients on the LT waiting list, indicating an increased prevalence with disease progression. Various precipitants of HRS include spontaneous bacterial peritonitis, large volume paracentesis with inadequate albumin replacement, use of nephrotoxic drugs and hypovolemia due to bleeding and or dehydration. With the help of early diagnosis and aggressive management with vasopressors the incidence of HRS may decrease with an improvement in overall outcomes<sup>[38]</sup>.

## MANAGEMENT OF HRS

Medical management of HRS has been shown to improve short-term outcomes; however, long term outcomes are dismal without LT. Current medical treatment includes avoidance of HRS precipitants and pharmacological management prior to considering transjugular intrahepatic portosystemic shunt (TIPS) and RRT. Pharmacological treatment serves as a bridge to transplantation to improve the patient's prognosis. There is a consensus on general measures in treating HRS including suspension of diuretic therapy, avoidance of nephrotoxic drugs and adjustment in doses of drugs. Moreover, per AASLD guidelines the role of albumin after large volume paracentesis (8 g of albumin for each liter of ascites removed) has been the standard of care.

### Role of terlipressin in HRS

Given the significance of arterial vasodilatation in the pathophysiology of HRS, vasoconstrictors along with albumin have improved renal function in approximately 40%-60% of patients with type 1 HRS (Table 1)<sup>[39]</sup>. Terlipressin plus albumin has been shown to improve renal function in 35%-40% of patients with type 1 HRS, with initial IV boluses of 0.5-1 mg every 4 h that can be titrated to 3 mg every 4 h if there is limited response<sup>[40-42]</sup>. A study comparing terlipressin bolus vs continuous infusion found that while the rate of response was not statistically significant, the rate of adverse of events was lower in the infusion group with lower associated dosing<sup>[43]</sup>.

While many studies demonstrate the use of terlipressin as a bridge to transplantation, it is important to note that fewer than 50% of patients who used terlipressin in the setting of HRS recover from a renal standpoint. One study assessed the efficacy of terlipressin plus albumin vs albumin alone for treatment of HRS-1 in the setting of LT. The 6-mo survival rate for those in the terlipressin group was 100% for transplanted patients and 34% for non-transplanted patients, while in the control group survival was 94% for transplanted patients and 17% for non-transplanted patients<sup>[44]</sup>. This study was able to show that terlipressin likely improved pre-transplant renal function while having no significant impact on post-transplant survival. On the other hand, Sagi *et al*<sup>[45]</sup> concluded improved transplant-free survival at 90 d (RR = 1.86, 95%CI: 1.0-3.4,  $P = 0.05$ ) in those in the terlipressin arm when studying 223 patients in 4 separate trials. A prospective, randomized, double-blind, placebo-controlled clinical trial showed that terli-

pressin group showed Cr improvement from baseline to day 14 while on the treatment<sup>[46]</sup>. It appears that terlipressin treatment beyond one week and up to 20 d has the potential for further improvement<sup>[47]</sup>. Moreover, a recent meta-analysis of randomized trials (5 trials,  $n = 243$  patients), showed the overall rate of patients on terlipressin with HRS who recovered renal function was 8.09 (95%CI: 3.52-18.59,  $P < 0.001$ )<sup>[48]</sup>.

One study found a better response to terlipressin in the setting of higher serum sodium concentrations and lower serum bilirubin at the beginning of treatment<sup>[49]</sup>, which would indicate that the early identification and treatment of HRS-1 may improve outcomes. A larger study was able to identify independent predictors of survival in the setting of terlipressin including age, duration of treatment, MELD score, and alcoholic cirrhosis<sup>[50]</sup>, while an additional study was able to identify low urinary sodium prior to treatment being associated with poor survival<sup>[51]</sup>.

Similar to the type 1 HRS patient population, terlipressin has been shown to improve renal function in type 2 HRS (Cr improvement in 8 out of 11 patients) when compared to organic renal disease<sup>[52]</sup>. Interestingly, a recent study examined 56 patients awaiting LT who were diagnosed with type 2 HRS. A subset of patients were being treated with terlipressin and albumin, but no differences were found in mortality in peri-operative setting or in post-transplantation outcomes (AKI, need for RRT, or development of CKD) when compared to the control group<sup>[53]</sup>. Moreover, another study also showed no benefit in using terlipressin in the setting of type 2 HRS<sup>[54]</sup>. Furthermore, while LT helps reverse type 2 HRS, there may be an association with longer intensive care stays and early-post-transplant CKD stage 3<sup>[55]</sup>.

### Role of other vasoconstrictors in HRS

Terlipressin is not available in United States; therefore midodrine, octreotide, omipressin and noradrenaline with albumin have been used in uncontrolled studies to treat HRS. It was found that HRS patients were more likely to improve while treated with AVP when compared to octreotide alone<sup>[56]</sup>. Another study assessed the effect of octreotide, midodrine, and albumin on survival compared to control populations and found improved renal function and short-term survival in the setting of both HRS-1 and HRS-2<sup>[57]</sup>. With use of a combo of octreotide, midodrine and albumin, reversal of HRS has been reported to be as high as 40%<sup>[58]</sup>.

Ornipressin is another potent splanchnic vasoconstrictor, but has been shown to have a higher incidence of vascular complications when compared to terlipressin<sup>[59]</sup>. In regards to noradrenaline, an unblinded study in 2007 was able to show that noradrenaline is an effective alternative to terlipressin in the setting of HRS type 1<sup>[60]</sup>. A more recent meta-analysis looked at 4 smaller studies where 154 patients were included and found that there was no difference between noradrenaline and terlipressin in regards to mortality at 30 d (RR = 0.89, 95%CI: 0.68 to 1.17) and reversal of HRS (RR = 0.97, 95%CI: 0.76 to 1.23)<sup>[61]</sup>.

Table 1 The role of terlipressin and albumin in hepatorenal syndrome-1

Ref.	Terlipressin dose	Albumin	Length	Terlipressin group: Cr (mg/dL) or Cr Cl (mL/min)	Control group: Cr (mg/dL) or Cr Cl (mL/min)	30 d survival (terlipressin <i>vs</i> control)	Transplant free outcome
Hadengue <i>et al</i> <sup>[13]</sup>	1 mg twice daily	No	2 d	Cr Cl: 27 ± 4	Cr Cl: 15 ± 2	N/A	N/A
Halimi <i>et al</i> <sup>[49]</sup>	4 mg/d	Yes	7 d (mean)	Decline in Cr from 31%-75% from day 0 to day 5	N/A	13/18 (72%) patient response	N/A
Danaliloglu <i>et al</i> <sup>[52]</sup>	2-4 mg/d	Yes	6 d	N/A	N/A	20% <i>vs</i> 0%	N/A
Testro <i>et al</i> <sup>[54]</sup>	1 mg every 6 h (max of 8 mg/d)	Yes	12 d	N/A	N/A	17/49 HRS type 1, 4/20 HRS type 2	All transplant free outcomes responded to terlipressin
Sanyal <i>et al</i> <sup>[46]</sup>	1 mg every 6 h (doubled on 4 d if Cr did not < 30%)	No (control group received albumin)	14 d	Cr < 1.5 mg/dL (19/59, 33.9%)	Cr < 1.5 mg/dL (7/56, 12.5%)	N/A	42.9% (24/56) <i>vs</i> 37.5% (21/56) in terlipressin <i>vs</i> control group at 180 d
von Kalckreuth <i>et al</i> <sup>[67]</sup>	3.9 mg ± 1.3 mg (responders) <i>vs</i> 3.4 mg ± 1.4 mg (nonresponders)	Yes	6 ± 4.9 d (responder) <i>vs</i> 8 ± 6.3 d (nonresponders)	N/A	N/A	Complete response by day 7 was 52%, while at day 17 it was 84%	25/38 (66%) of treatment complete response was achieved
Boyer <i>et al</i> <sup>[44]</sup>	1 mg every 6 h	Yes	6.3 d (mean)	Cr: 2.8 mg/dL	Cr: 3.8 mg/dL	N/A	34% non-transplanted survival 100% transplant survival at 180 d
Hinz <i>et al</i> <sup>[51]</sup>	2-6 mg/d	Yes	N/A	N/A	N/A	57% of patients (12/21) responded to terlipressin. Age was a negative predictor for treatment response	No difference seen in mortality between responders and non-responders at 60 d
Heidemann <i>et al</i> <sup>[50]</sup>	26.43 ± 30.86 (total dose for responders) <i>vs</i> 32.11 ± 31.57 (total dose for non-responders)	Yes	9 d (responders) <i>vs</i> 10.5 d (non-responders)	N/A	N/A	One month survival was longer in responders <i>vs</i> non-responders ( <i>P</i> = 0.048)	N/A
Sagi <i>et al</i> <sup>[45]</sup> (meta-analysis)	N/A	Yes	Minimum of 3 d of terlipressin	Cr must have been < 1.5 mg/dL at treatment end	N/A	Four trials ( <i>n</i> = 223) with RR for reversal in type 1 HRS with terlipressin was 3.66 (95%CI: 2.15-6.23)	N/A
Fabrizi <i>et al</i> <sup>[48]</sup> (meta-analysis)	N/A	N/A	N/A	N/A	N/A	Five trials ( <i>n</i> = 243 patients) with pooled OR of HRS reversal was 8.09 (95%CI: 3.52; 18.59)	Recovery of renal function occurs in less than 50% of patients with HRS even with terlipressin

Cr: Creatinine; Cr Cl: Creatinine clearance; HRS: Hepatorenal syndrome; N/A: Not available.

A recently published randomized study directly compared terlipressin with albumin to midodrine plus octreotide with albumin<sup>[62]</sup>. Terlipressin group was found to be significantly more effective in improving kidney function in HRS patients (70% *vs* 28%)<sup>[62]</sup>. Additionally, a small study that looked at three patients who were initially on terlipressin and attempted to switch treatment to midodrine plus octreotide on multiple attempts were found to have serum Cr elevation as well as diminished urine output<sup>[63]</sup>.

### Other treatment options for HRS

Among other options available for HRS management, TIPS has been increasingly utilized. While it is well documented that TIPS is effective treatment for refractory variceal bleeding and ascites, its role in patients with renal dysfunction is unclear. Few small studies on HRS indicate some clear benefit after TIPS<sup>[64,65]</sup>. A study examining non-

transplantable cirrhotic (14 type 1 HRS and 17 type 2 HRS) patients showed renal function improved within two weeks after TIPS with improved mortality over the course of 18 mo<sup>[66]</sup>. A recent study utilizing UNOS demonstrated that patients on the LT list status-post TIPS procedure had a lower mortality rate compared to patients without TIPS<sup>[67]</sup>. This study hypothesized that the TIPS plays a role in promoting survival by improving nutritional status and preventing variceal bleeding, refractory ascites, and HRS. It is important to remember that TIPS can increase the risk of hepatic encephalopathy as well as liver failure in rare occasions<sup>[68]</sup>.

Molecular absorbent recycling system (MARS) has the ability to remove both small- and medium-sized lipophilic toxins and may have a role in improving complications of liver disease such as hepatic encephalopathy and HRS. Multiple studies have shown MARS having the ability to reduce cholestatic parameters, improve mentation, as well as renal function especially in patients with a Model for End-Stage Liver Disease (MELD) between 20-29<sup>[69,70]</sup>. In 2002 a study showed when MARS was used there was improvement in mentation and hepatic encephalopathy in 14 out of 19 centers<sup>[71]</sup>. Interestingly, when MARS was directly compared to hemodiafiltration there was a decrease in Cr and bilirubin as well as a decrease in mortality at day 7<sup>[72]</sup>. Furthermore, a study looking at MARS use in the post-transplantation setting with HRS, HE, or intractable pruritis showed improvement in symptoms and laboratory findings<sup>[73]</sup>. However, none of these studies showed long term benefit in HRS patients including transplant free survival.

## PREDICTORS OF MORTALITY IN PATIENTS WITH HRS

Yang *et al.*<sup>[74]</sup> studied the predictors of mortality in type 1 HRS in a tertiary care center and formulated a time-dependent proportional hazards model. Contrary to other studies reporting on MELD score as predictor mortality, they found increased Cr by each point and total bilirubin levels during the admission increased mortality risk by 29% and 4%, respectively. Increasing albumin level during the admission showed its protective value<sup>[74]</sup>.

Sanchez *et al.*<sup>[75]</sup> looked at pre and peri-transplant predictors of renal dysfunction requiring either RRT or HD. This study looked at 724 LT patients where a clinical prediction model was constructed to assess the probability of requiring dialysis post-transplantation in a prospective manner. Pre-LT Cr > 1.9 mg/dL (OR = 3.57), pre-LT BUN > 27 mg/dL (OR = 2.68), ICU stay > 3 d (OR = 10.23), and MELD score > 21 (OR = 2.5) were significant<sup>[75]</sup>. Furthermore, changes in MELD scores (influenced by Cr and bilirubin) during the admission predict prognosis more so than the initial MELD<sup>[74]</sup>. A recent study was performed in attempts to assess renal impairment prior to overt HRS development by measuring renal arterial resistance indices (RI)<sup>[76]</sup>. Interestingly, RI was significantly higher in patients with ascites than those without ascites and may be an

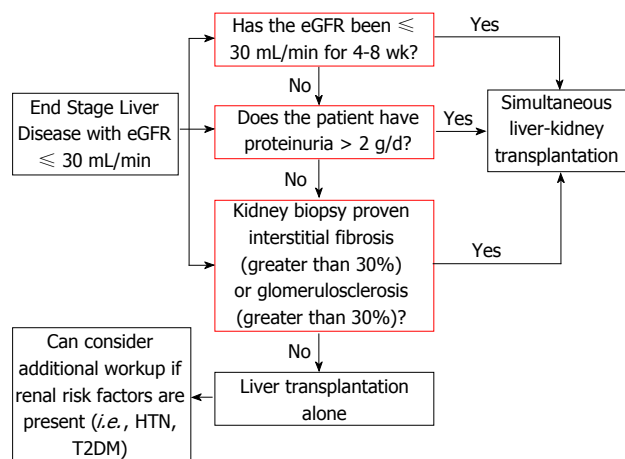
independent predictor of subsequent HRS development. Another study was able to show that "MAP responders" had improved response with better transplant-free survival in both the short-term and long-term settings<sup>[77]</sup>. However, these innovative modalities need further studies before being used in daily practice.

## LT ALONE VS SIMULTANEOUS LIVER KIDNEY TRANSPLANT FOR HRS

Since the introduction of the MELD scoring system there has been an increase in the number of simultaneous liver-kidney transplants (SLKT). From 2002 to 2013, the percentage of SLKT has increased from 4.2% to 8.1%, respectively. The most recent recommendations for SLKT include: (1) eGFR of 30 mL/min or less for 4-8 wk; (2) proteinuria > 2 g/d; and (3) biopsy proven interstitial fibrosis or glomerulosclerosis (Figure 1)<sup>[78]</sup>. An unintentional by product of SLKT has been a decrease number of kidney donors available for end stage renal disease (ESRD) patients. There are numerous studies indicating we should have stricter criteria for allocating two grafts to one patient as well as a debate on duration of renal dysfunction and duration of RRT in the setting of SLKT. A recent study proposed raising the dialysis requirement to greater than 12 wk (rather than current recommendations of 4-8 wk) to increase the number of kidney transplantations available for ESRD patients<sup>[79]</sup>. Table 2 outlines the outcomes of studies comparing liver transplantation alone (LTA) alone vs SLKT in the setting of HRS. One study retrospectively looked at 69 LT patients with a pre-transplantation Cr  $\geq$  1.5 and found that duration of pre-transplantation RRT rather than cause of renal dysfunction was a predictor of 6- and 12-mo kidney function post-LTA<sup>[80]</sup>. Interestingly, earlier studies have shown mixed data in regards to the utility of SLKT in the setting of HRS. A 1997 UNOS study looked at 414 SLKT vs 2442 LTA with a Cr > 2.0 and found a 5 year survival of 62.2% for SLKT patients and 50.4% for LTA recipients, suggesting SLKT may be beneficial for HRS patients<sup>[81]</sup>. Furthermore, another study including local center and UNOS database (2002-2008) compared LTA vs SLKT in the setting of renal impairment. Diagnosis of HRS was presumptive in UNOS database and confirmed on the local data. UNOS data showed a survival benefit of SLKT over LTA for those patients with poor renal function, specifically those with HRS, whereas results of local center suggest otherwise<sup>[82]</sup>.

On the other hand, a small study showed that in patients with HRS, SLKT did not confer a survival advantage over LTA (1-year patient survival was 72% vs 66%, *P*-value = 0.88)<sup>[83]</sup>. A much larger 2006 UNOS study that compared 1032 SLKT to 19137 LTA patients showed no mortality difference for patients with HRS (1 year survival was 72% vs 66%) unless the patient was receiving HD for longer than 8 wk, with a dialysis duration of > 12 wk that was a significant predictor for long-term outcomes<sup>[84]</sup>. Furthermore, one meta-analysis looked at 3536 SLKT





**Figure 1** Algorithm for evaluating for simultaneous liver-kidney transplantation in a liver transplant candidate with renal dysfunction. Modified from Saxena *et al.*<sup>[76]</sup>. eGFR: Estimation of glomerular filtration rate; T2DM: Type 2 diabetes mellitus; HTN: Hypertension.

(between 1984 to 2008) and found that the cumulative 1, 2, 3 and 5-year patient survival were 84.9%, 52.8%, 45.4% and 42.6%. It was concluded that there was no definitive evidence of better graft or patient survival in the SLKT population when compared to the LTA given the difficulty discerning irreversible kidney function in liver transplant candidates<sup>[85]</sup>. Additionally, one study found the rate of renal non-recovery within 6 mo of LTA for 2112 patients who underwent RRT within 90 d of their transplantation was only 8.9%, with risk factors for non-recovery including age, T2DM, and duration of RRT<sup>[86]</sup>. Because of this limitation as well as selection biases, the true survival benefit of SLKT in candidates without ESRD remains unproved<sup>[87]</sup>.

It appears that UNOS database studies have heterogeneous groups, including patients with renal impairment due to multiple reasons and hence a selection bias for patients with HRS. Single center studies have issue of small sample size. Nevertheless, chances of misclassification bias in small studies are less. These studies do not report added benefit of SLKT over LTA in patients with HRS, not on HD and duration of renal dysfunction < 8 wk.

Interestingly some studies address benefit of SKLT over LTA alone with respect to immune safety liver graft on kidney graft function due to immunogenic effect of liver. These studies justified SLKT over LTA for two additional reasons: (1) it is well documented there is significant decrease in graft rejection when a patient has a SLKT over an LTA (15% decreased reduction in graft loss); and (2) there is superior recipient and graft survival when compared to Kidney After LT or Liver After Kidney Transplantation<sup>[88,89]</sup>. Priority for allocation of kidneys to kidney-liver candidates follows the allocation priority for the non-renal organ. However, due to shortage of organ and justification of an equitable distribution of organ it is not possible to perform SLKT for this indication.

## HRS AND POST-TRANSPLANT MANAGEMENT/OUTCOMES

### Impact of HRS on outcomes of LT

The impact of LT on overall renal function has been well documented. Lafayette *et al.*<sup>[90]</sup> looked at renal function in the pre-transplantation setting and studied 115 liver transplant recipients by arbitrarily dividing them based on serum Cr into two groups (group 1 with Cr > 1.0, group 2 with < 1.0); they showed that group 1 patients had significantly longer ICU stays, higher hospital charges, and a greatly increased mortality rate<sup>[90]</sup>. Patients with HRS tend to require longer hospitalizations, increased intensive care duration, and further dialysis in the post-op setting<sup>[91]</sup>. Interestingly, when comparing HRS vs ATN post-transplant outcomes it was found that ATN was associated with higher mortality at 1 year post-LT along with increased incidence of CKD (stage 4 or 5) when compared to HRS<sup>[31]</sup>.

One of the first studies to address HRS in the post-operative setting was in reported in 1991 where Gonwa *et al.*<sup>[92]</sup> found close to 10% of HRS patients developed ESRD post-transplant when compared to 0.8% of non-HRS patients ( $P < 0.005$ ). However, a similar study revealed that while HRS patients were more likely to be dialyzed post-operatively, there was no difference between Cr levels at 24 wk between non-HRS vs HRS groups<sup>[93]</sup>. Park *et al.*<sup>[94]</sup> also confirmed this concept in a study that yielded similar results in 1-year patient survival after LT in the HRS patients vs those without HRS ( $P = 0.37$ ).

In regards to AKI in the post-LT setting, a large study looking at 1352 LT recipients found that 162 (12%) patients developed acute renal failure (ARF) within the first week. Type 2 HRS with GFR < 50 mL/min was reported to be one of major risk factor<sup>[95]</sup>. However, López Lago *et al.*<sup>[96]</sup> also looked at HRS vs non-HRS patients who developed ARF in the post-LT setting but found no differences in 1 year mortality, need for RRT, or rejection.

Many studies have aimed to identify the role of GFR following transplantation in stratifying risk of kidney impairment. Sato *et al.*<sup>[97]</sup> showed that an eGFR < 60 mL/min per 1.73 m<sup>2</sup> during the first month post LT can be associated with increased rate of development of CKD, 2 years post-OLT. Interestingly, a recent study assessed 191 LT patients who underwent intense post LT GFR measurements (especially at 1 and 3 years). The study concluded that a low GFR (< 40 mL/min per 1.73 m<sup>2</sup>) at 1 year was associated with higher risk for late renal dysfunction<sup>[98]</sup>. Moreover, Longenecker *et al.*<sup>[99]</sup> looked at the progression of GFR over 15 years post transplantation and found that eGFR < 60 mL/min per 1.73 m<sup>2</sup> and type 2 diabetes at the time of transplantation were associated with increased rates of progression to ESRD. When discussing long-term requirement of RRT post-transplantation, one study assessed 208 LT recipients and found 5.8% of surviving

Table 2 Comparing outcomes measures between liver transplantation alone *vs* simultaneous liver kidney transplantation including graft and patient survival as well as need for renal replacement therapy

Ref.	No. of LTA	No. of SLKT	Graft survival (LTA <i>vs</i> SLKT)	Patient survival (LTA <i>vs</i> SLKT)	Renal dysfunction post 1, 5 and 10 yr (LTA <i>vs</i> SLKT)	RRT post-transplantation (LTA <i>vs</i> SLKT)	Additional comments
Jeyarajah <i>et al</i> <sup>[81]</sup>	2442 (Cr > 2.0, nationwide)	29 (single center) + 414 (nationwide)	N/A	5 yr survival nationwide (50.4% <i>vs</i> 62.2%)	N/A	N/A	Interestingly, single center study had increased better survival in LTA than SLKT group
Campbell <i>et al</i> <sup>[80]</sup>	53	13	N/A	N/A	1 yr (1.4 mg/dL <i>vs</i> 1.5 mg/dL)	2% <i>vs</i> 0% (at 12 mo)	Adjusting for baseline characteristics, SLKT patients had lower Cr than LTA at 12 mo ( <i>P</i> = 0.01)
Ruiz <i>et al</i> <sup>[81]</sup>	80 (all with HRS)	98 (22 with HRS and 76 with primary renal disease)	1 yr SLKT survival (liver: 76% and kidney: 76%)	1 yr survival (66% LTA <i>vs</i> 72% SLKT)	N/A	Post-op dialysis: (89% LTA <i>vs</i> 55% SLKT pts for median 2.5 d)	1 yr acute kidney rejection in CLKT was 14% <i>vs</i> 23% in 5 yr LT cohort
Locke <i>et al</i> <sup>[81]</sup>	19137	1032	N/A	1 yr survival for pts with ≥ 3 mo RRT: (70.8% LTA <i>vs</i> 84.5% SLKT)	N/A	N/A	Even after matched-control analysis, there was no benefit in SLKT cohort <i>vs</i> LTA cohort outside of aforementioned RRT
Mehrab <i>et al</i> <sup>[82]</sup> (literature review)	N/A	3536	Cumulative 5 yr SLKT survival of both organs (60.9%)	Cumulative 5 yr survival 42.6%	N/A	N/A	It is concluded that there is no definitive evidence of better graft/patient survival in SLKT <i>vs</i> LTA
Chava <i>et al</i> <sup>[114]</sup>	N/A	39	5 yr SLKT survival (liver: 73.7% and kidney: 70%)	73.7% SLKT patient survival at 5 yr	N/A	N/A	15 surviving patients (53.6%) had mild/moderate kidney dysfunction
Fong <i>et al</i> <sup>[82]</sup>	2774	1501	5 yr survival (58.9% LTA <i>vs</i> 65.3% SLKT, <i>P</i> < 0.001)	5 yr survival (62.9% LTA <i>vs</i> 67.4% SLKT, <i>P</i> < 0.001)	0% with severe renal dysfunction	N/A	Liver graft survival and patient survival was better in SLKT <i>vs</i> LTA group
Martin <i>et al</i> <sup>[80]</sup> 2012	66026	2327	15% decreased risk of graft loss with SLKT <i>vs</i> LTA ( <i>P</i> = 0.02)	N/A	N/A	N/A	SLKT had higher graft survival rates than both KALT and LAKT
Sharma <i>et al</i> <sup>[86]</sup>	2112 (received RRT within 90 d before LT)	N/A	N/A	78% LTA survival at 6 mo (not associated with RRT duration)	N/A	8.90%	Risk for non-recovery increased by 3.6%/day of pre-LT RRT
Catalano <i>et al</i> <sup>[89]</sup>	74	37	10 yr survival (77% LTA <i>vs</i> 80% SLKT, <i>P</i> = 0.85)	10 yr survival (79% LTA <i>vs</i> 86% SLKT, <i>P</i> = 0.56)	N/A	N/A	Acute rejection episodes involving the liver were less in SLKT <i>vs</i> LTA

LTA: Liver transplantation alone; SLKT: Simultaneous liver kidney transplantation; RRT: Renal replacement therapy; HRS: Hepatorenal syndrome; KALT: Kidney after liver transplantation; LAKT: Liver after kidney transplantation; N/A: Not available.

patients required RRT at 3 mo. While there was no significant difference between underlying liver disease and immunosuppressive agents, patients who were on RRT at 3 mo were also on HD 2 years post-LT as well<sup>[1100]</sup>.

While the majority of studies seem to indicate HRS increases the risk for worse post-transplantation kidney function, there are certain exceptions found in the literature. One study looked at 419 LTA performed between 1995 to 2009 and found that MELD scoring system did not impact all-cause mortality in the post-transplantation setting; however, there was a 2-fold greater mortality risk if patients required the need for pre-transplant RRT and post-transplant kidney dysfunction<sup>[1101]</sup>.

Duration of pre-transplantation RRT and vasopressors for reversal of HRS

In regards to post-LT outcomes in patients with HRS who required vasopressor treatment one study compared 27 cases (triple therapy of octreotide, midodrine, and albumin)

vs 16 controls (no vasopressor treatment) and found the GFR was similar at 1 mo ( $P = 0.61$ ) and 1 year ( $P = 0.13$ )<sup>[58]</sup>. Moreover, 11 out of the 27 cases responded to triple therapy but there was no difference in GFR at 1 mo ( $P = 0.96$ ) and 1 year ( $P = 0.48$ ) between responders vs non-responders. A smaller study looked at 9 HRS patients on vasopressin vs 27 non-HRS patients and found there was no significant renal impairment between the two groups in regards to duration of hospitalizations, infections, or renal impairment post-transplantation<sup>[102]</sup>. These two studies are much different than the findings from Wong *et al.*<sup>[103]</sup>; they found that patients without HRS reversal from triple therapy were found to have longer duration of pre-transplant dialysis and increase in post-transplant mortality<sup>[103]</sup>.

One study assessed 253 living donor LT patients and compared survival between starting RRT in the pre-transplant setting vs post-transplant setting. It was found that the duration of RRT was significantly shorter in the RRT-pre group compared to the RRT-post group ( $5.3 \pm 2.1$  d vs  $17.8 \pm 14.1$  d,  $P = 0.02$ ) as well as higher graft survival ( $100\%$  vs  $51.9\%$ ,  $P < 0.01$ )<sup>[104]</sup>.

#### How to manage immunosuppression in immediate post-LT period with HRS

Acute or chronic rejection has become more of a rarity with the current immunosuppression therapies<sup>[105]</sup>. However, calcineurin inhibitors (CNI) have significant nephrotoxic effects by inducing interstitial fibrosis, chronic microangiopathy, and tubular atrophy *via* increased extracellular matrix production, vasoconstriction, and cyclosporine induced apoptosis<sup>[11,106]</sup>. The landmark study in 1994 comparing tacrolimus vs cyclosporine showed that both were comparable in patient and graft survival; however, tacrolimus had substantially more adverse events, including nephrotoxicity, requiring discontinuation of the drug<sup>[107]</sup>.

It is standard practice in majority of transplant centers to use different types of T-cell specific antibody induction in patients with post LT renal dysfunction. Commonly used agents are interleukin-2 receptor antagonists (daclizumab, or basiliximab) and polyclonal antibodies (rabbit anti-thymocyte globulin) based on center preference. Also it is practiced to use mycophenolate mofetil (MMF) and wait for improvement in kidney function post LT and introduce CNI.

Unfortunately, currently there is still no treatment for nephrotoxicity outside of dose reduction of current immunosuppressive regimen<sup>[108]</sup>. Patients who are more than 10 years post-transplant have a higher incidence of ESRD and chronic renal failure, which is related to increase in serum Cr at various stages post-operatively<sup>[109]</sup>. MMF has been used in situations where CNIs are held to improve renal function<sup>[110]</sup> but there exists greater risk for rejection when using MMF<sup>[111]</sup>. Cincinatti *et al.*<sup>[112]</sup> show that combined MMF and low dose CNI therapy may actually promote tolerance, as this combination seems to be nephroprotective.

## FURTHER RESEARCH AND CONCLUDING REMARKS

We aimed to review the literature on HRS in the setting of LT and focused on the critical issues that are barriers to improved outcomes. Many consistencies have remained as treatment options including albumin, vasoconstrictors, RRT, and eventual LT. One area that was not well addressed in our literature search was the utility of norepinephrine in the setting of type 1 HRS not responding to currently approved octreotide and terlipressin based pharmacotherapy.

While current guidelines for SLKT have been recently updated, there is still much debate regarding the utility of SLKT over LTA. Certain studies have shown improved graft and patient survival in the SLKT patient population, but the literature has not been consistent regarding long-term kidney benefit. This is a topic that we anticipate will need to be further explored given variable results seen at this time. Equity in organ allocation must be taken into consideration as SLKT unavoidably allocates multiple grafts to a single recipient and removes donor kidneys from the transplant pool otherwise meant for patients with primary renal disease.

Finally, in regards to post-transplantation kidney dysfunction an eGFR  $< 60$  mL/min per  $1.73$  m<sup>2</sup> seems to be associated with an increased risk of patients having long-term ESRD. While patients continue to have increased patient and graft survival rates, future studies may benefit from continuing to delineate risk factors that may result in post-transplant RRT.

## ACKNOWLEDGMENTS

We would like to acknowledge Sarah E Ginier (Master of Library and Information Science Candidate May 2016) and Stephanie Schulte, MLIS (Associate Professor, Head, Research and Education Services) for their help in formulating the literature search for this review.

## REFERENCES

- 1 Cárdenas A, Ginès P. Acute-on-chronic liver failure: the kidneys. *Curr Opin Crit Care* 2011; **17**: 184-189 [PMID: 21311322 DOI: 10.1097/MCC.0b013e328344b3da]
- 2 Biancofiore G, Davis CL. Renal dysfunction in the perioperative liver transplant period. *Curr Opin Organ Transplant* 2008; **13**: 291-297 [PMID: 18685320 DOI: 10.1097/MOT.0b013e328300a058]
- 3 Pipili C, Cholongitas E. Renal dysfunction in patients with cirrhosis: Where do we stand? *World J Gastrointest Pharmacol Ther* 2014; **5**: 156-168 [PMID: 25133044 DOI: 10.4292/wjgpt.v5.i3.156]
- 4 Allegretti AS, Ortiz G, Wenger J, Deferio JJ, Wibecan J, Kalim S, Tamez H, Chung RT, Karumanchi SA, Thadhani RI. Prognosis of Acute Kidney Injury and Hepatorenal Syndrome in Patients with Cirrhosis: A Prospective Cohort Study. *Int J Nephrol* 2015; **2015**: 108139 [PMID: 26266048 DOI: 10.1155/2015/108139]
- 5 McGuire BM, Julian BA, Bynon JS, Cook WJ, King SJ, Curtis JJ, Accortt NA, Eckhoff DE. Brief communication: Glomerulonephritis in patients with hepatitis C cirrhosis undergoing liver transplantation. *Ann Intern Med* 2006; **144**: 735-741 [PMID: 16702589 DOI: 10.7326/0003-4819-144-10-200605160-00007]
- 6 Davis CL, Feng S, Sung R, Wong F, Goodrich NP, Melton LB,

- Reddy KR, Guidinger MK, Wilkinson A, Lake J. Simultaneous liver-kidney transplantation: evaluation to decision making. *Am J Transplant* 2007; **7**: 1702-1709 [PMID: 17532752 DOI: 10.1111/j.1600-6143.2007.01856.x]
- 7 **Garcia-Tsao G**, Parikh CR, Viola A. Acute kidney injury in cirrhosis. *Hepatology* 2008; **48**: 2064-2077 [PMID: 19003880 DOI: 10.1002/hep.22605]
  - 8 **Baraldi O**, Valentini C, Donati G, Comai G, Cuna V, Capelli I, Angelini ML, Moretti MI, Angeletti A, Piscaglia F, La Manna G. Hepatorenal syndrome: Update on diagnosis and treatment. *World J Nephrol* 2015; **4**: 511-520 [PMID: 26558188 DOI: 10.5527/wjn.v4.i5.511]
  - 9 **Davenport A**, Cholongitas E, Xirouchakis E, Burroughs AK. Pitfalls in assessing renal function in patients with cirrhosis--potential inequity for access to treatment of hepatorenal failure and liver transplantation. *Nephrol Dial Transplant* 2011; **26**: 2735-2742 [PMID: 21690201 DOI: 10.1093/ndt/gfr354]
  - 10 **Cocchetto DM**, Tschanz C, Bjornsson TD. Decreased rate of creatinine production in patients with hepatic disease: implications for estimation of creatinine clearance. *Ther Drug Monit* 1983; **5**: 161-168 [PMID: 6879639 DOI: 10.1097/00007691-198306000-00002]
  - 11 **Francoz C**, Glotz D, Moreau R, Durand F. The evaluation of renal function and disease in patients with cirrhosis. *J Hepatol* 2010; **52**: 605-613 [PMID: 20185192 DOI: 10.1016/j.jhep.2009.11.025]
  - 12 **Moylan CA**, Brady CW, Johnson JL, Smith AD, Tuttle-Newhall JE, Muir AJ. Disparities in liver transplantation before and after introduction of the MELD score. *JAMA* 2008; **300**: 2371-2378 [PMID: 19033587 DOI: 10.1001/jama.2008.720]
  - 13 **Levey AS**, Perrone RD, Madias NE. Serum creatinine and renal function. *Annu Rev Med* 1988; **39**: 465-490 [PMID: 3285786 DOI: 10.1146/annurev.me.39.020188.002341]
  - 14 **Levey AS**, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**: 461-470 [PMID: 10075613 DOI: 10.7326/0003-4819-130-6-199903160-00002]
  - 15 **Sterner G**, Frennby B, Mansson S, Nyman U, Van Westen D, Almén T. Determining 'true' glomerular filtration rate in healthy adults using infusion of inulin and comparing it with values obtained using other clearance techniques or prediction equations. *Scand J Urol Nephrol* 2008; **42**: 278-285 [PMID: 17943640 DOI: 10.1080/00365590701701806]
  - 16 **Cruz DN**, Bagshaw SM, Maisel A, Lewington A, Thadhani R, Chakravarthi R, Murray PT, Mehta RL, Chawla LS. Use of biomarkers to assess prognosis and guide management of patients with acute kidney injury. *Contrib Nephrol* 2013; **182**: 45-64 [PMID: 23689655 DOI: 10.1159/000349965]
  - 17 **Qasem AA**, Farag SE, Hamed E, Emar M, Bihery A, Pasha H. Urinary biomarkers of acute kidney injury in patients with liver cirrhosis. *ISRN Nephrol* 2014; **2014**: 376795 [PMID: 24967242 DOI: 10.1155/2014/376795]
  - 18 **Aberg F**, Lempinen M, Hollmén M, Nordin A, Mäkisalo H, Isoniemi H. Neutrophil gelatinase-associated lipocalin associated with irreversibility of pre-liver transplant kidney dysfunction. *Clin Transplant* 2014; **28**: 869-876 [PMID: 24930480 DOI: 10.1111/ctr.12394]
  - 19 **Verna EC**, Brown RS, Farrand E, Pichardo EM, Forster CS, Soladell Valle DA, Adkins SH, Sise ME, Oliver JA, Radhakrishnan J, Barasch JM, Nickolas TL. Urinary neutrophil gelatinase-associated lipocalin predicts mortality and identifies acute kidney injury in cirrhosis. *Dig Dis Sci* 2012; **57**: 2362-2370 [PMID: 22562534 DOI: 10.1007/s10620-012-2180-x]
  - 20 **Seo YS**, Jung ES, An H, Kim JH, Jung YK, Kim JH, Yim HJ, Yeon JE, Byun KS, Kim CD, Ryu HS, Um SH. Serum cystatin C level is a good prognostic marker in patients with cirrhotic ascites and normal serum creatinine levels. *Liver Int* 2009; **29**: 1521-1527 [PMID: 19725889 DOI: 10.1111/j.1478-3231.2009.02105.x]
  - 21 **Sharawey MA**, Shawky EM, Ali LH, Mohammed AA, Hassan HA, Fouad YM. Cystatin C: a predictor of hepatorenal syndrome in patients with liver cirrhosis. *Hepatol Int* 2011; **5**: 927-933 [PMID: 21484118 DOI: 10.1007/s12072-011-9266-y]
  - 22 **Barbano B**, Sardo L, Gigante A, Gasperini ML, Liberatori M, Giraldo GD, Lacanna A, Amoroso A, Cianci R. Pathophysiology, diagnosis and clinical management of hepatorenal syndrome: from classic to new drugs. *Curr Vasc Pharmacol* 2014; **12**: 125-135 [PMID: 24678726 DOI: 10.2174/15701611201140327163930]
  - 23 **Bataller R**, Ginès P, Arroyo V, Rodés J. Hepatorenal syndrome. *Clin Liver Dis* 2000; **4**: 487-507 [PMID: 11232202 DOI: 10.1016/S1089-3261(05)70120-3]
  - 24 **Mijac D**, Kezić A, Stojimirović B. [Hepatorenal syndrome]. *Srp Arh Celok Lek* 2007; **135**: 98-104 [PMID: 17503577]
  - 25 **Cárdenas A**, Ginès P. Therapy insight: Management of hepatorenal syndrome. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 338-348 [PMID: 16741553 DOI: 10.1038/ncpgasthep0517]
  - 26 **Alessandria C**, Ozdogan O, Guevara M, Restuccia T, Jiménez W, Arroyo V, Rodés J, Ginès P. MELD score and clinical type predict prognosis in hepatorenal syndrome: relevance to liver transplantation. *Hepatology* 2005; **41**: 1282-1289 [PMID: 15834937 DOI: 10.1002/hep.20687]
  - 27 **Thomas ME**, Blaine C, Dawney A, Devonald MA, Ftouh S, Laing C, Latchem S, Lewington A, Milford DV, Ostermann M. The definition of acute kidney injury and its use in practice. *Kidney Int* 2015; **87**: 62-73 [PMID: 25317932 DOI: 10.1038/ki.2014.328]
  - 28 **Hartleb M**, Gutkowski K. Kidneys in chronic liver diseases. *World J Gastroenterol* 2012; **18**: 3035-3049 [PMID: 22791939 DOI: 10.3748/wjg.v18.i24.3035]
  - 29 **Fagundes C**, Barreto R, Guevara M, Garcia E, Solà E, Rodríguez E, Graupera I, Ariza X, Pereira G, Alfaro I, Cárdenas A, Fernández J, Poch E, Ginès P. A modified acute kidney injury classification for diagnosis and risk stratification of impairment of kidney function in cirrhosis. *J Hepatol* 2013; **59**: 474-481 [PMID: 23669284 DOI: 10.1016/j.jhep.2013.04.036]
  - 30 **Piano S**, Rosi S, Maresio G, Fasolato S, Cavallin M, Romano A, Morando F, Gola E, Frigo AC, Gatta A, Angeli P. Evaluation of the Acute Kidney Injury Network criteria in hospitalized patients with cirrhosis and ascites. *J Hepatol* 2013; **59**: 482-489 [PMID: 23665185 DOI: 10.1016/j.jhep.2013.03.039]
  - 31 **Nadim MK**, Genyk YS, Tokin C, Fieber J, Ananthapanyasut W, Ye W, Selby R. Impact of the etiology of acute kidney injury on outcomes following liver transplantation: acute tubular necrosis versus hepatorenal syndrome. *Liver Transpl* 2012; **18**: 539-548 [PMID: 22250075 DOI: 10.1002/lt.23384]
  - 32 **Junghare M**, Ibrahim HN. Not all types of acute kidney injury are equal in the setting of liver transplantation. *Liver Transpl* 2012; **18**: 507-508 [PMID: 22422672 DOI: 10.1002/lt.23428]
  - 33 **Russ KB**, Stevens TM, Singal AK. Acute Kidney Injury in Patients with Cirrhosis. *J Clin Transl Hepatol* 2015; **3**: 195-204 [PMID: 26623266 DOI: 10.14218/jcth.2015.00015]
  - 34 **Nair S**, Verma S, Thuluvath PJ. Pretransplant renal function predicts survival in patients undergoing orthotopic liver transplantation. *Hepatology* 2002; **35**: 1179-1185 [PMID: 11981768 DOI: 10.1053/jhep.2002.33160]
  - 35 **Martín-Llahí M**, Guevara M, Torre A, Fagundes C, Restuccia T, Gilabert R, Solà E, Pereira G, Marinelli M, Pavesi M, Fernández J, Rodés J, Arroyo V, Ginès P. Prognostic importance of the cause of renal failure in patients with cirrhosis. *Gastroenterology* 2011; **140**: 488-496.e4 [PMID: 20682324 DOI: 10.1053/j.gastro.2010.07.043]
  - 36 **Ginès P**, Guevara M, Arroyo V, Rodés J. Hepatorenal syndrome. *Lancet* 2003; **362**: 1819-1827 [PMID: 14654322 DOI: 10.1016/s0140-6736(03)14903-3]
  - 37 **Wong LP**, Blackley MP, Andreoni KA, Chin H, Falk RJ, Klemmer PJ. Survival of liver transplant candidates with acute renal failure receiving renal replacement therapy. *Kidney Int* 2005; **68**: 362-370 [PMID: 15954928 DOI: 10.1111/j.1523-1755.2005.00408.x]
  - 38 **EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis.** *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]
  - 39 **Arroyo V**, Fernández J. Management of hepatorenal syndrome in patients with cirrhosis. *Nat Rev Nephrol* 2011; **7**: 517-526 [PMID:



- 21826080 DOI: 10.1038/nrneph.2011.96]
- 40 **Angeli P**, Morando F, Cavallin M, Piano S. Hepatorenal syndrome. *Contrib Nephrol* 2011; **174**: 46-55 [PMID: 21921608 DOI: 10.1159/000329235]
  - 41 **Cavallin M**, Fasolato S, Marengo S, Piano S, Tonon M, Angeli P. The Treatment of Hepatorenal Syndrome. *Dig Dis* 2015; **33**: 548-554 [PMID: 26159272 DOI: 10.1159/000375346]
  - 42 **Danalioglu A**, Cakaloglu Y, Karaca C, Aksoy N, Akyuz F, Ozdil S, Demir K, Besisik F, Boztas G, Mungan Z, Kaymakoglu S, Okten A. Terlipressin and albumin combination treatment in hepatorenal syndrome. *Hepatogastroenterology* 2003; **50** Suppl 2: ccciii-ccccv [PMID: 15244209]
  - 43 **Cavallin M**, Piano S, Romano A, Fasolato S, Frigo AC, Benetti G, Gola E, Morando F, Stanco M, Rosi S, Sticca A, Cillo U, Angeli P. Terlipressin given by continuous intravenous infusion versus intravenous boluses in the treatment of hepatorenal syndrome: A randomized controlled study. *Hepatology* 2016; **63**: 983-992 [PMID: 26659927 DOI: 10.1002/hep.28396]
  - 44 **Boyer TD**, Sanyal AJ, Garcia-Tsao G, Regenstein F, Rossaro L, Appenrodt B, Güllberg V, Sigal S, Bexon AS, Teuber P. Impact of liver transplantation on the survival of patients treated for hepatorenal syndrome type 1. *Liver Transpl* 2011; **17**: 1328-1332 [PMID: 21837734 DOI: 10.1002/lt.22395]
  - 45 **Sagi SV**, Mittal S, Kasturi KS, Sood GK. Terlipressin therapy for reversal of type 1 hepatorenal syndrome: a meta-analysis of randomized controlled trials. *J Gastroenterol Hepatol* 2010; **25**: 880-885 [PMID: 20074149 DOI: 10.1111/j.1440-1746.2009.06132.x]
  - 46 **Sanyal AJ**, Boyer T, Garcia-Tsao G, Regenstein F, Rossaro L, Appenrodt B, Blei A, Güllberg V, Sigal S, Teuber P. A randomized, prospective, double-blind, placebo-controlled trial of terlipressin for type 1 hepatorenal syndrome. *Gastroenterology* 2008; **134**: 1360-1368 [PMID: 18471513 DOI: 10.1053/j.gastro.2008.02.014]
  - 47 **von Kalckreuth V**, Glowa F, Geibler M, Lohse AW, Denzer UW. Terlipressin in 30 patients with hepatorenal syndrome: results of a retrospective study. *Z Gastroenterol* 2009; **47**: 21-26 [PMID: 19156588 DOI: 10.1055/s-0028-1109084]
  - 48 **Fabrizi F**, Aghemo A, Messa P. Hepatorenal syndrome and novel advances in its management. *Kidney Blood Press Res* 2013; **37**: 588-601 [PMID: 24356549 DOI: 10.1159/000355739]
  - 49 **Halimi C**, Bonnard P, Bernard B, Mathurin P, Mofredj A, di Martino V, Demontis R, Henry-Biabaud E, Fievet P, Opolon P, Poynard T, Cadranet JF. Effect of terlipressin (Glypressin) on hepatorenal syndrome in cirrhotic patients: results of a multicentre pilot study. *Eur J Gastroenterol Hepatol* 2002; **14**: 153-158 [PMID: 11981339 DOI: 10.1097/00042737-200202000-00009]
  - 50 **Heidemann J**, Bartels C, Berssenbrügge C, Schmidt H, Meister T. Hepatorenal syndrome: outcome of response to therapy and predictors of survival. *Gastroenterol Res Pract* 2015; **2015**: 457613 [PMID: 25983746 DOI: 10.1155/2015/457613]
  - 51 **Hinz M**, Wree A, Jochum C, Bechmann LP, Saner F, Gerbes AL, Gerken G, Canbay A. High age and low sodium urine concentration are associated with poor survival in patients with hepatorenal syndrome. *Ann Hepatol* 2013; **12**: 92-99 [PMID: 23293199]
  - 52 **Alessandria C**, Venon WD, Marzano A, Barletti C, Fadda M, Rizzetto M. Renal failure in cirrhotic patients: role of terlipressin in clinical approach to hepatorenal syndrome type 2. *Eur J Gastroenterol Hepatol* 2002; **14**: 1363-1368 [PMID: 12468959 DOI: 10.1097/00042737-200212000-00013]
  - 53 **Rodriguez E**, Henrique Pereira G, Solà E, Elia C, Barreto R, Pose E, Colmenero J, Fernandez J, Navasa M, Arroyo V, Ginès P. Treatment of type 2 hepatorenal syndrome in patients awaiting transplantation: Effects on kidney function and transplantation outcomes. *Liver Transpl* 2015; **21**: 1347-1354 [PMID: 26178066 DOI: 10.1002/lt.24210]
  - 54 **Testro AG**, Wongseelashote S, Angus PW, Gow PJ. Long-term outcome of patients treated with terlipressin for types 1 and 2 hepatorenal syndrome. *J Gastroenterol Hepatol* 2008; **23**: 1535-1540 [PMID: 17784863 DOI: 10.1111/j.1440-1746.2007.05176.x]
  - 55 **Tan HK**, Marquez M, Wong F, Renner EL. Pretransplant Type 2 Hepatorenal Syndrome Is Associated With Persistently Impaired Renal Function After Liver Transplantation. *Transplantation* 2015; **99**: 1441-1446 [PMID: 25643142 DOI: 10.1097/tp.0000000000000557]
  - 56 **Kiser TH**, Fish DN, Obritsch MD, Jung R, MacLaren R, Parikh CR. Vasopressin, not octreotide, may be beneficial in the treatment of hepatorenal syndrome: a retrospective study. *Nephrol Dial Transplant* 2005; **20**: 1813-1820 [PMID: 15956066 DOI: 10.1093/ndt/gfh930]
  - 57 **Skagen C**, Einstein M, Lucey MR, Said A. Combination treatment with octreotide, midodrine, and albumin improves survival in patients with type 1 and type 2 hepatorenal syndrome. *J Clin Gastroenterol* 2009; **43**: 680-685 [PMID: 19238094 DOI: 10.1097/MCG.0b013e318188947c]
  - 58 **Rice JP**, Skagen C, Said A. Liver transplant outcomes for patients with hepatorenal syndrome treated with pretransplant vasoconstrictors and albumin. *Transplantation* 2011; **91**: 1141-1147 [PMID: 21544034 DOI: 10.1097/TP.0b013e31821690bf]
  - 59 **Mulkay JP**, Louis H, Donckier V, Bourgeois N, Adler M, Deviere J, Le Moine O. Long-term terlipressin administration improves renal function in cirrhotic patients with type 1 hepatorenal syndrome: a pilot study. *Acta Gastroenterol Belg* 2001; **64**: 15-19 [PMID: 11322061]
  - 60 **Alessandria C**, Ottobrelli A, Debernardi-Venon W, Todros L, Cerenzia MT, Martini S, Balzola F, Morgando A, Rizzetto M, Marzano A. Noradrenalin vs terlipressin in patients with hepatorenal syndrome: a prospective, randomized, unblinded, pilot study. *J Hepatol* 2007; **47**: 499-505 [PMID: 17560680 DOI: 10.1016/j.jhep.2007.04.010]
  - 61 **Nassar Junior AP**, Farias AQ, D' Albuquerque LA, Carrilho FJ, Malbouisson LM. Terlipressin versus norepinephrine in the treatment of hepatorenal syndrome: a systematic review and meta-analysis. *PLoS One* 2014; **9**: e107466 [PMID: 25203311 DOI: 10.1371/journal.pone.0107466]
  - 62 **Cavallin M**, Kamath PS, Merli M, Fasolato S, Toniutto P, Salerno F, Bernardi M, Romanelli RG, Colletta C, Salinas F, Di Giacomo A, Ridola L, Fornasiere E, Caraceni P, Morando F, Piano S, Gatta A, Angeli P. Terlipressin plus albumin versus midodrine and octreotide plus albumin in the treatment of hepatorenal syndrome: A randomized trial. *Hepatology* 2015; **62**: 567-574 [PMID: 25644760 DOI: 10.1002/hep.27709]
  - 63 **Caraceni P**, Santi L, Mirici F, Montanari G, Bevilacqua V, Pinna AD, Bernardi M. Long-term treatment of hepatorenal syndrome as a bridge to liver transplantation. *Dig Liver Dis* 2011; **43**: 242-245 [PMID: 20833118 DOI: 10.1016/j.dld.2010.08.001]
  - 64 **Guevara M**, Ginès P, Bandi JC, Gilabert R, Sort P, Jiménez W, Garcia-Pagan JC, Bosch J, Arroyo V, Rodés J. Transjugular intrahepatic portosystemic shunt in hepatorenal syndrome: effects on renal function and vasoactive systems. *Hepatology* 1998; **28**: 416-422 [PMID: 9696006 DOI: 10.1002/hep.510280219]
  - 65 **Bresing KA**, Textor J, Strunk H, Klehr HU, Schild H, Sauerbruch T. Transjugular intrahepatic portosystemic stent-shunt for hepatorenal syndrome. *Lancet* 1997; **349**: 697-698 [PMID: 9078203 DOI: 10.1016/S0140-6736(97)24010-9]
  - 66 **Bresing KA**, Textor J, Perz J, Schiedermaier P, Raab P, Strunk H, Klehr HU, Kramer HJ, Spengler U, Schild H, Sauerbruch T. Long term outcome after transjugular intrahepatic portosystemic stent-shunt in non-transplant cirrhotics with hepatorenal syndrome: a phase II study. *Gut* 2000; **47**: 288-295 [PMID: 10896924 DOI: 10.1136/gut.47.2.288]
  - 67 **Berry K**, Lerrigo R, Liou IW, Ioannou GN. Association Between Transjugular Intrahepatic Portosystemic Shunt and Survival in Patients With Cirrhosis. *Clin Gastroenterol Hepatol* 2016; **14**: 118-123 [PMID: 26192147 DOI: 10.1016/j.cgh.2015.06.042]
  - 68 **Brown RS**, Lake JR. Transjugular intrahepatic portosystemic shunt as a form of treatment for portal hypertension: indications and contraindications. *Adv Intern Med* 1997; **42**: 485-504 [PMID: 9048128]
  - 69 **Di Campli C**, Santoro MC, Gaspari R, Merra G, Zileri Dal Verme L, Zocco MA, Piscaglia AC, Di Gioacchino G, Novi M, Santoliquido A, Flore R, Tondi P, Proietti R, Gasbarrini G, Pola P, Gasbarrini A. Catholic university experience with molecular adsorbent recycling

- system in patients with severe liver failure. *Transplant Proc* 2005; **37**: 2547-2550 [PMID: 16182739 DOI: 10.1016/j.transproceed.2005.06.048]
- 70 **Mitzner SR**, Klammt S, Peszynski P, Hickstein H, Korten G, Stange J, Schmidt R. Improvement of multiple organ functions in hepatorenal syndrome during albumin dialysis with the molecular adsorbent recirculating system. *Ther Apher* 2001; **5**: 417-422 [PMID: 11778928]
  - 71 **Stange J**, Hassanein TI, Mehta R, Mitzner SR, Bartlett RH. The molecular adsorbents recycling system as a liver support system based on albumin dialysis: a summary of preclinical investigations, prospective, randomized, controlled clinical trial, and clinical experience from 19 centers. *Artif Organs* 2002; **26**: 103-110 [PMID: 11879237 DOI: 10.1046/j.1525-1594.2002.06822.x]
  - 72 **Mitzner SR**, Stange J, Klammt S, Risler T, Erley CM, Bader BD, Berger ED, Lauchart W, Peszynski P, Freytag J, Hickstein H, Looock J, Lohr JM, Liebe S, Emmrich J, Korten G, Schmidt R. Improvement of hepatorenal syndrome with extracorporeal albumin dialysis MARS: results of a prospective, randomized, controlled clinical trial. *Liver Transpl* 2000; **6**: 277-286 [PMID: 10827226 DOI: 10.1002/lt.500060326]
  - 73 **Gaspari R**, Cavaliere F, Sollazzi L, Perilli V, Melchionda I, Agnes S, Gasbarrini A, Avolio AW. Molecular adsorbent recirculating system (Mars) in patients with primary nonfunction and other causes of graft dysfunction after liver transplantation in the era of extended criteria donor organs. *Transplant Proc* 2009; **41**: 253-258 [PMID: 19249528 DOI: 10.1016/j.transproceed.2008.10.066]
  - 74 **Yang YW**, Wu CH, Hu RH, Ho MC, Tsai MK, Wu YM, Lee PH. Longitudinal assessment of prognostic factors for patients with hepatorenal syndrome in a tertiary center. *Hepatol Int* 2010; **4**: 507-510 [PMID: 20827408 DOI: 10.1007/s12072-010-9180-8]
  - 75 **Sanchez EQ**, Gonwa TA, Levy MF, Goldstein RM, Mai ML, Hays SR, Melton LB, Saracino G, Klintmalm GB. Preoperative and perioperative predictors of the need for renal replacement therapy after orthotopic liver transplantation. *Transplantation* 2004; **78**: 1048-1054 [PMID: 15480173 DOI: 10.1097/01.TP.0000137176.95730.5B]
  - 76 **Goyal S**, Dixit VK, Jain AK, Shukla RC, Ghosh J, Kumar V. Intrarenal resistance index (RI) as a predictor of early renal impairment in patients with liver cirrhosis. *Trop Gastroenterol* 2013; **34**: 235-239 [PMID: 25046885]
  - 77 **Maddukuri G**, Cai CX, Munigala S, Mohammadi F, Zhang Z. Targeting an early and substantial increase in mean arterial pressure is critical in the management of type 1 hepatorenal syndrome: a combined retrospective and pilot study. *Dig Dis Sci* 2014; **59**: 471-481 [PMID: 24146317 DOI: 10.1007/s10620-013-2899-z]
  - 78 **Saxena V**, Lai JC. Kidney Failure and Liver Allocation: Current Practices and Potential Improvements. *Adv Chronic Kidney Dis* 2015; **22**: 391-398 [PMID: 26311601 DOI: 10.1053/j.ackd.2015.05.002]
  - 79 **Chang Y**, Gallon L, Shetty K, Chang Y, Jay C, Levitsky J, Ho B, Baker T, Ladner D, Friedewald J, Abecassis M, Hazen G, Skaro AI. Simulation modeling of the impact of proposed new simultaneous liver and kidney transplantation policies. *Transplantation* 2015; **99**: 424-430 [PMID: 25099700 DOI: 10.1097/tp.0000000000000270]
  - 80 **Campbell MS**, Kotlyar DS, Brensing CM, Lewis JD, Shetty K, Bloom RD, Markmann JF, Olthoff KM, Shaked A, Reddy KR. Renal function after orthotopic liver transplantation is predicted by duration of pretransplantation creatinine elevation. *Liver Transpl* 2005; **11**: 1048-1055 [PMID: 16123966 DOI: 10.1002/lt.20445]
  - 81 **Jeyarajah DR**, Gonwa TA, McBride M, Testa G, Abbasoglu O, Husberg BS, Levy MF, Goldstein RM, Klintmalm GB. Hepatorenal syndrome: combined liver kidney transplants versus isolated liver transplant. *Transplantation* 1997; **64**: 1760-1765 [PMID: 9422417]
  - 82 **Fong TL**, Khemichian S, Shah T, Hutchinson IV, Cho YW. Combined liver-kidney transplantation is preferable to liver transplant alone for cirrhotic patients with renal failure. *Transplantation* 2012; **94**: 411-416 [PMID: 22805440 DOI: 10.1097/TP.0b013e3182590d6b]
  - 83 **Ruiz R**, Kunitake H, Wilkinson AH, Danovitch GM, Farmer DG, Ghobrial RM, Yersiz H, Hiatt JR, Busuttil RW. Long-term analysis of combined liver and kidney transplantation at a single center. *Arch Surg* 2006; **141**: 735-741; discussion 741-742 [PMID: 16924080 DOI: 10.1001/archsurg.141.8.735]
  - 84 **Locke JE**, Warren DS, Singer AL, Segev DL, Simpkins CE, Maley WR, Montgomery RA, Danovitch G, Cameron AM. Declining outcomes in simultaneous liver-kidney transplantation in the MELD era: ineffective usage of renal allografts. *Transplantation* 2008; **85**: 935-942 [PMID: 18408571 DOI: 10.1097/TP.0b013e318168476d]
  - 85 **Mehrabi A**, Fonouni H, Ayoub E, Rahbari NN, Müller SA, Morath Ch, Seckinger J, Sadeghi M, Golriz M, Esmaeilzadeh M, Hillebrand N, Weitz J, Zeier M, Büchler MW, Schmidt J, Schmied BM. A single center experience of combined liver kidney transplantation. *Clin Transplant* 2009; **23** Suppl 21: 102-114 [PMID: 19930323 DOI: 10.1111/j.1399-0012.2009.01146.x]
  - 86 **Sharma P**, Goodrich NP, Zhang M, Guidinger MK, Schaubel DE, Merion RM. Short-term pretransplant renal replacement therapy and renal nonrecovery after liver transplantation alone. *Clin J Am Soc Nephrol* 2013; **8**: 1135-1142 [PMID: 23449770 DOI: 10.2215/CJN.09600912]
  - 87 **Sung RS**, Wiseman AC. Simultaneous Liver-Kidney Transplant: Too Many or Just Enough? *Adv Chronic Kidney Dis* 2015; **22**: 399-403 [PMID: 26311602 DOI: 10.1053/j.ackd.2015.06.005]
  - 88 **Martin EF**, Huang J, Xiang Q, Klein JP, Bajaj J, Saeian K. Recipient survival and graft survival are not diminished by simultaneous liver-kidney transplantation: an analysis of the united network for organ sharing database. *Liver Transpl* 2012; **18**: 914-929 [PMID: 22467623 DOI: 10.1002/lt.23440]
  - 89 **Catalano G**, Tandoi F, Mazza E, Simonato F, Tognarelli G, Biancone L, Lupo F, Romagnoli R, Salizzoni M. Simultaneous Liver-Kidney Transplantation in Adults: A Single-center Experience Comparing Results With Isolated Liver Transplantation. *Transplant Proc* 2015; **47**: 2156-2158 [PMID: 26361666 DOI: 10.1016/j.transproceed.2014.11.073]
  - 90 **Lafayette RA**, Paré G, Schmid CH, King AJ, Rohrer RJ, Nasraway SA. Pretransplant renal dysfunction predicts poorer outcome in liver transplantation. *Clin Nephrol* 1997; **48**: 159-164 [PMID: 9342487]
  - 91 **Gonwa TA**, Klintmalm GB, Levy M, Jennings LS, Goldstein RM, Husberg BS. Impact of pretransplant renal function on survival after liver transplantation. *Transplantation* 1995; **59**: 361-365 [PMID: 7871566]
  - 92 **Gonwa TA**, Morris CA, Goldstein RM, Husberg BS, Klintmalm GB. Long-term survival and renal function following liver transplantation in patients with and without hepatorenal syndrome--experience in 300 patients. *Transplantation* 1991; **51**: 428-430 [PMID: 1994538]
  - 93 **Seu P**, Wilkinson AH, Shaked A, Busuttil RW. The hepatorenal syndrome in liver transplant recipients. *Am Surg* 1991; **57**: 806-809 [PMID: 1746799]
  - 94 **Park I**, Moon E, Hwang JA, Yu S, Kim BW, Wang HJ, Shin GT, Kim H. Does hepatorenal syndrome affect the result of liver transplantation? Clinical observations. *Transplant Proc* 2010; **42**: 2563-2566 [PMID: 20832544 DOI: 10.1016/j.transproceed.2010.04.049]
  - 95 **Junge G**, Schewior LV, Kohler S, Neuhaus R, Langrehr JM, Tullius S, Kahl A, Frei U, Neuhaus P. Acute renal failure after liver transplantation: incidence, etiology, therapy, and outcome. *Transplant Proc* 2006; **38**: 723-724 [PMID: 16647455 DOI: 10.1016/j.transproceed.2006.01.074]
  - 96 **López Lago AM**, Fernández Villanueva J, García Acuña JM, Paz ES, Vizoso EF, Pérez EV. Evolution of hepatorenal syndrome after orthotopic liver transplantation: comparative analysis with patients who developed acute renal failure in the early postoperative period of liver transplantation. *Transplant Proc* 2007; **39**: 2318-2319 [PMID: 17889176 DOI: 10.1016/j.transproceed.2007.07.070]
  - 97 **Sato K**, Kawagishi N, Fujimori K, Ohuchi N, Satomi S. Renal function status in liver transplant patients in the first month post-transplant is associated with progressive chronic kidney disease. *Hepatol Res* 2015; **45**: 220-227 [PMID: 24698087 DOI: 10.1111/hepr.12339]
  - 98 **Cohen AJ**, Stegall MD, Rosen CB, Wiesner RH, Leung N, Kremers WK, Zein NN. Chronic renal dysfunction late after liver transplantation. *Liver Transpl* 2002; **8**: 916-921 [PMID: 12360433]

- DOI: 10.1053/jlts.2002.35668]
- 99 **Longenecker JC**, Estrella MM, Segev DL, Atta MG. Patterns of Kidney Function Before and After Orthotopic Liver Transplant: Associations With Length of Hospital Stay, Progression to End-Stage Renal Disease, and Mortality. *Transplantation* 2015; **99**: 2556-2564 [PMID: 25989501 DOI: 10.1097/tp.0000000000000767]
  - 100 **Weigand K**, Bauer E, Encke J, Schmidt J, Stremmel W, Schwenger V. Prognostic value of standard parameters as predictors for long-term renal replacement therapy after liver transplantation. *Nephron Clin Pract* 2011; **119**: c342-c347 [PMID: 22135794]
  - 101 **Sethi A**, Estrella MM, Ugarte R, Atta MG. Kidney function and mortality post-liver transplant in the Model for End-Stage Liver Disease era. *Int J Nephrol Renovasc Dis* 2011; **4**: 139-144 [PMID: 22163170 DOI: 10.2147/ijnrd.s24812]
  - 102 **Restuccia T**, Ortega R, Guevara M, Ginès P, Alessandria C, Ozdogan O, Navasa M, Rimola A, Garcia-Valdecasas JC, Arroyo V, Rodés J. Effects of treatment of hepatorenal syndrome before transplantation on posttransplantation outcome. A case-control study. *J Hepatol* 2004; **40**: 140-146 [PMID: 14672625]
  - 103 **Wong F**, Leung W, Al Beshir M, Marquez M, Renner EL. Outcomes of patients with cirrhosis and hepatorenal syndrome type 1 treated with liver transplantation. *Liver Transpl* 2015; **21**: 300-307 [PMID: 25422261 DOI: 10.1002/lt.24049]
  - 104 **Ikegami T**, Shirabe K, Soejima Y, Taketomi A, Yoshizumi T, Uchiyama H, Harada N, Maehara Y. The impact of renal replacement therapy before or after living donor liver transplantation. *Clin Transplant* 2012; **26**: 143-148 [PMID: 21447144 DOI: 10.1111/j.1399-0012.2011.01450.x]
  - 105 **Jain A**, Reyes J, Kashyap R, Dodson SF, Demetris AJ, Ruppert K, Abu-Elmagd K, Marsh W, Madariaga J, Mazariegos G, Geller D, Bonham CA, Gayowski T, Cacciarelli T, Fontes P, Starzl TE, Fung JJ. Long-term survival after liver transplantation in 4,000 consecutive patients at a single center. *Ann Surg* 2000; **232**: 490-500 [PMID: 10998647]
  - 106 **Davis CL**, Gonwa TA, Wilkinson AH. Pathophysiology of renal disease associated with liver disorders: implications for liver transplantation. Part I. *Liver Transpl* 2002; **8**: 91-109 [PMID: 11862584 DOI: 10.1053/jlts.2002.31516]
  - 107 A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. The U.S. Multicenter FK506 Liver Study Group. *N Engl J Med* 1994; **331**: 1110-1115 [PMID: 7523946 DOI: 10.1056/nejm199410273311702]
  - 108 **Distant DA**, Gonwa TA. The kidney in liver transplantation. *J Am Soc Nephrol* 1993; **4**: 129-136 [PMID: 7691205]
  - 109 **Gonwa TA**, Mai ML, Melton LB, Hays SR, Goldstein RM, Levy MF, Klintmalm GB. End-stage renal disease (ESRD) after orthotopic liver transplantation (OLT) using calcineurin-based immunotherapy: risk of development and treatment. *Transplantation* 2001; **72**: 1934-1939 [PMID: 11773892]
  - 110 **de Mattos AM**, Olyaei AJ, Bennett WM. Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. *Am J Kidney Dis* 2000; **35**: 333-346 [PMID: 10676738]
  - 111 **Schlitt HJ**, Barkmann A, Böker KH, Schmidt HH, Emmanouilidis N, Rosenau J, Bahr MJ, Tusch G, Manns MP, Nashan B, Klempnauer J. Replacement of calcineurin inhibitors with mycophenolate mofetil in liver-transplant patients with renal dysfunction: a randomised controlled study. *Lancet* 2001; **357**: 587-591 [PMID: 11558484]
  - 112 **Cicinnati VR**, Yu Z, Klein CG, Sotiropoulos GC, Saner F, Malagó M, Frilling A, Gerken G, Broelsch CE, Beckebaum S. Clinical trial: switch to combined mycophenolate mofetil and minimal dose calcineurin inhibitor in stable liver transplant patients--assessment of renal and allograft function, cardiovascular risk factors and immune monitoring. *Aliment Pharmacol Ther* 2007; **26**: 1195-1208 [PMID: 17944734 DOI: 10.1111/j.1365-2036.2007.03466.x]
  - 113 **Hadengue A**, Gadano A, Moreau R, Giostra E, Durand F, Valla D, Erlinger S, Lebrec D. Beneficial effects of the 2-day administration of terlipressin in patients with cirrhosis and hepatorenal syndrome. *J Hepatol* 1998; **29**: 565-570 [PMID: 9824265]
  - 114 **Chava SP**, Singh B, Stangou A, Battula N, Bowles M, O'Grady J, Rela M, Heaton ND. Simultaneous combined liver and kidney transplantation: a single center experience. *Clin Transplant* 2010; **24**: E62-E68 [PMID: 20618811 DOI: 10.1111/j.1399-0012.2010.01168.x]

**P-Reviewer:** Boin IFSF, Coban M **S-Editor:** Ji FF  
**L-Editor:** A **E-Editor:** Li D



## Rethinking the role of non-selective beta blockers in patients with cirrhosis and portal hypertension

Alberto Ferrarese, Alberto Zanetto, Giacomo Germani, Patrizia Burra, Marco Senzolo

Alberto Ferrarese, Alberto Zanetto, Giacomo Germani, Patrizia Burra, Marco Senzolo, Multivisceral Transplant Unit, Department of Surgery, Oncology and Gastroenterology, Padua University Hospital, 35128 Padua, Italy

**Author contributions:** Ferrarese A wrote and reviewed the manuscript; Zanetto A and Germani G edited the manuscript; Burra P and Senzolo M supervised, drafted and reviewed the manuscript.

**Conflict-of-interest statement:** The authors do not have anything to disclose about this paper. The authors did not receive any funding for producing the manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Marco Senzolo, MD, PhD, Multivisceral Transplant Unit, Department of Surgery, Oncology and Gastroenterology, Padua University Hospital, via Giustiniani 2, 35128 Padua, Italy. [marcosenzolo@hotmail.com](mailto:marcosenzolo@hotmail.com)  
**Telephone:** +39-04-98218726  
**Fax:** +39-04-98218727

**Received:** April 30, 2016

**Peer-review started:** May 3, 2016

**First decision:** June 17, 2016

**Revised:** July 1, 2016

**Accepted:** July 14, 2016

**Article in press:** July 18, 2016

**Published online:** August 28, 2016

### Abstract

Non-selective beta blockers (NSBB) are commonly used to prevent portal hypertensive bleeding in cirrhotics.

Nevertheless, in the last years, the use of NSBB in critically decompensated patients, especially in those with refractory ascites, has been questioned, mainly for an increased risk of mortality and worsening of systemic hemodynamics. Moreover, even if NSBB have been reported to correlate with a higher risk of renal failure and severe infection in patients with advanced liver disease and hypotension, their use has been associated with a reduction of risk of spontaneous bacterial peritonitis, modification of gut permeability and reduction of bacterial translocation. This manuscript systematically reviews the published evidences about harms and benefits of the use of NSBB in patients with decompensated cirrhosis.

**Key words:** Beta blockers; Ascites; Cirrhosis; Portal hypertension

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In this review, we've critically analyzed the recent evidence on the role played by non-selective beta blockers in patients with decompensated liver disease.

Ferrarese A, Zanetto A, Germani G, Burra P, Senzolo M. Rethinking the role of non-selective beta blockers in patients with cirrhosis and portal hypertension. *World J Hepatol* 2016; 8(24): 1012-1018 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i24/1012.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i24.1012>

### INTRODUCTION

Cirrhosis is among the leading causes of death worldwide and hepatocellular carcinoma and complications of portal hypertension (PH) represent the most frequent causes of death.

PH is characterized by a systemic hyperdynamic



circulation, with increase of cardiac output (CO) and heart rate (HR), and reduction of mean arterial pressure (MAP) and systemic vascular resistances<sup>[1]</sup>. The degree of PH correlates with the severity of hyperdynamic circulation, while the absence of hemodynamic imbalance (*i.e.*, preserved right heart preload) is associated with better prognosis<sup>[2]</sup>.

Ascites, esophageal varices, encephalopathy and/or jaundice are the main features of decompensated cirrhosis. Ascites represents the first clinical sign of decompensation in 30%-50% of patients, being the incidence about 50% within 10 years<sup>[3]</sup>. Refractory ascites occurs in 5% to 10% of cases, leading to a significant shortening in survival<sup>[4]</sup>. Oesophageal varices occur in about 50% of cirrhotic patients<sup>[5]</sup> being the incidence of first variceal bleeding estimated to be about 12%-15% per year, and the mortality of 15%-20% for every episode<sup>[6]</sup>. Varices mainly develop due to increased PH, but Fernandez *et al.*<sup>[7]</sup> reported that their formation was also modulated by active angiogenesis, and not by a simple mechanism of vasodilation.

Moreover, several external factors, such surgery, bacterial infections or bleeding, represent severe trigger factors for derangement of hemodynamic; for instance, infection seemed more frequent in those patients who developed an acute-on-chronic liver failure (32.6% vs 21.8%,  $P < 0.01$ )<sup>[8]</sup>. Phillip *et al.*<sup>[9]</sup> showed that removal of > 5 L of ascites determined a significant reduction of MAP and SVR, which is usually associated with a counterbalancing increase of CO<sup>[10]</sup>. The hemodynamic imbalance after LVP led to an increased risk of renal dysfunction, and subsequently to an increased mortality, according to the well-defined Paracentesis Induced Circulatory Dysfunction (PICD)<sup>[11]</sup>.

Heart dysfunction has been shown in decompensated cirrhosis<sup>[12]</sup>, being caused both by organic (*i.e.*, alcoholic or septic cardiomyopathy) and/or functional [*i.e.*, cirrhotic cardiomyopathy (CM)] factors. CM is mainly due to chronic increase of pro-inflammatory cytokines, impairment of systemic and regional hemodynamic, and beta-adrenergic receptor desensitization, with reversible impairment of systolic contractility, diastolic function and electrophysiological activity<sup>[1,13]</sup>. The impaired CO may also contribute to a decrease in renal perfusion: For instance, Krag *et al.*<sup>[14]</sup> demonstrated that a lower cardiac index was associated with an increased development of hepatorenal syndrome within 3 mo (43% vs 5%,  $P = 0.04$ ). Although it's difficult to determine the prevalence of CM since it's usually masked at rest, it could be an important cause of multi-organ failure and death during stressing conditions, as infection or liver transplantation<sup>[15]</sup>.

## ROLE OF NON-SELECTIVE BETA BLOCKERS IN THE TREATMENT OF PH

### **Non-selective beta blockers and variceal bleeding**

Non-selective beta blockers (NSBB) act reducing portal

flow and PH by decreasing CO (through  $\beta_1$  receptors) and determining splanchnic vasoconstriction (through  $\beta_2$  receptors)<sup>[16]</sup>. In 1981 Lebrec *et al.*<sup>[17]</sup> demonstrated for the first time the effectiveness of NSBB for variceal bleeding; the re-bleeding rate was 4% in the treated group, compared to 50% in the placebo group.

Several randomized studies confirmed that NSBB represent the preferred option in primary prophylaxis against no intervention<sup>[18]</sup> and in preventing re-bleeding in combination with endoscopic band ligation<sup>[19]</sup>. Furthermore, a Cochrane metanalysis<sup>[20]</sup> confirmed that NSBB were as effective as endoscopic band ligation for reducing bleeding related mortality [29/567 (5.1%) vs 37/585 (6.3%); RR = 0.85; 95%CI: 0.53 to 1.39].

However, identification of hemodynamic response to NSBB still remains challenging for the hepatologists. Heebøll *et al.*<sup>[21]</sup> demonstrated that only 51/124 (40%) of patients with cirrhosis who underwent measurement of gradient between portal and hepatic veins (HVPG) presented a significant hemodynamic improvement (reduction greater than 20% or > 12 mmHg) after NSBB use. Moreover, authors did not demonstrate a significant association between improvement of HVPG and change of HR ( $P = 0.8$ ), which is commonly used parameter to tailor propranolol therapy.

Importantly, all the trials often ruled out cirrhotics with decompensated liver disease (*i.e.*, those with refractory ascites) from the analysis.

## NSBBS IN DECOMPENSATED CIRRHOTICS

Serstè *et al.*<sup>[22]</sup> showed for the first time in 2010 that the median survival was extremely reduced in 151 patients with cirrhosis and refractory ascites treated with propranolol (20.0 mo vs 5.0 mo;  $P = 0.00001$ ); other factors associated with higher mortality were Child-Pugh class C, hyponatremia and renal failure. These data raised several concerns amongst hepatologists<sup>[23-25]</sup> about the use of NSBB in cirrhotics with more advanced liver disease.

First, the group receiving NSBB comprises obviously sicker patients, because of higher prevalence of oesophageal varices (77/77 vs 3/74;  $P = 0.001$ ) and higher serum bilirubin (56 mg/dL vs 48 mg/dL,  $P = 0.01$ ). Second, the propranolol dose of 160 mg/d was significantly higher (in about half of the patients) than the mean dose used in the previous RCTs. Third, mortality was extremely higher in the NSBB group (63/77, 85.1%, median survival time was 5 mo), and there was an increased prevalence of sepsis related mortality, which remain difficult to explain<sup>[25]</sup>.

The French group hypothesized that NSBB use can worsen hemodynamic after LVP; thus, reduced survival could be due to an increased incidence of PICD. A cross-over study published in 2011<sup>[26]</sup> including 10 patients with refractory ascites, investigated the incidence of PICD after LVP when patients were taking NSBB and after

drug discontinuation. The authors showed that PICD was extremely decreased after propranolol discontinuation (1/10 vs 8/10;  $P = 0.01$ ). The hypothesis was that propranolol use determined a reduction of CO and consequently an increase of counter-regulatory vasoconstriction systems, as renin angiotensin aldosterone, whose permanent hyper-activation could be associated with poorer renal function and reduced paracentesis-free interval time.

The link between NSBB and hemodynamic impairment was explained with the reduced MAP, which is a known negative prognostic factor for hyperdynamic circulation and progression of liver disease<sup>[27]</sup>. For instance, in the French study by Serstè *et al.*<sup>[22]</sup>, the cohort receiving propranolol did have lower MAP (90 mmHg vs 83 mmHg). Nevertheless, NSBB have been shown not to reduce MAP after acute *i.v.* administration<sup>[28]</sup>, and the detrimental effects which were seen by the authors could have been due to the dose related side effect made by propranolol. CO is not usually reduced by NSBB introduction<sup>[29]</sup>.

The following clinical studies failed to find any association between the use of NSBB and increased risk of deaths in decompensated cirrhotics (Table 1). Leithead *et al.*<sup>[30]</sup> analyzed a subgroup of 117 patients with refractory ascites listed for LT, receiving a median dose of propranolol of 80 mg/d. They demonstrated that NSBB were independently associated with reduced waitlist death (adjusted HR = 0.35,  $P = 0.022$ ), without higher prevalence of sepsis related mortality. Moreover, an equal survival between patients with refractory ascites taking NSBB and patients without NSBB (12/38 vs 8/23;  $P = 0.79$ ) was shown in another smaller single center retrospective analysis<sup>[31]</sup>.

Bossen *et al.*<sup>[32]</sup> not only confirmed similar mid-term mortality between 258 patients with refractory ascites receiving NSBB and a control group of 330 patients (30.8% vs 30.5%; adjusted HR = 1.02, 95%CI: 0.74-1.39) retrospectively evaluated, but also showed that discontinuation of NSBB was associated with an higher mortality (adjusted HR = 5.13, 95%CI: 2.28-11.55).

In addition, new data seemed to confirm the absence of correlation between mortality and NSBB. Pereira *et al.*<sup>[33]</sup> included 163 patients with infection, of whom 104 were on NSBB. Use of NSBB was associated with lower frequency of sepsis (21% vs 42%,  $P = 0.03$ ), being 3-mo survival not different between cohorts (59% vs 63%;  $P = \text{ns}$ ). Mallawaarachchi *et al.*<sup>[34]</sup> showed that 75 patients treated with NSBB (67 with carvedilol and 8 propranolol) presented equal mortality after a median follow-up time of 28.0 mo (60.0% vs 66.7%;  $P = 0.10$ ); in those with moderate or severe ascites, survival was similar in both groups ( $P = 0.67$ ), while it was better in NSBB patients in mild ascites ( $P = 0.02$ ).

In a large multicentric cohort, Bhutta *et al.*<sup>[35]</sup> confirmed that survival was significantly greater in patients on NSBB at admission with a median survival of 58 d compared to 32 d in patients not on NSBB ( $P = 0.033$ ). No difference was found between those who did or did

not discontinue NSBB ( $P = 0.91$ ), being only systolic arterial pressure and acute renal failure independent predictors of death.

Onali *et al.*<sup>[36]</sup> evaluating 316 patients (126 with refractory ascites), showed that those on NSBB ( $n = 128$ , 40.5%) had a higher frequency of previous variceal bleeding (50% vs 21%,  $P < 0.001$ ) and spontaneous bacterial peritonitis (27% vs 17%,  $P = 0.025$ ), but were at lower risk of death (16% vs 32%;  $P = 0.002$ ). At multivariate analysis use of NSBB was associated with reduced mortality (HR = 0.511, 95%CI: 0.3-0.87,  $P = 0.014$ ).

Finally, in a recent study provided on 349 acute-on chronic patients with cirrhosis, Mookerjee *et al.*<sup>[37]</sup> demonstrated a significantly lower short term mortality in patients on NSBB compared to those without NSBB (24% vs 34%,  $P = 0.048$ ). Interestingly, patients on NSBB had less severe progression to the stages of acute-on-chronic liver failure, and those who discontinued NSBB had a higher mortality (37% vs 13%), even if it might be due to an independently higher presence of circulatory dysfunction.

The association between increased mortality and NSBB could be explained with the worsening of an already impaired hemodynamics, especially in those who experience a greater decrease of cardiac function (*i.e.*, of CO) and of MAP. However, in the study by Karagiannakis *et al.*<sup>[15]</sup> in which the decrease of CO (and subsequently of cardiac index) has been correlated with a lower survival, the used cut-off (1.5 L/m per square meter) is not diffusely seen in cirrhotics, even when decompensated<sup>[38]</sup>.

Simultaneous presence of several cofactors, as infection, could contribute to the change of clinical scenario, being patients at higher risk of hemodynamic derangement if NSBB are not withdrawn.

Mandorfer *et al.*<sup>[39]</sup> showed that 245 patients with refractory ascites but without infection, taking NSBB, experienced a significant reduction in hospitalization rate (19.4 d vs 23.9 d per person-year); at multivariate analysis, NSBB treatment correlated with higher transplant-free survival (HR = 0.771; 95%CI: 0.598-0.993;  $P = 0.04$ ). The Authors demonstrated a correlation between mortality and NSBB only in patients experiencing a previous episode of spontaneous bacterial peritonitis (SBP), with a significant difference in length of hospitalization (NSBB: 33.4 d per person-year; 95%CI: 31.9-34.9 vs no-NSBB: 28.8 d per person-year; 95%CI: 27.6-29.9), and impaired transplant-free survival (HR = 1.644; 95%CI: 1.145-2.361). These data may confirm that NSBB could negatively influence hemodynamic status in patients with infection, but not that NSBB represented a trigger for infection.

However, Galbois *et al.*<sup>[40]</sup> showed that cirrhotics admitted to intensive care unit for sepsis or septic shock who were receiving NSBB were not at increased risk of early or mid-term mortality (15/26 vs 26/42,  $P = 0.8$ ; and 21/26 vs 28/42;  $P = 0.27$ , respectively).

In summary, latest studies seem not to confirm correlation between NSBB and mortality. Another meta-

**Table 1** Available literature on the potential correlation between non-selective beta blockers and mortality in patients with cirrhosis

Ref.	Patients	Refractory ascites	Propranolol dose/day	Follow-up	Mortality	Sepsis
Serstè <i>et al</i> <sup>[22]</sup>	74	100%	40 mg (9); 80 mg (31); 120 mg (1); 160 mg (36)	8 mo	63/77 ( $P < 0.0001$ vs No NSBB)	NA
Galbois <i>et al</i> <sup>[40]</sup>	26	14 (53.8%)	NA	6 mo	21/26 (80.8%)	100%
Robins <i>et al</i> <sup>[60]</sup>	36	100%	48.9	10 mo	18/36 (50%) survival 18 mo	NA
Mandorfer <i>et al</i> <sup>[39]</sup>	245	100%	40 mg (20-120)	660 persons/year	Higher transplant free survival (HR = 0.771, $P = 0.044$ )	No correlation between NSBB and SBP (HR = 0.728, $P$ = 0.211)
Kimer <i>et al</i> <sup>[31]</sup>	23	100%	80 mg (40-200)	Retrospective	15/23 (65.2%)	NA
Leithhead <i>et al</i> <sup>[30]</sup>	159 (119 on propranolol)	NA	80 mg (10-240)	Retrospective	35/159 (22%)	NA
Bossen <i>et al</i> <sup>[32]</sup>	559	46%	NA	12 mo	125/559 (22.5%)	NA
Mookerjee <i>et al</i> <sup>[37]</sup>	164 (propranolol 111; nadolol 6; carvedilol 16; other 31)	NA	40 (20-80; propranolol)	NA	40/164 vs 63/184 (24.4% vs 34.1%, $P =$ 0.048) Similar 6 and 12-mo mortality between groups ( $P = 0.64$ and 0.35 respectively)	NA
Pereira <i>et al</i> <sup>[33]</sup>	104	NA	NA	NA	67% vs 69% ( $P =$ ns)	21% vs 42% ( $P =$ ns)
Mallawaarachchi <i>et al</i> <sup>[34]</sup>	75 (8 propranolol)	NA	NA	28 mo	60% vs 66% ( $P =$ ns)	NA
Bhutta <i>et al</i> <sup>[35]</sup>	308 (nadolol 155; propranolol 64; carvedilol 72, other 62)	NA	NA	NA	Mean survival: 58 d in NSBB group (vs 32 d of control group; $P$ = 0.033)	NA
Onali <i>et al</i> <sup>[36]</sup>	126	100%	NA	4 mo	20 vs 60 (16% vs 32%; $P = 0.002$ )	NA

NA: Not available; NSBB: Non-selective beta blockers; SBP: Spontaneous bacterial peritonitis; ns: No significance.

analysis<sup>[41]</sup>, which comprised 23 and 28 RCTs on primary and secondary prophylaxis for variceal bleeding, for a total of 4481 patients included (39.8% with ascites), extensively confirmed the absence of increased mortality for patients on NSBB. In primary prophylaxis, 215/955 patients died for bleeding-unrelated causes, in a proportion not different between those who were or were not on treatment with NSBB (OR = 0.91, 95%CI: 0.73-1.15). Similarly, in secondary prophylaxis RCTs, bleeding-unrelated deaths did not differ between groups (189/1143 vs 225/1208; OR = 0.90, 95%CI: 0.67-1.23). These data were confirmed in the subgroup taking 120 mg/d or more of propranolol (48/374 vs 57/309, OR = 1.01, 95%CI: 0.55-1.84), and in those with severe ascites (124/595 vs 151/627, OR = 0.93, CI: 0.61-1.43).

## SECOND GENERATION OF BETA BLOCKERS: CARVEDILOL

Carvedilol is a NSBB with mild anti- $\alpha$ 1-adrenergic activity. It has been shown to be more effective than propranolol in reducing HVPg due to the  $\alpha$ -1 blockage, which reduces intra-hepatic resistances. Its role was investigated for the first time more than 20 years ago<sup>[42]</sup>, as a potential tool for reducing PH in patients with cirrhosis, with promising results. Since then, several studies demonstrated its effectiveness in terms of HVPg decrease, after acute administration and after chronic treatment<sup>[43]</sup>.

In 2002, Bañares *et al*<sup>[44]</sup> demonstrated that 26

patients receiving carvedilol experienced a greater reduction of HVPg than 25 patients taking propranolol ( $-19\% \pm 2\%$  vs  $-12\% \pm 2\%$ ;  $P < 0.001$ ); the decrease of HVPg was higher in patients with more severe liver disease (Child-Pugh class B and C vs Child-Pugh class A:  $-25\% \pm 2\%$  vs  $-14\% \pm 3\%$  respectively).

Previous studies showed that, in patients with cirrhosis, acute administration of carvedilol could enhance hypotension and effective hypovolemia, reducing renal blood flow and consequently glomerular filtration rate. In the study by Bañares *et al*<sup>[44]</sup>, renal function remained stable (glomerular filtration rate from 90 mL/min  $\pm$  4 mL/min to 84 mL/min  $\pm$  5 mL/min;  $P =$  ns) in both groups, suggesting a potential chronic hemodynamic adjustment in response to arterial hypotension. Furthermore, the authors confirmed that reductions of HR and CO were lower with carvedilol than with propranolol. However, MAP was significantly reduced only in the carvedilol group (91.4 mmHg  $\pm$  2.5 mmHg vs 81.2 mmHg  $\pm$  2.9 mmHg;  $P < 0.05$ ; propranolol: 88.6 mmHg  $\pm$  4.5 mmHg vs 83.8 mmHg  $\pm$  3.1 mmHg;  $P =$  ns). Thus, despite promising data, the use of carvedilol as first choice drug remains controversial<sup>[19]</sup>, especially in those patients with severely impairment of hemodynamic (*i.e.*, refractory ascites), because further reduction of MAP could be detrimental for organ perfusion. In fact in a recent metanalysis<sup>[45]</sup> on 5 studies which analyzed the role of carvedilol in a total of 90 patients, the number of patients achieving a reduction in HVPg to  $\geq 20\%$  was markedly higher with carvedilol (57/94 vs 33/87), but hypotension occurred in one-third

more patients than with propranolol.

## NON-HEMODYNAMIC EFFECTS OF NSBBS IN PH

Several pleiotropic effects of NSBB have been recently demonstrated beyond their hemodynamic role<sup>[46]</sup>.

In 2003 Abrales *et al.*<sup>[47]</sup> compared the incidence of complications due to PH in 28 patients responders to NSBB; after a follow-up of 8 years, they found that the risk of developing ascites ( $P = 0.025$ ), hepatorenal syndrome ( $P = 0.026$ ), and encephalopathy ( $P = 0.024$ ) were significantly lower than in the 45 patients non-responders. Another study of Hernández-Gea *et al.*<sup>[48]</sup> demonstrated that an effective treatment (*i.e.*, significant reduction of HVP) with NSBB for primary prophylaxis was associated with reduced risk of ascites development (19% vs 57% at 3 years,  $P < 0.001$ ).

Since bacterial translocation has been widely considered an important trigger factor for worsening of PH, also for the lack of response of immune system in cirrhosis<sup>[49]</sup>, and since selective bacterial decontamination seems to partly reverse the hemodynamic derangement in cirrhosis<sup>[50]</sup>, several studies tried to investigate whether NSBB could contribute to PH reduction through a modification of the protean interactions between the gut and the liver.

Propranolol seems to play a role in reduction of bacterial translocation, probably increasing bowel motility through a sympatholytic action<sup>[51]</sup>. After the confirmation that intestinal permeability was significantly impaired in cirrhotic than in controls (lactulose/mannitol ratio: 0.026 vs 0.014,  $P = 0.001$ ); we demonstrated that NSBB introduction determined a significant improvement of intestinal permeability, and reduction of hyper-vascularization at confocal microscopy<sup>[52]</sup>. Also Reiberger *et al.*<sup>[53]</sup> showed a reduction of intestinal permeability after introduction of NSBB, and a contemporary reduction of bacterial translocation [LPS-binding protein: -16% ( $P = 0.018$ ); interleukin-6: -41% ( $P < 0.0001$ )]; interestingly, the Authors showed equal effectiveness also in those whose HVP did not significantly reduced after NSBB introduction.

Although a retrospective study on 134 patients with cirrhosis and ascites<sup>[54]</sup> did not show a reduction of SBP during therapy with NSBB (6/33 vs 33/101; OR = 0.46,  $P = 0.17$ ), a meta-analysis performed on 4 studies demonstrated a significant difference (12.1%,  $P < 0.001$ ) in favor of propranolol in preventing SBP<sup>[55]</sup>.

Bacterial translocation is the main trigger factor for infection in cirrhosis, and infection is a known trigger for variceal bleeding<sup>[46]</sup>. Merli *et al.*<sup>[56]</sup> demonstrated that in 140 patients with cirrhosis who experienced infection, those on NSBB showed a trend towards a lower incidence of sepsis (40% vs 57%), septic shock (8% vs 15%), hepatorenal syndrome (14% vs 17%) and mortality (15% vs 40%).

## CONCLUSION AND FUTURE PERSPECTIVES

To date, NSBB remain the treatment of choice for primary and secondary prophylaxis for portal hypertensive bleeding, even though new drugs, as statins<sup>[57]</sup>, or new generation beta blockers, as carvedilol, may increase the rate of hemodynamic response. NSBB use has been associated with several pleiotropic characteristics, *i.e.*, reduction of bacterial translocation, prevention of spontaneous bacterial peritonitis - different from prevention of bleeding, suggesting a pleiotropic role in decompensated cirrhosis. Contrasting data on the use of NSBB in sickest patients with decompensated cirrhosis made their use controversial. A recent survey<sup>[58]</sup> about 629 physicians highlighted the high heterogeneity across centers. For instance, refractory ascites was considered a contraindication to NSBB use for 36% of responders, while for the 61% NSBB have to be withdrawn during HRS, highlighting a general lack of consensus across all the issues of the survey. A window hypothesis for therapy with NSBB in the natural history of cirrhosis was made by Krag *et al.*<sup>[59]</sup>; according to this view, NSBB could play a detrimental role for cirrhotics at the earlier stage (*i.e.*, for pre-primary prophylaxis) and in the "extremely decompensated" phase, in those patients with MAP lower than 80 mmHg, decreased baseline CO of those with concomitant infections<sup>[19]</sup>.

Since infected cirrhotics are those at greater risk of variceal bleeding and HVP has been increased also after the resolution of infection<sup>[38]</sup>, attention should be paid to a potential increase in the risk of portal hypertensive bleeding. In addition, the interplay between propranolol and sepsis has to be further investigated with future larger studies.

## REFERENCES

- 1 Møller S, Henriksen JH. Cirrhotic cardiomyopathy. *J Hepatol* 2010; **53**: 179-190 [PMID: 20462649 DOI: 10.1016/j.jhep.2010.02.023]
- 2 Møller S, Hobolth L, Winkler C, Bendtsen F, Christensen E. Determinants of the hyperdynamic circulation and central hypovolaemia in cirrhosis. *Gut* 2011; **60**: 1254-1259 [PMID: 21504996 DOI: 10.1136/gut.2010.235473]
- 3 Pessione F, Ramond MJ, Peters L, Pham BN, Batel P, Rueff B, Valla DC. Five-year survival predictive factors in patients with excessive alcohol intake and cirrhosis. Effect of alcoholic hepatitis, smoking and abstinence. *Liver Int* 2003; **23**: 45-53 [PMID: 12640727 DOI: 10.1034/j.1600-0676.2003.01804.x]
- 4 EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]
- 5 D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]
- 6 Garcia-Tsao G, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology* 2007; **46**: 922-938 [PMID: 17879356 DOI: 10.1002/hep.21907]
- 7 Fernandez M, Vizzutti F, Garcia-Pagan JC, Rodes J, Bosch J. Anti-VEGF receptor-2 monoclonal antibody prevents portal-



- systemic collateral vessel formation in portal hypertensive mice. *Gastroenterology* 2004; **126**: 886-894 [PMID: 14988842]
- 8 **Moreau R**, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, Durand F, Gustot T, Saliba F, Domenicali M, Gerbes A, Wendon J, Alessandria C, Laleman W, Zeuzem S, Trebicka J, Bernardi M, Arroyo V. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; **144**: 1426-1437, 1437.e1-e9 [PMID: 23474284 DOI: 10.1053/j.gastro.2013.02.042]
- 9 **Phillip V**, Saugel B, Ernesti C, Hapfelmeier A, Schultheiß C, Thies P, Mayr U, Schmid RM, Huber W. Effects of paracentesis on hemodynamic parameters and respiratory function in critically ill patients. *BMC Gastroenterol* 2014; **14**: 18 [PMID: 24467993 DOI: 10.1186/1471-230X-14-18]
- 10 **Sagarad SV**, Chawla YK, Dhiman RK. Portal hemodynamics after large-volume paracentesis in patients with liver cirrhosis and tense ascites. *Dig Dis Sci* 1998; **43**: 2470-2472 [PMID: 9824136]
- 11 **Ginès A**, Fernández-Esparrach G, Monescillo A, Vila C, Domènech E, Abecasis R, Angeli P, Ruiz-Del-Arbol L, Planas R, Solà R, Ginès P, Terg R, Inglada L, Vaqué P, Salerno F, Vargas V, Clemente G, Quer JC, Jiménez W, Arroyo V, Rodés J. Randomized trial comparing albumin, dextran 70, and polygeline in cirrhotic patients with ascites treated by paracentesis. *Gastroenterology* 1996; **111**: 1002-1010 [PMID: 8831595 DOI: 10.1016/S0016-5085(96)70068-9]
- 12 **Farr M**, Schulze PC. Recent advances in the diagnosis and management of cirrhosis-associated cardiomyopathy in liver transplant candidates: advanced echo imaging, cardiac biomarkers, and advanced heart failure therapies. *Clin Med Insights Cardiol* 2014; **8**: 67-74 [PMID: 25657603]
- 13 **Krag A**, Möller S, Burroughs AK, Bendtsen F. Betablockers induce cardiac chronotropic incompetence. *J Hepatol* 2012; **56**: 298-299 [PMID: 22173037 DOI: 10.1016/j.jhep.2011.04.033]
- 14 **Krag A**, Bendtsen F, Henriksen JH, Möller S. Low cardiac output predicts development of hepatorenal syndrome and survival in patients with cirrhosis and ascites. *Gut* 2010; **59**: 105-110 [PMID: 19837678 DOI: 10.1136/gut.2009.180570]
- 15 **Karagiannakis DS**, Papatheodoridis G, Vlachogiannakos J. Recent advances in cirrhotic cardiomyopathy. *Dig Dis Sci* 2015; **60**: 1141-1151 [PMID: 25404411 DOI: 10.1007/s10620-014-3432-8]
- 16 **Kroeger RJ**, Groszmann RJ. Increased portal venous resistance hinders portal pressure reduction during the administration of beta-adrenergic blocking agents in a portal hypertensive model. *Hepatology* 1985; **5**: 97-101 [PMID: 2857150]
- 17 **Lebrec D**, Poynard T, Hillon P, Benhamou JP. Propranolol for prevention of recurrent gastrointestinal bleeding in patients with cirrhosis: a controlled study. *N Engl J Med* 1981; **305**: 1371-1374 [PMID: 7029276 DOI: 10.1056/NEJM198112033052302]
- 18 **Hayes PC**, Davis JM, Lewis JA, Bouchier IA. Meta-analysis of value of propranolol in prevention of variceal haemorrhage. *Lancet* 1990; **336**: 153-156 [PMID: 1973480]
- 19 **de Franchis R**. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol* 2015; **63**: 743-752 [PMID: 26047908 DOI: 10.1016/j.jhep.2015.05.022]
- 20 **Gluud LL**, Krag A. Banding ligation versus beta-blockers for primary prevention in oesophageal varices in adults. *Cochrane Database Syst Rev* 2012; **8**: CD004544 [PMID: 22895942 DOI: 10.1002/14651858.CD004544.pub2]
- 21 **Heebøll S**, Villadsen GE, Aagaard NK, Grønbaek H, Vilstrup H, Keiding S. Propranolol treatment of portal hypertension in cirrhosis patients is better the higher the untreated pressure: a single-centre prospective experience. *Scand J Gastroenterol* 2013; **48**: 969-973 [PMID: 23755897 DOI: 10.3109/00365521.2013.805811]
- 22 **Sersté T**, Melot C, Francoz C, Durand F, Rautou PE, Valla D, Moreau R, Lebrec D. Deleterious effects of beta-blockers on survival in patients with cirrhosis and refractory ascites. *Hepatology* 2010; **52**: 1017-1022 [PMID: 20583214 DOI: 10.1002/hep.23775]
- 23 **Efe C**, Purnak T, Ozaslan E. The deleterious effects of propranolol on patients with cirrhosis. *Hepatology* 2011; **53**: 371-372 [PMID: 20726015 DOI: 10.1002/hep.23881]
- 24 **Wong F**, Salerno F. Beta-blockers in cirrhosis: friend and foe? *Hepatology* 2010; **52**: 811-813 [PMID: 20812354 DOI: 10.1002/hep.23852]
- 25 **Senzolo M**, Nadal E, Cholongitas E, Burroughs AK. Is hydrophobia necessary for the hepatologist prescribing nonselective beta-blockers in cirrhosis? *Hepatology* 2011; **53**: 2149-2150 [PMID: 21400554 DOI: 10.1002/hep.24176]
- 26 **Sersté T**, Francoz C, Durand F, Rautou PE, Melot C, Valla D, Moreau R, Lebrec D. Beta-blockers cause paracentesis-induced circulatory dysfunction in patients with cirrhosis and refractory ascites: a cross-over study. *J Hepatol* 2011; **55**: 794-799 [PMID: 21354230 DOI: 10.1016/j.jhep.2011.01.034]
- 27 **Llach J**, Ginès P, Arroyo V, Rimola A, Titó L, Badalamenti S, Jiménez W, Gaya J, Rivera F, Rodés J. Prognostic value of arterial pressure, endogenous vasoactive systems, and renal function in cirrhotic patients admitted to the hospital for the treatment of ascites. *Gastroenterology* 1988; **94**: 482-487 [PMID: 3335320]
- 28 **Villanueva C**, Albillos A, Genescà J, Abalades JG, Calleja JL, Aracil C, Bañares R, Morillas R, Poca M, Peñas B, Augustin S, Garcia-Pagan JC, Pavel O, Bosch J. Development of hyperdynamic circulation and response to  $\beta$ -blockers in compensated cirrhosis with portal hypertension. *Hepatology* 2016; **63**: 197-206 [PMID: 26422126 DOI: 10.1002/hep.28264]
- 29 **Sharma P**, Kumar A, Jha S, Mishra SR, Sharma BC, Sarin SK. The haemodynamic response to propranolol in cirrhosis with arterial hypertension: a comparative analysis with normotensive cirrhotic patients. *Aliment Pharmacol Ther* 2010; **32**: 105-112 [PMID: 20345511 DOI: 10.1111/j.1365-2036.2010.04308.x]
- 30 **Leithead JA**, Rajoriya N, Tehami N, Hodson J, Gunson BK, Tripathi D, Ferguson JW. Non-selective  $\beta$ -blockers are associated with improved survival in patients with ascites listed for liver transplantation. *Gut* 2015; **64**: 1111-1119 [PMID: 25281417 DOI: 10.1136/gutjnl-2013-306502]
- 31 **Kimer N**, Feineis M, Möller S, Bendtsen F. Beta-blockers in cirrhosis and refractory ascites: a retrospective cohort study and review of the literature. *Scand J Gastroenterol* 2015; **50**: 129-137 [PMID: 25113796 DOI: 10.3109/00365521.2014.948053]
- 32 **Bossen L**, Krag A, Vilstrup H, Watson H, Jepsen P. Nonselective  $\beta$ -blockers do not affect mortality in cirrhosis patients with ascites: Post Hoc analysis of three randomized controlled trials with 1198 patients. *Hepatology* 2016; **63**: 1968-1976 [PMID: 26599983 DOI: 10.1016/S0168-8278(15)30087-8]
- 33 **Pereira GH**, Baldin C, Victor L, Piedade J, Guimarães L, Rocha T, Pereira L. Use of non-selective beta blockers (nsbb) in cirrhotic patients with bacterial infections is associated with lower frequency of sepsis, but not of acute-on-chronic liver failure (ACLF) or survival. Results of a prospective study. *J Hepatol* 2016; **64** (S2): S263
- 34 **Mallawaarachchi N**, Sinha R, Hayes P. Does the use of non-selective beta-blockers in cirrhosis patients with ascites result in increased mortality? *J Hepatol* 2016; **64** (S2): S278-279
- 35 **Bhutta AQ**, Garcia-Tsao G, Reddy R, Tandon P, Wong F, O'Leary JG, Bajaj J. Beta-blocker use in hospitalized cirrhotic patients with ascites is associated with a lower MELD, less inflammation and an improved survival. *J Hepatol* 2016; **64** (S2): S245
- 36 **Onali S**, Kalafateli M, Majumdar A, Westbrook M, O'Beirne J, Patch D, Tsochatzis E. Non-selective beta blockers (NSBBS) use is associated with improved survival in cirrhotic patients with ascites: a single centre retrospective study. *J Hepatol* 2016; **64** (S2): S668
- 37 **Mookerjee RP**, Pavesi M, Thomsen KL, Mehta G, Macnaughtan J, Bendtsen F, Coenraad M, Sperl J, Gines P, Moreau R, Arroyo V, Jalan R. Treatment with non-selective beta blockers is associated with reduced severity of systemic inflammation and improved survival of patients with acute-on-chronic liver failure. *J Hepatol* 2016; **64**: 574-582 [PMID: 26519600 DOI: 10.1016/j.jhep.2015.10.018]
- 38 **Ruiz-del-Arbol L**, Urman J, Fernández J, González M, Navasa M, Monescillo A, Albillos A, Jiménez W, Arroyo V. Systemic, renal, and hepatic hemodynamic derangement in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 2003; **38**: 1210-1218 [PMID: 14578859 DOI: 10.1053/jhep.2003.50447]

- 39 **Mandorfer M**, Bota S, Schwabl P, Bucsics T, Pfisterer N, Kruzik M, Hagmann M, Blacky A, Ferlitsch A, Sieghart W, Trauner M, Peck-Radosavljevic M, Reiberger T. Nonselective  $\beta$  blockers increase risk for hepatorenal syndrome and death in patients with cirrhosis and spontaneous bacterial peritonitis. *Gastroenterology* 2014; **146**: 1680-1690.e1 [PMID: 24631577 DOI: 10.1053/j.gastro.2014.03.005]
- 40 **Galbois A**, Das V, Thabut D, Maury E, Ait-Oufella H, Housset C, Guidet B. Beta-blockers have no effect on outcomes in patients with cirrhosis and severe infections. *Hepatology* 2011; **53**: 1412-1413 [PMID: 21480358 DOI: 10.1002/hep.24053]
- 41 **Ferrearese A**, Germani G, Rodriguez-Castro KI, Nadal E, Zanetto A, Bortoluzzi I, Russo FP, Burra P, Burroughs AK, Senzolo M. Bleeding-unrelated mortality is not increased in patients with cirrhosis and ascites on treatment with  $\beta$ -blockers: A meta-analysis. *Digest Liver Dis* 2014; **46** (Suppl 1): e31 [DOI: 10.1016/j.dld.2014.01.072]
- 42 **Forrest EH**, Bouchier IA, Hayes PC. Acute haemodynamic changes after oral carvedilol, a vasodilating beta-blocker, in patients with cirrhosis. *J Hepatol* 1996; **25**: 909-915 [PMID: 9007720]
- 43 **Berzigotti A**, Bosch J. Pharmacologic management of portal hypertension. *Clin Liver Dis* 2014; **18**: 303-317 [PMID: 24679496 DOI: 10.1016/j.cld.2013.12.003]
- 44 **Bañares R**, Moitinho E, Matilla A, García-Pagán JC, Lampreave JL, Piera C, Abrales JG, De Diego A, Albillos A, Bosch J. Randomized comparison of long-term carvedilol and propranolol administration in the treatment of portal hypertension in cirrhosis. *Hepatology* 2002; **36**: 1367-1373 [PMID: 12447861 DOI: 10.1053/jhep.2002.36947]
- 45 **Sinagra E**, Perricone G, D'Amico M, Tinè F, D'Amico G. Systematic review with meta-analysis: the haemodynamic effects of carvedilol compared with propranolol for portal hypertension in cirrhosis. *Aliment Pharmacol Ther* 2014; **39**: 557-568 [PMID: 24461301 DOI: 10.1111/apt.12634]
- 46 **Thalheimer U**, Bosch J, Burroughs AK. How to prevent varices from bleeding: shades of grey--the case for nonselective beta blockers. *Gastroenterology* 2007; **133**: 2029-2036 [PMID: 18054573 DOI: 10.1053/j.gastro.2007.10.028]
- 47 **Abrales JG**, Tarantino I, Turnes J, Garcia-Pagan JC, Rodés J, Bosch J. Hemodynamic response to pharmacological treatment of portal hypertension and long-term prognosis of cirrhosis. *Hepatology* 2003; **37**: 902-908 [PMID: 12668985 DOI: 10.1053/jhep.2003.50133]
- 48 **Hernández-Gea V**, Aracil C, Colomo A, Garupera I, Poca M, Torras X, Miñana J, Guarner C, Villanueva C. Development of ascites in compensated cirrhosis with severe portal hypertension treated with  $\beta$ -blockers. *Am J Gastroenterol* 2012; **107**: 418-427 [PMID: 22334252 DOI: 10.1038/ajg.2011.456]
- 49 **Mehta G**, Gustot T, Mookerjee RP, Garcia-Pagan JC, Fallon MB, Shah VH, Moreau R, Jalan R. Inflammation and portal hypertension - the undiscovered country. *J Hepatol* 2014; **61**: 155-163 [PMID: 24657399 DOI: 10.1016/j.jhep.2014.03.014]
- 50 **Rasaratnam B**, Kaye D, Jennings G, Dudley F, Chin-Dusting J. The effect of selective intestinal decontamination on the hyperdynamic circulatory state in cirrhosis. A randomized trial. *Ann Intern Med* 2003; **139**: 186-193 [PMID: 12899586]
- 51 **Pérez-Paramo M**, Muñoz J, Albillos A, Freile I, Portero F, Santos M, Ortiz-Berocal J. Effect of propranolol on the factors promoting bacterial translocation in cirrhotic rats with ascites. *Hepatology* 2000; **31**: 43-48 [PMID: 10613726]
- 52 **Nadal E**, Buda A, Pizzuti D, Nai L, Burra P, Senzolo M. Functional study of the intestinal barrier in patients with cirrhosis and portal hypertension. *J Hepatol* 2011; **54** (s1): 247-248 [DOI: 10.1016/S0168-8278(11)60612-0]
- 53 **Reiberger T**, Ferlitsch A, Payer BA, Mandorfer M, Heinisch BB, Hayden H, Lammert F, Trauner M, Peck-Radosavljevic M, Vogelsang H. Non-selective betablocker therapy decreases intestinal permeability and serum levels of LBP and IL-6 in patients with cirrhosis. *J Hepatol* 2013; **58**: 911-921 [PMID: 23262249 DOI: 10.1016/j.jhep.2012.12.011]
- 54 **Cholongitas E**, Papatheodoridis GV, Manesis EK, Burroughs AK, Archimandritis AJ. Spontaneous bacterial peritonitis in cirrhotic patients: Is prophylactic propranolol therapy beneficial? *J Gastroenterol Hepatol* 2006; **21**: 581-587 [PMID: 16638103]
- 55 **Senzolo M**, Cholongitas E, Burra P, Leandro G, Thalheimer U, Patch D, Burroughs AK. beta-Blockers protect against spontaneous bacterial peritonitis in cirrhotic patients: a meta-analysis. *Liver Int* 2009; **29**: 1189-1193 [PMID: 19508620 DOI: 10.1111/j.1478-3231.2009.02038.x]
- 56 **Merli M**, Riggio O. Interaction between infection and hepatic encephalopathy. *J Hepatol* 2015; **62**: 746-747 [PMID: 25450708 DOI: 10.1016/j.jhep.2014.10.028]
- 57 **Abrales JG**, Albillos A, Bañares R, Turnes J, González R, García-Pagán JC, Bosch J. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomized controlled trial. *Gastroenterology* 2009; **136**: 1651-1658 [PMID: 19208350 DOI: 10.1053/j.gastro.2009.01.043]
- 58 **Thorhauge KH**, Lindvig KP, Laleman W, Angeli P, Singh SP, Krag A. Lack of consensus for usage of  $\beta$ -blockers in end-stage liver disease. *Gut* 2016; **65**: 1058-1060 [PMID: 26933172]
- 59 **Krag A**, Wiest R, Albillos A, Gluud LL. The window hypothesis: haemodynamic and non-haemodynamic effects of  $\beta$ -blockers improve survival of patients with cirrhosis during a window in the disease. *Gut* 2012; **61**: 967-969 [PMID: 22234982 DOI: 10.1136/gutjnl-2011-301348]
- 60 **Robins A**, Bowden A, Watson W, Smith F, Gelson W, Griffiths W. Beta-blockers in cirrhosis patients with refractory ascites. *Hepatology* 2014; **59**: 2054-2055 [PMID: 23929786 DOI: 10.1002/hep.26676]

**P- Reviewer:** Grgurevic I, Mercado MA, Tovo CV

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D



Observational Study

## Hypolactasia is associated with insulin resistance in nonalcoholic steatohepatitis

Daniel Ferraz de Campos Mazo, Rejane Mattar, José Tadeu Stefano, Joyce Matie Kinoshita da Silva-Etto, Márcio Augusto Diniz, Sebastião Mauro Bezerra Duarte, Fabíola Rabelo, Rodrigo Vieira Costa Lima, Priscila Brizolla de Campos, Flair José Carrilho, Claudia P Oliveira

Daniel Ferraz de Campos Mazo, Rejane Mattar, José Tadeu Stefano, Joyce Matie Kinoshita da Silva-Etto, Márcio Augusto Diniz, Sebastião Mauro Bezerra Duarte, Fabíola Rabelo, Rodrigo Vieira Costa Lima, Priscila Brizolla de Campos, Flair José Carrilho, Claudia P Oliveira, Division of Gastroenterology and Hepatology, Department of Gastroenterology (LIM 07), University of São Paulo School of Medicine, São Paulo 05403-000, Brazil

**Author contributions:** de Campos Mazo DF, Mattar R and Oliveira CP conceived and designed the study, contributed to the data analysis and interpretation and wrote the manuscript; da Silva-Etto JMK and Mattar R performed the LCT-13910C>T genotyping; de Campos Mazo DF, Stefano JT, Duarte SMB, Rabelo F, Lima RVC and de Campos PB collected and assembled the data; Diniz MA performed the statistical analysis and analyzed the data; Carrilho FJ contributed to the data analysis and interpretation; all authors read and approved the final version of the manuscript.

**Institutional review board statement:** This study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of the Hospital das Clínicas (No. 448520).

**Informed consent statement:** All involved persons provided their informed consent prior to study inclusion.

**Conflict-of-interest statement:** The authors declare no conflicts of interest in this work.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Claudia P Oliveira, MD, PhD, Division of Gastroenterology and Hepatology, Department of Gastroenterology (LIM 07), University of São Paulo School of Medicine, Av Dr Eneas de Carvalho Aguiar 255, 9º Andar, Sala 9159, São Paulo 05403-000, Brazil. [cpm@usp.br](mailto:cpm@usp.br)  
 Telephone: +55-11-26616447  
 Fax: +55-11-26617830

Received: April 26, 2016  
 Peer-review started: April 28, 2016  
 First decision: May 17, 2016  
 Revised: June 26, 2016  
 Accepted: July 14, 2016  
 Article in press: July 18, 2016  
 Published online: August 28, 2016

## Abstract

### AIM

To assess lactase gene (*LCT*)-13910C>T polymorphisms in Brazilian non-alcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) patients in comparison with healthy controls.

### METHODS

This was a transverse observational clinical study with NAFLD patients who were followed at the Hepatology Outpatient Unit of the Hospital das Clínicas, São Paulo, Brazil. The polymorphism of lactase non-persistence/lactase persistence (*LCT*-13910C>T) was examined by PCR-restriction fragment length polymorphism technique in 102 liver biopsy-proven NAFLD patients (steatosis in 9 and NASH in 93) and compared to those of 501 unrelated healthy volunteers. Anthropometric, clinical, biochemical and liver histology data were analyzed. Continuous variables were compared using the *t* or Mann-Whitney tests, and categorical data were compared with the Fisher's exact test. Univariate logistic regression and

multivariate logistic regression adjusted for gender and age were performed.

## RESULTS

No differences in the *LCT*-13910 genotype frequencies were noted between the NAFLD patients (66.67% of the patients with steatosis were CC, 33.33% were CT, and none were TT; 55.91% of the patients with NASH were CC, 39.78% were CT, and 4.3% were TT;  $P = 0.941$ ) and the healthy controls (59.12% were CC, 35.67% were CT, and 5.21% were TT) or between the steatosis and NASH patients. That is, the distribution of the lactase non-persistence/lactase persistence polymorphism (*LCT*-13910C>T) in the patients with NAFLD was equal to that in the general population. In the NASH patients, the univariate analysis revealed that the lactase non-persistence (low lactase activity or hypolactasia) phenotype was associated with higher insulin levels ( $23.47 \pm 15.94 \mu\text{U/mL}$  *vs*  $15.8 \pm 8.33 \mu\text{U/mL}$ ,  $P = 0.027$ ) and a higher frequency of insulin resistance (91.84% *vs* 72.22%,  $P = 0.02$ ) compared with the lactase persistence phenotype. There were no associations between the *LCT* genotypes and diabetes ( $P = 0.651$ ), dyslipidaemia ( $P = 0.328$ ), hypertension ( $P = 0.507$ ) or liver histology in these patients. Moreover, in the NASH patients, hypolactasia was an independent risk factor for insulin resistance even after adjusting for gender and age [OR = 5.0 (95%CI: 1.35-20;  $P = 0.017$ )].

## CONCLUSION

The *LCT*-13910 genotype distribution in Brazilian NAFLD patients was the same as that of the general population, but hypolactasia increased the risk of insulin resistance in the NASH patients.

**Key words:** Lactose intolerance; Genetic polymorphism; Insulin resistance; Non-alcoholic fatty liver disease; Nonalcoholic steatohepatitis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Non-alcoholic fatty liver disease (NAFLD) exhibits a close relationship with metabolic syndrome (MetS), but the associations of the lactase non-persistence/lactase persistence genotypes with MetS components are controversial. Therefore, we assessed hypolactasia (*LCT*-13910CC) and lactase persistence genotypes in 102 Brazilian NAFLD patients in comparison with 501 healthy controls, the associations of these polymorphisms were verified with the results of biochemical tests, MetS and severity of liver histology in nonalcoholic steatohepatitis (NASH) patients. No differences in the *LCT*-13910C>T polymorphisms were noted between the NAFLD and controls, but hypolactasia increased the risk of insulin resistance in the NASH patients.

de Campos Mazo DF, Mattar R, Stefano JT, da Silva-ETTO JMK, Diniz MA, Duarte SMB, Rabelo F, Lima RVC, de Campos PB, Carrilho FJ, Oliveira CP. Hypolactasia is associated with insulin

resistance in nonalcoholic steatohepatitis. *World J Hepatol* 2016; 8(24): 1019-1027 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i24/1019.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i24.1019>

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of liver damage that ranges from steatosis to nonalcoholic steatohepatitis (NASH) and advanced fibrosis/cirrhosis in persons without significant alcohol consumption<sup>[1,2]</sup>. NAFLD is currently considered the most common liver disease and is associated to metabolic syndrome (MetS) components, such as obesity and diabetes<sup>[3-5]</sup>. Several studies have correlated the severity of liver injury with increased frequencies of such components, thus making these components important targets in the management of this condition<sup>[1,6-8]</sup>. However, while specific pharmacological therapy are still far from solving all of the issues related to fatty liver disease, the pursuit of high-risk individuals can be a strategy for concentrating efforts on its diagnosis and management.

Milk is the primary energy source for newborns and is rich in lactose. Lactase phlorizin hydrolase in the microvillus membrane of the small intestinal cells digests lactose. However, after 2-12 years of age, a physiological genetically programmed reduction in lactase activity occurs, hypolactasia or lactase non-persistence, which, when accompanied by symptoms, defines lactose intolerance<sup>[9]</sup>. In contrast some populations mainly from Northern Europe present lactase persistence during adulthood<sup>[10]</sup>. The most interesting report published in 2002<sup>[11]</sup> found that the polymorphisms in intron 13 [lactase gene (*LCT*)-13910C>T] and in intron 9 (*LCT*-22018G>A) of the *MCM6* gene conferred lactase persistence in several populations<sup>[9,12-14]</sup>. These genotypes render a person a lactose digester. The lactase-persistence phenotype has a prevalence of 43.4% in Caucasian Brazilians, and there is no difference between genders<sup>[12]</sup>.

Recent studies have raised concerns regarding the possible associations of lactase persistence with the components of MetS. In Europeans those with hypolactasia genotype (*LCT*-13910CC) had lower body mass indices and waist circumferences than those with lactase persistence genotypes<sup>[15,16]</sup>. Likewise, in the Canary Islands, those with lactase persistence genotypes exhibit higher odds ratios for MetS than do subjects with the *LCT*-13910CC genotype<sup>[17]</sup>.

However, other studies have demonstrated that dairy food consumption showed lower susceptibility to type 2 diabetes or worsening of glucose homeostasis indices<sup>[18-20]</sup>. Nicklas *et al*<sup>[21]</sup> applied a questionnaire to a sample of 3452 American adults and reported that diagnosis of diabetes and hypertension were higher in individuals that considered themselves lactose intolerant with lower ingestion of calcium from dairies. Additionally, Samara *et al*<sup>[22]</sup> assessed a French population and



reported that better metabolic profiles in men was associated with more dairies intake.

As noted, the role of milk in MetS is not clearly defined at this moment, and the literature is controversial<sup>[23]</sup>. Moreover, publications regarding the *LCT-13910C>T* polymorphism in patients with NAFLD are scarce. Therefore, the purpose was to assess expression profiles of the *LCT-13910* genotypes in Brazilians with NAFLD compared to those of healthy individuals to investigate whether the *LCT-13910C>T* variant could be a predictor of NASH. An additional goal was to analyze the associations of the lactase-persistence genotype with biochemical tests, components of MetS and the severity of liver histology in NASH patients.

## MATERIALS AND METHODS

### Ethical considerations

The Ethics Committee of the Hospital das Clínicas (number 448520) approved this study that was conducted following the ethical guidelines of the 1975 Declaration of Helsinki.

### Patients and clinical design

This was a transverse study with NAFLD patients who were followed at the Hepatology Outpatient Unit of the Hospital das Clínicas, São Paulo, Brazil. *LCT-13910C>T* polymorphism was investigated in 102 liver biopsy-proven NAFLD patients and 501 unrelated healthy volunteers. All NAFLD patients were previously evaluated for other liver diseases, being excluded viral hepatitis, autoimmune hepatitis, hemochromatosis, Wilson disease and alpha 1-antitrypsin deficiency. MetS components identification followed the recommendations of the Adult Treatment Panel III Report as follows: Triglycerides  $\geq 150$  mg/dL, high-density lipoproteins (HDL)  $< 40$  mg/dL in men and  $< 50$  mg/dL in women, fasting glucose  $\geq 110$  mg/dL,  $\geq 130$  mmHg systolic or  $\geq 85$  mmHg diastolic pressure, and abdominal obesity<sup>[24]</sup>. The study inclusion criteria were patients 18-75 years old with NAFLD diagnoses based on liver histology. Exclusion criteria were any other liver disease, significant alcohol intake ( $> 100$  g/wk), previous exposure to drugs associated with liver steatosis or not accepting to participate in the study.

Liver histology were scored according to the macro- and micro-vacuolar steatosis, the inflammation and the hepatocyte ballooning. Fibrosis pattern and zonal distributions of the analysed variables were also recorded. The slides were classified according to the NASH Clinical Research Network<sup>[25]</sup>. The biochemical investigations included the following: Fasting glucose, plasma insulin, total cholesterol and fractions, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transferase (GGT), which were collected after a 12-h overnight fast and evaluated at the time of the liver biopsy. Homeostatic Model of Assessment (HOMA-IR) was used to evaluate insulin resistance [ $22.5 \times \text{fasting insulin (mU/mL)} \times \text{glucose (mmol/L)}$ ]<sup>[26]</sup>. A HOMA-IR  $\geq 2.5$  was used as the cutoff

point to define insulin resistance<sup>[27,28]</sup>. Retrospective information regarding co-morbidities was also collected.

### Genotyping

Leukocytes were used for genomic DNA extraction (Miller *et al.*<sup>[29]</sup> 1988). The technique for *LCT-13910* genotyping was described elsewhere<sup>[11,30-32]</sup>.

### Statistical analysis

The continuous variables are presented as the means  $\pm$  the standard deviations and were compared using the *t* test (the assumption of normality was verified using the Anderson-Darling test). When appropriate, the Mann-Whitney test was used. The categorical variables are expressed as the percentages (frequencies) of affected individuals and were compared using Fisher's exact test. Univariate logistic regression was performed to evaluate the odds ratios with the respective 95% CIs. Multivariate logistic regression adjusted for gender and age was performed. The best predictive cut-offs for the continuous variables were determined using conditional trees when the traditional cut-offs did not provide interesting information<sup>[33]</sup>. *P* values below 0.05 were considered statistically significant. The R Project for Statistical Computing ver. 3.1.1 (R Core Team, Vienna, Austria, 2014) software package was used for the statistical analyses<sup>[34]</sup>. A statistical review of the study was performed by a biomedical statistician (Márcio Augusto Diniz).

## RESULTS

The anthropometric, clinical, and biochemical characteristics of the patients are provided in Table 1. We evaluated 102 NAFLD patients, including 9 steatosis and 93 with NASH. All of the steatosis patients were women, whereas in the NASH group, 32 patients (34.41%) were men ( $P = 0.04$ ). The NASH patients had higher fasting glucose levels than did the patients with steatosis only ( $123.14 \pm 48.28$  vs  $91.71 \pm 9.2$ , respectively,  $P = 0.033$ ). There were no differences between the groups in terms of age, MetS components, BMI, insulin, HOMA-IR values  $\geq 2.5$ , AST, ALT, GGT, total cholesterol, HDL, LDL or triglycerides (Table 1).

The distributions of alleles and genotypes are presented in Table 2. No differences in *LCT-13910* genotype frequencies were noted between the NAFLD patients (66.67% patients with steatosis were CC, 33.33% were CT and none were TT; 55.91% of those with NASH were CC, 39.78% were CT and 4.3% were TT;  $P = 0.941$ ) and the healthy controls (59.12% were CC, 35.67% CT, 5.21% TT). Likewise, no differences in the *LCT-13910C>T* allele frequencies were noted between the groups (76.95% of the controls, 83.33% of those with steatosis and 75.81% of the NASH patients had the *LCT-13910C* allele;  $P = 0.764$ ). That is, the distribution of the *LCT-13910C>T* polymorphism in the patients with NAFLD was equal to that in the general population.

**Table 1** Demographic, clinical and biochemical characteristics of the non-alcoholic fatty liver disease patients

	Steatosis ( <i>n</i> = 9)	NASH ( <i>n</i> = 93)	<i>P</i> value
Age	55.11 ± 10.3	56.51 ± 10.13	0.692
Men/women ( <i>n</i> )	0% (0)/100% (9)	34.41% (32)/65.59% (61)	0.04 <sup>a</sup>
Type 2 diabetes ( <i>n</i> )	33.33% (2)	60.67% (54)	0.224
Dyslipidaemia ( <i>n</i> )	83.33% (5)	79.78% (71)	1
High-blood pressure ( <i>n</i> )	66.67% (4)	64.04% (89)	1
BMI	31.28 ± 5.79	31.25 ± 5.93	0.969
Fasting glucose (mg/dL)	91.71 ± 9.2	123.14 ± 48.28	0.033 <sup>a</sup>
Insulin (μU/mL)	12.44 ± 4.2	19.92 ± 13.29	0.102
HOMA-IR value ≥ 2.5	57.14%	83.53%	0.115
AST (U/L)	25.14 ± 6.89	38.8 ± 37.99	0.159
ALT (U/L)	40 ± 16.74	50.65 ± 54.99	0.934
GGT (U/L)	56.57 ± 59.9	87.36 ± 96.33	0.185
Total cholesterol (mg/dL)	203.29 ± 54.39	195.31 ± 45.71	0.863
HDL (mg/dL)	53 ± 6.58	46.15 ± 13.42	0.067
LDL (mg/dL)	124.14 ± 51.87	114.89 ± 39.74	0.72
Triglycerides (mg/dL)	130.43 ± 59.19	167.25 ± 82.02	0.258

<sup>a</sup>*P* value < 0.05. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; HDL: High-density lipoprotein; HOMA-IR: Homeostatic Model of Assessment; LDL: Low-density lipoprotein; GGT: Gamma glutamyl transferase; NASH: Nonalcoholic steatohepatitis.

Analysis *via* simple logistic regressions of the associations of the *LCT*-13910C>T polymorphisms with the results of the biochemical tests, components of MetS and severity of liver histology in the NAFLD patients (steatosis and NASH groups) did not reveal any associations (data not shown). Subsequently, we evaluated the patients with NASH (Table 3). In this group (*n* = 93), univariate analysis revealed that the hypolactasia phenotype was associated with higher insulin levels (*P* = 0.027) and greater insulin resistance (*P* = 0.02). No associations were noted between the liver histology parameters (*i.e.*, steatosis, inflammation and fibrosis) and the *LCT*-13910 genotype or phenotype. Moreover, no associations were found between the components of MetS or MetS diagnosis (*P* = 1.0) and the *LCT*-13910 genotype or phenotype.

Table 4 illustrates the logistic regression analysis that was adjusted for gender and age and assessed the independent associations of the *LCT*-13910C>T polymorphism with HOMA-IR, BMI ≥ 30, insulin value and MetS in the NASH patients. Hypolactasia phenotype was associated with a 5-fold increase in insulin resistance (95%CI: 1.35-20; *P* = 0.017). The *LCT*-13910CT genotype conferred a 6.25-fold decrease in insulin resistance (95%CI: 0.04-0.64; *P* = 0.009). In this multivariate regression analysis, we no longer observed an association between hypolactasia and insulin level (even when using the cut-off of > 29.8 μU/mL, *P* = 0.197) after adjusting for gender and age. Similarly, the MetS diagnosis and a BMI ≥ 30 were not associated with the *LCT*-13910C>T polymorphism.

## DISCUSSION

### Key findings

In this transverse clinical study, we were unable to find any differences in the *LCT*-13910C>T polymorphism

expression profile between Brazilian NAFLD patients and healthy controls (*P* = 0.941). Moreover, the presence of the T allele was not able to differentiate steatosis from NASH in NAFLD patients (*P* = 0.764). However, in NASH patients, the hypolactasia phenotype (*i.e.*, the *LCT*-13910CC genotype) was associated with insulin resistance, and conversely, the *LCT*-13910CT genotype conferred protection against its occurrence.

The *LCT*-13910C>T polymorphism prevalence varies among different populations across the globe. The lactase-persistence phenotype (*i.e.*, the *LCT*-13910-CT and *LCT*-13910-TT genotypes) can occur at rates as high as 72% and 73.7% in New Zealand and Sweden, respectively<sup>[13,35]</sup>. In Hungary, the prevalence is 35.9%, and in Caucasian Brazilians, the prevalence is 43.4%<sup>[12,36]</sup>. In contrast, in Chinese and Japanese Brazilians, the lactase-persistence phenotype was not found at all in some published studies<sup>[12,37]</sup>. The *LCT* genotype distribution was also the same in NAFLD patients regardless of the presence of NASH or steatosis only.

In a recent European meta-analysis with 31720 individuals, Kettunen *et al.*<sup>[16]</sup> found that the *LCT*-13910CC genotype was associated with a decreased body mass index (BMI), when compared to *LCT*-13910CT/TT. In an analysis of 17374 Finns, it was observed that when the lactase persistent allele was present, BMI was 0.3 kg/m<sup>2</sup> higher, which corresponds to approximately 1 kg<sup>[16]</sup>. These findings were reproduced by Corella *et al.*<sup>[15]</sup> in a Mediterranean population in which *LCT*-13910CC individuals exhibited a lower risk of obesity, lower body weights, lower BMIs and smaller waist circumferences than *LCT*-13910T-allele carriers. Although the association between the *LCT*-13910C>T genotypes and the diagnosis of full-blown MetS was not significant in the overall analysis in the study, a subgroup analysis revealed a significant association in the subjects with a lactose intake higher than 8 g/d<sup>[15]</sup>. In a cross-sectional

**Table 2** Allele and genotype frequencies of the lactase-13910C>T polymorphisms

		Allele frequency % (n) <sup>a</sup>		Total (%)	Genotype frequency % (n) <sup>b</sup>			Total (%)
		C	T		CC	CT	TT	
<i>LCT</i> -13910	Control (n = 501)	76.95 (768)	23.05 (230)	100	59.12 (295)	35.67 (178)	5.21 (26)	100
	Steatosis (n = 9)	83.33 (15)	16.67 (3)	100	66.67 (6)	33.33 (3)	0 (0)	100
	NASH (n = 93)	75.81 (141)	24.19 (43)	100	55.91 (52)	39.78 (37)	4.3 (3)	100

<sup>a</sup>P = 0.764; <sup>b</sup>P = 0.941. NASH: Nonalcoholic steatohepatitis; *LCT*: Lactase gene.

**Table 3** Associations of the lactase-13910 phenotype in nonalcoholic steatohepatitis patients (n = 93)

	Hypolactasia	Lactase persistence	P value
Age	55.96 ± 10.91	57.61 ± 9.33	0.443
Gender: Female % (n)	70.83 (34)	60.98 (25)	0.51
Type 2 diabetes % (n)	66.67 (30)	57.5 (23)	0.664
Dyslipidaemia % (n)	82.22 (37)	77.5 (31)	0.792
High-blood pressure % (n)	68.89 (31)	57.5 (23)	0.273
BMI	31.39 ± 6.55	31.28 ± 5.37	0.714
BMI ≥ 30 % (n)	58.14 (25)	65 (26)	0.388
Fasting glucose (mg/dL)	122.61 ± 50.14	123.83 ± 46.43	0.892
Insulin (μU/mL)	23.47 ± 15.94	15.8 ± 8.33	0.027 <sup>a</sup>
HOMA-IR value ≥ 2.5 (n)	91.84 (45)	72.22 (26)	0.02 <sup>a</sup>
AST (U/L)	38.94 ± 37.66	42.67 ± 40.15	0.121
ALT (U/L)	51.47 ± 69.44	52.12 ± 35.02	0.072
GGT (U/L)	97.49 ± 118.27	80.51 ± 65.6	0.427
Total cholesterol (mg/dL)	196.62 ± 47.13	195.55 ± 44.06	0.965
HDL (mg/dL)	46.38 ± 12.26	46.05 ± 15.06	0.698
LDL (mg/dL)	117.19 ± 39.47	114.25 ± 39.9	0.893
Triglycerides (mg/dL)	169.11 ± 82.97	162.3 ± 83.11	0.477
Steatosis			
1	21.28 (10)	24.39 (10)	0.453
2	51.06 (24)	39.02 (16)	
3	27.66 (13)	36.59 (15)	
Inflammation			
0	2.13 (1)	7.32 (3)	0.133
1	61.7 (29)	46.34 (19)	
2	23.4 (11)	39.02 (16)	
3	12.77 (6)	7.32 (3)	
Fibrosis			
0	18.75 (9)	14.63 (6)	0.804
1	39.58 (19)	43.9 (18)	
2	16.67 (8)	17.07 (7)	
3	20.83 (10)	19.51 (8)	
4	4.17 (2)	4.88 (2)	
MetS	51.92 (27)	53.66 (19)	1

<sup>a</sup>P value < 0.05. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; HDL: High-density lipoprotein; HOMA-IR: Homeostatic Model of Assessment; LDL: Low-density lipoprotein; GGT: Gamma glutamyl transferase; MetS: Metabolic syndrome.

work conducted in the Canary Islands, Almon *et al.*<sup>[17]</sup> demonstrated that subjects with the *LCT*-13910CT and *LCT*-13910TT genotypes exhibited higher odds ratio for MetS than subjects with the *LCT*-13910CC genotype. The authors concluded that the T allele might constitute a nutrigenetic factor that increases the susceptibility to MetS development, and this susceptibility was particularly noted in women<sup>[17]</sup>.

Despite the aforementioned studies that have demonstrated correlations of the CC genotype with decreased BMI, a lower risk of obesity, a lower body weight, and smaller waist circumference compared with the CT and TT genotypes<sup>[15,16]</sup> and the even further increased

higher odds ratio for MetS in individuals with the T allele<sup>[17]</sup>, we could not corroborate these findings in our NAFLD population. Studying only the NASH patients in the univariate analysis, we did not find associations between the *LCT*-13910C>T polymorphism and BMI or MetS diagnoses even after adjusting for gender and age in the multivariate analysis. In fact, the patients with NASH and a genetic profile of persistent lactase activity exhibited less insulin resistance than the patients with hypolactasia. These divergences in our findings could be related to differences in the studied populations and possible positive effects of dairy ingestion on the metabolic profiles of these individuals.

**Table 4** Multivariate logistic regression analysis in non-alcoholic steatohepatitis patients

Factor	OR	95%CI	P value
HOMA-IR value $\geq 2.5$			
Hypolactasia phenotype	5	1.35-20	0.017 <sup>a</sup>
CT genotype	0.16	0.04-0.64	0.009 <sup>a</sup>
TT genotype	-	-	0.994
BMI $\geq 30$			
Hypolactasia phenotype	0.49	0.13-1.81	0.285
CT genotype	1.73	0.69-4.35	0.244
TT genotype	1.01	0.12-8.39	0.991
Insulin $> 29.8$			
Hypolactasia phenotype	2.04	0.68-6.25	0.197
CT genotype	0.52	0.17-1.56	0.25
TT genotype	-	-	0.991
MetS			
Hypolactasia phenotype	0.94	0.47-2.42	0.89
CT genotype	1.07	0.46-2.49	0.866
TT genotype	0.91	0.11-7.3	0.929

<sup>a</sup>P value < 0.05. HOMA-IR: Homeostatic Model of Assessment; OR: Odds ratio; BMI: Body mass index; MetS: Metabolic syndrome.

Our studied population consisted only of NAFLD patients, among which the prevalences of MetS components are expected to be higher than those of the overall population. Therefore, firm direct comparisons are precluded. However, a recently published Brazilian study demonstrated that in the general population, the lactase non-persistence genotype subjects exhibit higher prevalences of hypertension ( $P = 0.032$ ) and MetS ( $P = 0.01$ ) than lactase-persistence genotype individuals based on univariate analysis<sup>[38]</sup>. Furthermore, multivariate analyses revealed that lactase persistence was associated with a lower risk for MetS after adjusting for gender, age, BMI and physical activity (OR = 0.462;  $P = 0.009$ ). These data are in line with our findings that demonstrated a favourable profile of MetS components and glucose homeostasis in the NASH patients with lactase persistence. Moreover, in a longitudinal French study encompassing 3575 subjects, Lamri *et al.*<sup>[39]</sup> demonstrated that the C allele was associated with a higher frequency of impaired fasting glycaemia and type 2 diabetes. However, Enattah *et al.*<sup>[40]</sup> were unable to demonstrate that lactase persistence polymorphisms were risk factors for type 1 or type 2 diabetes in the Finnish study. Similar to NASH, polycystic ovary syndrome is also frequently associated with metabolic disturbances, including dyslipidaemia, insulin resistance and central obesity, and NASH often coexists in these patients<sup>[41]</sup>. Lerchbaum *et al.*<sup>[42]</sup> demonstrated a significantly higher prevalence of hypolactasia in polycystic ovary syndrome women, which also corroborates our findings.

Ultimately, we believe that dairy consumption appears to modulate the metabolic profiles of these different populations because of the strong association of the *LCT*-13910 genotype with dairies intake and lactose malabsorption<sup>[11,31,39,43]</sup>. Several studies have highlighted the benefits of dairy and dairy components on MetS components<sup>[18-22,44-46]</sup> and cardiovascular health<sup>[47]</sup>. The

benefits of dairy products may be mediated through several mechanisms, including the following<sup>[23,48]</sup>: The insulinotropic role of whey and its beneficial effect on body weight and fat; the favorable effects of amino acids, medium chain fatty acids, calcium and other minerals found in milk and its derivatives; improvements in insulin sensitivity due to medium chain fatty acids; reductions in the absorption of cholesterol and other fats from fermented products; the probiotic bacteria present in these foods and the associated proteins and peptides; and improvements in weight control, blood pressure and plasma lipids due to lactose, citrate, proteins and peptides. Specifically addressing glucose homeostasis, a hypothetical explanation is that milk and dairy consumption may be associated with an enhanced insulinaemic response, decreased glycemic fluctuations, and increased secretion of glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide<sup>[49]</sup>.

Experimental models also provide some mechanistic explanations that link dairy consumption with lower incidences of insulin resistance and diabetes<sup>[50]</sup>. Milk components such as rumenic acid, vaccenic acid, phytanic acid and its derivative pristanic acid have been demonstrated to improve insulin resistance through PPAR signalling activation in different rat models<sup>[51-54]</sup>. These findings suggest that dairy consumption could have a role in insulin resistance and NASH management.

However, in our study, there was no association between *LCT*-13910 genotype and the severity of liver histology in the NASH patients. The reason for this finding may be that the pathogenesis of NASH involves a complex multiple parallel hits process in which a number of different events may contribute to liver injury<sup>[55]</sup>. Lifestyle and genetic predisposition remain relevant disease determinants. The consumption of high-calorie diets rich in lipids results in weight gain, obesity and insulin resistance. Moreover, a diet high in carbohydrates (mainly fructose) and saturated fatty acids contributes to the production of excess free fatty acids, whose safe disposal is impaired, which results in oxidative stress and NASH<sup>[56]</sup>. Recent data have also demonstrated a potential role of the microbiota in the induction of insulin resistance and the development of NAFLD/NASH<sup>[57-59]</sup>. The major components of the gut microbiota at the phylum level are *Bacteroidetes* and *Firmicutes*<sup>[60]</sup>. It has been demonstrated that *Firmicutes* levels are elevated in obesity and related diseases, whereas *Bacteroidetes* levels are decreased, which leads to an increase in the *Firmicutes/Bacteroidetes* ratio<sup>[61,62]</sup>. Interestingly, it has been shown that lysozyme-rich milk consumption results in a decline in *Firmicutes* levels (mainly *Clostridia* spp.) and in an increase in *Bacteroidetes* levels over time<sup>[63,64]</sup>. Despite the absence of high levels of lysozyme in the milk of dairy animals, these studies highlighted the potential role of milk and its components in the composition of the microbiome in health and disease.

The main limitations of our study are the lack of alimentary reports from the NAFLD patients to quantify the dairy intakes and the absence of ethnic data because



the prevalence of LCT-13910C>T polymorphisms may vary widely, as has been previously demonstrated<sup>[12]</sup>.

In conclusion, we demonstrate that hypolactasia (*i.e.*, the LCT-13910CC genotype) is associated with a higher insulin resistance frequency in NASH patients. However, further studies that include dairy ingestion reports are needed to elucidate the associations of the lactase-persistence phenotype with MetS and NAFLD/NASH in different populations.

## COMMENTS

### Background

Non-alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of liver damage that ranges from steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis in persons without significant alcohol consumption and has a close relationship with metabolic syndrome (MetS). The lactase gene (LCT)-13910C>T polymorphism located upstream of the LCT is tightly associated with lactase persistence. The LCT-13910CT and LCT-13910TT genotypes are associated with the lactase-persistence phenotype, *i.e.*, they render a person a lactose digester, whereas the LCT-13910CC genotype is associated with lactose malabsorption.

### Research frontiers

The role of milk in MetS is not currently clearly defined, and the literature is controversial. Moreover, to our knowledge, there are no published data regarding the LCT-13910C>T polymorphism in patients with NAFLD. Therefore, the authors assessed the expression profile of LCT-13910 genotypes in Brazilian patients with NAFLD in comparison with those of healthy controls to investigate whether the LCT-13910C>T variant could be a predictor of NASH. Furthermore, in NASH patients, the authors analyzed the associations of the lactase-persistence genotype with the results of biochemical tests, components of MetS and the severity of liver histology.

### Innovations and breakthroughs

The authors were unable to find any differences in the LCT-13910C>T polymorphism expression profiles between Brazilian NAFLD patients and healthy controls. Moreover, the presence of the T allele was not able to discriminate steatosis from NASH in NAFLD patients. However, in NASH patients, the hypolactasia phenotype (*i.e.*, the LCT-13910CC genotype) was associated with insulin resistance, and conversely, the LCT-13910CT genotype conferred protection against its occurrence.

### Applications

Specific pharmacological therapy for NASH is still lacking, so the pursuit of high-risk individuals can be a strategy for concentrating efforts on its diagnosis and management. Dairy consumption appears to modulate the metabolic profile because hypolactasia was found to be an independent risk factor for insulin resistance in NASH patients. Further studies that include dairy ingestion reports are needed to elucidate the associations of the lactase-persistence phenotype with MetS and NAFLD/NASH in different populations.

### Terminology

NAFLD: Non-alcoholic fatty liver disease, which encompasses a wide spectrum of liver damage that ranges from steatosis to NASH and cirrhosis in persons without significant alcohol consumption. The MetS components include the following: Fasting glucose  $\geq 110$  mg/dL, triglyceride  $\geq 150$  mg/dL, high-density lipoprotein  $< 40$  mg/dL in men or  $< 50$  mg/dL in women,  $\geq 130$  mmHg systolic or  $\geq 85$  mmHg diastolic pressure and abdominal obesity. The LCT-13910CT and LCT-13910TT genotypes are associated with the lactase-persistence phenotype, *i.e.*, these genotypes render a person a lactose digester, whereas the LCT-13910CC genotype is associated with hypolactasia, *i.e.*, lactose malabsorption.

### Peer-review

The paper indicated that among nonalcoholic steatohepatitis patients, hypo-

lactasia is associated with insulin resistance in Brazil. It is a very interesting and well-written paper.

## REFERENCES

- 1 **Ratziu V**, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010; **53**: 372-384 [PMID: 20494470 DOI: 10.1016/j.jhep.2010.04.008]
- 2 **Farrell GC**, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**: S99-S112 [PMID: 16447287 DOI: 10.1002/hep.20973]
- 3 **Younossi ZM**, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, Srishord M. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin Gastroenterol Hepatol* 2011; **9**: 524-530.e1; quiz e60 [PMID: 21440669 DOI: 10.1016/j.cgh.2011.03.020]
- 4 **Gaggini M**, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. *Nutrients* 2013; **5**: 1544-1560 [PMID: 23666091 DOI: 10.3390/nu5051544]
- 5 **Wong RJ**, Ahmed A. Obesity and non-alcoholic fatty liver disease: Disparate associations among Asian populations. *World J Hepatol* 2014; **6**: 263-273 [PMID: 24868320 DOI: 10.4254/wjh.v6.i5.263]
- 6 **Bettermann K**, Hohensee T, Haybaeck J. Steatosis and steatohepatitis: complex disorders. *Int J Mol Sci* 2014; **15**: 9924-9944 [PMID: 24897026 DOI: 10.3390/ijms15069924]
- 7 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]
- 8 **Nascimbeni F**, Pais R, Bellentani S, Day CP, Ratziu V, Loria P, Lonardo A. From NAFLD in clinical practice to answers from guidelines. *J Hepatol* 2013; **59**: 859-871 [PMID: 23751754 DOI: 10.1016/j.jhep.2013.05.044]
- 9 **Mattar R**, de Campos Mazo DF, Carrilho FJ. Lactose intolerance: diagnosis, genetic, and clinical factors. *Clin Exp Gastroenterol* 2012; **5**: 113-121 [PMID: 22826639 DOI: 10.2147/CEG.S32368]
- 10 **Ingram CJ**, Mulcare CA, Itan Y, Thomas MG, Swallow DM. Lactose digestion and the evolutionary genetics of lactase persistence. *Hum Genet* 2009; **124**: 579-591 [PMID: 19034520 DOI: 10.1007/s00439-008-0593-6]
- 11 **Enattah NS**, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Järvelä I. Identification of a variant associated with adult-type hypolactasia. *Nat Genet* 2002; **30**: 233-237 [PMID: 11788828 DOI: 10.1038/ng826]
- 12 **Mattar R**, Monteiro MS, Villares CA, Santos AF, Silva JM, Carrilho FJ. Frequency of LCT -13910C & gt; T single nucleotide polymorphism associated with adult-type hypolactasia/lactase persistence among Brazilians of different ethnic groups. *Nutr J* 2009; **8**: 46 [PMID: 19799794 DOI: 10.1186/1475-2891-8-46]
- 13 **Almon R**, Engfeldt P, Tysk C, Sjöström M, Nilsson TK. Prevalence and trends in adult-type hypolactasia in different age cohorts in Central Sweden diagnosed by genotyping for the adult-type hypolactasia-linked LCT -13910C & gt; T mutation. *Scand J Gastroenterol* 2007; **42**: 165-170 [PMID: 17327935 DOI: 10.1080/00365520600825257]
- 14 **Khabarova Y**, Torniaainen ST, Nurmi HA, Järvelä IE, Isokoski MK, Mattila KJ. Prevalence of lactase persistent/non-persistent genotypes and milk consumption in a young population in north-west Russia. *World J Gastroenterol* 2009; **15**: 1849-1853 [PMID: 19370782 DOI: 10.3748/wjg.15.1849]
- 15 **Corella D**, Arregui M, Coltell O, Portolés O, Guillem-Sáiz P, Carrasco P, Sorlí JV, Ortega-Azorín C, González JI, Ordovás JM. Association of the LCT-13910C & gt; T polymorphism with obesity and its modulation by dairy products in a Mediterranean population. *Obesity* (Silver Spring) 2011; **19**: 1707-1714 [PMID: 21193851]

DOI: 10.1038/oby.2010.320]

- 16 **Kettunen J**, Silander K, Saarela O, Amin N, Müller M, Timpson N, Surakka I, Ripatti S, Laitinen J, Hartikainen AL, Pouta A, Lahermo P, Anttila V, Männistö S, Jula A, Virtamo J, Salomaa V, Lehtimäki T, Raitakari O, Gieger C, Wichmann EH, Van Duijn CM, Smith GD, McCarthy MI, Järvelin MR, Perola M, Peltonen L. European lactase persistence genotype shows evidence of association with increase in body mass index. *Hum Mol Genet* 2010; **19**: 1129-1136 [PMID: 20015952 DOI: 10.1093/hmg/ddp561]
- 17 **Almon R**, Alvarez-Leon EE, Engfeldt P, Serra-Majem L, Magnuson A, Nilsson TK. Associations between lactase persistence and the metabolic syndrome in a cross-sectional study in the Canary Islands. *Eur J Nutr* 2010; **49**: 141-146 [PMID: 19844753 DOI: 10.1007/s00394-009-0058-2]
- 18 **Aune D**, Norat T, Romundstad P, Vatten LJ. Dairy products and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. *Am J Clin Nutr* 2013; **98**: 1066-1083 [PMID: 23945722 DOI: 10.3945/ajcn.113.059030]
- 19 **Kalergis M**, Leung Yinko SS, Nedelcu R. Dairy products and prevention of type 2 diabetes: implications for research and practice. *Front Endocrinol (Lausanne)* 2013; **4**: 90 [PMID: 23888154 DOI: 10.3389/fendo.2013.00090]
- 20 **Hirahatake KM**, Slavin JL, Maki KC, Adams SH. Associations between dairy foods, diabetes, and metabolic health: potential mechanisms and future directions. *Metabolism* 2014; **63**: 618-627 [PMID: 24636056 DOI: 10.1016/j.metabol.2014.02.009]
- 21 **Nicklas TA**, Qu H, Hughes SO, He M, Wagner SE, Foushee HR, Shewchuk RM. Self-perceived lactose intolerance results in lower intakes of calcium and dairy foods and is associated with hypertension and diabetes in adults. *Am J Clin Nutr* 2011; **94**: 191-198 [PMID: 21525197 DOI: 10.3945/ajcn.110.009860]
- 22 **Samara A**, Herbeth B, Ndiaye NC, Fumeron F, Billod S, Siest G, Visvikis-Siest S. Dairy product consumption, calcium intakes, and metabolic syndrome-related factors over 5 years in the STANISLAS study. *Nutrition* 2013; **29**: 519-524 [PMID: 23274089 DOI: 10.1016/j.nut.2012.08.013]
- 23 **Pfeuffer M**, Schrezenmeir J. Milk and the metabolic syndrome. *Obes Rev* 2007; **8**: 109-118 [PMID: 17300277 DOI: 10.1111/j.1467-789X.2006.00265.x]
- 24 **Grundy SM**, Brewer HB, Cleeman JI, Smith SC, Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004; **109**: 433-438 [PMID: 14744958 DOI: 10.1161/01.CIR.0000111245.75752.C6]
- 25 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]
- 26 **Matthews DR**, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412-419 [PMID: 3899825]
- 27 **Vasques AC**, Rosado LE, Cássia GAlfenas Rd, Geloneze B. [Critical analysis on the use of the homeostasis model assessment (HOMA) indexes in the evaluation of the insulin resistance and the pancreatic beta cells functional capacity]. *Arq Bras Endocrinol Metabol* 2008; **52**: 32-39 [PMID: 18345394 DOI: 10.1590/S0004-27302008000100006]
- 28 **Madeira IR**, Carvalho CN, Gazolla FM, de Matos HJ, Borges MA, Bordallo MA. [Cut-off point for Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index established from Receiver Operating Characteristic (ROC) curve in the detection of metabolic syndrome in overweight pre-pubertal children]. *Arq Bras Endocrinol Metabol* 2008; **52**: 1466-1473 [PMID: 19197455 DOI: 10.1590/S0004-27302008000900010]
- 29 **Miller SA**, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215 [PMID: 3344216]
- 30 **Mulcare CA**, Weale ME, Jones AL, Connell B, Zeitlyn D, Tarekegn A, Swallow DM, Bradman N, Thomas MG. The T allele of a single-nucleotide polymorphism 13.9 kb upstream of the lactase gene (LCT) (C-13.9kbT) does not predict or cause the lactase-persistence phenotype in Africans. *Am J Hum Genet* 2004; **74**: 1102-1110 [PMID: 15106124 DOI: 10.1086/421050]
- 31 **Mattar R**, Monteiro Mdo S, Villares CA, dos Santos AF, Carrilho FJ. Single nucleotide polymorphism C/T(-13910), located upstream of the lactase gene, associated with adult-type hypolactasia: validation for clinical practice. *Clin Biochem* 2008; **41**: 628-630 [PMID: 18237552 DOI: 10.1016/j.clinbiochem.2008.01.006]
- 32 **Büning C**, Genschel J, Jurga J, Fiedler T, Voderholzer W, Fiedler EM, Worm M, Weltrich R, Lochs H, Schmidt H, Ockenga J. Introducing genetic testing for adult-type hypolactasia. *Digestion* 2005; **71**: 245-250 [PMID: 16024930 DOI: 10.1159/000087050]
- 33 **Hothorn T**, Hornik K, Zeileis A. Unbiased Recursive Partitioning: A Conditional Inference Framework. *J Comput Graph Stat* 2006; **15**: 651-674 [DOI: 10.1198/106186006X133933]
- 34 **R Core Team**. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2014. Available from: URL: <http://www.R-project.org/>
- 35 **Upton J**, George P. The prevalence of lactose intolerance (adult hypolactasia) in a randomly selected New Zealand population. *N Z Med J* 2010; **123**: 123 [PMID: 20173814]
- 36 **Nagy D**, Tömöry G, Csányi B, Bogácsi-Szabó E, Czibula Á, Priskin K, Bede O, Bartosiewicz L, Downes CS, Raskó I. Comparison of lactase persistence polymorphism in ancient and present-day Hungarian populations. *Am J Phys Anthropol* 2011; **145**: 262-269 [PMID: 21365615 DOI: 10.1002/ajpa.21490]
- 37 **Enattah NS**, Trudeau A, Pimenoff V, Maiuri L, Auricchio S, Greco L, Rossi M, Lentze M, Seo JK, Rahgozar S, Khalil I, Alifrangis M, Natah S, Groop L, Shaat N, Kozlov A, Verschubskaya G, Comas D, Bulayeva K, Mehdi SQ, Terwilliger JD, Sahi T, Savilahti E, Perola M, Sajantila A, Järvelä I, Peltonen L. Evidence of still-ongoing convergence evolution of the lactase persistence T-13910 alleles in humans. *Am J Hum Genet* 2007; **81**: 615-625 [PMID: 17701907 DOI: 10.1086/520705]
- 38 **Friedrich DC**, de Andrade FM, Fiegenbaum M, de Almeida S, Mattevi VS, Callegari-Jacques SM, Hutz MH. The lactase persistence genotype is a protective factor for the metabolic syndrome. *Genet Mol Biol* 2014; **37**: 611-615 [PMID: 25505833 DOI: 10.1590/S1415-47572014005000012]
- 39 **Lamri A**, Poli A, Emery N, Bellili N, Velho G, Lantieri O, Balkau B, Marre M, Fumeron F. The lactase persistence genotype is associated with body mass index and dairy consumption in the D.E.S.I.R. study. *Metabolism* 2013; **62**: 1323-1329 [PMID: 23647908 DOI: 10.1016/j.metabol.2013.04.006]
- 40 **Enattah NS**, Forsblom C, Rasinperä H, Tuomi T, Groop PH, Järvelä I. The genetic variant of lactase persistence C (-13910) T as a risk factor for type I and II diabetes in the Finnish population. *Eur J Clin Nutr* 2004; **58**: 1319-1322 [PMID: 15054412 DOI: 10.1038/sj.ejcn.1601971]
- 41 **Vassilatou E**. Nonalcoholic fatty liver disease and polycystic ovary syndrome. *World J Gastroenterol* 2014; **20**: 8351-8363 [PMID: 25024594 DOI: 10.3748/wjg.v20.i26.8351]
- 42 **Lerchbaum E**, Giuliani A, Gruber HJ, Pieber TR, Obermayer-Pietsch B. Adult-type hypolactasia and calcium intake in polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2012; **77**: 834-843 [PMID: 22233423 DOI: 10.1111/j.1365-2265.2012.04334.x]
- 43 **Högenauer C**, Hammer HF, Mellitzer K, Renner W, Krejs GJ, Toplak H. Evaluation of a new DNA test compared with the lactose hydrogen breath test for the diagnosis of lactase non-persistence. *Eur J Gastroenterol Hepatol* 2005; **17**: 371-376 [PMID: 15716664]
- 44 **Calton EK**, James AP, Pannu PK, Soares MJ. Certain dietary patterns are beneficial for the metabolic syndrome: reviewing the evidence. *Nutr Res* 2014; **34**: 559-568 [PMID: 25150114 DOI: 10.1016/j.nutres.2014.06.012]
- 45 **Shin H**, Yoon YS, Lee Y, Kim CI, Oh SW. Dairy product intake is inversely associated with metabolic syndrome in Korean adults:

- Anseong and Ansan cohort of the Korean Genome and Epidemiology Study. *J Korean Med Sci* 2013; **28**: 1482-1488 [PMID: 24133353 DOI: 10.3346/jkms.2013.28.10.1482]
- 46 **Martins ML**, Kac G, Silva RA, Bettiol H, Barbieri MA, Cardoso VC, Silva AA. Dairy consumption is associated with a lower prevalence of metabolic syndrome among young adults from Ribeirão Preto, Brazil. *Nutrition* 2015; **31**: 716-721 [PMID: 25837218 DOI: 10.1016/j.nut.2014.12.017]
  - 47 **Crichton GE**, Alkerwi A. Dairy food intake is positively associated with cardiovascular health: findings from Observation of Cardiovascular Risk Factors in Luxembourg study. *Nutr Res* 2014; **34**: 1036-1044 [PMID: 25476191 DOI: 10.1016/j.nutres.2014.04.002]
  - 48 **Da Silva MS**, Rudkowska I. Dairy products on metabolic health: current research and clinical implications. *Maturitas* 2014; **77**: 221-228 [PMID: 24445013 DOI: 10.1016/j.maturitas.2013.12.007]
  - 49 **Visioli F**, Strata A. Milk, dairy products, and their functional effects in humans: a narrative review of recent evidence. *Adv Nutr* 2014; **5**: 131-143 [PMID: 24618755 DOI: 10.3945/an.113.005025]
  - 50 **Parodi PW**. Cooperative action of bioactive components in milk fat with PPARs may explain its anti-diabetogenic properties. *Med Hypotheses* 2016; **89**: 1-7 [PMID: 26968898 DOI: 10.1016/j.mehy.2015.12.028]
  - 51 **Belury MA**, Moya-Camarena SY, Lu M, Shi L, Leesnitzer LA, Blanchard SG. Conjugated linoleic acid is an activator and ligand for peroxisome proliferator-activated receptor-gamma (PPAR $\gamma$ ). *Nutr Res* 2002; **2002**: 817-824 [DOI: 10.1016/S0271-5317(02)00393-7]
  - 52 **Moya-Camarena SY**, Van den Heuvel JP, Belury MA. Conjugated linoleic acid activates peroxisome proliferator-activated receptor alpha and beta subtypes but does not induce hepatic peroxisome proliferation in Sprague-Dawley rats. *Biochim Biophys Acta* 1999; **1436**: 331-342 [PMID: 9989264]
  - 53 **Wang Y**, Jacome-Sosa MM, Ruth MR, Lu Y, Shen J, Reaney MJ, Scott SL, Dugan ME, Anderson HD, Field CJ, Proctor SD, Vine DF. The intestinal bioavailability of vaccenic acid and activation of peroxisome proliferator-activated receptor- $\alpha$  and - $\gamma$  in a rodent model of dyslipidemia and the metabolic syndrome. *Mol Nutr Food Res* 2012; **56**: 1234-1246 [PMID: 22714958 DOI: 10.1002/mnfr.201100517]
  - 54 **Jacome-Sosa MM**, Borthwick F, Mangat R, Uwiera R, Reaney MJ, Shen J, Quiroga AD, Jacobs RL, Lehner R, Proctor SD, Nelson RC. Diets enriched in trans-11 vaccenic acid alleviate ectopic lipid accumulation in a rat model of NAFLD and metabolic syndrome. *J Nutr Biochem* 2014; **25**: 692-701 [PMID: 24775093 DOI: 10.1016/j.jnutbio.2014.02.011]
  - 55 **Tilg H**, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 2010; **52**: 1836-1846 [PMID: 21038418 DOI: 10.1002/hep.24001]
  - 56 **Peverill W**, Powell LW, Skoien R. Evolving concepts in the pathogenesis of NASH: beyond steatosis and inflammation. *Int J Mol Sci* 2014; **15**: 8591-8638 [PMID: 24830559 DOI: 10.3390/ijms15058591]
  - 57 **Paolella G**, Mandato C, Pierri L, Poeta M, Di Stasi M, Vajro P. Gut-liver axis and probiotics: their role in non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 15518-15531 [PMID: 25400436 DOI: 10.3748/wjg.v20.i42.15518]
  - 58 **Miura K**, Ohnishi H. Role of gut microbiota and Toll-like receptors in nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 7381-7391 [PMID: 24966608 DOI: 10.3748/wjg.v20.i23.7381]
  - 59 **Imajo K**, Yoneda M, Ogawa Y, Wada K, Nakajima A. Microbiota and nonalcoholic steatohepatitis. *Semin Immunopathol* 2014; **36**: 115-132 [PMID: 24337650 DOI: 10.1007/s00281-013-0404-6]
  - 60 **Bäckhed F**, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* 2005; **307**: 1915-1920 [PMID: 15790844 DOI: 10.1126/science.1104816]
  - 61 **Mouzaki M**, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, McGilvray ID, Allard JP. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* 2013; **58**: 120-127 [PMID: 23401313 DOI: 10.1002/hep.26319]
  - 62 **Turnbaugh PJ**, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480-484 [PMID: 19043404 DOI: 10.1038/nature07540]
  - 63 **Maga EA**, Desai PT, Weimer BC, Dao N, Kültz D, Murray JD. Consumption of lysozyme-rich milk can alter microbial fecal populations. *Appl Environ Microbiol* 2012; **78**: 6153-6160 [PMID: 22752159 DOI: 10.1128/AEM.00956-12]
  - 64 **Donovan SM**, Wang M, Li M, Friedberg I, Schwartz SL, Chapkin RS. Host-microbe interactions in the neonatal intestine: role of human milk oligosaccharides. *Adv Nutr* 2012; **3**: 450S-455S [PMID: 22585924 DOI: 10.3945/an.112.001859]

**P- Reviewer:** Huang C, Qu BG **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Li D



Observational Study

## Diagnostic non-invasive model of large risky esophageal varices in cirrhotic hepatitis C virus patients

Hatem Elalfy, Walid Elsherbiny, Ashraf Abdel Rahman, Dina Elhammady, Shaker Wagih Shaltout, Ayman Z Elsamanoudy, Bassem El Deek

Hatem Elalfy, Walid Elsherbiny, Dina Elhammady, Shaker Wagih Shaltout, Tropical Medicine Department, Mansoura Faculty of Medicine, Mansoura University Hospital, Mansoura 35516, Egypt

Ashraf Abdel Rahman, Diagnostic Radiology Department, Mansoura Faculty of Medicine, Mansoura University Children Hospital, Mansoura 35516, Egypt

Ayman Z Elsamanoudy, Medical Biochemistry and Molecular Biology, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt

Bassem El Deek, Department of Community Medicine, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt

**Author contributions:** Elalfy H, Elsherbiny W, Abdel Rahman A and Shaltout SW designed the research; Elalfy H, Elsherbiny W and Shaltout SW performed patients selection and clinical evaluation with endoscopy practice; Abdel Rahman A performed and analyzed the CT; Elhammady D and Elalfy H wrote the paper; El Deek B analyzed the data; Elsamanoudy AZ performed and analyzed the laboratory tests.

**Institutional review board statement:** This study was reviewed and approved by the Ethics Committee of the Faculty of medicine, Mansoura University.

**Informed consent statement:** Written informed consent was signed by the patients for the treatment and sample usage in this study.

**Conflict-of-interest statement:** We have no financial relationships to disclose.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and

the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Dr. Hatem Elalfy, Assistant Professor, Tropical Medicine Department, Mansoura Faculty of Medicine, Mansoura University Hospital, Algomhoria Street, Mansoura 35516, Egypt. [elalfy\\_hatem66@yahoo.com](mailto:elalfy_hatem66@yahoo.com)  
**Telephone:** +20-12-24790518  
**Fax:** +20-50-2267563

**Received:** March 23, 2016

**Peer-review started:** March 24, 2016

**First decision:** May 23, 2016

**Revised:** June 4, 2016

**Accepted:** July 20, 2016

**Article in press:** July 22, 2016

**Published online:** August 28, 2016

### Abstract

#### AIM

To build a diagnostic non-invasive model for screening of large varices in cirrhotic hepatitis C virus (HCV) patients.

#### METHODS

This study was conducted on 124 post-HCV cirrhotic patients presenting to the clinics of the Endemic Medicine Department at Mansoura University Hospital for evaluation before HCV antiviral therapy: 78 were Child A and 46 were Child B (score  $\leq 8$ ). Inclusion criteria for patients enrolled in this study was presence of cirrhotic HCV (diagnosed by either biopsy or fulfillment of clinical basis). Exclusion criteria consisted of patients with other etiologies of liver cirrhosis, *e.g.*, hepatitis B virus and patients with high MELD score on transplant list. All patients were subjected to full medical record, full basic investigations, endoscopy, and computed tomography



(CT), and then divided into groups with no varices, small varices, or large risky varices. In addition, values of Fibrosis-4 score (FIB-4), aminotransferase-to-platelet ratio index (APRI), and platelet count/splenic diameter ratio (PC/SD) were also calculated.

### RESULTS

Detection of large varices is a multi-factorial process, affected by many variables. Choosing binary logistic regression, dependent factors were either large or small varices while independent factors included CT variables such as coronary vein diameter, portal vein (PV) diameter, lieno-renal shunt and other laboratory non-invasive variables namely FIB-4, APRI, and platelet count/splenic diameter. Receiver operating characteristic (ROC) curve was plotted to determine the accuracy of non-invasive parameters for predicting the presence of large esophageal varices and the area under the ROC curve for each one of these parameters was obtained. A model was established and the best model for prediction of large risky esophageal varices used both PC/SD and PV diameter (75% accuracy), while the logistic model equation was shown to be  $(PV \text{ diameter} \times -0.256) \text{ plus } (PC/SD \times -0.006) \text{ plus } (8.155)$ . Values nearing 2 or more denote large varices.

### CONCLUSION

This model equation has 86.9% sensitivity and 57.1% specificity, and would be of clinical applicability with 75% accuracy.

**Key words:** Diagnostic model; Large varices; Cirrhotic hepatitis C virus; Computed tomography; Noninvasive variceal diagnosis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatitis C virus infection is a major global health problem, with over 14% of the Egyptian population currently infected. End-stage liver disease with cirrhosis is commonly complicated by potentially life-threatening esophageal varices, which require regular screening by endoscopy. However, this invasive procedure is burdened by patient non-compliance and possible complications, thus prompting the search for alternative non-invasive yet accurate means of diagnosis. This study group aimed to assess the use of computed tomography to evaluate and grade variceal size, and to compare its diagnostic value with other non-invasive predictors of portal hypertension, such as platelet count to splenic diameter ratio, aminotransferase-to-platelet ratio index, and Fibrosis-4 score.

Elalfy H, Elsherbiny W, Abdel Rahman A, Elhammady D, Shaltout SW, Elsamanoudy AZ, El Deek B. Diagnostic non-invasive model of large risky esophageal varices in cirrhotic hepatitis C virus patients. *World J Hepatol* 2016; 8(24): 1028-1037 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i24/1028.htm> DOI: <http://dx.doi.org/10.4254/wjgh.v8.i24.1028>

## INTRODUCTION

Hepatitis C virus (HCV) represents one of the major health problems affronting the medical community today, with chronic HCV infection affecting approximately 130-170 million people globally, or about 2%-3% of the world's population<sup>[1]</sup>. The largest HCV epidemic is currently found in Egypt, with an estimated national prevalence reported to be 14.7%<sup>[2]</sup>. As with any chronic liver disease, the end stage of chronic HCV infection is cirrhosis, ultimately complicated by portal hypertension, an established contributing factor in the evolution of a variety of complications of cirrhosis including ascites, hepatic encephalopathy, and esophageal varices<sup>[3]</sup>.

Portal hypertension generates development of porto-systemic collaterals, giving rise to esophageal varices (OV), most notably gastroesophageal varices because of their enhanced tendency for bleeding<sup>[4]</sup>. Esophageal varices can be found in 60%-80% of cirrhotic patients<sup>[5]</sup>, with variceal hemorrhage presenting as the most devastating complication of cirrhosis. Because of this dramatic course of events, it is imperative to prevent variceal bleeding either with non-selective beta-blockers or endoscopic variceal ligation<sup>[6]</sup>. However, in spite of recent progress, mortality rate due to bleeding from ruptured esophageal varices remains between 10%-20%<sup>[7]</sup>.

Current guidelines advocate screening for esophageal varices in all cirrhotic patients at the time of diagnosis. Lack of detection of esophageal varices at the first endoscopic evaluation warrants repeat endoscopy annually in patients with decompensated liver cirrhosis and every 2-3 years in patients with compensated cirrhosis<sup>[8]</sup>. Although upper endoscopy is regularly performed and conveys a diminished risk of adverse effects<sup>[9]</sup>, repeated endoscopies are associated with several side effects including aspiration, perforation, and bacteremia<sup>[10]</sup>. Furthermore, these recommendations impose a huge burden on medical resources and branch from expert assumption rather than being evidence-based. In addition to the invasive nature of the procedure and lack of patient compliance restricting its use, there is also a cost-ineffectiveness of this policy in lack of actual detection of varices in many of the patients<sup>[11]</sup>. These considerations have spurred several attempts to identify non-invasive clinical, radiological, and biochemical parameters, used either separately or in conjunction, to determine the presence of portal hypertension and esophageal varices.

Perhaps the best predictor of esophageal varices developed to date is the platelet count to splenic diameter ratio, which proposes linking thrombocytopenia to spleen size by considering that diminished platelet count is probably the result of hypersplenism due to splenomegaly caused by portal hypertension<sup>[12]</sup>. Other parameters have attempted to determine the state of liver tissue with good accuracy by evaluating the extent of fibrosis and cirrhosis as a predictive indicator of progression of portal hypertension, these including

aminotransferase-to-platelet ratio index (APRI), Fibro-index, and Fibrosis-4 score (FIB-4)<sup>[13]</sup>.

Several radiological techniques have also been suggested for evaluation of esophageal varices. Doppler ultrasonography has been used for investigating portal and hepatic hemodynamics but its value in assessment of portal hypertension remains obscure. Although several indices for portal hypertension have been commonly used, inaccuracy remains due to fluctuating variations related to both observer and equipment<sup>[14]</sup>.

Computed tomography (CT) has also been proposed as an evaluation tool for esophageal varices<sup>[15]</sup>. Examination of the correlation between CT findings and endoscopy from previous studies has shown better agreement between variceal size and radiological assessment than with endoscopic interpretation<sup>[16]</sup>. In addition, CT was found to be more desirable in initial screening of esophageal varices in comparison to endoscopy when considering patient preference and cost-effectiveness<sup>[17]</sup>.

Therefore, considering these findings, we aimed to evaluate the use of CT in the diagnosis of esophageal varices, differentiating between small and large varices, and assessing its use in grading the size of varices. In addition, we aimed to compare the value of CT in diagnosis of esophageal varices with other non-invasive predictors of portal hypertension including laboratory indices such as platelet count to splenic diameter ratio, APRI, and FIB-4.

The objective of the study was to build a diagnostic non-invasive model for screening of large esophageal varices in cirrhotic HCV patients.

## MATERIALS AND METHODS

### Ethical approval

Informed consent was taken from each patient. The research protocol was approved by the Ethical Committee of Faculty of Medicine, Mansoura University.

### Study design

This comparative cross sectional study included subjects presenting to the Endemic Medicine Department clinic at Mansoura University Hospital for evaluation before HCV antiviral therapy during the period between December 2014 and June 2015. Inclusion criteria for patients enrolled in this study was presence of cirrhotic HCV as diagnosed either by biopsy (F4) or on the basis of clinical evaluation combined with laboratory findings and ultrasonography. Exclusion criteria consisted of patients with other etiologies of liver cirrhosis or those ineligible for the HCV therapy program, *e.g.*, HBV, Child C decompensated patients, and patients with high MELD score on transplant list. Patients with liver cirrhosis were then stratified according to endoscopic findings into groups with either no varices, small varices, or large varices.

The indication for CT imaging in the majority of cases was for evaluation of focal lesions for hepatocellular

carcinoma while the entire laboratory assessment was done as a part of the HCV therapeutic evaluation program.

### Clinical and laboratory workup

All subjects were HCV infected and thus subjected to complete laboratory assessment before antiviral therapy, including complete blood picture, PCR for HCV, alpha fetal protein (AFP), alanine and aspartate transaminases, albumin, bilirubin, INR, creatinine, TSH, as well as abdominal ultrasound and biopsy in selected cases.

Those with findings of F4 on biopsy or showed clinical, laboratory, or ultrasonographic features of cirrhosis were selected for this study, to be then classified into case and control groups.

Cases were patients with post-HCV liver cirrhosis with esophageal varices on endoscopy, divided into two groups with either small or large varices, while the control group was patients with post-HCV liver cirrhosis without varices.

### Gastroscopy for varices evaluation and therapy

Using slight sedation with IV midazolam administered just before the session, patients were stratified by risk of first variceal hemorrhage into either high-risk patients, *i.e.*, those with medium/large varices, or low risk patients, *i.e.*, those with small varices occurring in a Child A or B patient. Trials have shown that patients with medium/large varices can be treated with either non-selective  $\beta$ -blockers (propranolol, nadolol) or esophageal band ligation.

### Calculation of non-invasive parameters (APRI, FIB4, platelet count/splenic diameter)

$APRI = \{[AST \text{ Level (IU/L)}] / [AST \text{ (upper limit of normal) (IU/L)}] \times 100\} / \text{Platelet count (} 10^9/\text{L)}^{[18]}$

$FIB4 = [\text{age (years)} \times AST \text{ (IU/L)}] / [\text{PLT (} \times 10^9/\text{L)}] \times [\sqrt{ALT \text{ (IU/L)}}]^{[19,20]}$

$\text{Platelet count (PC) to spleen diameter (SD) ratio} = \text{PC (N}/\mu\text{L)} / \text{the maximum bipolar diameter of the spleen (mm)}^{[21]}$

These parameters were selected based on the criteria of being simple routine laboratory tests that are also inexpensive.

### Multi-slice detector CT

For all patients, multi-slice detector CT (MDCT) scan of the abdomen and pelvis was performed on a 16-MDCT scanner (Brilliance, Philips) using a tube collimation of 16 mm  $\times$  1.5 mm with overlapping reconstruction at 2 mm slice thickness and 0.8 mm increment.

Examination was carried out using a multiphasic liver protocol starting with non-contrast examination. Arterial phase examination was carried out using bolus tracking technique and post-threshold delay of 12 s. Low osmolar iodinated intravenous contrast [Omnipaque™ (iohexol) 350, GE Healthcare] was injected using a power injector [MEDRAD Vistron CT® Injector, Medrad] administered in a dose of 1.5 mL/kg at a flow rate of 4-5 mL/s. Portal phase examination was carried out 40 s after threshold

and delayed phase examination after 5 min.

Images were reviewed on a dedicated workstation (Extended brilliance work space, Philips) in axial, coronal and sagittal planes. Images were evaluated for the following parameters: Maximum short axis diameter of the largest visible esophageal varix, diameter of coronary vein, diameter of the paraumbilical vein, maximum short axis diameter of the portal vein at the portahepatis, presence of ascites, and maximum height of the spleen. An esophageal varix was defined as an enhancing intramural nodular tubular structure (which may be bulging into the lumen of the esophagus or runs within the inner esophageal mucosa).

### Statistical analysis

Data were statistically analyzed using the Statistical Package for Social Science (SPSS) version 20. The quantitative data were presented in the form of mean and standard deviation. One-way Anova was used to compare between the three groups.  $\chi^2$  test was used to compare the qualitative data. Receiver operating curve (ROC) was done to determine a cut-off point predicting large varices. Logistic regression was done to construct a model for predicting the occurrence of large varices. Significance was considered at *P* value of 0.05.

## RESULTS

### Patient characteristics

A total of 124 patients with hepatic cirrhosis were included in this study. The mean age of the included patients were  $56.52 \pm 5.759$  (range 37-66) years with 26 patients (52%) being males. The etiology of cirrhosis in all included patients was HCV. Most patients (59.7%) had esophageal varices and 50 patients (40.3%) had no varices. According to gastroscopy, among those who had esophageal varices, 28 patients (22.6%) were classified as having small varices and 46 patients (37.1%) had large varices. According to Child-Turcotte-Pugh Classification, 78 patients (62.9%) were classified as class A, proven by liver biopsy and 46 (37.1%) as class B. There were 34 patients with diuretic responsive ascites (27.5%) and 90 patients (72.5%) without ascites. Ten patients (8.06%) had hepatocellular carcinoma less than 3 cm (Table 1).

### Comparison between non-invasive parameters in the studied groups

The values of Fib4 and APRI were significantly higher in cirrhotic patients with large esophageal varices than those in cirrhotic patients without varices or with small esophageal varices (*P* = 0.001). Comparison of values of the PC/SD ratio between groups demonstrated a significant decrease in cirrhotic patients with large esophageal varices in comparison to cirrhotic patients without varices or with small esophageal varices (*P* = 0.001).

Regarding the values of CT parameters, there was a demonstrable difference between groups, as the

cirrhotic patients with esophageal varices had higher values of portal vein diameter (PVD) and splenic vein diameter (SVD) than cirrhotic patients without varices (*P* = 0.012 vs 0.284, respectively). A coronary vein threshold  $\geq 7$  mm as measured by CT was present in 16 of these cirrhotic patients (12.7%), of which 4 patients were without varices, 4 patients had small varices, and 8 patients had large varices (*P* = 0.026). While the measurement of lieno-renal shunt by CT was  $\geq 12$  mm, there were 8 cirrhotic patients (6.45%) without esophageal varices (*P* = 0.006). In addition, CT significantly differentiated between presence and absence of OV. When CT reported that there were no variceal findings in 50 patients (40.3%), 46 of these were actually without varices and 4 patients had esophageal varices; CT indication of varices in 74 patients (59.7%) was confirmed in 70 of these patients who actually had OV (*P* = 0.001) (Table 2).

### Non-invasive prediction of large risky esophageal varices

ROC curve was plotted to determine the accuracy of non-invasive parameters for predicting the presence of large esophageal varices rather than presence of varices and the area under the ROC curve for each one of these parameters was obtained. A FIB-4  $\geq 3.13$  had a sensitivity of 71.7% and a specificity of 50% with an area under the ROC curve of 0.585 (95%CI: 0.442-0.728). The area under the ROC curve for APRI was 0.558 (95%CI: 0.417-0.699). An APRI value of  $\geq 1.083$  had a sensitivity of 63% and specificity of 46.4%. A PC/SD ratio of  $\leq 806.93$  had 75% sensitivity and 47.8% specificity, with the area under the ROC curve being 0.558 (95%CI: 0.417-0.699). In addition, the PVD as measured by CT had a sensitivity of 71.1% and specificity of 37% at cutoff  $\geq 12.5$  mm with an area under the ROC curve of 0.560 (95%CI: 0.425-0.630) (Table 3). ROC curves are demonstrated in Figure 1.

### Model for detecting large risky varices

The detection of large risky esophageal varices on the verge of rupture is a multi-factorial process affected by many variables. Statistically, the research team chose to use binary logistic regression. Dependent factors were either large or small varices while the independent factors measured by CT were coronary vein diameter, PVD and lieno-renal shunt in addition to various laboratory parameters including FIB-4, APRI, and PC/SD. The accuracy of this model was about 62.2%. After removal of insignificant predictors, *i.e.*, APRI, FIB-4, coronary vein diameter, and lieno-renal shunt, the accuracy of the model becomes 75%. If only PC/SD was used, the accuracy was 73%, while use of both PC/SD and PVD raised the accuracy to 75.7% (Tables 4-6).

## DISCUSSION

A major health problem facing the medical community

**Table 1** Patients characteristics *n* (%)

Variables	All patients ( <i>n</i> = 124)	No varices ( <i>n</i> = 50)	Small varices ( <i>n</i> = 28)	Large varices ( <i>n</i> = 46)	<i>P</i> value
Age, mean ± SD	56.52 ± 5.759	57.28 ± 4.513	58.57 ± 3.072	54.43 ± 7.423	0.005
Gender					
Female	52 (41.9)	24 (48.0)	12 (42.9)	16 (34.8)	0.421
Male	72 (58.1)	26 (52)	16 (57.1)	30 (65.2)	
Laboratory data (mean ± SD)					
Serum albumin (g/dL)	3.329 ± 0.43	3.452 ± 0.404	3.271 ± 0.352	3.229 ± 0.474	0.028
Serum bilirubin (mg/dL)	1.312 ± 0.572	1.093 ± 0.534	1.414 ± 0.591	1.489 ± 0.532	0.001
INR	1.326 ± 0.238	1.249 ± 0.225	1.431 ± 0.24	1.347 ± 0.226	0.003
Serum creatinine (mg/dL)	0.999 ± 0.179	0.956 ± 0.169	0.994 ± 0.178	1.047 ± 0.180	0.044
AST (IU/L)	68.00 ± 40.086	65.84 ± 44.072	63.71 ± 22.993	72.96 ± 43.797	0.561
ALT (IU/L)	55.18 ± 23.607	51.84 ± 19.844	57.43 ± 24.848	57.43 ± 26.519	0.436
Platelet count (cells/mm <sup>3</sup> )	152.94 ± 22.012	145.96 ± 20.833	156.5 ± 27.941	1548.35 ± 17.071	0.001
AFP (ng/mL)	2.687 ± 2.687	3.646 ± 60.527	5.868 ± 1.854	3.274 ± 811.945	0.064
Clinical data					
Spleen size (mm)	152.94 ± 22.012	145.96 ± 20.833	156.5 ± 27.941	158.35 ± 17.071	0.013
Presence of ascites					0.017
Mild (respo-nsive)	34 (27.5)	6 (12)	8 (28.5)	20 (43.4)	
No	90 (72.6)	44 (88)	20 (71.4)	26 (56.5)	
MELD score (mean ± SD)	1.103 ± 2.828	9.852 ± 2.480	1.2 ± 2.55	1.171 ± 2.942	0.001
History of encephalo-lopathy					
No	124 (100)	50 (100)	28 (100)	46 (100)	
Yes	0 (0)	0 (0)	0 (0)	0 (0)	
Child Pugh Classification					
A	78 (62.9)	42 (84.3)	18 (64.3)	18 (39.1)	0.001
B	46 (37.1)	8 (16)	10 (35.7)	28 (60.9)	

AST: Aspartate transaminase; ALT: Alanine aminotransferase; AFP: Alpha fetal protein.

**Table 2** Comparison of multiple variables between patients groups (mean ± SD) *n* (%)

Variables	All patients ( <i>n</i> = 124)	No varices ( <i>n</i> = 50)	Small varices ( <i>n</i> = 28)	Large varices ( <i>n</i> = 46)	<i>P</i> value
FIB-4	5.526 ± 3.239	4.432 ± 2.334	4.814 ± 2.457	7.149 ± 3.844	0.001
APRI	1.836 ± 1.256	1.408 ± 0.84	1.606 ± 1.02	2.442 ± 1.519	0.001
PC/SD	722.235 ± 316.5	891.133 ± 317.027	765.016 ± 326.324	512.611 ± 150.784	0.001
PVD by CT	14.116 ± 2.967	13.142 ± 2.959	14.929 ± 3.366	14.639	0.012
SVD by CT	10.903 ± 2.857	10.42 ± 2.989	11.071 ± 3.62	11.326 ± 2.063	0.284
Coronary vein ≥ 7 mm by CT	16 (12.7)	4 (8.0)	4 (14.29)	8 (17.39)	0.026
Lienorenal shunt ≥ 12 mm by CT	8 (6.45)	8 (28.57)	0 (0)	0 (0)	0.006
Presence of varices in CT					0.001
Yes	74 (59.7)	4 (8.0)	28 (100)	42 (91.3)	
No	50 (40.3)	46 (92.0)	0 (0)	4 (8.7)	

FIB-4: Fibrosis-4 score; APRI: Aminotransferase-to-platelet ratio index; PC/SD: Platelet count/splenic diameter ratio; PVD: Portal vein diameter; SVD: Splenic vein diameter; CT: Computed tomography.

**Table 3** Sensitivity and specificity of noninvasive parameters

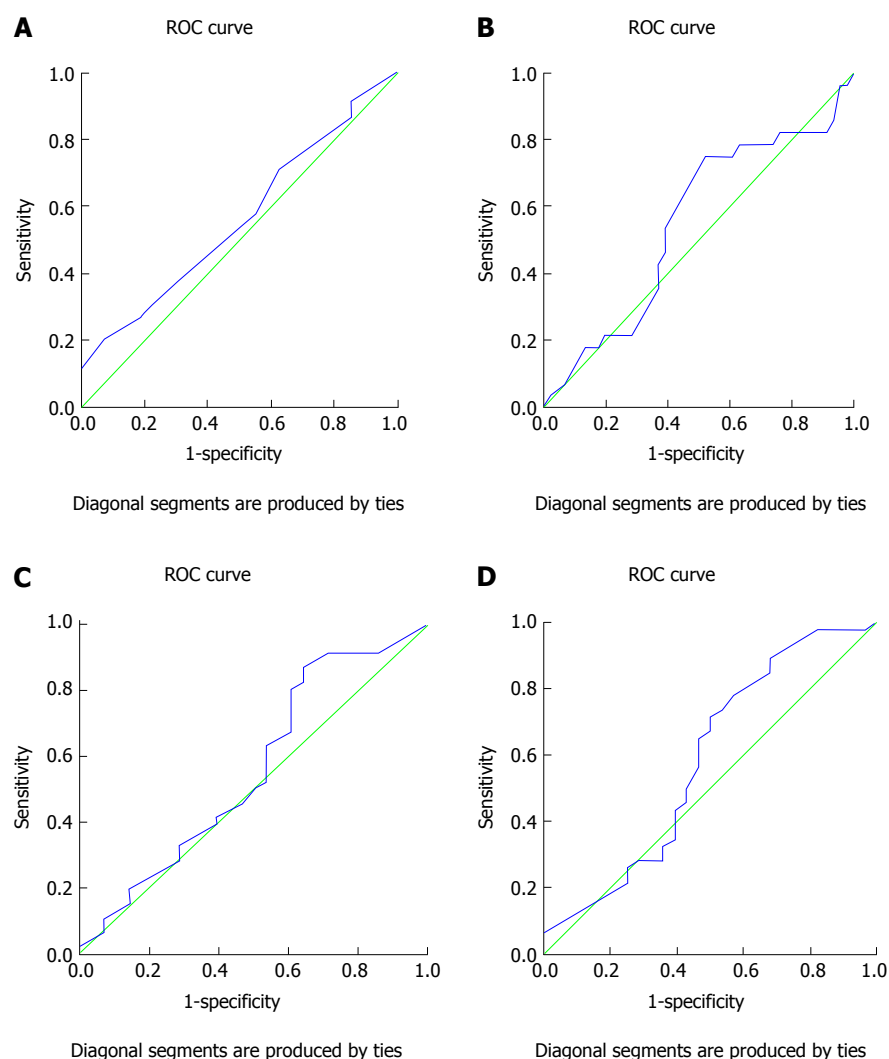
Parameters	Sensitivity	Specificity	AUC	95%CI	Cut-off	Significance
FIB-4	71.70%	50%	0.585	0.442-0.728	3.13	0.222
APRI	63%	46.40%	0.558	0.417-0.699	1.083	0.406
PC/SD	75%	47.80%	0.550	0.412-0.688	806.93	0.472
PVD by CT	71%	37%	0.560	0.425-0.695	12.5	0.396

FIB-4: Fibrosis-4 score; APRI: Aminotransferase-to-platelet ratio index; PC/SD: Platelet count/splenic diameter ratio; PVD: Portal vein diameter; CT: Computed tomography; AUC: Area under the curve.

today is chronic HCV infection, which affects about 2%-3% of the global population, or from 130-170 million people worldwide<sup>[1]</sup>, with Egypt currently bearing the largest HCV epidemic which affects 14.7% of its national population<sup>[2]</sup>. Cirrhosis represents the end-stage of chronic liver dis-

ease, ultimately complicated by portal hypertension<sup>[3]</sup>, the main inducing factor in the formation of esophageal varices<sup>[4]</sup>, found in more than half (60%-80%) of cirrhotic patients<sup>[5]</sup>. These varices are ensuingly prone to consequent rupture and bleeding, with a devastatingly high





**Figure 1** Receiver operating characteristic curve of portal vein diameter (A), platelet counts/splenic diameter ratio (B), aminotransferase-to-platelet ratio index (C), and Fibrosis-4 score (D). ROC: Receiver operating characteristic.

**Table 4** Diagnostic model of large varices

Variables in the Equation		B	SE	Wald	df	Sig.	Exp (B)	95.0%CI for Exp (B)	
								Lower	Upper
Step 1 <sup>1</sup>	APRI	-0.444	0.813	0.298	1	0.585	0.641	0.130	3.155
	FIB-4	0.236	0.340	0.484	1	0.487	1.267	0.651	2.465
	PVD by CT	-0.257	0.123	4.398	1	0.036	0.773	0.608	0.983
	PC/SD	-0.006	0.002	8.327	1	0.004	0.994	0.990	0.998
	Coronary vein diameter by CT	-0.853	0.687	1.544	1	0.214	0.426	0.111	1.637
	Lieno-renal vein diameter by CT	-0.747	0.805	0.860	1	0.354	0.474	0.098	2.297
	Constant	10.125	3.838	6.959	1	0.008	2.497E4		

<sup>1</sup>Variable(s) entered: APRI, FIB-4, PVD by CT, PC/SD, coronary vein by CT, lien-renal shunt diameter by CT. FIB-4: Fibrosis-4 score; APRI: Aminotransferase-to-platelet ratio index; PC/SD: Platelet count/splenic diameter ratio; PVD: Portal vein diameter; CT: Computed tomography; Exp: Exponential; SE: Standard error.

mortality rate of 10%-20%<sup>[7]</sup>.

Current guidelines advocate screening for esophageal varices using endoscopy in all cirrhotic patients at the time of diagnosis<sup>[8]</sup>. However, the invasive nature and subsequent complications associated with this maneuver have prompted the search for further accurate and

non-invasive techniques to evaluate the presence of esophageal varices resulting from portal hypertension in these cirrhotic patients.

Founded on the basis that liver fibrosis is the primary factor enhancing hepatic resistance resulting in portal hypertension, use of non-invasive serum markers of liver

**Table 5** Diagnostic model of large varices

Variables in the equation		B	SE	Wald	df	Sig.	Exp (B)	95%CI for Exp (B)	
								Lower	Upper
PC/SD <sup>1</sup>	PC/SD	-0.005	0.001	10.721	1	0.001	0.995	0.992	0.998
	Constant	3.457	0.926	13.935	1	0.000	31.707		
PVD by CT and PC/SD <sup>2</sup>	PVD by CT	-0.256	0.116	4.879	1	0.027	0.774	0.617	0.972
	PC/SD	-0.006	0.002	13.057	1	0.000	0.994	0.990	0.997
	Constant	8.155	2.465	10.942	1	0.001	3.480E3		

<sup>1</sup>Variable entered: PC/SD; <sup>2</sup>Variable entered: PVD by CT and PC/SD. PC/SD: Platelet count/splenic diameter ratio; PVD: Portal vein diameter; CT: Computed tomography; Exp: Exponential; SE: Standard error.

**Table 6** Diagnostic model of large varices

Classification table <sup>1</sup>					
Observed			Predicted		
			ROC size		Percentage correct
			1	2	
PC/SD	ROC size	1	12	16	42.9%
		2	4	42	91.3%
	Overall percentage				73%
PC/SD plus PVD by CT	ROC size	1	16	12	57.1%
		2	6	40	87%
	Overall percentage				75.7%

<sup>1</sup>The cut-off value is 0.500. PC/SD: Platelet count/splenic diameter ratio; PVD: Portal vein diameter; CT: Computed tomography; ROC: Receiver operating characteristic curve.

fibrosis has shown favorable outcomes when predicting presence of esophageal varices<sup>[21]</sup>. Expected findings from previous studies have demonstrated that scores of FIB-4 and APRI were significantly higher in cirrhotic patients with or without portal hypertension when compared to healthy volunteers or patients with chronic liver disease<sup>[3]</sup>. In our study, significantly higher values of FIB-4 and APRI were also found in cirrhotic patients with large esophageal varices in comparison to those without varices or with small esophageal varices ( $P = 0.001$ ).

Although several studies have previously demonstrated a strong relation between platelet count and splenic diameter with presence of esophageal varices<sup>[22,23]</sup>, the decreased platelet count present in chronic liver disease may be the result of several factors other than portal hypertension, including diminished mean platelet life span, reduced production of thrombopoietin, or myelotoxic effects of hepatitis viruses<sup>[24]</sup>. An additional proposed underlying mechanism of "platelet exhaustion" states that hyperdynamic circulation causes platelet damage during intravascular activation with consequent hypofunction. However, the presence of splenomegaly in patients with cirrhosis is, in all likelihood, derived from vascular derangement mainly resulting from portal hypertension<sup>[25]</sup>.

Consequently, Giannini *et al*<sup>[26]</sup> aimed to chart a new parameter bridging thrombocytopenia to splenomegaly so as to originate a variable that takes into account the diminished platelet count probably due to hypersplenism attributed to portal hypertension. A study performed by Giannini *et al* demonstrated that a PC/SD ratio cutoff

< 909 had a positive predictive value of 96% and negative predictive value of 100%<sup>[26]</sup>. These data have been subsequently confirmed in a number of recent studies<sup>[27-30]</sup>; however, these studies focused mainly on presence of varices as a whole.

In the current study, our main target was detection of large risky varices subject to impending rupture. Comparison of values of PS/SD ratio between studied groups showed significant decrease in cirrhotic patients with large esophageal varices compared to those without varices or with small esophageal varices ( $P = 0.001$ ). These findings are in concordance with several previous studies demonstrating a similar significant correlation between platelet count/splenic size ratio with stages according to Child-Turcotte-Pugh classification, extent of ascites, and size of esophageal varices<sup>[31,32]</sup>.

Findings indicative of portal hypertension can also be commonly detected with use of CT imaging, these including, in addition to splenomegaly and ascites, the presence esophageal varices, augmentation of portal vein, and existence of collateral vessel enlargement<sup>[33]</sup>. Several previous studies have investigated the interconnection between findings from both CT and endoscopy, and have demonstrated an agreement between variceal size and radiologic interpretations rather than between variceal size and endoscopic valuation<sup>[16,34]</sup>. However, CT scanning cannot adequately differentiate between small and large varices nor can it detect red signs on small varices that are also subject to a higher risk of bleeding<sup>[35]</sup>.

Comparison of CT parameters between groups in this study demonstrated evident differences, as cirr-

hotic patients with esophageal varices had higher values for PVD as well as SVD when compared with cirrhotic patients without varices (0.0012 and 0.284 respectively). In addition, CT significantly differentiated between presence and absence of esophageal varices. Interpretation of CT imaging showing no varices in 50 patients (40.2%) proved accurate in 46 of these patients who truly had no varices while only 4 patients indeed had esophageal varices as detected by endoscopy. Furthermore, demonstration of varices by CT in 74 patients (59.7%) was correct in 70 of these patients who had endoscopic evidence of esophageal varices. These results indicate that CT is almost as effective in detection of esophageal varices as endoscopy, hence possibly providing an acceptable substitute to endoscopy in detection of esophageal varices in cirrhotic patients.

To evaluate the efficacy of these non-invasive parameters in detecting presence of large esophageal varices, our study group plotted a ROC curve and the area under the curve was obtained for each individual parameter. FIB-4 score of  $\geq 3.13$  was shown to have a sensitivity of 71.7% and a specificity of 50% with an area under the ROC curve of 0.585 (95%CI: 0.442-0.728), which are higher than those for APRI which at a value of  $\geq 1.083$  had a sensitivity of 63% and a specificity of 46.4%, with the area under the ROC curve being 0.550 (95%CI: 0.442-0.728). In addition, a PC/SD ratio of  $\leq 806.93$  had a sensitivity of 75% and specificity of 47.8%, while PVD measurement by CT had 71.1% sensitivity and 37% specificity at a cutoff of  $\geq 12.5$  mm, with area under the ROC curve of 0.560 (95%CI: 0.425-0.630). These results indicate that use of CT in detection of large esophageal varices offers results comparable to those provided by both FIB-4 and APRI values, as well by evaluation of PC/SD ratio.

Based on these data, we proposed a non-invasive model for the prediction of large esophageal varices in patients with cirrhosis. Being a multi-factorial process, the detection of large varices is affected by many variables. In order to construct a model for the prediction of large esophageal varices, the research team chose binary logistic regression as a statistical means of evaluation. Dependent factors were either large or small varices, while independent factors were coronary vein diameter, PVD, lienorenal shunt, FIB-4, APRI, and PC/SD. The accuracy of this model was shown to be about 62.2%. However, after removal of insignificant factors such as APRI, FIB-4, coronary vein diameter, and lienorenal shunt, accuracy of the model becomes 75%. With use of PC/SD alone, the model accuracy was shown to be 73%, but combined use of both PC/SD and PVD offered an accuracy of 75.7% for prediction of large risky esophageal varices.

In conclusion, endoscopy continues to be the mainstay in diagnosis of esophageal varices, in spite of its invasive nature, unacceptability by a large number of patients, and diverse side effects and complications; however, there remains a need for further non-invasive, effective tools for detection of large esophageal varices

which may be subject to imminent rupture and hemorrhage in patients with cirrhosis. Thus, CT scanning may afford an adequate alternative to endoscopy in diagnosis of esophageal varices in patients afflicted with cirrhosis, as it appears to offer similar diagnostic value for large esophageal varices as other non-invasive parameters, with the added benefit of detection of other pathology of the liver, such as various hepatic lesions or masses, most notably hepatocellular carcinoma. In addition, parameters easily detectable by CT, such as PC/SD and PVD, form the basis for the model proposed by this study group, which provides accuracy of 75% for detection of large risky esophageal varices threatening to rupture in cirrhotic patients.

## COMMENTS

### Background

Chronic hepatitis C virus (HCV) infection currently affects approximately 130-170 million people globally, or about 2%-3% of the world's population, with the largest HCV epidemic currently found in Egypt, affecting about 14.7% of the Egyptian population. The end stage of chronic HCV infection is cirrhosis, often complicated by esophageal varices. While upper gastroscopy remains the gold standard for diagnosis of esophageal varices, several disadvantages of this invasive procedure have prompted the search for non-invasive parameters to determine the presence of esophageal varices.

### Research frontiers

Several parameters have emerged as predictors of esophageal varices including platelet count to splenic diameter ratio, aminotransferase-to-platelet ratio index (APRI), Fibroindex, and Fibrosis-4 score (FIB-4) as well as a number of radiological techniques including Doppler ultrasonography and computed tomography (CT). However, all of these elements are plagued by limitations. The research hotspot is to acknowledge these various parameters and their limitations to help other peers understand the background behind the search for an accurate.

### Innovations and breakthroughs

The search for a non-invasive method to accurately diagnose the presence of esophageal varices has been advancing in recent years. The present study involved a significant number of cirrhotic patients who underwent upper gastroscopy followed by CT imaging in addition to a series of simple inexpensive investigations to determine values for APRI, FIB-4, and platelet count/splenic diameter ratio (PC/SD). Patients undergoing the latter procedures were much more willing to comply when compared to those consenting for endoscopy, giving further support that endoscopy, in spite of its established benefits, remains a costly, uncomfortable procedure for many patients who prefer to avoid this invasive maneuver in any way possible, particularly when other accurate diagnostic tools are readily available.

### Applications

Data from this study suggest that CT scanning may afford an adequate alternative to endoscopy in diagnosis of esophageal varices in cirrhotic patients. In addition, parameters easily detectable by CT, such as PC/SD and portal vein diameter, form the basis for the model proposed by this study group.

### Terminology

Chronic HCV infection is a long-standing infection of the liver with HCV culminating into development of cirrhosis and its associated complications, including portal hypertension and esophageal varices. Esophageal varices are abnormally enlarged veins in the lower part of the esophagus, which may leak or even rupture, possibly causing life-threatening bleeding. Endoscopy continues to be the mainstay in diagnosis of esophageal varices.

### Peer-review

The work is really valuable occult blood in stool is always a diagnostic challenge.

Besides esophageal varices, acute mucosa lesions, venous bleeding caused by portal hypertension in any site of the gastrointestinal tract are equally causes.

## REFERENCES

- 1 Cuadros DF, Branscum AJ, Miller FD, Abu-Raddad LJ. Spatial epidemiology of hepatitis C virus infection in Egypt: analyses and implications. *Hepatology* 2014; **60**: 1150-1159 [PMID: 24913187 DOI: 10.1002/hep.27248]
- 2 Guerra J, Garenne M, Mohamed MK, Fontanet A. HCV burden of infection in Egypt: results from a nationwide survey. *J Viral Hepat* 2012; **19**: 560-567 [PMID: 22762140 DOI: 10.1111/j.1365-2893.2011.01576.x]
- 3 Zhang W, Wang L, Wang L, Li G, Huang A, Yin P, Yang Z, Ling C, Wang L. Liver stiffness measurement, better than APRI, Fibroindex, Fib-4, and NBI gastroscopy, predicts portal hypertension in patients with cirrhosis. *Cell Biochem Biophys* 2015; **71**: 865-873 [PMID: 25417057 DOI: 10.1007/s12013-014-0275-z]
- 4 Peñaloza-Posada MA, Pérez-Torres E, Pérez-Hernández JL, Higuera-de la Tijera F. Non-invasive parameters as predictors of high risk of variceal bleeding in cirrhotic patients. *Rev Med Hosp Gen Mex* 2014; **77**: 179-184 [DOI: 10.1016/j.hgmx.2014.09.002]
- 5 Jensen DM. Endoscopic screening for varices in cirrhosis: findings, implications, and outcomes. *Gastroenterology* 2002; **122**: 1620-1630 [PMID: 12016427 DOI: 10.1053/gast.2002.33419]
- 6 Bosch J, Abraldes JG, Berzigotti A, Garcia-Pagan JC. Portal hypertension and gastrointestinal bleeding. *Semin Liver Dis* 2008; **28**: 3-25 [PMID: 18293274 DOI: 10.1055/s-2008-1040318]
- 7 de Franchis R. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol* 2015; **63**: 743-752 [PMID: 26047908 DOI: 10.1016/j.jhep.2015.05.022]
- 8 de Franchis R. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2010; **53**: 762-768 [PMID: 20638742 DOI: 10.1016/j.jhep.2010.06.004]
- 9 ASGE Standards of Practice Committee; Ben-Menachem T, Decker GA, Early DS, Evans J, Fanelli RD, Fisher DA, Fisher L, Fukami N, Hwang JH, Ikenberry SO, Jain R, Jue TL, Khan KM, Krinsky ML, Malpas PM, Maple JT, Sharaf RN, Dominitz JA, Cash BD. Adverse events of upper GI endoscopy. *Gastrointest Endosc* 2012; **76**: 707-718 [PMID: 22985638 DOI: 10.1016/j.gie.2012.03.252]
- 10 Spiegel BM, Targownik L, Dulai GS, Karsan HA, Gralnek IM. Endoscopic screening for esophageal varices in cirrhosis: Is it ever cost effective? *Hepatology* 2003; **37**: 366-377 [PMID: 12540787 DOI: 10.1053/jhep.2003.50050]
- 11 Talwalkar JA, Kamath PS. Screening for esophageal varices among patients with cirrhosis of the liver. *Am J Gastroenterol* 2001; **96**: 3039-3040 [PMID: 11693352 DOI: 10.1111/j.1572-0241.2001.04692.x]
- 12 Giannini E, Botta F, Borro P, Risso D, Romagnoli P, Fasoli A, Mele MR, Testa E, Mansi C, Savarino V, Testa R. Platelet count/spleen diameter ratio: proposal and validation of a non-invasive parameter to predict the presence of oesophageal varices in patients with liver cirrhosis. *Gut* 2003; **52**: 1200-1205 [PMID: 12865282 DOI: 10.1136/gut.52.8.1200]
- 13 Crisan D, Radu C, Lupsor M, Sparchez Z, Grigorescu MD, Grigorescu M. Two or more synchronous combination of noninvasive tests to increase accuracy of liver fibrosis assesment in chronic hepatitis C; results from a cohort of 446 patients. *Hepat Mon* 2012; **12**: 177-184 [PMID: 22550525 DOI: 10.5812/hepatmon.853]
- 14 Choi YJ, Baik SK, Park DH, Kim MY, Kim HS, Lee DK, Kwon SO, Kim YJ, Park JW. Comparison of Doppler ultrasonography and the hepatic venous pressure gradient in assessing portal hypertension in liver cirrhosis. *J Gastroenterol Hepatol* 2003; **18**: 424-429 [PMID: 12653891 DOI: 10.1046/j.1440-1746.2003.02992.x]
- 15 Kim H, Choi D, Gwak GY, Lee JH, Park MK, Lee Hie, Kim SH, Nam S, Yoo EY, Do YS. Evaluation of esophageal varices on liver computed tomography: receiver operating characteristic analyses of the performance of radiologists and endoscopists. *J Gastroenterol Hepatol* 2009; **24**: 1534-1540 [PMID: 19486446 DOI: 10.1111/j.1440-1746.2009.05849.x]
- 16 Yu NC, Margolis D, Hsu M, Raman SS, Lu DS. Detection and grading of esophageal varices on liver CT: comparison of standard and thin-section multiplanar reconstructions in diagnostic accuracy. *AJR Am J Roentgenol* 2011; **197**: 643-649 [PMID: 21862806 DOI: 10.2214/AJR.10.5458]
- 17 Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
- 18 Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]
- 19 Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325 [PMID: 16729309 DOI: 10.1002/hep.21178]
- 20 Koda M, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. *Hepatology* 2007; **45**: 297-306 [PMID: 17256741 DOI: 10.1002/hep.21520]
- 21 Stefanescu H, Procopet B. Noninvasive assessment of portal hypertension in cirrhosis: liver stiffness and beyond. *World J Gastroenterol* 2014; **20**: 16811-16819 [PMID: 25492995 DOI: 10.3748/wjg.v20.i45.16811]
- 22 Thomopoulos KC, Labropoulou-Karatza C, Mimidis KP, Katsakoulis EC, Ionomou G, Nikolopoulou VN. Non-invasive predictors of the presence of large oesophageal varices in patients with cirrhosis. *Dig Liver Dis* 2003; **35**: 473-478 [PMID: 12870732 DOI: 10.1016/S1590-8658(03)00219-6]
- 23 Madhotra R, Mulcahy HE, Willner I, Reuben A. Prediction of esophageal varices in patients with cirrhosis. *J Clin Gastroenterol* 2002; **34**: 81-85 [PMID: 11743252 DOI: 10.1097/00004836-200201000-00016]
- 24 Peck-Radosavljevic M. Thrombocytopenia in liver disease. *Can J Gastroenterol* 2000; **14** Suppl D: 60D-66D [PMID: 11110614]
- 25 Witters P, Freson K, Verslype C, Peerlinck K, Hoylaerts M, Nevens F, Van Geet C, Cassiman D. Review article: blood platelet number and function in chronic liver disease and cirrhosis. *Aliment Pharmacol Ther* 2008; **27**: 1017-1029 [PMID: 18331464 DOI: 10.1111/j.1365-2036.2008.03674.x]
- 26 Giannini EG, Botta F, Borro P, Dulbecco P, Testa E, Mansi C, Savarino V, Testa R. Application of the platelet count/spleen diameter ratio to rule out the presence of oesophageal varices in patients with cirrhosis: a validation study based on follow-up. *Dig Liver Dis* 2005; **37**: 779-785 [PMID: 15996912 DOI: 10.1016/j.dld.2005.05.007]
- 27 Giannini EG, Zaman A, Kreil A, Floreani A, Dulbecco P, Testa E, Sohaey R, Verhey P, Peck-Radosavljevic M, Mansi C, Savarino V, Testa R. Platelet count/spleen diameter ratio for the noninvasive diagnosis of esophageal varices: results of a multicenter, prospective, validation study. *Am J Gastroenterol* 2006; **101**: 2511-2519 [PMID: 17029607 DOI: 10.1111/j.1572-0241.2006.00874.x]
- 28 Sharma J, Yadav MK, Gupta A, Arya TS. A study of the role of platelet count/splenic diameter ratio as a predictor of esophageal varices in patients with chronic liver disease. *Nat J Med Res* 2014; **4**: 232-234
- 29 Amin K, Muhammad D, Anjum A, Jamil K, Hassan A. Platelet count to splenic diameter ratio as a predictor of esophageal varices in patients of liver cirrhosis due to hepatitis C virus. *JUMDC* 2012; **3**: 6-11
- 30 González-Ojeda A, Cervantes-Guevara G, Chávez-Sánchez M, Dávalos-Cobián C, Ornelas-Cázares S, Macías-Amezcu MD, Chávez-Tostado M, Ramírez-Campos KM, Ramírez-Arce Adel R, Fuentes-Orozco C. Platelet count/spleen diameter ratio to predict esophageal varices in Mexican patients with hepatic cirrhosis. *World J Gastroenterol* 2014; **20**: 2079-2084 [PMID: 24616574]



- DOI: 10.3748/wjg.v20.i8.2079]
- 31 **Mahassadi AK**, Bathaix FY, Assi C, Bangoura AD, Allah-Kouadio E, Kissi HY, Touré A, Doffou S, Konaté I, Attia AK, Camara MB, Ndri-Yoman TA. Usefulness of Noninvasive Predictors of Oesophageal Varices in Black African Cirrhotic Patients in Côte d'Ivoire (West Africa). *Gastroenterol Res Pract* 2012; **2012**: 216390 [PMID: 22888334 DOI: 10.1155/2012/216390]
  - 32 **Ying L**, Lin X, Xie ZL, Hu YP, Shi KQ. Performance of platelet count/spleen diameter ratio for diagnosis of esophageal varices in cirrhosis: a meta-analysis. *Dig Dis Sci* 2012; **57**: 1672-1681 [PMID: 22367112 DOI: 10.1007/s10620-012-2058-y]
  - 33 **Somsouk M**, To'o K, Ali M, Vittinghoff E, Yeh BM, Yee J, Monto A, Inadomi JM, Aslam R. Esophageal varices on computed tomography and subsequent variceal hemorrhage. *Abdom Imaging* 2014; **39**: 251-256 [PMID: 24366107 DOI: 10.1007/s00261-013-0057-x]
  - 34 **Shen M**, Zhu KS, Meng XC, Zhang JS, Liu LY, Shan H. [Evaluation of esophageal varices and predicting the risk of esophageal varices bleeding with multi-detector CT in patients with portal hypertension]. *Zhonghua Yi Xue Za Zhi* 2010; **90**: 2911-2915 [PMID: 21211396]
  - 35 **Perri RE**, Chiorean MV, Fidler JL, Fletcher JG, Talwalkar JA, Stadheim L, Shah ND, Kamath PS. A prospective evaluation of computerized tomographic (CT) scanning as a screening modality for esophageal varices. *Hepatology* 2008; **47**: 1587-1594 [PMID: 18393388 DOI: 10.1002/hep.22219]

**P- Reviewer:** Bordas JM, Caboclo JL, Yoshida H  
**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Li D



## Prospective Study

# Liver resections can be performed safely without Pringle maneuver: A prospective study

Christoph A Maurer, Mikolaj Walensi, Samuel A Käser, Beat M Künzli, René Löttscher, Anne Zuse

Christoph A Maurer, Department of Surgery, Hirslanden-Clinic Beau-Site, 3013 Bern, Switzerland

Christoph A Maurer, Mikolaj Walensi, Samuel A Käser, Beat M Künzli, Anne Zuse, Department of Surgery, Hospital of Baselland, Affiliated with the University of Basel, 4410 Liestal, Switzerland

Samuel A Käser, Department of Visceral and Transplant Surgery, Rämistrasse 100, 8091 Zurich, Switzerland

René Löttscher, Department of Anesthesia and Intensive Care, Hospital of Baselland, Affiliated with the University of Basel, 4410 Liestal, Switzerland

**Author contributions:** Maurer CA made the conception and design of the study; Maurer CA, Walensi M, Käser SA, Künzli BM and Löttscher R acquired the data; Maurer CA, Künzli BM and Zuse A analyzed the data; Maurer CA, Walensi M, Käser SA, Künzli BM, Löttscher R and Zuse A did the interpretation of the data; Maurer CA, Künzli BM and Zuse A drafted the manuscript; Maurer CA, Walensi M, Käser SA, Künzli BM, Löttscher R and Zuse A revised and approved the final version of the manuscript.

**Institutional review board statement:** The study was approved by the ethical committee of Basel and Baselland (EKBB No. 188/13).

**Informed consent statement:** All patients gave their informed consent for surgery prior to study inclusion.

**Conflict-of-interest statement:** Nothing to declare. No potential or real conflicts of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Christoph A Maurer, MD, FACS, FRCS (Hon.), FEBS, Professor of Surgery, Department of Surgery, Hirslanden-Clinic Beau-Site, Schänzlihalde 1, CH-3000 Bern-25, 3013 Bern, Switzerland. [christoph.maurer@hin.ch](mailto:christoph.maurer@hin.ch)  
Telephone: +41-32-6215113  
Fax: +41-32-6215112

Received: March 20, 2016  
Peer-review started: March 23, 2016  
First decision: April 20, 2016  
Revised: May 4, 2016  
Accepted: July 14, 2016  
Article in press: July 18, 2016  
Published online: August 28, 2016

## Abstract

### AIM

To evaluate liver resections without Pringle maneuver, *i.e.*, clamping of the portal triad.

### METHODS

Between 9/2002 and 7/2013, 175 consecutive liver resections ( $n = 101$  major anatomical and  $n = 74$  large atypical  $> 5$  cm) without Pringle maneuver were performed in 127 patients (143 surgeries). Accompanying, 37 wedge resections (specimens  $< 5$  cm) and 43 radiofrequency ablations were performed. Preoperative volumetric calculation of the liver remnant preceded all anatomical resections. The liver parenchyma was dissected by water-jet. The median central venous pressure was 4 mmHg (range: 5-14). Data was collected prospectively.

### RESULTS

The median age of patients was 60 years (range: 16-85). Preoperative chemotherapy was used in 70 cases (49.0%). Liver cirrhosis was present in 6.3%, and liver steatosis of  $\geq 10\%$  in 28.0%. Blood loss was median 400 mL (range 50-5000 mL). Perioperative blood transfusions were given in 22/143 procedures (15%). The median weight of anatomically resected liver specimens

was 525 g (range: 51-1850 g). One patient died post-operatively. Biliary leakages ( $n = 5$ ) were treated conservatively. Temporary liver failure occurred in two patients.

# CONCLUSION

Major liver resections without Pringle maneuver are feasible and safe. The avoidance of liver inflow clamping might reduce liver damage and failure, and shorten the hospital stay.

**Key words:** Liver resection; Pringle maneuver; Blood loss

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This retrospective cohort study on 175 consecutive liver resections ( $n = 101$  major anatomical and  $n = 74$  large atypical  $> 5$  cm) shows that major liver resections without Pringle maneuver are feasible and safe. The avoidance of liver inflow clamping might reduce liver damage and failure, and shorten the hospital stay.

Maurer CA, Walensi M, Käser SA, Künzli BM, Lötscher R, Zuse A. Liver resections can be performed safely without Pringle maneuver: A prospective study. *World J Hepatol* 2016; 8(24): 1038-1046 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i24/1038.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i24.1038>

# INTRODUCTION

Massive haemorrhage is a key factor associated with poorer prognosis and outcome of patients undergoing liver resection<sup>[1,2]</sup>. The amount of blood loss correlates with postoperative morbidity and mortality<sup>[3]</sup>. Moreover, blood transfusion is linked to a decrease in cancer free survival<sup>[4]</sup>. Hence, it is a major goal to minimize the blood loss during liver resection. There are three main phases during liver resections when bleeding may occur: The liver mobilisation phase, the parenchymal dissection phase and the revascularization phase<sup>[1]</sup>. Portal triad clamping (PTC), also known as Pringle maneuver<sup>[5]</sup>, is the most widely used technique to reduce bleeding during the parenchymal dissection phase. In addition, vascular clamping can also be applied to control venous backflow<sup>[6,7]</sup>. Thus, total hepatic vascular exclusion can be achieved when combining PTC with clamping of the liver veins or the inferior vena cava cranial and caudad of the liver<sup>[8]</sup>. Further techniques to minimize intraoperative blood loss such as hypoventilation<sup>[9]</sup> and reduction of the central venous pressure (CVP)<sup>[10]</sup> have been developed over the last decades.

Although partial or complete vascular clamping results in reduction of blood loss, there are concerns regarding ischemia/reperfusion (I/R) injury to the liver remnant mediated by cytokines and reactive oxygen species<sup>[11,12]</sup>. Therefore, various attempts have been made to decrease

the I/R-injury associated with prolonged clamping of liver vessels: Use of drugs<sup>[13]</sup>, *in situ* cooling<sup>[14]</sup>, intermittent clamping<sup>[15,16]</sup>, ischemic preconditioning<sup>[17]</sup> and ischemic postconditioning<sup>[18]</sup>. Ischemic preconditioning involves I/R for a short period of time before exposure to prolonged I/R. The molecule nitric oxide plays a critical role in the early<sup>[11,12]</sup> and late phases<sup>[11]</sup> of ischemic preconditioning. Furthermore, during I/R-injury neutrophil and kupffer cell-induced oxidative stress, hepatic circular disturbance as well as inflammatory processes occur. Circular dysfunction is based on sinusoidal endothelial damage<sup>[19]</sup> as well as unbalance of vasoconstrictive and vasodilating transmitters such as endothelin<sup>[20]</sup>, tumor necrosis factor  $\alpha$ <sup>[21]</sup>, and interleukins<sup>[22]</sup>. Other mediators and pathways, *e.g.*, CD39 and purinergic signalling, are believed to play a role in hepatic ischemia and reperfusion injury<sup>[23]</sup>.

Thus, the molecular hepatic system is far better understood today and recent advances in surgical strategies and perioperative care have made liver resections much safer, allowing low mortality and morbidity in experienced hands. However, the question remains whether the risk of resective liver surgery can be further reduced by complete avoidance of any vascular clamping of the liver remnant and hence by minimizing the I/R injury.

The purpose of this retrospective single center data analysis was to assess the feasibility and safety of major liver resections without any Pringle maneuver or its variations. In the second step, we were interested in any differences in outcome between three subgroups: Anatomical resections, atypical resections and the combination of both, *i.e.*, the combination of anatomical and atypical resection.

# MATERIALS AND METHODS

## Study population

From September 2002 through July 2013, a prospective database was established including 175 liver resections [anatomical resections ( $n = 101$ ) and large atypical resections (specimens  $> 5$  cm in at least one diameter,  $n = 74$ )] which were performed at the occasion of 143 consecutive liver surgeries. Twenty-five patients had two stage procedures, 2 patients had 3 or more staged liver resections. The indications for these 143 liver surgeries were liver metastases ( $n = 91$ , from the following primaries: 73 colorectal cancer, 2 ovarian, 5 breast, 1 gallbladder, 1 esophageal, 1 stomach, 1 leiomyosarcoma, 1 melanoma, 2 gastrointestinal stroma tumor, and 4 with unknown primary), hepatocellular carcinoma ( $n = 11$ ), follicular nodular hyperplasia ( $n = 4$ ), liver hemangioma ( $n = 9$ ), carcinoma of the gallbladder ( $n = 4$ ), cholangiolar carcinomas ( $n = 8$ ), liver adenomas ( $n = 4$ ), hepaticolithiasis ( $n = 4$ ), echinococcal cysts ( $n = 5$ ), benign liver cysts ( $n = 2$ ) and one sclerotic steatohepatitis. Patients' characteristics were summarized in Table 1. The extent of hepatectomy was depending on tumor size and localization, severity of liver steatosis and cirrhosis, age, nutritional status, preoperatively determined liver function and preopera-

**Table 1** Patients' characteristics shown as total and as subgroups according to the types of resection *n* (%)

Patient characteristics	Total	Anatomical resections	Atypical resections > 5 cm	Combination of ana-tomical and atypical resections > 5 cm	P-values <sup>3</sup>
No. of liver resections	175	84	54	37	n.d.
No. of liver surgeries	143	77	50	16	n.d.
No. of surgeries with ≥ 2 similar resections	14 (9.8)	7 (9.1)	4 (8.0)	3 (18.8)	n.d.
No. of surgeries with ≥ 1 additional wedge resection <sup>5</sup>	29 (20.3)	10 (13.0)	14 (28)	5 (31.3)	n.d.
No. of surgeries with ≥ 1 additional radiofrequency ablation	25 (17.5)	7 (9.1)	11 (22)	7 (43.8)	n.d.
Demographics					
Gender (female/male) <sup>1</sup>	74/69	41/36	24/26	9/7	0.4804 <sup>3</sup>
BMI (kg/m <sup>2</sup> ) <sup>2</sup>	25.5 (17.4-53.2)	24.8 (17.4-53.2)	27.1 (18.1-36.0)	25.2 (18.8-29.6)	0.3660 <sup>4</sup>
Age (yr) <sup>2</sup>	60.0 (16-85)	59.0 (16-85)	61.5 (28-84)	63.5 (22-78)	0.4952 <sup>4</sup>
Preoperative ASA scores 1/2/3/4 <sup>1</sup>	8/77/58/0	3/42/32/0	2/28/20/0	3/7/6/0	0.4247 <sup>3</sup>
	5/54/41/0	4/54/42/0	4/56/40.0/0	19/44/37/0	
Indications for liver surgery					< 0.0001 <sup>3</sup>
Malignant primary liver tumors	23 (16.1)	15 (19.5)	8 (16.0)	0	
Liver metastases	91 (63.6)	44 (57.1)	34 (68.0)	13 (81.2)	
Benign liver tumors	19 (13.3)	10 (13.0)	6 (12.0)	3 (18.8)	
Others	10 (7.0)	8 (10.4)	2 (4.0)	0	
Preoperative chemotherapy <sup>1</sup>	70 (49.0)	33 (42.9)	26 (52)	11 (68.8)	0.4281 <sup>3</sup>
Steatosis grade of normal liver <sup>2</sup>					0.9195 <sup>3</sup>
Steatosis 0%-9% (grade 0)	103 (72.0)	56 (72.7)	37 (74.0)	10 (62.5)	
Steatosis 10%-29% (grade 1)	26 (18.2)	14 (18.2)	8 (16.0)	4 (25.0)	
Steatosis ≥ 30% (grade 2)	14 (9.8)	7 (9.1)	5 (10.0)	2 (12.5)	
Cirrhosis (Child-Pugh A) <sup>1</sup>	9 (6.3)	5 (6.5)	4 (8.0)	0	0.8568 <sup>3</sup>

<sup>1</sup>Values are total number of patients (%); <sup>2</sup>Continuous variables are expressed as median (range); <sup>3</sup>P-values of categorical variables; <sup>4</sup>Calculated by  $\chi^2$  test and continuous ones by One-way Anova analysis of variance. No significance between the group of anatomical, atypical, and combined resections for selected variables was found, except for indications for surgery; <sup>5</sup>Liver wedge resection is defined as obtaining a liver specimen with a maximum diameter of less than 5 cm. n.d.: Not determined; BMI: Body mass index.

**Table 2** Extent of anatomical resections based on segmental and sectorial anatomy of the liver according to Brisbane classification

Type of anatomical liver resection	<i>n</i>
Extended right hemihepatectomy	6
Extended left hemihepatectomy	3
Right hemihepatectomy	31
Left hemihepatectomy	12
Right posterior sectorectomy	4
Right anterior sectorectomy	1
Left lateral sectionectomy	19
Segmentectomy	19
Bisegmentectomy	24
Trisegmentectomy	2
Total of anatomical liver resections	121

tive chemotherapy. The various extents of anatomical resections were classified according to Brisbane nomenclature<sup>[24]</sup> and were shown in Table 2.

### Intraoperative anesthesia management

Surgery was generally performed under low central venous pressure (LCVP). Therefore, the patient's internal jugular vein was cannulated using a dual-channel catheter and CVP was continuously measured. Values below 5 mmHg were targeted by limiting the volume of crystalloid infusion (lactated Ringer) and stimulating diuresis with furosemide (10-20 mg *i.v.*). At the same time, mean arterial blood pressure, determined within the radial artery, was maintained above 60 mmHg by intravenous

infusion of norepinephrine (0-10  $\mu$ g/min). During dissection of liver parenchyma intermittent positive pressure ventilation was reduced to an end-expiratory level of zero mmHg to further minimize the CVP.

### Surgical procedures

Following an intravenous antibiotic single shot prophylaxis, either a roof-top or midline abdominal incision without thoracotomy was used in all patients. After exclusion of extrahepatic intraabdominal tumor spread by exploration of the abdominal cavity and the hepatoduodenal ligament, careful visual and bimanual examination of the liver was performed. At least partial mobilization of the liver including dissection of round and falciforme ligament was done in almost all procedures. Inferior hepatic veins were dissected for hemihepatectomies and/or segment 1 resections, and as necessary in other types of resection. Intraoperative ultrasonography of the liver was systematically done to accurately determine the number and location of liver tumors and their relation to hepatic blood vessels and bile ducts. A Tru-Cut<sup>®</sup>-needle (CareFusion Temno needle 14G, 11 cm, distributed by Admedics, Zuchwil, Switzerland) biopsy of grossly normal liver was sent to frozen section to assess the grades of steatosis and cirrhosis.

Blood vessels of the liver were clamped and dissected from the later liver specimen, only. Temporary or intermittent clamping of vascular structures of the liver remnant or of the liver hilum has been strictly avoided in all patients. And, neither ischemic preconditioning nor



ischemic postconditioning has been used in any of the patients. Only twice, an anterior approach according to Launois<sup>[25]</sup> was necessary due to a large tumor mass of the right liver lobe.

In all surgeries, the liver parenchyma was cut by means of water-jet dissection. The hence visualized intrahepatic blood vessels and bile ducts were dissected between ligatures or metal clips, small ones were electro-coagulated. The resection surface was treated punctually by argon plasma coagulation and checked for small bile leaks using white gauzes. The resection surface was then covered by the fibrin-based hemostyptic Tachosil® or Beriplast® (Takeda/Nycomed, Basel, Switzerland). In all patients a silicone drain (EasyFlow®, Teleflex Medical GmbH, Kernen, Germany) without suction was inserted.

### Perioperative assessment of liver function

The liver function was assessed by measurement of indocyanine green (ICG) clearance<sup>[26]</sup>. The dye ICG is metabolized and eliminated by the liver, only. Therefore, their elimination velocity is directly corresponding with the functional capacity of the liver. Plasma disappearance rate (range of normal values from 18%-25%) of ICG and the residual ICG after 15 min (R15, normal range between 0%-10%) were examined pre-, intra- and post-operatively. At the beginning of the series, 6 patients had measurement of galactose elimination capacity (GEC) instead of ICG-clearance. No intra- or postoperative controls of GEC were performed at that time. Additionally, various serum parameters were measured repeatedly, most of them daily.

The volumina of total functional liver and the anticipated functional liver remnant (FLR) were calculated by computed tomography (CT), when a resection volume of more than 40% of the total functional liver volume was anticipated. Twenty percent to 25% of total functional liver volume was regarded as a sufficient FLR in an otherwise healthy and non-steatotic liver, and 30%-40% in a steatotic or chemotherapeutically pretreated liver, respectively. In advance of 8 anatomical liver resections, induction of an atrophy-hypertrophy complex by embolization or ligation of right or left portal vein was regarded necessary. One patient underwent preoperative chemoembolization. Patients with liver cirrhosis Child-Pugh stage B were not considered candidates for surgery, and stage A patients ( $n = 9$ ) had  $\leq 2$  liver segments resected.

### Outcome measures and perioperative management

Intraoperative blood loss was calculated by adding the blood volume in the suction device plus the blood kept in towels. The indications for blood transfusion were determined individually, according to patients' preoperative heart status and haemoglobin. Generally, patients with ASA-scores 1 or 2 did not receive blood transfusions before the haemoglobin decreased below a value of 80 g/L. For patients with coronary heart disease or hemodynamic instability, the administration of blood

transfusion was less restrictive. Blood transfusions referred to the total time of hospital stay.

Postoperatively, patients were closely monitored at the intensive care unit (ICU). The Simplified Acute Physiology Score (SAPS) II was used to assess the severity of illness in intensive care patients<sup>[27]</sup>. The SAPS II predicts the risk of hospital mortality and provides an reliable estimation of the risk of death<sup>[27]</sup>.

Bilirubin content was measured from the silicon drainage tube at days 2 and 4, or daily when the drained fluid was suspicious for bile leak. Bile leakage was defined as suggested by Koch *et al.*<sup>[28]</sup> as bilirubin concentration in the drain fluid at least 3 times the serum bilirubin concentration on or after postoperative day 3; or further as the need for radiologic or operative intervention resulting from biliary collections or biliary peritonitis<sup>[28]</sup>.

Resected specimens were weighed immediately after removal. Specimens of malignant neoplasias were sent to the department of pathology for marking the resection margins with ink before formaline fixation.

Liver cirrhosis was defined as F4 fibrosis according to the METAVIR score<sup>[29]</sup>.

### Statistical analysis

Data in this study are presented as median and range or as mean  $\pm$  standard error of mean. Statistical analysis of data was performed using the GraphPad PRISM6 software (GraphPad Software Inc., San Diego, CA, United States). Comparisons of continuous variables between groups were analyzed using one-way ANOVA analysis for multiple comparisons. Categorical variables were compared by chi-square test ( $\chi^2$  test). Values of  $P < 0.05$  are considered statistically significant.

## RESULTS

Data related to the operative procedure such as operation time, CVP, blood loss and substitution, length of ICU stay, SAPS, and specimen weight is summarized in Table 3. Data are presented as total of the  $n = 143$  liver surgeries and as subgroups according to the types of resection. From the 22 patients needing perioperative blood transfusions, 7 received them intraoperatively, 1 preoperatively and 14 postoperatively.

In patients with provided preoperative volumetry of the liver, *i.e.*, patients with anticipated minimum resected volume of  $\geq 40\%$  of total functional liver volume, the median effectively resected functional volume was 53% (20%-76%). A R0-resection at the liver site could be achieved in 98/114 (86.0%) procedures for malignant liver disease. No local R2-resection did occur.

### Laboratory results

Perioperative increases or decreases of relevant laboratory parameters are shown in Table 4, as total and as subgroups according to the types of resection. Table 5 summarizes the ICG-measurements preoperatively, intraoperatively immediately upon removal of the speci-

**Table 3** Perioperative parameters and characteristics of hepatic resections, shown as total and as subgroups according to the types of resection

Perioperative data	Total (n = 143)	Anatomical resections (n = 77)	Atypical resections > 5 cm (n = 50)	Combination of anatomical and atypical resections (n = 16)	P-values
<b>Intraoperative parameters</b>					
Median operation time (min)	361 (78-726)	386 (134-726)	299 (78-692)	362 (120-567)	0.0061 <sup>3</sup>
Median CVP <sub>min</sub> during liver resection (mmHg)	4 (-5 to 14)	3 (-5 to 12)	5 (-3 to 14)	4 (-4 to 12)	0.0511
Median total blood loss per procedure (n = 143) (mL)	500 (50-5000)	500 (50-5000)	400 (50-1500)	700 (150-2400)	0.0214 <sup>3</sup>
No. of patients needing ECs (% of n = 143 procedures) <sup>1</sup>	22 (15%)	14 (18%)	6 (12%)	2 (13%)	0.9854
Mean number of ECU during total hospital stay, per procedure (n = 143) <sup>2</sup>	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.4844
<b>Postoperative parameters</b>					
Median length of ICU stay (d)	3 (0-44)	3 (0-15)	3 (0-44)	3 (2-5)	0.2960
Median length of hospital stay (d)	13 (3-99)	14 (3-95)	12 (4-99)	12 (7-32)	0.0450 <sup>3</sup>
Maximum SAPS, median (range)	27 (7-40)	26 (14-40)	27 (7-40)	27 (14-39)	0.6001
Median weight of resected liver tissue (g) per procedure (n = 143)	340 (8-1850)	525 (51-1850)	53 (8-490)	352 (40-1018)	< 0.0001 <sup>3</sup>

<sup>1</sup>Since some patients had simultaneously more than 1 resection, the percentage of the perioperative need for ECs is calculated per number of procedures. P-values were calculated, comparing the variable of interest in between the different resection groups (Anova one-way analysis Kruskal-Wallis);

<sup>2</sup>Continuous variables are expressed as median (range), except presented as mean ± SEM; <sup>3</sup>Denote statistical significance among resections in the group of anatomical, atypical, and combined resections. CVP: Central venous pressure; EC: Erythrocyte concentrate; ECU: Erythrocyte concentrate unit; ICU: Intensive care unit; SAPS: Simplified acute physiology score.

mens and on postoperative day 2, again as total and as subgroups according to the type of resection. All 6 patients with preoperatively measured GEC showed values within the normal range. From further 22 patients, only the preoperative ICG-testing was available and resulted as normal (data not shown).

### Morbidity and mortality

There was one death in our series due to a preoperatively unknown high-grade stenosis at the origin of the superior mesenteric artery with consecutive extended mesenteric infarction in the postoperative course. Hence, in-hospital mortality was 1/143 procedures (0.7%).

The following major procedure-specific complications (9/143 procedures, 6.3%) occurred: 1 hemorrhage on postoperative day 9 after right hemihepatectomy in a patient needing therapeutic dosages of heparin, 5 biliary leakages treated conservatively and 2 temporary liver failures. From the later, one occurred in a patient after right hemihepatectomy who suffered from ischemic colon perforation, fecal peritonitis and multiorgan dysfunction. Another patient with extended left hemihepatectomy including segment 1 and includes hepatic artery and bile duct reconstruction for a Klatskin tumor developed intercurrent portal vein thrombosis with prolonged hepatic insufficiency. Relief was achieved by insertion of a portal stent. Finally, 1 patient with right hemihepatectomy developed postoperative peritonitis from an accidental small bowel leak, needing reintervention and laparostomy. No hepato-renal syndrome did occur.

Overall, the following advanced grades of complications according Dindo *et al.*<sup>[30]</sup> were encountered: 2 patients with grade IIIA, 4 with grade IVB and 1 with grade V complication.

## DISCUSSION

During hepatectomy, portal triad clamping developed by Pringle<sup>[5]</sup> is still commonly applied today as a routine procedure and gold standard to limit haemorrhage worldwide<sup>[18,31-35]</sup>. Clamping of the hepatoduodenal ligament and hence control of the hepatic vascular inflow is thought to reduce blood loss and to avoid blood transfusions<sup>[5]</sup>, both associated with increased perioperative morbidity and mortality<sup>[4,36,37]</sup> as well as impaired long-term outcome<sup>[34]</sup>.

Albeit the huge importance of this topic, only few studies investigated the value of Pringle maneuver in the past. No randomized study using a standard Pringle maneuver could be found in literature. And to our knowledge, only three randomized trials comparing liver resections with or without intermittent Pringle maneuver were performed so far<sup>[38-40]</sup>. The value of the intermittent Pringle maneuver is even more questionable, since these studies report conflicting results. Therefore, a very recent paper from Hoekstra *et al.*<sup>[6]</sup> was entitled "vascular occlusion or not during liver resection: The continuing story".

### Feasibility and safety of liver resections without Pringle maneuver

In the present paper, a consecutive series of major liver resections is reported without any Pringle maneuver during the total operation time in all procedures. Accordingly, a conversion to Pringle maneuver as a salvage clamping was necessary in none of the patients. Furthermore, only a minor number of patients needed perioperative blood transfusions and in-hospital-mortality was minimal with 0.7%. Hence, the feasibility and safety

**Table 4 Perioperative alterations of laboratory parameters, shown as total and as subgroups according to the types of resection**

Serum parameters	Total (n = 143)	Anatomical resections (n = 77)	Atypical resections > 5 cm (n = 50)	Combination of ana-tomical and atypical resections > 5 cm (n = 16)	P-values
	Median $\Delta$ -values <sup>1</sup> (ranges)	Median $\Delta$ -values <sup>1</sup> (ranges)	Median $\Delta$ -values <sup>1</sup> (ranges)	Median $\Delta$ -values <sup>1</sup> (ranges)	
ASAT (U/L, norm < 41)	304 (-486 to 9885)	346 (-486 to 9885)	285 (-137 to 2361)	463 (-5 to 1270)	0.1747
ALAT (U/L, norm < 41)	299 (-356 to 3909)	300 (-356 to 3909)	245 (-250 to 2200)	421 (-27 to 1093)	0.2635
Bilirubin ( $\mu$ mol/L, norm < 20)	7 (-130 to 234)	9 (-130 to 234)	4 (-36 to 152)	7 (-0.1 to 33.4)	0.4605
Ammonia ( $\mu$ mol/L, norm 12-48)	39 <sup>3</sup> (14 to 155)	41 <sup>3</sup> (14 to 155)	39 <sup>3</sup> (20 to 90)	37 <sup>3</sup> (25 to 152)	0.5026 <sup>4</sup>
Albumin (g/L, norm 35-50)	-8 (-41 to 192)	-8 (-19 to 12)	-6 (-20 to 192)	-8 (-18 to 1)	0.2262
Hemoglobin (g/L, norm 130-180)	-37 (-83 to 0)	-35 (-83 to 0)	-37 (-71 to -4)	-39 (-68 to -22)	0.4654
Prothrobine time: Quick (% , norm > 70)	-27 (-108 to 62)	-32 (-81 to -9)	-22 (-53 to 13)	-35 (-63 to -7)	0.0005 <sup>2</sup>

<sup>1</sup>Medians and ranges of  $\Delta$ -values are presented.  $\Delta$ -values are calculated by the difference between preoperative value and the maximum postoperative value or postoperative nadir. P-values were calculated, comparing the  $\Delta$ -value of each serum marker among the different resection groups (One-way Anova analysis of variance); <sup>2</sup>Denote statistical significance among resections in the group of anatomical, atypical, and combined resections; <sup>3</sup>Variable is presented as median value and range of postoperative maximum, since no preoperative values were available; <sup>4</sup>P-value was calculated, comparing postoperatively determined ammonia levels (maximum) among the different resection groups (One-way Anova analysis of variance). ASAT: Aspartate transaminase; ALAT: Alanine aminotransferase.

**Table 5 Pre-, intra- and postoperative values of Indocyanine-green-clearance testing were available in 45 liver surgeries and were presented as total as well as subgroups according to the type of liver resection**

ICG data <sup>1</sup>	Total (n = 45)	Anatomical resections (n = 27)	Atypical resections > 5 cm (n = 13)	Combination of anatomical and atypical resections > 5 cm (n = 5)	P-values
R15 (% , norm 0-10)					
Preoperative	3.6 (0.1 to 28.4)	3.6 (0.1 to 28.4)	3.6 (0.2 to 16.3)	2.7 (0.8 to 15)	0.4573
Intraop. after resection	12.4 (0.5 to 69.8)	17.3 (0.5 to 69.8)	6.5 (0.9 to 15.3)	22.4 (1.3 to 28.8)	0.0302 <sup>3</sup>
Postoperative day 2	5.8 (0.2 to 26.7)	7.8 (0.2 to 26.7)	3.2 (0.7 to 13.4)	7.6 (0.9 to 12.1)	0.0420 <sup>3</sup>
R15 $\Delta$ <sup>2</sup>	1.8 (-8.4 to 14.1)	4.2 (-8.4 to 14.1)	-0.2 (-5.2 to 9.8)	0.5 (-0.9 to 8.4)	0.0693
PDR (% , norm 18-25)					
Preoperative	22.2 (8.4 to 48)	21.4 (8.4 to 48)	22.2 (12.1 to 40.1)	24.1 (12.0 to 31.0)	0.6772
Intraop. after resection	14.4 (2.4 to 35.3)	11.7 (2.4 to 35.3)	18.2 (12.5 to 31.2)	9.5 (8.3 to 26.0)	0.0047 <sup>3</sup>
Postoperative day 2	19.0 (8.8 to 40.3)	17.5 (8.8 to 40.3)	22.5 (13.4 to 28.4)	17.5 (14.1 to 31.3)	0.5732
PDR $\Delta$ <sup>2</sup>	-1.4 (-15 to 37.1)	-3.7 (-12.9 to 37.1)	0.1 (-15 to 7.4)	-2.2 (-24.6 to 4.7)	0.5534

<sup>1</sup>Median values and range of data are presented. As sensitive indicator for liver function the retention rate after 15 min (R15) and the plasma disappearance rate (PDR) were evaluated; <sup>2</sup> $\Delta$  values of R15 and PDR were determined by the difference of preoperative and postoperative day 2 values. P values were calculated, comparing the variable of interest in between the different resection group (one-way Anova analysis of variance); <sup>3</sup>Denote statistical significance among resections in the group of anatomical, atypical, and combined resections. ICG: Indocyanine green clearance.

to principally avoid the Pringle maneuver seems to be demonstrated.

### Comparison of blood loss and blood transfusions without Pringle maneuver in the present series vs the literature with Pringle maneuver

In the present series, having used water jet dissection but no Pringle maneuver for all hepatic resections, the median blood loss of 500 mL was comparable with other reported series using a Pringle maneuver<sup>[38,40]</sup>, varying between 370 and 610 mL. Additionally, the percentage of patients who needed perioperative blood transfusions was 15 in this data and again comparable with data from studies having used Pringle maneuver, ranging from 13% to 36%<sup>[35,39,40]</sup>. It is noteworthy that excessive intraoperative blood losses in this series, in one patient up to 5000 mL, were exceptional and resulted all from bleeding from the inferior vena cava or the liver veins that would not have been improved by the use of a Pringle maneuver.

### Conditions facilitating the avoidance of Pringle maneuver

The following points are regarded as crucial if avoidance of Pringle maneuver is intended: Good exposure of the liver, careful planning of the dissection plane(s) on behalf of the preoperative imaging procedures and the intraoperative ultrasound, knowledge of the liver anatomy and its variants, low CVP during parenchyma dissection phase<sup>[38,41,42]</sup> and a completed learning curve in major hepatic surgery<sup>[43]</sup>. Furthermore, various dissection tools such as water jet, harmonic knife, ultrasound, humid bipolar clamp and other devices are thought to facilitate a well controlled parenchyma dissection and avoidance of major blood loss<sup>[43,44]</sup>.

### How to obtain low CVP?

The goal is a CVP below 5 mmHg at the time point of hepatic parenchyma dissection. There is a direct relation between the pressure of the hepatic sinusoidal system with CVP. Bleeding during resection phase is proportional

to the pressure gradient across vascular walls and diameter of injured vessels. Therefore, lowering of the CVP contributes to minimizing the blood loss during dissection phase<sup>[45]</sup>. Besides a close cooperation and communication between surgeon and anesthesiologist, the following measures may support lowering the CVP: Omission of any positive endexpiratory pressure during ventilation, restrictive intravenous fluid administration, forced diuresis, and a liberal use of drugs sustaining arterial blood pressure.

#### **Advantages of liver resections without Pringle maneuver**

The most important advantage of abstaining from Pringle maneuver is the fact that the I/R injury to the liver remnant is almost nihil. This is especially relevant in patients with pre-existing liver damage since the toxic effects of liver ischemia with consecutive liver dysfunction lead to morbidity and mortality<sup>[15]</sup>.

Furthermore, PTC may lead to significant higher systemic vascular resistance combined with decrease in cardiac index as well as increase in mean arterial pressure and, thus, increasing risk of perioperative cardiovascular complications<sup>[16]</sup>.

Although various modifications of Pringle maneuver such as intermittent PTC, ischemic preconditioning and more recently pharmacological preconditioning have been developed to limit these disadvantages<sup>[16,46-48]</sup>, excessive bleeding during reperfusion period partially counterbalances the positive effects regarding minimizing damage of residual liver tissue.

#### **Perioperative monitoring of I/R-injury and liver function**

Ischemia/reperfusion (I/R)-injury is usually monitored by measuring levels of aminotransaminases, bilirubin and prothrombin. The trauma during liver surgery caused by manipulation and parenchyma dissection usually result in a mild to moderate increase of transaminases in the serum (not more than 10-fold normal values), with a quick tendency to recover from postoperative day 1 or 2 on. Such mild increases in liver enzymes are usually not relevant for clinical outcome. However, strong elevation of transaminases (more than 20-fold normal level) with a continuous increase over at least 3 postoperative days may be the result of I/R-injury or decreased blood supply to the liver remnant. Levels of transaminases are well correlating with the ischemic damage<sup>[49]</sup>. I/R-injury may cause postoperative liver failure, mainly in preconditioned patients (*e.g.*, steatosis) with lower tolerance towards ischemia. In the present series without Pringle maneuver, no death occurred due to postoperative liver failure. Only 2 patients experienced temporary liver insufficiency, one due to a septic complication, and another due to postoperative thrombosis of portal vein. It is supposed that these favorable results with regard to postoperative liver failure may be attributed to the maintenance of optimum blood supply to the liver remnant at any time and hence the avoidance of I/R-injury. Accordingly, only moderate increases of transaminases (AST and ALT) in

this series were noticed (Table 4).

Additional serum markers that are thought to have stronger validity and more sensitive indication for liver failure and prognosis are increased bilirubin and ammonia as well as decreased prothrombin levels<sup>[50]</sup>. No serious changes in these parameters were observed with the exception of the 2 mentioned patients with severe complications.

#### **Comparison of anatomical vs atypical resection**

As expected, no significant difference in perioperative and laboratory parameters was observed between the group of anatomical resections vs the group of atypical resections, with two exceptions: Operation time was significantly shorter and prothrombin time was significantly less reduced in the atypically resected group when compared to the group with anatomical resections. Especially, blood loss, blood transfusions and the length of stay in the ICU were similar in both groups.

#### **Limitations of the study**

Data of this study originates from a single center. However, it is a consecutive series with prospective data recording. Large atypical liver resections were also included in this study although they would not belong to major liver resections per definition. However, with view on the study aim, we considered the inclusion of atypical liver resections of at least 5 cm diameter as appropriate, since atypical resections may be accompanied by technical difficulties and inadvertent blood loss similar to segment oriented liver resections.

In conclusion, the data of this study suggests that major liver resections may be performed safely without Pringle maneuver. The low morbidity and mortality rate might be due to minimizing the postoperative liver failure rate by avoidance of the I/R injury to the liver. Anatomical and large atypical liver resections may attempted to be performed without portal triad clamping.

## **ACKNOWLEDGMENTS**

The authors thank the co-workers of the department of anesthesia and intensive care unit for the appreciated help in optimizing the intra- and post-operative management for the patients undergoing liver surgery.

## **COMMENTS**

### **Background**

The role of Pringle maneuver in liver resection is under debate.

### **Research frontiers**

Different techniques of Pringle maneuver have been compared.

### **Innovations and breakthroughs**

The present study shows that major liver resections may be performed safely without Pringle maneuver.

### **Applications**

Major liver resections can be done avoiding Pringle maneuver.



## Peer-review

This study suggests that major liver resections may be performed safely without Pringle maneuver.

## REFERENCES

- 1 **Belghiti J**, Noun R, Zante E, Ballet T, Sauvanet A. Portal triad clamping or hepatic vascular exclusion for major liver resection. A controlled study. *Ann Surg* 1996; **224**: 155-161 [PMID: 8757378 DOI: 10.1097/0000658-199608000-00007]
- 2 **Makuuchi M**, Takayama T, Gunvén P, Kosuge T, Yamazaki S, Hasegawa H. Restrictive versus liberal blood transfusion policy for hepatectomies in cirrhotic patients. *World J Surg* 1989; **13**: 644-648 [PMID: 2554598 DOI: 10.1007/BF01658893]
- 3 **Matsumata T**, Ikeda Y, Hayashi H, Kamakura T, Taketomi A, Sugimachi K. The association between transfusion and cancer-free survival after curative resection for hepatocellular carcinoma. *Cancer* 1993; **72**: 1866-1871 [PMID: 8395966]
- 4 **de Boer MT**, Molenaar IQ, Porte RJ. Impact of blood loss on outcome after liver resection. *Dig Surg* 2007; **24**: 259-264 [PMID: 17657150 DOI: 10.1159/000103656]
- 5 **Pringle JH**. V. Notes on the Arrest of Hepatic Hemorrhage Due to Trauma. *Ann Surg* 1908; **48**: 541-549 [PMID: 17862242 DOI: 10.1097/0000658-190810000-00005]
- 6 **Hoekstra LT**, van Trigt JD, Reiniers MJ, Busch OR, Gouma DJ, van Gulik TM. Vascular occlusion or not during liver resection: the continuing story. *Dig Surg* 2012; **29**: 35-42 [PMID: 22441618 DOI: 10.1159/000335724]
- 7 **Chouillard EK**, Gumbs AA, Cherqui D. Vascular clamping in liver surgery: physiology, indications and techniques. *Ann Surg Innov Res* 2010; **4**: 2 [PMID: 20346153 DOI: 10.1186/1750-1164-4-2]
- 8 **Heaney JP**, Stanton WK, Halbert DS, Seidel J, Vice T. An improved technic for vascular isolation of the liver: experimental study and case reports. *Ann Surg* 1966; **163**: 237-241 [PMID: 4286023 DOI: 10.1097/0000658-196602000-00013]
- 9 **Hasegawa K**, Takayama T, Orii R, Sano K, Sugawara Y, Imamura H, Kubota K, Makuuchi M. Effect of hypoventilation on bleeding during hepatic resection: a randomized controlled trial. *Arch Surg* 2002; **137**: 311-315 [PMID: 11888456]
- 10 **Wang WD**, Liang LJ, Huang XQ, Yin XY. Low central venous pressure reduces blood loss in hepatectomy. *World J Gastroenterol* 2006; **12**: 935-939 [PMID: 16521223 DOI: 10.3748/wjg.v12.i6.935]
- 11 **Banga NR**, Homer-Vanniasinkam S, Graham A, Al-Mukhtar A, White SA, Prasad KR. Ischaemic preconditioning in transplantation and major resection of the liver. *Br J Surg* 2005; **92**: 528-538 [PMID: 15852422 DOI: 10.1002/bjs.5004]
- 12 **Koti RS**, Seifalian AM, Davidson BR. Protection of the liver by ischemic preconditioning: a review of mechanisms and clinical applications. *Dig Surg* 2003; **20**: 383-396 [PMID: 12840597 DOI: 10.1159/000072064]
- 13 **Bartels M**, Biesalski HK, Engelhart K, Sendlhofer G, Rehak P, Nagel E. Pilot study on the effect of parenteral vitamin E on ischemia and reperfusion induced liver injury: a double blind, randomized, placebo-controlled trial. *Clin Nutr* 2004; **23**: 1360-1370 [PMID: 15556258 DOI: 10.1016/j.clnu.2004.05.003]
- 14 **Azoulay D**, Eshkenazy R, Andreani P, Castaing D, Adam R, Ichai P, Naili S, Vinet E, Saliba F, Lemoine A, Gillon MC, Bismuth H. In situ hypothermic perfusion of the liver versus standard total vascular exclusion for complex liver resection. *Ann Surg* 2005; **241**: 277-285 [PMID: 15650638 DOI: 10.1097/01.sla.0000152017.62778.2f]
- 15 **Brooks AJ**, Hammond JS, Girling K, Beckingham IJ. The effect of hepatic vascular inflow occlusion on liver tissue pH, carbon dioxide, and oxygen partial pressures: defining the optimal clamp/release regime for intermittent portal clamping. *J Surg Res* 2007; **141**: 247-251 [PMID: 17512550 DOI: 10.1016/j.jss.2006.10.054]
- 16 **Belghiti J**, Noun R, Malafosse R, Jagot P, Sauvanet A, Pierangeli F, Marty J, Farges O. Continuous versus intermittent portal triad clamping for liver resection: a controlled study. *Ann Surg* 1999; **229**: 369-375 [PMID: 10077049 DOI: 10.1097/0000658-199903000-00010]
- 17 **Lesurtel M**, Lehmann K, de Rougemont O, Clavien PA. Clamping techniques and protecting strategies in liver surgery. *HPB* (Oxford) 2009; **11**: 290-295 [PMID: 19718355 DOI: 10.1111/j.1477-2574.2009.00066.x]
- 18 **Beck-Schimmer B**, Breitenstein S, Bonvini JM, Lesurtel M, Ganter M, Weber A, Puhan MA, Clavien PA. Protection of pharmacological postconditioning in liver surgery: results of a prospective randomized controlled trial. *Ann Surg* 2012; **256**: 837-844; discussion 844-845 [PMID: 23095629 DOI: 10.1097/SLA.0b013e318272df7c]
- 19 **Natori S**, Selzner M, Valentino KL, Fritz LC, Srinivasan A, Clavien PA, Gores GJ. Apoptosis of sinusoidal endothelial cells occurs during liver preservation injury by a caspase-dependent mechanism. *Transplantation* 1999; **68**: 89-96 [PMID: 10428274 DOI: 10.1097/00007890-199907150-00018]
- 20 **Zhang JX**, Pegoli W, Clemens MG. Endothelin-1 induces direct constriction of hepatic sinusoids. *Am J Physiol* 1994; **266**: G624-G632 [PMID: 8179001]
- 21 **Funaki H**, Shimizu K, Harada S, Tsuyama H, Fushida S, Tani T, Miwa K. Essential role for nuclear factor kappaB in ischemic preconditioning for ischemia-reperfusion injury of the mouse liver. *Transplantation* 2002; **74**: 551-556 [PMID: 12352918 DOI: 10.1097/00007890-200208270-00021]
- 22 **Suzuki S**, Toledo-Pereyra LH. Interleukin 1 and tumor necrosis factor production as the initial stimulants of liver ischemia and reperfusion injury. *J Surg Res* 1994; **57**: 253-258 [PMID: 7518017 DOI: 10.1006/jsre.1994.1140]
- 23 **Beldi G**, Banz Y, Kroemer A, Sun X, Wu Y, Graubardt N, Rellstab A, Nowak M, Enjyoji K, Li X, Junger WG, Candinas D, Robson SC. Deletion of CD39 on natural killer cells attenuates hepatic ischemia/reperfusion injury in mice. *Hepatology* 2010; **51**: 1702-1711 [PMID: 20146261 DOI: 10.1002/hep.23510]
- 24 **Strasberg SM**. Nomenclature of hepatic anatomy and resections: a review of the Brisbane 2000 system. *J Hepatobiliary Pancreat Surg* 2005; **12**: 351-355 [PMID: 16258801 DOI: 10.1007/s00534-005-0999-7]
- 25 **Launois B**, Jamieson GG. The posterior intrahepatic approach for hepatectomy or removal of segments of the liver. *Surg Gynecol Obstet* 1992; **174**: 155-158 [PMID: 1734576]
- 26 **Hsieh CB**, Chen CJ, Chen TW, Yu JC, Shen KL, Chang TM, Liu YC. Accuracy of indocyanine green pulse spectrophotometry clearance test for liver function prediction in transplanted patients. *World J Gastroenterol* 2004; **10**: 2394-2396 [PMID: 15285026 DOI: 10.3748/wjg.v10.i16.2394]
- 27 **Agha A**, Bein T, Fröhlich D, Höfler S, Krenz D, Jauch KW. ["Simplified Acute Physiology Score" (SAPS II) in the assessment of severity of illness in surgical intensive care patients]. *Chirurg* 2002; **73**: 439-442 [PMID: 12089827 DOI: 10.1007/s00104-001-0374-4]
- 28 **Koch M**, Garden OJ, Padbury R, Rahbari NN, Adam R, Capussotti L, Fan ST, Yokoyama Y, Crawford M, Makuuchi M, Christophi C, Banting S, Brooke-Smith M, Usatoff V, Nagino M, Maddern G, Hugh TJ, Vauthey JN, Greig P, Rees M, Nimura Y, Figueras J, DeMatteo RP, Büchler MW, Weitz J. Bile leakage after hepatobiliary and pancreatic surgery: a definition and grading of severity by the International Study Group of Liver Surgery. *Surgery* 2011; **149**: 680-688 [PMID: 21316725 DOI: 10.1016/j.surg.2010.12.002]
- 29 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832 [PMID: 9121257 DOI: 10.1016/S0140-6736(96)07642-8]
- 30 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213 [PMID: 15273542 DOI: 10.1097/01.sla.0000133083.54934.ae]
- 31 **Belghiti J**, Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O. Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. *J Am Coll Surg* 2000; **191**: 38-46 [PMID: 10898182 DOI: 10.1016/

- S1072-7515(00)00261-1]
- 32 **Jarnagin WR**, Gonen M, Fong Y, DeMatteo RP, Ben-Porat L, Little S, Corvera C, Weber S, Blumgart LH. Improvement in perioperative outcome after hepatic resection: analysis of 1,803 consecutive cases over the past decade. *Ann Surg* 2002; **236**: 397-406; discussion 406-7 [PMID: 12368667 DOI: 10.1097/0000658-200210000-00001]
  - 33 **Imamura H**, Seyama Y, Kokudo N, Maema A, Sugawara Y, Sano K, Takayama T, Makuuchi M. One thousand fifty-six hepatectomies without mortality in 8 years. *Arch Surg* 2003; **138**: 1198-1206; discussion 1206 [PMID: 14609867 DOI: 10.1001/archsurg.138.11.1198]
  - 34 **Poon RT**, Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Improving perioperative outcome expands the role of hepatectomy in management of benign and malignant hepatobiliary diseases: analysis of 1222 consecutive patients from a prospective database. *Ann Surg* 2004; **240**: 698-708; discussion 708-710 [PMID: 15383797 DOI: 10.1097/01.sla.0000141195.66155.0c]
  - 35 **van der Bilt JD**, Livestro DP, Borren A, van Hillegersberg R, Borel Rinkes IH. European survey on the application of vascular clamping in liver surgery. *Dig Surg* 2007; **24**: 423-435 [PMID: 17855781 DOI: 10.1159/000108325]
  - 36 **Kooby DA**, Stockman J, Ben-Porat L, Gonen M, Jarnagin WR, DeMatteo RP, Tuorto S, Wuest D, Blumgart LH, Fong Y. Influence of transfusions on perioperative and long-term outcome in patients following hepatic resection for colorectal metastases. *Ann Surg* 2003; **237**: 860-869; discussion 869-870 [PMID: 12796583 DOI: 10.1097/0000658-200306000-00015]
  - 37 **Wei AC**, Tung-Ping Poon R, Fan ST, Wong J. Risk factors for perioperative morbidity and mortality after extended hepatectomy for hepatocellular carcinoma. *Br J Surg* 2003; **90**: 33-41 [PMID: 12520572 DOI: 10.1002/bjs.4018]
  - 38 **Lee KF**, Cheung YS, Wong J, Chong CC, Wong JS, Lai PB. Randomized clinical trial of open hepatectomy with or without intermittent Pringle manoeuvre. *Br J Surg* 2012; **99**: 1203-1209 [PMID: 22828986 DOI: 10.1002/bjs.8863]
  - 39 **Capussotti L**, Muratore A, Ferrero A, Massucco P, Ribero D, Polastri R. Randomized clinical trial of liver resection with and without hepatic pedicle clamping. *Br J Surg* 2006; **93**: 685-689 [PMID: 16703653 DOI: 10.1002/bjs.5301]
  - 40 **Man K**, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Prospective evaluation of Pringle maneuver in hepatectomy for liver tumors by a randomized study. *Ann Surg* 1997; **226**: 704-711; discussion 711-713 [PMID: 9409569 DOI: 10.1097/0000658-199712000-00007]
  - 41 **Lee KF**, Wong J, Ng W, Cheung YS, Lai P. Feasibility of liver resection without the use of the routine Pringle manoeuvre: an analysis of 248 consecutive cases. *HPB (Oxford)* 2009; **11**: 332-338 [PMID: 19718361 DOI: 10.1111/j.1477-2574.2009.00053.x]
  - 42 **Chau GY**, Lui WY, King KL, Wu CW. Evaluation of effect of hemihepatic vascular occlusion and the Pringle maneuver during hepatic resection for patients with hepatocellular carcinoma and impaired liver function. *World J Surg* 2005; **29**: 1374-1383 [PMID: 16240064 DOI: 10.1007/s00268-005-7766-4]
  - 43 **Rau HG**, Duessel AP, Wurzbacher S. The use of water-jet dissection in open and laparoscopic liver resection. *HPB (Oxford)* 2008; **10**: 275-280 [PMID: 18773110 DOI: 10.1080/13651820802167706]
  - 44 **Hassanain M**, Metrakos P, Fisette A, Doi SA, Schricker T, Lattermann R, Carvalho G, Wykes L, Molla H, Cianflone K. Randomized clinical trial of the impact of insulin therapy on liver function in patients undergoing major liver resection. *Br J Surg* 2013; **100**: 610-618 [PMID: 23339047 DOI: 10.1002/bjs.9034]
  - 45 **Melendez JA**, Arslan V, Fischer ME, Wuest D, Jarnagin WR, Fong Y, Blumgart LH. Perioperative outcomes of major hepatic resections under low central venous pressure anesthesia: blood loss, blood transfusion, and the risk of postoperative renal dysfunction. *J Am Coll Surg* 1998; **187**: 620-625 [PMID: 9849736 DOI: 10.1016/S1072-7515(98)00240-3]
  - 46 **Choukèr A**, Schachtner T, Schauer R, Dugas M, Löhe F, Martignoni A, Pollwein B, Niklas M, Rau HG, Jauch KW, Peter K, Thiel M. Effects of Pringle manoeuvre and ischaemic preconditioning on haemodynamic stability in patients undergoing elective hepatectomy: a randomized trial. *Br J Anaesth* 2004; **93**: 204-211 [PMID: 15194628 DOI: 10.1093/bja/aei195]
  - 47 **Clavien PA**, Yadav S, Sindram D, Bentley RC. Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans. *Ann Surg* 2000; **232**: 155-162 [PMID: 10903590 DOI: 10.1097/0000658-200008000-00001]
  - 48 **Rahbari NN**, Wente MN, Schemmer P, Diener MK, Hoffmann K, Motschall E, Schmidt J, Weitz J, Büchler MW. Systematic review and meta-analysis of the effect of portal triad clamping on outcome after hepatic resection. *Br J Surg* 2008; **95**: 424-432 [PMID: 18314921 DOI: 10.1002/bjs.6141]
  - 49 **Bismuth H**, Castaing D, Garden OJ. Major hepatic resection under total vascular exclusion. *Ann Surg* 1989; **210**: 13-19 [PMID: 2742411 DOI: 10.1097/0000658-198907000-00002]
  - 50 **Balzan S**, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D, Durand F. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**: 824-828; discussion 828-829 [PMID: 16327492]

P- Reviewer: Chen JL, Morales-Gonzalez J

S- Editor: Qiu S L- Editor: A E- Editor: Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

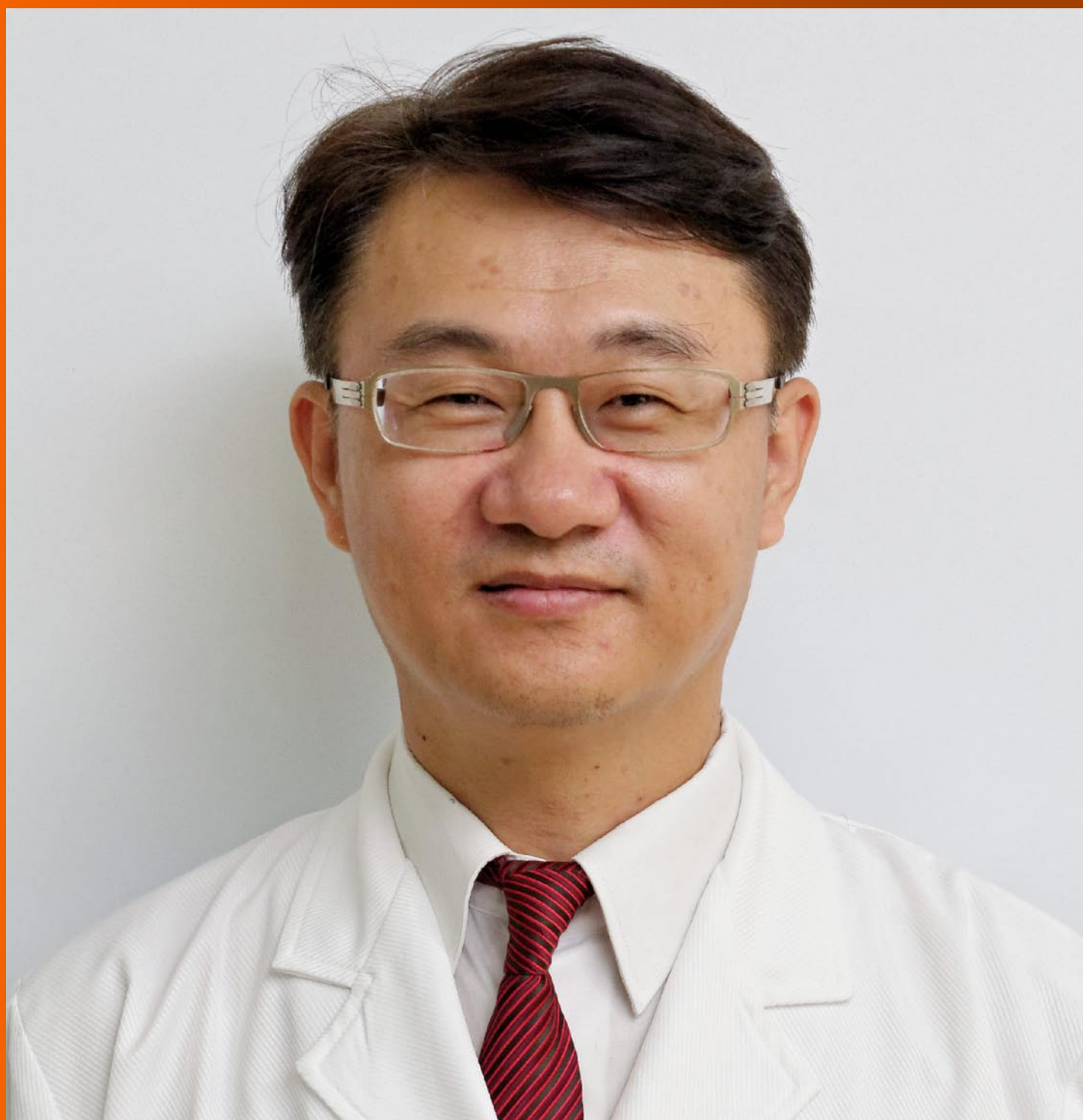
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 September 8; 8(25): 1047-1092







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



**REVIEW**

- 1047** Systemic hemodynamics in advanced cirrhosis: Concerns during perioperative period of liver transplantation

*Hori T, Ogura Y, Onishi Y, Kamei H, Kurata N, Kainuma M, Takahashi H, Suzuki S, Ichikawa T, Mizuno S, Aoyama T, Ishida Y, Hirai T, Hayashi T, Hasegawa K, Takeichi H, Ota A, Kodera Y, Sugimoto H, Iida T, Yagi S, Taniguchi K, Uemoto S*

**MINIREVIEWS**

- 1061** Inhibition of apoptosis by oncogenic hepatitis B virus X protein: Implications for the treatment of hepatocellular carcinoma

*Chao CCK*

**ORIGINAL ARTICLE****Retrospective Study**

- 1067** *CD36* genetic variation, fat intake and liver fibrosis in chronic hepatitis C virus infection

*Ramos-Lopez O, Roman S, Martinez-Lopez E, Fierro NA, Gonzalez-Aldaco K, Jose-Abrego A, Panduro A*

**EVIDENCE-BASED MEDICINE**

- 1075** Therapeutic alternatives for the treatment of type 1 hepatorenal syndrome: A Delphi technique-based consensus

*Arab JP, Claro JC, Arancibia JP, Contreras J, Gómez F, Muñoz C, Nazal L, Roessler E, Wolff R, Arrese M, Benítez C*

**SYSTEMATIC REVIEWS**

- 1087** Hydatid cyst of the gallbladder: A systematic review of the literature

*Gómez R, Allaoua Y, Colmenares R, Gil S, Roquero P, Ramia JM*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Dr. Chih-Wen Lin, MD, Lecturer, Division of Gastroenterology and Hepatology, Department of Medicine, E-DA Hospital/ I-SHOU University, Kaohsiung 82445, Taiwan

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/ ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xin-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Department of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL BOARD MEMBERS**  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

**EDITORIAL OFFICE**  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
September 8, 2016

**COPYRIGHT**  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
<http://www.wjgnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esps/>

## Systemic hemodynamics in advanced cirrhosis: Concerns during perioperative period of liver transplantation

Tomohide Hori, Yasuhiro Ogura, Yasuharu Onishi, Hideya Kamei, Nobuhiko Kurata, Motoshi Kainuma, Hideo Takahashi, Shogo Suzuki, Takashi Ichikawa, Shoko Mizuno, Tadashi Aoyama, Yuki Ishida, Takahiro Hirai, Tomoko Hayashi, Kazuko Hasegawa, Hiromu Takeichi, Atsunobu Ota, Yasuhiro Kodaera, Hiroyuki Sugimoto, Taku Iida, Shintaro Yagi, Kentaro Taniguchi, Shinji Uemoto

Tomohide Hori, Yasuhiro Ogura, Yasuharu Onishi, Hideya Kamei, Nobuhiko Kurata, Department of Transplant Surgery, Nagoya University Hospital, Nagoya 466-8550, Japan

Motoshi Kainuma, Hideo Takahashi, Shogo Suzuki, Takashi Ichikawa, Shoko Mizuno, Tadashi Aoyama, Yuki Ishida, Takahiro Hirai, Tomoko Hayashi, Kazuko Hasegawa, Hiromu Takeichi, Atsunobu Ota, Department of Intensive Care Unit, Nagoya University Hospital, Nagoya 466-8550, Japan

Yasuhiro Kodaera, Hiroyuki Sugimoto, Second Department of Surgery, Nagoya University Hospital, Nagoya 466-8550, Japan

Taku Iida, Shintaro Yagi, Shinji Uemoto, Department of Transplant Surgery, Kyoto University Hospital, Kyoto 624-0802, Japan

Kentaro Taniguchi, First Department of Surgery, Mie University Hospital, Tsu 437-6001, Japan

**Author contributions:** Hori T wrote this review; Ogura Y, Onishi Y, Kamei H, Kurata N, Kainuma M, Takahashi H, Suzuki S, Ichikawa T, Mizuno S, Aoyama T, Ishida Y, Hirai T, Hayashi T, Hasegawa K, Takeichi H, Ota A and Sugimoto H gave academic opinions for peri-operative period and helped to review the papers; Kodaera Y and Uemoto S supervised this review; Iida T, Yagi S and Taniguchi K helped to collect clinical data and to review important papers.

**Conflict-of-interest statement:** No potential conflicts of interest. No financial support.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Tomohide Hori, MD, PhD, Department of Transplant Surgery, Nagoya University Hospital, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. [horit@kuhp.kyoto-u.ac.jp](mailto:horit@kuhp.kyoto-u.ac.jp)  
Telephone: +81-52-7442248  
Fax: +81-52-7441911

Received: March 23, 2016

Peer-review started: March 23, 2016

First decision: May 16, 2016

Revised: May 16, 2016

Accepted: July 14, 2016

Article in press: July 18, 2016

Published online: September 8, 2016

### Abstract

Advanced liver cirrhosis is usually accompanied by portal hypertension. Long-term portal hypertension results in various vascular alterations. The systemic hemodynamic state in patients with cirrhosis is termed a hyperdynamic state. This peculiar hemodynamic state is characterized by an expanded blood volume, high cardiac output, and low total peripheral resistance. Vascular alterations do not disappear even long after liver transplantation (LT), and recipients with cirrhosis exhibit a persistent systemic hyperdynamic state even after LT. Stability of optimal systemic hemodynamics is indispensable for adequate portal venous flow (PVF) and successful LT, and reliable parameters for optimal systemic hemodynamics and adequate PVF are required. Even a subtle disorder in systemic hemodynamics is precisely indicated by the balance between cardiac output and blood volume. The indocyanine green (ICG) kinetics reflect the patient's functional hepatocytes and effective PVF, and PVF is a major determinant of the ICG elimination

constant (*k*ICG) in the well-preserved allograft. The *k*ICG value is useful to set the optimal PVF during living-donor LT and to evaluate adequate PVF after LT. Perioperative management has a large influence on the postoperative course and outcome; therefore, key points and unexpected pitfalls for intensive management are herein summarized. Transplant physicians should fully understand the peculiar systemic hemodynamic behavior in LT recipients with cirrhosis and recognize the critical importance of PVF after LT.

**Key words:** Liver cirrhosis; Portal hypertension; Liver transplantation; Indocyanine green; Hyperdynamic

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In patients with advanced cirrhosis who undergo liver transplantation (LT), perioperative management greatly influences the postoperative course and outcome. This review covers key points and unexpected pitfalls of intensive management in these patients. A peculiar systemic hemodynamic state (hyperdynamic state) persists in recipients with cirrhosis even after LT, and stability of optimal systemic hemodynamics is important for adequate portal venous flow (PVF) and successful LT. Reliable parameters for optimal systemic hemodynamics (a balance between cardiac output and blood volume) and adequate PVF (indocyanine clearance) during and after LT are herein described. Transplant physicians should fully understand these peculiar hemodynamic phenomena.

Hori T, Ogura Y, Onishi Y, Kamei H, Kurata N, Kainuma M, Takahashi H, Suzuki S, Ichikawa T, Mizuno S, Aoyama T, Ishida Y, Hirai T, Hayashi T, Hasegawa K, Takeichi H, Ota A, Kodera Y, Sugimoto H, Iida T, Yagi S, Taniguchi K, Uemoto S. Systemic hemodynamics in advanced cirrhosis: Concerns during perioperative period of liver transplantation. *World J Hepatol* 2016; 8(25): 1047-1060 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i25/1047.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i25.1047>

## INTRODUCTION

Advanced liver cirrhosis (LC) is usually accompanied by portal hypertension (PH). Long-term PH results in various vascular alterations, such as venous dilatation, endothelial damage, collateral pathway formation, and shunt development<sup>[1-3]</sup>. Some pathognomonic findings (e.g., varices, splanchnic congestion, intractable ascites, hepatic encephalopathy, and hepatorenal syndrome) are directly related to PH<sup>[3,4]</sup>, and the pathophysiology of PH involves a complex of humoral and neural mechanisms<sup>[3]</sup>. These mechanisms determine hemodynamic changes and lead to a peculiar systemic circulation pattern<sup>[3]</sup>. The clinical implications of these peculiar systemic hemo-

dynamics in patients with LC have been described as a hyperdynamic state (so-called "hyperdynamic syndrome")<sup>[3]</sup>. Specific manifestations that have been described include high cardiac output (CO), a large blood volume (BV), low total peripheral resistance (TPR), hyponatremic electrolyte abnormalities, and a lower potassium level due to secondary aldosteronism<sup>[5]</sup>.

Here, we reviewed the peculiar systemic hemodynamics in patients with advanced LC. We focused particularly on the systemic hemodynamic phenomena in liver transplantation (LT) recipients with LC because such LT recipients usually have long-term PH due to advanced LC. Adequate portal venous flow (PVF) to acquire satisfactory graft function is attributed to continuous optimal systemic hemodynamic stability beginning immediately after LT<sup>[1,2]</sup>. Therefore, we herein review the optimal state of the systemic hemodynamics after LT for excellent outcomes and discuss key points and unexpected pitfalls in the perioperative intensive managements of recipients with LC. We also demonstrate the usefulness of indocyanine green (ICG) during and after LT to estimate optimal systemic hemodynamics and adequate PVF.

## SYSTEMIC HEMODYNAMICS IN PATIENTS WITH ADVANCED LC

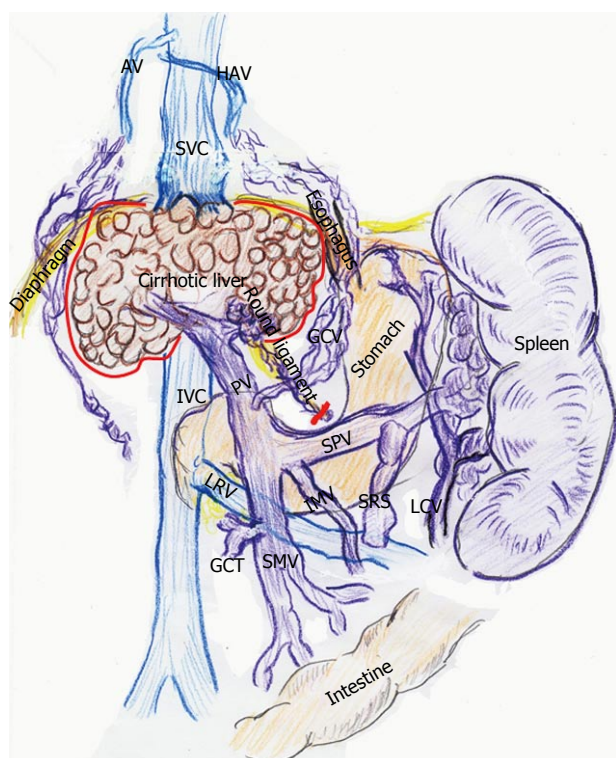
The systemic hemodynamic state in patients with LC has been characterized as hyperdynamic<sup>[3,6,7]</sup>. Cirrhotic hemodynamics are characterized as hyperdynamic by a high CO, large BV, low TPR, mildly tachycardic heart rate (HR), and low or normal mean arterial pressure (MAP)<sup>[1-4,6,8-10]</sup>. Parameters of peripheral resistance, such as TPR, clearly reflect various vascular alterations<sup>[1-3,9,11,12]</sup>.

## NONINVASIVE METHODOLOGY FOR REAL-TIME ASSESSMENT OF SYSTEMIC HEMODYNAMIC STATE

The ICG dye dilution curve can be used to measure hemodynamic parameters<sup>[13,14]</sup>. The currently available noninvasive method for measuring systemic hemodynamic parameters is pulse dye densitometry (PDD). Its basic principles have been described in detail elsewhere<sup>[13-16]</sup>. This noninvasive method is more reliable than invasive methods<sup>[13-15]</sup> and is suitable for clinical use because of its simplicity for bedside use, real-time presentation of results, and cost-effectiveness<sup>[15-17]</sup>.

The principles of BV measurement using radioactive isotopes have already been established<sup>[18-20]</sup>. However, these techniques are associated with potential biohazards due to the use of radioactive indicators and require complex management. Indeed, these invasive methods using radioactive isotopes are completely unsuitable for BV monitoring during the perioperative period<sup>[14,15]</sup>. BV measurement by noninvasive PDD is considerably correlated with BV measurement by radioactive isotope





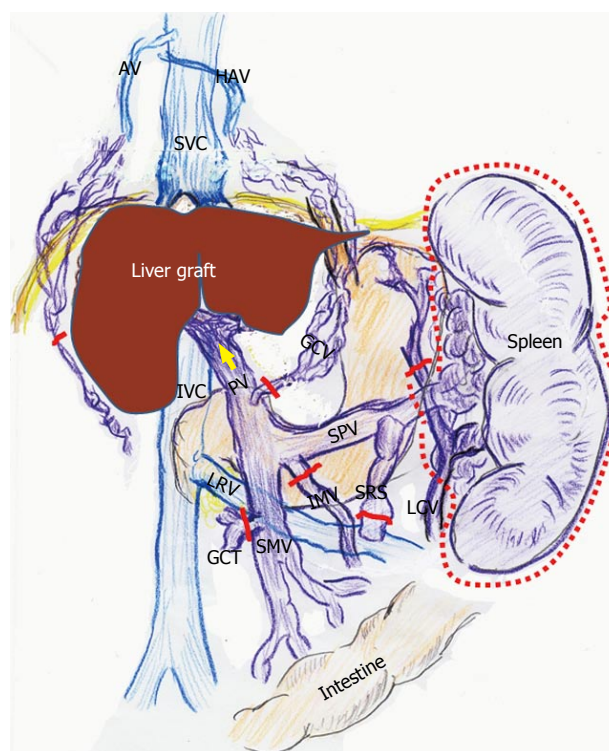
**Figure 1 Vascular alterations in advanced liver cirrhosis.** Collaterals along the round ligament are removed with native liver (red line). Collaterals developed around the native liver are also ligated (red line). AV: Azygos vein; GCT: Gastro-colic trunk; GCV: Gastric coronary vein; HAV: Hemi-azygos vein; IMV: Inferior mesenteric vein; IVC: Inferior vena cava; LCV: Left colic vein; LRV: Left renal vein; PV: Portal vein; SMV: Superior mesenteric vein; SPV: Splenic vein; SRS: Splenoportal shunt; SVC: Superior vena cava.

methods<sup>[21-23]</sup>; it is thus advantageous for real-time evaluation of BV<sup>[1,15]</sup>.

## SYSTEMIC HEMODYNAMIC BEHAVIOR AFTER LT

Adult LT recipients often develop peculiar hemodynamics due to advanced LC<sup>[1,2]</sup> (Figure 1). Mainly in the 1990s, various researchers focused on systemic hemodynamics after LT<sup>[8,9,11,12,24-28]</sup>. Controversial opinions exist regarding these systemic hemodynamic behaviors after LT. While several investigators found persistence of hyperdynamic state<sup>[8,11,24-26]</sup>, others insisted on a decrease toward normal ranges<sup>[12,27,28]</sup>. This discrepancy is believed to be due to the peculiarity of cirrhotic hemodynamics<sup>[9,11]</sup>.

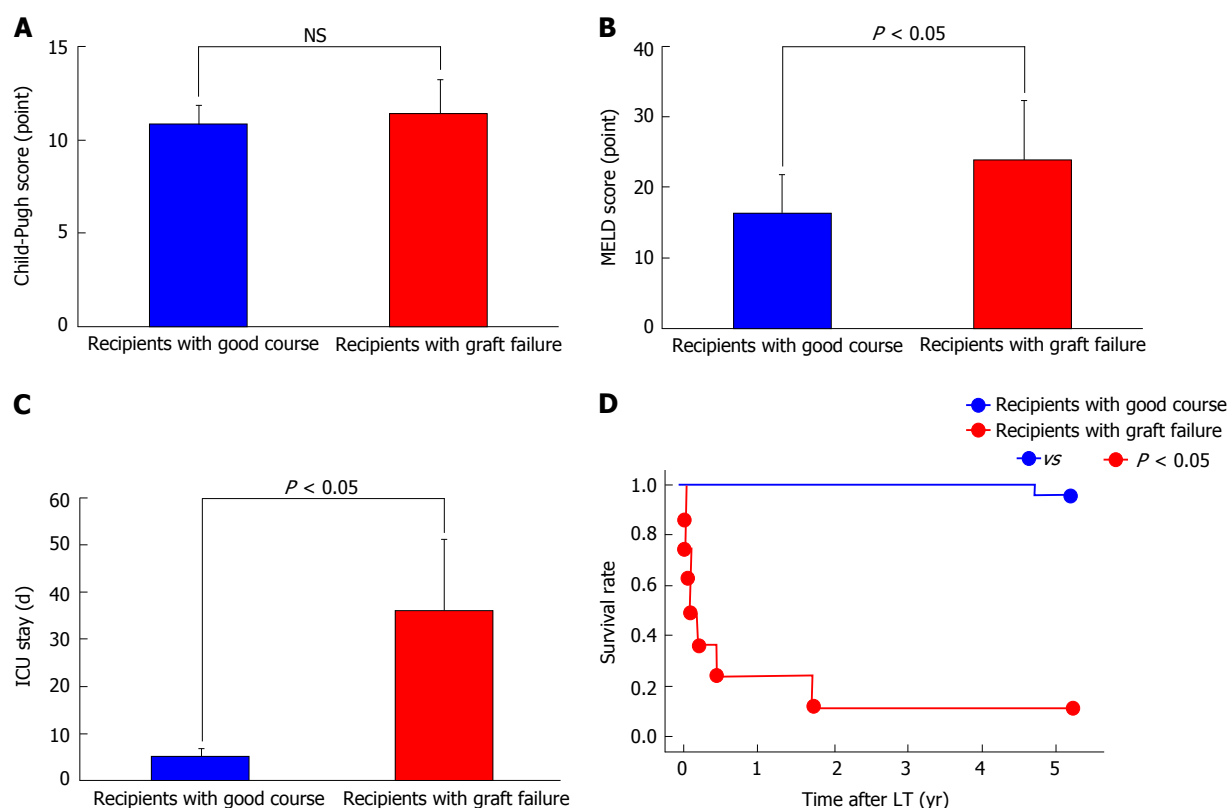
According to studies of TPR in recipients with LC, vascular alterations including venous dilatation and the development of collateral vessels and shunts do not disappear within the first month after LT<sup>[1,2]</sup>. These vascular alterations remain on imaging studies even several years after LT<sup>[29,30]</sup>. Various alterations in systemic hemodynamics in recipients with LC should be maintained despite restoration of the liver function and portal venous pressure (PVP) after LT<sup>[8,11,24-26]</sup>, and most systemic parameters are very slowly restored to the normal range after LT<sup>[9,11]</sup> (Figure 2).



**Figure 2 Intentional modulation of portal venous pressure during living-donor liver transplantation.** Splenectomy is chosen to reduce PVP (red dotted line). Ligations (red lines) of vessels (GCV, IMV, and GCT), collaterals (along LCV and around the native liver) and shunt (SRS) prevent a steal of PVF, and thereafter, PVF will increase (yellow arrow). AV: Azygos vein; GCT: Gastro-colic trunk; GCV: Gastric coronary vein; HAV: Hemi-azygos vein; IMV: Inferior mesenteric vein; IVC: Inferior vena cava; LCV: Left colic vein; LRV: Left renal vein; PV: Portal vein; SMV: Superior mesenteric vein; SPV: Splenic vein; SRS: Splenoportal shunt; SVC: Superior vena cava.

## ACTUAL CHANGES IN SYSTEMIC AND SPLANCHNIC HEMODYNAMIC PARAMETERS AFTER LT

Hemodynamic and splanchnic systemic parameters were analyzed in 35 adult recipients who underwent living-donor LT (LDLT). All patients had advanced LC based on imaging studies and histopathological assessments. ABO blood groups were identical or compatible. Combinations of lymphoid cross-matches were all negative. The CO, CI, BV, central blood volume (CBV), and HR were measured with a PDD apparatus. The TPR was measured simultaneously with the PDD examination; calculation of the TPR has been described in detail elsewhere<sup>[2,9]</sup>. Splanchnic circulatory parameters were simultaneously assessed using Doppler ultrasound. Measurements of splanchnic parameters including PVF has been described in detail elsewhere<sup>[2]</sup>. Measurements were performed before LDLT and from 1 to 14 d after LDLT. Measurements were repeated every 12 h until 72 h after LDLT. To establish the normal ranges of each parameter, the variables were investigated in 16 healthy individuals (live donors before LDLT). Our 35 recipients were retrospectively classified into 2 groups based on graft functions that corresponded to outcomes<sup>[31,32]</sup>.



**Figure 3** Pre-transplant factors and post-transplant course. A: Child-Pugh score; B: MELD score; C: Duration of ICU stay; D: Survival rate. ICU: Intensive care unit; LT: Liver transplantation; MELD: Model for end-stage liver disease; NS: Not significant.

Twenty-seven recipients had good clinical courses after LDLT, although eight recipients developed graft failure. No significant differences were found in the Child-Pugh score (Figure 3A), graft-to-recipient weight ratio (GRWR), operative time, or intraoperative blood loss between the two groups; however, significant differences were found in the Model for End-Stage Liver Disease score (Figure 3B), duration of intensive care unit stay (Figure 3C), and survival rate (Figure 3D). In addition, in the patients who survived, the above-mentioned parameters were measured 3 mo after LDLT. All protocols used in the present study were approved by our institutional review board (approved No. C-297) and were based on the ethical guidelines of the Helsinki Declaration. Informed consent was obtained from all patients before enrollment. For individually, temporally, and repeatedly measured data, differences in the changes over time after LDLT between the two groups were analyzed by repeated-measures analysis of variance. Differences in unpaired discontinuous data between the two groups were analyzed by the Mann-Whitney *U* test. Survival rates were calculated by the Kaplan-Meier method, and the log-rank test was used for between-group comparisons of recipient survival. Values of  $P < 0.05$  were considered statistically significant.

There were no significant differences in the absolute CO (Figure 4A), CI, BV (Figure 4B), CBV (Figure 4C), or MAP between the two groups, although the absolute HR showed differences (Figure 4D). There were also

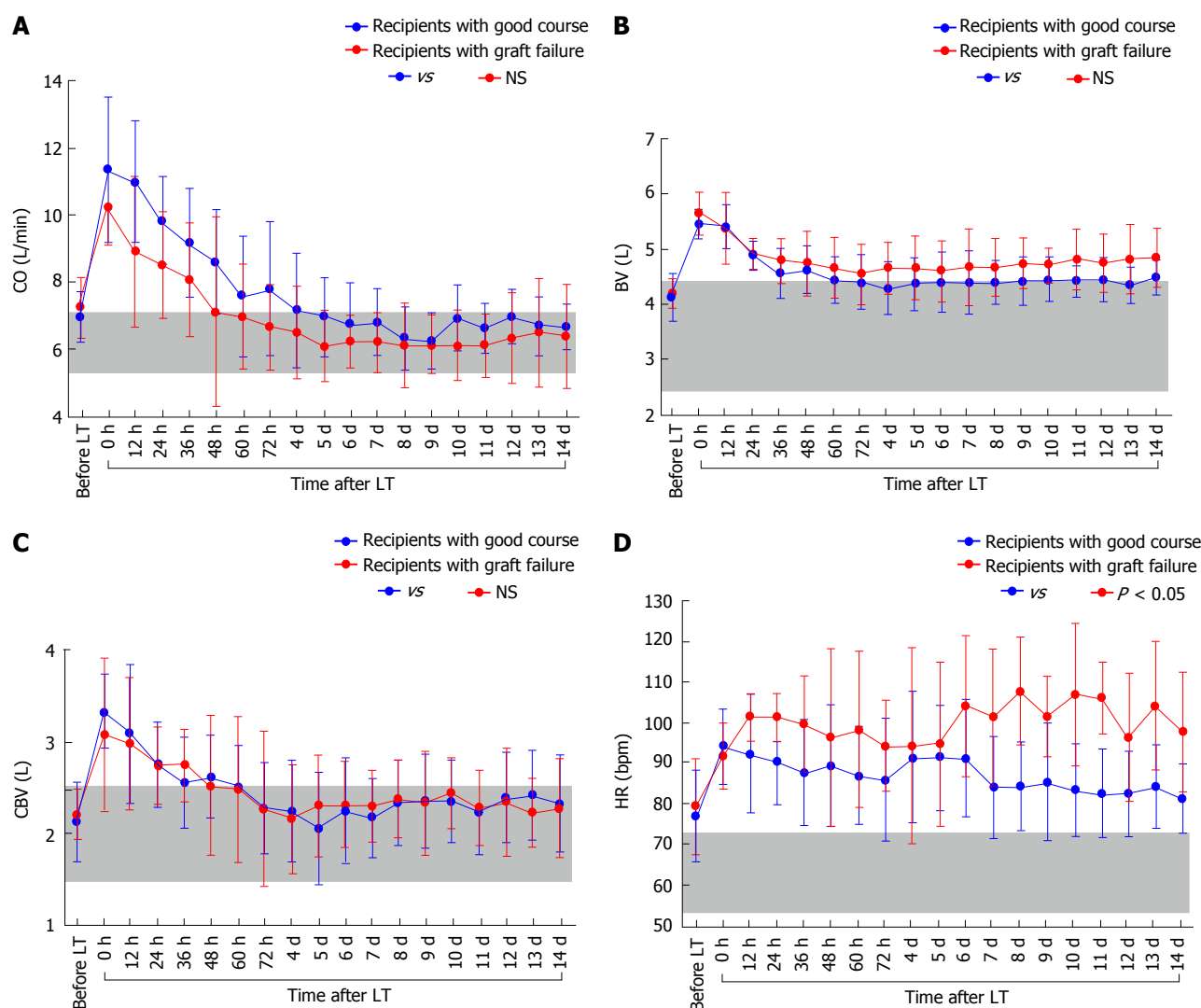
no significant differences in the absolute TPR, which closely reflected vascular alterations (Figure 5A). The balance between CO and BV (*i.e.*, CO/BV) clearly showed significant differences between the groups (Figure 5B). There were significant differences in the PVF velocity (Figure 6A) and PVF volume (Figure 6B) between the groups, although the variables for hepatic arterial flow showed no differences. There were also significant differences in the ICG elimination constant ( $k_{ICG}$ ), which mainly reflects PVF in the early postoperative period<sup>[1,2,32]</sup>.

The CBV reflects the greater circulatory system, and some researchers have suggested that this greater circulation in patients with LC may be slightly lower than that in healthy individuals<sup>[33]</sup>, although the total BV is significantly higher in patients with LC. Our data also demonstrated no remarkable differences in the greater circulation itself between patients with LC and healthy individuals.

The absolute CO, BV, CBV, HR, TPR, and  $k_{ICG}$  in LT recipients who were still alive 3 mo after LDLT are summarized in Figure 7. Our data support the previous opinion that cirrhotic vascular alterations still remain long after LT<sup>[29,30]</sup>.

## OPTIMAL HEMODYNAMIC STATE IN RECIPIENTS WITH LC AFTER LT

As described above, recipients with LC exhibit a persistent systemic hyperdynamic state even after LT<sup>[1,2,8,11,24-26]</sup>.



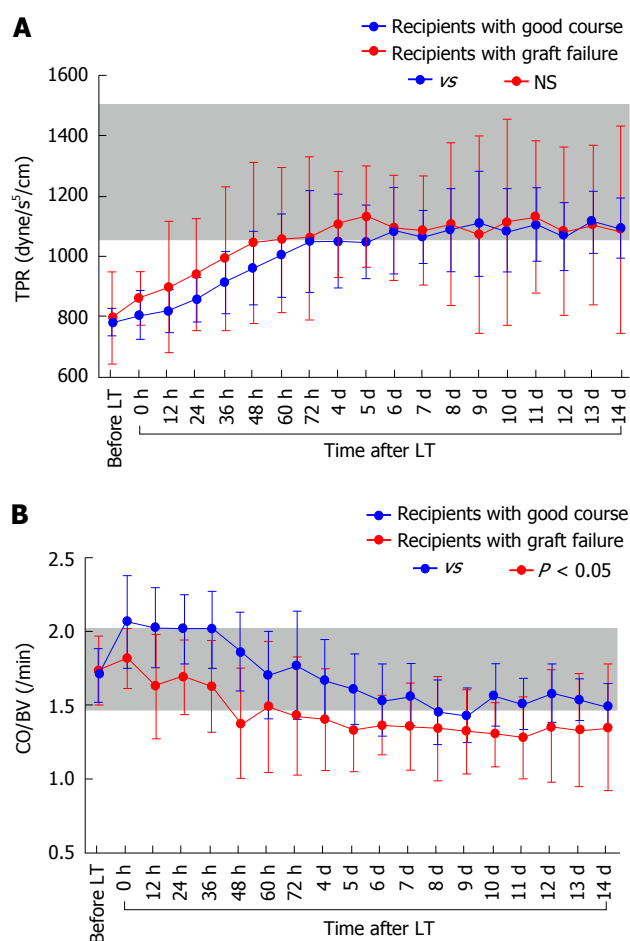
**Figure 4** Actual changes in systemic hemodynamic parameters. A: CO; B: BV; C: CBV; D: HR. Gray zones represent normal ranges. BV: Blood volume; CBV: Central blood volume; CO: Cardiac output; HR: Heart rate; LT: Liver transplantation; NS: Not significant.

Stability of characteristic systemic hyperdynamic parameters after LT is necessary for successful LT in recipients with LC<sup>[1,2]</sup>. Because recipients with LC exhibit these peculiar systemic hyperdynamics even after LT<sup>[8,9,11,24-26]</sup>, an accurate real-time evaluation is necessary to ensure appropriate intensive management after LT<sup>[1,2,15,32]</sup>. The optimal systemic hemodynamics needed for excellent outcomes and the precise parameters for the most appropriate clinical strategy remain unclear<sup>[1,32]</sup> because the absolute values themselves, such as CO, CI, BV, CBV, and MAP, are not necessarily satisfactory for the detection of the subtle instabilities of these patients' peculiar hyperdynamic state<sup>[1,2]</sup>.

## CONCEPT OF CO STANDARDIZATION AGAINST BV

Several investigators have used CO and/or CI to assess hemodynamics after LT<sup>[8,11,12,25]</sup>. Use of the CI, an index that concisely standardizes CO against the body surface area, has been popularized as a standardized CO value

for better assessment. Similar to CO, BV is also one of the most important factors affecting cardiac preload<sup>[14,34]</sup>. Intrinsically, preload is a concept that represents the blood load in the left ventricle and considers the left ventricle as the center of blood ejection<sup>[1,15]</sup>. Therefore, the left ventricular end-diastolic volume becomes a quantitative parameter<sup>[1,15]</sup>. The preload usually replaces actual clinical assessment with parameters representing pressures such as the pulmonary capillary wedge pressure and central venous pressure<sup>[14,35]</sup>. The central venous pressure can be a useful indicator of the filling status of the right ventricle; it is especially useful when followed over time and combined with a measurement of cardiac output<sup>[36]</sup>. Pressure-expressing parameters including the pulmonary capillary wedge pressure and central venous pressure are mainly provided by the CO and BV<sup>[35]</sup>. Therefore, although pressure-expressing parameters do not necessarily reflect the left ventricular end-diastolic volume<sup>[14,15]</sup>, pressure-expressing parameters that reduce the precision of assessment of the systemic hemodynamics have been paradoxically used to judge

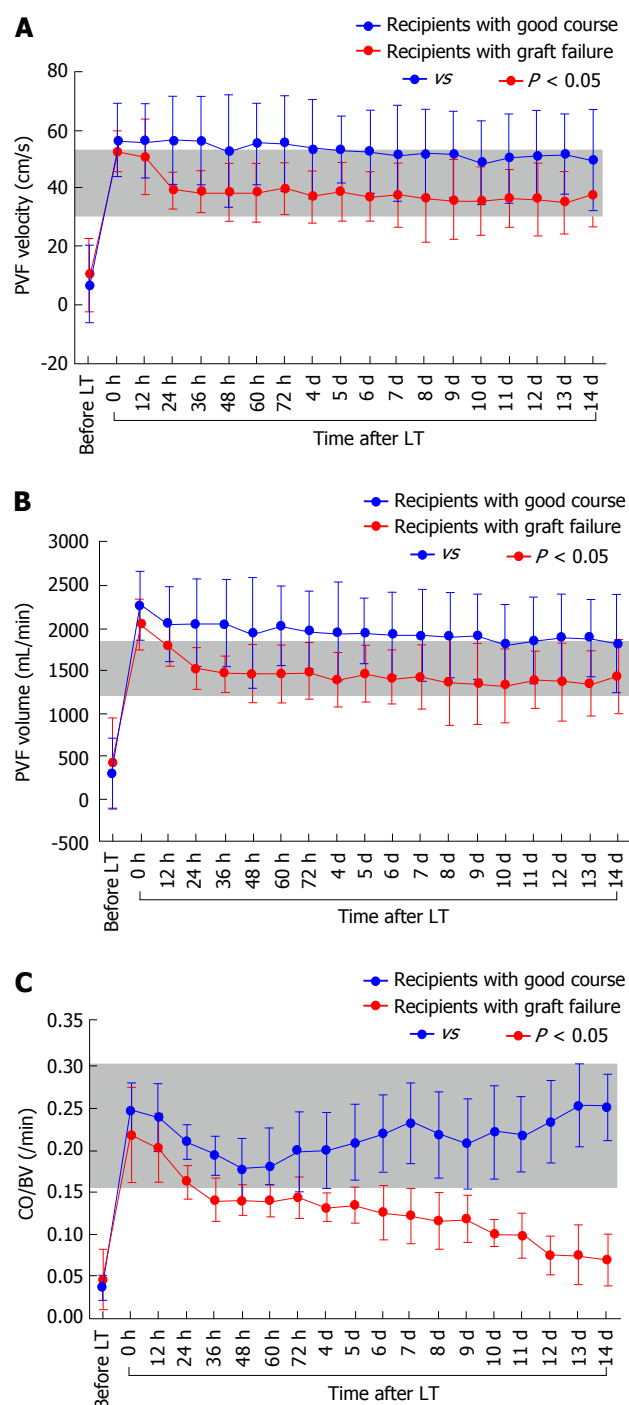


**Figure 5 Actual changes in systemic hemodynamic parameters.** A: TPR; B: CO/BV. Gray zones represent normal ranges. BV: Blood volume; CO: Cardiac output; LT: Liver transplantation; NS: Not significant; TPR: Total peripheral resistance.

distinct factors that represent the amount of BV and strength of CO clinically because BV monitoring has been impossible in the past<sup>[14,15]</sup>. It is necessary to standardize CO against BV, but not against the body surface area, for precise evaluation of preload<sup>[1]</sup>. Currently, the PDD guarantees noninvasive vigilance of the balance between CO and BV as an index for precise assessment of the systemic hemodynamic state<sup>[1,15]</sup>. The CO/BV ratio is a reliable indicator of the optimal systemic hemodynamic state after LT<sup>[1,2]</sup>. Preload focuses on the balance between CO and BV, and cirrhotic systemic hemodynamics are characterized by a high CO and large BV<sup>[6-9,12,24-26,35]</sup>. Real-time assessment of CO and BV by making the best use of noninvasive PDD may become an effective strategy for evaluating the systemic hemodynamic state in LT recipients with LC.

## IMPACT OF SYSTEMIC HEMODYNAMIC STATE ON PVF AFTER LT

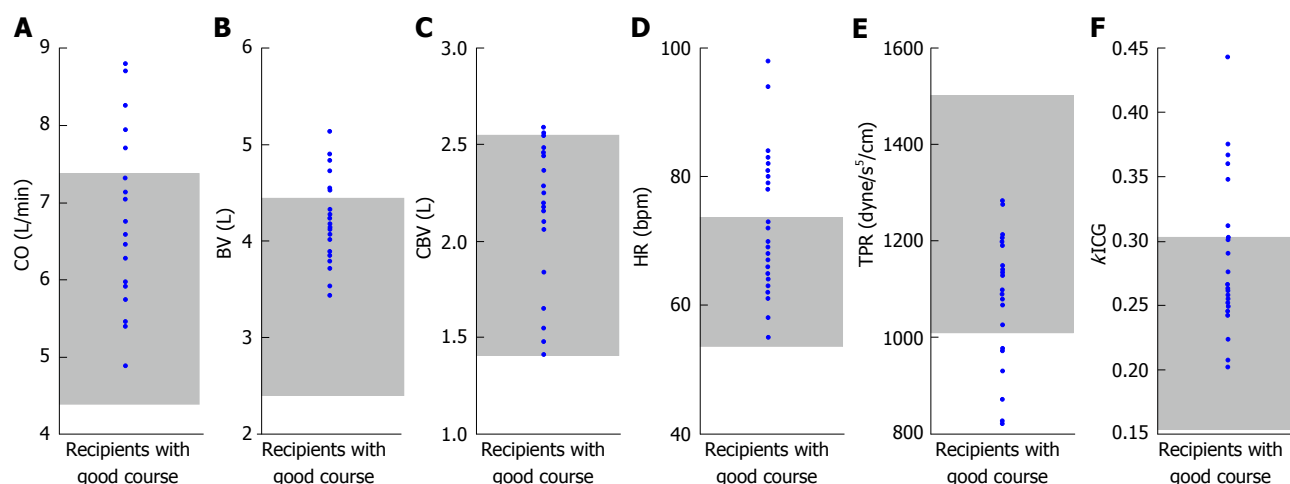
Postoperatively, LT recipients with LC show a clear tendency toward PVF overflow compared with healthy individuals<sup>[2]</sup>. The systemic hemodynamics impact the local graft circulation after LT<sup>[1,2]</sup>, and even a subtle systemic



**Figure 6 Actual changes in portal venous flow and indocyanine green elimination constant.** A: PVF velocity; B: PVF volume; C: kICG. Gray zones represent normal ranges. ICG: Indocyanine green; kICG: Indocyanine green elimination constant; LT: Liver transplantation; PVF: Portal venous flow.

hyperdynamic disorder strongly affects the splanchnic circulation. An imbalance between CO and BV decreases the PVF, which results in critical outcomes<sup>[1,2]</sup>. In brief, an optimal balance between CO and BV guarantees adequate PVF after LT<sup>[1,2]</sup>. Interestingly, subtle disorders in the optimal systemic hyperdynamic state more easily influence the PVF than the hepatic arterial flow<sup>[2]</sup>. Vascular alterations secondary to PH develop in the vessels that originally flow into the portal vein under normal PVP. Such alterations are one cause of a large BV<sup>[2]</sup>. The





**Figure 7 Systemic hemodynamic parameters 3 mo after liver transplantation.** A-F: CO, BV, CBV, HR, TPR, and *k*ICG in patients who survived are shown. Gray zones represent normal ranges. BV: Blood volume; CBV: Central blood volume; CO: Cardiac output; HR: Heart rate; ICG: Indocyanine green; *k*ICG: Indocyanine green elimination constant; LT: Liver transplantation; TPR: Total peripheral resistance.

intestine and spleen become a pool for the large BV<sup>[37]</sup>. Postoperative imbalance between the greater CO and larger BV cause stagnation of the tributary blood flow in the dilated veins and collateral pathways, resulting in a decrease in PVF<sup>[2]</sup>. Transplant physicians should never forget that the systemic hyperdynamic state persists in recipients with LC even after LT<sup>[1,2,8,11,24-26]</sup> and that this peculiar systemic hemodynamic stability is indispensable for adequate PVF after LT<sup>[1,2]</sup>.

Actual images of Doppler ultrasound in cases without stability of systemic hemodynamic state (*i.e.*, an imbalance of CO and BV in the lower TPR) are shown in Figure 8. The PVF should be detected as a stationary wave. However, in a case of unstable systemic hyperdynamic state, the waveform of PVF may seem to be undulant. Moreover, HA waveform may blend into the background of a decreased PVF.

## INTENTIONAL MODULATION OF PVP DURING LDLT

Partial liver grafting is inevitable in the LDLT setting, and the allograft size from the live donor is therefore insufficient. Intentional modulation of the PVP to  $\leq 15$  mmHg is a simple and sure strategy during LDLT<sup>[38-42]</sup>. Detailed surgical procedures for intentional modulation of PVP have been described elsewhere<sup>[40,41]</sup>. Paradoxically, the acceptable minimum GRWR of  $< 0.7$  is possible at graft selection<sup>[40]</sup> because intentional PVP modulation during LDLT will prevent small-for-size syndrome after LDLT<sup>[38-42]</sup>. Although intentional PVP control seems to overcome an GRWR of  $< 0.7$ , these grafts still cause critical problems when evaluated retrospectively<sup>[40]</sup>. Selection of a graft with an GRWR of  $\geq 0.8$  and establishment of a target PVP of  $\leq 15$  mmHg during LDLT are considered keys for successful LDLT<sup>[40]</sup>. Optimal PVF is required for successful LDLT<sup>[2,43]</sup>. Ligation of collaterals and shunts often require an advanced surgical technique because these vessels are always abnormal<sup>[41,42]</sup>.

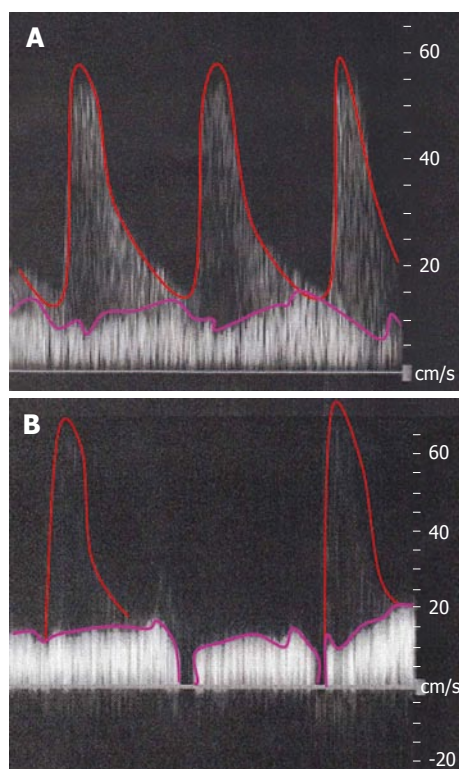
However, intentional setting of the PVF during LDLT is effective not only to trigger liver regeneration after LT, but also to prevent steal of PVF after LDLT.

## STRATEGIC VALUE OF ICG KINETICS DURING LT

ICG is widely used for analysis of liver functions because it is exclusively eliminated by the liver without involvement of the enterohepatic circulation and does not accumulate in the body<sup>[44]</sup>. Asialoglycoprotein receptors on hepatocytes are characteristic of functional liver cells<sup>[45]</sup>, and liver scintigraphy using <sup>99m</sup>Tc-galactosyl human serum albumin has been used as a reliable method of assessment of the hepatic functional reserve in hepatectomy and graft parenchymal function after LT<sup>[46-48]</sup>. There is a correlation between ICG clearance and the hepatic uptake ratio assayed by liver scintigraphy<sup>[45,46]</sup>.

ICG kinetics reflect the functional hepatocytes (cell volume) and effective PVF (clearance)<sup>[31,49-52]</sup>, and PVF is a major determinant of *k*ICG in the normal liver<sup>[32,34,49,51,53]</sup>. The PVF has a large influence on liver regeneration after LT<sup>[32,43]</sup>, and reversible damage to hepatocytes begins immediately after graft recirculation<sup>[32,38,39,43]</sup>. Some researchers have focused on ICG kinetics as a liver function test after LT<sup>[31,32]</sup>, and *k*ICG values can predict clinical outcomes in the early postoperative period after LDLT by closely reflecting the influence of systemic dynamics on the splanchnic circulation<sup>[32]</sup>.

Hepatocytes are well preserved in LDLT because the cold storage time (CIT) is shorter. The *k*ICG reflects the optimum PVF value during LT and in the early postoperative period<sup>[41,42]</sup>. Hence, a division by graft weight is a simple resolution to ensure that the *k*ICG reflects only the PVF based on the advantage of well-preserved hepatocytes during LDLT<sup>[41,42]</sup>. Intentional PVP modulation based on real-time PVP monitoring and the confirmation of an optimal *k*ICG/graft weight value reflecting the PVF are useful procedures used by transplant surgeons



**Figure 8 Decreased portal venous flow due to unstable systemic hyperdynamic state (A and B).** Even a subtle instability of systemic hyperdynamic state (i.e., an imbalance of CO and BV in the lower TPR) results in a decreased PVF. During the PVF measurement by Doppler ultrasound, HA waveform (Red line) blends into the background of a decreased PVF (purple lines). BV: Blood volume; CO: Cardiac output; HA: Hepatic artery; PVF: Portal venous flow; TPR: Total peripheral resistance.

during LDLT<sup>[41,42]</sup>. Actually, in some cases, the *k*ICG value did not change even with intentional controls to decrease or increase the PVP<sup>[41]</sup>. In other cases, the *k*ICG values improved with an increased PVP by ligation of portosystemic collaterals or a decrease in the PVP by splenectomy<sup>[41]</sup>. Thus, these factors seemed to show some discrepancies in some cases<sup>[41,42]</sup>. The relationship between PVP and PVF remains unclear<sup>[42]</sup>. The usefulness of ICG kinetics during LT was first described in 2012<sup>[41]</sup>. Simultaneous fulfillment of a final PVP of  $\leq 15$  mmHg and a final *k*ICG of  $> 4 \times 10^{-4}/g \times$  the graft weight (g) is a sure strategy for achieving the optimal PVF during LDLT<sup>[41]</sup>. Thereafter, the cut-off level of the final *k*ICG/graft weight was demonstrated as  $3.1175 \times 10^{-4}/g$ <sup>[42]</sup>. The final *k*ICG/graft weight during LT has potential as an accurate parameter for the optimal PVF and as a reliable predictor of the postoperative course and outcome after LT<sup>[41,42]</sup>.

## KEY POINTS AND UNEXPECTED PITFALLS IN PERIOPERATIVE MANAGEMENT OF LT RECIPIENTS WITH ADVANCED LC

Liver allografts are at risk of problems such as cold

ischemia/warm reperfusion injury, acute rejection, disease recurrence and hepatic blood flow disorders<sup>[32]</sup>. Transplant physicians should consider many factors simultaneously.

Eventration of the diaphragm because of intractable ascites, or easily broken ribs, often disrupts ventilation<sup>[54]</sup>. Vascular alteration due to long-term PH causes endothelial injury and permeant breakdown and subsequently results in large amounts of ascites, pleural effusion, and gastric fluid<sup>[55]</sup>. The electrolyte composition of these third-space fluids may not be similar to that of the extracellular fluid, and the electrolyte composition of third-space fluids should be checked once if the quantity is large<sup>[56]</sup>. Replenishment for third-space loss should be performed using not Ringer's solution but bicarbonated Ringer's solution<sup>[57-59]</sup> if the electrolyte composition is similar to that of the extracellular fluid and if the third-space loss is quantitatively large.

Careless management techniques, such as rapid increases or decreases of transfusions and medications, are detrimental<sup>[60,61]</sup>. Effects of increases or decreases of transfusions are usually reflected on a day-to-day basis because of the peculiar cirrhotic hemodynamics<sup>[55,60,61]</sup>, and a roller-coaster management technique that repeatedly changes within a single day will trigger poor clinical courses with unexpected complications<sup>[60,61]</sup>. All transfusion management plans should be handled with great caution, and transplant physicians should very carefully evaluate the effects of increases or decreases of transfusions<sup>[60,62]</sup>. A response time lag due to endothelial injury and permeant breakdown should be considered in LC recipients with long-term PH<sup>[63-65]</sup>. Adequate hydration is also required; dehydration should be avoided because of these patients' peculiar hemodynamics. Even temporal dehydration causes unexpected thrombosis, renal failure, and impaired drug metabolism<sup>[60-62]</sup>. Plans to stay within stable systemic hemodynamics (e.g., noradrenaline to maintain CO and well-hydration with human atrial natriuretic peptide) should be considered. Tachycardia may lower the CO. A lower CO that is insufficient to circulate the larger BV decreases the PVF, and a lower PVF results in a poor outcome. As described above, vascular alterations cause the large BV in these patients<sup>[2]</sup>, and the intestine and spleen become pools for the large BV<sup>[37]</sup>. Even a subtle imbalance between the greater CO and larger BV induced by roller-coaster management triggers a decrease in the PVF<sup>[2,60,61]</sup>.

Long-term PH causes splanchnic congestion and intractable ascites. Splanchnic congestion results in breakdown of the enteric barrier<sup>[66]</sup>, and portal venous gas and/or abdominal compartment syndrome may be temporally observed<sup>[66-68]</sup>. Induction of drugs with fibrolytic activity (not heparin, but urokinase and warfarin) should be initiated without hesitation based on the endothelial damage in patients with LC, although heparin induction may be effective from the viewpoint of thromboprophylaxis<sup>[69]</sup>. Notably, long-term biliary drainage may cause coagulopathy due to impaired absorption of vitamins<sup>[70]</sup>. Massive ascites is usually intractable due to endothelial injury and

permeant breakdown, and systemic arterial pressure may be effected even by body motion<sup>[63-65]</sup>. Diuretics (e.g., furosemide and potassium-conserving diuretics) and a water-clearance mediator (e.g., tolvaptan) are available<sup>[71]</sup>. Hemodynamic disorders such as hepatic venous obstruction and portal thrombosis may develop if no response is observed after diuretic induction<sup>[72]</sup>.

The most frequent cause of morbidity and mortality after LT is not immunological rejection but infection-related complications<sup>[73-75]</sup>. Some infections are usually intractable in patients with LC, including bacterial cholangitis<sup>[76]</sup>, spontaneous bacterial peritonitis<sup>[77]</sup>, spontaneous bacterial empyema<sup>[78]</sup>, viral infection<sup>[79]</sup>, aspergillosis<sup>[80]</sup>, and *Pneumocystis jirovecii* pneumonia (formerly known as *Pneumocystis carinii* pneumonia)<sup>[81]</sup>. Because the postoperative risk of complications is associated with the pretransplant conditions<sup>[82,83]</sup>, these infections should be ruled-out and/or treated beforehand. Even a subtle infection will trigger severe complications after LT<sup>[73-75,83,84]</sup>. Evaluation of LT candidates should be carefully performed<sup>[83,85,86]</sup>; pretransplant infections may greatly impair the clinical course and outcomes after LT<sup>[83,87,88]</sup>. Transplant physicians should never forget that intentional pretransplant control of infections, including bacterial, viral, and fungal infections, has a large influence on allograft function and survival<sup>[89,90]</sup>. Uncontrolled infections will have catastrophic effects<sup>[83,87,88]</sup>, and any infections should therefore be treated before LT.

Glycemic control also has an influence on the clinical course after LT<sup>[91]</sup>. Good glycolytic activity and glycemic control in the perioperative period will help to ensure adequate liver regeneration<sup>[92,93]</sup>.

## DISTINCTION BETWEEN HEPATOPULMONARY SYNDROME AND PORTOPULMONARY HYPERTENSION

Hepatopulmonary syndrome (HPS) and portopulmonary hypertension (PPHTN) are cardiopulmonary complications<sup>[3,94-97]</sup> that are frequently seen in patients with LC<sup>[54,94-98]</sup>. Both conditions result from a lack of hepatic clearance of vasoactive substances produced in the splanchnic territory<sup>[95]</sup>. These substances mainly cause subsequent pulmonary vascular remodeling. In previous studies, some degree of vasoconstriction in patients with PPHTN resulted in pulmonary arterial hypertension (PAH) and right ventricular dysfunction<sup>[54,98]</sup>. The current definition of PPHTN includes secondary PAH due to portosystemic shunts<sup>[98]</sup>. In patients with HPS, these vasoactive mediators cause intrapulmonary shunts with hypoxemia<sup>[97]</sup>. The HPS is accompanied by abnormal pulmonary gas exchange and evidence of intrapulmonary vascular dilatation that results in a right-to-left intrapulmonary shunt<sup>[98]</sup>. These entities are both clinically and pathophysiologically distinct<sup>[3,94,95]</sup>, and PPHTN and HPS should be considered as different pathological states<sup>[98]</sup>. HPS is characterized by abnormal pulmonary vasodilation and right-to-left shunting that result in gas

exchange abnormalities<sup>[3,54,94,95,97,98]</sup>, whereas PPHTN is caused by pulmonary artery vasoconstriction that leads to hemodynamic failure<sup>[3,94-96]</sup>. Both HPS and PPHTN are associated with significantly increased morbidity and mortality<sup>[3,94,95,97]</sup>, although these patients are commonly asymptomatic. All candidates for LT should be actively screened for the presence of these two complications<sup>[54,94,95,97,98]</sup>.

Although LT results in the disappearance of HPS within 1 year<sup>[95,99]</sup>, the effect of LT on PPHTN is highly unpredictable<sup>[54,95,98-101]</sup>. PPHTN with PAH has historically been a contraindication for LT<sup>[54,98-100]</sup>. However, the diagnosis and treatment of PPHTN have advanced during the past two decades<sup>[54]</sup>. Assessment of patients' preoperative reactivity and response to pharmacological therapies for moderate-to-severe PPHTN is important to ensure excellent survival rates after LT<sup>[102]</sup>. Prostaglandin *I*<sub>2</sub> has drastically improved outcomes<sup>[103]</sup> and is currently considered a key drug in the control of PPHTN<sup>[103]</sup>. Modern strategies in managing HPS and PPHTN rely on a thorough screening and grading of the disease severity to tailor the appropriate therapy and select only the patients who will benefit from LT<sup>[54,95,97-101]</sup>. Hemodynamic and respiratory modifications in the perioperative period must be avoided through continuation of the preoperatively initiated drugs, appropriate intraoperative monitoring, and proper hemodynamic and respiratory therapies<sup>[54,95,98,99]</sup>. The most reliable monitoring factor for PPHTN with PAH during the perioperative period is the mean pulmonary arterial pressure<sup>[54,98]</sup>, though supplemental oxygen and monitoring of oxygen saturation during the perioperative period are adequate for monitoring of HPS<sup>[97,104,105]</sup>.

## COAGULOPATHY AND ENDOTHELIAL INJURY

The systemic hyperdynamic state causes vessel dilation and collateral development, and the venous endothelium becomes damaged<sup>[4,65]</sup>. An intact endothelial barrier is important, especially in critical situations such as sepsis and thrombotic microangiopathy<sup>[106,107]</sup>. High mobility group box 1 (HMGB1) is an evolutionarily conserved nuclear protein that is passively released by almost all cells during cellular necrosis and is actively secreted from activated macrophages, monocytes, and endothelial cells<sup>[108]</sup>. Once secreted into the extracellular space, HMGB1 serves as a dangerous signal that stimulates inflammatory reactions<sup>[108]</sup>. Thrombomodulin (TM) is an endothelial anticoagulant cofactor that promotes thrombin-mediated formation of activated protein C<sup>[109]</sup>. TM plays an anti-inflammatory role through inactivation of HMGB1<sup>[109,110]</sup>. Recombinant human soluble TM (rTM) has recently become available<sup>[111]</sup>, and this novel drug is effective for sepsis<sup>[110]</sup>. Thrombotic microangiopathy and a positive lymphoid cross-match combination will result in poor outcomes after LT, especially in adult recipients<sup>[112,113]</sup>. Intrahepatic and vascular conditions pathophysiologically overlap. Pathophysiologically, rTM



is effective for sepsis and thrombotic microangiopathy in LT recipients<sup>[107,111]</sup>, although there are no reports of its usefulness for ABO incompatibility in patients undergoing LT. Vascular alterations including endothelial injury still remain even after LT. Based on our experience, the dose of rTM should be reduced to two-thirds of the regular dose in LT recipients with LC, although one-half of the regular dose loses any effects.

## MEDICAL ECONOMY

Insurance systems are different in each country<sup>[114,115]</sup>, and every country has its own limitations of medical resources<sup>[116]</sup>. Hence, transplant physicians should always consider a cost-benefit analysis if they want to continue an effective LT program<sup>[116,117]</sup>. Dialysis treatment, plasma exchange, blood derivatives, and direct-acting antivirals are very expensive<sup>[62,118,119]</sup>. Notably, attempts to perform blood transfusion and infusion of fraction products are ill-advised because they are very detrimental to the medical economy<sup>[62,116,117,119]</sup>. A shorter intensive care unit (ICU) stay has benefits for patients<sup>[120]</sup>, although expensive and intensive care during the ICU stay is needed for post-transplant management. Longer hospital stay impairs quality of life and spoils social status after hospital discharge<sup>[121,122]</sup>.

## DISCUSSION

It is necessary to standardize CO against BV for precise evaluation of preload<sup>[1]</sup>. Considering that cirrhotic hyperdynamics are consolidated in patients with a large BV and high CO under a low TPR<sup>[3,6,8-10]</sup> and that the concept of preload is focused on the balance between CO and BV<sup>[35]</sup>, we can now use the new concept of the CO/BV ratio by making the best use of available devices that can noninvasively measure BV<sup>[1,2,15]</sup>. The PDD guarantees noninvasive vigilance of the balance between CO and BV as an index for precise assessment of the systemic hemodynamic state in LT recipients with LC<sup>[1]</sup>. The CO/BV ratio expresses the CO per min corresponding to a fraction of the BV, which represents how the heart efficiently ejects the BV that should be circulated<sup>[1,2]</sup>. Interestingly, previous studies revealed no differences in the CO/BV among recipients with LC, recipients without LC, and healthy individuals<sup>[1,2]</sup>. This variable has potential as a reliable clinical marker after LT. Subtle instabilities that do not appear when comparing absolute values themselves are simply indicated by the balance between CO and BV<sup>[1,2]</sup>. It seems reasonable that tachycardia resulted in a lower CO in recipients with poor outcomes (Figure 4D) and that the decreased CO could not circulate the large BV in these recipients (Figure 5B).

In LDLT, the CIT is short and the hepatocytes are well preserved<sup>[41]</sup>. Therefore, division by the graft weight is a simple method that allows the kICG to reflect only the PVF, by taking advantage of the shorter CIT in LDLT<sup>[41]</sup>. Strategic values in ICG kinetics are used to set the optimal PVF during LDLT and to evaluate the optimal

systemic hemodynamics after LT<sup>[1,2,32,41,42]</sup>. ICG kinetics reflects the functional hepatocyte volume and effective PVF<sup>[31,49-52]</sup>. Advanced selection criteria of a graft with an GRWR of  $\geq 0.6$  and establishment of a target PVP of  $\leq 15$  mmHg during LDLT are currently documented for successful LDLT<sup>[123-126]</sup>. This defiant set-up with lower GRWR has advantages for donor pool and safety, although these grafts may cause critical problems<sup>[40]</sup>. ICG kinetics is useful to set-up of adequate PVF during LDLT with lower GRWR. Conversely, in deceased-donor LT, although PVF is a major determinant of kICG in the normal liver<sup>[32,34,49,51,53]</sup>, the kICG value may be affected by damaged hepatocytes due to the longer CIT. The decreased kICG may not indicate only an inadequate PVF in deceased-donor LT because ICG kinetics is dually factorial.

## CONCLUSION

LT recipients with LC exhibit peculiar hemodynamics (*i.e.*, systemic hyperdynamic syndrome and PH). Vascular alterations do not easily disappear despite restorations of PH and liver function in recipients with LC, and PVF impacts liver regeneration after LT<sup>[43]</sup>. Stability of characteristic systemic hyperdynamics is indispensable for adequate PVF and successful LT<sup>[1,2]</sup>. Even a subtle disorder of the systemic hyperdynamics dictates PVF<sup>[1,2]</sup>. ICG kinetics is useful to set an adequate PVF during LDLT and evaluate the optimal systemic hemodynamics after LT<sup>[1,2,32,41,42]</sup>. Perioperative management has a large influence on the postoperative course and outcome. Transplant physicians should fully understand the peculiarities of cirrhotic hemodynamics. We hope that this review will be informative for transplant physicians.

## REFERENCES

- 1 Hori T, Yagi S, Iida T, Taniguchi K, Yamagiwa K, Yamamoto C, Hasegawa T, Yamakado K, Kato T, Saito K, Wang L, Torii M, Hori Y, Takeda K, Maruyama K, Uemoto S. Optimal systemic hemodynamic stability for successful clinical outcomes after adult living-donor liver transplantation: prospective observational study. *J Gastroenterol Hepatol* 2008; **23**: e170-e178 [PMID: 18422962 DOI: 10.1111/j.1440-1746.2008.05394.x]
- 2 Hori T, Yagi S, Iida T, Taniguchi K, Yamagiwa K, Yamamoto C, Hasegawa T, Yamakado K, Kato T, Saito K, Wang L, Torii M, Hori Y, Takeda K, Maruyama K, Uemoto S. Stability of cirrhotic systemic hemodynamics ensures sufficient splanchnic blood flow after living-donor liver transplantation in adult recipients with liver cirrhosis. *World J Gastroenterol* 2007; **13**: 5918-5925 [PMID: 17990357 DOI: 10.3748/wjg.v13.i44.5918]
- 3 Licata A, Mazzola A, Ingrassia D, Calvaruso V, Cammà C, Craxi A. Clinical implications of the hyperdynamic syndrome in cirrhosis. *Eur J Intern Med* 2014; **25**: 795-802 [PMID: 25245607 DOI: 10.1016/j.ejim.2014.09.004]
- 4 Ho HL, Huang HC. Molecular mechanisms of circulatory dysfunction in cirrhotic portal hypertension. *J Chin Med Assoc* 2015; **78**: 195-203 [PMID: 25769934 DOI: 10.1016/j.jcma.2014.10.004]
- 5 Stanley MM. Pathogenesis of ascites in cirrhosis. A unitary hypothesis. *ASAIO Trans* 1989; **35**: 161-163 [PMID: 2659055]
- 6 Kowalski HJ, Abelmann WH. The cardiac output at rest in Laennec's cirrhosis. *J Clin Invest* 1953; **32**: 1025-1033 [PMID: 13096569 DOI: 10.1172/JCI102813]
- 7 Vorobioff J, Bredfeldt JE, Groszmann RJ. Increased blood flow



- through the portal system in cirrhotic rats. *Gastroenterology* 1984; **87**: 1120-1126 [PMID: 6479534]
- 8 **Henderson JM**, Mackay GJ, Hooks M, Chezmar JL, Galloway JR, Dodson TF, Kutner MH. High cardiac output of advanced liver disease persists after orthotopic liver transplantation. *Hepatology* 1992; **15**: 258-262 [PMID: 1735528 DOI: 10.1002/hep.1840150214]
  - 9 **Piscaglia F**, Zironi G, Gaiani S, Mazziotti A, Cavallari A, Gramantieri L, Valgimigli M, Bolondi L. Systemic and splanchnic hemodynamic changes after liver transplantation for cirrhosis: a long-term prospective study. *Hepatology* 1999; **30**: 58-64 [PMID: 10385639 DOI: 10.1002/hep.510300112]
  - 10 **Murray JF**, Dawson AM, Sherlock S. Circulatory changes in chronic liver disease. *Am J Med* 1958; **24**: 358-367 [PMID: 13520736]
  - 11 **Gadano A**, Hadengue A, Widmann JJ, Vachieri F, Moreau R, Yang S, Soupison T, Sogni P, Degott C, Durand F. Hemodynamics after orthotopic liver transplantation: study of associated factors and long-term effects. *Hepatology* 1995; **22**: 458-465 [PMID: 7635413]
  - 12 **Navasa M**, Feu F, García-Pagán JC, Jiménez W, Llach J, Rimola A, Bosch J, Rodés J. Hemodynamic and humoral changes after liver transplantation in patients with cirrhosis. *Hepatology* 1993; **17**: 355-360 [PMID: 8444409 DOI: 10.1002/hep.1840170302]
  - 13 **Iijima T**, Aoyagi T, Iwao Y, Masuda J, Fuse M, Kobayashi N, Sankawa H. Cardiac output and circulating blood volume analysis by pulse dye-densitometry. *J Clin Monit* 1997; **13**: 81-89 [PMID: 9112203 DOI: 10.1023/A:1007339924083]
  - 14 **Haruna M**, Kumon K, Yahagi N, Watanabe Y, Ishida Y, Kobayashi N, Aoyagi T. Blood volume measurement at the bedside using ICG pulse spectrophotometry. *Anesthesiology* 1998; **89**: 1322-1328 [PMID: 9856705 DOI: 10.1097/00000542-199812000-00008]
  - 15 **Hori T**, Yamamoto C, Yagi S, Iida T, Taniguchi K, Hasegawa T, Yamakado K, Hori Y, Takeda K, Maruyama K, Uemoto S. Assessment of cardiac output in liver transplantation recipients. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 362-366 [PMID: 18693170]
  - 16 **Fujita Y**, Yamamoto T, Fuse M, Kobayashi N, Takeda S, Aoyagi T. Pulse dye densitometry using indigo carmine is useful for cardiac output measurement, but not for circulating blood volume measurement. *Eur J Anaesthesiol* 2004; **21**: 632-637 [PMID: 15473618 DOI: 10.1097/00003643-200408000-00008]
  - 17 **Ishigami Y**, Masuzawa M, Miyoshi E, Kato M, Tamura K, Kanda M, Awazu K, Taniguchi K, Kurita M, Hayashi N. Clinical applications of ICG Finger Monitor in patients with liver disease. *J Hepatol* 1993; **19**: 232-240 [PMID: 8301056]
  - 18 **Erickson JR**, McCormick JB, Seed L. An improved method for the determination of blood volume using radioactive iodinated human serum albumen. *Science* 1953; **118**: 595-596 [PMID: 13113188]
  - 19 **Strumia MM**, Colwell LS, Dugan A. The measure of erythropoiesis in anemias. I. The mixing time and the immediate post-transfusion disappearance of T-1824 dye and of Cr-51-tagged erythrocytes in relation to blood volume determination. *Blood* 1958; **13**: 128-145 [PMID: 13510291]
  - 20 **Reba RC**, Eckelman WC, Albert SN. Tc-99m labeled red blood cells: a new radiopharmaceutical for the determination of total blood volume and blood pool scanning. *Med Ann Dist Columbia* 1973; **42**: 1-3 [PMID: 4511104]
  - 21 **Bradley EC**, Barr JW. Determination of blood volume using indocyanine green (cardio-green) dye. *Life Sci* 1968; **7**: 1001-1007 [PMID: 4898425 DOI: 10.1016/0024-3205(68)90108-2]
  - 22 **Iijima T**, Iwao Y, Sankawa H. Circulating blood volume measured by pulse dye-densitometry: comparison with (131)I-HSA analysis. *Anesthesiology* 1998; **89**: 1329-1335 [PMID: 9856706]
  - 23 **Imai T**, Mitaka C, Nosaka T, Koike A, Ohki S, Isa Y, Kunimoto F. Accuracy and repeatability of blood volume measurement by pulse dye densitometry compared to the conventional method using 51Cr-labeled red blood cells. *Intensive Care Med* 2000; **26**: 1343-1349 [PMID: 11089762 DOI: 10.1007/s001340000618]
  - 24 **Paulsen AW**, Klintmalm GB. Direct measurement of hepatic blood flow in native and transplanted organs, with accompanying systemic hemodynamics. *Hepatology* 1992; **16**: 100-111 [PMID: 1618464 DOI: 10.1002/hep.1840160118]
  - 25 **Hadengue A**, Lebrech D, Moreau R, Sogni P, Durand F, Gaudin C, Bernuau J, Belghiti J, Gayet B, Erlinger S. Persistence of systemic and splanchnic hyperkinetic circulation in liver transplant patients. *Hepatology* 1993; **17**: 175-178 [PMID: 8428714]
  - 26 **Henderson JM**, Mackay GJ, Kutner MH, Noe B. Volumetric and functional liver blood flow are both increased in the human transplanted liver. *J Hepatol* 1993; **17**: 204-207 [PMID: 8445233]
  - 27 **Plevak DJ**. Hyperdynamic circulatory state after liver transplantation. *Transplant Proc* 1993; **25**: 1839 [PMID: 8470191]
  - 28 **Textor SC**, Wiesner R, Wilson DJ, Porayko M, Romero JC, Burnett JC, Gores G, Hay E, Dickson ER, Krom RA. Systemic and renal hemodynamic differences between FK506 and cyclosporine in liver transplant recipients. *Transplantation* 1993; **55**: 1332-1339 [PMID: 7685934 DOI: 10.1097/00007890-199306000-00023]
  - 29 **Chezmar JL**, Redvanly RD, Nelson RC, Henderson JM. Persistence of portosystemic collaterals and splenomegaly on CT after orthotopic liver transplantation. *AJR Am J Roentgenol* 1992; **159**: 317-320 [PMID: 1632346 DOI: 10.2214/ajr.159.2.1632346]
  - 30 **Liang YY**, Wang J, Shan H, Yan RH, Hu B, Jiang ZB, He BJ, Liu JJ, Ren LL, Shao S. [To evaluate the role of OLT on splenomegaly of portal hypertension by the radiological changes of splenic morphology and collaterals]. *Zhonghua Yi Xue Za Zhi* 2012; **92**: 3058-3061 [PMID: 23328378]
  - 31 **Tsubono T**, Todo S, Jabbour N, Mizoe A, Warty V, Demetris AJ, Starzl TE. Indocyanine green elimination test in orthotopic liver recipients. *Hepatology* 1996; **24**: 1165-1171 [PMID: 8903393]
  - 32 **Hori T**, Iida T, Yagi S, Taniguchi K, Yamamoto C, Mizuno S, Yamagiwa K, Isaji S, Uemoto S. K(ICG) value, a reliable real-time estimator of graft function, accurately predicts outcomes in adult living-donor liver transplantation. *Liver Transpl* 2006; **12**: 605-613 [PMID: 16555326 DOI: 10.1002/lt.20713]
  - 33 **Wong F**, Liu P, Tobe S, Morali G, Blendis L. Central blood volume in cirrhosis: measurement with radionuclide angiography. *Hepatology* 1994; **19**: 312-321 [PMID: 8294089]
  - 34 **Hashimoto M**, Watanabe G. Simultaneous measurement of effective hepatic blood flow and systemic circulation. *Hepato-gastroenterology* 2000; **47**: 1669-1674 [PMID: 11149029]
  - 35 **Sakka SG**, Reinhart K, Wegscheider K, Meier-Hellmann A. Comparison of cardiac output and circulatory blood volumes by transpulmonary thermo-dye dilution and transcutaneous indocyanine green measurement in critically ill patients. *Chest* 2002; **121**: 559-565 [PMID: 11834672 DOI: 10.1378/chest.121.2.559]
  - 36 **Magder S**. Understanding central venous pressure: not a preload index? *Curr Opin Crit Care* 2015; **21**: 369-375 [PMID: 26348416 DOI: 10.1097/MCC.0000000000000238]
  - 37 **Hartleb M**, Rudzki K, Karpel E, Becker A, Waluga M, Boldys H, Nowak A, Nowak S. Cardiovascular status after postural change in compensated cirrhosis: an argument for vasodilatory concept. *Liver* 1997; **17**: 1-6 [PMID: 9062872]
  - 38 **Yagi S**, Iida T, Hori T, Taniguchi K, Yamamoto C, Yamagiwa K, Uemoto S. Optimal portal venous circulation for liver graft function after living-donor liver transplantation. *Transplantation* 2006; **81**: 373-378 [PMID: 16477223]
  - 39 **Yagi S**, Iida T, Taniguchi K, Hori T, Hamada T, Fujii K, Mizuno S, Uemoto S. Impact of portal venous pressure on regeneration and graft damage after living-donor liver transplantation. *Liver Transpl* 2005; **11**: 68-75 [PMID: 15690538 DOI: 10.1002/lt.20317]
  - 40 **Ogura Y**, Hori T, El Moghazy WM, Yoshizawa A, Oike F, Mori A, Kaido T, Takada Y, Uemoto S. Portal pressure < 15 mm Hg is a key for successful adult living donor liver transplantation utilizing smaller grafts than before. *Liver Transpl* 2010; **16**: 718-728 [PMID: 20517905 DOI: 10.1002/lt.22059]
  - 41 **Hori T**, Ogura Y, Ogawa K, Kaido T, Segawa H, Okajima H, Kogure T, Uemoto S. How transplant surgeons can overcome the inevitable insufficiency of allograft size during adult living-donor liver transplantation: strategy for donor safety with a smaller-size graft and excellent recipient results. *Clin Transplant* 2012; **26**: E324-E334 [PMID: 22686957 DOI: 10.1111/j.1399-0012.2012.01664.x]
  - 42 **Hori T**, Ogura Y, Yagi S, Iida T, Taniguchi K, El Moghazy WM, Hedaya MS, Segawa H, Ogawa K, Kogure T, Uemoto S. How do

- transplant surgeons accomplish optimal portal venous flow during living-donor liver transplantation? Noninvasive measurement of indocyanine green elimination rate. *Surg Innov* 2014; **21**: 43-51 [PMID: 23703675 DOI: 10.1177/1553350613487803]
- 43 **Eguchi S**, Yanaga K, Sugiyama N, Okudaira S, Furui J, Kanematsu T. Relationship between portal venous flow and liver regeneration in patients after living donor right-lobe liver transplantation. *Liver Transpl* 2003; **9**: 547-551 [PMID: 12783393]
  - 44 **Wheeler HO**, Cranston WI, Meltzer JI. Hepatic uptake and biliary excretion of indocyanine green in the dog. *Proc Soc Exp Biol Med* 1958; **99**: 11-14 [PMID: 13601749]
  - 45 **Ashwell G**, Harford J. Carbohydrate-specific receptors of the liver. *Annu Rev Biochem* 1982; **51**: 531-554 [PMID: 6287920]
  - 46 **Kwon AH**, Ha-Kawa SK, Uetsuji S, Inoue T, Matsui Y, Kamiyama Y. Preoperative determination of the surgical procedure for hepatectomy using technetium-99m-galactosyl human serum albumin (99mTc-GSA) liver scintigraphy. *Hepatology* 1997; **25**: 426-429 [PMID: 9021958]
  - 47 **de Graaf W**, Bennink RJ, Veteläinen R, van Gulik TM. Nuclear imaging techniques for the assessment of hepatic function in liver surgery and transplantation. *J Nucl Med* 2010; **51**: 742-752 [PMID: 20395336 DOI: 10.2967/jnumed.109.069435]
  - 48 **Kaibori M**, Ha-Kawa SK, Maehara M, Ishizaki M, Matsui K, Sawada S, Kwon AH. Usefulness of Tc-99m-GSA scintigraphy for liver surgery. *Ann Nucl Med* 2011; **25**: 593-602 [PMID: 21800021 DOI: 10.1007/s12149-011-0520-0]
  - 49 **Groszmann RJ**. The measurement of liver blood flow using clearance techniques. *Hepatology* 1983; **3**: 1039-1040 [PMID: 6629317]
  - 50 **Jiao LR**, El-Desoky AA, Seifalian AM, Habib N, Davidson BR. Effect of liver blood flow and function on hepatic indocyanine green clearance measured directly in a cirrhotic animal model. *Br J Surg* 2000; **87**: 568-574 [PMID: 10792311]
  - 51 **Niemann CU**, Yost CS, Mandell S, Henthorn TK. Evaluation of the splanchnic circulation with indocyanine green pharmacokinetics in liver transplant patients. *Liver Transpl* 2002; **8**: 476-481 [PMID: 12004348]
  - 52 **Niemann CU**, Roberts JP, Ascher NL, Yost CS. Intraoperative hemodynamics and liver function in adult-to-adult living liver donors. *Liver Transpl* 2002; **8**: 1126-1132 [PMID: 12474151]
  - 53 **Huet PM**, Villeneuve JP. Determinants of drug disposition in patients with cirrhosis. *Hepatology* 1983; **3**: 913-918 [PMID: 6629320]
  - 54 **Ogawa E**, Hori T, Doi H, Segawa H, Uemoto S. Living-donor liver transplantation for congenital biliary atresia with porto-pulmonary hypertension and moderate or severe pulmonary arterial hypertension: Kyoto University experience. *Clin Transplant* 2014; **28**: 1031-1040 [PMID: 24986560 DOI: 10.1111/ctr.12415]
  - 55 **McCullough AJ**, Mullen KD, Kalhan SC. Measurements of total body and extracellular water in cirrhotic patients with and without ascites. *Hepatology* 1991; **14**: 1102-1111 [PMID: 1959861]
  - 56 **Vitale GC**, Neill GD, Fenwick MK, Stewart WW, Cuschieri A. Body composition in the cirrhotic patient with ascites: assessment of total exchangeable sodium and potassium with simultaneous serum electrolyte determination. *Am Surg* 1985; **51**: 675-681 [PMID: 4073676]
  - 57 **Nakayama M**, Yamauchi M, Kanaya N, Namiki A. [Utility of bicarbonated Ringer's solution as an intraoperative fluid during long-term laparotomy]. *Masui* 2007; **56**: 1334-1338 [PMID: 18027603]
  - 58 **Fukuta Y**, Kumamoto T, Matsuda A, Kataoka M, Kokuba Y. [Effects of various Ringer's solutions on acid-base balance in rats in hemorrhagic shock and with hepatic dysfunction]. *Masui* 1998; **47**: 22-28 [PMID: 9492494]
  - 59 **Satoh K**, Ohtawa M, Okamura E, Satoh T, Matsuura A. Pharmacological study of BRS, a new bicarbonated Ringer's solution, in partially hepatectomized rabbits. *Eur J Anaesthesiol* 2005; **22**: 624-629 [PMID: 16119600]
  - 60 **Bernardi M**, Ricci CS, Santi L. Hyponatremia in Patients with Cirrhosis of the Liver. *J Clin Med* 2014; **4**: 85-101 [PMID: 26237020 DOI: 10.3390/jcm4010085]
  - 61 **Liu H**, Gaskari SA, Lee SS. Cardiac and vascular changes in cirrhosis: pathogenic mechanisms. *World J Gastroenterol* 2006; **12**: 837-842 [PMID: 16521209]
  - 62 **Alessandria C**, Elia C, Mezzabotta L, Risso A, Andrealli A, Spandre M, Morgando A, Marzano A, Rizzetto M. Prevention of paracentesis-induced circulatory dysfunction in cirrhosis: standard vs half albumin doses. A prospective, randomized, unblinded pilot study. *Dig Liver Dis* 2011; **43**: 881-886 [PMID: 21741331 DOI: 10.1016/j.dld.2011.06.001]
  - 63 **Bolognesi M**, Di Pascoli M, Verardo A, Gatta A. Splanchnic vasodilation and hyperdynamic circulatory syndrome in cirrhosis. *World J Gastroenterol* 2014; **20**: 2555-2563 [PMID: 24627591 DOI: 10.3748/wjg.v20.i10.2555]
  - 64 **Gracia-Sancho J**, Maeso-Díaz R, Bosch J. Pathophysiology and a Rational Basis of Therapy. *Dig Dis* 2015; **33**: 508-514 [PMID: 26159267 DOI: 10.1159/000374099]
  - 65 **Iwakiri Y**, Shah V, Rockey DC. Vascular pathobiology in chronic liver disease and cirrhosis - current status and future directions. *J Hepatol* 2014; **61**: 912-924 [PMID: 24911462 DOI: 10.1016/j.jhep.2014.05.047]
  - 66 **Vincent JG**. Use of autologous pericardium for ventricular aneurysm closure. *Ann Thorac Surg* 1989; **48**: 146-147 [PMID: 2535603]
  - 67 **Hayakawa M**, Gando S, Kameue T, Morimoto Y, Kemmotsu O. Abdominal compartment syndrome and intrahepatic portal venous gas: a possible complication of endoscopy. *Intensive Care Med* 2002; **28**: 1680-1681 [PMID: 12415460]
  - 68 **Ahmed K**, Atiq M, Richer E, Neff G, Kemmer N, Safdar K. Careful observation of hepatic portal venous gas following esophageal variceal band ligation. *Endoscopy* 2008; **40** Suppl 2: E103 [PMID: 19085707 DOI: 10.1055/s-2007-966850]
  - 69 **Li G**, Thabane L, Cook DJ, Lopes RD, Marshall JC, Guyatt G, Holbrook A, Akhtar-Danesh N, Fowler RA, Adhikari NK, Taylor R, Arabi YM, Chittock D, Dodek P, Freitag AP, Walter SD, Heels-Ansdell D, Levine MA. Risk factors for and prediction of mortality in critically ill medical-surgical patients receiving heparin thromboprophylaxis. *Ann Intensive Care* 2016; **6**: 18 [PMID: 26921148 DOI: 10.1186/s13613-016-0116-x]
  - 70 **Kloek JJ**, Heger M, van der Gaag NA, Beuers U, van Gulik TM, Gouma DJ, Levi M. Effect of preoperative biliary drainage on coagulation and fibrinolysis in severe obstructive cholestasis. *J Clin Gastroenterol* 2010; **44**: 646-652 [PMID: 20142756 DOI: 10.1097/MCG.0b013e3181ce5b36]
  - 71 **Kogiso T**, Tokushige K, Hashimoto E, Ikarashi Y, Kodama K, Taniai M, Torii N, Shiratori K. Safety and efficacy of long-term tolvaftan therapy for decompensated liver cirrhosis. *Hepatol Res* 2016; **46**: E194-E200 [PMID: 26123753 DOI: 10.1111/hepr.12547]
  - 72 **Thomas MN**, Sauter GH, Gerbes AL, Stangl M, Schiergens TS, Angele M, Werner J, Guba M. Automated low flow pump system for the treatment of refractory ascites: a single-center experience. *Langenbecks Arch Surg* 2015; **400**: 979-983 [PMID: 26566989 DOI: 10.1007/s00423-015-1356-1]
  - 73 **Arsalan H**. Infections in liver transplant recipients. *Exp Clin Transplant* 2014; **12** Suppl 1: 24-27 [PMID: 24635787]
  - 74 **Kim SI**. Bacterial infection after liver transplantation. *World J Gastroenterol* 2014; **20**: 6211-6220 [PMID: 24876741 DOI: 10.3748/wjg.v20.i20.6211]
  - 75 **Shepherd RW**, Turmelle Y, Nadler M, Lowell JA, Narkewicz MR, McDiarmid SV, Anand R, Song C. Risk factors for rejection and infection in pediatric liver transplantation. *Am J Transplant* 2008; **8**: 396-403 [PMID: 18162090]
  - 76 **van Delden C**. Bacterial biliary tract infections in liver transplant recipients. *Curr Opin Organ Transplant* 2014; **19**: 223-228 [PMID: 24752064 DOI: 10.1097/MOT.0000000000000083]
  - 77 **Coons SJ**. Promoting the appropriate use of medications by older adults; the pharmacist's role. *J Ky Med Assoc* 1989; **87**: 571-573 [PMID: 2584846]
  - 78 **Chen TA**, Lo GH, Lai KH. Risk factors for spontaneous bacterial empyema in cirrhotic patients with hydrothorax. *J Chin Med Assoc*

- 2003; **66**: 579-586 [PMID: 14703274]
- 79 **Takino T**, Ogasawara T, Okuno T, Takahashi T. Disseminated cytomegalic inclusion disease in an adult with cirrhosis of liver and review of literatures. *Gastroenterol Jpn* 1976; **11**: 347-355 [PMID: 190080]
  - 80 **Jeurissen S**, Vogelaers D, Sermijn E, Van Dycke K, Geerts A, Van Vlierberghe H, Colle I. Invasive aspergillosis in patients with cirrhosis, a case report and review of the last 10 years. *Acta Clin Belg* 2013; **68**: 368-375 [PMID: 24579244]
  - 81 **Valand AG**, Deshpande V, Pandya BS. Pneumocystis carinii pneumonia in immunocompromised host--an autopsy report of three cases. *Indian J Pathol Microbiol* 2007; **50**: 38-40 [PMID: 17474255]
  - 82 **Mueller AR**, Platz KP, Kremer B. Early postoperative complications following liver transplantation. *Best Pract Res Clin Gastroenterol* 2004; **18**: 881-900 [PMID: 15494284]
  - 83 **Wiklund RA**. Preoperative preparation of patients with advanced liver disease. *Crit Care Med* 2004; **32**: S106-S115 [PMID: 15064669]
  - 84 **Paya CV**, Hermans PE. Bacterial infections after liver transplantation. *Eur J Clin Microbiol Infect Dis* 1989; **8**: 499-504 [PMID: 2504588]
  - 85 **Mah A**, Wright A. Infectious Considerations in the Pre-Transplant Evaluation of Cirrhotic Patients Awaiting Orthotopic Liver Transplantation. *Curr Infect Dis Rep* 2016; **18**: 4 [PMID: 26743200 DOI: 10.1007/s11908-015-0514-5]
  - 86 **Carrión AF**, Aye L, Martin P. Patient selection for liver transplantation. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 571-579 [PMID: 23985006 DOI: 10.1586/17474124.2013.824701]
  - 87 **Petrovsky H**, Rana A, Kaldas FM, Sharma A, Hong JC, Agopian VG, Durazo F, Honda H, Gornbein J, Wu V, Farmer DG, Hiatt JR, Busuttill RW. Liver transplantation in highest acuity recipients: identifying factors to avoid futility. *Ann Surg* 2014; **259**: 1186-1194 [PMID: 24263317 DOI: 10.1097/SLA.0000000000000265]
  - 88 **Morell B**, Dufour JF. [Liver transplantation - when and for whom it should be performed]. *Ther Umsch* 2011; **68**: 707-713 [PMID: 22139986 DOI: 10.1024/0040-5930/a000234]
  - 89 **Martin-Gandul C**, Mueller NJ, Pascual M, Manuel O. The Impact of Infection on Chronic Allograft Dysfunction and Allograft Survival After Solid Organ Transplantation. *Am J Transplant* 2015; **15**: 3024-3040 [PMID: 26474168 DOI: 10.1111/ajt.13486]
  - 90 **Balogh J**, Gordon Burroughs S, Boktour M, Patel S, Saharia A, Ochoa RA, McFadden R, Victor DW, Ankoma-Sey V, Galati J, Monsour HP, Fainstein V, Li XC, Grimes KA, Gaber AO, Aloia T, Ghobrial RM. Efficacy and cost-effectiveness of voriconazole prophylaxis for prevention of invasive aspergillosis in high-risk liver transplant recipients. *Liver Transpl* 2016; **22**: 163-170 [PMID: 26515643 DOI: 10.1002/lt.24365]
  - 91 **Lv C**, Zhang Y, Chen X, Huang X, Xue M, Sun Q, Wang T, Liang J, He S, Gao J, Zhou J, Yu M, Fan J, Gao X. New-onset diabetes after liver transplantation and its impact on complications and patient survival. *J Diabetes* 2015; **7**: 881-890 [PMID: 25676209 DOI: 10.1111/1753-0407.12275]
  - 92 **Burnstock G**, Vaughn B, Robson SC. Purinergic signalling in the liver in health and disease. *Purinergic Signal* 2014; **10**: 51-70 [PMID: 24271096 DOI: 10.1007/s11302-013-9398-8]
  - 93 **Amaya MJ**, Oliveira AG, Guimarães ES, Castaluber MC, Carvalho SM, Andrade LM, Pinto MC, Mennone A, Oliveira CA, Resende RR, Menezes GB, Nathanson MH, Leite MF. The insulin receptor translocates to the nucleus to regulate cell proliferation in liver. *Hepatology* 2014; **59**: 274-283 [PMID: 23839970 DOI: 10.1002/hep.26609]
  - 94 **Raevens S**, Geerts A, Van Steenkiste C, Verhelst X, Van Vlierberghe H, Colle I. Hepatopulmonary syndrome and portopulmonary hypertension: recent knowledge in pathogenesis and overview of clinical assessment. *Liver Int* 2015; **35**: 1646-1660 [PMID: 25627425 DOI: 10.1111/liv.12791]
  - 95 **Aldenkortt F**, Aldenkortt M, Caviezel L, Waeber JL, Weber A, Schiffer E. Portopulmonary hypertension and hepatopulmonary syndrome. *World J Gastroenterol* 2014; **20**: 8072-8081 [PMID: 25009379 DOI: 10.3748/wjg.v20.i25.8072]
  - 96 **Porres-Aguilar M**, Mukherjee D. Cardiopulmonary hemodynamics for accurate diagnosis of portopulmonary hypertension: a redefinition to consider. *Hepatology* 2015; **61**: 733-734 [PMID: 24849250 DOI: 10.1002/hep.27234]
  - 97 **Pastor CM**, Schiffer E. Therapy Insight: hepatopulmonary syndrome and orthotopic liver transplantation. *Nat Clin Pract Gastroenterol Hepatol* 2007; **4**: 614-621 [PMID: 17978818]
  - 98 **Ogawa E**, Hori T, Doi H, Segawa H, Uemoto S. Living-donor liver transplantation for moderate or severe porto-pulmonary hypertension accompanied by pulmonary arterial hypertension: a single-centre experience over 2 decades in Japan. *J Hepatobiliary Pancreat Sci* 2012; **19**: 638-649 [PMID: 22086457 DOI: 10.1007/s00534-011-0453-y]
  - 99 **Krowka MJ**, Mandell MS, Ramsay MA, Kawut SM, Fallon MB, Manzarbeitia C, Pardo M, Marotta P, Uemoto S, Stoffel MP, Benson JT. Hepatopulmonary syndrome and portopulmonary hypertension: a report of the multicenter liver transplant database. *Liver Transpl* 2004; **10**: 174-182 [PMID: 14762853]
  - 100 **Kuo PC**, Plotkin JS, Gaine S, Schroeder RA, Rustgi VK, Rubin LJ, Johnson LB. Portopulmonary hypertension and the liver transplant candidate. *Transplantation* 1999; **67**: 1087-1093 [PMID: 10232556]
  - 101 **Krowka MJ**, Plevak DJ, Findlay JY, Rosen CB, Wiesner RH, Krom RA. Pulmonary hemodynamics and perioperative cardiopulmonary-related mortality in patients with portopulmonary hypertension undergoing liver transplantation. *Liver Transpl* 2000; **6**: 443-450 [PMID: 10915166]
  - 102 **Ashfaq M**, Chinnakotla S, Rogers L, Ausloos K, Saadeh S, Klintmalm GB, Ramsay M, Davis GL. The impact of treatment of portopulmonary hypertension on survival following liver transplantation. *Am J Transplant* 2007; **7**: 1258-1264 [PMID: 17286619]
  - 103 **Krowka MJ**. Pulmonary hypertension: diagnostics and therapeutics. *Mayo Clin Proc* 2000; **75**: 625-630 [PMID: 10852424]
  - 104 **Møller S**, Bendtsen F. Complications of cirrhosis. A 50 years flashback. *Scand J Gastroenterol* 2015; **50**: 763-780 [PMID: 25881709 DOI: 10.3109/00365521.2015.1021709]
  - 105 **Grace JA**, Angus PW. Hepatopulmonary syndrome: update on recent advances in pathophysiology, investigation, and treatment. *J Gastroenterol Hepatol* 2013; **28**: 213-219 [PMID: 23190201 DOI: 10.1111/jgh.12061]
  - 106 **Opal SM**, van der Poll T. Endothelial barrier dysfunction in septic shock. *J Intern Med* 2015; **277**: 277-293 [PMID: 25418337 DOI: 10.1111/joim.12331]
  - 107 **Iwase H**, Ekser B, Satyananda V, Bhama J, Hara H, Ezzelrab M, Klein E, Wagner R, Long C, Thacker J, Li J, Zhou H, Jiang M, Nagaraju S, Zhou H, Veroux M, Bajona P, Wijkstrom M, Wang Y, Phelps C, Klymiuk N, Wolf E, Ayares D, Cooper DK. Pig-to-baboon heterotopic heart transplantation--exploratory preliminary experience with pigs transgenic for human thrombomodulin and comparison of three costimulation blockade-based regimens. *Xenotransplantation* 2015; **22**: 211-220 [PMID: 25847282 DOI: 10.1111/xen.12167]
  - 108 **Matthay MA**. Severe sepsis--a new treatment with both anticoagulant and antiinflammatory properties. *N Engl J Med* 2001; **344**: 759-762 [PMID: 11236781]
  - 109 **Abeyama K**, Stern DM, Ito Y, Kawahara K, Yoshimoto Y, Tanaka M, Uchimura T, Ida N, Yamazaki Y, Yamada S, Yamamoto Y, Yamamoto H, Iino S, Taniguchi N, Maruyama I. The N-terminal domain of thrombomodulin sequesters high-mobility group-B1 protein, a novel antiinflammatory mechanism. *J Clin Invest* 2005; **115**: 1267-1274 [PMID: 15841214]
  - 110 **Li YH**, Kuo CH, Shi GY, Wu HL. The role of thrombomodulin lectin-like domain in inflammation. *J Biomed Sci* 2012; **19**: 34 [PMID: 22449172 DOI: 10.1186/1423-0127-19-34]
  - 111 **Martin FA**, Murphy RP, Cummins PM. Thrombomodulin and the vascular endothelium: insights into functional, regulatory, and therapeutic aspects. *Am J Physiol Heart Circ Physiol* 2013; **304**: H1585-H1597 [PMID: 23604713 DOI: 10.1152/ajpheart.00096.2013]



- 112 **Hori T**, Uemoto S, Takada Y, Oike F, Ogura Y, Ogawa K, Miyagawa-Hayashino A, Yurugi K, Nguyen JH, Hori Y, Chen F, Egawa H. Does a positive lymphocyte cross-match contraindicate living-donor liver transplantation? *Surgery* 2010; **147**: 840-844 [PMID: 20096431 DOI: 10.1016/j.surg.2009.11.022]
- 113 **Hori T**, Kaido T, Oike F, Ogura Y, Ogawa K, Yonekawa Y, Hata K, Kawaguchi Y, Ueda M, Mori A, Segawa H, Yurugi K, Takada Y, Egawa H, Yoshizawa A, Kato T, Saito K, Wang L, Torii M, Chen F, Baine AM, Gardner LB, Uemoto S. Thrombotic microangiopathy-like disorder after living-donor liver transplantation: a single-center experience in Japan. *World J Gastroenterol* 2011; **17**: 1848-1857 [PMID: 21528059 DOI: 10.3748/wjg.v17.i14.1848]
- 114 **de Paiva Haddad LB**, Decimoni TC, Turri JA, Leandro R, de Soárez PC. Economic evaluations in gastroenterology in Brazil: A systematic review. *World J Gastrointest Pharmacol Ther* 2016; **7**: 162-170 [PMID: 26855823 DOI: 10.4292/wjgpt.v7.i1.162]
- 115 **Dan YY**, Wong JB, Hamid SS, Han KH, Jia JD, Liu CJ, Piratvisuth T, Lok AS, Lim SG. Consensus cost-effectiveness model for treatment of chronic hepatitis B in Asia Pacific countries. *Hepatol Int* 2014; **8**: 382-394 [PMID: 26202640 DOI: 10.1007/s12072-014-9549-1]
- 116 **Neff GW**, Duncan CW, Schiff ER. The current economic burden of cirrhosis. *Gastroenterol Hepatol* (N Y) 2011; **7**: 661-671 [PMID: 22298959]
- 117 **Axelrod DA**. Economic and financial outcomes in transplantation: whose dime is it anyway? *Curr Opin Organ Transplant* 2013; **18**: 222-228 [PMID: 23449346 DOI: 10.1097/MOT.0b013e32835f0757]
- 118 **Cortesi PA**, Mantovani LG, Ciaccio A, Rota M, Mazzarelli C, Cesana G, Strazzabosco M, Belli LS. Cost-Effectiveness of New Direct-Acting Antivirals to Prevent Post-Liver Transplant Recurrent Hepatitis. *Am J Transplant* 2015; **15**: 1817-1826 [PMID: 26086300 DOI: 10.1111/ajt.13320]
- 119 **Katz PP**, Showstack JA, Lake JR, Brown RS, Dudley RA, Colwell ME, Wiesner RH, Zetterman RK, Everhart J. Methods to estimate and analyze medical care resource use. An example from liver transplantation. *Int J Technol Assess Health Care* 1999; **15**: 366-379 [PMID: 10507195]
- 120 **Mor E**, Cohen J, Erez E, Grozovsky A, Shaharabani E, Bar-Nathan N, Yussim A, Micowiz R, Shapira Z, Zinger P. Short intensive care unit stay reduces septic complications and improves outcome after liver transplantation. *Transplant Proc* 2001; **33**: 2939-2940 [PMID: 11543799]
- 121 **Head SJ**, Osnabrugge RL, Howell NJ, Freemantle N, Bridgewater B, Pagano D, Kappetein AP. A systematic review of risk prediction in adult cardiac surgery: considerations for future model development. *Eur J Cardiothorac Surg* 2013; **43**: e121-e129 [PMID: 23423916 DOI: 10.1093/ejcts/ezt044]
- 122 **Baztán JJ**, Gálvez CP, Socorro A. Recovery of functional impairment after acute illness and mortality: one-year follow-up study. *Gerontology* 2009; **55**: 269-274 [PMID: 19141990 DOI: 10.1159/000193068]
- 123 **Uemura T**, Wada S, Kaido T, Mori A, Ogura Y, Yagi S, Fujimoto Y, Ogawa K, Hata K, Yoshizawa A, Okajima H, Uemoto S. How far can we lower graft-to-recipient weight ratio for living donor liver transplantation under modulation of portal venous pressure? *Surgery* 2016; **159**: 1623-1630 [PMID: 26936527 DOI: 10.1016/j.surg.2016.01.009]
- 124 **Hammad A**, Kaido T, Ogawa K, Fujimoto Y, Tomiyama K, Mori A, Uemura T, Uemoto S. Perioperative changes in nutritional parameters and impact of graft size in patients undergoing adult living donor liver transplantation. *Liver Transpl* 2014; **20**: 1486-1496 [PMID: 25205246 DOI: 10.1002/lt.23992]
- 125 **Kaido T**, Mori A, Ogura Y, Hata K, Yoshizawa A, Iida T, Yagi S, Uemoto S. Lower limit of the graft-to-recipient weight ratio can be safely reduced to 0.6% in adult-to-adult living donor liver transplantation in combination with portal pressure control. *Transplant Proc* 2011; **43**: 2391-2393 [PMID: 21839274 DOI: 10.1016/j.transproceed.2011.05.037]
- 126 **Kaido T**, Ogawa K, Fujimoto Y, Ito T, Tomiyama K, Mori A, Ogura Y, Uemoto S. Section 7. A new therapeutic strategy on portal flow modulation that increases donor safety with good recipient outcomes. *Transplantation* 2014; **97** Suppl 8: S30-S32 [PMID: 24849829 DOI: 10.1097/01.tp.0000446271.28557.e8]

**P- Reviewer:** Bubnov RV, Giorgio A **S- Editor:** Qiu S

**L- Editor:** A **E- Editor:** Li D





## Inhibition of apoptosis by oncogenic hepatitis B virus X protein: Implications for the treatment of hepatocellular carcinoma

Chuck C K Chao

Chuck C K Chao, Department of Biochemistry and Molecular Biology, College of Medicine, Chang Gung University, Taoyuan 33302, Taiwan

Chuck C K Chao, Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan 33302, Taiwan

Chuck C K Chao, Chang Gung Memorial Hospital at Linkuo, Liver Research Center, Taoyuan 33305, Taiwan

**Author contributions:** The author solely contributed to this paper.

**Conflict-of-interest statement:** No conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Chuck C K Chao, PhD, Department of Biochemistry and Molecular Biology, College of Medicine, Chang Gung University, No.259, Wenhua 1st Rd., Guishan Dist, Taoyuan 33302, Taiwan. [ckchao@mail.cgu.edu.tw](mailto:ckchao@mail.cgu.edu.tw)  
**Telephone:** +886-32-118800-5151  
**Fax:** +886-32-118700

**Received:** April 21, 2016

**Peer-review started:** April 22, 2016

**First decision:** June 6, 2016

**Revised:** June 27, 2016

**Accepted:** July 20, 2016

**Article in press:** July 22, 2016

**Published online:** September 8, 2016

### Abstract

Hepatitis B virus X protein (HBx) plays an important role in the development of hepatocellular carcinoma (HCC). In addition, hepatoma upregulated protein (HURP) is a cellular oncogene that is upregulated in a majority of HCC cases. We highlight here recent findings demonstrating a link between HBx, HURP and anti-apoptosis effects observed in cisplatin-treated HCC cells. We observed that Hep3B cells overexpressing HBx display increased HURP mRNA and protein levels, and show resistance to cisplatin-induced apoptosis. Knockdown of HURP in HBx-expressing cells reverses this effect, and sensitizes cells to cisplatin. The anti-apoptotic effect of HBx requires activation of the p38/MAPK pathway as well as expression of SATB1, survivin and HURP. Furthermore, silencing of HURP using short-hairpin RNA promotes accumulation of p53 and reduces cell proliferation in SK-Hep-1 cells (p53<sup>+/+</sup>), whereas these effects are not observed in p53-mutant Mahlavu cells. Similarly, HURP silencing does not affect the proliferation of H1299 lung carcinoma cells or Hep3B HCC cells which lack p53. Silencing of HURP sensitizes SK-Hep-1 cells to cisplatin. While HURP overexpression promotes p53 ubiquitination and degradation by the proteasome, HURP silencing reverses these effects. Inoculation of SK-Hep-1 cancer cells in which HURP has been silenced produces smaller tumors than control in nude mice. Besides, gankyrin, a positive regulator of the E3 ubiquitin ligase MDM2, is upregulated following HURP expression, and silencing of gankyrin reduces HURP-mediated downregulation of p53. In addition, we observed a positive correlation between HURP and gankyrin protein levels in HCC patients ( $r^2 = 0.778$ ;  $n = 9$ ). These findings suggest a role for the viral protein HBx and the host protein HURP in preventing p53-mediated apoptosis during cancer progression and establishment of chemoresistance.

**Key words:** Hepatitis B virus X protein; Hepatocellular

carcinoma; Hepatitis B virus; Hepatoma upregulated protein; p53; Gankyrin; SATB1

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatitis B virus X protein (HBx) plays a critical role in the development of hepatocellular carcinoma (HCC). Hepatoma upregulated protein (HURP) is an oncogene that is upregulated in a majority of HCC cases. However, the role of these proteins in the response of HCC cells to chemotherapeutic drugs remains unclear. We show here that the HBx/SATB1/HURP axis plays a critical role in down-regulating p53 and upregulating anti-apoptotic proteins *in vitro* and *in vivo*. We discuss the regulation of this novel pathway and its implications in resistance of HCC cells to chemotherapy.

Chao CCK. Inhibition of apoptosis by oncogenic hepatitis B virus X protein: Implications for the treatment of hepatocellular carcinoma. *World J Hepatol* 2016; 8(25): 1061-1066 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i25/1061.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i25.1061>

## INTRODUCTION

Chronic hepatitis B virus (HBV) infection represents an important risk factor for the development of hepatocellular carcinoma (HCC)<sup>[1-3]</sup>. The hepatitis B virus X protein (HBx) is produced by HBV and is required for viral replication in host cells<sup>[4,5]</sup>. HBx interferes with a variety of cellular functions in host cells. It forms a heterodimeric complex with the host protein HBx interacting protein, and this interaction dysregulates centrosome dynamics and chromosomal stability<sup>[6]</sup>. HBx also interacts with the tumor suppressor p53 and modulates cellular apoptosis in the presence of various stimuli<sup>[7-9]</sup>. A recent study indicates that HBx binds to the DNA-repair protein damaged DNA binding protein 1 (DDB1), and redirects the CUL4-DDB1 E3 complex, a protein complex with ubiquitin ligase activity that is involved in regulating DNA replication and repair, transcription and signal transduction in host cells<sup>[10]</sup>.

Recent studies suggest that HBx plays a role in HCC pathogenesis<sup>[11-14]</sup>. However, the effect of HBx on apoptosis remains incompletely understood as some authors have reported pro-apoptotic<sup>[15-19]</sup> as well as anti-apoptotic effects<sup>[8,20-23]</sup>. Importantly, experiments performed in laboratory animals indicate that the HBx protein may induce resistance to the anti-cancer drug cisplatin in hepatoma cells<sup>[16]</sup>. Here, I present recent experimental evidence highlighting a prominent pathway used by HBx to upregulate hepatoma upregulated protein (HURP) and avoid apoptosis in HCC cells.

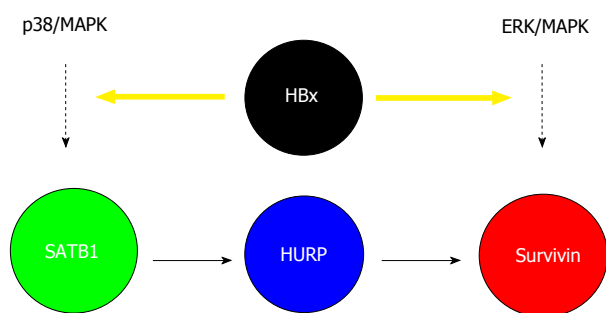
## HURP AS A MARKER IN HCC

HURP was initially shown to be overexpressed in HCC

based on a bioinformatics analysis of sequence tags expressed in the human liver<sup>[24]</sup>. Also known as discs large homolog 7 or disks large-associated protein 5<sup>[25,26]</sup>, HURP was previously thought to represent a stem cell marker as this protein is not detected in fully differentiated cells<sup>[27]</sup>. Previous reports indicate that HURP overexpression in differentiated cells blocks apoptosis and increases cell growth in response to serum starvation<sup>[24,28]</sup>. HURP also appears to regulate the cell cycle and act specifically during mitosis. More specifically, HURP represents a kinetochore protein that stabilizes microtubules in the vicinity of chromosomes<sup>[29-31]</sup>. That is, HURP is associated with the mitotic spindle where it helps to determine spindle bipolarity and participates in the growth of microtubules toward chromosomes during mitosis. Furthermore, HURP forms a Ran-dependent complex<sup>[29]</sup>, and is a target of the serine/threonine kinase aurora-A, which possesses oncogenic properties<sup>[28]</sup>. Aurora-A thus phosphorylates HURP and this process may represent a cellular mechanism that controls mitotic spindle assembly and functions<sup>[32]</sup>. Therefore, HURP is implicated in stem cell functions<sup>[25-27]</sup> and carcinogenesis in cancer cells of human origins<sup>[24,28]</sup>. Analysis of gene expression showed that HURP represents a marker of cancer prognosis that can be used to distinguish between benign and malignant adrenocortical tumors<sup>[33,34]</sup>. In addition, HURP undergoes proteolysis following phosphorylation by Cdk1-cyclin B and recognition by the Fbx7-associated SCF complex that functions as an E3 ubiquitin ligase<sup>[35]</sup>. These results indicate that HURP is involved in control of the cell cycle, specifically during mitosis, suggesting that this protein may regulate apoptosis and be involved in tumor development. However, the role of HURP in HCC and apoptosis, and how this protein is regulated is incompletely understood.

## HBx UPREGULATES HURP EXPRESSION IN HCC CELLS

Given that the viral protein HBx plays a critical role in the development of HCC and HURP is upregulated in a majority of HCC cases, we examined the possible link between HBx, HURP, and cisplatin resistance in HCC cells. Hep3B cells expressing HBx showed not only elevated HURP mRNA and protein levels but also resistance to apoptosis induced by cisplatin. HURP silencing in cells expressing HBx reversed this process and enhanced sensitization of Hep3B cells to apoptosis. Notably, HBx overexpression induced SATB1, a global gene regulator that is upregulated in breast cancer. However, the role of SATB1 in the regulation of cell survival is unclear. We found that the anti-apoptotic effect of HBx requires p38/MAPK pathway activation in Hep3B cells. HBx also induced the expression of the anti-apoptotic protein survivin in an HURP-dependent manner<sup>[36]</sup>. We observed that the HBx produces anti-apoptotic effects in HCC cells, a process that may lead to chemoresistance. Enhanced chemoresistance of HCC cells that express



**Figure 1** Proposed model to explain the link between hepatitis B virus X protein, p38/MAPK, SATB1, hepatoma upregulated protein, and survivin in mediating anti-apoptotic effects during cisplatin treatment. HBx upregulates the anti-apoptotic protein survivin through induction of p38/MAPK and ERK/MAPK pathways. Another less defined ERK/MAPK pathway which may regulate survivin independently of HURP is also shown. HBx: Hepatitis B virus X protein; HURP: Hepatoma upregulated protein.

HBx was associated with increased activity of several proteins, including SATB1, HURP, and survivin. Previous reports indicate that PKC negatively regulates SATB1 transactivation activity<sup>[37]</sup>. Our group showed that SATB1 and the p38/MAPK pathway mediates the anti-apoptotic activity of HBx. Therefore, it appears that HBx upregulates SATB1 and MAPK or HURP transcription. HURP induced survivin expression in HBx-expressing cells. ERK inhibition also inhibited surviving activity<sup>[36,38]</sup>; however, HURP protein levels remained constant in the presence of ERKi, an observation which suggested that HBx may induce survivin *via* another pathway that requires the ERK kinase (Figure 1). Our results may explain, at least in part, the cellular mechanism underlying the anti-apoptotic effect of HBx during the development of HBV-associated HCC. In agreement with our results, previous studies have shown that stable expression of HBx can stimulate PI3-kinase activity and suppress TGF-beta-induced apoptosis in Hep3B cells<sup>[8,22]</sup>.

SATB1, a chromatin organizer, is involved in gene regulation and the formation of chromosome loops, in addition to its role in the organization of transcriptionally poised chromatin<sup>[39]</sup>. SATB1 was initially described as a protein mediator of apoptosis<sup>[40]</sup>. We have shown the role of SATB1 in upregulating surviving and preventing apoptosis during cancer progression and establishment of chemoresistance<sup>[36]</sup>. SATB1 phosphorylation also appears to control interleukin-2 transcription as shown based on results obtained in a T-cell activation model; a similar mechanism may potentially be associated with SATB1 and its gene regulation activity<sup>[37]</sup>. In addition, SATB1 cleavage *via* sumoylation-directed caspase activity appears to regulate gene expression or may lead to clearance of immune cells<sup>[41]</sup>. Furthermore, SATB1 promotes cancer cell metastasis and overexpression of this protein increases resistance to chemotherapeutic drugs in breast cancer cells<sup>[42,43]</sup>. These observations suggest that HBx induces HURP expression by activating the p38/MAPK pathway and SATB1, leading to accumulation of survivin. We conclude that

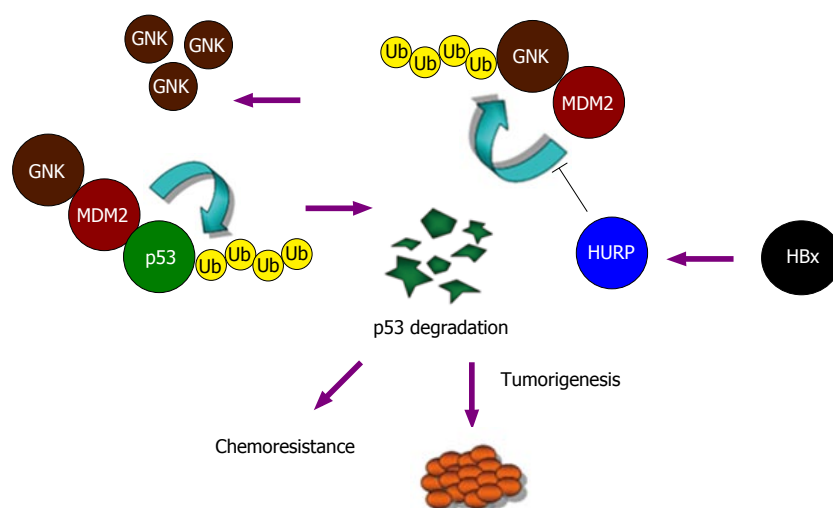
activation of the HBx/SATB1/HURP axis may increase chemoresistance in hepatic cancer cells.

## HURP/GANKYRIN/p53 AXIS IN REGULATING HCC APOPTOSIS

The tumor suppressor p53 inhibits cancer development by inducing cell cycle arrest and apoptosis<sup>[44,45]</sup>. Some human tumors (10%) are characterized by overexpression of MDM2, an E3 ubiquitin ligase known for its role in the ubiquitination of p53 and its subsequent degradation by the proteasome<sup>[46]</sup>; this phenomenon may account for the development of many cancers, even those in which the *p53* gene is no longer functional<sup>[47]</sup>. We found that overexpressing HURP in HEK293 cells induces p53 ubiquitination and degradation of the protein by the proteasome<sup>[48]</sup>. Conversely, HURP silencing with short-hairpin RNA reverses these processes. Knockdown of HURP promotes p53 accumulation and reduces cell proliferation in SK-Hep-1 cells (p53<sup>+/−</sup>), while Mahlavu cells (p53-mutant) are not affected. HURP silencing showed no effect on cellular proliferation in Hep3B and H1299 cells (lung carcinoma) (both lack p53 activity). In comparison, HURP silencing sensitized SK-Hep-1 cells to cisplatin. HURP overexpression not only reduced exogenous p53 expression in H1299 and Hep3B cells but also reduced sensitivity of these cells to cisplatin. Notably, HURP expression induced HEK293 cell proliferation in an anchorage-independent manner; moreover, injection of SK-Hep-1 cancer cells in which HURP had been silenced produced tumors of smaller size in immunocompromised mice compared to control<sup>[48]</sup>.

The ankyrin-repeat oncoprotein gankyrin<sup>[49]</sup> has also been shown to be upregulated in HCC. Previous work indicated that this protein interacts with the product of retinoblastoma (*Rb*) gene as well as a subunit of the 26S proteasome subunit (S6 ATPase), a process that increases degradation of *Rb*<sup>[50,51]</sup>. Gankyrin is part of the 19S cap of the proteasome. This protein has an ankyrin repeat that forms alpha helices<sup>[51]</sup>. Gankyrin can increase the E3 ubiquitin ligase activity of MDM2, which regulates the degradation of the tumor suppressors *p53* and *Rb*, which are both often mutated in human tumors<sup>[52,53]</sup>. Gankyrin regulates the cell cycle by mediating protein-protein interactions involving CDK4 (a cyclin-dependent kinase). *Rb* may inhibit the activity of gankyrin and lead to inhibition of MDM2-mediated p53 ubiquitination in HCC cells<sup>[54]</sup>. In our study<sup>[48]</sup>, we observed that HURP represents an oncogene that reduces the level of p53 in normal cells and cancerous cells. Gankyrin was upregulated following HURP overexpression, and silencing of gankyrin reduced downregulation of p53 mediated by HURP. Importantly, high HURP levels positively correlated with gankyrin protein levels in HCC patients ( $n = 9$ ;  $r^2 = 0.778$ ).

We propose a mechanism to explain the activity of HURP and its action on gankyrin accumulation in cancer cells (Figure 2). In this model, HURP prevents the ubiqui-



**Figure 2** Simplified model illustrating the oncogenic properties of hepatitis B virus X protein and hepatoma upregulated protein in human liver cancer. In this cycle of gankyrin/MDM2-enhanced p53 degradation, HURP reduces MDM2-mediated ubiquitination of gankyrin, leading to accumulation of gankyrin in both normal and tumorigenic cells. Downstream effects of HURP appear to include malignant cell transformation and prevention of apoptosis induced by chemotherapeutic drug, processes which may in turn lead to the development of a chemoresistant cellular phenotype. HBx: Hepatitis B virus X protein; HURP: Hepatoma upregulated protein.

tion and degradation of gankyrin but in a process that does not involve the disruption of the interaction between MDM2 and gankyrin. Alternatively, HURP may regulate the activity of other deubiquitination enzymes by inducing binding to the gankyrin/MDM2 protein complex (not illustrated in the model shown in Figure 2), which may subsequently inhibit MDM2's effects on gankyrin degradation. More experimental data are needed to determine if HURP affects deubiquitination enzymes which interact with the MDM2/gankyrin protein complex. The degradation of p53 mediated by HURP may therefore be relevant to the development of HCC. Our findings identify a novel pathway for the malignant transformation induced by HURP and involving degradation of p53 and accumulation of gankyrin.

## CONCLUSION

Our observations suggest that HBx induces HURP expression via the p38/MAPK pathway and SATB1 activity. This process leads to accumulation survivin, which possesses anti-apoptotic properties. Our results also identify a novel cellular pathway in which the oncogenic protein HURP induces cancer transformation by inducing p53 degradation and gankyrin accumulation. The processes of cell survival and apoptosis have been shown to be regulated by differential activation of p53 target genes<sup>[55]</sup>. For instance, CAS may bind to p53 on chromatin and this process may induce expression of a set of genes that facilitate apoptosis<sup>[56]</sup>. In contrast, interaction between the zinc-finger protein Hzf and p53 activates expression of growth-arrest genes and promotes cell survival<sup>[56,57]</sup>. HURP-mediated p53 degradation thus appears to be relevant for the development of HCC. In conclusion, recent advances regarding the oncogenic proteins HBx and HURP as described here offer new strategies to defeat human

liver cancer.

## ACKNOWLEDGMENTS

The author would like to thank Ms. Chiaying Yang for technical assistance as well Dr. Kuo TC (Kuo JY) for helpful discussions.

## REFERENCES

- 1 Bouchard MJ, Schneider RJ. The enigmatic X gene of hepatitis B virus. *J Virol* 2004; **78**: 12725-12734 [PMID: 15542625 DOI: 10.1128/JVI.78.23.12725-12734.2004]
- 2 Tang H, Oishi N, Kaneko S, Murakami S. Molecular functions and biological roles of hepatitis B virus x protein. *Cancer Sci* 2006; **97**: 977-983 [PMID: 16984372 DOI: 10.1111/j.1349-7006.2006.00299.x]
- 3 Yen TS. Hepadnaviral X Protein: Review of Recent Progress. *J Biomed Sci* 1996; **3**: 20-30 [PMID: 11725079 DOI: 10.1007/BF02253575]
- 4 McClain SL, Clippinger AJ, Lizzano R, Bouchard MJ. Hepatitis B virus replication is associated with an HBx-dependent mitochondrion-regulated increase in cytosolic calcium levels. *J Virol* 2007; **81**: 12061-12065 [PMID: 17699583 DOI: 10.1128/JVI.00740-07]
- 5 Bouchard MJ, Puro RJ, Wang L, Schneider RJ. Activation and inhibition of cellular calcium and tyrosine kinase signaling pathways identify targets of the HBx protein involved in hepatitis B virus replication. *J Virol* 2003; **77**: 7713-7719 [PMID: 12829810 DOI: 10.1128/JVI.77.14.7713-7719.2003]
- 6 Wen Y, Golubkov VS, Strongin AY, Jiang W, Reed JC. Interaction of hepatitis B viral oncoprotein with cellular target HBXIP dysregulates centrosome dynamics and mitotic spindle formation. *J Biol Chem* 2008; **283**: 2793-2803 [PMID: 18032378 DOI: 10.1074/jbc.M708419200]
- 7 Kim H, Lee H, Yun Y. X-gene product of hepatitis B virus induces apoptosis in liver cells. *J Biol Chem* 1998; **273**: 381-385 [PMID: 9417092 DOI: 10.1074/jbc.273.1.381]
- 8 Shih WL, Kuo ML, Chuang SE, Cheng AL, Doong SL. Hepatitis B virus X protein inhibits transforming growth factor-beta-induced apoptosis through the activation of phosphatidylinositol 3-kinase pathway. *J Biol Chem* 2000; **275**: 25858-25864 [PMID: 10835427 DOI: 10.1074/jbc.M003578200]
- 9 Su F, Schneider RJ. Hepatitis B virus HBx protein sensitizes cells



- to apoptotic killing by tumor necrosis factor alpha. *Proc Natl Acad Sci USA* 1997; **94**: 8744-8749 [PMID: 9238048 DOI: 10.1073/pnas.94.16.8744]
- 10 **Li T**, Robert EI, van Breugel PC, Strubin M, Zheng N. A promiscuous alpha-helical motif anchors viral hijackers and substrate receptors to the CUL4-DDB1 ubiquitin ligase machinery. *Nat Struct Mol Biol* 2010; **17**: 105-111 [PMID: 19966799 DOI: 10.1038/nsmb.1719]
  - 11 **Feitelson M**. Hepatitis B virus infection and primary hepatocellular carcinoma. *Clin Microbiol Rev* 1992; **5**: 275-301 [PMID: 1323384 DOI: 10.1128/CMR.5.3.275]
  - 12 **Murakami S**. Hepatitis B virus X protein: structure, function and biology. *Intervirology* 1999; **42**: 81-99 [PMID: 10516464 DOI: 10.1159/000024969]
  - 13 **Robinson WS**. Molecular events in the pathogenesis of hepadnavirus-associated hepatocellular carcinoma. *Annu Rev Med* 1994; **45**: 297-323 [PMID: 8198385 DOI: 10.1146/annurev.med.45.1.297]
  - 14 **Kew MC**. Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 144-152 [PMID: 21199526 DOI: 10.1111/j.1440-1746.2010.06546.x]
  - 15 **Kim SY**, Kim JK, Kim HJ, Ahn JK. Hepatitis B virus X protein sensitizes UV-induced apoptosis by transcriptional transactivation of Fas ligand gene expression. *IUBMB Life* 2005; **57**: 651-658 [PMID: 16203685 DOI: 10.1080/15216540500239697]
  - 16 **Koike K**, Moriya K, Yotsuyanagi H, Iino S, Kurokawa K. Induction of cell cycle progression by hepatitis B virus HBx gene expression in quiescent mouse fibroblasts. *J Clin Invest* 1994; **94**: 44-49 [PMID: 8040286 DOI: 10.1172/JCI117343]
  - 17 **Lin N**, Chen HY, Li D, Zhang SJ, Cheng ZX, Wang XZ. Apoptosis and its pathway in X gene-transfected HepG2 cells. *World J Gastroenterol* 2005; **11**: 4326-4331 [PMID: 16038029 DOI: 10.3748/wjg.v11.i28.4326]
  - 18 **Miao J**, Chen GG, Chun SY, Lai PP. Hepatitis B virus X protein induces apoptosis in hepatoma cells through inhibiting Bcl-xL expression. *Cancer Lett* 2006; **236**: 115-124 [PMID: 15990224 DOI: 10.1016/j.canlet.2005.05.014]
  - 19 **Su F**, Theodosios CN, Schneider RJ. Role of NF-kappaB and myc proteins in apoptosis induced by hepatitis B virus HBx protein. *J Virol* 2001; **75**: 215-225 [PMID: 11119591 DOI: 10.1128/JVI.75.1.215-225.2001]
  - 20 **Cheng AS**, Wong N, Tse AM, Chan KY, Chan KK, Sung JJ, Chan HL. RNA interference targeting HBx suppresses tumor growth and enhances cisplatin chemosensitivity in human hepatocellular carcinoma. *Cancer Lett* 2007; **253**: 43-52 [PMID: 17296261 DOI: 10.1016/j.canlet.2007.01.004]
  - 21 **Murata M**, Matsuzaki K, Yoshida K, Sekimoto G, Tahashi Y, Mori S, Uemura Y, Sakaida N, Fujisawa J, Seki T, Kobayashi K, Yokote K, Koike K, Okazaki K. Hepatitis B virus X protein shifts human hepatic transforming growth factor (TGF)-beta signaling from tumor suppression to oncogenesis in early chronic hepatitis B. *Hepatology* 2009; **49**: 1203-1217 [PMID: 19263472 DOI: 10.1002/hep.22765]
  - 22 **Shih WL**, Kuo ML, Chuang SE, Cheng AL, Doong SL. Hepatitis B virus X protein activates a survival signaling by linking SRC to phosphatidylinositol 3-kinase. *J Biol Chem* 2003; **278**: 31807-31813 [PMID: 12805382 DOI: 10.1074/jbc.M302580200]
  - 23 **Wu BK**, Li CC, Chen HJ, Chang JL, Jeng KS, Chou CK, Hsu MT, Tsai TF. Blocking of G1/S transition and cell death in the regenerating liver of Hepatitis B virus X protein transgenic mice. *Biochem Biophys Res Commun* 2006; **340**: 916-928 [PMID: 16403455 DOI: 10.1016/j.bbrc.2005.12.089]
  - 24 **Tsou AP**, Yang CW, Huang CY, Yu RC, Lee YC, Chang CW, Chen BR, Chung YF, Fann MJ, Chi CW, Chiu JH, Chou CK. Identification of a novel cell cycle regulated gene, HURP, overexpressed in human hepatocellular carcinoma. *Oncogene* 2003; **22**: 298-307 [PMID: 12527899 DOI: 10.1038/sj.onc.1206129]
  - 25 **Nomura N**, Miyajima N, Sazuka T, Tanaka A, Kawarabayashi Y, Sato S, Nagase T, Seki N, Ishikawa K, Tabata S. Prediction of the coding sequences of unidentified human genes. I. The coding sequences of 40 new genes (K1AA0001-K1AA0040) deduced by analysis of randomly sampled cDNA clones from human immature myeloid cell line KG-1. *DNA Res* 1994; **1**: 27-35 [PMID: 7584026 DOI: 10.1093/dnares/1.1.27]
  - 26 **Bassal S**, Nomura N, Venter D, Brand K, McKay MJ, van der Spek PJ. Characterization of a novel human cell-cycle-regulated homologue of Drosophila dlgl. *Genomics* 2001; **77**: 5-7 [PMID: 11543626 DOI: 10.1006/geno.2001.6570]
  - 27 **Gudmundsson KO**, Thorsteinsson L, Sigurjonsson OE, Keller JR, Olafsson K, Egeland T, Gudmundsson S, Rafnar T. Gene expression analysis of hematopoietic progenitor cells identifies Dlg7 as a potential stem cell gene. *Stem Cells* 2007; **25**: 1498-1506 [PMID: 17322106 DOI: 10.1634/stemcells.2005-0479]
  - 28 **Yu CT**, Hsu JM, Lee YC, Tsou AP, Chou CK, Huang CY. Phosphorylation and stabilization of HURP by Aurora-A: implication of HURP as a transforming target of Aurora-A. *Mol Cell Biol* 2005; **25**: 5789-5800 [PMID: 15987997 DOI: 10.1128/MCB.25.14.5789-5800.2005]
  - 29 **Koffa MD**, Casanova CM, Santarella R, Köcher T, Wilm M, Mattaj JW. HURP is part of a Ran-dependent complex involved in spindle formation. *Curr Biol* 2006; **16**: 743-754 [PMID: 16631581 DOI: 10.1016/j.cub.2006.03.056]
  - 30 **Silljé HH**, Nagel S, Körner R, Nigg EA. HURP is a Ran-importin beta-regulated protein that stabilizes kinetochore microtubules in the vicinity of chromosomes. *Curr Biol* 2006; **16**: 731-742 [PMID: 16631580 DOI: 10.1016/j.cub.2006.02.070]
  - 31 **Wong J**, Fang G. HURP controls spindle dynamics to promote proper interkinetochore tension and efficient kinetochore capture. *J Cell Biol* 2006; **173**: 879-891 [PMID: 16769820 DOI: 10.1083/jcb.200511132]
  - 32 **Wong J**, Lerrigo R, Jang CY, Fang G. Aurora A regulates the activity of HURP by controlling the accessibility of its microtubule-binding domain. *Mol Biol Cell* 2008; **19**: 2083-2091 [PMID: 18321990 DOI: 10.1091/mbc.E07-10-1088]
  - 33 **Betz MJ**, Beuschlein F. Diagnosis: Novel molecular signatures for adrenocortical carcinoma. *Nat Rev Endocrinol* 2009; **5**: 297-299 [PMID: 19465894 DOI: 10.1038/nrendo.2009.93]
  - 34 **de Reyniès A**, Assié G, Rickman DS, Tissier F, Groussin L, René-Corail F, Dousset B, Bertagna X, Clauser E, Bertherat J. Gene expression profiling reveals a new classification of adrenocortical tumors and identifies molecular predictors of malignancy and survival. *J Clin Oncol* 2009; **27**: 1108-1115 [PMID: 19139432 DOI: 10.1200/JCO.2008.18.5678]
  - 35 **Hsu JM**, Lee YC, Yu CT, Huang CY. Fbx7 functions in the SCF complex regulating Cdk1-cyclin B-phosphorylated hepatoma up-regulated protein (HURP) proteolysis by a proline-rich region. *J Biol Chem* 2004; **279**: 32592-32602 [PMID: 15145941 DOI: 10.1074/jbc.M404950200]
  - 36 **Kuo TC**, Chao CC. Hepatitis B virus X protein prevents apoptosis of hepatocellular carcinoma cells by upregulating SATB1 and HURP expression. *Biochem Pharmacol* 2010; **80**: 1093-1102 [PMID: 20541537 DOI: 10.1016/j.bcp.2010.06.003]
  - 37 **Pavan Kumar P**, Purbey PK, Sinha CK, Notani D, Limaye A, Jayani RS, Galande S. Phosphorylation of SATB1, a global gene regulator, acts as a molecular switch regulating its transcriptional activity in vivo. *Mol Cell* 2006; **22**: 231-243 [PMID: 16630892 DOI: 10.1016/j.molcel.2006.03.010]
  - 38 **Wiesener CA**, Yip-Schneider MT, Wang Y, Schmidt CM. Multiple anticancer effects of blocking MEK-ERK signaling in hepatocellular carcinoma. *J Am Coll Surg* 2004; **198**: 410-421 [PMID: 14992744 DOI: 10.1016/j.jamcollsurg.2003.10.004]
  - 39 **Kumar PP**, Bischof O, Purbey PK, Notani D, Urlaub H, Dejean A, Galande S. Functional interaction between PML and SATB1 regulates chromatin-loop architecture and transcription of the MHC class I locus. *Nat Cell Biol* 2007; **9**: 45-56 [PMID: 17173041 DOI: 10.1038/ncb1516]
  - 40 **Zweyer M**, Riederer BM, Ochs RL, Fackelmayer FO, Kohwi-Shigematsu T, Bareggi R, Narducci P, Martelli AM. Association of nuclear matrix proteins with granular and threaded nuclear bodies in cell lines undergoing apoptosis. *Exp Cell Res* 1997; **230**: 325-336 [PMID: 9024791 DOI: 10.1006/excr.1996.3415]

- 41 **Tan JA**, Sun Y, Song J, Chen Y, Krontiris TG, Durrin LK. SUMO conjugation to the matrix attachment region-binding protein, special AT-rich sequence-binding protein-1 (SATB1), targets SATB1 to promyelocytic nuclear bodies where it undergoes caspase cleavage. *J Biol Chem* 2008; **283**: 18124-18134 [PMID: 18408014 DOI: 10.1074/jbc.M800512200]
- 42 **Han HJ**, Russo J, Kohwi Y, Kohwi-Shigematsu T. SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis. *Nature* 2008; **452**: 187-193 [PMID: 18337816 DOI: 10.1038/nature06781]
- 43 **Li QQ**, Chen ZQ, Xu JD, Cao XX, Chen Q, Liu XP, Xu ZD. Overexpression and involvement of special AT-rich sequence binding protein 1 in multidrug resistance in human breast carcinoma cells. *Cancer Sci* 2010; **101**: 80-86 [PMID: 19860849 DOI: 10.1111/j.1349-7006.2009.01372.x]
- 44 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]
- 45 **Vogelstein B**, Lane D, Levine AJ. Surfing the p53 network. *Nature* 2000; **408**: 307-310 [PMID: 11099028 DOI: 10.1038/35042675]
- 46 **Yang Y**, Li CC, Weissman AM. Regulating the p53 system through ubiquitination. *Oncogene* 2004; **23**: 2096-2106 [PMID: 15021897 DOI: 10.1038/sj.onc.1207411]
- 47 **Michael D**, Oren M. The p53-Mdm2 module and the ubiquitin system. *Semin Cancer Biol* 2003; **13**: 49-58 [PMID: 12507556 DOI: 10.1016/S1044-579X(02)00099-8]
- 48 **Kuo TC**, Chang PY, Huang SF, Chou CK, Chao CC. Knockdown of HURP inhibits the proliferation of hepatocellular carcinoma cells via downregulation of gankyrin and accumulation of p53. *Biochem-Pharmacol* 2012; **83**: 758-768 [PMID: 22230478 DOI: 10.1016/j.bcp.2011.12.034]
- 49 **Higashitsuji H**, Itoh K, Nagao T, Dawson S, Nonoguchi K, Kido T, Mayer RJ, Arai S, Fujita J. Reduced stability of retinoblastoma protein by gankyrin, an oncogenic ankyrin-repeat protein overexpressed in hepatomas. *Nat Med* 2000; **6**: 96-99 [PMID: 10613832 DOI: 10.1038/71600]
- 50 **Dawson S**, Apcher S, Mee M, Higashitsuji H, Baker R, Uhle S, Dubiel W, Fujita J, Mayer RJ. Gankyrin is an ankyrin-repeat oncoprotein that interacts with CDK4 kinase and the S6 ATPase of the 26 S proteasome. *J Biol Chem* 2002; **277**: 10893-10902 [PMID: 11779854 DOI: 10.1074/jbc.M107313200]
- 51 **Krzywda S**, Brzozowski AM, Higashitsuji H, Fujita J, Welchman R, Dawson S, Mayer RJ, Wilkinson AJ. The crystal structure of gankyrin, an oncoprotein found in complexes with cyclin-dependent kinase 4, a 19 S proteasomal ATPase regulator, and the tumor suppressors Rb and p53. *J Biol Chem* 2004; **279**: 1541-1545 [PMID: 14573599 DOI: 10.1074/jbc.M310265200]
- 52 **Higashitsuji H**, Liu Y, Mayer RJ, Fujita J. The oncoprotein gankyrin negatively regulates both p53 and RB by enhancing proteasomal degradation. *Cell Cycle* 2005; **4**: 1335-1337 [PMID: 16177571 DOI: 10.4161/cc.4.10.2107]
- 53 **Higashitsuji H**, Higashitsuji H, Itoh K, Sakurai T, Nagao T, Sumitomo Y, Masuda T, Dawson S, Shimada Y, Mayer RJ, Fujita J. The oncoprotein gankyrin binds to MDM2/HDM2, enhancing ubiquitylation and degradation of p53. *Cancer Cell* 2005; **8**: 75-87 [PMID: 16023600 DOI: 10.1016/j.ccr.2005.06.006]
- 54 **Qiu W**, Wu J, Walsh EM, Zhang Y, Chen CY, Fujita J, Xiao ZX. Retinoblastoma protein modulates gankyrin-MDM2 in regulation of p53 stability and chemosensitivity in cancer cells. *Oncogene* 2008; **27**: 4034-4043 [PMID: 18332869 DOI: 10.1038/onc.2008.43]
- 55 **Aylon Y**, Oren M. Living with p53, dying of p53. *Cell* 2007; **130**: 597-600 [PMID: 17719538 DOI: 10.1016/j.cell.2007.08.005]
- 56 **Tanaka T**, Ohkubo S, Tatsuno I, Prives C. hCAS/CSE1L associates with chromatin and regulates expression of select p53 target genes. *Cell* 2007; **130**: 638-650 [PMID: 17719542 DOI: 10.1016/j.cell.2007.08.001]
- 57 **Das S**, Raj L, Zhao B, Kimura Y, Bernstein A, Aaronson SA, Lee SW. Hzf Determines cell survival upon genotoxic stress by modulating p53 transactivation. *Cell* 2007; **130**: 624-637 [PMID: 17719541 DOI: 10.1016/j.cell.2007.06.013]

P- Reviewer: Piiper A, Tomizawa M S- Editor: Gong ZM

L- Editor: A E- Editor: Li D



Retrospective Study

## CD36 genetic variation, fat intake and liver fibrosis in chronic hepatitis C virus infection

Omar Ramos-Lopez, Sonia Roman, Erika Martinez-Lopez, Nora A Fierro, Karina Gonzalez-Aldaco, Alexis Jose-Abrego, Arturo Panduro

Omar Ramos-Lopez, Sonia Roman, Erika Martinez-Lopez, Nora A Fierro, Karina Gonzalez-Aldaco, Alexis Jose-Abrego, Arturo Panduro, Department of Molecular Biology in Medicine, Civil Hospital of Guadalajara "Fray Antonio Alcalde", Guadalajara, Jalisco 44280, Mexico

Omar Ramos-Lopez, Sonia Roman, Erika Martinez-Lopez, Nora A Fierro, Karina Gonzalez-Aldaco, Alexis Jose-Abrego, Arturo Panduro, Health Sciences University Center, University of Guadalajara, Guadalajara, Jalisco 44340, Mexico

**Author contributions:** Ramos-Lopez O performed the genotyping experiments, statistical analysis and prepared the first draft of the manuscript; Roman S wrote, integrated the final version and critically revised the content of this article; Martinez-Lopez E provided the biochemical tests and critically revised the manuscript; Fierro NA wrote and critically revised the article; Gonzalez-Aldaco K and Jose-Abrego A wrote, revised statistical analysis and critically reviewed the manuscript; Panduro A conceived the study, performed clinical studies and transient elastography, wrote and critically revised the content of this article; all authors critically reviewed all drafts and approved the final manuscript.

**Supported by** Promep-University of Guadalajara to Arturo Panduro, No. UDG-CA-478.

**Institutional review board statement:** The study was reviewed and approved by the Ethics Committee of the Health Sciences University Center.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** The authors declare that they have no conflict of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative

Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Arturo Panduro, MD, PhD, FAASLD, Department of Molecular Biology in Medicine, Civil Hospital of Guadalajara "Fray Antonio Alcalde", Hospital # 278, Col. El Retiro, Guadalajara, Jalisco 44280, Mexico. [apanduro@prodigy.net.mx](mailto:apanduro@prodigy.net.mx)  
**Telephone:** +52-33-36147743  
**Fax:** +52-33-36147743

**Received:** March 29, 2016  
**Peer-review started:** March 31, 2016  
**First decision:** June 12, 2016  
**Revised:** June 28, 2016  
**Accepted:** August 11, 2016  
**Article in press:** August 15, 2016  
**Published online:** September 8, 2016

## Abstract

### AIM

To analyze the association of the *CD36* polymorphism (rs1761667) with dietary intake and liver fibrosis (LF) in chronic hepatitis C (CHC) patients.

### METHODS

In this study, 73 patients with CHC were recruited. The *CD36* genotype (G > A) was determined by a TaqMan real-time PCR system. Dietary assessment was carried out using a three-day food record to register the daily intake of macronutrients. Serum lipids and liver enzymes were measured by a dry chemistry assay. LF evaluated by transient elastography (Fibroscan®)

and APRI score was classified as mild LF (F1-F2) and advanced LF (F3-F4).

## RESULTS

Overall, the *CD36* genotypic frequencies were AA (30.1%), AG (54.8%), and GG (15.1%), whereas the allelic A and G frequencies were 57.5% and 42.5%, respectively. CHC patients who were carriers of the *CD36* AA genotype had a higher intake of calories attributable to total fat and saturated fatty acids than those with the non-AA genotypes. Additionally, aspartate aminotransferase (AST) serum values were higher in AA genotype carriers compared to non-AA carriers (91.7 IU/L vs 69.8 IU/L,  $P = 0.02$ ). Moreover, the AA genotype was associated with an increase of 30.23 IU/L of AST ( $\beta = 30.23$ , 95%CI: 9.0-51.46,  $P = 0.006$ ). Likewise, the AA genotype was associated with advanced LF compared to the AG (OR = 3.60, 95%CI: 1.16-11.15,  $P = 0.02$ ) or AG + GG genotypes (OR = 3.52, 95%CI: 1.18-10.45,  $P = 0.02$ ).

## CONCLUSION

This study suggests that the *CD36* (rs1761667) AA genotype is associated with higher fat intake and more instances of advanced LF in CHC patients.

**Key words:** Hepatitis C virus infection; *CD36* receptor; Lipids; Liver fibrosis; Mexico

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In this study, chronically infected hepatitis C patients who were carriers of the AA genotype of the *CD36* receptor polymorphism (rs1761667) showed a higher risk of advanced liver fibrosis compared to patients with an AG/GG genotype. This liver damage was associated with the consumption of a hepatopathogenic diet, high-calories and excessive intake of total and saturated fat, typical of the population of West Mexico. Thus, preventive nutritional intervention strategies based on the *CD36* genotype may be a useful tool to avoid further liver damage due to alterations in liver lipid metabolism and inflammation in patients with chronic hepatitis C infection.

Ramos-Lopez O, Roman S, Martinez-Lopez E, Fierro NA, Gonzalez-Aldaco K, Jose-Abrego A, Panduro A. *CD36* genetic variation, fat intake and liver fibrosis in chronic hepatitis C virus infection. *World J Hepatol* 2016; 8(25): 1067-1074 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i25/1067.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i25.1067>

## INTRODUCTION

The hepatitis C virus (HCV) is a hepatotropic human RNA virus, member of the *Flaviviridae* family<sup>[1]</sup>. Globally, it is estimated that nearly 170 million individuals are infected with HCV, causing yearly 350000 deaths<sup>[2]</sup>. Liver cirrhosis

causes a high burden of liver disease in Mexico, and HCV infection represents one of its primary etiologies<sup>[3,4]</sup>. Approximately two million Mexican individuals are infected with HCV<sup>[5,6]</sup> and up to 64% of patients with acute HCV infection fail to undergo spontaneous viral clearance<sup>[7]</sup>. Thus, chronically infected patients may be at risk of liver fibrosis (LF), cirrhosis, and hepatocellular carcinoma during a period of 20 to 30 years<sup>[4,8]</sup>.

Regardless of etiology, the pathogenesis of LF is influenced both by genetic and environmental factors<sup>[9,10]</sup>. High-fat diets, which have a significant content of saturated fatty acids (SFA), have been associated with the pathological processes known to be involved in liver fibrogenesis, including steatosis, inflammation, and insulin resistance<sup>[11-13]</sup>. Recently, we reported that in West Mexico, the general population and patients with liver disease consume an excessive amount of red meat, fried foods, sausages, and pastry products<sup>[14]</sup>. Consequently, these dietary trends have increased the proportional intake of calories, total fat, and SFA, which could eventually lead to liver damage in individuals that consume this type of hepatopathogenic diet.

In addition to the textural, olfactory, neural and hormonal mechanisms involved in food intake, taste perception is considered a critical determinant of dietary preferences<sup>[15,16]</sup>. There is growing evidence of the existence of a new taste modality related to fat preference<sup>[17]</sup>. Experimental studies suggest that the lingual cluster of differentiation 36 (*CD36*) receptor regulates the motivation for fatty food consumption in rodents<sup>[18,19]</sup>. This effect is carried out through the cellular capture of long-chain fatty acids by the *CD36* receptors on the taste buds<sup>[20]</sup>; subsequently, lipid signals are transduced into the gustatory nervous pathway<sup>[21]</sup>. Therefore, genetic variations that lead to changes in the expression of *CD36* could explain the interindividual differences in fat linking<sup>[15]</sup>. *CD36* protein levels are modulated by several single nucleotide polymorphisms (SNPs) in the *CD36* gene on chromosome 7<sup>[22,23]</sup>. One SNP consists of a nucleotide substitution of guanine for adenine in the *CD36* gene promoter sequence (-31118G > A, rs1761667)<sup>[24]</sup>. This SNP has been associated with a significant reduction in the *CD36* expression in several tissues<sup>[25,26]</sup>.

Recently, we reported an association between *CD36* with a higher intake of fat portions and high serum cholesterol among the general population of West Mexico<sup>[27]</sup>. However, its role in dietary intake and HCV-related liver damage is currently unknown. Therefore, this study aimed to analyze the association of the rs1761667 *CD36* polymorphism with dietary intake and LF in patients chronically infected with hepatitis C.

## MATERIALS AND METHODS

### Study design

In this retrospective study, 73 chronic hepatitis C (CHC) patients were recruited at the Department of Molecular Biology in Medicine from January 2012 to December 2014. Chronic HCV infection was defined as a positive



anti-HCV test result (ELISA Third-Generation, AxSYM, Abbott Laboratories, Illinois, United States) and the presence of serum HCV RNA for more than six months (COBAS® AmpliPrep/COBAS® Taqman® HCV Test; Roche Diagnostics, Pleasanton, CA, United States)<sup>[28,29]</sup>. Duration of infection (years) was estimated by the self-reported date of exposure to any known risk factor for HCV infection including the history of surgeries, blood transfusions, hemodialysis, acupuncture, injection drug use and tattooing<sup>[30]</sup>. Patients co-infected with the hepatitis B virus or human immunodeficiency virus, as well as alcohol abusers were excluded. Based on the pattern of alcohol intake in West Mexico, alcohol abusers were defined as those individuals that consumed more than two drinks per occasion, as previously described<sup>[31]</sup>. None of the CHC patients in the study group had received antiviral treatment for HCV infection.

### **Viral genotyping**

A VERSANT HCV Genotype 2.0 line probe assay was used to determine the HCV genotypes (Innogenetics, Ghent, Belgium).

### **Body mass index measurement**

An electrical bioimpedance apparatus was used to assess body mass index (BMI, kg/m<sup>2</sup>) (INBODY 3.0, Analyzer Body Composition, Biospace, South Korea).

### **Dietary assessment**

A three-day food record (two weekdays and one weekend day) was used as a tool to assess the patient's dietary intake, which has been previously used for our population<sup>[27,32-34]</sup>. This methodology provides accurate data concerning intake of food and nutrients<sup>[35]</sup>. Briefly, each subject was instructed on how to register the type, amount, and mode of preparation of all foods using food models<sup>[32]</sup>. The food records were coded by a qualified dietitian using a specialized software (Nutrikal VO®, Mexico). This program calculated the total amount of calories, fat, protein, and carbohydrates as well as the daily intake of food group servings such as sugars, meat, fruits, vegetables, fats, milk, legumes, and cereals. Dietary data were averaged over the three-day food records and were compared with the recommended dietary intakes based on the Mexican System of Food and Equivalents<sup>[36,37]</sup>.

### **Biochemical tests**

Serum was obtained from 10 mL blood samples after a 12-h overnight fast. Biochemical tests included glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-c). The Friedewald formula was selected to estimate low-density lipoprotein cholesterol (LDL-c)<sup>[38]</sup>. The concentration of very low-density lipoprotein cholesterol (VLDL-c) was calculated as Total Cholesterol - (LDL-c + HDL-c). All biochemical tests were performed using a dry chemistry assay on a Vitros

250 Analyzer (Ortho-Clinical Diagnostics, Johnson and Johnson Co, Rochester, NY).

### **Liver fibrosis evaluation**

Liver stiffness (fibrosis) was evaluated by transient elastography (TE) (FibroScan® Echosens, Paris, France). The average value of ten successful readings expressed in kilopascals (kPa) was used as an indicator of LF according to the following classification: F1 (< 7 kPa), F1-F2 (7 kPa-8.49 kPa), F2 (8.5 kPa-9.49 kPa), F3 (9.5 kPa-12.49 kPa) and F3-F4 (12.5 kPa-14.49 kPa) and F4 (> 14.5 kPa)<sup>[39]</sup>. For this study, patients in either the F1 or F2 stages were classified as having mild LF and those in the F3 or F4 stages were classified as having advanced LF<sup>[40]</sup>. This classification was corroborated by calculating the aspartate aminotransferase-to-platelet ratio index (APRI score), as previously described<sup>[41]</sup>.

### **CD36 genotyping**

Leukocyte genomic DNA was extracted by a modified salting-out method<sup>[42]</sup>. The rs1761667 *CD36* polymorphism was detected by an allelic discrimination assay (TaqMan, Applied Biosystems, ID C\_8314999\_10; Foster City, CA, United States) in a 96-well format (StepOnePlus thermocycler (Applied Biosystems, Foster City, CA, United States) as previously described<sup>[27,34]</sup>.

### **Statistical analysis**

The sample size was estimated by a formula for the comparison of proportions<sup>[43]</sup> resulting in a statistical power of 80% ( $\beta = 0.20$ ) with a reliability of 95% ( $\alpha = 0.05$ ) based on the rs1761667 *CD36* allelic frequency in our population<sup>[24,27]</sup>. Quantitative variables were expressed as mean  $\pm$  SD and analyzed by one-way ANOVA adjusted for age, gender, and BMI. Subsequently, post hoc tests were run (Bonferroni's test and Dunnett's T3 test). Finally, to quantify the effect of the *CD36* genotypes on quantitative variables, linear regression was performed. The Hardy-Weinberg equilibrium (HWE) and qualitative variables were evaluated by the  $\chi^2$  test. The association of the *CD36* genotypes with LF was assessed by odds ratio (OR) as well as logistic regression tests considering a confidence interval (CI) of 95%. A *P*-value of < 0.05 was considered significant. Statistical analyses were performed using Arlequin (version 3.1), Epi Info™ 7 (CDC, Atlanta, GA) and SPSS Statistics, Version 20.0 (IBM Corp, Armonk, NY). All statistical analyses were reviewed and approved by an expert biomedical statistician.

### **Ethical guidelines**

This study was in compliance with the ethical guidelines defined by the Declaration of Helsinki 2013 and was approved by the Institutional Board Review (CI-01913). All patients who agreed to enter this study signed a written informed consent.

## **RESULTS**

In this study, the genotypic frequencies were AA (30.1%),

**Table 1** Demographical and clinical characteristics of the chronic hepatitis C patients classified by cluster of differentiation 36 genotype

Variable	<i>CD36</i> genotype			<i>P</i> -value
	AA	AG	GG	
No. of patients, <i>n</i> (%)	22 (30.1)	40 (54.8)	11 (15.1)	---
Age (yr)	48.1 ± 11.7	51.4 ± 11.1	53.7 ± 15.3	0.38
Gender (F/M)	(12/10)	(21/19)	(7/4)	0.68
BMI (kg/m <sup>2</sup> )	26.6 ± 4.1	24.9 ± 4.2	24.4 ± 3.1	0.52
Duration of infection (yr)	26.9 ± 10.1	25.2 ± 8.1	25.4 ± 7.4	0.62
HCV genotype 1, <i>n</i> (%)	15 (68.2)	27 (67.5)	8 (72.7)	0.40
HCV genotype 2, <i>n</i> (%)	5 (22.7)	9 (22.5)	3 (27.3)	
HCV genotype 3, <i>n</i> (%)	2 (9.1)	4 (10)	0 (0)	

Quantitative values are expressed as mean ± SD. Frequencies are expressed as percentage. CHC: Chronic hepatitis C; F/M: Female/male; BMI: Body mass index; HCV: Hepatitis C virus; *CD36*: Cluster of differentiation 36.

AG (54.8%), and GG (15.1%), whereas the allelic A and G frequencies were 57.5% and 42.5%, respectively. These genotypes were concordant with the HWE ( $P = 0.50$ ). In Table 1, the demographical and clinical characteristics of the CHC patients by *CD36* genotype are shown. No significant differences for the variables of age, gender, BMI, years of infection, and HCV genotypes between *CD36* genotypes were found. Only the CHC patients who were carriers of the AA genotype were overweight according to the WHO classification (BMI = 26.6 kg/m<sup>2</sup>). HCV genotype 1 was the most frequent with 68.4% of the total cases, followed by HCV genotype 2 (23.3%) and HCV genotype 3 (8.2%).

The daily dietary intake of the CHC patients classified by *CD36* genotype is shown in Table 2. CHC patients who were carriers of the *CD36* AA genotype had a higher caloric intake relative to total fat, and SFA than those with the AG and GG genotypes. No differences in protein and CH intakes between *CD36* genotypes were observed. Subsequently, the daily intake of several food groups classified by *CD36* genotype is shown in Table 3. Fats were the only food group associated with the *CD36* genotype. The lipid and liver profiles of the CHC patients by *CD36* genotype are shown in Table 4. CHC patients with the *CD36* AA genotype had more elevated serum levels of AST than the AG genotype carriers (91.7 IU/L vs 69.8 IU/L,  $P = 0.02$ ). Furthermore, an increase of 30.23 IU/L of AST was attributed to the AA genotype when compared with the AG genotype ( $\beta = 30.23$ , 95%CI: 9.0–51.46,  $P = 0.006$ ). No differences for ALT and GGT were observed (Table 4).

According to the categories of LF established in this study, 47.9% of the CHC patients had mild fibrosis, whereas 52.1% presented advanced fibrosis (Table 5). Among the CHC patients, the kPa values and APRI score were higher in those with advanced fibrosis compared to those with mild fibrosis (22.7 kPa vs 6.5 kPa,  $P < 0.001$  and 1.78 vs 0.81,  $P < 0.001$ , respectively). CHC patients with advanced fibrosis had a higher frequency of the *CD36* AA genotype than those with mild fibrosis (42.1% vs 17.1%,  $P = 0.002$ ), respectively (Table 6). Additionally, patients who were AA genotype carriers had

a higher risk for advanced fibrosis than those with the AG genotype (OR = 3.60, 95%CI: 1.16–11.15,  $P = 0.02$ ) and AG + GG genotypes (OR = 3.51 95%CI: 1.18–10.45,  $P = 0.02$ ). A logistic regression test was used to corroborate this association (OR = 2.23 95%CI: 1.03–4.81,  $P = 0.041$ ).

## DISCUSSION

Genetic polymorphisms in fat taste perception may partially explain the interindividual variability in fat intake<sup>[15]</sup> and their association with the risk of developing chronic diseases<sup>[15,44]</sup>. Over recent years, it has been proposed that the *CD36* receptor is an oral fat sensor that may influence an individual's preference for high-fat foods<sup>[15–18]</sup>. Specifically, it has been shown that the *CD36* AA genotype decreases fat taste perception<sup>[45–48]</sup>. In this study, the frequency of *CD36* AA genotype was 30.1%. In regards to food consumption, despite that the three-day food record may not be representative of the long-term food variety, the amount of fat intake represented over 30% of the total daily calories. It has been documented that the prolonged ingestion of high-fat diets increases the risk for metabolic disorders<sup>[49]</sup>. These data were consistent with previous results found in overweight patients from the general population of West Mexico<sup>[27]</sup>.

The association of high-fat diets with LF has been well documented in animal models<sup>[11–13]</sup> as well as in humans in different populations<sup>[50,51]</sup>. In this study, among the *CD36* AA genotype carriers, more cases of advanced LF were detected. This disease stage is characterized by steatosis and persistent inflammation<sup>[4]</sup>. Also, they exhibited significantly higher levels of AST, which is a better predictor of progression of LF than ALT or GGT<sup>[52]</sup>. Furthermore, two validated non-invasive methods (TE and APRI score) were used to evaluate LF<sup>[41,53]</sup>. Since no differences in demographic and viral characteristics between *CD36* genotypes were found, the likelihood of HCV-related LF seems to be enhanced because of the higher consumption of fat portions observed among the *CD36* AA genotype carriers.

The immunological mechanisms that regulate LF progression during HCV infection have been extensively studied<sup>[54–56]</sup>. However, alterations in lipid and lipoprotein metabolism have been reported to play a key role<sup>[9]</sup>, considering that chronic HCV infection is characterized by hypocholesterolemia and reduced levels of LDL-c, TG and apolipoprotein B (apoB)<sup>[57]</sup>. Recently, a novel interaction of the *CD36* receptor in liver VLDL-c metabolism has been proposed<sup>[58]</sup>. Findings in a further study, concurring with this hypothesis, have demonstrated that *CD36* deletion can reduce VLDL output and liver fat in obese mice<sup>[59]</sup>. This finding was related to the enhanced production of the series-2 liver prostaglandins, which have been shown to suppress VLDL output and increase the hepatocyte triglyceride content in an inflammatory condition-dependent manner<sup>[60]</sup>. Thus, it is plausible that the AA genotype carriers may have a lower expression of the *CD36* receptor that could contribute to liver steatosis and consequently to fibrosis similar to the effects of

**Table 2** Daily dietary intake of the chronic hepatitis C patients classified by cluster of differentiation 36 genotype

Variable	Reference values	<i>CD36</i> genotype			<i>P</i> -value
		AA	AG	GG	
Total calories	-	2531.3 ± 301.3	1902.5 ± 396.1	1873.5 ± 345.7	0.021 <sup>a</sup>
CH (%)	50-60	55.4 ± 10.5	54.3 ± 8.9	53.2 ± 6.4	0.76
Protein (%)	15	17.2 ± 4.6	16.3 ± 3.9	16.4 ± 2.9	0.81
Fat (%)	< 30	34.9 ± 7.5	27.5 ± 7.2	24.9 ± 1.1	0.001299 <sup>a</sup>
SFA (%)	< 7	16.1 ± 6.1	8.1 ± 3.2	8.4 ± 2.7	0.2 × 10 <sup>-6a</sup>
MUFA (%)	20	13.1 ± 3.4	12.8 ± 7.6	12.1 ± 5.4	0.94
PUFA (%)	10	8.8 ± 6.5	5.6 ± 4.2	5.2 ± 1.3	0.11

<sup>a</sup>By post hoc tests: Total calories: AA genotype *vs* AG and GG genotypes, *P* = 0.027. Fat: AA *vs* AG, *P* = 0.006; AA *vs* GG, *P* = 0.002; SFA: AA *vs* AG, *P* = 0.2 × 10<sup>-6</sup>, AA *vs* GG, *P* = 0.185 × 10<sup>-4</sup>. Quantitative values are expressed as mean ± SD. CH: Carbohydrates; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFAs: Polyunsaturated fatty acids; *CD36*: Cluster of differentiation 36.

**Table 3** Daily intake of food group servings in chronic hepatitis C patients classified by cluster of differentiation 36 genotype

Variable	Reference values	<i>CD36</i> genotype			<i>P</i> -value
		AA	AG	GG	
Sugars	0-3	5.7 ± 4.3	5.5 ± 4.8	5.2 ± 4.1	0.85
Meat	2-3	5.7 ± 1.6	5.1 ± 2.8	4.4 ± 2.2	0.15
Fruits	2-4	2.0 ± 1.8	1.7 ± 0.9	1.4 ± 1.1	0.43
Vegetables	3-5	2.1 ± 1.6	1.9 ± 1.1	1.6 ± 0.8	0.42
Fats	0-3	6.5 ± 1.7	4.3 ± 3.1	3.9 ± 2.2	0.003207 <sup>1</sup>
Milk	1-3	1.0 ± 0.7	0.8 ± 0.7	0.8 ± 0.9	0.86
Legumes	1-2	1.0 ± 0.7	0.9 ± 0.7	0.8 ± 0.7	0.88
Cereals	6-11	10.3 ± 5.4	9.6 ± 5.8	9.0 ± 5.1	0.77

Quantitative values are expressed as mean ± SD. <sup>1</sup>By Post hoc tests: Fats: AA *vs* GG, *P* = 0.011608. *CD36*: Cluster of differentiation 36.

**Table 4** Biochemical profile of the chronic hepatitis C patients classified by cluster of differentiation 36 genotype

Variable	<i>CD36</i> genotype			<i>P</i> -value
	AA	AG	GG	
Glucose (mg/dL)	109.5 ± 59.3	106.7 ± 42.9	97.4 ± 19.8	0.78
TC (mg/dL)	146.8 ± 35.1	162.2 ± 44.2	157.8 ± 51.1	0.40
TG (mg/dL)	112.8 ± 43.3	140.8 ± 60.8	142.3 ± 51.1	0.30
HDL-c (mg/dL)	42.7 ± 15.1	40.4 ± 13.1	33.8 ± 9.8	0.21
LDL-c (mg/dL)	83.1 ± 28.8	95.4 ± 42.6	101.1 ± 42.6	0.44
VLDL-c (mg/dL)	22.6 ± 8.7	28.2 ± 12.1	28.9 ± 10.1	0.27
ALT (IU/L)	93.8 ± 42.6	73.4 ± 73.1	71.5 ± 46.4	0.38
AST (IU/L)	91.7 ± 41.3	61.5 ± 40.3	69.8 ± 53.9	0.028 <sup>1</sup>
GGT (IU/L)	85.9 ± 56.2	66.4 ± 40.8	43.1 ± 33.2	0.18

<sup>1</sup>By post hoc tests: AA genotype *vs* AG genotype, *P* = 0.024. Quantitative values are expressed as mean ± SD. TC: Total cholesterol; TG: Triglycerides; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; VLDL-c: Very low-density lipoprotein cholesterol; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl-transferase; *CD36*: Cluster of differentiation 36.

a *CD36* deletion. Nonetheless, further investigation is required to elucidate the correlation between the *CD36* genotype and liver steatosis and clarify its interaction with other key molecules involved in this metabolic alteration, such as the microsomal triglyceride transfer protein (MTTP), apolipoprotein E and apoB<sup>[61,62]</sup>.

Concerning the nutritional management of liver disease,

**Table 5** Kilopascals and aspartate aminotransferase to platelet ratio index score values by the severity of liver fibrosis among chronic hepatitis C patients

Variable	Mild fibrosis	Advanced fibrosis	<i>P</i> -value
No. of patients, <i>n</i> (%)	35 (47.9)	38 (52.1)	-
kPa	6.5 ± 1.7	22.7 ± 13.4	< 0.001
APRI score	0.81 ± 0.33	1.78 ± 0.53	< 0.001

Quantitative values are expressed as mean ± SD. kPa: Kilopascals; APRI: Aspartate aminotransferase to platelet ratio index.

including HCV infection, the majority of international guidelines focus on the reduction of total fat and SFA intake<sup>[51,63]</sup> without taking into account the nutrigenetics and food cultures of individual populations. We advocate shifting towards a genome-based nutrition approach as a preventive and intervention strategy for chronic diseases given the fact that, worldwide, human populations differ<sup>[64]</sup>. Specifically, in the case of Mexico and most of Latin America, the people in these regions are genetically an admixture of Amerindian, Caucasian, and African ancestries with a heterogeneous inter-regional distribution<sup>[65,66]</sup>. Furthermore, 70% of the Mexican general population is overweight or obese due to the consumption of an obesogenic and hepatopatogenic diet that was previously described<sup>[4,14,64]</sup>. Thus, based on the gene-environmental interactions that currently prevail in the Mexican population, specific preventive strategies are crucial to diminish the progression of liver damage caused by alterations in lipid metabolism and inflammation.

In this study, the frequency of the *CD36* AA genotype (30.1%) was comparable to the pattern of distribution (28.4%) observed in non-diabetic individuals of Caucasian origin<sup>[24]</sup>. These findings are consistent with the high Caucasian ancestry that prevails among Mexican-Mestizos and HCV patients that have been previously reported<sup>[7]</sup>, whereas different frequencies have been reported elsewhere<sup>[67-69]</sup>. Thus, we consider that the detection of the *CD36* genotype, as well as other nutrient-interacting genes<sup>[31-34]</sup> could be used as auxiliary tools to predict the adherence to dietary regimens and for the implementation of genome-based intervention

**Table 6 Association of the cluster of differentiation 36 genotype with the severity of liver fibrosis among chronic hepatitis C patients**

CD36 genotype	Mild fibrosis n (%)	Advanced fibrosis n (%)	Genotype comparison	Odds ratio (95%CI)	P-value
AA	6 (17.1)	16 (42.1)	AA vs GG	3.20 (0.70-14.52)	0.12
AG	23 (65.7)	17 (44.7)	AA vs AG	3.60 (1.16-11.15)	0.02
GG	6 (17.1)	5 (13.2)	AA vs AG/GG	3.51 (1.18-10.45)	0.02

CD36: Cluster of differentiation 36.

strategies<sup>[64]</sup> aimed at reducing fat intake and dyslipidemia in our population<sup>[27]</sup>.

In conclusion, the AA genotype of the rs1761667 CD36 polymorphism was associated with higher fat intake and more instances of advanced LF in CHC patients. However, further genomic studies are needed to analyze the role of the CD36 polymorphism on liver disease in other populations within Mexico and worldwide.

## COMMENTS

### Background

Regardless of etiology, liver fibrosis (LF) pathogenesis is influenced by genetic and environmental factors, such as dietary intake. Diets that are high in saturated fatty acids have been associated with the pathological processes involved in liver fibrogenesis, including steatosis, inflammation, and insulin resistance. There is growing evidence that suggest that the lingual cluster of differentiation 36 (CD36) receptor regulates the motivation for fatty food consumption. Therefore, genetic variations in CD36 expression could explain the global heterogeneity of fat linking and its association with chronic diseases. This study aimed to analyze the association of the CD36 polymorphism (rs1761667) with dietary fat intake and LF in chronically infected hepatitis C patients.

### Research frontiers

The results of this study contribute to the understanding of the specific gene-environmental interactions that occur among a population with an admixture genome. The role of CD36 genetic variation on hepatitis C virus (HCV)-related liver disease or other chronic diseases in distinct populations worldwide requires further studies.

### Innovations and breakthroughs

In this study, the authors provide evidence regarding the effect of the CD36 (AA) risk genotype on the consumption of a high-fat diet and its association with LF in HCV patients.

### Applications

The detection of the CD36 genotype together with other nutrient-sensing genes could be useful for the implementation of genome-based intervention strategies aimed at reducing fat intake and dyslipidemia in chronic hepatitis C patients.

### Peer-review

The authors of this paper evaluated the dietary fat intake and the degree of LF in patients chronically infected with hepatitis C based on the CD36 genotypes. The results suggest that the risk AA genotype of the CD36 polymorphism was associated with higher dietary fat intake and more instances of advanced LF in chronic hepatitis C patients.

## REFERENCES

- Zaltron S, Spinetti A, Biasi L, Baiguera C, Castelli F. Chronic HCV infection: epidemiological and clinical relevance. *BMC Infect Dis* 2012; **12** Suppl 2: S2 [PMID: 23173556 DOI: 10.1186/1471-2334-12-S2-S2]
- Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]
- Méndez-Sánchez N, Aguilar-Ramírez JR, Reyes A, Dehesa M, Juárez A, Castañeda B, Sánchez-Avila F, Poo JL, Guevara González L, Lizardi J, Valdovinos MA, Uribe M, Contreras AM, Tirado P, Aguirre J, Rivera-Benítez C, Santiago-Santiago R, Bosques-Padilla F, Muñoz L, Guerrero A, Ramos M, Rodríguez-Hernández H, Jacobo-Karam J. Etiology of liver cirrhosis in Mexico. *Ann Hepatol* 2004; **3**: 30-33 [PMID: 15118577]
- Ramos-Lopez O, Martinez-Lopez E, Roman S, Fierro NA, Panduro A. Genetic, metabolic and environmental factors involved in the development of liver cirrhosis in Mexico. *World J Gastroenterol* 2015; **21**: 11552-11566 [PMID: 26556986 DOI: 10.3748/wjg.v21.i41.11552]
- Panduro A, Escobedo Meléndez G, Fierro NA, Ruiz Madrigal B, Zepeda-Carrillo EA, Román S. [Epidemiology of viral hepatitis in Mexico]. *Salud Publica Mex* 2011; **53** Suppl 1: S37-S45 [PMID: 21877071 DOI: 10.1590/S0036-36342011000700008]
- Panduro A, Roman S. Need of righteous attitudes towards eradication of hepatitis C virus infection in Latin America. *World J Gastroenterol* 2016; **22**: 5137-5142 [PMID: 27298556 DOI: 10.3748/wjg.v22.i22.5137]
- Gonzalez-Aldaco K, Rebello Pinho JR, Roman S, Gleyzer K, Fierro NA, Oyakawa L, Ramos-Lopez O, Ferraz Santana RA, Sitnik R, Panduro A. Association with Spontaneous Hepatitis C Viral Clearance and Genetic Differentiation of IL28B/IFNL4 Haplotypes in Populations from Mexico. *PLoS One* 2016; **11**: e0146258 [PMID: 26741362 DOI: 10.1371/journal.pone.0146258]
- Roman S, Panduro A. Genomic medicine in gastroenterology: A new approach or a new specialty? *World J Gastroenterol* 2015; **21**: 8227-8237 [PMID: 26217074 DOI: 10.3748/wjg.v21.i27.8227]
- Fierro NA, Gonzalez-Aldaco K, Torres-Valadez R, Martinez-Lopez E, Roman S, Panduro A. Immunologic, metabolic and genetic factors in hepatitis C virus infection. *World J Gastroenterol* 2014; **20**: 3443-3456 [PMID: 24707127 DOI: 10.3748/wjg.v20.i13.3443]
- Papandreou D, Andreou E. Role of diet on non-alcoholic fatty liver disease: An updated narrative review. *World J Hepatol* 2015; **7**: 575-582 [PMID: 25848481 DOI: 10.4254/wjh.v7.i3.575]
- Wang D, Wei Y, Pagliassotti MJ. Saturated fatty acids promote endoplasmic reticulum stress and liver injury in rats with hepatic steatosis. *Endocrinology* 2006; **147**: 943-951 [PMID: 16269465 DOI: 10.1210/en.2005-0570]
- Ha SK, Chae C. Inducible nitric oxide distribution in the fatty liver of a mouse with high fat diet-induced obesity. *Exp Anim* 2010; **59**: 595-604 [PMID: 21030787 DOI: 10.1538/expanim.59.595]
- Longato L, Tong M, Wands JR, de la Monte SM. High fat diet induced hepatic steatosis and insulin resistance: Role of dysregulated ceramide metabolism. *Hepatol Res* 2012; **42**: 412-427 [PMID: 22176347 DOI: 10.1111/j.1872-034X.2011.00934.x]
- Ramos-López O, Román S, Ojeda-Granados C, Sepúlveda-Villegas M, Martínez-López E, Torres-Valadez R, Trujillo-Trujillo E, Arturo Panduro. Patrón de ingesta alimentaria y actividad física en pacientes hepatópatas en el Occidente de México. *Rev Endocrinol Nutr* 2013; **21**: 7-15
- Garcia-Bailo B, Toguri C, Eny KM, El-Sohehy A. Genetic variation in taste and its influence on food selection. *OMICS* 2009; **13**: 69-80 [PMID: 18687042 DOI: 10.1089/omi.2008.0031]
- Dransfield E. The taste of fat. *Meat Sci* 2008; **80**: 37-42 [PMID: 18687042 DOI: 10.1089/omi.2008.0031]



- 22063168 DOI: 10.1016/j.meatsci.2008.05.030]
- 17 **Degrace-Passilly P**, Besnard P. CD36 and taste of fat. *Curr Opin Clin Nutr Metab Care* 2012; **15**: 107-111 [PMID: 22248592 DOI: 10.1097/MCO.0b013e32834ff19c]
- 18 **Laugerette F**, Passilly-Degrace P, Patris B, Niot I, Febbraio M, Montmayeur JP, Besnard P. CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. *J Clin Invest* 2005; **115**: 3177-3184 [PMID: 16276419 DOI: 10.1172/JCI25299]
- 19 **Martin C**, Passilly-Degrace P, Gaillard D, Merlin JF, Chevrot M, Besnard P. The lipid-sensor candidates CD36 and GPR120 are differentially regulated by dietary lipids in mouse taste buds: impact on spontaneous fat preference. *PLoS One* 2011; **6**: e24014 [PMID: 21901153 DOI: 10.1371/journal.pone.0024014]
- 20 **Su X**, Abumrad NA. Cellular fatty acid uptake: a pathway under construction. *Trends Endocrinol Metab* 2009; **20**: 72-77 [PMID: 19185504 DOI: 10.1016/j.tem.2008.11.001]
- 21 **Aly R**, Maibach HI, Bagatell FK, Dittmar W, Hänel H, Falanga V, Leyden JJ, Roth HL, Stoughton RB, Willis I. Ciclopirox olamine lotion 1%: bioequivalence to ciclopirox olamine cream 1% and clinical efficacy in tinea pedis. *Clin Ther* 2016; **11**: 290-303 [PMID: 2663159 DOI: 10.1152/physrev.00002.2015]
- 22 **Rač ME**, Safranow K, Poncyljusz W. Molecular basis of human CD36 gene mutations. *Mol Med* 2007; **13**: 288-296 [PMID: 17673938 DOI: 10.2119/2006-00088.Rac]
- 23 **Fernández-Ruiz E**, Armesilla AL, Sánchez-Madrid F, Vega MA. Gene encoding the collagen type I and thrombospondin receptor CD36 is located on chromosome 7q11.2. *Genomics* 1993; **17**: 759-761 [PMID: 7503937 DOI: 10.1006/geno.1993.1401]
- 24 **Ma X**, Bacci S, Mlynarski W, Gottardo L, Soccio T, Menzaghi C, Iori E, Lager RA, Shroff AR, Gervino EV, Nesto RW, Johnstone MT, Abumrad NA, Avogaro A, Trischitta V, Doria A. A common haplotype at the CD36 locus is associated with high free fatty acid levels and increased cardiovascular risk in Caucasians. *Hum Mol Genet* 2004; **13**: 2197-2205 [PMID: 15282206 DOI: 10.1093/hmg/ddh233]
- 25 **Love-Gregory L**, Sherva R, Schappe T, Qi JS, McCrea J, Klein S, Connelly MA, Abumrad NA. Common CD36 SNPs reduce protein expression and may contribute to a protective atherogenic profile. *Hum Mol Genet* 2011; **20**: 193-201 [PMID: 20935172 DOI: 10.1093/hmg/ddq449]
- 26 **Ghosh A**, Murugesan G, Chen K, Zhang L, Wang Q, Febbraio M, Anselmo RM, Marchant K, Barnard J, Silverstein RL. Platelet CD36 surface expression levels affect functional responses to oxidized LDL and are associated with inheritance of specific genetic polymorphisms. *Blood* 2011; **117**: 6355-6366 [PMID: 21478428 DOI: 10.1182/blood-2011-02-338582]
- 27 **Ramos-Lopez O**, Panduro A, Martinez-Lopez E, Fierro NA, Ojeda-Granados C, Sepulveda-Villegas M, Roman S. Genetic variant in the CD36 gene (rs1761667) is associated with higher fat intake and high serum cholesterol among the population of West Mexico. *J Nutr Food Sci* 2015; **5**: 353 [DOI: 10.4172/2155-9600.1000353]
- 28 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; **60**: 392-420 [PMID: 24331294 DOI: 10.1016/j.jhep.2013.11.003]
- 29 **Ghany MG**, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374 [PMID: 19330875 DOI: 10.1002/hep.22759]
- 30 **Muñoz-Espinosa LE**, Trujillo-Trujillo ME, Martínez-Macias RF, Panduro A, Rivas-Estilla AM, Fierro NA, Silvera-Linares AL, Torres-Valadez R, Cordero-Pérez P, González-Aldaco K, Chen-López CY, José-Abrego A, Zuñiga-Noriega JR, Gutiérrez-Ruiz MC, Roman S. Increase of drug use and genotype 3 in HCV-infected patients from Central West and Northeast Mexico. *Ann Hepatol* 2015; **14**: 642-651 [PMID: 26256892]
- 31 **Ramos-Lopez O**, Roman S, Martinez-Lopez E, Gonzalez-Aldaco K, Ojeda-Granados C, Sepulveda-Villegas M, Panduro A. Association of a novel TAS2R38 haplotype with alcohol intake among Mexican-Mestizo population. *Ann Hepatol* 2015; **14**: 729-734 [PMID: 26256902]
- 32 **Martinez-Lopez E**, Garcia-Garcia MR, Gonzalez-Avalos JM, Maldonado-Gonzalez M, Ruiz-Madrigril B, Vizmanos B, Hernandez-Nazara Z, Roman S, Panduro A. Effect of Ala54Thr polymorphism of FABP2 on anthropometric and biochemical variables in response to a moderate-fat diet. *Nutrition* 2013; **29**: 46-51 [PMID: 22817827 DOI: 10.1016/j.nut.2012.03.002]
- 33 **Garcia-Garcia MR**, Morales-Lanuza MA, Campos-Perez WY, Ruiz-Madrigril B, Maldonado-Gonzalez M, Vizmanos B, Hernandez-Cañaveral I, Yañez-Sanchez I, Roman S, Panduro A, Martinez-Lopez E. Effect of the ADIPOQ Gene -11391G/A Polymorphism Is Modulated by Lifestyle Factors in Mexican Subjects. *J Nutrigenet Nutrigenomics* 2014; **7**: 212-224 [PMID: 25790965 DOI: 10.1159/000371801]
- 34 **Ramos-Lopez O**, Panduro A, Martinez-Lopez E, Roman S. Sweet Taste Receptor TAS1R2 Polymorphism (Val191Val) Is Associated with a Higher Carbohydrate Intake and Hypertriglyceridemia among the Population of West Mexico. *Nutrients* 2016; **8**: 101 [PMID: 26907331 DOI: 10.3390/nu8020101]
- 35 **Thompson FE**, Byers T. Dietary assessment resource manual. *J Nutr* 1994; **124**: 2245S-2317S [PMID: 7965210]
- 36 **Marvan Laborde L**, Perez Lizaur AB, Palacios Gonzalez B. Sistema Mexicano de Alimentos Equivalentes. 2nd ed. Fomento de Nutricion y Salud, 2000: 1-84
- 37 **Perez Lizaur AB**, Marvan LL. Manual de dietas normales y terapéuticas: los alimentos en la salud y en la enfermedad. 5th ed. Mexico: DF La Prensa Médica Mexicana, 2005: 1-281
- 38 **Tremblay AJ**, Morrisette H, Gagné JM, Bergeron J, Gagné C, Couture P. Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol compared with beta-quantification in a large population. *Clin Biochem* 2004; **37**: 785-790 [PMID: 15329317 DOI: 10.1016/j.clinbiochem.2004.03.008]
- 39 **Foucher J**, Chanteloup E, Vergniol J, Castéra L, Le Bail B, Adhoute X, Bertet J, Couzigou P, de Lédinghen V. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006; **55**: 403-408 [PMID: 16020491 DOI: 10.1136/gut.2005.069153]
- 40 **do Carmo RF**, Vasconcelos LR, Mendonça TF, de Mendonça Cavalcanti Mdo S, Pereira LM, Moura P. Myeloperoxidase gene polymorphism predicts fibrosis severity in women with hepatitis C. *Hum Immunol* 2014; **75**: 766-770 [PMID: 24882572 DOI: 10.1016/j.humimm.2014.05.008]
- 41 **Lin ZH**, Xin YN, Dong QJ, Wang Q, Jiang XJ, Zhan SH, Sun Y, Xuan SY. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatology* 2011; **53**: 726-736 [PMID: 21319189 DOI: 10.1002/hep.24105]
- 42 **Miller SA**, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215 [PMID: 3344216]
- 43 **Fleiss JL**, Levin B, Cho-Paik M. Statistical Methods for Rates and Proportions. 3rd ed. New York: John Wiley & Sons, 2003: 1-800
- 44 **Ramos-López O**, Ojeda-Granados C, Román S, Panduro A. Influencia genética en las preferencias alimentarias. *Rev Endocrinol Nutr* 2013; **21**: 74-83
- 45 **Pepino MY**, Love-Gregory L, Klein S, Abumrad NA. The fatty acid translocase gene CD36 and lingual lipase influence oral sensitivity to fat in obese subjects. *J Lipid Res* 2012; **53**: 561-566 [PMID: 22210925 DOI: 10.1194/jlr.M021873]
- 46 **Mrizak I**, Šerý O, Plesnik J, Arfa A, Fekih M, Bouslema A, Zaouali M, Tabka Z, Khan NA. The A allele of cluster of differentiation 36 (CD36) SNP 1761667 associates with decreased lipid taste perception in obese Tunisian women. *Br J Nutr* 2015; **113**: 1330-1337 [PMID: 25822988 DOI: 10.1017/S0007114515000343]
- 47 **Sayed A**, Šerý O, Plesnik J, Daoudi H, Rouabah A, Rouabah L, Khan NA. CD36 AA genotype is associated with decreased lipid taste perception in young obese, but not lean, children. *Int J Obes (Lond)* 2015; **39**: 920-924 [PMID: 25687220 DOI: 10.1038/ijo.2015.20]

- 48 **Melis M**, Sollai G, Muroi P, Crnjar R, Barbarossa IT. Associations between orosensory perception of oleic acid, the common single nucleotide polymorphisms (rs1761667 and rs1527483) in the CD36 gene, and 6-n-propylthiouracil (PROP) tasting. *Nutrients* 2015; **7**: 2068-2084 [PMID: 25803547 DOI: 10.3390/nu7032068]
- 49 **Zivkovic AM**, German JB, Sanyal AJ. Comparative review of diets for the metabolic syndrome: implications for nonalcoholic fatty liver disease. *Am J Clin Nutr* 2007; **86**: 285-300 [PMID: 17684197]
- 50 **Corrao G**, Ferrari PA, Galatola G. Exploring the role of diet in modifying the effect of known disease determinants: application to risk factors of liver cirrhosis. *Am J Epidemiol* 1995; **142**: 1136-1146 [PMID: 7485060]
- 51 **Freedman ND**, Cross AJ, McGlynn KA, Abnet CC, Park Y, Hollenbeck AR, Schatzkin A, Everhart JE, Sinha R. Association of meat and fat intake with liver disease and hepatocellular carcinoma in the NIH-AARP cohort. *J Natl Cancer Inst* 2010; **102**: 1354-1365 [PMID: 20729477 DOI: 10.1093/jnci/djq301]
- 52 **Stránský J**, Ryzlová M, Striteský J, Horák J. [Aspartate aminotransferase (AST) more than alanine aminotransferase (ALT) levels predict the progression of liver fibrosis in chronic HCV infection]. *Vnitr Lek* 2002; **48**: 924-928 [PMID: 16737138]
- 53 **Guéhot J**. [Noninvasive assessment of liver fibrosis in patients with chronic hepatitis virus C]. *Presse Med* 2006; **35**: 1317-1326 [PMID: 16969327 DOI: 10.1016/S0755-4982(06)74811-4]
- 54 **Fierro NA**, Castro-Garcia FP, Panduro A. Rethinking cytokine function during hepatitis A and hepatitis C infections. *Adv Biosci Biotechnol* 2013; **4**: 13-18 [DOI: 10.4236/abb.2013.47A1003]
- 55 **Fierro NA**, González-Aldaco K, Torres-Valadez R, Trujillo-Trujillo ME, Roman S, Trujillo-Ochoa JL, Panduro A. Spontaneous hepatitis C viral clearance and hepatitis C chronic infection are associated with distinct cytokine profiles in Mexican patients. *Mem Inst Oswaldo Cruz* 2015; **110**: 267-271 [PMID: 25946254 DOI: 10.1590/0074-02760140377]
- 56 **Oshiumi H**, Matsumoto M, Seya T. [Chronic hepatitis C virus infection attenuates host antiviral innate immune response]. *Nihon Rinsho* 2015; **73**: 234-238 [PMID: 25764676]
- 57 **Chang ML**. Metabolic alterations and hepatitis C: From bench to bedside. *World J Gastroenterol* 2016; **22**: 1461-1476 [PMID: 26819514 DOI: 10.3748/wjg.v22.i4.1461]
- 58 **Pepino MY**, Kuda O, Samovski D, Abumrad NA. Structure-function of CD36 and importance of fatty acid signal transduction in fat metabolism. *Annu Rev Nutr* 2014; **34**: 281-303 [PMID: 24850384 DOI: 10.1146/annurev-nutr-071812-161220]
- 59 **Nassir F**, Adewole OL, Brunt EM, Abumrad NA. CD36 deletion reduces VLDL secretion, modulates liver prostaglandins, and exacerbates hepatic steatosis in ob/ob mice. *J Lipid Res* 2013; **54**: 2988-2997 [PMID: 23964120 DOI: 10.1194/jlr.M037812]
- 60 **Pérez S**, Aspichueta P, Ochoa B, Chico Y. The 2-series prostaglandins suppress VLDL secretion in an inflammatory condition-dependent manner in primary rat hepatocytes. *Biochim Biophys Acta* 2006; **1761**: 160-171 [PMID: 16545597 DOI: 10.1016/j.bbalip.2006.02.003]
- 61 **Mirandola S**, Bowman D, Hussain MM, Alberti A. Hepatic steatosis in hepatitis C is a storage disease due to HCV interaction with microsomal triglyceride transfer protein (MTP). *Nutr Metab (Lond)* 2010; **7**: 13 [PMID: 20178560 DOI: 10.1186/1743-7075-7-13]
- 62 **Bassendine MF**, Sheridan DA, Bridge SH, Felmlee DJ, Neely RD. Lipids and HCV. *Semin Immunopathol* 2013; **35**: 87-100 [PMID: 23111699 DOI: 10.1007/s00281-012-0356-2]
- 63 **Dietitians of Canada**. Hepatitis C: nutrition care Canadian guidelines for health care providers. *Can J Diet Pract Res* 2003; **64**: 139-141 [PMID: 12959661]
- 64 **Roman S**, Ojeda-Granados C, Ramos-Lopez O, Panduro A. Genome-based nutrition: an intervention strategy for the prevention and treatment of obesity and nonalcoholic steatohepatitis. *World J Gastroenterol* 2015; **21**: 3449-3461 [PMID: 25834309 DOI: 10.3748/wjg.v21.i12.3449]
- 65 **Aceves D**, Ruiz B, Nuño P, Roman S, Zepeda E, Panduro A. Heterogeneity of apolipoprotein E polymorphism in different Mexican populations. *Hum Biol* 2006; **78**: 65-75 [PMID: 16900882 DOI: 10.1353/hub.2006.0021]
- 66 **Martínez-Cortés G**, Salazar-Flores J, Haro-Guerrero J, Rubi-Castellanos R, Velarde-Félix JS, Muñoz-Valle JF, López-Casamichana M, Carrillo-Tapia E, Canseco-Avila LM, Bravi CM, López-Armenta M, Rangel-Villalobos H. Maternal admixture and population structure in Mexican-Mestizos based on mtDNA haplogroups. *Am J Phys Anthropol* 2013; **151**: 526-537 [PMID: 23754474 DOI: 10.1002/ajpa.22293]
- 67 **Bayoumy NM**, El-Shabrawi MM, Hassan HH. Association of cluster of differentiation 36 gene variant rs1761667 (G > A) with metabolic syndrome in Egyptian adults. *Saudi Med J* 2012; **33**: 489-494 [PMID: 22588808]
- 68 **Keller KL**, Liang LC, Sakimura J, May D, van Belle C, Breen C, Driggin E, Tepper BJ, Lanzano PC, Deng L, Chung WK. Common variants in the CD36 gene are associated with oral fat perception, fat preferences, and obesity in African Americans. *Obesity (Silver Spring)* 2012; **20**: 1066-1073 [PMID: 22240721 DOI: 10.1038/oby.2011.374]
- 69 **Banerjee M**, Gautam S, Saxena M, Kumar Bid H, Agrawal CG. Association of CD36 gene variants rs1761667 (G > A) and rs1527483 (C > T) with Type 2 diabetes in North Indian population. *Int J Diabetes Mellit* 2010; **2**: 179-183 [DOI: 10.1016/j.ijdm.2010.08.002]

P- Reviewer: Sunami Y, Trovato FM S- Editor: Qiu S

L- Editor: A E- Editor: Li D



## Therapeutic alternatives for the treatment of type 1 hepatorenal syndrome: A Delphi technique-based consensus

Juan P Arab, Juan C Claro, Juan P Arancibia, Jorge Contreras, Fernando Gómez, Cristian Muñoz, Leyla Nazal, Eric Roessler, Rodrigo Wolff, Marco Arrese, Carlos Benítez

Juan P Arab, Rodrigo Wolff, Marco Arrese, Carlos Benítez, Departamento de Gastroenterología, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago 833-0024, Chile

Juan C Claro, Departamento de Medicina Interna and Programa de Salud Basada en la Evidencia Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago 833-0024, Chile

Juan P Arancibia, Sección de Gastroenterología, Departamento de Medicina, Hospital Clínico Universidad de Chile and Clínica Santa María, Santiago 838-0456, Chile

Jorge Contreras, Unidad de Gastroenterología, Universidad del Desarrollo, Clínica Alemana, Santiago 765-0568, Chile

Fernando Gómez, Cristian Muñoz, Servicio de Gastroenterología, Unidad de Trasplante Hepático, Hospital del Salvador, Facultad de Medicina, Universidad de Chile and Unidad de Gastroenterología, Clínica Alemana de Santiago, Clínica Indisa, Santiago 750-0922, Chile

Leyla Nazal, Clínica Las Condes y Servicio de Gastroenterología, Hospital Fuerza Aérea de Chile, Santiago 756-0171, Chile

Eric Roessler, Departamento de Nefrología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago 833-0024, Chile

**Author contributions:** Arab JP and Benítez C designed the survey and coordinated the expert panel; Arab JP, Claro JC, Arancibia JP, Contreras J, Gómez F, Muñoz C, Nazal L, Roessler E, Wolff R and Benítez C participated in the panel; Arab JP, Arrese M and Benítez C wrote the manuscript; all of the authors reviewed and approved the final draft.

**Supported by** The Sociedad Chilena de Gastroenterología (SCHGE) and the Asociación Chilena de Hepatología (ACHHEP).

**Conflict-of-interest statement:** There are no conflicts of interest arising from this work.

**Data sharing statement:** No further data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative

Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Carlos Benítez, MD, Departamento de Gastroenterología, Escuela de Medicina, Pontificia Universidad Católica de Chile, Marcoleta #367, Santiago 833-0024, Chile. [benitezc@gmail.com](mailto:benitezc@gmail.com)  
**Telephone:** +56-2-23543820  
**Fax:** +56-2-26397780

**Received:** May 28, 2016

**Peer-review started:** May 30, 2016

**First decision:** July 6, 2016

**Revised:** July 17, 2016

**Accepted:** July 29, 2016

**Article in press:** August 1, 2016

**Published online:** September 8, 2016

### Abstract

#### AIM

To propose several alternatives treatment of type 1 hepatorenal syndrome (HRS-1) what is the most severe expression of circulatory dysfunction on patients with portal hypertension.

#### METHODS

A group of eleven gastroenterologists and nephrologists performed a structured analysis of available literature. Each expert was designated to review and answer a question. They generated draft statements for evaluation by all the experts. Additional input was obtained from medical community. In order to reach consensus, a modified three-round Delphi technique method was used. According to United States

Preventive Services Task Force criteria, the quality of the evidence and level of recommendation supporting each statement was graded.

## RESULTS

Nine questions were formulated. The available evidence was evaluated considering its quality, number of patients included in the studies and the consistency of its results. The generated questions were answered by the expert panel with a high level of agreement. Thus, a therapeutic algorithm was generated. The role of terlipressin and norepinephrine was confirmed as the pharmacologic treatment of choice. On the other hand the use of the combination of octreotide, midodrine and albumin without vasoconstrictors was discouraged. The role of several other options was also evaluated and the available evidence was explored and discussed. Liver transplantation is considered the definitive treatment for HRS-1. The present consensus is an important effort that intends to organize the available strategies based on the available evidence in the literature, the quality of the evidence and the benefits, adverse effects and availability of the therapeutic tools described.

## CONCLUSION

Based on the available evidence the expert panel was able to discriminate the most appropriate therapeutic alternatives for the treatment of HRS-1.

**Key words:** Hepatorenal syndrome; Delphi; Consensus; Evidence-based medicine; Treatment

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The available evidence for the treatment of type 1 hepatorenal syndrome (HRS-1) was evaluated. The role of terlipressin and norepinephrine was confirmed as the pharmacologic treatment of choice. On the other hand the use of the combination of octreotide, midodrine and albumin without vasoconstrictors was discouraged. The role of several other options was also evaluated and the available evidence was explored and discussed. Liver transplantation is considered the definitive treatment for HRS-1 and the necessary conditions to optimize the recovery of renal function was also discussed.

Arab JP, Claro JC, Arancibia JP, Contreras J, Gómez F, Muñoz C, Nazal L, Roessler E, Wolff R, Arrese M, Benítez C. Therapeutic alternatives for the treatment of type 1 hepatorenal syndrome: A Delphi technique-based consensus. *World J Hepatol* 2016; 8(25): 1075-1086 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i25/1075.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i25.1075>

## INTRODUCTION

Hepatorenal syndrome (HRS) is a severe disease strictly

related to the presence of portal hypertension (PHT). The cumulative probability of HRS in patients with cirrhosis and ascites was initially reported as 18% at one year and 39% at five years<sup>[1]</sup>. More recently and employing the Ascites Club criteria, the incidence of HRS was estimated as 7.1% in a cohort that was followed for  $41 \pm 3$  mo<sup>[2]</sup>. Table 1 depicts the diagnostic criteria proposed by the Ascites Club for the diagnosis of HRS<sup>[3]</sup>. These criteria are the result of a consensus based on clinical knowledge of the condition, and at this time, there are no biochemical tests clinically available for the diagnosis of HRS. Two types of HRS have been defined by the Ascites Club. Type 2 (HRS-2) is a slowly progressive disease, clinically expressed as refractory ascites with an average median survival of approximately six months. In contrast, type 1 HRS (HRS-1) is a catastrophic disease characterized by a rapid decrease in glomerular filtration rate<sup>[3]</sup>, and it is considered the most extreme expression of circulatory dysfunction secondary to PHT. Its diagnosis requires the exclusion of intrinsic renal disease, hypovolemia, shock and exposure to nephrotoxic drugs. It has a very poor prognosis with a mean survival of only two weeks without treatment<sup>[4]</sup>. Thus, HRS-1, is considered one of the most severe complications of cirrhosis and PHT, and it represents a therapeutic challenge for hepatologists.

Several alternatives have been proposed for the treatment of HRS-1. Although liver transplantation (LT) appears to be the logical definitive treatment for this condition, other interventions are needed while the patient is on the waitlist, sometimes for several weeks before LT. Moreover, for a myriad of reasons, some patients cannot be considered appropriate candidates for LT. Hence, LT it is not a proper alternative for every patient, and other therapeutic alternatives must be considered.

Recently, the Chilean Gastroenterology Society provided a consensus for the treatment of HRS-1. The aim of this article is to show the results of the consensus and to suggest a therapeutic approach based on the best available evidence.

## MATERIALS AND METHODS

### Participants and literature search

The consensus organizing committee [under the sponsorship of the Chilean Society of Gastroenterology (<http://sociedadgastro.cl>)] assembled a group of adult gastroenterologists and nephrologists with expertise in the management of advanced liver disease patients and evidence-based medicine. The panel generated a list of questions relevant for the treatment of HRS-1. To address these questions, two members of the panel (JCC and JPA) performed separate searches in PubMed®, retrieving reports published in English or Spanish through June 2014. The search results were distributed. Simultaneously, each individual panelist contributed additional data and abstracts presented at meetings. Each expert was designated to review and answer a question. They generated draft statements for evaluation by all the experts; the answers were to



**Table 1** Criteria for the diagnosis of hepatorenal syndrome

<b>HRS</b>	
Presence of cirrhosis and ascites	
Serum creatinine > 1.5 mg/dL (or 133 micromoles/L)	
No improvement of serum creatinine (decrease equal to or less than 1.5 mg/dL) after at least 48 h of diuretic withdrawal and volume expansion with albumin (recommended dose: 1 g/kg per day up to a maximum of 100 g of albumin/day)	
Absence of shock	
No current or recent treatment with nephrotoxic drugs	
Absence of parenchymal kidney disease as indicated by proteinuria > 500 mg/d, microhematuria (> 50 RBCs/high power field, and/or abnormal renal ultrasound scanning)	
<b>HRS-1</b>	
Rapidly progressive renal failure defined by a doubling of the initial serum creatinine to a level greater than 2.5 mg/dL or 220 $\mu$ mol/L in less than 2 wk	
Although it may appear spontaneously, HRS-1 often develops with a precipitating event, particularly spontaneous bacterial peritonitis	
HRS-1 occurs in the setting of an acute deterioration of circulatory function (arterial hypotension and activation of the endogenous vasoconstrictor systems) and is frequently associated to rapid impairment in liver function and encephalopathy	
<b>HRS-2</b>	
Characterized by a moderate renal failure (serum creatinine greater than 1.5 mg/dL) which follows a steady or slowly progressive course. It appears spontaneously in most cases	
HRS-2 is frequently associated with refractory ascites. Survival of patients with HRS-2 is shorter than that of patients with ascites but without renal failure	

HRS: Hepatorenal syndrome; HRS-2: Type-2 HRS; HRS-1: Type-1 HRS.

**Table 2** Levels of evidence according to the study design

Level of evidence	Description
Type I	Evidence obtained at least from one well-designed, randomized, controlled <sup>1</sup> trial or from a systematic review of randomized clinical studies
Type II	II -1 evidence obtained from non-randomized, prospective, controlled <sup>1</sup> studies II -2 evidence obtained from cohort observational studies <sup>2</sup> or case-control studies, preferably multi-centric II -3 evidence obtained from case series
Type III	Opinion of authorities on the subject matter based on expertise, expert committees, case reports, pathophysiological studies or basic science studies

<sup>1</sup>A controlled study is a study where the intervention is managed by the researcher; <sup>2</sup>An observational study is a study where the intervention is not controlled by the researcher.

be supported through a review of the literature. The quality of the evidence (Table 2) and the level of recommendation (Table 3) were graded following the United States Preventive Services Task Force criteria<sup>[5,6]</sup>.

### Consensus methodology

Initially, each expert wrote a draft recommendation statement and sent to organizing committee for evaluation and distribution among the entire panel. A 1 to 5 Likert scale (where 1 means "totally disagree" and 5 "totally agree") was used to measure agreement. To reach a final consensus a modified three-round Delphi technique method was used as described by Arab *et al.*<sup>[7]</sup>.

The final statements and recommendations were exposed during the XLI Chilean Congress of Gastroenterology and the I Chilean Symposium on HRS-1 treatment in Coquimbo, Chile, in November 2014. The audience of approximately 450 physicians voted in real-time. The approved final recommendations (those with average scores  $\geq 4$  on the Likert scale) are presented below.

### Statistical analysis

Statistical review of the study was performed by a

biomedical statistician. Level of agreement from the Delphi panel was expressed in mean  $\pm$  SD.

## RESULTS

### Are vasoconstrictors effective in the treatment of HRS-1?

**Terlipressin:** Terlipressin is the vasoconstrictor of choice for the treatment of HRS-1, due to the large number of studies (and enrolled subjects) showing its effectiveness and its positive effects on survival. Terlipressin is a synthetic analogue of vasopressin acting through V1 receptors, increasing effective circulating volume, and by means of an increase in resistance in the splanchnic territory (which reduces portal pressure), it allows for the redistribution of the bloodstream, increasing renal perfusion<sup>[8]</sup>. Two important controlled, randomized and multicenter trials showed that terlipressin associated with albumin resulted in an improvement in renal function and could also reverse HRS-1<sup>[9,10]</sup>. A recently published meta-analysis, which included 320 subjects, proved 50% effectiveness with an OR of 7.5<sup>[11]</sup>.

An Italian trial reported on the impact of terlipressin on the survival rate of HRS-1 patients. This randomized

**Table 3** Levels of recommendation according to the available evidence

Recommendation	Description
A	The consensus strongly recommends the mentioned intervention or service. This recommendation is based on high quality evidence, with a benefit that significantly exceeds the risks
B	The consensus recommends the regular clinical use of the mentioned intervention or service. This recommendation is based on moderate quality evidence, with a benefit that exceeds the risks
C	The consensus does not make any positive or negative recommendation regarding the mentioned intervention or service. A categorical recommendation is not provided, because the evidence (of at least moderate quality) does not show a satisfactory risk/benefit relationship. The decision has to be made on a case-by-case basis
D	The consensus makes a negative recommendation against the mentioned intervention or service. The recommendation is based on at least moderate quality evidence, not showing any benefit or where the risk or damage exceeds the benefits of the intervention
I	The consensus concludes that the evidence is insufficient, due to low-quality studies, heterogeneous results or because the risk/benefit balance cannot be determined

trial included 52 subjects and showed a higher and more significant probability of survival in the group treated with terlipressin<sup>[12]</sup>. These findings were confirmed in a recent meta-analysis published by Cochrane, including 6 trials and 309 subjects, in which a statistically significant reduction in the mortality rate was observed in HRS-1 patients treated with terlipressin (RR = 0.76, 95%CI: 0.61-0.95)<sup>[13]</sup>.

For the treatment of HRS-1, it is recommended to start the administration of terlipressin at an initial dose of 0.5-1 mg every 4-6 h as an IV bolus, with the possibility of increasing the dose up to 2 mg every 4-6 h if there is no proper response after 3 d; a proper response is defined as a reduction > 25% from basal plasma creatinine. It is recommended to maintain the administration of terlipressin until creatinine levels decrease to less than 1.5 mg/dL or for a maximum of 14 d<sup>[14]</sup>. Recurrence can occur after discontinuation of the therapy (< 20%)<sup>[15]</sup>, in which case, the recommendation is to repeat a new cycle. The most frequent side effects are abdominal pain, diarrhea, arrhythmia and ischemic complications. The incidence of serious effects requiring suspension of terlipressin is close to 7%<sup>[16]</sup>. The presence of coronary, vascular or peripheral arterial ischemic disease must be considered a contraindication for the use of terlipressin and other systemic vasoconstrictors.

**Recommendation:** Treatment with terlipressin associated with albumin represents the drug therapy of choice in HRS-1 patients, and it is capable of reversing this condition and reducing the associated mortality rate (Evidence Level I, grade of recommendation A, Agreement 5 ± 0).

**Norepinephrine:** Norepinephrine, an adrenergic agonist widely available in critical care units, is regarded as an alternative therapy in association with albumin, and it is effective and safe for the treatment of HRS-1<sup>[17]</sup>. Two randomized trials compared norepinephrine to terlipressin, reporting similar efficacy levels for reversal of HRS-1, as well as a comparable safety profile<sup>[18,19]</sup>. A recent study conducted in India enrolled 46 HRS-1 patients and reported results that confirmed previously published results<sup>[20]</sup>, in addition to a recently published meta-analysis<sup>[21]</sup>. The effects of both drugs on mortality

rates after 30 d and the probability of HRS-1 recurrence were also similar. In this meta-analysis, adverse effects were less common with the use of norepinephrine; however, only four studies were considered<sup>[21]</sup>.

Norepinephrine is used as a continuous intravenous infusion at a 0.5 mg/h initial dose, with the purpose of achieving a > 10 mmHg increase in basal mean blood pressure (MBP). Accordingly, the dose can be adjusted by 0.5 mg/h every 4 h until a maximum dose of 3 mg/h is attained<sup>[20]</sup>.

The significantly reduced cost and broad availability of norepinephrine are attractive<sup>[18,20]</sup>. It must be considered, however, that the number of cases treated with norepinephrine remains low, compared to the number of cases treated with terlipressin.

**Recommendation:** The use of norepinephrine associated with albumin represents an alternative to the use of terlipressin for the treatment of HRS-1; however, the currently available information is not as abundant in comparison to terlipressin (Evidence Level I, grade of recommendation B, Agreement 5 ± 0).

**Octreotide plus midodrine:** The most studied vasoconstrictor used for the treatment of HRS-1 is terlipressin, and it results in complete reversal of the disease and a reduction in the associated mortality rate. However, many studies have assessed the use of other vasoconstrictors, with or without volume expansion agents, with variable results<sup>[22]</sup>. The association of midodrine, a systemic vasoconstrictor acting on alpha-adrenergic receptors, and octeotride, a synthetic analogue of vasopressin that inhibits the release of endogenous vasodilators, has shown a benefit to mortality rates in some small studies<sup>[23,24]</sup>. Regarding reversal, some studies have shown complete response with reduction in creatinine to values less than 1.5 mg/dL; nevertheless, other studies have shown contradictory results<sup>[25]</sup>. These two points were assessed in a meta-analysis published in 2012<sup>[26]</sup> that included 256 subjects from 3 separate observational studies. This meta-analysis showed a reduction in mortality rates at 30 d (OR = 0.33; 95%CI: 0.18-0.60) and 90 d (OR = 0.17; 95%CI: 0.03-0.96) but no conclusive results in terms of HRS-1 reversal; however, a delay in progression was observed, based on

a reduction in creatinine levels that was not statistically significant. It is important to note that, in the cases of the control groups in these three studies, two studies used dopamine, and one study used albumin. This reduction in the progression of renal function would be the mechanism believed to reduce the mortality rate, even without achieving HRS-1 reversal.

A randomized, monocentric trial including 23 subjects compared the association of midodrine and octeotride with noradrenaline<sup>[27]</sup>, with complete response in 73% and 75% of subjects, respectively, without significant differences between the two therapeutic options. However, the small number of subjects included in this study limited the interpretation of the results. In contrast, a recent prospective, randomized trial compared the use of terlipressin plus albumin (27 subjects) with the combination of midodrine/octreotide plus albumin (22 subjects). This study showed a 70.4% response rate in the terlipressin branch vs a 28.6% response rate in the midodrine/octreotide branch. Moreover, the complete response rate was significantly higher in the terlipressin branch [55.5% vs 4.8% in the midodrine/octreotide group ( $P < 0.001$ )], showing low efficacy in terms of complete response in the midodrine/octreotide group<sup>[28]</sup>. These results were consistent with the low reversal rate described in previous studies.

Some studies have suggested that an increase in MBP is necessary for reverting alterations in renal hemodynamics specific to HRS-1; this increase is greater in patients responding to vasoconstrictor treatment compared with non-responding patients, regardless of the vasoconstrictor used. In a joint analysis of 501 patients from 21 studies, Velez *et al.*<sup>[29]</sup> proved a significant correlation between an increase of 10 to 15 mm in MBP and the HRS-1 patient's response to treatment, with improvement in renal function. Other studies have not been able to prove this association. The main limitation of the study by Velez *et al.*<sup>[29]</sup> is that it gathered information from previous studies that were not designed to assess the measured result. Therefore, their study cannot be regarded as having sufficient evidence for issuing a recommendation.

With regard to the safety and efficacy of the use of midodrine and octeotride, these data were assessed in a retrospective study<sup>[30]</sup> including 60 HRS-1 patients, compared to 21 patients treated only with albumin. Midodrine treatment combined with octeotride was not associated with significant adverse effects.

**Recommendation:** Although the midodrine-octeotride combination is a safe treatment with easy administration, its beneficial effects on survival and improvement in renal function have not been consistent across trials. Therefore, we do not recommend its use for the treatment of HRS-1 (Evidence Level I, grade of recommendation B, Agreement  $4.6 \pm 0.5$ ).

**Vasopressin:** Vasopressin has been proposed as a vasoconstrictor for the treatment of HRS-1 in some

countries where no other therapies are available.

A retrospective study<sup>[31]</sup> compared the use of vasopressin alone and in combination with octeotride in HRS-1 patients vs the use of octeotride. This study showed a reduction in creatinine to values  $< 1.5$  mg/dL with the use of vasopressin with or without octeotride vs octeotride alone (42% vs 38% vs 0%, respectively,  $P = 0.001$ ), with an OR of 6.4 as well as an improvement in the survival rate and the possibility of being candidate for LT.

The dose required for achieving this objective has not yet been established. The aforementioned study required a dose of  $0.23 + 0.19$  U/min for a period of 5 to 9 d. In contrast, the use of low doses of vasopressin (1 U/h)<sup>[32]</sup> was effective for the restoration of urine volume in HRS-1 patients and patients with congestive heart failure, without improving the overall prognosis of the patients or their creatinine levels.

The use of vasopressin requires strict monitoring to avoid adverse effects associated with ischemic phenomena.

**Recommendation:** We do not recommend the use of vasopressin for the treatment of HRS-1, due to several adverse effects and the lack of randomized, clinical trials supporting its use (Evidence Level II -2, grade of recommendation I, Agreement  $4.8 \pm 0.3$ ).

### **Efficacy of the use of albumin**

The use of human albumin in cirrhosis is based mainly on its hemodynamic properties, improving oncotic pressure in patients with circulatory disorders, and it is characterized by dilatation of the splanchnic territory, effective hypovolemia and activation of the renin-angiotensin-aldosterone system. In addition, albumin has antioxidant and immunomodulatory functions; albumin also has the capacity to transport and metabolize other substances and has hemostatic and endothelial stabilization effects. In cirrhotic patients, both plasma albumin concentration and its functional properties are diminished, becoming even more severe depending on the level of the patient's renal failure<sup>[33]</sup>.

The aforementioned situation has been the rationale for the use of albumin in decompensated cirrhosis. The main evidence in favor of its use is in the prevention of renal failure, both in the presence of spontaneous bacterial peritonitis and after a large-volume paracentesis.

In HRS-1, circulatory disorders are established at its highest levels, and by definition, these disorders are not reversed by the administration of albumin alone, the use of which is commonly suggested to expand intravascular volume and to improve cases of prerenal failure, thus disregarding the HRS-1 diagnosis in these patients<sup>[3]</sup>.

Vasoconstrictors are the basis of HRS-1 treatment, and in the majority of well-designed, prospective and randomized studies, they have been associated with the use of albumin as a plasma expander and compared to the isolated use of albumin<sup>[9,12]</sup>. In these studies, there has been evidence of a significant difference in favor of combined therapy regarding HRS-1 reversal,

improvement of renal function, MBP and diuresis.

The purpose of a meta-analysis performed by Dobre *et al.*<sup>[11]</sup> was to prove the usefulness of terlipressin with or without albumin, compared with placebo with or without albumin. The study showed that HRS-1 reversal using the first alternative was significantly more common, with an OR of 7.47. Of the six studies included in the meta-analysis, only 1 did not use albumin, and it was the oldest (1998) and had the smallest number of subjects<sup>[11]</sup>, proving that the majority of researchers have considered albumin to be a part of the basic treatment for HRS-1 patients.

Regarding the assumption that albumin provides an additional benefit to the use of vasoconstrictors alone, we only found evidence of an observational study designed to answer this question. Seventy-seven percent of the patients who used terlipressin and albumin experienced a resolution of HRS-1, compared to 25% in the group that used terlipressin alone. In addition, the group of patients treated with terlipressin plus albumin showed significant improvement in MBP and a reduction in the activation of the renin-aldosterone system<sup>[34]</sup>.

Along these same lines, the retrospective study by Moreau *et al.*<sup>[35]</sup> assessing the usefulness of terlipressin in HRS-1 showed that the respondent group used albumin in 79% of cases vs 68% in the non-respondent group, which was not a significant difference.

There is no evidence available that other fluids (such as crystalloids) can have a similar effect to albumin associated with vasoconstrictors. No studies have been designed for this purpose, and no such studies are likely to be performed because the use of crystalloids increases ascites and, therefore, intra-abdominal pressure, which in turn affects renal perfusion and reduces the likelihood of improvement of renal and circulatory failure. An observational study, in which a large-volume paracentesis with reposition of albumin was performed in HRS-1 patients, showed partial improvement of renal function, supporting the hypothesis that a reduction in intra-abdominal pressure could be useful for renal perfusion recovery<sup>[36]</sup>.

**Recommendation:** The use of albumin with vasoconstrictors is the therapy of choice for treating HRS-1. The use of albumin without vasoconstrictors is only recommended in the stage prior to HRS-1 diagnosis to exclude patients with prerenal failure (Evidence Level II-2, grade of recommendation B, Agreement  $4.8 \pm 0.3$ ).

### **Efficacy of the use of a trans-jugular intrahepatic portosystemic shunt**

There is scarce evidence for the use of trans-jugular intrahepatic portosystemic shunt (TIPS) in HRS-1. In a study including 16 patients, 6 with HRS-1 and 10 with HRS-2 and Child-Pugh scores of 7-9, a duplication of creatinine clearance in serum and increased sodium concentration excreted in the urine were observed two weeks after the TIPS procedure. Three of the HRS-1 patients required hemodialysis during the progression of

the disease, and 12 and 18 d after the TIPS procedure, two patients were able to stop hemodialysis. There was an improvement in renal function, even after 6-8 wk. Three of the 16 HRS-1 and HRS-2 patients did not respond, and they died within a 6-wk period<sup>[37]</sup>. A prospective, non-randomized, phase II study included 41 patients with cirrhosis and HRS without indications for transplantation: 21 with HRS-1 and 20 with HRS-2. Thirty-one of these patients (14 type 1 and 17 type 2) received TIPS and were followed for a mean of 24 mo. The use of TIPS in HRS-1 and HRS-2 patients reduced significantly ( $P < 0.001$ ) the hepatic venous pressure gradient and increased creatinine clearance and sodium excretion. Those patients who received TIPS showed higher survival rates than those who did not. There was only one death related to the procedure (3.2%). It is important to note that the HRS-2 patients had a significantly greater benefit and were identified as a variable independently correlated with survival<sup>[38]</sup>. Another uncontrolled study assessed 7 patients with cirrhosis and HRS-1. The TIPS procedure was associated with gradual improvements in the glomerular filtration rate (9 to 27 mL/min) and blood urea nitrogen and creatinine reduction. The majority of patients also showed a reduction in the activity of the renin-angiotensin system and in the sympathetic nervous system, suggesting an improvement in hemodynamic parameters. The average survival after the TIPS procedure was approximately 5 mo, which was longer than the survival rate expected for these patients<sup>[39]</sup>.

Unfortunately, many HRS-1 patients are too sick to undergo a TIPS procedure, mainly because the procedure can present complications such as deterioration of hepatic encephalopathy and liver function (increased bilirubin levels), bleeding and intravenous contrast-induced nephropathy<sup>[40]</sup>. In a study that designed a predictive model for determining the survival rate after a TIPS procedure, patients with HRS-1 due to alcoholic cirrhosis or chronic cholestatic disease showed a 25% mortality rate 90 d after the procedure and a mortality rate of 80% in patients with cirrhosis due to other causes<sup>[41]</sup>.

In general, these results suggest that TIPS could be considered for patients with relatively preserved liver function and as a bridge therapy to LT. However, due to the risks associated with the procedure and the lack of well-designed studies, this procedure should be considered only as a last resource.

**Recommendation:** Due to the lack of evidence, the consensus considers that the use of TIPS shall not be recommended in HRS-1 patients (Evidence Level II-2, grade of recommendation I, Agreement  $4.7 \pm 0.4$ ).

### **Extracorporeal substitution therapies**

#### **Role of renal replacement therapies for the management of acute renal failure associated with HRS-1:**

If there is no response to proven pharmacological strategies, acute renal failure (ARF) in HRS-1 takes an irreversible course unless the patient undergoes LT. At this point, patients develop oliguria, hydrosaline balance



disorders and severe metabolic disorders that can lead to the prescription of renal replacement therapies (RRTs).

For patients who will not undergo LT, the potential benefit of RRT is controversial due to the high morbidity-mortality associated with RRT, basically determined by poor hemodynamic tolerance and/or hemorrhages associated with liver failure complications<sup>[42]</sup>.

For patients who are non-respondent to pharmacological therapy or TIPS, who are waiting for a LT or who are under evaluation to undergo the surgery, the use of RRT is advised as a bridge to LT. In a retrospective study developed by Keller *et al.*<sup>[43]</sup>, the survival rate of patients with HRS-1 was 44% in the group that received RRT vs 10% in the group that did not receive the therapy. However, this higher survival rate could be related to reduced RRT tolerance, which could increase the number of hospitalizations. In a report of 4 patients who received hemodialysis while waiting for LT, Capling *et al.*<sup>[44]</sup> observed an average survival of 236 d (31 to 460 d). All of the patients survived the initial event and were discharged, but 33% of the days gained were then spent in hospitalization due to intercurrent diseases. The most common cause of hospitalization was hepatic encephalopathy; the authors believe that avoiding lactulose during the days when the patient undergoes dialysis, to prevent diarrhea events, might have been a contributing factor<sup>[44]</sup>.

Efficacy, safety and the best RRT modality in HRS-1 patients have not been systematically assessed. Potential advantages of continuous vs intermittent RRT include slower removal of fluids with higher hemodynamic stability and slower control of solute concentrations, which is why many clinicians prefer continuous RRT in patients with hemodynamic instability and in patients with evidence of cerebral edema<sup>[45]</sup>. In two studies, Davenport and Detry proved that continuous RRT was better tolerated than intermittent hemodialysis in patients with liver failure, evidenced by greater cardiovascular stability, gradual correction of hyponatremia and less variation in intracranial pressure<sup>[46,47]</sup>.

**Recommendation:** We recommend the initiation of RRT in patients with HRS-1 refractory to pharmacological therapy who are candidates for LT. In patients with hemodynamic instability and/or evidence of cerebral edema, we recommend the use of other continuous RRTs; We recommend maintaining RRT in patients with HRS-1 who are candidates for LT and who must be temporarily removed from the waiting list because they have developed an intercurrent disease (Evidence Level II-3, grade of recommendation C, Agreement 4.6 ± 0.5).

**Extracorporeal liver support with albumin dialysis (molecular adsorbent recirculating system):** The molecular adsorbent recirculating system (MARS) is an extracorporeal liver support that, by means of recirculating albumin dialysis, helps to remove water-soluble substances, as well as protein-bound substances. The removal function has been shown to reduce

bilirubin, ammonium, urea nitrogen, creatinine, fatty acids and bile salts, which are all substances that, in high concentrations, are related to liver and renal failure. As expected, the effect is temporary, and if there is no improvement in liver function, these parameters change again in the short term<sup>[48,49]</sup>. This system does not improve hepatic synthesis; it is used as a depuration system, comparable to renal hemodialysis. In contrast, some small studies have shown an improvement in the hemodynamics of these patients, with increased blood pressure that had been reduced due to the hyperdynamic circulation characteristic of liver failure. The mechanism of the aforementioned benefit would be depuration and the reduction of substances such as renin, angiotensin and aldosterone, which are responsible for the hemodynamic disorders related to liver failure<sup>[48]</sup>. A recent retrospective, uncontrolled study showed that, of 32 HRS-1 patients receiving MARS therapy for an average of 3.5 ± 1.5 sessions, 13 (40%) experienced an improvement in renal function, but only 9 (28%) showed complete response in the form of renal function recovery. In contrast, of the 15 patients who survived > 28 d, only 9 achieved this stage without transplantation, and of these 9 patients, only 2 showed complete renal response using MARS therapy<sup>[50]</sup>. Therefore, it has been suggested that MARS therapy is capable of improving HRS-1 through the removal of vasodilators; however, this effect would more probably be caused by MARS's hemofiltration function, which is an effect that is similar to conventional hemodiafiltration.

As mentioned, non-treated HRS-1 patients show high mortality rates in the short term. The effect of MARS therapy in this stage is controversial. There have been three studies that have not shown any benefit in terms of survival<sup>[51-53]</sup>, and another two studies, both from the same author, did not show benefits<sup>[49,54]</sup>.

Finally, a recent (2013) multi-center, randomized study<sup>[51]</sup> included patients with acute or chronic liver failure and randomized a total of 189 patients to a group with standard medical therapy plus MARS therapy (95 patients) vs another group using only standard medical therapy (94 patients); this study observed no benefit in survival at 28 d in the MARS group. A sub-analysis of the HRS-1 patients that were included (48 in the MARS group and 47 in the control group) also did not show differences in the survival rates.

**Recommendation:** We do not recommend the use of extracorporeal liver support with albumin dialysis (MARS) for the treatment of HRS-1 (Evidence Level I, grade of recommendation D, Agreement 4.8 ± 0.3).

#### **Efficacy of LT: Survival and renal function**

LT is the therapy of choice for HRS-1 patients because it not only improves renal failure but also improves the underlying diseases, *i.e.*, cirrhosis and PHT. Post-transplantation survival in HRS-1 patients seems to be lower than for transplanted patients without HRS-1; however, survival is considerably higher compared with

that in HRS-1 patients without transplantation. In a retrospective study, the survival of HRS-1 transplanted patients after 1 and 3 years was 80.3% and 76.6%, respectively, and it was 90.7% and 85.3%, respectively, for recipients without HRS-1<sup>[55]</sup>.

Although we can consider that treating HRS-1 with vasoconstrictor agents can improve post-transplant results by improving renal function before the procedure, there is no clear evidence of this effect. In a clinical study, 99 patients were randomized to receive terlipressin or placebo. Of these patients, 35% received LT. Subjects receiving albumin plus terlipressin showed a 100% survival rate among transplanted patients and a 34% survival in non-transplanted patients after 6 mo. In contrast, subjects receiving only albumin showed 94% survival in transplanted patients and only 17% survival in non-transplanted patients after 6 mo. The authors concluded that the use of terlipressin has no impact on post-transplant survival. The sole benefit of the use of terlipressin in patients who will undergo transplantation seems to be the facilitation of the use of calcineurin inhibitors post-transplant, reducing the need for anti-IL2 antibodies<sup>[56]</sup>.

The majority of HRS-1 patients experience an improvement in renal function post-transplant; therefore, there seems to be no advantage in performing double liver and renal transplantation vs single LT. In a Chinese observational study, 32 HRS-1 patients received transplantation, and of these patients, 8 received dialysis, showing that 94% of the patients recovered renal function in an average of 24 d, with 65% survival after 1 year<sup>[57]</sup>. In another observational study with 28 patients, with an average MELD score of  $30 \pm 6$ , only 58% of patients recovered renal function. Four patients died, of whom 3 showed resolution of HRS-1<sup>[58]</sup>. However, patients that did not experience any improvement in renal function post-transplant showed poorer survival rates<sup>[59]</sup>.

In a recent retrospective study, 62 HRS-1 patients received transplantation, with an average basal creatinine of 3.35 mg/dL and an average MELD score of  $35 \pm 1$ . The progression time of HRS-1 before transplantation was 18 d. Eleven patients continued dialysis after the surgery, and 5 patients died. Survival after 1 year in the patients who recovered renal function was 97% vs 60% in the group that did not show any improvement in renal function. After one year, the creatinine levels in the group with HRS-1 resolution were similar to the creatinine levels in the group of transplanted patients without HRS-1. The only factor associated with the non-resolution of HRS-1 after transplantation was the period of time on dialysis pre-transplantation. For each day of dialysis, the patient has a 6% increase in the risk of non-resolution of HRS-1. A patient who is on dialysis for more than 14 d has a 9.2 times greater relative risk of non-resolution of HRS-1<sup>[60]</sup>.

Despite these findings, apart from the duration of dialysis time before transplantation, predictive factors for the improvement of renal function after transplantation have not been clearly established. Patients with ARF

requiring dialysis more than two times per week for more than 4 wk must be assessed for double liver and renal transplantation, considering the risk factors at the time of the surgery, such as hypertension, diabetes and older age<sup>[60,61]</sup>.

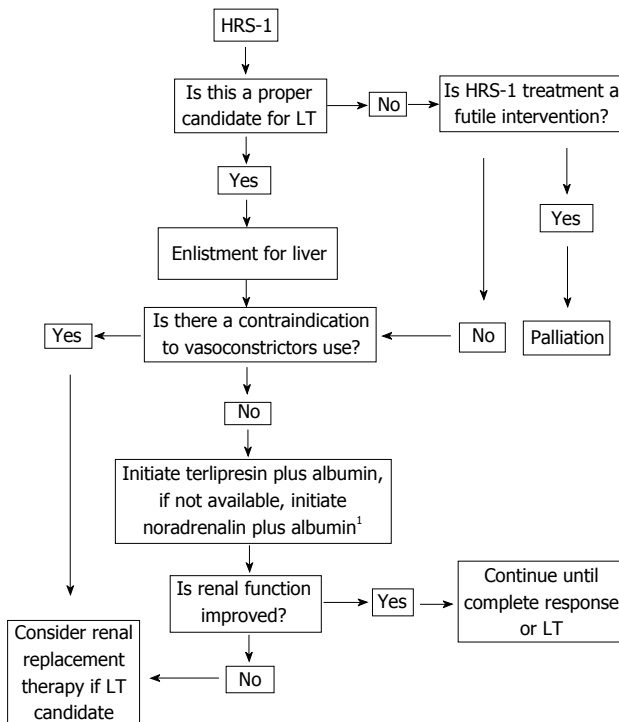
**Recommendation:** LT can be considered the definitive treatment for HRS-1 patients. HRS-1 patients must receive treatment with vasopressors before LT because it could improve the subsequent results. Patients requiring dialysis for long periods of time ( $> 4$  wk) must be considered for combined liver-kidney transplantation (Evidence Level II-2, grade of recommendation B, Agreement  $4.6 \pm 0.5$ ).

## DISCUSSION

LT is considered the treatment of choice for HRS-1<sup>[62]</sup>. It is the only therapy able to reverse this condition completely, resolving circulatory dysfunction and the consequences of cirrhosis and liver failure<sup>[63]</sup>. Thus, survival can be dramatically improved after LT<sup>[55]</sup>. In fact, the 180 d survival rate was 97% in a recent study<sup>[56]</sup>. In another recent study, the one- and three-year survival rates were 80.3% and 76.6%, respectively<sup>[55]</sup>. Interestingly, the impact of pharmacologic treatment on the outcomes after LT was recently evaluated. In the study by Boyer *et al.*<sup>[56]</sup>, the use of terlipressin plus albumin had no impact on post-transplant survival. However, this is not an argument for neglecting the importance of the treatment of HRS-1, especially considering that the time elapsed between enlistment and transplantation could be weeks. In this regard, an effort to recover renal function before LT is advisable. Although it seems to be the most frequent scenario, not every patient recovers renal function after LT. In a very recent study, Wong *et al.*<sup>[64]</sup> evaluated the survival of liver recipients who experienced reversal of HRS-1 after LT, compared to the survival of patients who did not. In this study, 75.8% of the recipients had a reversal of HRS-1 after LT. The one-year survival rate after LT was 97% for patients who had a reversal of HRS-1 and 60% for those who did not<sup>[64]</sup>. Thus, for all of the aforementioned reasons, LT is a desirable approach for HRS-1 patients.

Nonetheless, there are at least two obstacles that render access to LT difficult: (1) the scarcity of liver grafts, which dramatically reduces the likelihood of these patients receiving a transplant as rapidly as they should; and (2) the existence of severe comorbidities or conditions that make LT not plausible. Hence, there is an important role for different therapeutic approaches than could be used as alternatives or "bridges" to LT.

Thus, several therapeutic tools have been evaluated. As expected, the quality of these studies, the efficacy of the interventions, and their availability, costs and adverse effects are, of course, very different. It was the purpose of this panel of experts to determine the treatments with greater efficacy, based on studies with the highest quality available.



**Figure 1 Therapeutic algorithm for the treatment of type 1 hepatorenal syndrome.** <sup>1</sup>Doses must be adjusted according to diuresis or creatinine levels. HRS-1: Type 1 hepatorenal syndrome; LT: Liver transplantation.

Based on the available evidence, the expert panel agrees that the best evidence for the treatment of HRS-1 supports the use of vasoconstrictors as a treatment of the choice, specifically terlipressin, based on a recent systematic review<sup>[13]</sup>. On the other hand, noradrenalin seems to be as effective as terlipressin. In fact, a recent systematic review evaluated the efficacy of noradrenalin compared to terlipressin. Only four studies were included (154 randomized patients). The authors report a similar rate of reversal of HRS-1, 30 d mortality and recurrence. Thus, in this study, its effect on renal function seemed to be completely comparable to that of terlipressin<sup>[21]</sup>, and its use seems to be adequate when terlipressin is not available. Nonetheless, these findings are more difficult to interpret because two studies included patients with HRS-2. In this regard, the expert panel recommends the use of noradrenalin as a second choice if terlipressin is not available. Another strategy based on the use of vasoconstrictors is the combination of octreotide plus midodrine (also in combination with albumin). Very interestingly, a recent study compared the use of terlipressin plus albumin with the combination of octreotide, midodrine and albumin. Notably, the rate of complete response was 55.5% in the terlipressin group and 4.8% in the octreotide-midodrine group ( $P < 0.001$ )<sup>[28]</sup>. Based on these results, the panel of experts did not recommend the use of octreotide plus midodrine for the treatment of HRS-1.

The use of vasopressin was not recommended by the expert panel due to the scarcity and poor quality of the evidence, in addition to the incidence of ischemic side effects<sup>[31]</sup>.

Another issue evaluated by the panel was the use of albumin as a plasma expander. Most of the studies that evaluated the use of vasoconstrictors combined them with albumin. However, Ortega *et al.*<sup>[34]</sup> conducted a prospective, non-randomized study that compared the use of terlipressin with and without albumin. A complete response was observed in 77% of patients receiving albumin and in 25% of those who did not receive albumin. In contrast, there is a lack of evidence suggesting that the apparent benefit of using albumin combined with vasoconstrictors cannot be substituted for another colloid or crystalloids. However, considering that the benefit of vasoconstrictors has been proved in combination with albumin, this panel decided to recommend its use every time that vasoconstrictors are indicated.

The use of a TIPS has been tested in only a few patients; however, there have been no randomized, controlled studies, and it has not been compared to the use of vasoconstrictors. In contrast, the associated adverse effects, mostly hepatic encephalopathy, have made the use of the TIPS procedure difficult. For these reasons, the expert panel does not recommend its use.

The MARS has also been tested as an alternative for the treatment of HRS-1. However, its benefits have not been consistently demonstrated. In fact, in a recent randomized, controlled trial, MARS was employed for patients with acute or chronic liver disease, including 95 patients with HRS-1. No benefit on survival was demonstrated<sup>[51]</sup>. Hence, the panel of experts does not recommend its use.

RRT use is considered controversial in cirrhotic patients with HRS-1 when LT is not considered an option because of the morbidity and mortality associated with the procedure and with liver failure<sup>[42]</sup>. Although the literature is scarce in this topic, RRT seems to prolong short term survival<sup>[43]</sup>, potentially improving the probability of receiving a liver graft. Thus, although disputable, the expert panel decided to recommend the use of RRT only in those patients listed for LT.

The present consensus is an important effort that intends to organize the available strategies based on the available evidence in the literature, the quality of the evidence and the benefits, adverse effects and availability of the therapeutic tools described. This attempt has been synthesized in the algorithm described in Figure 1. We hope that it will be a useful tool for guiding the management of HRS-1 patients.

## COMMENTS

### Background

Hepatorenal syndrome (HRS) is a severe condition strictly related to the presence of portal hypertension and ascites. It has a very poor prognosis with a mean survival that only reaches two weeks. Several therapeutics alternatives have been evaluated. Based on the best available evidence the authors attempt to define the best therapeutic choice considering its availability and the clinical characteristics of each patient.

### Research frontiers

Although several therapeutic alternatives have been proposed the quality of the

evidence is heterogeneous and it can be difficult to discriminate the best option on each circumstance. A careful evaluation of the evidence is necessary to make appropriate recommendations.

### Innovations and breakthroughs

This is the first published consensus about the treatment of HRS. A significant effort has been made to evaluate the quality of the available literature to generate appropriate recommendations.

### Applications

The information included on this consensus will be a valuable tool to determine the best therapeutic option for HRS based on the best available evidence, the availability of each intervention and the particular condition of the patient.

### Terminology

Delphi-technique method was used to reach consensus. A panel of experts voted on a 1 to 5 Likert scale (where 1 means "totally disagree" and 5 "totally agree"). Approved recommendations (those with average score  $\geq 4$  on the Likert scale) are presented; Level of evidence: The quality of the evidence is classified in three types. Thus, type I is obtained from well design, randomized trials or systematic reviews. Type II is obtained from studies of lower quality (*i.e.*, non-randomized trials, case-control studies or case series). Type III is obtained from opinion of authorities on the subject matter based on expertise, expert committees, case reports, pathophysiological studies or basic science studies; Levels of recommendation are classified in five categories (A, B, C, D, I) where A corresponds to the stronger recommendation to support a determined intervention and I classifies situations where the evidence is insufficient to generate a recommendation (see Table 3 on the manuscript).

### Peer-review

The study is well organized and properly processed. The conclusion is reasonable.

## REFERENCES

- Ginès A, Escorsell A, Ginès P, Saló J, Jiménez W, Inglada L, Navasa M, Clària J, Rimola A, Arroyo V. Incidence, predictive factors, and prognosis of the hepatorenal syndrome in cirrhosis with ascites. *Gastroenterology* 1993; **105**: 229-236 [PMID: 8514039 DOI: 10.1016/0016-5085(93)90031-7]
- Montoliu S, Ballesté B, Planas R, Alvarez MA, Rivera M, Miquel M, Masnou H, Cirera I, Morillas RM, Coll S, Sala M, García-Retortillo M, Cañete N, Solà R. Incidence and prognosis of different types of functional renal failure in cirrhotic patients with ascites. *Clin Gastroenterol Hepatol* 2010; **8**: 616-622; quiz e80 [PMID: 20399905 DOI: 10.1016/j.cgh.2010.03.029]
- Salerno F, Gerbes A, Ginès P, Wong F, Arroyo V. Diagnosis, prevention and treatment of hepatorenal syndrome in cirrhosis. *Gut* 2007; **56**: 1310-1318 [PMID: 17389705]
- Alessandria C, Ozdogan O, Guevara M, Restuccia T, Jiménez W, Arroyo V, Rodés J, Ginès P. MELD score and clinical type predict prognosis in hepatorenal syndrome: relevance to liver transplantation. *Hepatology* 2005; **41**: 1282-1289 [PMID: 15834937 DOI: 10.1002/hep.20687]
- Harris RP, Helfand M, Woolf SH, Lohr KN, Mulrow CD, Teutsch SM, Atkins D. Current methods of the US Preventive Services Task Force: a review of the process. *Am J Prev Med* 2001; **20**: 21-35 [PMID: 11306229 DOI: 10.1016/S0749-3797(01)00261-6]
- Sawaya GF, Guirguis-Blake J, LeFevre M, Harris R, Petitti D. Update on the methods of the U.S. Preventive Services Task Force: estimating certainty and magnitude of net benefit. *Ann Intern Med* 2007; **147**: 871-875 [PMID: 18087058 DOI: 10.7326/0003-4819-147-12-200712180-00007]
- Arab JP, Candia R, Zapata R, Muñoz C, Arancibia JP, Poniachik J, Soza A, Fuster F, Brahm J, Sanhueza E, Contreras J, Cuellar MC, Arrese M, Riquelme A. Management of nonalcoholic fatty liver disease: an evidence-based clinical practice review. *World J Gastroenterol* 2014; **20**: 12182-12201 [PMID: 25232252 DOI: 10.3748/wjg.v20.i34.12182]
- Mazur JE, Cooper TB, Dasta JF. Terlipressin in hepatorenal syndrome. *Ann Pharmacother* 2011; **45**: 380-387 [PMID: 21386023 DOI: 10.1345/aph.1P195]
- Martín-Llahí M, Pépin MN, Guevara M, Díaz F, Torre A, Monescillo A, Soriano G, Terra C, Fábrega E, Arroyo V, Rodés J, Ginès P. Terlipressin and albumin vs albumin in patients with cirrhosis and hepatorenal syndrome: a randomized study. *Gastroenterology* 2008; **134**: 1352-1359 [PMID: 18471512 DOI: 10.1053/j.gastro.2008.02.024]
- Sanyal AJ, Boyer T, Garcia-Tsao G, Regenstein F, Rossaro L, Appenrodt B, Blei A, Gülberg V, Sigal S, Teuber P. A randomized, prospective, double-blind, placebo-controlled trial of terlipressin for type 1 hepatorenal syndrome. *Gastroenterology* 2008; **134**: 1360-1368 [PMID: 18471513 DOI: 10.1053/j.gastro.2008.02.014]
- Dobre M, Demirjian S, Sehgal AR, Navaneethan SD. Terlipressin in hepatorenal syndrome: a systematic review and meta-analysis. *Int Urol Nephrol* 2011; **43**: 175-184 [PMID: 20306131 DOI: 10.1007/s11255-010-9725-8]
- Neri S, Pulvirenti D, Malaguarnera M, Cosimo BM, Bertino G, Ignaccolo L, Siringo S, Castellino P. Terlipressin and albumin in patients with cirrhosis and type I hepatorenal syndrome. *Dig Dis Sci* 2008; **53**: 830-835 [PMID: 17939047 DOI: 10.1007/s10620-007-9919-9]
- Gluud LL, Christensen K, Christensen E, Krag A. Terlipressin for hepatorenal syndrome. *Cochrane Database Syst Rev* 2012; **9**: CD005162 [PMID: 22972083 DOI: 10.1002/14651858.cd005162.pub3]
- Fagundes C, Ginès P. Hepatorenal syndrome: a severe, but treatable, cause of kidney failure in cirrhosis. *Am J Kidney Dis* 2012; **59**: 874-885 [PMID: 22480795 DOI: 10.1053/j.ajkd.2011.12.032]
- Barbano B, Sardo L, Gigante A, Gasperini ML, Liberatori M, Giraldi GD, Lacanna A, Amoroso A, Ciani R. Pathophysiology, diagnosis and clinical management of hepatorenal syndrome: from classic to new drugs. *Curr Vasc Pharmacol* 2014; **12**: 125-135 [PMID: 24678726 DOI: 10.2174/15701611201140327163930]
- Sagi SV, Mittal S, Kasturi KS, Sood GK. Terlipressin therapy for reversal of type 1 hepatorenal syndrome: a meta-analysis of randomized controlled trials. *J Gastroenterol Hepatol* 2010; **25**: 880-885 [PMID: 20074149 DOI: 10.1111/j.1440-1746.2009.06132.x]
- Duvoux C, Zanditenas D, Hézode C, Chauvat A, Monin JL, Roudot-Thoraval F, Mallat A, Dhumeaux D. Effects of noradrenalin and albumin in patients with type I hepatorenal syndrome: a pilot study. *Hepatology* 2002; **36**: 374-380 [PMID: 12143045 DOI: 10.1053/jhep.2002.34343]
- Alessandria C, Ottobrelli A, Debernardi-Venon W, Todros L, Cerenzia MT, Martini S, Balzola F, Morgando A, Rizzetto M, Marzano A. Noradrenalin vs terlipressin in patients with hepatorenal syndrome: a prospective, randomized, unblinded, pilot study. *J Hepatol* 2007; **47**: 499-505 [PMID: 17560680 DOI: 10.1016/j.jhep.2007.04.010]
- Sharma P, Kumar A, Shrama BC, Sarin SK. An open label, pilot, randomized controlled trial of noradrenaline versus terlipressin in the treatment of type 1 hepatorenal syndrome and predictors of response. *Am J Gastroenterol* 2008; **103**: 1689-1697 [PMID: 18557715 DOI: 10.1111/j.1572-0241.2008.01828.x]
- Singh V, Ghosh S, Singh B, Kumar P, Sharma N, Bhalla A, Sharma AK, Choudhary NS, Chawla Y, Nain CK. Noradrenaline vs. terlipressin in the treatment of hepatorenal syndrome: a randomized study. *J Hepatol* 2012; **56**: 1293-1298 [PMID: 22322237 DOI: 10.1016/j.jhep.2012.01.012]
- Nassar Junior AP, Farias AQ, D'Albuquerque LA, Carrilho FJ, Malbouisson LM. Terlipressin versus norepinephrine in the treatment of hepatorenal syndrome: a systematic review and meta-analysis. *PLoS One* 2014; **9**: e107466 [PMID: 25203311 DOI: 10.1371/journal.pone.0107466]
- Tandon P, Bain VG, Tsuyuki RT, Klarenbach S. Systematic review: renal and other clinically relevant outcomes in hepatorenal syndrome trials. *Aliment Pharmacol Ther* 2007; **25**: 1017-1028 [PMID: 17439502 DOI: 10.1111/j.1365-2036.2007.03303.x]



- 23 **Hassanein TI**, Abdeen O, El-Tahawi M, Hart M, Khanna A, R. M. Octeotide, midodrine and albumin triple therapy is effective in reversing hepatorenal syndrome. *Hepatology* 2001; **34**: A54
- 24 **Salerno F**, Cazzaniga M, Merli M, Spinzi G, Saibeni S, Salmi A, Fagiuoli S, Spadaccini A, Trotta E, Laffi G, Koch M, Riggio O, Boccia S, Felder M, Balzani S, Bruno S, Angeli P. Diagnosis, treatment and survival of patients with hepatorenal syndrome: a survey on daily medical practice. *J Hepatol* 2011; **55**: 1241-1248 [PMID: 21703199 DOI: 10.1016/j.jhep.2011.03.012]
- 25 **Karwa R**, Woodis CB. Midodrine and octreotide in treatment of cirrhosis-related hemodynamic complications. *Ann Pharmacother* 2009; **43**: 692-699 [PMID: 19299324 DOI: 10.1345/aph.1L373]
- 26 **Hiremath SB**, Lokikere SD, Madalageri NK. Efficacy of midodrine plus octeotide in hepatorenal syndrome: A meta-analysis. *IJRAP* 2012; **3**: 576-581
- 27 **Tavakkoli H**, Yazdanpanah K, Mansourian M. Noradrenalin versus the combination of midodrine and octreotide in patients with hepatorenal syndrome: randomized clinical trial. *Int J Prev Med* 2012; **3**: 764-769 [PMID: 23189227]
- 28 **Cavallin M**, Kamath PS, Merli M, Fasolato S, Toniutto P, Salerno F, Bernardi M, Romanelli RG, Colletta C, Salinas F, Di Giacomo A, Ridola L, Fornasiero E, Caraceni P, Morando F, Piano S, Gatta A, Angeli P. Terlipressin plus albumin versus midodrine and octreotide plus albumin in the treatment of hepatorenal syndrome: A randomized trial. *Hepatology* 2015; **62**: 567-574 [PMID: 25644760 DOI: 10.1002/hep.27709]
- 29 **Velez JC**, Nietert PJ. Therapeutic response to vasoconstrictors in hepatorenal syndrome parallels increase in mean arterial pressure: a pooled analysis of clinical trials. *Am J Kidney Dis* 2011; **58**: 928-938 [PMID: 21962618 DOI: 10.1053/j.ajkd.2011.07.017]
- 30 **Esraïlian E**, Pantangco ER, Kyulo NL, Hu KQ, Runyon BA. Octreotide/Midodrine therapy significantly improves renal function and 30-day survival in patients with type 1 hepatorenal syndrome. *Dig Dis Sci* 2007; **52**: 742-748 [PMID: 17235705 DOI: 10.1007/s10620-006-9312-0]
- 31 **Kiser TH**, Fish DN, Obritsch MD, Jung R, MacLaren R, Parikh CR. Vasopressin, not octreotide, may be beneficial in the treatment of hepatorenal syndrome: a retrospective study. *Nephrol Dial Transplant* 2005; **20**: 1813-1820 [PMID: 15956066 DOI: 10.1093/ndt/gfh930]
- 32 **Eisenman A**, Armali Z, Enat R, Bankir L, Baruch Y. Low-dose vasopressin restores diuresis both in patients with hepatorenal syndrome and in anuric patients with end-stage heart failure. *J Intern Med* 1999; **246**: 183-190 [PMID: 10447787 DOI: 10.1046/j.1365-2796.1999.00556.x]
- 33 **Garcia-Martinez R**, Caraceni P, Bernardi M, Gines P, Arroyo V, Jalan R. Albumin: pathophysiologic basis of its role in the treatment of cirrhosis and its complications. *Hepatology* 2013; **58**: 1836-1846 [PMID: 23423799 DOI: 10.1002/hep.26338]
- 34 **Ortega R**, Ginès P, Uriz J, Cárdenas A, Calahorra B, De Las Heras D, Guevara M, Bataller R, Jiménez W, Arroyo V, Rodés J. Terlipressin therapy with and without albumin for patients with hepatorenal syndrome: results of a prospective, nonrandomized study. *Hepatology* 2002; **36**: 941-948 [PMID: 12297842 DOI: 10.1016/S0270-9139(02)00101-5]
- 35 **Moreau R**, Durand F, Poynard T, Duhamel C, Cervoni JP, Ichai P, Abergel A, Halimi C, Pauwels M, Bronowicki JP, Giostra E, Fleurot C, Gurnot D, Nouel O, Renard P, Rivoal M, Blanc P, Coumaros D, Ducloux S, Levy S, Pariente A, Perarnau JM, Roche J, Scribe-Outtas M, Valla D, Bernard B, Samuel D, Butel J, Hadengue A, Platek A, Lebre C, Cadranet JF. Terlipressin in patients with cirrhosis and type 1 hepatorenal syndrome: a retrospective multicenter study. *Gastroenterology* 2002; **122**: 923-930 [PMID: 11910344 DOI: 10.1053/gast.2002.32364]
- 36 **Umgefter A**, Reindl W, Wagner KS, Franzen M, Stock K, Schmid RM, Huber W. Effects of plasma expansion with albumin and paracetamol on haemodynamics and kidney function in critically ill cirrhotic patients with tense ascites and hepatorenal syndrome: a prospective uncontrolled trial. *Crit Care* 2008; **12**: R4 [PMID: 18197961 DOI: 10.1186/cc6765]
- 37 **Bresing KA**, Textor J, Strunk H, Klehr HU, Schild H, Sauerbruch T. Transjugular intrahepatic portosystemic stent-shunt for hepatorenal syndrome. *Lancet* 1997; **349**: 697-698 [PMID: 9078203 DOI: 10.1016/S0140-6736(97)24010-9]
- 38 **Bresing KA**, Textor J, Perz J, Schiedermaier P, Raab P, Strunk H, Klehr HU, Kramer HJ, Spengler U, Schild H, Sauerbruch T. Long term outcome after transjugular intrahepatic portosystemic stent-shunt in non-transplant cirrhotics with hepatorenal syndrome: a phase II study. *Gut* 2000; **47**: 288-295 [PMID: 10896924 DOI: 10.1136/gut.47.2.288]
- 39 **Guevara M**, Ginès P, Bandi JC, Gilabert R, Sort P, Jiménez W, Garcia-Pagan JC, Bosch J, Arroyo V, Rodés J. Transjugular intrahepatic portosystemic shunt in hepatorenal syndrome: effects on renal function and vasoactive systems. *Hepatology* 1998; **28**: 416-422 [PMID: 9696006 DOI: 10.1002/hep.510280219]
- 40 **Rössle M**, Gerbes AL. TIPS for the treatment of refractory ascites, hepatorenal syndrome and hepatic hydrothorax: a critical update. *Gut* 2010; **59**: 988-1000 [PMID: 20581246 DOI: 10.1136/gut.2009.193227]
- 41 **Malinchoc M**, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871 [PMID: 10733541 DOI: 10.1053/he.2000.5852]
- 42 **Wilkinson SP**, Weston MJ, Parsons V, Williams R. Dialysis in the treatment of renal failure in patients with liver disease. *Clin Nephrol* 1977; **8**: 287-292 [PMID: 884909]
- 43 **Keller F**, Heinze H, Jochimsen F, Passfall J, Schuppan D, Büttner P. Risk factors and outcome of 107 patients with decompensated liver disease and acute renal failure (including 26 patients with hepatorenal syndrome): the role of hemodialysis. *Ren Fail* 1995; **17**: 135-146 [PMID: 7644764 DOI: 10.3109/08860229509026250]
- 44 **Capling RK**, Bastani B. The clinical course of patients with type 1 hepatorenal syndrome maintained on hemodialysis. *Ren Fail* 2004; **26**: 563-568 [PMID: 15526916 DOI: 10.1081/JDI-200035988]
- 45 The 2011 kidney disease: Improving global outcomes (kdigo) clinical practice guideline for acute kidney injury (aki). *Kidney Inter* 2012; **2** Suppl: 107-110
- 46 **Davenport A**, Will EJ, Davidson AM. Improved cardiovascular stability during continuous modes of renal replacement therapy in critically ill patients with acute hepatic and renal failure. *Crit Care Med* 1993; **21**: 328-338 [PMID: 8440100 DOI: 10.1097/00003246-199303000-00007]
- 47 **Detry O**, Arkadopoulos N, Ting P, Kahaku E, Margulies J, Arnaout W, Colquhoun SD, Rozga J, Demetriou AA. Intracranial pressure during liver transplantation for fulminant hepatic failure. *Transplantation* 1999; **67**: 767-770 [PMID: 10096539 DOI: 10.1097/00007890-199903150-00024]
- 48 **Mitzner SR**, Stange J, Klammt S, Peszynski P, Schmidt R, Nöldge-Schomburg G. Extracorporeal detoxification using the molecular adsorbent recirculating system for critically ill patients with liver failure. *J Am Soc Nephrol* 2001; **12** Suppl 17: S75-S82 [PMID: 11251037]
- 49 **Mitzner SR**, Stange J, Klammt S, Risler T, Erley CM, Bader BD, Berger ED, Lauchart W, Peszynski P, Freytag J, Hickstein H, Looek J, Löhr JM, Liebe S, Emmrich J, Korten G, Schmidt R. Improvement of hepatorenal syndrome with extracorporeal albumin dialysis MARS: results of a prospective, randomized, controlled clinical trial. *Liver Transpl* 2000; **6**: 277-286 [PMID: 10827226 DOI: 10.1002/lt.500060326]
- 50 **Lavayssière L**, Kallab S, Cardeau-Desangles I, Nogier MB, Cointault O, Barange K, Muscari F, Rostaing L, Kamar N. Impact of molecular adsorbent recirculating system on renal recovery in type-1 hepatorenal syndrome patients with chronic liver failure. *J Gastroenterol Hepatol* 2013; **28**: 1019-1024 [PMID: 23425070 DOI: 10.1111/jgh.12159]
- 51 **Bañares R**, Nevens F, Larsen FS, Jalan R, Albillos A, Dollinger M, Saliba F, Sauerbruch T, Klammt S, Ockenga J, Pares A, Wendon J, Brünner T, Kramer L, Mathurin P, de la Mata M, Gasbarrini A, Mühlhaupt B, Wilmer A, Laleman W, Eefsen M, Sen S, Zipprich A, Tenorio T, Pavesi M, Schmidt HH, Mitzner S, Williams R, Arroyo

- V. Extracorporeal albumin dialysis with the molecular adsorbent recirculating system in acute-on-chronic liver failure: the RELIEF trial. *Hepatology* 2013; **57**: 1153-1162 [PMID: 23213075 DOI: 10.1002/hep.26185]
- 52 **Cholongitas E**, Senzolo M, Patch D, Shaw S, O'Beirne J, Burroughs AK. Cirrhotics admitted to intensive care unit: the impact of acute renal failure on mortality. *Eur J Gastroenterol Hepatol* 2009; **21**: 744-750 [PMID: 20160527 DOI: 10.1097/MEG.0b013e328308bb9c]
- 53 **Wolff B**, Machill K, Schumacher D, Schulzki I. MARS dialysis in decompensated alcoholic liver disease: a single-center experience. *Liver Transpl* 2007; **13**: 1189-1192 [PMID: 17663393 DOI: 10.1002/lt.21235]
- 54 **Mitzner SR**, Klammt S, Peszynski P, Hickstein H, Korten G, Stange J, Schmidt R. Improvement of multiple organ functions in hepatorenal syndrome during albumin dialysis with the molecular adsorbent recirculating system. *Ther Apher* 2001; **5**: 417-422 [PMID: 11778928 DOI: 10.1046/j.1526-0968.2001.00388.x]
- 55 **Lee JP**, Kwon HY, Park JI, Yi NJ, Suh KS, Lee HW, Kim M, Oh YK, Lim CS, Kim YS. Clinical outcomes of patients with hepatorenal syndrome after living donor liver transplantation. *Liver Transpl* 2012; **18**: 1237-1244 [PMID: 22714872 DOI: 10.1002/lt.23493]
- 56 **Boyer TD**, Sanyal AJ, Garcia-Tsao G, Regenstein F, Rossaro L, Appenrodt B, Gülberg V, Sigal S, Bexon AS, Teuber P. Impact of liver transplantation on the survival of patients treated for hepatorenal syndrome type 1. *Liver Transpl* 2011; **17**: 1328-1332 [PMID: 21837734 DOI: 10.1002/lt.22395]
- 57 **Xu X**, Ling Q, Zhang M, Gao F, He Z, You J, Zheng S. Outcome of patients with hepatorenal syndrome type 1 after liver transplantation: Hangzhou experience. *Transplantation* 2009; **87**: 1514-1519 [PMID: 19461488 DOI: 10.1097/TP.0b013e3181a4430b]
- 58 **Marik PE**, Wood K, Starzl TE. The course of type 1 hepato-renal syndrome post liver transplantation. *Nephrol Dial Transplant* 2006; **21**: 478-482 [PMID: 16249201 DOI: 10.1093/ndt/gfi212]
- 59 **Nadim MK**, Genyk YS, Tokin C, Fieber J, Ananthapanyasut W, Ye W, Selby R. Impact of the etiology of acute kidney injury on outcomes following liver transplantation: acute tubular necrosis versus hepatorenal syndrome. *Liver Transpl* 2012; **18**: 539-548 [PMID: 22250075 DOI: 10.1002/lt.23384]
- 60 **Nadim MK**, Sung RS, Davis CL, Andreoni KA, Biggins SW, Danovitch GM, Feng S, Friedewald JJ, Hong JC, Kellum JA, Kim WR, Lake JR, Melton LB, Pomfret EA, Saab S, Genyk YS. Simultaneous liver-kidney transplantation summit: current state and future directions. *Am J Transplant* 2012; **12**: 2901-2908 [PMID: 22822723 DOI: 10.1111/j.1600-6143.2012.04190.x]
- 61 **Ruiz R**, Kunitake H, Wilkinson AH, Danovitch GM, Farmer DG, Ghobrial RM, Yersiz H, Hiatt JR, Busuttil RW. Long-term analysis of combined liver and kidney transplantation at a single center. *Arch Surg* 2006; **141**: 735-741; discussion 741-742 [PMID: 16924080 DOI: 10.1001/archsurg.141.8.735]
- 62 **Arroyo V**, Terra C, Ginès P. Advances in the pathogenesis and treatment of type-1 and type-2 hepatorenal syndrome. *J Hepatol* 2007; **46**: 935-946 [PMID: 17391801 DOI: 10.1016/j.jhep.2007.02.001]
- 63 **Gonwa TA**, Morris CA, Goldstein RM, Husberg BS, Klintmalm GB. Long-term survival and renal function following liver transplantation in patients with and without hepatorenal syndrome--experience in 300 patients. *Transplantation* 1991; **51**: 428-430 [PMID: 1994538 DOI: 10.1097/00007890-199102000-00030]
- 64 **Wong F**, Leung W, Al Beshir M, Marquez M, Renner EL. Outcomes of patients with cirrhosis and hepatorenal syndrome type 1 treated with liver transplantation. *Liver Transpl* 2015; **21**: 300-307 [PMID: 25422261 DOI: 10.1002/lt.24049]

**P- Reviewer:** Coban M, Ishibashi H, Mocellin S

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D



## Hydatid cyst of the gallbladder: A systematic review of the literature

Roberto Gómez, Yousef Allaoua, Rafael Colmenares, Sergio Gil, Pilar Roquero, José M Ramia

Roberto Gómez, Yousef Allaoua, Rafael Colmenares, Sergio Gil, Pilar Roquero, Faculty of Medicine, Universidad de Alcalá, 28805 Alcalá de Henares, Spain

José M Ramia, Servicio de Cirugía General y del Aparato Digestivo, Hospital Universitario de Guadalajara, 19002 Guadalajara, Spain

Author contributions: Gómez R, Allaoua Y, Colmenares R, Gil S and Roquero P analyzed data; Gómez R, Allaoua Y, Colmenares R, Gil S, Roquero P and Ramia JM wrote the manuscript; Ramia JM performed research.

Conflict-of-interest statement: Authors declare no conflict of interest.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: José M Ramia, MD, PhD, FACS, FRCS, Servicio de Cirugía General y del Aparato Digestivo, Hospital Universitario de Guadalajara, C/Donantes de Sangre s/n. 7ª planta, 19002 Guadalajara, Spain. [jose\\_ramia@hotmail.com](mailto:jose_ramia@hotmail.com)  
Telephone: +34-61-6292056  
Fax: +34-94-9209218

Received: April 11, 2016

Peer-review started: April 13, 2016

First decision: May 17, 2016

Revised: July 4, 2016

Accepted: July 29, 2016

Article in press: August 1, 2016

Published online: September 8, 2016

### Abstract

#### AIM

To evaluate all the references about primary gallbladder hidatidosis looking for best treatment evidence.

#### METHODS

Search: 1966-2015 in MEDLINE, Cochrane Library, SciELO, and Tripdatabase. Key words: "gallbladder hydatid disease" and "gallbladder hydatid cyst". We found 124 papers in our searches but only 14 papers including 16 cases were about hydatid cyst of the gallbladder (GBHC).

#### RESULTS

Eight cases of GBHC were women and seven men. One not mentioned. Median age was 48.3 years. The most frequent clinical symptom was abdominal pain (94%) usually in the right upper quadrant. Ultrasound was performed in ten patients (62.5%) but in most cases a combination of several techniques was performed. The location of the cysts was intravesicular in five patients. Five patients presented GBHC and liver hydatid cysts. Two patients presented cholelithiasis and one choledocholithiasis. The most frequent surgical technique was cholecystectomy by laparotomy (81.25%). Simultaneous surgery of liver cysts was carried out in five cases. Eleven patients did not present postoperative complications, but one died. The mean hospital stay was seven days. No recurrence of GBHC was recorded.

#### CONCLUSION

In GBHC, the most frequent symptom is right hypochondrium pain (evidence level V). Best diagnostic methods are ultrasound and computed tomography (level V, grade D). Suggested treatment is open cholecystectomy and postoperative albendazole (level V, grade D) obtaining good clinical results and none relapses.

**Key words:** Hydatid cyst; Gallbladder; Cholecystectomy; Review; Hydatidosis

© The Author(s) 2016. Published by Baishideng Publishing

Group Inc. All rights reserved.

**Core tip:** Systematic review of gallbladder hydatidosis has not previously done. We have performed a systematic search trying to define best diagnostic procedures and best therapeutical strategies.

Gómez R, Allaoua Y, Colmenares R, Gil S, Roquero P, Ramia JM. Hydatid cyst of the gallbladder: A systematic review of the literature. *World J Hepatol* 2016; 8(25): 1087-1092 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i25/1087.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i25.1087>

## INTRODUCTION

Hydatid disease is a zoonotic infection found all over the world, which is caused by the larval stage of parasites of the *Echinococcus* species. *Echinococcus granulosus* is the most frequent (95% of cases); other species such as *Echinococcus multilocularis* are rare (5%). Hydatid disease is endemic in cattle-raising regions like the Mediterranean countries, Africa, South America, Middle East, Australia and New Zealand<sup>[1,2]</sup>.

*Echinococcus granulosus* lives in the intestine of dogs and other wild canines, which are the definitive hosts. Humans are accidentally infected *via* the fecal-oral route. Larval embryos pass through the intestinal wall and reach the liver through the portal system. Subsequently, through the liver and lungs, parasites reach the arterial circulation and may spread through the rest of the organs<sup>[1-3]</sup>. The larvae can remain and develop into hydatid cyst anywhere in the body, but liver (70%) and lungs (20%) are the most commonly affected sites.

Primary hydatid cyst of the gallbladder (GBHC) is an exceptional location for hydatidosis, and its pathogenesis is not completely clear. While the literature on liver hydatid disease is abundant, references to the primary involvement of the gallbladder are limited to clinical cases and so it is difficult to reach meaningful conclusions<sup>[3-16]</sup>. In this paper we present a systematic review of the literature on GBHC published to date.

## MATERIALS AND METHODS

### Search strategy

We introduced the following keywords in the MEDLINE (PubMed), Tripdatabase, SciELO and Cochrane Library databases: "gallbladder hydatid disease (GHD)" and "gallbladder hydatid cyst (GHC)" without restrictions on publication date or author until 31 December 2015<sup>[17]</sup>. The first selection of papers was made after reading title and abstract, and in case of doubt, after reading the full text. A flowchart is shown in Figure 1.

Our results were as follows: (1) zero results in SciELO; (2) 2 results for both searches (GHD and GHC) in the Cochrane Library: Neither met the inclusion criteria; (3) 21 results for GHD and 17 for GHC in Tripdatabase. After

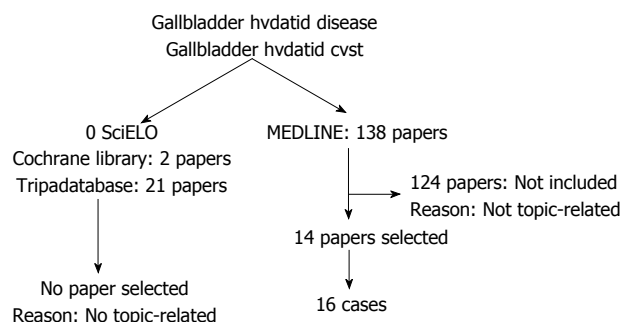


Figure 1 Search flowchart.

review, none were found to be related to the topic; and (4) 137 results for GHD and 138 for GHC in MEDLINE. Since the overlap between search results was 99%, we used the latter search with 138 results; of them, only 14 (10.14%) met the selection criteria for this study.

These 14 papers included 16 clinical cases covering a wide range of clinical, diagnostic and therapeutic aspects of GBHC. These characteristics are summarized in Tables 1-4.

In the next step, to assess the quality of the selected studies we used the rating scale described by Manterola *et al.*<sup>[18]</sup>, which assesses each publication individually depending on the type of study, the size of the sample and whether it is justified, and the methodology used. A mean score of all the selected studies is produced ranging from 6 to 36 points, with a quality cut-off score of 18 points. The mean score in our review was 10.3; however, due to the rarity of GBHC and the few studies of this issue published, we selected all the papers available.

We also carried out a qualitative analysis of the selected papers and their conclusions, based on the classical levels of evidence and grades of recommendation proposed in Cook *et al.*<sup>[19]</sup> and Sackett<sup>[20]</sup>.

## RESULTS

Eight cases of GBHC were women and seven men. The sex of one patient was not specified. Median age was 48.3 years (range: 27-76). The most frequent clinical manifestation was abdominal pain (15/16) (94%) (Table 1), in the right upper quadrant in 13 patients) (81.25%), in the epigastrium in four (25%), (three of whom combined upper quadrant pain in right hypochondrium and epigastric pain), and finally diffuse abdominal pain in two (12.5%). In one case, no data on abdominal pain were included (6.25%). Three patients presented vomiting and two had nausea; no information on nausea or vomiting was reported in the rest of patients. Three patients had fever, four were fever-free, and no data on fever were available for the remaining nine patients. Four patients had jaundice, five did not, and no data were available in seven cases. As regards past medical history, two patients had been previously diagnosed with hydatid disease and one had had hepatitis.

On physical examination (Table 1), four patients presented abdominal tenderness, three hepatomegaly,



Table 1 Clinical data

Ref.	Sex	Age	Abdominal pain	Nausea and vomiting	Fever	Jaundice	Abdominal exploration	Past medical history
Noomene <i>et al</i> <sup>[3]</sup> , 2013	Male	48	Diffuse		No (36.7 °C)	Yes	Painful palpation in right hypocondrium	
Ertem <i>et al</i> <sup>[4]</sup> , 2012	Male	32	Right hyponcondrium and epigastrium	Nausea	No	No	Painful palpation in right hypocondrium	
Krasniqi <i>et al</i> <sup>[5]</sup> , 2010	Female	39	Right hypocondrium (18 mo)	Nausea		No	Painful palpation in right hypocondrium	
Murtaza <i>et al</i> <sup>[6]</sup> , 2008	Female	32	Right hyponcondrium and epigastrium (3 mo)		No	No	Hepatomegaly	Liver hydatid surgery 8 yr ago
Sabat <i>et al</i> <sup>[7]</sup> , 2008	Female	35	Right hyponcondrium and epigastrium		Yes	Yes		
Wani <i>et al</i> <sup>[8]</sup> , 2005	Female	51	Right hyponcondrium		Yes (38 °C-39.5 °C)		Abdominal distension	
Pitiakoudis <i>et al</i> <sup>[9]</sup> , 2006	Male	60	Right hyponcondrium (10 d)	Vomiting				
Safioleas <i>et al</i> <sup>[10]</sup> , 2004	Female	65	Right hyponcondrium and epigastrium	Vomiting				
Safioleas <i>et al</i> <sup>[10]</sup> , 2004	Female	51	Right hyponcondrium (6 mo)				Normal	
Safioleas <i>et al</i> <sup>[10]</sup> , 2004	Male	63	Right hyponcondrium and epigastrium					
Kumar <i>et al</i> <sup>[11]</sup> , 2004	Female	27	Diffuse					Relapsed liver hydatid cyst
Raza <i>et al</i> <sup>[12]</sup> , 2003	Male	27	Right hyponcondrium (4 mo)		No	No	Hepatomegaly	
Kapoor <i>et al</i> <sup>[13]</sup> , 2000	Male	53	Right hyponcondrium (2 mo)		Yes (high fever, 10 d)	Yes	Abdominal distension, ascitis, gallbladder mass	
Cangiotti <i>et al</i> <sup>[14]</sup> , 1994	Male							
Rigas <i>et al</i> <sup>[15]</sup> , 1979	Female	65	Right hyponcondrium	Vomiting		No	Normal	
Barón Urbano <i>et al</i> <sup>[16]</sup> , 1978	-	76	Right hyponcondrium			Sí	Hepatomegaly, rubi spots in thorax and abdomen	Hepatitis

Table 2 Radiological and analitical studies

Ref.	Alkaline phosphatase (UI/L)	Bilirubin (mg/dL)	Ultrasound	CT	MRI	Cysts inside gallbladder	Cholelithiasis	Cholelithiasis	Serology <i>E. granulosus</i>
Noomene <i>et al</i> <sup>[3]</sup> , 2013	220	7.1	Yes	Yes	Cholangio MRI			Yes	Positive
Ertem <i>et al</i> <sup>[4]</sup> , 2012			Yes	Yes	Yes	Yes	No	No	Negative
Krasniqi <i>et al</i> <sup>[5]</sup> , 2010			Yes	Yes				No	
Murtaza <i>et al</i> <sup>[6]</sup> , 2008	140	10.2	Yes						
Sabat <i>et al</i> <sup>[7]</sup> , 2008			Yes	Yes					
Wani <i>et al</i> <sup>[8]</sup> , 2005			Yes	Yes			Yes		
Pitiakoudis <i>et al</i> <sup>[9]</sup> , 2006		0.9	Yes	Yes	Yes	Yes			
Safioleas <i>et al</i> <sup>[10]</sup> , 2004							Dude	Dude	
Safioleas <i>et al</i> <sup>[10]</sup> , 2004			Yes						
Safioleas <i>et al</i> <sup>[10]</sup> , 2004				Yes		Yes			Positive
Kumar <i>et al</i> <sup>[11]</sup> , 2004				Yes		Yes			
Raza <i>et al</i> <sup>[12]</sup> , 2003			Yes				Yes		
Kapoor <i>et al</i> <sup>[13]</sup> , 2000	465	5.6	Yes			Yes			Positive
Cangiotti <i>et al</i> <sup>[14]</sup> , 1994									
Rigas <i>et al</i> <sup>[15]</sup> , 1979				Yes				No	
Barón Urbano <i>et al</i> <sup>[16]</sup> , 1978	266	8.8							

CT: Computed tomography; MRI: magnetic resonance imaging; *E. granulosus*: *Echinococcus granulosus*.

two abdominal distension, and one a palpable mass. Serological information was available in only five cases (Table 2). Levels of alkaline phosphatase and bilirubin were high in four patients, normal in one, and no information was recorded for the other eleven. In the cases in which they were specified, alkaline phosphatase levels were between 140 and 465 IU/L and bilirubin between 5.6 and 10.2 mg/dL. *Echinococcus* serology was performed in four cases, being positive in three and negative in one.

Image diagnostic methods are described in Table 2. Abdominal ultrasound (US) was performed in ten patients (62.5%), abdominal computed tomography (CT) in nine (56.25%), and magnetic resonance imaging (MRI) in three (18.75%). In most cases a combination of several techniques was performed: US + CT + MRI in three cases, US + TC in three others; so four cases underwent US alone and three CT alone. The location of the cysts was intravesicular in five patients. Five patients presented

**Table 3** Therapeutical strategies

Ref.	Preoperative albendazole	Treatment	Liver hydatidosis	Intraoperative treatment cyst	Intraoperative findings
Noomene <i>et al</i> <sup>[3]</sup> , 2013	No	ERCP + Stent Laparoscopy cholecystectomy	No	No	Biliary sludge and stones in ampulla seen in ERCP
Ertem <i>et al</i> <sup>[4]</sup> , 2012	No	Cholecystectomy by laparotomy	No	No	Galbladder cyst with inflammatory changes
Krasniqi <i>et al</i> <sup>[5]</sup> , 2010	No	Cholecystectomy by laparotomy	Yes Cystopericystectomy	No	Calcified primary gallbladder cyst
Murtaza <i>et al</i> <sup>[6]</sup> , 2008	Yes (2 wk)	Subtotal Cholecystectomy by laparotomy	No	Yes	Biliary communication into the cyst closed with sutures
Sabat <i>et al</i> <sup>[7]</sup> , 2008	No	Cholecystectomy by laparotomy	No	Yes (aspiration + hypertonic solution cleaning)	-
Wani <i>et al</i> <sup>[8]</sup> , 2005	No	Cholecystectomy by laparotomy	No	No	-
Pitiakoudis <i>et al</i> <sup>[9]</sup> , 2006	No	Cholecystectomy by laparotomy	No	Yes	-
Safioleas <i>et al</i> <sup>[10]</sup> , 2004	No	Cholecystectomy by laparotomy	No	No	5 cm × 4 cm cyst
Safioleas <i>et al</i> <sup>[10]</sup> , 2004	No	Cholecystectomy by laparotomy	No	No	3 cm × 4 cm cyst
Safioleas <i>et al</i> <sup>[10]</sup> , 2004	No	Cholecystectomy by laparotomy	No	No	5 cm × 4 cm cyst
Kumar <i>et al</i> <sup>[11]</sup> , 2004	No	Cholecystectomy by laparotomy	Yes Cysts segment IV and VIII. Cystopericystectomy segment IV + PAIR segment VII	Yes (aspiration + hypertonic solution cleaning) segment VII cyst	Cyst invading segment IV. Communication between cyst and gallbladder
Raza <i>et al</i> <sup>[12]</sup> , 2003	No	Cholecystectomy by laparotomy	Yes Right Lobe Enucleation	No	In gallbladder: Stones and daughter vesicles
Kapoor <i>et al</i> <sup>[13]</sup> , 2000	No	NO. ERCP + Stent	No	No	-
Cangiotti <i>et al</i> <sup>[14]</sup> , 1994	No	Cholecystectomy by laparotomy	SI. Right lobe. Cystopericystectomy	No	-
Rigas <i>et al</i> <sup>[15]</sup> , 1979	No	Cholecystectomy by laparotomy	No	No	-
Barón Urbano <i>et al</i> <sup>[16]</sup> , 1978	No	Cholecystectomy by laparotomy	Yes Segment IV. Done by thoracotomy	-	Enlarged liver. Cholangitis. Daughter vesicles in cystic conduct lumen

ERCP: Endoscopic retrograde cholangiopancreatography.

GBHC and liver hydatid cysts. Two patients presented cholelithiasis and one choledocholithiasis.

The data on therapeutic management are displayed in Table 3. One patient received preoperative albendazole for two weeks, but no data on the other fifteen were available. The most frequent surgical technique was cholecystectomy by laparotomy (81.25%), performed in 13 patients; laparoscopic cholecystectomy was performed in two cases (12.5%), in one of them a previous endoscopic retrograde cholangiopancreatography (ERCP) was done and received a biliary stent; in the last patient, cholecystectomy was not performed, only ERCP and biliary stenting (6.25%). Cholecystectomies were total in 14 cases (93.3%) and subtotal in the patient treated preoperatively with albendazole (6.7%). Simultaneous surgery of liver hydatid cysts was carried out in five cases: Cystopericystectomy in three cases, enucleation in one, and in the other the surgical technique was not specified except for the fact that access was made by thoracotomy. Eleven patients did not present postoperative complications: One presented fever, atelectasis

and pleural effusion, and another multiple organ failure and death. No data regarding postoperative outcome were recorded in three cases. The pathological examination (Table 4) was performed in nine patients. In three, the presence of *Echinococcus granulosus* was confirmed microscopically.

The mean hospital stay was seven days (range: 1-12 d). Seven patients were treated postoperatively with varying doses of albendazole. In nine cases follow-up after the postoperative period was recorded, for a mean period of 38 mo (range: 1-120 mo); no recurrence of GBHC was recorded.

## DISCUSSION

Hydatidosis is a disease caused by the larva of the genus *Echinococcus*, within which *Echinococcus granulosus* is the most common species. Although cases have been diagnosed all over the world as a result of increased intercontinental migration, areas in which the incidence is significantly higher include the Mediterranean Sea,

**Table 4** Pathology, postoperative course and follow-up

Ref.	Pathologic study	Stay	Postoperative treatment	Morbidity	Follow-up
Noomene <i>et al</i> <sup>[3]</sup> , 2013	Cysts in gallbladder. Chronic inflammation	1	Albendazole 400 mg/d	No	
Ertem <i>et al</i> <sup>[4]</sup> , 2012	Cyst in gallbladder	4		No	6 mo
Krasniqi <i>et al</i> <sup>[5]</sup> , 2010	Calcified cyst 7 cm × 5 cm located in gallbladder mucosa	7	Albendazole 400 mg/d, 42 d	No	5 yr
Murtaza <i>et al</i> <sup>[6]</sup> , 2008				No	2 mo
Sabat <i>et al</i> <sup>[7]</sup> , 2008			Albendazole 10 mg/kg, 9 mo	No	
Wani <i>et al</i> <sup>[8]</sup> , 2005					
Pitiakoudis <i>et al</i> <sup>[9]</sup> , 2006	Echinococcus in gallbladder	12	Albendazole 800 mg/d, 4 mo	No	2 yr
Safioleas <i>et al</i> <sup>[10]</sup> , 2004	Echinococcus in gallbladder			No	10 yr
Safioleas <i>et al</i> <sup>[10]</sup> , 2004	Cyst with wall of 5 mm. Daughter vesicles	7		No	6 yr
Safioleas <i>et al</i> <sup>[10]</sup> , 2004	Calcified cyst with daughter vesicles	10	Albendazole 2 mo	Yes: Fever, atelectasis and pleural effusion	4 yr
Kumar <i>et al</i> <sup>[11]</sup> , 2004			Albendazole	No	1 yr
Raza <i>et al</i> <sup>[12]</sup> , 2003			Albendazole 10 mg/kg per day	No	1 mo
Kapoor <i>et al</i> <sup>[13]</sup> , 2000	Postmortem: Cholangitis, chronic liver obstruction			Yes: Sepsis, Multiorgan failure. Death	
Cangiotti <i>et al</i> <sup>[14]</sup> , 1994					
Rigas <i>et al</i> <sup>[15]</sup> , 1979	Cyst 5 cm × 4 cm with membranes. <i>Echinococcus</i> in gallbladder	9		No	
Barón <i>et al</i> <sup>[16]</sup> , 1978					

Africa, South America, Middle East, Australia and New Zealand. Hydatid disease is prevalent in pastoral areas where cattle and dogs are in close contact. Dogs are the definitive hosts; they excrete eggs in their feces, and humans become intermediate hosts through accidental fecal-oral infection<sup>[2,21]</sup>.

The reviews of Dziri *et al*<sup>[21,22]</sup> and Gomez I Gavara *et al*<sup>[1]</sup> concluded that many questions about liver hydatidosis still lack evidence-based answers. In 2016, PAIR or surgery, systematic or selective preoperative ERCP, the best surgical approach (conservative or radical), type of technique (laparoscopic or laparotomy), and the use of albendazole all remain topics for debate<sup>[1,21,22]</sup>.

GBHC is an extremely rare entity, even in places where hydatid disease is endemic. Primary involvement is even less common. It is essential to differentiate primary GBHC from secondary invasion of the gallbladder caused by daughter vesicles of primary liver hydatid disease. GBHC can be located within the vesicle or on its outer surface. GBHC pathogenesis is not very well documented; one of the most accepted hypotheses is infestation through the bile duct, although this explanation is unconvincing in cases of superficial cysts, and also often requires prior hepatic involvement. Larval spread through the lymphatic system after intestinal absorption is possible and may explain the intraluminal cysts. Other routes, such as contamination of gallbladder after surgery for hepatic hydatid cyst, should also be considered<sup>[3]</sup>.

In this evidence-based systematic review we have attempted to answer questions about the symptoms, diagnosis and treatment of GBHC. The main limitation is the lack of published series; all the reviewed papers are clinical cases, and so we are unable to reach an acceptable level of evidence. The most common symptom in GBHC is pain in the right upper quadrant<sup>[4-10,12-16]</sup>. Suspicion of GBHC is established by ultrasound and/or CT<sup>[3-13,15]</sup>. The involvement of the gallbladder is usually an incidental

finding in patients being examined for liver hydatid cysts<sup>[4-6,8,10-12,14-16]</sup>. The most common therapeutic approach is cholecystectomy by laparotomy and postoperative albendazole<sup>[4-12,14-16]</sup>. Few cases present postoperative complications, and the recurrence of hydatid disease is practically zero<sup>[3-12,15]</sup>.

In conclusion, three main conclusions can be drawn regarding the clinical diagnosis and treatment of GBHC: (1) the most common clinical finding is right upper quadrant pain with a very low level of evidence (level V, grade D recommendation); (2) the most useful diagnostic methods are diagnostic ultrasound and CT with a very low level of evidence (level V, grade D recommendation); and (3) the recommended treatment is cholecystectomy by laparotomy plus albendazole in the postoperative period. This strategy achieves good results: There is no postoperative recurrence in the subsequent months of follow-up, with a very low level of evidence (level V, grade D recommendation). To our knowledge, this is the first literature review that focuses on the clinical, diagnostic and therapeutic aspects of GBHC. The lack of published cases on the topic and the fact that all the papers included deal with clinical cases impeded us from achieving a higher level of evidence in the results. More studies are needed, especially randomized controlled trials, in order to reach meaningful conclusions.

## COMMENTS

### Background

Primary gallbladder hidatidosis is an unfrequent disease. No systematic reviews have been done before.

### Research frontiers

Obtaining best clinical evidence to treat primary gallbladder hydatidosis.

### Applications

Future cases and publications will have a systematic review to treat these

patients.

### Peer-review

Hydatid disease of the gallbladder is very rare, from this point of view this systematic review has some interest.

## REFERENCES

- Gomez I Gavara C, López-Andújar R, Belda Ibáñez T, Ramia Angel JM, Moya Herraiz Á, Orbis Castellanos F, Pareja Ibars E, San Juan Rodríguez F. Review of the treatment of liver hydatid cysts. *World J Gastroenterol* 2015; **21**: 124-131 [PMID: 25574085 DOI: 10.3748/wjg.v21.i1.124]
- Ramía-Angel JM, Gasz A, de la Plaza-Llamas R, Quinones-Sampedro J, Sancho E, García Parreno J. Hidatidosis of the spleen. *Pol Przegl Chir* 2011; **83**: 271-275 [PMID: 22166480 DOI: 10.2478/v10035-011-0042-4]
- Noomene R, Ben Maamer A, Bouhafa A, Haoues N, Oueslati A, Cherif A. Primary hydatid cyst of the gallbladder: an unusual localization diagnosed by magnetic resonance imaging (MRI). *Pan Afr Med J* 2013; **14**: 15 [PMID: 23504393 DOI: 10.11604/pamj.2013.14.15.1424]
- Ertem M, Aytaç E, Karaduman Z. Cystic hydatid disease of the gallbladder. *Turk J Gastroenterol* 2012; **23**: 825-826 [PMID: 23864475 DOI: 10.4318/tjg.2012.0440]
- Krasniqi A, Limani D, Gashi-Luci L, Spahija G, Dreshaj IA. Primary hydatid cyst of the gallbladder: a case report. *J Med Case Rep* 2010; **4**: 29 [PMID: 20205877 DOI: 10.1186/1752-1947-4-29]
- Murtaza B, Malik IB, Mahmood A, Sharif MA, Saeed S, Satti AA. Cholecysto-hydatid cyst fistula. *J Coll Physicians Surg Pak* 2008; **18**: 778-780 [PMID: 19032895]
- Sabat SB, Barhate KP, Deshmukh MP. Cholecysto-hydatid cyst fistula. *J Ultrasound Med* 2008; **27**: 299-301 [PMID: 18204023]
- Wani RA, Malik AA, Chowdri NA, Wani KA, Naqash SH. Primary extrahepatic abdominal hydatidosis. *Int J Surg* 2005; **3**: 125-127 [PMID: 17462273 DOI: 10.1016/j.ijsu.2005.06.004]
- Pitiakoudis MS, Tsaroucha AK, Deftereos S, Laftsidis P, Prassopoulos P, Simopoulos CE. Primary hydatid disease in a retroplaced gallbladder. *J Gastrointest Liver Dis* 2006; **15**: 383-385 [PMID: 17205152]
- Safioleas M, Stamoulis I, Theocharis S, Moulakakis K, Makris S, Kostakis A. Primary hydatid disease of the gallbladder: a rare clinical entity. *J Hepatobiliary Pancreat Surg* 2004; **11**: 352-356 [PMID: 15549437 DOI: 10.1007/s00534-004-0915-6]
- Kumar A, Upadhyaya DN, Singh S, Kumar M, Ansari MA. Cholecysto-hydatid cyst fistula. *Indian J Gastroenterol* 2004; **23**: 76-77 [PMID: 15176546]
- Raza MH, Harris SH, Khan R. Hydatid cyst of gall bladder. *Indian J Gastroenterol* 2003; **22**: 67-68 [PMID: 12696832]
- Kapoor A, Sarma D, Gandhi D. Sonographic diagnosis of a ruptured primary hydatid cyst of the gallbladder. *J Clin Ultrasound* 2000; **28**: 51-52 [PMID: 10602107 DOI: 10.1002/(SICI)1097-0096(200001)28:1<51::AID-JCU9>3.0.CO;2-8]
- Cangiotti L, Muiesan P, Begni A, de Cesare V, Pouchè A, Giulini SM, Tiberio G. Unusual localizations of hydatid disease: a 18 year experience. *G Chir* 1994; **15**: 83-86 [PMID: 8060784]
- Rigas AM, Karatzas GM, Markidis NC, Bonikos DS, Sotiropoulou GG, Skalkas G. Primary hydatid cyst of the gallbladder. *Br J Surg* 1979; **66**: 406 [PMID: 466022 DOI: 10.1002/bjs.1800660609]
- Barón Urbano C, Diego Estévez M, Pascual Montero J, Suberviola Gómez E. [Ectopia of the gallbladder associated with hepatic hydatidosis]. *Rev Esp Enferm Apar Dig* 1978; **53**: 691-698 [PMID: 725197]
- Manterola C, Astudillo P, Arias E, Claros N. [Systematic reviews of the literature: what should be known about them]. *Cir Esp* 2013; **91**: 149-155 [PMID: 22035847 DOI: 10.1016/j.ciresp.2011.07.009]
- Manterola C, Vial M, Pineda V, Sanhueza A. Systematic Review of Literature with Different Types of Designs. *Int J Morphol* 2009; **27**: 1179-1186 [DOI: 10.4067/S0717-95022009000400035]
- Cook DJ, Guyatt GH, Laupacis A, Sackett DL. Rules of evidence and clinical recommendations on the use of antithrombotic agents. *Chest* 1992; **102**: 305S-311S [PMID: 1395818]
- Sackett DL. Rules of evidence and clinical recommendations on the use of antithrombotic agents. *Chest* 1989; **95**: 2S-4S [PMID: 2914516]
- Dziri C, Haouet K, Fingerhut A. Treatment of hydatid cyst of the liver: where is the evidence? *World J Surg* 2004; **28**: 731-736 [PMID: 15457348 DOI: 10.1007/s00268-004-7516-z]
- Dziri C, Haouet K, Fingerhut A, Zaoouche A. Management of cystic echinococcosis complications and dissemination: where is the evidence? *World J Surg* 2009; **33**: 1266-1273 [PMID: 19350321 DOI: 10.1007/s00268-009-9982-9]

P- Reviewer: Abbasoglu O, Nari GA, Roman A

S- Editor: Gong ZM L- Editor: A E- Editor: Li D







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 September 18; 8(26): 1093-1118





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**EDITORIAL**

- 1093** Cholangiocarcinoma, gone without the Wnt?

*Noll ATR, Cramer T, Olde Damink SWM, Schaap FG*

**ORIGINAL ARTICLE****Retrospective Study**

- 1097** Role of epidural anesthesia in a fast track liver resection protocol for cirrhotic patients - results after three years of practice

*Siniscalchi A, Gamberini L, Bardi T, Laici C, Gamberini E, Francorsi L, Faenza S*

**Prospective Study**

- 1105** Immune response to hepatitis B virus vaccine in celiac subjects at diagnosis

*Filippelli M, Garozzo MT, Capizzi A, Spina M, Manti S, Tardino L, Salpietro C, Leonardi S*

**CASE REPORT**

- 1110** Contrast-enhanced ultrasonographic findings of serum amyloid A-positive hepatocellular neoplasm: Does hepatocellular adenoma arise in cirrhotic liver?

*Kumagawa M, Matsumoto N, Watanabe Y, Hirayama M, Miura T, Nakagawara H, Ogawa M, Matsuoka S, Moriyama M, Takayama T, Sugitani M*

**LETTERS TO THE EDITOR**

- 1116** Predictive potential of *IL-28B* genetic testing for interferon based hepatitis C virus therapy in Pakistan: Current scenario and future perspective

*Afzal MS*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Rui-An Xu, PhD, Director, Professor, Engineering Research Center of Molecular Medicine, Ministry of Education, Xiamen 361021, Fujian Province, China

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xin-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL BOARD MEMBERS  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

EDITORIAL OFFICE  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
September 18, 2016

COPYRIGHT  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS  
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION  
<http://www.wjgnet.com/esps/>



## Cholangiocarcinoma, gone without the Wnt?

Anne T R Noll, Thorsten Cramer, Steven W M Olde Damink, Frank G Schaap

Anne T R Noll, Steven W M Olde Damink, Frank G Schaap, Department of Surgery, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, 6200 MD Maastricht, The Netherlands

Thorsten Cramer, Molecular Tumor Biology, Department of General, Visceral and Transplantation Surgery, RWTH Aachen University, D-52074 Aachen, Germany

Thorsten Cramer, Steven W M Olde Damink, Frank G Schaap, Euregional HPB collaboration Aachen-Maastricht, Aachen-Maastricht, Germany-The Netherlands

**Author contributions:** Noll ATR and Schaap FG drafted the manuscript; Cramer T and Olde Damink SWM critically reviewed the manuscript and provided important intellectual content; all authors approved the final version of the manuscript.

**Conflict-of-interest statement:** None of the authors has declared any conflict of interest related to this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Frank G Schaap, PhD, Department of Surgery, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, PO BOX 616, 6200 MD Maastricht, The Netherlands. [frank.schaap@maastrichtuniversity.nl](mailto:frank.schaap@maastrichtuniversity.nl)  
 Telephone: +31-43-3884502

Received: January 15, 2016

Peer-review started: January 19, 2016

First decision: February 29, 2016

Revised: March 18, 2016

Accepted: August 6, 2016

Article in press: August 8, 2016

Published online: September 18, 2016

### Abstract

Cholangiocarcinoma (CCA) is a relatively rare malignancy of the intra- or extra-hepatic bile ducts that is classified according to its anatomical localization as intrahepatic, perihilar or distal. Overall, CCA has a dismal prognosis due to typical presentation at an advanced irresectable stage, lack of effective non-surgical treatments, and a high rate of disease recurrence. CCA frequently arises on a background of chronic liver inflammation and cholestasis. Chronic inflammation is accompanied by enhanced cell turnover with generation of additional inflammatory stimuli, and a microenvironment rich in pro-inflammatory mediators and proliferative factors that enable accumulation of mutations, transformation and expansion of mutated cells. A recent study by Boulter *et al* implicates the Wnt signaling cascade in cholangiocarcinogenesis. Wnt ligands Wnt7B and Wnt10A were found to be highly overexpressed in human CCA tissue. Wnt7B protein was present throughout the tumor stroma, and often co-localized with a subset of CD68<sup>+</sup> macrophages. To address in a direct manner whether Wnt signaling is engaged in development of CCA, Boulter *et al* explored the Wnt signaling pathway in an experimental model that recapitulates the multi-stage progression of human CCA. Wnt ligands found to be elevated in human CCA were also upregulated during the course of CCA development following thioacetamide treatment. Wnt10a increased during the (pre-cancerous) regenerative phase, while Wnt7b induction paralleled tumor growth. Along with upregulation of target genes, the findings demonstrate that the canonical Wnt pathway is progressively activated during cholangio-carcinogenesis. Macrophage depletion, eliminating a major source of Wnt7b, prevented activation of the canonical Wnt cascade, and resulted in reduced number and volume of tumors in this model. Moreover, specific inhibitors of the canonical Wnt pathway (ICG-001 and C-59) caused reduction of tumor area and number, in xenograft and thioacetamide models of CCA. The aggregated findings show that experimental, and presumably human CCA, is a Wnt-driven tumor. Modulation of Wnt signaling, alone or in combination with surgical

or chemotherapy approaches, holds promise in the management of this fatal malignancy.

**Key words:** Intrahepatic cholangiocarcinoma; Liver neoplasms; Carcinogenesis; Wnt signaling pathway; Wnt7B protein; Wnt proteins

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Cholangiocarcinoma (CCA) is a relatively rare malignancy of the intra- or extra-hepatic bile ducts with dismal prognosis. CCA frequently arises on a background of chronic liver inflammation and cholestasis, which creates a microenvironment rich in pro-inflammatory mediators and proliferative factors that enable accumulation of mutations, transformation and expansion of mutated cells. A recent elaborate study by Boulter *et al* (*J Clin Invest* 125:1269) has provided novel insights into the molecular pathogenesis of CCA. Involvement of the Wnt signaling pathway in cholangiocarcinogenesis, and effect of Wnt inhibitors on CCA development *in vivo* are discussed in this Editorial.

Noll ATR, Cramer T, Olde Damink SWM, Schaap FG. Cholangiocarcinoma, gone without the Wnt? *World J Hepatol* 2016; 8(26): 1093-1096 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i26/1093.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i26.1093>

## INTRODUCTION

Cholangiocarcinoma (CCA) is a relatively rare malignancy of the intra- or extrahepatic bile ducts that is classified according to its anatomical localization as intrahepatic, perihilar or distal<sup>[1,2]</sup>. CCA accounts for 10%-20% of the primary liver malignancies, with perihilar (50%-67%) and distal extrahepatic tumors (27%-42%) comprising the majority of CCA cases<sup>[1]</sup>. Tumor biology (*e.g.*, growth pattern, mutation spectrum) and clinical presentation, management and outcomes are different for the three CCA types. Overall, CCA has a dismal prognosis due to typical presentation at an advanced irresectable stage, lack of non-surgical potentially curative treatments, and a high rate of disease recurrence. The five-year survival rate is 5%-10%.

Our understanding of the molecular pathogenesis of CCA is limited. CCA frequently arises on a background of chronic liver inflammation and cholestasis, as reflected by risk factors of cholangiocarcinogenesis (*e.g.*, liver cirrhosis, viral hepatitis, hepatolithiasis, liver fluke infestation, primary sclerosing cholangitis). Chronic inflammation is accompanied by enhanced cell turnover with generation of additional inflammatory stimuli, and a microenvironment rich in pro-inflammatory mediators and proliferative factors that enable accumulation of mutations, transformation and expansion of mutated cells<sup>[3,4]</sup>. Cholestasis may contribute to cholangiocarcinogenesis through effects of (conjugated)

bile salts on proliferation and invasion of cholangiocytes<sup>[5,6]</sup>.

The overall incidence of CCA has increased over the past decades and this is attributed to a global rise in the incidence of intrahepatic CCA. Liver transplantation is generally not considered for treatment of CCA due to frequent tumor recurrence and poor five-year survival rates after liver transplantation for intrahepatic CCA. Hence, resection is the only potentially curative treatment of CCA. The majority of patients with CCA, however, do not qualify for surgery and have to resort to palliative therapies. Molecular-targeted therapies hold potential for personalized treatment of malignancies including CCA<sup>[7]</sup>. A recent study by Boulter *et al*<sup>[8]</sup> implicates the Wnt signaling cascade in cholangiocarcinogenesis. Importantly, specific inhibitors of this pathway prevented tumor development in animal models of CCA.

## THE WNT SIGNALING CASCADE

Wnt signaling is initiated by binding of membrane-bound Wnt ligand to a transmembrane receptor of the Frizzled family, and can operate in autocrine and paracrine modes<sup>[9-11]</sup>. Wnt ligands are a family of secreted glycoproteins that have undergone a lipid modification (Cys-palmitoylation) that is essential for biological activity. The Frizzled family are G-protein coupled receptors that, alone or in conjunction with co-receptors (*e.g.*, Lrp5/6), serve as binding sites for Wnt ligands. Canonical Wnt signaling results in a transcriptional response in which the transcription factor  $\beta$ -catenin plays a central role, whereas non-canonical Wnt signaling cascades control the cytoskeletal structure or intracellular  $\text{Ca}^{2+}$  content through  $\beta$ -catenin-independent non-genomic actions. The canonical Wnt signaling pathway is the focus of the studies of Boulter *et al*<sup>[8]</sup>. In the absence of Wnt signaling,  $\beta$ -catenin is targeted for proteasomal degradation by a multi-protein complex. Formation of this degradation complex is abrogated by activation of Wnt signaling, resulting in cytoplasmic accumulation and subsequent nuclear translocation of  $\beta$ -catenin. There,  $\beta$ -catenin acts in concert with other transcription factors (*e.g.*, TCF/LEF family members) to activate expression of target genes including cell cycle-related genes (*e.g.*, *CCND2*, *CDKN2A*).

Wnt signaling was identified through its role in carcinogenesis<sup>[10]</sup>, but not surprisingly found to participate in normal development and adult tissue homeostasis as well. Mutations in downstream components of the Wnt signaling pathway have been identified in various types of human cancers<sup>[11,12]</sup>. For example, adenomatous polyposis coli (APC), a tumor suppressor that is part of the  $\beta$ -catenin degradation complex, is frequently mutated in colorectal and gastric cancers. Mutations in  $\beta$ -catenin that enhance protein stability, exemplifying a gain-of-function mutation, have been found in hepatocellular carcinoma<sup>[11]</sup>. Hepatic adenomas that are positive for  $\beta$ -catenin have a high risk for malignant conversion and are typically resected, whereas other adenoma types are generally left untreated<sup>[13]</sup>. Targeting of the Wnt

pathway is being explored as treatment of Wnt-driven malignancies<sup>[14]</sup>.

## WNT SIGNALING IN CCA

By analyzing tumoral and matched unaffected liver tissue of patients with intrahepatic or perihilar CCA, Boulter *et al.*<sup>[8]</sup> demonstrate that Wnt ligands Wnt7B and Wnt10A are highly overexpressed in tumor tissue. Wnt7B protein was present throughout the tumor stroma, often co-localizing with a subset of CD68<sup>+</sup> macrophages. Moreover, tumors displayed elevated levels of transcripts of known  $\beta$ -catenin targets (*e.g.*, *CCND2*, *CDKN2A*, *BIRC5*), and cancerous biliary epithelium showed increased immunohistochemical positivity for a number of  $\beta$ -catenin targets. These findings suggest that canonical Wnt signaling is activated in human CCA.

To address in a direct manner whether Wnt signaling is engaged in development of CCA, this pathway was further explored in an experimental model that recapitulates the multi-stage progression (*i.e.*, chronic cholangiocyte damage, inflammation, biliary repair/regeneration, tumorigenesis) of human CCA. For this purpose, rats were treated with thioacetamide (TAA) and sacrificed at pre-cancerous (0-16 wk of treatment) and cancerous (20-26 wk of treatment) stages. Mirroring end-stage CCA in humans, strong nuclear  $\beta$ -catenin staining was observed in cancerous epithelium. In the pre-cancerous stage, regenerating ductules showed a membranous staining pattern. Wnt ligands found to be elevated in human CCA were also upregulated during the course of CCA development following TAA treatment. Wnt10a increased during the (pre-cancerous) regenerative phase, while Wnt7b induction paralleled tumor growth. Along with upregulation of target genes, above findings demonstrate that the canonical Wnt pathway is progressively activated during cholangiocarcinogenesis.

Through an elegant set of experiments Boulter *et al.*<sup>[8]</sup> demonstrate that Wnt7B in tumor stroma is largely derived from recruited bone marrow-derived macrophages rather than from resident Kupffer cells, and that these cells play a key role in CCA progression. The role of macrophages in CCA growth was initially assessed in mice xenografted with three different human CCA cell lines. Groups of mice with established palpable subcutaneous tumors received vehicle or treatments to deplete phagocytic macrophages or prevent differentiation of monocytes into macrophages. Xenograft characteristics were determined after a further growth period of 3 wk. Loss of macrophages by either of the two strategies, resulted in reduced number of CD68<sup>+</sup> macrophages and decreased Wnt7b expression in all xenografts. In two out of three xenografted CCA cell lines (*i.e.*, CC-LP-1 and SNU-1079, derived from intrahepatic CCA) this was accompanied by decreased expression of (human) proliferative genes, increased apoptosis and lowered tumor burden. The lack of functional consequences, despite loss

of Wnt signal, in xenografts derived from the third cell line (*i.e.*, WITT-1) may relate to its different origin (distal extrahepatic CCA) and/or distinct growth requirements. As a model more representative in terms of tumor micro-environment (stroma) and disease progression, Boulter *et al.*<sup>[8]</sup> then studied the consequences of macrophage depletion (liposomal clodronate) in TAA-induced CCA. Strikingly, macrophage ablation prevented activation of the canonical Wnt cascade (loss of tumoral Wnt7B signal) and resulted in reduced number and volume of tumors.

The aggregated findings show that experimental, and presumably human CCA, is a Wnt-driven tumor. Since general macrophage depletion is not feasible in clinical practice, Boulter *et al.*<sup>[8]</sup> explored the impact of specific inhibitors of the canonical Wnt pathway in the xenograft- and TAA-model of CCA. For this, they chose two targets that were elevated in human CCA, namely *CTBP1* and *PORCN*. CTBP1 interacts with  $\beta$ -catenin to drive expression of growth-stimulating Wnt target genes, and their interaction can be targeted by ICG-001<sup>[15]</sup>. As mentioned above, Wnt ligands require palmitoylation for biological activity and this lipid modification can be prevented by the PORCN inhibitor C-59<sup>[16,17]</sup>. Both ICG-001 and C-59 were effective in reducing *in vitro* growth of five human CCA cell lines with presumed autocrine activation of canonical Wnt signaling (constitutive Wnt7B and  $\beta$ -catenin expression). Similar to macrophage ablation, ICG-001 and C-59 reduced tumor volume and mass in two CCA cell lines of intrahepatic origin when xenografted in mice, but did not affect WITT-1 xenograft growth. The reliance on Wnt signaling for proliferation and survival of CCA cells was confirmed in TAA-induced CCA, with ICG-001 and C-59 causing reduction of tumor area and number. Importantly, neither treatment affected body weight or caused liver test abnormalities, side effects observed with use of earlier generation Wnt inhibitors<sup>[14]</sup>.

## PERSPECTIVE

The work of Boulter *et al.*<sup>[8]</sup> demonstrates that the canonical Wnt pathway is activated in intrahepatic and perihilar CCA. Inhibition of canonical Wnt signaling, either by depleting the macrophage source of Wnt ligand or *via* pharmacological blockage, reduces CCA formation in a rat model that closely resembles human CCA. This is achieved through stimulation of apoptosis and reduced cell cycle entry. Thus, Wnt signaling is important for proliferation and survival of CCA cells in the TAA-model. It remains to be determined whether human CCA growth/progression is Wnt-dependent, and hence amenable to targeting by Wnt pathway inhibitors. More detailed insight into the interaction of Wnt signaling with the complex cellular surrounding, and its integration with other cellular signaling cascades, is warranted. Time will tell if systemic or CCA-directed Wnt inhibition, alone or in combination with surgical or chemotherapy approaches, will improve clinical outcomes of this fatal malignancy.

## REFERENCES

- 1 **Ghouri YA**, Mian I, Blechacz B. Cancer review: Cholangiocarcinoma. *J Carcinog* 2015; **14**: 1 [PMID: 25788866 DOI: 10.4103/1477-3163.151940]
- 2 **Rizvi S**, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. *Gastroenterology* 2013; **145**: 1215-1229 [PMID: 24140396 DOI: 10.1053/j.gastro.2013.10.013]
- 3 **Sia D**, Tovar V, Moeini A, Llovet JM. Intrahepatic cholangiocarcinoma: pathogenesis and rationale for molecular therapies. *Oncogene* 2013; **32**: 4861-4870 [PMID: 23318457 DOI: 10.1038/onc.2012.617]
- 4 **Zabron A**, Edwards RJ, Khan SA. The challenge of cholangiocarcinoma: dissecting the molecular mechanisms of an insidious cancer. *Dis Model Mech* 2013; **6**: 281-292 [PMID: 23520144 DOI: 10.1242/dmm.010561]
- 5 **Liu R**, Zhao R, Zhou X, Liang X, Campbell DJ, Zhang X, Zhang L, Shi R, Wang G, Pandak WM, Sirica AE, Hylemon PB, Zhou H. Conjugated bile acids promote cholangiocarcinoma cell invasive growth through activation of sphingosine 1-phosphate receptor 2. *Hepatology* 2014; **60**: 908-918 [PMID: 24700501 DOI: 10.1002/hep.27085]
- 6 **Werneburg NW**, Yoon JH, Higuchi H, Gores GJ. Bile acids activate EGF receptor via a TGF- $\alpha$ -dependent mechanism in human cholangiocyte cell lines. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G31-G36 [PMID: 12606307 DOI: 10.1152/ajpgi.00536.2002]
- 7 **Sia D**, Losic B, Moeini A, Cabellos L, Hao K, Revill K, Bonal D, Miltiadous O, Zhang Z, Hoshida Y, Cornella H, Castillo-Martin M, Pinyol R, Kasai Y, Roayaie S, Thung SN, Fuster J, Schwartz ME, Waxman S, Cordon-Cardo C, Schadt E, Mazzaferro V, Llovet JM. Massive parallel sequencing uncovers actionable FGFR2-PPHLN1 fusion and ARAF mutations in intrahepatic cholangiocarcinoma. *Nat Commun* 2015; **6**: 6087 [PMID: 25608663 DOI: 10.1038/ncomms7087]
- 8 **Boulter L**, Guest RV, Kendall TJ, Wilson DH, Wojtacha D, Robson AJ, Ridgway RA, Samuel K, Van Rooijen N, Barry ST, Wigmore SJ, Sansom OJ, Forbes SJ. WNT signaling drives cholangiocarcinoma growth and can be pharmacologically inhibited. *J Clin Invest* 2015; **125**: 1269-1285 [PMID: 25689248 DOI: 10.1172/JCI76452]
- 9 **Niehrs C**. The complex world of WNT receptor signalling. *Nat Rev Mol Cell Biol* 2012; **13**: 767-779 [PMID: 23151663 DOI: 10.1038/nrm3470]
- 10 **Nusse R**, Varmus H. Three decades of Wnts: a personal perspective on how a scientific field developed. *EMBO J* 2012; **31**: 2670-2684 [PMID: 22617420 DOI: 10.1038/emboj.2012.146]
- 11 **Polakis P**. Wnt signaling in cancer. *Cold Spring Harb Perspect Biol* 2012; **4**: pii: a008052 [PMID: 22438566 DOI: 10.1101/cshperspect.a008052]
- 12 **McMillan M**, Kahn M. Investigating Wnt signaling: a chemogenomic safari. *Drug Discov Today* 2005; **10**: 1467-1474 [PMID: 16243267 DOI: 10.1016/S1359-6446(05)03613-5]
- 13 **Bioulac-Sage P**, Laumonier H, Couchy G, Le Bail B, Sa Cunha A, Rullier A, Laurent C, Blanc JF, Cubel G, Trillaud H, Zucman-Rossi J, Balabaud C, Saric J. Hepatocellular adenoma management and phenotypic classification: the Bordeaux experience. *Hepatology* 2009; **50**: 481-489 [PMID: 19585623 DOI: 10.1002/hep.22995]
- 14 **Kahn M**. Can we safely target the WNT pathway? *Nat Rev Drug Discov* 2014; **13**: 513-532 [PMID: 24981364 DOI: 10.1038/nrd4233]
- 15 **Gang EJ**, Hsieh YT, Pham J, Zhao Y, Nguyen C, Huantes S, Park E, Naing K, Klemm L, Swaminathan S, Conway EM, Pelus LM, Crispino J, Mullighan CG, McMillan M, Mischen M, Kahn M, Kim YM. Small-molecule inhibition of CBP/catenin interactions eliminates drug-resistant clones in acute lymphoblastic leukemia. *Oncogene* 2014; **33**: 2169-2178 [PMID: 23728349 DOI: 10.1038/onc.2013.169]
- 16 **Proffitt KD**, Madan B, Ke Z, Pendharkar V, Ding L, Lee MA, Hannoush RN, Virshup DM. Pharmacological inhibition of the Wnt acyltransferase PORCN prevents growth of WNT-driven mammary cancer. *Cancer Res* 2013; **73**: 502-507 [PMID: 23188502 DOI: 10.1158/0008-5472.CAN-12-2258]
- 17 **Takada R**, Satomi Y, Kurata T, Ueno N, Norioka S, Kondoh H, Takao T, Takada S. Monounsaturated fatty acid modification of Wnt protein: its role in Wnt secretion. *Dev Cell* 2006; **11**: 791-801 [PMID: 17141155 DOI: 10.1016/j.devcel.2006.10.003]

**P- Reviewer:** Abdel-Wahab M, Chetty R, Qin JM, Xu R  
**S- Editor:** Kong JX **L- Editor:** A **E- Editor:** Li D





Retrospective Study

# Role of epidural anesthesia in a fast track liver resection protocol for cirrhotic patients - results after three years of practice

Antonio Siniscalchi, Lorenzo Gamberini, Tommaso Bardi, Cristiana Laici, Elisa Gamberini, Letizia Francorsi, Stefano Faenza

Antonio Siniscalchi, Lorenzo Gamberini, Tommaso Bardi, Cristiana Laici, Elisa Gamberini, Letizia Francorsi, Stefano Faenza, Division of Anesthesiology, Alma Mater Studiorum University of Bologna, 40138 Bologna, Italy

**Author contributions:** All the authors contributed to this manuscript.

**Institutional review board statement:** Approved by the Policlinico S. Orsola Malpighi review board.

**Informed consent statement:** Approved by the Policlinico S. Orsola Malpighi review board.

**Conflict-of-interest statement:** The authors of this study certify that they have no affiliations with, or involvement in any organization or entity with any financial or non-financial interest, relating to the subject matter or the materials discussed in this manuscript. This study was fully supported by the Department of Anesthesiology of the University of Bologna.

**Data sharing statement:** No data were created so no data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Antonio Siniscalchi, MD, Division of Anesthesiology, Alma Mater Studiorum University of Bologna, Via Massarenti 9, 40138 Bologna, Italy. [sinianest@libero.it](mailto:sinianest@libero.it)  
 Telephone: +39-05-12143440

Received: May 8, 2016  
 Peer-review started: May 8, 2016  
 First decision: June 13, 2016  
 Revised: June 22, 2016  
 Accepted: August 11, 2016  
 Article in press: August 15, 2016  
 Published online: September 18, 2016

## Abstract

### AIM

To evaluate the potential benefits and risks of the use of epidural anaesthesia within an enhanced recovery protocol in this specific subpopulation.

### METHODS

A retrospective review was conducted, including all cirrhotic patients who underwent open liver resection between January 2013 and December 2015 at Bologna University Hospital. Patients with an abnormal coagulation profile contraindicating the placement of an epidural catheter were excluded from the analysis. The control group was composed by patients refusing epidural anaesthesia.

### RESULTS

Of the 183 cirrhotic patients undergoing open liver resections, 57 had contraindications to the placement of an epidural catheter; of the remaining 126, 86 patients received general anaesthesia and 40 combined anaesthesia. The two groups presented homogeneous characteristics. Intraoperatively the metabolic data did not differ between the two groups, whilst the epidural group had a lower mean arterial pressure ( $P = 0.041$ ) and received more colloid infusions ( $P = 0.007$ ). Postoperative liver and kidney function did not differ significantly.

Length of mechanical ventilation ( $P = 0.003$ ) and hospital stay ( $P = 0.032$ ) were significantly lower in the epidural group. No complications related to the epidural catheter placement or removal was recorded.

## CONCLUSION

The use of Epidural Anaesthesia within a fast track protocol for cirrhotic patients undergoing liver resections had a positive impact on the patient's outcomes and comfort as demonstrated by a significantly lower length of mechanical ventilation and hospital stay in the epidural group. The technique appears to be safely manageable in this fragile population even though these results need confirmation in larger studies.

**Key words:** Anesthesia; Postoperative care; Analgesia; Epidural; Postoperative; Liver cirrhosis; Liver function tests; Complication

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This retrospective study evaluates the potential benefits and risks of the use of epidural anaesthesia within an enhanced recovery protocol in the subpopulation of cirrhotic patients undergoing liver resection. We included all cirrhotic patients who underwent open liver resection between January 2013 and December 2015 at our Unit. The study included 126 cirrhotic patients, 86 patients received general anaesthesia and 40 combined anaesthesia. The two groups presented homogeneous characteristics. The epidural group had a lower intraoperative mean arterial pressure ( $P = 0.041$ ) and received more colloid infusions ( $P = 0.007$ ). Postoperative liver and kidney function did not differ significantly. Length of mechanical ventilation ( $P = 0.003$ ) and hospital stay were significantly lower ( $P = 0.032$ ) in the epidural group. No complications related to the epidural catheter management were recorded.

Siniscalchi A, Gamberini L, Bardi T, Laici C, Gamberini E, Francorsi L, Faenza S. Role of epidural anesthesia in a fast track liver resection protocol for cirrhotic patients - results after three years of practice. *World J Hepatol* 2016; 8(26): 1097-1104 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i26/1097.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i26.1097>

## INTRODUCTION

Fast track surgery or Enhanced Recovery after Surgery (ERAS®) programmes have been first described in the 1990s in the field of colo-rectal surgery<sup>[1]</sup>. These programmes entail a number of evidence based actions aimed at reducing unnecessary perioperative stress and inflammation, and restoring as quickly as possible the normal preoperative physiology. Since their first introduction ERAS programmes are being implemented in different surgical specialties, and in more recent

times also in the field of liver surgery<sup>[2-6]</sup>. A recent meta-analysis<sup>[7]</sup> evaluating five randomized controlled trials, has consolidated the evidence indicating that ERAS applied to liver resection surgery has a positive impact on post-operative complications and length of hospital stay.

The use of epidural anesthesia and analgesia is a vital part of any enhanced recovery program, mostly because it blunts the neuroendocrine response to surgical stress and allows better postoperative pain control and faster mobilization. Epidural analgesia has been widely applied in the field of open liver surgery with very positive results in terms of reduction in pain scores<sup>[8]</sup>. However cirrhotic patients undergoing liver resection represent a special subpopulation with a high risk of developing perioperative complications. In these patients the preoperative liver function, and the future remnant liver volume, are critical factors in determining perioperative morbidity<sup>[9]</sup> and the placement of an epidural catheter, and its management, could present potential risks, most of which related to coagulation disorders<sup>[10]</sup>.

Another aspect to be taken into consideration is hemodynamics, in fact the cirrhotic hyperdynamic circulation could be particularly influenced by the sympathetic blockade produced by an epidural block, potentially leading to splanchnic malperfusion, which could be reflected in postoperative organ dysfunction.

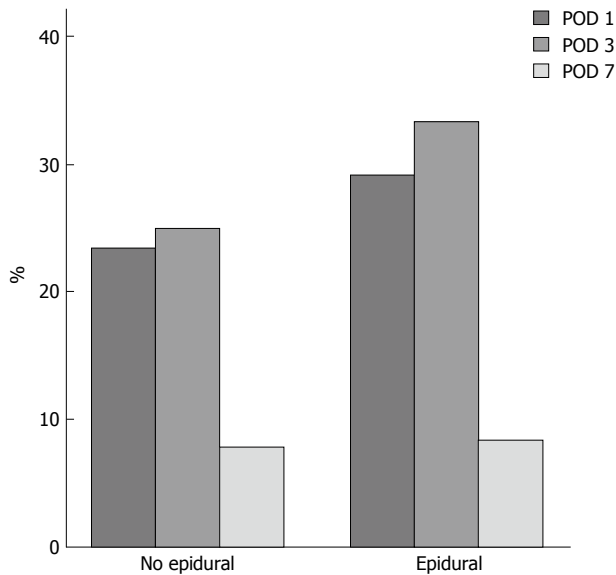
In a previous study<sup>[11]</sup> we evaluated the incidence of post liver resection coagulopathy in cirrhotic patients, and discussed its hypothetic impact on the management of an epidural catheter (Figure 1). Following the results of this study, we have implemented a wider use of Epidural analgesia and anaesthesia also in a selected population of cirrhotic patients undergoing Liver resections. To date there are no studies considering the application of ERAS protocols to cirrhotic patients and the importance of epidural anesthesia within these protocols. Moreover most of the studies considering ERAS protocols applied to liver surgery populations have included patients undergoing liver resections for colorectal metastasis<sup>[3,5,6,12]</sup>, in whom underlying liver function is expected to be normal.

The primary objective of this retrospective observational study was to evaluate the use of epidural analgesia in an ERAS program dedicated to cirrhotic patients undergoing liver resection for hepatocellular carcinoma (HCC) in terms of length of hospital stay, and incidence of complications.

Secondary objectives of the study were to evaluate the differences in terms of intraoperative hemodynamic stability, fluid management and postoperative liver and kidney function tests.

## MATERIALS AND METHODS

Following the approval of our Hospital Ethics Committee (approval number: 100/2014/O/OssN), we conducted a retrospective observational review including all cirrhotic patients who underwent open liver resection between January 2013 and December 2015. Inclusion criteria



**Figure 1 Course of post-operative coagulopathy.** The figure displays the percentage of the patients in the two study groups presenting significant alterations of coagulation exams. Platelets count  $< 100000/\mu\text{L}$  or INR  $> 1.5$  post operatively at day 1, 3, 7. INR: International normalized ratio; POD: Postoperative days.

were: Age  $> 18$  years, histologically proven liver cirrhosis, open liver resection surgery for HCC. Exclusion criteria were: Abnormal preoperative coagulation profile contraindicating an epidural catheter placement [international normalized ratio (INR) values  $\geq 1.5$  and/or platelet count  $< 100.000/\mu\text{L}$ <sup>[13]</sup>], laparoscopic liver resection.

Major hepatic resection was defined as a resection of three or more hepatic segments, whilst a minor hepatic resection was defined as a resection of two or fewer hepatic segments in accordance to the IHPBA classification<sup>[14]</sup>. All the liver resections were performed to achieve a tumor-free margin of at least 1 cm based on intraoperative examination and ultrasonography.

Patients were divided into two groups on the basis of the placement of an epidural catheter. The control group was composed by the patients who refused the placement of an epidural catheter, at the pre operative interview with the anesthetist. The same team of surgeons performed all of the surgical procedures. The ERAS protocol was applied to each patient included in this study. The main features of the ERAS protocol for cirrhotic patients used at our unit are described in Table 1. The anesthetic management for liver resection at our unit includes: General endotracheal anaesthesia, arterial line and central venous catheter placement for fluid infusions, hemodynamic monitoring (EKG, arterial blood pressure, CVP), and acid-base parameter measurement (blood gas analysis data).

General anesthesia is induced with propofol (2-2.5 mg/kg), fentanyl (1-2 mcg/kg) and rocuronium (0.6 mg/kg), while Sevoflurane 0.7-1.0 MAC and boluses of rocuronium and fentanyl are used for maintenance.

For combined anesthesia a T8-T9 epidural catheter

**Table 1 Fast track protocol for cirrhotic patients undergoing liver resection**

**Fast track protocol for cirrhotic patients undergoing liver resection**

Preoperative counseling
Regular diet on the day before surgery
No bowel preparation
Intraoperative CVP target $< 6$ mmHg, restricted fluids administration
ICU admission for at least the first post-operative night (or on POD 0)
Maintenance fluids discontinued on POD 3
Nasogastric probe removal on POD 1
Liquid diet on POD 1
Regular diet on POD 3
Urinary catheter discontinued on POD 3
Drains removal on POD 3
Ambulation on POD 3
Discharge criteria: Liver and kidney function tests compatible with preoperative data or decreasing, able to tolerate food intake, able to ambulate, good pain control (NRS $< 3$ )

ICU: Intensive care unit; CVP: Central venous pressure; POD: Postoperative days; NRS: Numerical rating scale.

is positioned before anesthesia induction. Anesthesia is induced with propofol, fentanyl and rocuronium, at the same dosages mentioned above, the epidural anesthesia is induced with an initial bolus of L-bupivacaine 7.5-10 mg and 10 mcg sufentanil, followed by a continuous infusion at 5 to 7 mL/h of L-bupivacaine 2.5 mg/mL. Narcosis is maintained with Sevoflurane at a concentration of 0.5-0.7 MAC, adequate muscle paralysis is maintained with boluses of rocuronium.

Postoperative pain control in patients without epidural is maintained with a PCA system with intravenous morphine (1-2 mg/h continuous infusion, bolus 1 mg, lock-out 15 min, maximum dose in 4 h 18 mg) and boluses of paracetamol (1 g intravenous, max 3 g per day), when oral intake is possible, morphine is substituted with oxycodone.

Postoperative pain control in patients without epidural is maintained with a PCA system with intravenous morphine (1-2 mg/h, bolus 1 mg, lockout 15 s, maximum dose in 4 h 18 mg) and boluses of paracetamol (1 g intravenous, max 3 g/d), when oral intake is possible, morphine is substituted with oxycodone.

In patients with the epidural catheter, postoperative analgesia is maintained with a continuous epidural infusion of L-bupivacaine 1.25 mg/mL and sufentanil 0.5 mcg/mL at a rate of 5-7 mL/h. After the first 36 hour post operatively only the local anesthetic infusion was maintained and the opioid stopped. Intravenous paracetamol (1 g iv, max 3 g/d) is added if more analgesia is needed.

Fluid infusions during hepatic dissection follow the units protocol and target a low central venous pressure ( $\leq 6$  mmHg). Red blood cells in cirrhotic patients are transfused when hematocrit is lower than 24% and/or hemoglobin is lower than 8 g/dL. The occurrence of hypothermia was prevented by infusion of warm fluids, forced-air warming and the use of warm water on the surgical field.

**Table 2** Preoperative data

	Group no epidural (n = 86)	Group epidural (n = 40)	P
Sex male (%)	69 (80.2%)	30 (75%)	0.655
Age (yr)	63.28 ± 11.38	62.8 ± 11.92	0.832
BMI	26.65 ± 4.36	25.23 ± 5.50	0.155
Cirrhosis etiology			
HBV	19	10	0.947
HCV	49	27	0.434
Alcohol	9	2	0.501
Other	13	3	0.341
Type of resection			
Major	19	12	0.461
Minor	67	28	
Preoperative data			
AST (UI/L)	50.8 ± 47.4	46.9 ± 29.1	0.586
ALT (UI/L)	47.4 ± 43.1	52.3 ± 38.5	0.541
Bilirubin (mg/dL)	0.80 ± 0.43	0.77 ± 0.40	0.710
INR	1.14 ± 0.12	1.10 ± 0.79	0.092
Creatinine (mg/dL)	1.08 ± 1.24	0.87 ± 0.26	0.164
Urea (mg/dL)	37.25 ± 13.74	36.84 ± 11.79	0.867
Platelet count	180134 ± 83856	211079 ± 94262	0.088

HBV: Hepatitis B virus; BMI: Body mass index; HCV: Hepatitis C virus; AST: Aspartate aminotransferase; ALT: Alanine transaminase; INR: International normalized ratio.

Perioperative coagulation alterations are corrected according to POC coagulation testing using a tromboelastograph (TEG®).

All patients at end of surgery were admitted to the intensive care unit (ICU). The routine ICU admission for at least the first post-operative night is a part of the ERAS protocol for cirrhotic patients used at our unit (Table 1).

Data collected preoperatively included patient characteristics, underlying surgical pathology, etiology of cirrhosis, MELD score, baseline coagulation profile and blood tests.

Intraoperative data analyzed included type of hepatic resection, fluid infusions and transfusion of blood products, while hemodynamics and blood gas analysis data were registered at the beginning of the intervention, after resection and at the end of surgery.

Postoperative blood tests collected were liver and kidney function tests on postoperative days (POD) 1, 3 and 7. Postoperative complications were also evaluated using Clavien-Dindo classification, acute kidney injury was classified following AKI network criteria.

### Statistical analysis

Statistical analysis was carried out with IBM SPSS 21. Categorical data were expressed as numbers (percentages), continuous variables as mean and standard deviation. Differences in perioperative data between groups were evaluated with *t*-test for continuous variables and  $\chi^2$  test or Fisher exact test for nominal variables. A general linear model for repeated measures was used to compare postoperative function tests and intraoperative measures of arterial pressure, central venous pressure and blood gas analysis data. For Clavien-Dindo classification and postoperative kidney

injury evaluated with AKIN score, Mann-Whitney test was used.

## RESULTS

From January 2013 to December 2015, 183 cirrhotic patients underwent elective open hepatic resection for hepatocellular carcinoma at the Department of Surgery and Transplantation of Bologna University. Fifty-six of these were excluded because their preoperative coagulation profile was incompatible with the placement of an epidural catheter. The remaining 126 patients were included in the study and divided into two groups on the basis of the presence of an epidural catheter during surgery; 86 patients received a general endotracheal anesthesia (group no epidural) while 40 patients received a combined anesthesia (group epidural). All of the patients who received epidural anaesthesia, could effectively control post-operative pain with the epidural protocol and did not require intravenous opioids, also no catheter displacement occurred. The two groups were homogeneous for the demographic aspects, and etiology of cirrhosis, Table 2 shows preoperative data. Intraoperative data showed a significantly lower mean arterial pressure during resection and higher hypotension time and colloids infusions in the epidural group (Table 3), whilst central venous pressure (CVP) and metabolic data in terms of pH, lactate and base excess were not significantly different. Postoperative liver and kidney function tests, as well as platelet count did not significantly differ between the two groups (Table 4).

The course of postoperative coagulopathy is shown in Graph 1, we have to highlight that on POD 7, 6 patients out of 126 still had a measurable coagulopathy (INR > 1.5 and/or Plt < 100000/ $\mu$ L). Amongst these patients 3 had undergone a minor resection and one a major resection under general anaesthesia. The remaining 2 patients with coagulopathy had undergone a major liver resection with a combined anesthesia and had to have their coagulations profiles corrected before a safe removal of the epidural catheter could be performed. The correction was performed with the infusion of FFP and there were no complications after the removal of the catheter.

The length of ICU stay did not significantly differ between the two groups. The duration of mechanical ventilation and length of hospital stay were significantly lower in the epidural group (Table 5).

The rate of complications and their severity classified following Clavien-Dindo score and postoperative acute kidney injury did not differ, however 9 cases of post-operative delirium were recorded, all of which occurred in the general anesthesia group.

In the epidural group no complications related to epidural catheter placement or removal were recorded. Epidural catheters were usually removed between POD 3 and 5 and there was no need for major analgesics adjuncts in these patients.



**Table 3** Intraoperative data

		Group no epidural (n = 86)	Group epidural (n = 40)	P
Hemodynamic parameters				
MAP	Baseline	94.4 ± 12	89.5 ± 11.8	0.035
(mmHg)	Post-resection	77.3 ± 16.9	71.2 ± 9.7	0.041
(P = 0.004)	End of surgery	74.9 ± 10.7	74.9 ± 10.7	0.048
CVP	Baseline	8.26 ± 3.4	8.92 ± 3.15	0.323
(mmHg)	Post-resection	6.0 ± 3.25	5.76 ± 3.13	0.704
(P = 0.991)	End of surgery	7.35 ± 3.13	6.92 ± 2.57	0.466
Metabolic parameters				
pH	Baseline	7.44 ± 0.043	7.44 ± 0.055	0.717
(P = 0.627)	Post-resection	7.40 ± 0.053	7.39 ± 0.053	0.608
	End of surgery	7.38 ± 0.573	7.39 ± 0.062	0.258
Lac (mmol/L)	Baseline	2.07 ± 3.15	1.98 ± 3.11	0.925
(P = 0.894)	Post-resection	4.22 ± 6.11	4.68 ± 7.40	0.800
	End of surgery	2.59 ± 2.20	2.62 ± 1.88	0.958
BE (mEq/L)	Baseline	1.27 ± 2.10	1.6 ± 1.94	0.563
(P = 0.343)	Post-resection	-1.93 ± 2.37	-1.31 ± 2.61	0.354
	End of surgery	-2.72 ± 2.88	-2.56 ± 2.92	0.499
Other data				
Lenght of surgery (min)		250.4 ± 93.48	267.6 ± 88.97	0.326
Hypotension duration (min)		2.28 ± 4.52	5.43 ± 6.68	0.006
Cristalloids infusions (mL)		2768 ± 1213	2574 ± 1022	0.354
Colloids infusions (mL)		259 ± 320	428 ± 312	0.007
RBC transfusions (U)		0.06 ± 0.239	0.01 ± 0.304	0.470
Total diuresis (mL)		467 ± 376	552 ± 384	0.248

MAP: Mean arterial pressure; RBC: Red blood count; Lac: Lactate; BE: Base excess; CVP: Central venous pressure.

**Table 4** Post-operative data

		Group no epidural (n = 86)	Group epidural (n = 40)	P
Hepatic function tests				
AST (UI/L)	POD 1	205 ± 141	238 ± 168	0.239
(P = 0.451)	POD 3	97 ± 66	96 ± 54	0.334
	POD 7	49 ± 29	52 ± 26	0.636
ALT (UI/L)	POD 1	195 ± 161	229 ± 210	0.144
(P = 0.605)	POD 3	157 ± 126	157 ± 106	0.391
	POD 7	67 ± 47	73 ± 40	0.884
Bilirubin (mg/dL)	POD 1	1.60 ± 1.0	1.60 ± 0.88	0.994
(P = 0.557)	POD 3	1.8 ± 1.05	1.57 ± 0.81	0.306
	POD 7	1.38 ± 1.06	1.26 ± 1.31	0.636
INR	POD 1	1.34 ± 0.18	1.31 ± 0.20	0.593
(P = 0.544)	POD 3	1.31 ± 0.16	1.30 ± 0.25	0.899
	POD 7	1.26 ± 0.14	1.12 ± 0.15	0.319
Platelet count	POD 1	163649 ± 78332	148015 ± 72007	0.647
(P = 0.532)	POD 3	148015 ± 72007	132275 ± 43514	0.277
	POD 7	191073 ± 74978	187586 ± 63602	0.827
Kidney function tests				
Creatinine (mg/dL)	POD 1	0.96 ± 0.87	0.87 ± 0.42	0.579
(P = 0.417)	POD 3	0.98 ± 1.04	0.79 ± 0.29	0.331
	POD 7	0.96 ± 1.15	0.78 ± 0.28	0.410
Urea (mg/dL)	POD 1	33.37 ± 13.87	32.86 ± 12.17	0.866
(P = 0.315)	POD 3	38.0 ± 21.17	33.79 ± 14.27	0.332
	POD 7	35.57 ± 20.13	29.76 ± 11.67	0.151

For repeated measures, the *P* value expressed under the variable is referred to the between subjects effect test. AST: Aspartate aminotransferase; ALT: Alanine transaminase; INR: International normalized ratio; POD: Postoperative days.

## DISCUSSION

The results of this study suggest that the use of epidural anaesthesia and analgesia in the context of ERAS<sup>®</sup> protocols for cirrhotic patients undergoing liver surgery is feasible. In fact none of the patients in the epidural

group had complications related to the positioning or the removal of the epidural catheter. However, the incidence of an epidural complication requiring an elective surgical treatment varies between 1 event in 22189 and 1 event in 4330 epidural placements in the general population<sup>[15]</sup>. Hence to consistently rule out the potential

**Table 5** Hospital length of stay and complications

	Group no epidural (n = 86)	Group epidural (n = 40)	P
Post operative MV length (h)	7.34 ± 18.11	1.29 ± 1.74	0.003
ICU stay (d)	2.78 ± 2.35	2.43 ± 1.57	0.183
Total PO hospital stay (d)	11.49 ± 7.95	8.65 ± 3.26	0.032
AKIN (grade)			
0	81	40	0.121
1	3	0	
2	1	0	
3	1	0	
DINDO (grade)			
1	30	15	0.262
2	19	9	
3	10	1	
4	0	0	
5	0	0	

MV: Mechanical ventilation; PO: Postoperative; AKIN: Acute kidney injury network classification; DINDO: Clavien dindo classification of surgical complications.

safety issues relating to epidural catheters in the specific subpopulation of cirrhotic patients, a larger sample should be considered.

Postoperative coagulopathy is considered another great risk in cirrhotic patients, often limiting the use of regional anesthesia techniques in this subpopulation. However the incidence of the postoperative coagulopathy, especially in minor resections, appears to be compatible with the safe management of an epidural catheter.

It also must be underlined that hemostasis alterations in cirrhotic patients are more complex than a simple increase in hemorrhagic risk due to coagulation factors deficiency<sup>[16]</sup>. Hence laboratory values such as the INR and platelet count do not describe entirely the wide array of alterations, which constitute the hemorrhagic risk of these patients. Probably in the near future thromboelastometry will have a major role in better defining the individual coagulation profile. Moreover, neuraxial blocks are safely undertaken even in patients assuming platelets inhibitors such as ASA and undergoing surgical interventions in which systemic anticoagulation is prescribed in the postoperative period, such as peripheral vascular surgery<sup>[17]</sup>.

Combined anesthesia had significant intraoperative hemodynamic effects in terms of lower mean arterial pressure and longer hypotension duration, which required more colloid infusions but had no metabolic effects on base excess and lactate concentration, even CVP was not significantly affected by the sympathetic blockade.

Postoperative data showed slightly higher AST and ALT values in the epidural group, however it must be noted that, in this group, major resections were more frequent than minor resections, hence these data are difficult to interpret. Finally these differences in postoperative transaminase levels did not have any clinical impact, as no cases of postoperative liver failure were observed, and the postoperative courses of INR bilirubin and kidney

functions were substantially comparable between the two groups.

A recent large retrospective study by Kambakamba *et al.*<sup>[18]</sup> postulated that epidural anesthesia could have a role in jeopardizing postoperative kidney function in major, but not in minor liver resections. The difference in our results could be explained primarily by the fact that cirrhotic patients were excluded from the analysis in the Kambakamba study; also our sample is much smaller in size and we did not register a use of vasoactive drugs to correct intraoperative hypotension as extensive as the one in their study group. Postoperative complications were not significantly different between the two groups, however it is interesting to note that in the group without epidural anesthesia we observed 9 cases of postoperative delirium, while none was observed in the group receiving epidural anesthesia.

Also respiratory complications were observed only in patients treated with general anesthesia and postoperative systemic opiates. Patients receiving epidural analgesia in 50% of the cases were extubated at the end of surgery in the operating theatre, and in general required fewer hours of mechanical ventilation. These results indicate a beneficial role of epidural anesthesia with regard to the respiratory system function and its possible postoperative complications.

The shorter postoperative hospital length of stay observed in the epidural group could be related to a better analgesia, faster ambulation and a better postoperative intestinal function. We registered a longer mean hospital length of stay than the one enounced in other studies; the composition of our study population considering only cirrhotic patients has contributed in altering our results in this sense.

Another important aspect to underline is the large number of patients which were considered not eligible for neuraxial analgesia (57 out of 183 patients), in which other analgesic techniques to reduce postoperative opiates use, such as continuous wound infusion of local anesthetics<sup>[19]</sup>, intercostal nerve blocks<sup>[20]</sup>, intrathecal morphine administration<sup>[21]</sup> and TAP block<sup>[22]</sup> could find an indication. In a recent review by Hughes *et al.*<sup>[23]</sup> these techniques appear to be in some cases even superior to epidurals in terms of reduction of postoperative complications, despite providing less relief from pain. Another recent RCT from Hughes *et al.*<sup>[24]</sup> has compared epidural anaesthesia and analgesia with a combination of TAP and rectus sheath block with continuous wound infiltration, confirming the superiority of this alternative technique to TEA in terms of post operative complications and recovery and also achieving comparable pain scores. These results are particularly promising especially because to our knowledge this is the first trial achieving comparable pain scores with a technique alternative to TEA, and need to be confirmed by larger multicenter trials. Finally it is our belief that, based on the most solid evidence available at the moment, the use of TEA still represents the technique providing the most comfort to the patient whilst accelerating post operative

recovery compared to standard general anesthesia and opiate analgesia; alternative analgesic techniques find their correct indication in those patients not eligible for an epidural catheter positioning making a complete avoidance of systemic opiates in this population achievable.

The main limitations of the present study lay in its retrospective design, and the limited numerosity of the sample, which originated from a single center.

In conclusion, the main results of this study show that the known benefits of thoracic epidural anaesthesia and analgesia within an ERAS protocol for perioperative management, seem to be reproducible in a subpopulation including only cirrhotic patients undergoing open liver surgery. Epidural anaesthesia plays a major role in accomplishing many of these benefits, and its systematic use has important effects on patient outcomes and comfort. Our results also show that, in a selected population of cirrhotic patients, the technique can be performed safely without complications even if this aspect needs to be confirmed in larger populations.

## COMMENTS

### Background

Enhanced recovery after surgery is a solid reality in most surgical specialties and has been successfully applied to liver surgery. The subpopulation of cirrhotic patients undergoing liver resections has been poorly studied and represents a challenge for the application of such protocols. Moreover the use of epidural anaesthesia and analgesia in this subpopulation is still a matter of debate.

### Research frontiers

Defining the possible benefits of using epidural anaesthesia within an Enhanced Recovery after Surgery (ERAS) protocol for cirrhotic patients undergoing liver resection surgery is of great relevance in order to further implement the use of such protocols.

### Innovations and breakthroughs

This is the first retrospective study showing improved post operative outcomes using an ERAS protocol and epidural anaesthesia in a population including only cirrhotic patients undergoing liver resection surgery.

### Applications

These data suggest that the implementation of an ERAS protocol for cirrhotic patients using epidural anaesthesia is feasible, safe and provides positive clinical outcomes. This could be of great value in spreading the implementation of ERAS protocols to this particular subpopulation of patients.

### Peer-review

The manuscript describes the findings of a retrospective review to determine if there are benefits with the use of ERAS and epidural during liver resection surgery. The study is reasonably large and could provide useful information to the readers.

## REFERENCES

- 1 **Kehlet H.** Multimodal approach to control postoperative pathophysiology and rehabilitation. *Br J Anaesth* 1997; **78**: 606-617 [PMID: 9175983]
- 2 **Page AJ, Ejaz A, Spolverato G, Zavadsky T, Grant MC, Galante DJ, Wick EC, Weiss M, Makary MA, Wu CL, Pawlik TM.** Enhanced recovery after surgery protocols for open hepatectomy--physiology, immunomodulation, and implementation. *J Gastrointest Surg* 2015; **19**: 387-399 [PMID: 25472030 DOI: 10.1007/s11605-014-2712-0]
- 3 **Jones C, Kelliher L, Dickinson M, Riga A, Worthington T, Scott MJ, Vandrevale T, Fry CH, Karanjia N, Quiney N.** Randomized clinical trial on enhanced recovery versus standard care following open liver resection. *Br J Surg* 2013; **100**: 1015-1024 [PMID: 23696477 DOI: 10.1002/bjs.9165]
- 4 **Lin DX, Li X, Ye QW, Lin F, Li LL, Zhang QY.** Implementation of a fast-track clinical pathway decreases postoperative length of stay and hospital charges for liver resection. *Cell Biochem Biophys* 2011; **61**: 413-419 [PMID: 21556940 DOI: 10.1007/s12013-011-9203-7]
- 5 **Schultz NA, Larsen PN, Klarskov B, Plum LM, Frederiksen HJ, Christensen BM, Kehlet H, Hillingsø JG.** Evaluation of a fast-track programme for patients undergoing liver resection. *Br J Surg* 2013; **100**: 138-143 [PMID: 23165484 DOI: 10.1002/bjs.8996]
- 6 **van Dam RM, Hendry PO, Coolsen MM, Bemelmans MH, Lassen K, Revhaug A, Fearon KC, Garden OJ, Dejong CH.** Initial experience with a multimodal enhanced recovery programme in patients undergoing liver resection. *Br J Surg* 2008; **95**: 969-975 [PMID: 18618897 DOI: 10.1002/bjs.6227]
- 7 **Ni TG, Yang HT, Zhang H, Meng HP, Li B.** Enhanced recovery after surgery programs in patients undergoing hepatectomy: A meta-analysis. *World J Gastroenterol* 2015; **21**: 9209-9216 [PMID: 26290648 DOI: 10.3748/wjg.v21.i30.9209]
- 8 **Ganapathi S, Roberts G, Mogford S, Bahlmann B, Ateleanu B, Kumar N.** Epidural analgesia provides effective pain relief in patients undergoing open liver surgery. *Br J Pain* 2015; **9**: 78-85 [PMID: 26516562 DOI: 10.1177/2049463714525140]
- 9 **Cucchetti A, Cescon M, Trevisani F, Pinna AD.** Current concepts in hepatic resection for hepatocellular carcinoma in cirrhotic patients. *World J Gastroenterol* 2012; **18**: 6398-6408 [PMID: 23197885 DOI: 10.3748/wjg.v18.i44.6398]
- 10 **Tzimas P, Prout J, Papadopoulos G, Mallett SV.** Epidural anaesthesia and analgesia for liver resection. *Anaesthesia* 2013; **68**: 628-635 [PMID: 23662750 DOI: 10.1111/anae.12191]
- 11 **Antonio S, Lorenzo G, Andrea C, Cristiana TS.** Platelet Count and INR Profile after Hepatic Resection in Cirrhotic Patients: Implications for Epidural Analgesia. *Int J Anesth Anesthesiol* 2014; **1**: 12
- 12 **Dunne DF, Yip VS, Jones RP, McChesney EA, Lythgoe DT, Psarelli EE, Jones L, Lacasia-Purroy C, Malik HZ, Poston GJ, Fenwick SW.** Enhanced recovery in the resection of colorectal liver metastases. *J Surg Oncol* 2014; **110**: 197-202 [PMID: 24715651 DOI: 10.1002/jso.23616]
- 13 **Horlocker TT.** Regional anaesthesia in the patient receiving antithrombotic and antiplatelet therapy. *Br J Anaesth* 2011; **107** Suppl 1: i96- i106 [PMID: 22156275 DOI: 10.1093/bja/aer381]
- 14 **Belghiti J, Clavien PA, Gadzijev.** The Brisbane 2000 terminology of liver anatomy and resections. *HPB (Oxford)* 2000; **(2)**: 333-339
- 15 **Bateman BT, Mhyre JM, Ehrenfeld J, Kheterpal S, Abbey KR, Argalious M, Berman MF, Jacques PS, Levy W, Loeb RG, Paganelli W, Smith KW, Wethington KL, Wax D, Pace NL, Tremper K, Sandberg WS.** The risk and outcomes of epidural hematomas after perioperative and obstetric epidural catheterization: a report from the Multicenter Perioperative Outcomes Group Research Consortium. *Anesth Analg* 2013; **116**: 1380-1385 [PMID: 22504213 DOI: 10.1213/ANE.0b013e318251daed]
- 16 **Tripodi A.** Hemostasis abnormalities in cirrhosis. *Curr Opin Hematol* 2015; **22**: 406-412 [PMID: 26203733 DOI: 10.1097/MOH.0000000000000164]
- 17 **Bertini L, Savoia G, De Nicola A, Ivani G, Gravino E, Albani A, Alemanno F, Barbati A, Borghi B, Borrometi F, Casati A, Celleno D, Ciaschi A, Corcione A, De Negri P, Di Benedetto P, Evangelista M, Fanelli G, Grossi P, Loreto M, Margaria E, Mastronardi P, Mattia C, Nicosia F, Noll M, Rutili A, Santangelo E, Sucre J, Tagariello V, Varrassi G, Paoletti F, Tufano R.** SIAARTI guidelines for safety in locoregional anaesthesia. *Minerva Anesthesiol* 2006; **72**: 689-722 [PMID: 16871153]
- 18 **Kambakamba P, Slankamenac K, Tschuor C, Kron P, Wirsching A, Maurer K, Petrowsky H, Clavien PA, Lesurtel M.** Epidural analgesia and perioperative kidney function after major liver resection. *Br J Surg* 2015; **102**: 805-812 [PMID: 25877255 DOI: 10.1002/bjs.12111]

- 10.1002/bjs.9810]
- 19 **Ventham NT**, Hughes M, O'Neill S, Johns N, Brady RR, Wigmore SJ. Systematic review and meta-analysis of continuous local anaesthetic wound infiltration versus epidural analgesia for postoperative pain following abdominal surgery. *Br J Surg* 2013; **100**: 1280-1289 [PMID: 24244968]
- 20 **Finnerty O**, Carney J, McDonnell JG. Trunk blocks for abdominal surgery. *Anaesthesia* 2010; **65** Suppl 1: 76-83 [PMID: 20377549 DOI: 10.1111/j.1365-2044.2009.06203.x]
- 21 **De Pietri L**, Siniscalchi A, Reggiani A, Masetti M, Begliomini B, Gazzi M, Gerunda GE, Pasetto A. The use of intrathecal morphine for postoperative pain relief after liver resection: a comparison with epidural analgesia. *Anesth Analg* 2006; **102**: 1157-1163 [PMID: 16551916 DOI: 10.1213/01.ane.0000198567.85040.ce]
- 22 **Niraj G**, Kelkar A, Jeyapalan I, Graff-Baker P, Williams O, Darbar A, Maheshwaran A, Powell R. Comparison of analgesic efficacy of subcostal transversus abdominis plane blocks with epidural analgesia following upper abdominal surgery. *Anaesthesia* 2011; **66**: 465-471 [PMID: 21457153 DOI: 10.1111/j.1365-2044.2011.06700.x]
- 23 **Hughes MJ**, Ventham NT, McNally S, Harrison E, Wigmore S. Analgesia after open abdominal surgery in the setting of enhanced recovery surgery: a systematic review and meta-analysis. *JAMA Surg* 2014; **149**: 1224-1230 [PMID: 25317633 DOI: 10.1001/jamasurg.2014.210]
- 24 **Hughes MJ**, Harrison EM, Peel NJ, Stutchfield B, McNally S, Beattie C, Wigmore SJ. Randomized clinical trial of perioperative nerve block and continuous local anaesthetic infiltration via wound catheter versus epidural analgesia in open liver resection (LIVER 2 trial). *Br J Surg* 2015; **102**: 1619-1628 [PMID: 26447461 DOI: 10.1002/bjs.9949]

**P- Reviewer:** Celikbilek M, Lalor P **S- Editor:** Qiu S

**L- Editor:** A **E- Editor:** Li D





Prospective Study

## Immune response to hepatitis B virus vaccine in celiac subjects at diagnosis

Martina Filippelli, Maria Teresa Garozzo, Antonino Capizzi, Massimo Spina, Sara Manti, Lucia Tardino, Carmelo Salpietro, Salvatore Leonardi

Martina Filippelli, Maria Teresa Garozzo, Antonino Capizzi, Massimo Spina, Lucia Tardino, Salvatore Leonardi, Department of Medical and Pediatric Sciences, University of Catania, 95100 Catania, Italy

Sara Manti, Carmelo Salpietro, Department of Pediatric Sciences, University of Messina, 98100 Messina, Italy

**Author contributions:** Filippelli M, Garozzo MT and Leonardi S revised the manuscript for final submission; Filippelli M, Capizzi A, Manti S, Tardino L and Salpietro C contributed to writing of the article; Garozzo MT analyzed the data; Spina M performed the research; Leonardi S designed the study; all authors read and approved the final manuscript.

**Institutional review board statement:** The study was reviewed and approved by the Institutional Review Board of the University Hospital "Policlinico Vittorio Emanuele".

**Informed consent statement:** All study participants, or their legal guardians, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** The authors have no conflicts of interest related to the manuscript.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Salvatore Leonardi, Professor, Department of Medical and Pediatric Sciences, University of

Catania, Via S Sofia 78, 95100 Catania, Italy. [leonardi@unict.it](mailto:leonardi@unict.it)  
 Telephone: +39-9-53782764  
 Fax: +39-9-53782895

Received: May 20, 2016

Peer-review started: May 20, 2016

First decision: July 4, 2016

Revised: July 14, 2016

Accepted: July 29, 2016

Article in press: August 1, 2016

Published online: September 18, 2016

### Abstract

#### AIM

To evaluate hepatitis B virus (HBV) vaccine response and correlation with human leukocyte antigens (HLA) and/or gluten intake in celiac patients at diagnosis.

#### METHODS

Fifty-one patients affected by celiac disease, diagnosed at the Department of Pediatrics of the University of Catania (Italy), were recruited. All patients were tested at admission for immunization against HBV, according to findings from analysis of quantitative HBV surface antibody (anti-HBs). The anti-HBs titer was measured by enzyme-linked immunosorbent assay. Following the international standards, subjects with antibody titer < 10 IU/L were defined as non-responders. The prevalence of responders and non-responders among celiac subjects and the distribution of immunization for age were examined. In addition, the prevalence of responders and non-responders was assessed for correlation to HLA and clinical features at diagnosis of celiac disease.

#### RESULTS

The entire study population was divided into three groups according to age: 24 patients aged between 0

to 5.5 years (48.9%, group A); 16 aged between 5.5 and 9.5 years (30.61%, group B); 9 aged between 9.5 and 17 years (18.75%, group C). Comparison of the percentage of responders and non-responders between the youngest and the oldest age group showed no significant difference between the two groups ( $P > 0.05$ ). With regard to the HLA haplotype, comparison of the distribution of vaccination response showed no statistically significant difference between the different genotypes (homozygosity for the HLADQ2 haplotype compared with HLADQ2/DQ8 heterozygosity or other haplotypes;  $P > 0.05$ ). Moreover, distribution of the responders according to clinical features of celiac disease showed no statistically significant differences ( $P > 0.05$ ).

## CONCLUSION

This prospective study confirmed the lower percentage of response to HBV vaccine in celiac subjects. However, the underlying mechanism remains unclear and further studies are needed.

**Key words:** Celiac disease; Hepatitis B virus vaccination; Human leukocyte antigens; Gluten; Poor response

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Correlation between celiac disease and lower response to hepatitis B virus (HBV) vaccine has been demonstrated, but the causes remain unclear. The lack of prospective data represents an extensive gap between the time of vaccination and development of the immune response, contributing to select “false non-responders” (*i.e.*, those who are destined to lose the antibody titer over time). The originality of our prospective study is that of analyzing the response to HBV vaccine in a group of celiac patients at the time of diagnosis in an attempt to nullify the percentage of error related to confounding factors.

Filippelli M, Garozzo MT, Capizzi A, Spina M, Manti S, Tardino L, Salpietro C, Leonardi S. Immune response to hepatitis B virus vaccine in celiac subjects at diagnosis. *World J Hepatol* 2016; 8(26): 1105-1109 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i26/1105.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i26.1105>

## INTRODUCTION

Celiac disease (CD) is a permanent immune-mediated enteropathy, triggered by gluten in genetically predisposed individuals. The genetic predisposition consists of the presence of alleles encoding for the molecules DQ2 or DQ8 of the human leukocyte antigen (HLA)<sup>[1]</sup>. A significant correlation between CD and a lower response to the hepatitis B virus (HBV) vaccine was demonstrated several years ago, but the causes of this phenomenon remain unclear. Many authors have postulated the role of

HLA molecules (DQ2 and DQ8) in affecting an impaired immune response to HBV vaccine in CD<sup>[2]</sup>. On the other hand, it has been theorized that gluten intake could represent the main factor involved, because according to some studies the percentage of responders among celiac patients who are compliant with a gluten-free diet (GFD) is similar to that among healthy subjects<sup>[3,4]</sup>.

Despite the many hypotheses, the debate on poor response to hepatitis B vaccination in CD remains largely open. It could be hypothesized that many confounding factors in some of the previous studies have contributed to maintaining this uncertainty. First of all, the lack of prospective data determines a more extensive gap between time of vaccination and development of the immune response, contributing to select “false non-responders” (*i.e.*, those who are destined to lose the antibody titer over time)<sup>[5]</sup>. Moreover, it could be easier to evaluate the effective role of HLA in influencing HBV vaccine response when CD has just been diagnosed and no other factors have yet intervened.

For all these reasons, the aim of our prospective study was to eliminate or reduce such confounding factors and to evaluate hepatitis B vaccination response in celiac patients at diagnosis of the disease and its possible correlation with HLA and/or gluten intake.

## MATERIALS AND METHODS

In this prospective study we recruited 51 patients affected by CD, diagnosed at the Department of Pediatrics of the University of Catania (Italy). The diagnosis of CD was made according to the European Society for Paediatric Gastroenterology, Hepatology and Nutrition criteria updated in 2012<sup>[6]</sup>. The total serum IgA levels were measured in all patients in order to exclude the presence of a selective deficit of IgA. Inclusion criteria required that subjects must have completed obligatory vaccinations, including the HBV vaccine. All patients were tested at admission for immunization against HBV, according to finding from quantitative analysis of the HBV surface antibody (anti-HBs). The anti-HBs titer was measured by enzyme-linked immunosorbent assay. Following the international standards, subjects with antibody titer < 10 IU/L were defined as non-responders<sup>[7]</sup>.

Two of the 51 celiac patients were excluded because of insufficiency of their serum samples for analysis of the anti-HBs titer.

We examined the prevalence of responders and non-responders among celiac subjects and the distribution of immunization for age. For this, all patients were divided into three groups on the basis of their age at diagnosis: Group A children were aged between 1.5 and 5.5 years; group B children were aged between 5.5 and 9.5 years; group C children were aged between 9.5 and 17 years.

Moreover, we divided all 49 patients on the basis of clinical features at diagnosis of CD and distinguished them in the following three groups: Group 1 patients had typical form (onset with diarrhea, abdominal pain, cramping or distension, dyspepsia, vomiting or failure to

**Table 1** Serologic and histologic findings of the duodenal biopsies for celiac disease diagnosis

	TTG IgA ( $\mu$ A/mL)			Marsh score	
	70-200	201-300	> 300	3C-B2	3B-B1
Patients, <i>n</i>	20	10	19	26	23

thrive); group 2 patients had atypical form (onset with other symptoms such as deficiency iron-anemia, chronic fatigue, behavior change, dermatitis and joint pain); group 3 patients had silent form (asymptomatic onset). The prevalence of responders and non-responders was assessed for correlation to HLA and the clinical features at diagnosis of CD (typical or atypical onset).

At the end, we compared the results obtained by the present observational study with the results of a retrospective study previously conducted in our Department of Pediatrics.

### Statistical analysis

The statistical analysis of data was performed with the use of SPSS version 21.0 software (SPSS Inc. Chicago, IL, United States). The results for quantitative variables were expressed as mean  $\pm$  SD, and those of qualitative variables were expressed as frequencies and percentages. Differences between groups were compared using the Mann-Whitney *U* test for two independent samples. The Fisher's exact test was used to compare frequencies. For all analyses, statistical significance was defined as  $P < 0.05$ .

## RESULTS

Data for the serologic and histologic findings of duodenal biopsies (according to Marsh classification) used for the diagnosis of CD are summarized in Table 1, while characteristics of the 49 patients included in the study (sex, age, percentage of responders, HLA haplotype) are summarized in Table 2.

When we divided the entire study population into the three age groups, we found 24 patients were aged between 0 to 5.5 years (48.9%, group A), 16 were aged between 5.5 and 9.5 years (30.61%, group B) and 9 were aged between 9.5 and 17 years (18.75%, group C). The responders were distributed into the three age groups as follows: 19 (38.77%) in group A; 11 (22.44%) in group B; 4 (8.16%) in group C. Comparing the percentage of responders and non-responders between the youngest and the oldest group, no significant difference was found ( $P > 0.05$ ).

With regard to the HLA haplotype, comparison of the distribution of vaccination response showed no statistically significant difference between the different genotypes (Table 2). Moreover, the distribution of responders according to clinical features of CD was as follows: 20 out of 26 patients in group 1; 11 out of 17 in group 2; 3 out of 6 in group 3. The typical form showed significant association with the presence of

**Table 2** Patient characteristics and distribution of human leukocyte antigens and clinical features

	Responders	Non-responders	<i>P</i> value
HBV vaccination	34 (69.4%)	15 (30.6%)	
Female sex	22 (66.7%)	11 (33.3%)	
Male sex	12 (75%)	4 (25%)	
Median age	5.55 ( $\pm$ 3.25 SDS)	8.04 ( $\pm$ 4.3 SDS)	> 0.05
HLA			
DQ2/DQ2	8	4	
DQ2/DQ8	6	1	
Other HLA <sup>1</sup>	19	10	
Distribution according HLA			> 0.05
Clinical form of CD			
Typical form	20	6	
Atypical form	11	6	
Silent form	3	3	
Distribution according clinical form			> 0.05

<sup>1</sup>Includes heterozygosis for HLA DQ2, heterozygosis for HLA DQ8 and homozygosis for HLA DQ8 patients. HBV: Hepatitis B virus; CD: Celiac disease; SDS: Standard deviation score; HLA: Human leukocyte antigens.

HLADQ2 ( $P < 0.05$ ). Comparison of the immunological vaccine response between the three groups showed no statistically significant differences related to the clinical features (Table 2).

Finally, we found a statistically significant difference in the vaccination response for patients in the present observational study as compared to patients analyzed in the previous retrospective study. In the present study, 34 out of 49 patients were responders compared to 30 out of 60 patients in the retrospective study ( $P < 0.01$ ).

## DISCUSSION

CD is defined as an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically-susceptible individuals and is characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 or DQ8 haplotypes and enteropathy<sup>[6]</sup>.

The reasons why CD could be related to an inadequate response to hepatitis B vaccination have long been discussed. Some previous studies have suggested a genetically-related failure of response, attributed to particular HLA antigens, mainly the DQ2 haplotype, which is also involved in autoimmunity<sup>[8,9]</sup>. In fact, while DQ2 is present in only approximately 40% of the general population, it is expressed in up to 81% of CD patients. The HLA-DQ2 status would induce an inadequate Th2 response, leading to inefficient B cell differentiation and formation of memory T cells<sup>[8,10,11]</sup>. In 2007, a study by Park *et al.*<sup>[2]</sup> demonstrated that more than 50% of the enrolled children with CD did not show a response to standard vaccination regimens for HBV, in contrast to a physiological response that was observed with other vaccinations (tetanus, rubella, and *Haemophilus influenzae* type b). This finding supported the hypothesis that HLA haplotype played a specific role in response

to HBV vaccine. One year later, a subsequent study conducted by Ahishali *et al.*<sup>[12]</sup> confirmed this theory by finding responsiveness to hepatitis B vaccination in 68% of celiac patients, in contrast to the 100% response observed for the controls, emphasizing the genotypic coincidence.

In 2009, Leonardi *et al.*<sup>[13]</sup> published a case control retrospective study about the prevalence of HBV vaccine non-responders among celiac and healthy subjects. The anti-HBs titer was measured after a successful period of time on a GFD, as demonstrated by the normalization of serum markers of CD. The study confirmed that celiac patients have a lower percentage of response to hepatitis B vaccination than healthy controls. However, the authors also found a significantly higher number of responders among the celiac patients that were younger than 18-month-old at diagnosis and a significantly lower number of responders in adolescent patients older than 14-year-old at diagnosis. The drawback of the study was that the HLA typing was performed in few patients, so that the study could not demonstrate the correlation of the phenomenon observed with HLA-DQ2 or HLA-DQ8, and that there was a long interval between the time of hepatitis B vaccination and the time of collecting samples for analysis of the anti-HBs titer. In this regard, a recent case control retrospective study by Zanoni *et al.*<sup>[11]</sup> investigated the serological response to HBV and measles-containing vaccines in three groups of individuals: Diabetes mellitus type 1 (T1DM) patients, celiac patients and controls. No significant differences were found in the percentage of responders to HBV and measles vaccines among the T1DM and CD patients and the control group, and there was also a lack of correlation between HBV vaccine response and DQ2. According to the authors, these conflicting results between their findings and the data reported in the literature may be due to differences in ages of the examined subjects at time of vaccination and in time intervals between vaccination and blood sample collection for testing. They concluded that prospective studies of pathological and healthy groups, with same age at hepatitis B vaccination and same time interval for blood sample collection to determine antibody levels, are necessary to provide more conclusive data.

For these reasons, the originality of our prospective study is that of analyzing the response to hepatitis B vaccination in a group of 49 celiac patients at the time of diagnosis, helping us to nullify the percentage of error related to a long interval from time of hepatitis B vaccination to time of serum anti-HBs analysis. In fact, when we compared the results of our prospective study (based upon patients at time diagnosis of CD) with those retrospectively obtained by Leonardi *et al.*<sup>[13]</sup> in 2009 (based upon celiac patients on a GFD), we found a higher percentage of responders among the celiac subjects, probably due to our study design having eliminated more of the potential confounding factors related to loss of immunity over the time, which have been documented extensively in the literature<sup>[14-16]</sup>.

Meanwhile, we also observed that whereas more than half of our celiac population represented responders (69.39%), the percentage still remained lower than in the general population (90%), suggesting a role of genetic predisposition. However, comparison of the distribution of vaccination response showed no statistically significant difference between the different genotypes, providing an argument against the theory that homozygosity for the HLA-DQ2 haplotype could act in isolation to negatively influence the response to vaccination, in comparison with the HLA-DQ2/DQ8 heterozygosity or other haplotypes.

In this regard, several studies hypothesized that gluten intake at the time of vaccination could influence immune response, *via* competition of both gliadin peptides and hepatitis B surface antigen protein fragments for binding to HLA-DQ2 molecules, which could result in defective antibody production<sup>[3,17,18]</sup>. In support of this hypothesis, Nemes *et al.*<sup>[19]</sup> showed that seroconversion after hepatitis B vaccination was 95.5% in CD patients vaccinated during dietary treatment; in contrast, in a second group of CD patients that were either untreated or with a diet status ranging from strict to non-strict, the response was 50.9%. The HLA-DQ alleles did not seem to play a primary role because all of the patients carried the HLA-DQ2. In our study, patients were enrolled at diagnosis of CD, when their diet contained gluten; although, we do not know the exact period of exposure. It could be of interest to administer a booster dose of HBV vaccine in these subjects after a period of GFD and to subsequently evaluate the effects on the immune response. However, since our study did not reveal a significant correlation between HBV vaccine response and HLA alone, we now question whether it is possible that impaired immune response in CD is the result of a combination of several factors. Indeed, it could be possible that genetic predisposition, gluten intake and phenotype of the disease interact to influence a lower HBV vaccine response in CD.

In conclusion, our study is the first prospective study on HBV vaccine response in CD. The findings confirm the lower percentage of response to hepatitis B vaccination in the celiac population, as compared with healthy subjects. The mechanism that causes this phenomenon, however, remains unclear. According to our results, the mechanism does not appear to be related to HLA haplotype alone but could result from several variables working in combination. Further studies are needed to support this hypothesis and to establish the best surveillance program of response to HBV vaccine in CD.

## COMMENTS

### Background

The correlation between celiac disease and a lower response to the hepatitis B virus (HBV) vaccine has been demonstrated, but the causes remain unclear. Many confounding factors identified by previous studies have contributed to this uncertainty; moreover, the lack of prospective data represents a more extensive gap between the time of vaccination and the development of an immune response, contributing to select false non-responders (*i.e.*, those who are destined to lose the antibody titer over time). The originality of the authors' prospective



study lies in the authors' analysis of the response to hepatitis B vaccination in a group of celiac patients at the time of diagnosis, which allowed the authors' study to nullify the percentage of error related to these confounding factors.

### Research frontiers

In this study, there is suggestion that genetic predisposition, gluten intake and phenotype of celiac disease could work in conjunction to influence a lower HBV vaccine response.

### Innovations and breakthroughs

This study is the first prospective study in literature on the topic of lower HBV vaccine response in patients with celiac disease. This study confirms the lower percentage of response to hepatitis B vaccination in the celiac population, as compared with healthy subjects. According to the authors' results, the mechanism that causes this phenomenon is unlikely to be related to human leukocyte antigens haplotype alone but could be a result of several variables together.

### Applications

This study provides additional evidence that, along with the collective data in the literature, will help to establish an optimal surveillance program of response to HBV vaccine in celiac disease.

### Terminology

Non-responders are all subjects with a titer of hepatitis B surface antibody < 10 IU/mL after the primary vaccination cycle.

### Peer-review

The authors have studied antibody response to HBV vaccine in celiac patients. This is an interesting study, well designed and performed.

## REFERENCES

- 1 **Sollid LM**, Jabri B. Triggers and drivers of autoimmunity: lessons from coeliac disease. *Nat Rev Immunol* 2013; **13**: 294-302 [PMID: 23493116 DOI: 10.1038/nri3407]
- 2 **Park SD**, Markowitz J, Pettei M, Weinstein T, Sison CP, Swiss SR, Levine J. Failure to respond to hepatitis B vaccine in children with celiac disease. *J Pediatr Gastroenterol Nutr* 2007; **44**: 431-435 [PMID: 17414139 DOI: 10.1097/MPG.0b013e3180320654]
- 3 **Zingone F**, Capone P, Tortora R, Rispo A, Morisco F, Caporaso N, Imperatore N, De Stefano G, Iovino P, Ciacci C. Role of gluten intake at the time of hepatitis B virus vaccination in the immune response of celiac patients. *Clin Vaccine Immunol* 2013; **20**: 660-662 [PMID: 23446217 DOI: 10.1128/CI.00729-12]
- 4 **Leonardi S**, Del Giudice MM, Spicuzza L, Spina M, La Rosa M. Hepatitis B vaccine administered by intradermal route in patients with celiac disease unresponsive to the intramuscular vaccination schedule: a pilot study. *Am J Gastroenterol* 2010; **105**: 2117-2119 [PMID: 20818367 DOI: 10.1038/ajg.2010.195]
- 5 **European Consensus Group on Hepatitis B Immunity**. Are booster immunisations needed for lifelong hepatitis B immunity? *Lancet* 2000; **355**: 561-565 [PMID: 10683019 DOI: 10.1016/S0140-6736(99)07239-6]
- 6 **Husby S**, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C, Lelgeman M, Mäki M, Ribes-Koninckx C, Ventura A, Zimmer KP. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012; **54**: 136-160 [PMID: 22197856 DOI: 10.1097/MPG.0b013e31821a23d0]
- 7 **Jack AD**, Hall AJ, Maine N, Mendy M, Whittle HC. What level of hepatitis B antibody is protective? *J Infect Dis* 1999; **179**: 489-492 [PMID: 9878036 DOI: 10.1086/314578]
- 8 **Martinetti M**, De Silvestri A, Belloni C, Pasi A, Tinelli C, Pistorio A, Salvaneschi L, Rondini G, Avanzini MA, Cuccia M. Humoral response to recombinant hepatitis B virus vaccine at birth: role of HLA and beyond. *Clin Immunol* 2000; **97**: 234-240 [PMID: 11112362 DOI: 10.1006/clim.2000.4933]
- 9 **Lin HH**, Liao HW, Lin SK, Wang LY. HLA and response to booster hepatitis B vaccination in anti-HBs-seronegative adolescents who had received primary infantile vaccination. *Vaccine* 2008; **26**: 3414-3420 [PMID: 18501999 DOI: 10.1016/j.vaccine.2008.04.038]
- 10 **Noh KW**, Poland GA, Murray JA. Hepatitis B vaccine nonresponse and celiac disease. *Am J Gastroenterol* 2003; **98**: 2289-2292 [PMID: 14572581 DOI: 10.1111/j.1572-0241.2003.07701.x]
- 11 **Zanoni G**, Contreas G, Valletta E, Gabrielli O, Mengoli C, Veneri D. Normal or defective immune response to Hepatitis B vaccine in patients with diabetes and celiac disease. *Hum Vaccin Immunother* 2015; **11**: 58-62 [PMID: 25483516 DOI: 10.4161/hv.34309]
- 12 **Ahishali E**, Boztas G, Akyuz F, Ibrisim D, Poturoglu S, Pinarbasi B, Ozdil S, Mungan Z. Response to hepatitis B vaccination in patients with celiac disease. *Dig Dis Sci* 2008; **53**: 2156-2159 [PMID: 18157638 DOI: 10.1007/s10620-007-0128-3]
- 13 **Leonardi S**, Spina M, Spicuzza L, Rotolo N, La Rosa M. Hepatitis B vaccination failure in celiac disease: is there a need to reassess current immunization strategies? *Vaccine* 2009; **27**: 6030-6033 [PMID: 19682619 DOI: 10.1016/j.vaccine.2009.07.099]
- 14 **Filippelli M**, Lionetti E, Pulvirenti A, Gennaro A, Lanzafame A, Marseglia GL, Salpietro C, Rosa ML, Leonardi S. New approaches in hepatitis B vaccination for celiac disease. *Immunotherapy* 2014; **6**: 945-952 [PMID: 25313572 DOI: 10.2217/IMT.14.64]
- 15 **Filippelli M**, Lionetti E, Gennaro A, Lanzafame A, Arrigo T, Salpietro C, La Rosa M, Leonardi S. Hepatitis B vaccine by intradermal route in non responder patients: an update. *World J Gastroenterol* 2014; **20**: 10383-10394 [PMID: 25132754 DOI: 10.3748/wjg.v20.i30.10383]
- 16 **McMahon BJ**, Bruden DL, Petersen KM, Bulkow LR, Parkinson AJ, Nainan O, Khristova M, Zanis C, Peters H, Margolis HS. Antibody levels and protection after hepatitis B vaccination: results of a 15-year follow-up. *Ann Intern Med* 2005; **142**: 333-341 [PMID: 15738452 DOI: 10.7326/0003-4819-142-5-200503010-00008]
- 17 **Ertem D**, Gonen I, Tanidir C, Ugras M, Yildiz A, Pehlivanoglu E, Eksioğlu-Demiralp E. The response to hepatitis B vaccine: does it differ in celiac disease? *Eur J Gastroenterol Hepatol* 2010; **22**: 787-793 [PMID: 19584738 DOI: 10.1097/MEG.0b013e32832e9d41]
- 18 **Ertekin V**, Tosun MS, Selimoglu MA. Is there need for a new hepatitis B vaccine schedule for children with celiac disease? *Hepat Mon* 2011; **11**: 634-637 [PMID: 22140387 DOI: 10.5812/kowsar.1735143X.1129]
- 19 **Nemes E**, Lefler E, Szegedi L, Kapitány A, Kovács JB, Balogh M, Szabados K, Tumpek J, Sipka S, Korponay-Szabó IR. Gluten intake interferes with the humoral immune response to recombinant hepatitis B vaccine in patients with celiac disease. *Pediatrics* 2008; **121**: e1570-e1576 [PMID: 18519462 DOI: 10.1542/peds.2007-2446]

**P- Reviewer:** Balaban YH, Saez LR, Sener AG  
**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Li D



## Contrast-enhanced ultrasonographic findings of serum amyloid A-positive hepatocellular neoplasm: Does hepatocellular adenoma arise in cirrhotic liver?

Mariko Kumagawa, Naoki Matsumoto, Yukinobu Watanabe, Midori Hirayama, Takao Miura, Hiroshi Nakagawara, Masahiro Ogawa, Shunichi Matsuoka, Mitsuhiko Moriyama, Tadatoshi Takayama, Masahiko Sugitani

Mariko Kumagawa, Naoki Matsumoto, Yukinobu Watanabe, Midori Hirayama, Takao Miura, Hiroshi Nakagawara, Masahiro Ogawa, Shunichi Matsuoka, Mitsuhiko Moriyama, Division of Gastroenterology and Hepatology, Department of Medicine, Nihon University School of Medicine, Tokyo 173-8610, Japan

Tadatoshi Takayama, Division of Gastroenterology and Hepatology, Department of Digestive Surgery, Nihon University School of Medicine, Tokyo 173-8610, Japan

Masahiko Sugitani, Department of Pathology, Nihon University School of Medicine, Tokyo 173-8610, Japan

**Author contributions:** All authors contributed to the acquisition of data, writing, and revision of this manuscript.

**Institutional review board statement:** This case report exempt from the Institutional Review Board of Nihon University School of Medicine and Nihon University Itabashi Hospital.

**Informed consent statement:** The patient involved in this study gave his written informed consent authorizing use and disclosure of his protected health information.

**Conflict-of-interest statement:** All authors have no conflict-of-interest statement.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Mariko Kumagawa, MD, Division of

Gastroenterology and Hepatology, Department of Medicine, Nihon University School of Medicine, 30-1, Oyaguchi Kami-cho, Itabashi-ku, Tokyo 173-8610, Japan. [m\\_hayashida05@yahoo.co.jp](mailto:m_hayashida05@yahoo.co.jp)  
 Telephone: +81-3-39728111  
 Fax: +81-3-39720015

Received: April 29, 2016

Peer-review started: May 3, 2016

First decision: June 17, 2016

Revised: July 12, 2016

Accepted: July 20, 2016

Article in press: July 22, 2016

Published online: September 18, 2016

### Abstract

Hepatocellular adenoma (HCA) was recently classified into four pathological subtypes. There have been few studies describing the findings of contrast-enhanced ultrasonography (CEUS) of each type. Our case concerns a 78-year-old man who had undergone routine medical check-ups for hepatitis C for 11 years. Abdominal ultrasonography showed a 28 mm, hypo-echoic mass in the segment 4 of the liver. His integrating amount of drinking was 670 kg convert into ethanol. CEUS with Sonazoid demonstrated mild uniform hypo-enhancement with inflow of microbubbles from the periphery of the tumor in the arterial phase, and heterogeneously hypo-enhancement in the post vascular phase. Because the mass increased in size within 3 mo, a well differentiated hepatocellular carcinoma was suspected, and hepatic resection was performed. Microscopic findings showed homogeneous cell proliferation with low grade atypia, infiltration of inflammatory cells, ductular reactions, fatty deposit in part, and sinusoidal dilation. Immunohistochemistry revealed geographic positive for serum amyloid A (SAA), focal positive for glutamine

synthetase, diffuse and strong positive for C-reactive protein, and positive for liver-type fatty acid binding protein. These pathological features corresponded to that of an inflammatory HCA. However, we could not make a clear diagnosis, because HCAs were defined as not to arise in cirrhotic liver. Finally, this tumor was diagnosed as a SAA positive hepatocellular neoplasm.

**Key words:** Hepatocellular adenoma; Contrast-enhanced ultrasonography; Serum amyloid A; Serum amyloid A-positive hepatocellular neoplasms; Alcoholic cirrhosis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatocellular adenoma (HCA) was classified into four pathological subtypes. And HCA usually arises in the absence of significant fibrosis. Recently, some reports about serum amyloid A (SAA) positive hepatocellular neoplasm were published. All tumors shared features with inflammatory HCA arising in alcoholic cirrhosis. We describe the contrast-enhanced ultrasonographic findings of SAA positive HCA.

Kumagawa M, Matsumoto N, Watanabe Y, Hirayama M, Miura T, Nakagawara H, Ogawa M, Matsuoka S, Moriyama M, Takayama T, Sugitani M. Contrast-enhanced ultrasonographic findings of serum amyloid A-positive hepatocellular neoplasm: Does hepatocellular adenoma arise in cirrhotic liver? *World J Hepatol* 2016; 8(26): 1110-1115 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i26/1110.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i26.1110>

## INTRODUCTION

Hepatocellular adenoma (HCA) was recently classified into four pathological subtypes; hepatocyte nuclear factor 1 alpha inactivated HCA, beta catenin activated HCA, inflammatory HCA, and unclassified HCA<sup>[1-4]</sup>. Although contrast-enhanced ultrasonographic features of HCA have been reported in several literatures till now<sup>[5-7]</sup>, there have been little studies that described those of each type of HCA<sup>[8]</sup>. HCAs usually arise in the liver without steatosis, because a nodule arising in fibrotic/cirrhotic liver was not to be a HCA according to World Health Organization classification 2010<sup>[1]</sup>. Recently, some reports about serum amyloid A (SAA) positive hepatocellular neoplasm were published. All nodules shared features with inflammatory HCA arising in alcoholic cirrhosis<sup>[9-11]</sup>. In this report, we describe contrast-enhanced ultrasonographic findings of SAA positive hepatocellular neoplasm which had features similar to inflammatory HCAs.

## CASE REPORT

A 78-year-old man had undergone routine medical check-ups for hepatitis C over 21 years. He received interferon therapy 21 years ago, but could not achieve

complete remission. In these years, he had compensatory hepatic cirrhosis and was given medication of glycyrrhizin formulation. Abdominal ultrasonography showed 20 mm, hypo-echoic in the segment 4 of the liver 3 mo ago. Because the tumor increased in diameter to 28 mm, he was admitted to our hospital for further examinations. He had no history of other disease. He drank two glasses of whisky and one glass of beer from 20 till 66-year-old. His integrating amount of drinking was 670 kg convert into ethanol. He had no symptoms. Physical examination showed untoward features. Blood examination demonstrated thrombocytopenia, mild hyper-bilirubinemia, elevated liver enzymes (aspartate aminotransferase, 36 IU/L; alanine aminotransferase, 35 IU/L), positive for HCV-antibody, and 6.4 log IU/mL for HCV-RNA (Table 1).

Sonographic examination showed a homogenous, hypo-echoic, round mass in the segment 4b of the liver (Figure 1A). Color Doppler sonography revealed no signals in the lesion (Figure 1B). Contrast-enhanced ultrasonography (CEUS) with 0.5 mL of Sonazoid (Daiichi Sankyo, Tokyo, Japan) demonstrated mild global hyper-enhancement with inflow of microbubbles from the periphery of the tumor in the arterial phase (Figure 1C and D), persist enhancement in the portal venous phase (Figure 1E), and heterogeneous hypo-enhancement in the post vascular phase (Figure 1F). Plain computed tomography (CT) showed a hypodense tumor (Figure 2A). Contrast-enhanced CT showed iso-enhancement in the arterial phase (Figure 2B) and slight hypo-enhancement in the portal phase (Figure 2C). Magnetic resonance imaging (MRI) demonstrated slightly high intensity in the T1 weighted image (Figure 3A), and slightly low intensity in the T2 weighted image (Figure 3B). Contrast-enhanced MRI using Gadolinium ethoxybenzyl diethylene triamine pentaacetic acid revealed slightly high intensity in the hepatobiliary phase (Figure 3C).

Because the mass increased in size, it was suspected as being a well differentiated hepatocellular carcinoma. Considering the risk of hemorrhage and dissemination, partial segment 4 resection was performed without biopsy. Because the mass was adjacent to horizontal portion of the left portal vein and pathological diagnosis was needed, percutaneous ablation was not chosen. Microscopic findings showed homogeneous cell proliferation with low grade atypia, infiltration of inflammatory cells, ductular reactions, fatty deposit in part, and sinusoidal dilation (Figure 4). Immunohistochemistry revealed geographic positive for SAA, focal positive for glutamine synthetase, diffuse and strong positive for C-reactive protein, and positive for liver-type fatty acid binding protein (Figure 5). These pathological features corresponded to that of an inflammatory HCA.

## DISCUSSION

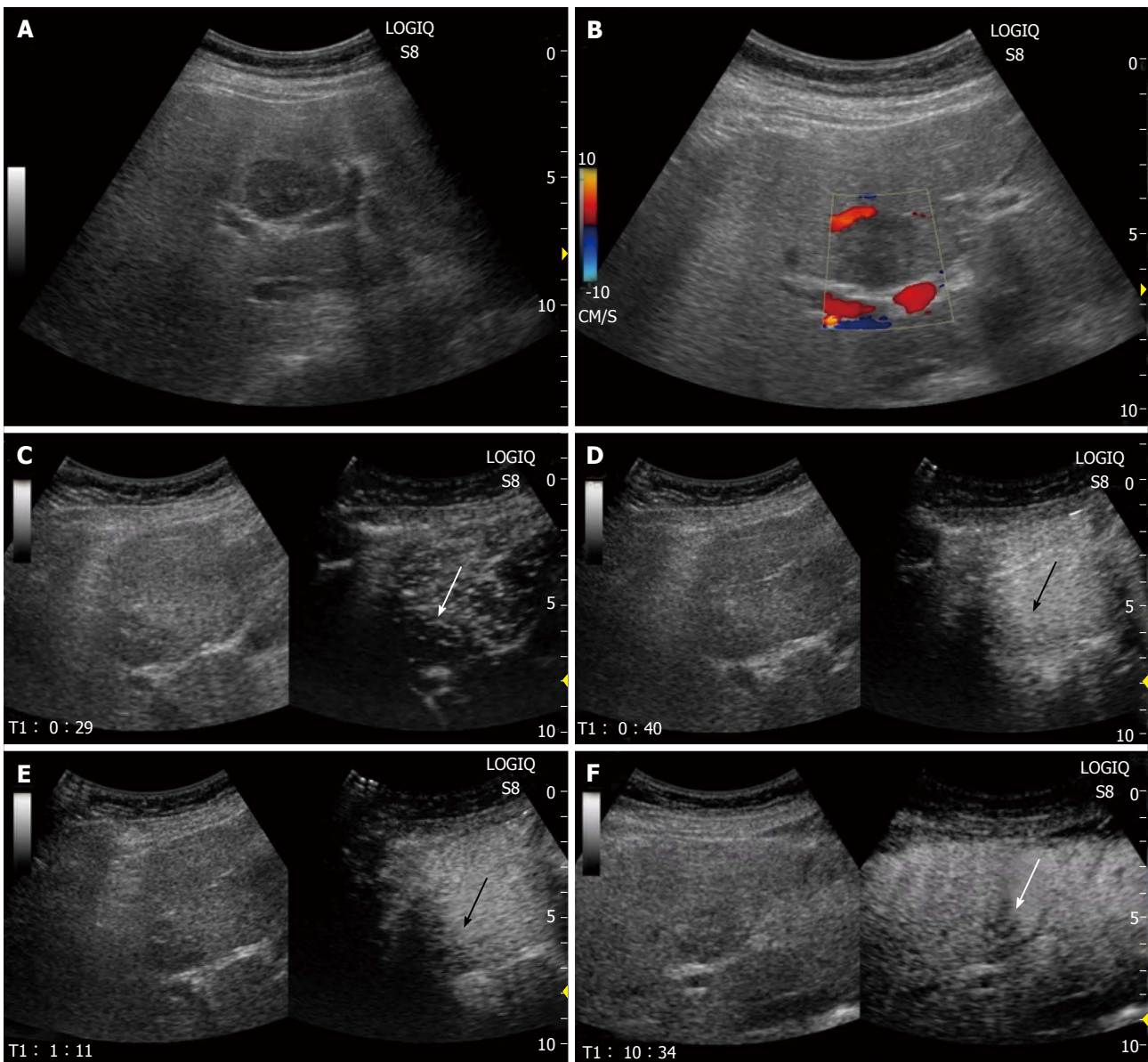
Classification of HCA is based on molecular, pathologic, and immunohistochemical features<sup>[1]</sup>. Inflammatory HCA accounts for 45%-60% of all HCAs<sup>[1-4]</sup>, and has



**Table 1** Patient's laboratory results (the normal ranges)

WBC (3.3-8.6)	6200/ $\mu$ L	Total protein (6.6-8.1)	8.0 g/dL
Hgb (13.7-16.8)	15.0 g/dL	Albumin (4.1-5.1)	4.7 g/dL
Platelet (148-348)	$108 \times 10^3$ / $\mu$ L	HBs antibody	(-)
PT% (> 80)	99%	HBc antigen	(-)
INR	0.92	HCV antibody	(+)
Total bilirubin (0.4-1.5)	1.41 mg/dL	HCV-RNA	6.4 logIU/mL
Direct bilirubin (0.05-0.4)	0.37 mg/dL	Alpha-fetoprotein (< 20)	6.4 ng/mL
Aspartate transaminase (13-30)	36 IU/L	PIVKA-II (< 40)	91 mAU/mL
Alanine transaminase (10-42)	35 IU/L	ICG (15 s) (< 15.0)	14.0%

WBC: White blood cell; INR: International normalized ratio; HCV: Hepatitis C virus; PIVKA-II: Prothrombin induced by vitamin K absence-II; ICG: Indocyanine green.

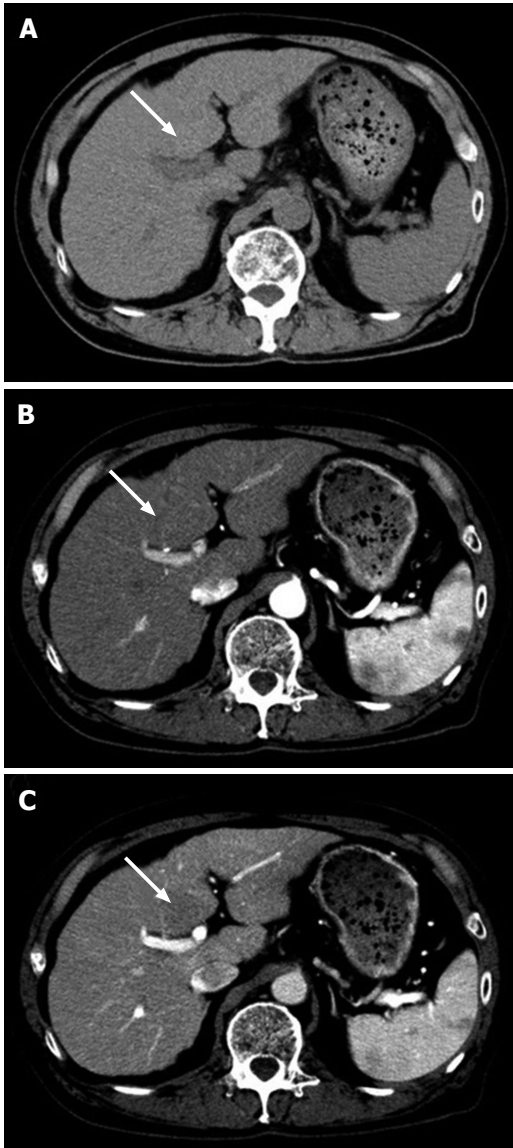


**Figure 1** Sonography. Sonographic examination showed a homogenous, hypo-echoic, round mass in segment 4 of the liver (A). Color Doppler sonography revealed no signals in the lesion (B). CEUS demonstrated mild global hypo-enhancement (D, arrow) with inflow of microbubbles from peripheral of the tumor (C, arrow) in the arterial phase, persist enhancement in the portal venous phase (E, arrow), and heterogeneous hypo-enhancement in the post vascular phase (F, arrow). CEUS: Contrast-enhanced ultrasonography.

mutations of the *IL6ST* gene<sup>[12-14]</sup>. Alcohol intake and obesity are association with inflammatory HCA<sup>[9,15-17]</sup>.

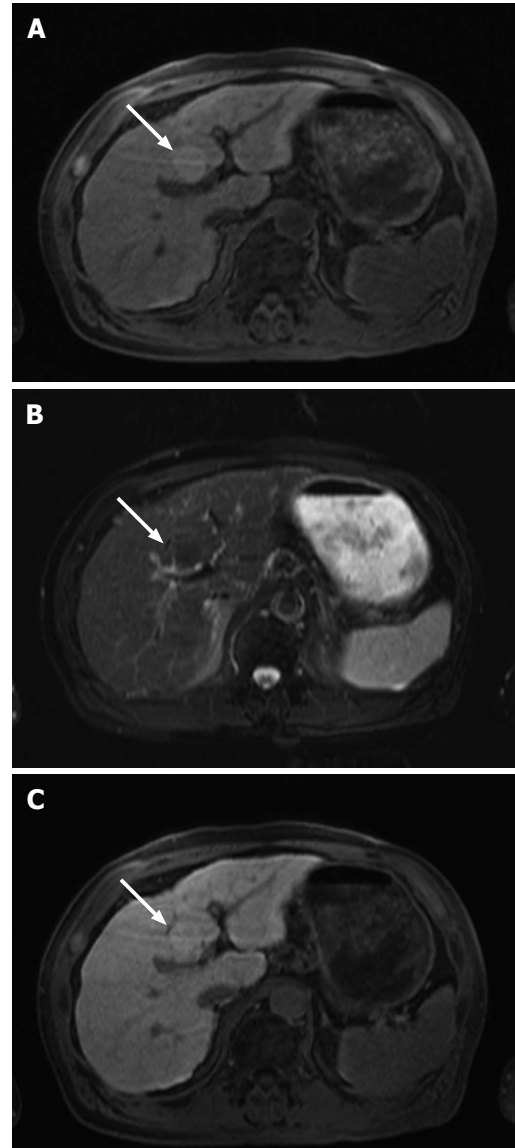
The rate of malignant transformation is unknown. CEUS findings of HCA were described in previous



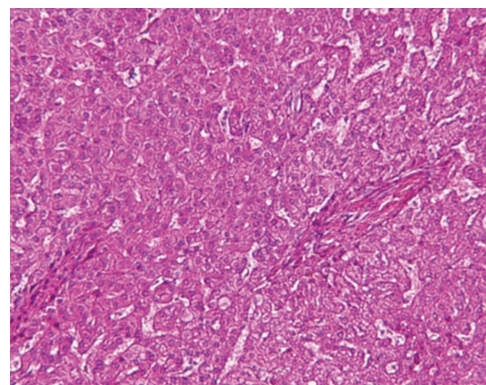


**Figure 2 Computed tomography.** Plain computed tomography (CT) showed a hypodense tumor (A, arrow); Contrast-enhanced CT showed iso-enhancement in the arterial phase (B, arrow); and slightly hypo-enhancement in the portal phase (C, arrow).

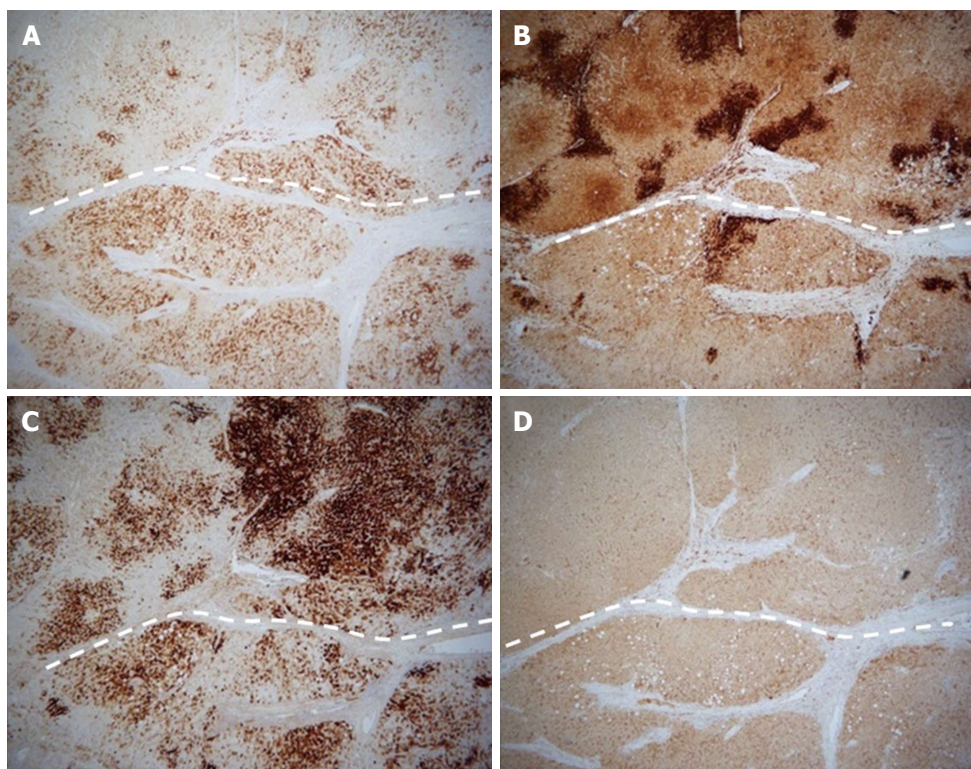
studies<sup>[5-8,18]</sup>. In one study which investigated 18 lesions, which were iso-hypoechoic in 9, hyper-enhancing in all in the arterial phase, and iso or hypo-enhancement in all in the late phase<sup>[18]</sup>. Ricci *et al.*<sup>[19]</sup> emphasized homogeneous and centripetal enhancement during artery phase was showed in almost all. In accordance with previous studies<sup>[19-21]</sup>, HCA lesions had some typical features, including early, homogeneous, centripetal, and strong enhancement in the arterial phase and the lack of a portal vein supply. According to a study<sup>[22]</sup>, a number of HCAs demonstrated persistent enhancement. Dong *et al.*<sup>[22]</sup> said “slow wash-out” (persistent enhancement during portal venous and late phase) may be a discriminant sign for HCAs in CEUS. In our case, the arterial phase findings were not so strong but homogenous and centripetal hyper-enhancement and some peripheral vessels were showed. The contrast medium that used in that study



**Figure 3 Magnetic resonance imaging.** Magnetic resonance imaging (MRI) demonstrated slightly high intensity in T1 weighted image (A, arrow), and slightly low intensity in T2 weighted image (B, arrow). Contrast-enhanced MRI using Gd-EOB-DTPA revealed slightly high intensity in the hepatobiliary phase (C, arrow). Gd-EOB-DTPA: Gadolinium ethoxybenzyl diethylene triamine pentaacetic acid.



**Figure 4 Microscopy** showed homogeneous cell proliferation with low grade atypia, infiltration of inflammatory cells, ductular reactions, fatty deposit in part, and sinusoidal dilation.



**Figure 5** Immunohistochemistry revealed geometric positive staining for serum amyloid A (A), focal positive for glutamine synthetase (B), diffuse and strong positive for C-reactive protein (C), positive for liver-type fatty acid binding protein (D). Upper side is tumor area.

was Sonovue (Bracco, Milan, Italy) whereas Sonazoid was administered to our patient. Sonovue and Sonazoid are phagocytosed by Kupffer cells, and visualize clearly malignant tumor as defect. CEUS using Sonazoid revealed hypo-enhancement in the post vascular phase in our patient which was interpreted as lack of Kupffer cells in the tumor.

Recently SAA-positive hepatocellular neoplasms were proposed<sup>[10,11]</sup>. Generally, HCA arises from normal liver<sup>[1,23]</sup>. Although SAA-positive hepatocellular neoplasms have similar features to inflammatory HCA, they arise from alcoholic cirrhosis. Sasaki *et al.*<sup>[10,11]</sup> suggested, considering that the patient exposed to alcohol in inflammatory HCA, it may be not be surprising that inflammatory HCA arise in alcoholic hepatic disease or cirrhosis. Our case had liver cirrhosis with HCV infection, moreover had a history of excessive amounts-alcohol consumption. We considered that our case is SAA-positive hepatocellular neoplasm.

In conclusion, CEUS revealed homogeneous mild hyper-enhancement in the arterial phase and heterogeneous hypo-enhancement in the post vascular phase in our case. Some of CEUS findings corresponded to features of HCA. Our patient had both HCV infection and alcohol abuse, and it was not typical for inflammatory HCA. It may be a case of so-called SAA-positive hepatocellular neoplasm.

## COMMENTS

### Case characteristics

A 78-year-old man with hepatitis C and hepatic cirrhosis received abdominal

ultrasonography, in which 20 mm, homogenous, hypo-echoic, round mass was shown in the segment 4b of the liver.

### Clinical diagnosis

Because the mass increased in size and he had hepatic cirrhosis, a well differentiated hepatocellular carcinoma was suspected.

### Differential diagnosis

Dysplastic nodule, large regenerative nodule, hepatocellular adenoma (HCA), and focal nodular hyperplasia.

### Laboratory diagnosis

Hepatic pre-cirrhosis with early hepatocellular carcinoma.

### Imaging diagnosis

A well differentiated hepatocellular carcinoma was suspected.

### Pathological diagnosis

Inflammatory HCA.

### Treatment

Segment 4 partial resection.

### Related reports

HCA was classified into four pathological subtypes and usually arises in the absence of significant fibrosis. Serum amyloid A (SAA)-positive hepatocellular neoplasm shares features with inflammatory HCA arising in alcoholic cirrhosis.

### Term explanation

SAA-positive hepatocellular neoplasms have similar features to inflammatory HCA, they arise from alcoholic cirrhosis.

### Experience and lessons

Considering that the patient exposed to alcohol in inflammatory HCA, it may



be not be surprising that it arise in alcoholic hepatic disease or cirrhosis. Recognizing SAA-positive hepatocellular neoplasm as differential diagnosis is important in the case that had both HCV infection and alcohol abuse.

### Peer-review

This is a very interesting case, well investigated and presented with also pathological comparison images.

## REFERENCES

- 1 **Bioulac-Sage P**, Balabaud C, Wanless I. Focal nodular hyperplasia and hepatocellular adenoma, In: WHO classification of tumours of the digestive system, Edited by F Bosman, F Carneiro, H Hruban, et al. 4th ed. IARC, Lyon, 2010: 198-204
- 2 **van Aalten SM**, Verheij J, Terkivatan T, Dwarkasing RS, de Man RA, Ijzermans JN. Validation of a liver adenoma classification system in a tertiary referral centre: implications for clinical practice. *J Hepatol* 2011; **55**: 120-125 [PMID: 21145863 DOI: 10.1016/j.jhep.2010.10.030]
- 3 **Zucman-Rossi J**, Jeannot E, Nhieu JT, Scoazec JY, Guettier C, Rebouissou S, Bacq Y, Leteurtre E, Paradis V, Michalak S, Wendum D, Chiche L, Fabre M, Mellottee L, Laurent C, Partensky C, Castaing D, Zafrani ES, Laurent-Puig P, Balabaud C, Bioulac-Sage P. Genotype-phenotype correlation in hepatocellular adenoma: new classification and relationship with HCC. *Hepatology* 2006; **43**: 515-524 [PMID: 16496320 DOI: 10.1002/hep.21068]
- 4 **Bioulac-Sage P**, Rebouissou S, Thomas C, Blanc JF, Saric J, Sa Cunha A, Rullier A, Cubel G, Couchy G, Imbeaud S, Balabaud C, Zucman-Rossi J. Hepatocellular adenoma subtype classification using molecular markers and immunohistochemistry. *Hepatology* 2007; **46**: 740-748 [PMID: 17663417 DOI: 10.1002/hep.21743]
- 5 **Kong WT**, Wang WP, Huang BJ, Ding H, Mao F, Si Q. Contrast-enhanced ultrasound in combination with color Doppler ultrasound can improve the diagnostic performance of focal nodular hyperplasia and hepatocellular adenoma. *Ultrasound Med Biol* 2015; **41**: 944-951 [PMID: 25701530 DOI: 10.1016/j.ultrasmedbio.2014.11.012]
- 6 **Ricci P**, Cantisani V, D'Onofrio M, Sahani D, Pagliara E, Calliada F, Mehmet E, Sanjeva K, Faccioli N, Pozzi-Mucelli R, D'Ambrosio U, Passariello R. Behavior of hepatocellular adenoma on real-time low-mechanical index contrast-enhanced ultrasonography with a second-generation contrast agent. *J Ultrasound Med* 2008; **27**: 1719-1726 [PMID: 19022997]
- 7 **Roche V**, Pigneur F, Tselikas L, Roux M, Baranes L, Djabbari M, Costentin C, Calderaro J, Laurent A, Rahmouni A, Luciani A. Differentiation of focal nodular hyperplasia from hepatocellular adenomas with low-mechanical-index contrast-enhanced sonography (CEUS): effect of size on diagnostic confidence. *Eur Radiol* 2015; **25**: 186-195 [PMID: 25120205 DOI: 10.1007/s00330-014-3363-y]
- 8 **Laumonier H**, Cailliez H, Balabaud C, Possenti L, Zucman-Rossi J, Bioulac-Sage P, Trillaud H. Role of contrast-enhanced sonography in differentiation of subtypes of hepatocellular adenoma: correlation with MRI findings. *AJR Am J Roentgenol* 2012; **199**: 341-348 [PMID: 22826395 DOI: 10.2214/AJR.11.7046]
- 9 **Sasaki M**, Yoneda N, Kitamura S, Sato Y, Nakanuma Y. A serum amyloid A-positive hepatocellular neoplasm arising in alcoholic cirrhosis: a previously unrecognized type of inflammatory hepatocellular tumor. *Mod Pathol* 2012; **25**: 1584-1593 [PMID: 22766792 DOI: 10.1038/modpathol.2012.114]
- 10 **Sasaki M**, Kondo F, Sawai Y, Imai Y, Kadowaki S, Sano K, Fukusato T, Matsui O, Nakanuma Y. Serum amyloid A-positive hepatocellular neoplasms in the resected livers from 3 patients with alcoholic cirrhosis. *Histol Histopathol* 2013; **28**: 1499-1505 [PMID: 23690168]
- 11 **Sasaki M**, Yoneda N, Sawai Y, Imai Y, Kondo F, Fukusato T, Yoshikawa S, Kobayashi S, Sato Y, Matsui O, Nakanuma Y. Clinicopathological characteristics of serum amyloid A-positive hepatocellular neoplasms/nodules arising in alcoholic cirrhosis. *Histopathology* 2015; **66**: 836-845 [PMID: 25318388 DOI: 10.1111/his.12588]
- 12 **Nault JC**, Fabre M, Couchy G, Pilati C, Jeannot E, Tran Van Nhieu J, Saint-Paul MC, De Muret A, Redon MJ, Buffet C, Salenave S, Balabaud C, Prevot S, Labrune P, Bioulac-Sage P, Scoazec JY, Chanson P, Zucman-Rossi J. GNAS-activating mutations define a rare subgroup of inflammatory liver tumors characterized by STAT3 activation. *J Hepatol* 2012; **56**: 184-191 [PMID: 21835143 DOI: 10.1016/j.jhep.2011.07.018]
- 13 **Rebouissou S**, Amessou M, Couchy G, Poussin K, Imbeaud S, Pilati C, Izard T, Balabaud C, Bioulac-Sage P, Zucman-Rossi J. Frequent in-frame somatic deletions activate gp130 in inflammatory hepatocellular tumours. *Nature* 2009; **457**: 200-204 [PMID: 19020503 DOI: 10.1038/nature07475]
- 14 **Pilati C**, Amessou M, Bihl MP, Balabaud C, Nhieu JT, Paradis V, Nault JC, Izard T, Bioulac-Sage P, Couchy G, Poussin K, Zucman-Rossi J. Somatic mutations activating STAT3 in human inflammatory hepatocellular adenomas. *J Exp Med* 2011; **208**: 1359-1366 [PMID: 21690253 DOI: 10.1084/jem.20110283]
- 15 **Sasaki M**, Yoneda N, Kitamura S, Sato Y, Nakanuma Y. Characterization of hepatocellular adenoma based on the phenotypic classification: The Kanazawa experience. *Hepatol Res* 2011; **41**: 982-988 [PMID: 21883740 DOI: 10.1111/j.1872-034X.2011.00851.x]
- 16 **Farges O**, Ferreira N, Dokmak S, Belghiti J, Bedossa P, Paradis V. Changing trends in malignant transformation of hepatocellular adenoma. *Gut* 2011; **60**: 85-89 [PMID: 21148580 DOI: 10.1136/gut.2010.222109]
- 17 **Paradis V**, Champault A, Ronot M, Deschamps L, Valla DC, Vidaud D, Vilgrain V, Belghiti J, Bedossa P. Telangiectatic adenoma: an entity associated with increased body mass index and inflammation. *Hepatology* 2007; **46**: 140-146 [PMID: 17596890 DOI: 10.1002/hep.21684]
- 18 **Forsberg F**, Piccoli CW, Liu JB, Rawool NM, Merton DA, Mitchell DG, Goldberg BB. Hepatic tumor detection: MR imaging and conventional US versus pulse-inversion harmonic US of NC100100 during its reticuloendothelial system-specific phase. *Radiology* 2002; **222**: 824-829 [PMID: 11867808 DOI: 10.1148/radiol.2223001786]
- 19 **Ricci P**, Laghi A, Cantisani V, Paolantonio P, Pacella S, Pagliara E, Arduini F, Pasqualini V, Trippa F, Filipo M, Passariello R. Contrast-enhanced sonography with SonoVue: enhancement patterns of benign focal liver lesions and correlation with dynamic gadobenate dimeglumine-enhanced MRI. *AJR Am J Roentgenol* 2005; **184**: 821-827 [PMID: 15728603 DOI: 10.2214/ajr.184.3.01840821]
- 20 **Quaia E**, Calliada F, Bertolotto M, Rossi S, Garioni L, Rosa L, Pozzi-Mucelli R. Characterization of focal liver lesions with contrast-specific US modes and a sulfur hexafluoride-filled microbubble contrast agent: diagnostic performance and confidence. *Radiology* 2004; **232**: 420-430 [PMID: 15286314 DOI: 10.1148/radiol.2322031401]
- 21 **Leen E**, Ceccotti P, Kalogeropoulou C, Angerson WJ, Moug SJ, Horgan PG. Prospective multicenter trial evaluating a novel method of characterizing focal liver lesions using contrast-enhanced sonography. *AJR Am J Roentgenol* 2006; **186**: 1551-1559 [PMID: 16714643 DOI: 10.2214/AJR.05.0138]
- 22 **Dong Y**, Zhu Z, Wang WP, Mao F, Ji ZB. Ultrasound features of hepatocellular adenoma and the additional value of contrast-enhanced ultrasound. *Hepatobiliary Pancreat Dis Int* 2016; **15**: 48-54 [PMID: 26818543 DOI: 10.1016/S1499-3872(15)60039-X]
- 23 **Rooks JB**, Ory HW, Ishak KG, Strauss LT, Greenspan JR, Hill AP, Tyler CW. Epidemiology of hepatocellular adenoma. The role of oral contraceptive use. *JAMA* 1979; **242**: 644-648 [PMID: 221698 DOI: 10.1001/jama.242.7.644]

**P- Reviewer:** Cosgrove D, Lagadinou M, Lee SY, Mauri G, Negrei C  
**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D



## Predictive potential of *IL-28B* genetic testing for interferon based hepatitis C virus therapy in Pakistan: Current scenario and future perspective

Muhammad Sohail Afzal

Muhammad Sohail Afzal, Department of Chemistry, School of Science, University of Management and Technology, Lahore 54000, Pakistan

**Author contributions:** Afzal MS designed the research, analyzed the data, wrote the letter, and revised the letter.

**Conflict-of-interest statement:** Afzal MS declares that there is no conflict of interest in this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Muhammad Sohail Afzal, PhD, Department of Chemistry, School of Science, University of Management and Technology, C-II Johar Town, Lahore 54000, Pakistan. [sohail.ncvi@gmail.com](mailto:sohail.ncvi@gmail.com)  
Telephone: +92-321-5244808

Received: April 5, 2016

Peer-review started: April 5, 2016

First decision: May 17, 2016

Revised: June 29, 2016

Accepted: July 20, 2016

Article in press: July 22, 2016

Published online: September 18, 2016

### Abstract

In Pakistan which ranked second in terms of hepatitis C virus (HCV) infection, it is highly needed to have an established diagnostic test for antiviral therapy response

prediction. Interleukin 28B (*IL-28B*) genetic testing is widely used throughout the world for interferon based therapy prediction for HCV patients and is quite helpful not only for health care workers but also for the patients. There is a strong relationship between single nucleotide polymorphisms at or near the *IL-28B* gene and the sustained virological response with pegylated interferon plus ribavirin treatment for chronic hepatitis C. Pakistan is a resource limited country, with very low per capita income and there is no proper social security (health insurance) system. The allocated health budget by the government is very low and is used on other health emergencies like polio virus and dengue virus infection. Therefore it is proposed that there should be a well established diagnostic test on the basis of *IL-28B* which can predict the antiviral therapy response to strengthen health care set-up of Pakistan. This test once established will help in better management of HCV infected patients.

**Key words:** Diagnostics; Hepatitis C virus; Interferon therapy; Polymorphisms; *IL-28B*; Genetic testing; Pakistan

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Pakistan has a very heavy burden of hepatitis C virus (HCV) infection with around 11 million positive cases; however, in spite of well established prognostic value, the data regarding the role of interleukin 28B (*IL-28B*) single nucleotide polymorphisms (SNPs) in HCV antiviral therapy response are very limited. There are only six reports on the topic and it can be concluded from this limited information that *IL-28B* could be a good prognosis marker for HCV patient management in Pakistan. The major prevalent HCV genotype in Pakistan is 3a and *IL-28B* SNP rs12979860 showed a good prediction for interferon based antiviral therapy response against this viral genotype. It can be predicted that inclusion of *IL-28B* genetic testing in



routine diagnostic set-up of Pakistan will help in better management of the disease. A well directed antiviral therapy based on personalized *IL-28B* genotyping along with virus genotyping will help in lessening of therapy cost and better management of the disease.

Afzal MS. Predictive potential of *IL-28B* genetic testing for interferon based hepatitis C virus therapy in Pakistan: Current scenario and future perspective. *World J Hepatol* 2016; 8(26): 1116-1118 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i26/1116.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i26.1116>

## TO THE EDITOR

Recent advancements in molecular biology techniques help in identification of various host and pathogenic factors influencing the disease prognosis and therapeutic outcomes. One example is identification of various genetic factors through genome wide analysis studies (GWAS). In the field of gastroenterology and hepatology, an example is the discovery of an association between single nucleotide polymorphisms (SNPs) at or near the interleukin 28B (*IL-28B*) gene and the sustained virological response (SVR) rate with pegylated interferon (IFN) plus ribavirin treatment for chronic hepatitis C (CH-C)<sup>[1-3]</sup>. *IL-28B* (IFN- $\lambda$ 3) is produced by many immune cells like neuronal cells, alveolar epithelial cells, and hepatocytes in response to viral infection. IFN- $\lambda$  showed antiviral activity against many viruses. It not only inhibits viral replication but also has immune-modulatory functions<sup>[4]</sup>. It has been shown by four autonomous GWAS that SNPs of the *IL-28B* gene, which is located on chromosome 19q13, are strongly associated with treatment response to interferon based therapy and spontaneous viral clearance in chronic hepatitis C virus (HCV)-infected patients<sup>[4]</sup>. After these studies, the predictive potential of *IL-28B* genetic variations has been investigated and verified throughout the world in patients infected with HCV of all viral genotypes and currently *IL-28B* SNPs are in commercial use for antiviral therapy response prediction around the world.

In Pakistan, data regarding the role of *IL-28B* SNPs in HCV antiviral therapy response are very limited. To our knowledge, there are only six studies that investigated the role of *IL-28B* in HCV patients regarding interferon therapy response and disease prognosis (Table 1)<sup>[5-10]</sup>. These studies investigated the predictive potential of either *IL-28B* protein level or *IL-28B* SNPs (rs12979860, rs8099917, rs12980275). It can be concluded from existing limited data that *IL-28B* could be a good prognosis marker for HCV patient management. Recent studies by Shaikh *et al*<sup>[9]</sup> (2014) and Imran *et al*<sup>[8]</sup>

(2015) reported significant existence (47.5% and 6.4%, respectively) of circulation of diagnostically untypable HCV variants in local populations of Sindh Province of Pakistan. We have lately highlighted the issue of diagnostically untypable HCV circulation in Pakistan and recommended immediate need to resolve this problem for the better management of HCV patients as course and fate of antiviral therapy are viral genotype dependent<sup>[11]</sup>. The resolution of this problem will also help in understanding the potential role of *IL-28B* SNPs in antiviral therapy response prediction against each viral genotype.

HCV is highly endemic in Pakistan with around 11 million infections<sup>[12-14]</sup>. The major prevalent viral genotype is 3a along with 2a, 3b, 1b, 2b, 2a and a large number of untypable ones<sup>[11,15,16]</sup>. It is observed that irrespective of the HCV genotype, SVR rate of interferon plus ribavirin is quite good (80%-97%) in Pakistan<sup>[7,8,17,18]</sup>. Pakistan is a resource limited country with much low per capita income in the general population. According to the World Health Organization, the total expenditure on health is only 2.8% of GDP, which means total expenditure on health per capita is only 126 \$<sup>[19]</sup>. Other medical emergencies like polio virus and dengue virus endemics shift the government priorities and funds are becoming less available for HCV management. There is no health insurance for the general population in Pakistan, which also affect the patient's ability to bear therapy cost. *IL-28B* genetic test is an established diagnostic test for interferon based antiviral therapy response prediction across the world. In the current scenario of Pakistan, it is highly needed to have an established diagnostic test on the basis of *IL-28B* which can predict the antiviral therapy response.

The currently available literature on the role of *IL-28B* in HCV interferon therapy response in Pakistan shows that rs12979860 is a good predictor of therapy response against HCV 3a genotype. In the era of direct acting antivirals (DAAs), interferon based therapy against HCV will remain the major choice in Pakistan due to higher SVR and low cost compared with DAAs<sup>[20]</sup>. On the basis of the above discussion, we propose future studies across the country on different ethnic groups infected with all viral genotypes so that the results could be generalized for diagnostic purpose. It is also suggested that the forthcoming studies should include a comparatively larger number of patients so that the results could be applicable for commercial purpose. It is highly anticipated that inclusion of *IL-28B* genetic testing in routine diagnostic tests will help health care professionals in better management of the patients. Well directed antiviral therapy on the basis of personalized *IL-28B* genotyping along with viral genotyping will help in reduction of therapy cost and better management of the disease.

**Table 1 Summary of interleukin 28B and interferon based therapy response in hepatitis C virus patients in Pakistan**

Year	Viral genotype	Patients (n)	Objective/SNP investigated	Findings/conclusion	Ref.
2015	3a	66	IL-28B protein levels	IL-28B protein levels were significantly associated with therapy response	[5]
2015	3	105	rs8099917	TT genotype favors RVR	[6]
			rs12979860	CC genotype favors SVR	
2015	1a,1b, 3a	111	rs12979860	CC genotype favors SVR in HCV 3a genotype	[7]
2015	1a, 1b, 3a, 3b, 4, UT	140	rs8099917	No association was observed with therapy response	[8]
			rs12979860	CC genotype favors SVR	
2014	(2a, 3a, UT)	220	rs8099917	No association was observed with therapy response	[9]
			rs12979860	No association was observed with therapy response	
			rs12980275	AA genotype favors SVR	
2014	3a	200	rs12979860	TT genotype favors SVR	[10]

Genotyping performed only for non-responders patients. SVR: Sustained virological response; RVR: Rapid virological response; UT: Untypable; IL-28B: Interleukin 28B; HCV: Hepatitis C virus; SNP: Single nucleotide polymorphism.

## REFERENCES

- Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]
- Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100-1104 [PMID: 19749758 DOI: 10.1038/ng.447]
- Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105-1109 [PMID: 19749757 DOI: 10.1038/ng.449]
- Imran M, Manzoor S, Ashraf J, Khalid M, Tariq M, Khaliq HM, Azam S. Role of viral and host factors in interferon based therapy of hepatitis C virus infection. *Virol J* 2013; **10**: 299 [PMID: 24079723 DOI: 10.1186/1743-422X-10-299]
- Resham S, Manzoor S, Imran M, Saalim M, Naseem S, Azam S. Interleukin- 28B: a prognostic marker in interferon based therapy of chronic HCV patients of the Pakistan with variable treatment response. *APMIS* 2015; **123**: 765-771 [PMID: 26177560 DOI: 10.1111/apm.12414]
- Aziz H, Raza A, Ali K, Khattak JZ, Irfan J, Gill ML. Polymorphism of the IL28B gene (rs8099917, rs12979860) and virological response of Pakistani hepatitis C virus genotype 3 patients to pegylated interferon therapy. *Int J Infect Dis* 2015; **30**: 91-97 [PMID: 25462177 DOI: 10.1016/j.ijid.2014.09.021]
- Khubaib B, Saleem S, Idrees M, Afzal S, Wasim M. The genotype CC of IL-28B SNP rs12979860 is significantly associated with a sustained virological response in chronic HCV-infected Pakistani patients. *J Dig Dis* 2015; **16**: 293-298 [PMID: 25708904 DOI: 10.1111/1751-2980.12238]
- Imran M, Manzoor S, Azam S, Resham S. Genetic variant of IL28B rs12979860, as predictive marker of interferon-based therapy in Pakistani population. *APMIS* 2015; **123**: 342-349 [PMID: 25703417 DOI: 10.1111/apm.12365]
- Shaikh N, Waryah AM, Devrajani BR, Rajput MI, Hayat AS, Shaikh S. IL28B rs12980275 polymorphism shows association with response to treatment in Pakistani patients with chronic hepatitis C. *J Med Virol* 2015; **87**: 814-820 [PMID: 25652367 DOI: 10.1002/jmv.24100]
- Hashmi AH, Ahmad N, Riaz S, Ali L, Siddiqi S, Khan KM, Shakoori AR, Mansoor A. Genotype CC of rs12979860 is providing protection against infection rather than assisting in treatment response for HCV genotype 3a infection. *Genes Immun* 2014; **15**: 430-432 [PMID: 24898388 DOI: 10.1038/gene.2014.31]
- Afzal MS, Khan MY, Ammar M, Anjum S, Zaidi NU. Diagnostically untypable hepatitis C virus variants: it is time to resolve the problem. *World J Gastroenterol* 2014; **20**: 17690-17692 [PMID: 25516688 DOI: 10.3748/wjg.v20.i46.17690]
- Afzal MS, Anjum S, Zaidi NU. Changing of HCV clade pattern in Iran; the possible means for something good. *Hepat Mon* 2014; **14**: e11879 [PMID: 24497875 DOI: 10.5812/hepatmon.11879]
- Afzal MS, Ahmed T, Zaidi NU. Comparison of HCV prevalence in Pakistan and Iran; an insight into future. *Hepat Mon* 2014; **14**: e11466 [PMID: 24497874 DOI: 10.5812/hepatmon.11466]
- Afzal MS. Are efforts up to the mark? A cirrhotic state and knowledge about HCV prevalence in general population of Pakistan. *Asian Pac J Trop Med* 2016; **9**: 616-618 [PMID: 27262079 DOI: 10.1016/j.apjtm.2016.04.013]
- Afzal MS, Shah ZH, Ahmed H. Recent HCV genotype changing pattern in the Khyber Pakhtunkhwa province of Pakistan; is it pointing out a forthcoming problem? *Braz J Infect Dis* 2016; **20**: 312-313 [PMID: 26963150 DOI: 10.1016/j.bjid.2015.12.011]
- Anjum S, Afzal MS, Ahmad T, Aslam B, Waheed Y, Shafi T, Qadri I. Mutations in the STAT1-interacting domain of the hepatitis C virus core protein modulate the response to antiviral therapy. *Mol Med Rep* 2013; **8**: 487-492 [PMID: 23799612 DOI: 10.3892/mmr.2013.1541]
- Akhtar N, Bilal M, Rizwan M, Khan MA, Khan A. Genotypes of hepatitis C virus in relapsed and non-respondent patients and their response to anti-viral therapy in district Mardan, Khyber Pakhtunkhwa, Pakistan. *Asian Pac J Cancer Prev* 2015; **16**: 1037-1040 [PMID: 25735327]
- Ahmad B, Ali S, Ali I, Azam S, Bashir S. Response rates of standard interferon therapy in chronic HCV patients of Khyber Pakhtunkhwa (KPK). *Virol J* 2012; **9**: 18 [PMID: 22244529 DOI: 10.1186/1743-422X-9-18]
- World health Organization. Pakistan. Available from: URL: <http://www.who.int/countries/pak/en/>
- Raza H, Ahmad T, Afzal MS. HCV, Interferon Therapy Response, Direct Acting Antiviral Therapy Revolution and Pakistan: Future Perspectives. *Asian Pac J Cancer Prev* 2015; **16**: 5583-5584 [PMID: 26225714]

P- Reviewer: Sirin G, Souza-Mello V S- Editor: Qi Y

L- Editor: Wang TQ E- Editor: Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 September 28; 8(27): 1119-1156







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**Stephen DH Malnick, *Rehovot***Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**Kamal E Bani-Hani, *Zarqa***Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**Angela Peltec, *Chishinev***Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**CA Asabamaka Onyekwere, *Lagos***Pakistan**Bikha Ram Devrajani, *Jamshoro***Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**Jacek Zielinski, *Gdansk***Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**Reem Al Olaby, *Doha***Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**Ser Yee Lee, *Singapore***South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



**REVIEW**

- 1119 Molecular pathological epidemiology in diabetes mellitus and risk of hepatocellular carcinoma  
*Gao C*

**MINIREVIEWS**

- 1128 Implication of the intestinal microbiome in complications of cirrhosis  
*Bhat M, Arendt BM, Bhat V, Renner EL, Humar A, Allard JP*

**ORIGINAL ARTICLE****Clinical Trials Study**

- 1137 Independent effects of diet and exercise training on fat oxidation in non-alcoholic fatty liver disease  
*Croci I, Byrne NM, Chachay VS, Hills AP, Clouston AD, O'Moore-Sullivan TM, Prins JB, Macdonald GA, Hickman IJ*

**Prospective Study**

- 1149 Ohio solid organ transplantation consortium criteria for liver transplantation in patients with alcoholic liver disease  
*Hajifathalian K, Humberson A, Hanouneh MA, Barnes DS, Arora Z, Zein NN, Eghtesad B, Kelly D, Hanouneh IA*

**LETTERS TO THE EDITOR**

- 1155 Is MELD score failing patients with liver disease and hepatorenal syndrome?  
*Sibulesky L, Leca N, Blosser C, Rahnamaï-Azar AA, Bhattacharya R, Reyes J*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Danny Ka-Ho Wong, BSc, MSc, PhD, Research Assistant Professor, Department of Medicine, the University of Hong Kong, Hong Kong, China

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/  
ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR  
THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xin-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Department of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL BOARD MEMBERS**  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

**EDITORIAL OFFICE**  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
September 28, 2016

**COPYRIGHT**  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
<http://www.wjgnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esps/>

## Molecular pathological epidemiology in diabetes mellitus and risk of hepatocellular carcinoma

Chun Gao

Chun Gao, Department of Gastroenterology, China-Japan Friendship Hospital, Ministry of Health, Beijing 100029, China

**Author contributions:** Gao C conceived the topic, performed research, retrieved concerned literatures and wrote the paper.

**Supported by** Beijing NOVA Programme of Beijing Municipal Science and Technology Commission, No. Z13110.7000413067.

**Conflict-of-interest statement:** No conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Chun Gao, MD, Department of Gastroenterology, China-Japan Friendship Hospital, Ministry of Health, No. 2 Yinghua East Road, Beijing 100029, China. [gaochun@bjmu.edu.cn](mailto:gaochun@bjmu.edu.cn)  
 Telephone: +86-10-84205313  
 Fax: +86-10-64481924

Received: April 2, 2016  
 Peer-review started: April 7, 2016  
 First decision: June 6, 2016  
 Revised: June 28, 2016  
 Accepted: August 6, 2016  
 Article in press: August 8, 2016  
 Published online: September 28, 2016

### Abstract

Molecular pathological epidemiology (MPE) is a multi-disciplinary and transdisciplinary study field, which has emerged as an integrated approach of molecular patho-

logy and epidemiology, and investigates the relationship between exogenous and endogenous exposure factors, tumor molecular signatures, and tumor initiation, progression, and response to treatment. Molecular epidemiology broadly encompasses MPE and conventional-type molecular epidemiology. Hepatocellular carcinoma (HCC) is the third most common cause of cancer-associated death worldwide and remains as a major public health challenge. Over the past few decades, a number of epidemiological studies have demonstrated that diabetes mellitus (DM) is an established independent risk factor for HCC. However, how DM affects the occurrence and development of HCC remains as yet unclearly understood. MPE may be a promising approach to investigate the molecular mechanisms of carcinogenesis of DM in HCC, and provide some useful insights for this pathological process, although a few challenges must be overcome. This review highlights the recent advances in this field, including: (1) introduction of MPE; (2) HCC, risk factors, and DM as an established independent risk factor for HCC; (3) molecular pathology, molecular epidemiology, and MPE in DM and HCC; and (4) MPE studies in DM and risk of HCC. More MPE studies are expected to be performed in future and I believe that this field can provide some very important insights on the molecular mechanisms, diagnosis, personalized prevention and treatment for DM and risk of HCC.

**Key words:** Diabetes mellitus; Molecular pathological epidemiology; Hepatocellular carcinoma; Risk factor; Molecular mechanism

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Diabetes mellitus (DM) is an established independent risk factor for hepatocellular carcinoma (HCC); however, how DM affects the occurrence and development of HCC remains as yet unclearly understood. Molecular pathological epidemiology (MPE) may be a promising approach to investigate the molecular mechanisms of carcinogenesis of DM in HCC, and provide some

useful insights for this pathological process. This review highlights the recent advances in this field and more MPE studies are expected to be performed for this question in future.

Gao C. Molecular pathological epidemiology in diabetes mellitus and risk of hepatocellular carcinoma. *World J Hepatol* 2016; 8(27): 1119-1127 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i27/1119.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i27.1119>

## INTRODUCTION

Molecular pathology examines the expression of molecular markers within bodily fluids, tissues or organs, and focuses on the diagnosis and studies of diseases, such as tumors<sup>[1,2]</sup>. Epidemiology is focused upon the studies of distributions and determinants of diseases and health conditions in specific populations<sup>[3,4]</sup>. Molecular pathological epidemiology (MPE) is a multidisciplinary and transdisciplinary study field, which has emerged as an integrated approach of molecular pathology and epidemiology, and investigates the relationship between tumour molecular markers, exposure of endogenous and exogenous factors, and development, progression and prognosis of tumors<sup>[5-8]</sup>. Molecular epidemiology broadly encompasses MPE and conventional-type molecular epidemiology.

In MPE, researchers investigate the relationships between: (1) changes of extracellular or cellular molecules (disease molecular signatures); (2) genetic, dietary, environmental and lifestyle factors; and (3) development and progression of diseases, such as tumors<sup>[6]</sup>. In 2010, Professor Shuji Ogino and Professor Meir Stampfer<sup>[5]</sup> were the first to introduce the concept of MPE. They consolidated this concept mainly based on the researches of colorectal cancer (CRC), particularly the prototypical study in the evolving field of MPE, which was conducted by Professor Peter T Campbell and others<sup>[9]</sup>.

This case-control study of Campbell *et al*<sup>[9]</sup> was conducted to determine the relationships between CRC microsatellite instability (MSI) status, risk of CRC, and human body mass index (BMI). The results showed that an increased CRC risk was found in those patients with a high BMI; however, this risk of CRC was associated with the MSI status. For patients with MS-stable, the adjusted odds ratio (OR) was 1.38 and 95%CI was 1.24-1.54, for an increment of 5 kg/m<sup>2</sup> of BMI; for patients with MSI-low, the OR was 1.33 and 95%CI was 1.04-1.72; however, for patients with MSI-high tumours, the value of OR and 95%CI were 1.05 and 0.84-1.31, respectively<sup>[9]</sup>. The authors concluded that the relationship between the high BMI and increased CRC risk was related to the tumor MSI status<sup>[9]</sup>. According to the concept and principle of MPE, this prototypical study addressed the relationship between exposure factor (high BMI), molecular change (CRC MSI status) and tumor initiation (risk of CRC)<sup>[5,10]</sup>.

MPE addresses two questions: (1) the association of particular exposure factors with specific molecular changes; and (2) the interaction of particular exposure factors with specific molecular changes to affect development, progression and prognosis of tumors. The typical research of cancer MPE is used to examine the relationship between exposure factors and risk of tumors according to the status of tumor signatures<sup>[9,10]</sup>. Cancer MPE techniques and studies can help us understand the carcinogenesis of certain exposure factors, through the examination of molecular pathological signatures associated with initiation and progression of tumors, and the exposures<sup>[5,6]</sup>.

## HEPATOCELLULAR CARCINOMA AND RISK FACTORS

Hepatocellular carcinoma (HCC) has been confirmed as the third leading cause globally, among all the cancer-related deaths<sup>[10-12]</sup>. For primary liver cancers, more than 80% are HCC and the incidence rate annually of HCC is 4.9 per 100000 persons. Although some advances have been gained in the diagnosis and treatment of HCC, the prognosis remains very poor. Similarly, the annual mortality rate remains very high and HCC has also been ranked as one of the most lethal cancers<sup>[13]</sup>.

With the using and popularization of hepatitis B virus (HBV) vaccination, the improvement of people's living standard and life style, and advancement of early diagnosis and treatment of premalignant lesions, the incidence of HCC had been anticipated to be decreased. However, the incidence rate of HCC has already been found to be increased significantly in the past thirty years in some countries, including the United States, China and Japan<sup>[14,15]</sup>. For example, during the period of 1981-1983 in the United States, the age adjusted incidence rate was 1.3 per 100000; however, this rate increased to 3.0 per 100000 during the period of 1996-1998<sup>[14,15]</sup>. Although more than fifty percent of this increase has been attributed to hepatitis virus C (HCV), other hepatitis viruses and alcoholic liver disease<sup>[16]</sup>, the reason remains as unclear.

The identified risk factors of HCC include liver cirrhosis, HBV, HCV, heavy alcoholic consumption, aflatoxin exposure, non-alcoholic steatohepatitis, positive family history, male sex, and increasing age<sup>[17-19]</sup>. Over the past few decades, a number of epidemiological studies have demonstrated that diabetes mellitus (DM) is an established independent risk factor for HCC<sup>[12,20-23]</sup>.

## DM AS AN ESTABLISHED INDEPENDENT RISK FACTOR FOR HCC

In the year of 1986, for the first time, Lawson *et al*<sup>[24]</sup> proposed accidentally the positive association of DM with HCC. The authors observed that, in Western Europe, the incidence rate of primary liver cancers was increased, and deduced that this increase might in part be associated



with the induction of hepatic microsomal enzyme caused by long-term usage of some drugs. On the basis of this assumption, the authors designed and performed an observational case-control study, which included 105 patients with HCC and long-term drug use, and 105 age and sex-matched patients with colorectal tumors and with fractures of femur<sup>[24]</sup>. Surprisingly, the results demonstrated that compared to the control group, the HCC group patients had four-fold excess of diabetic cases, and this association was independent of those pre-existing diseases, for example viral hepatitis, alcoholic cirrhosis and haemochromatosis<sup>[24]</sup>. The relationship between DM and HCC was proposed clearly although some limitations could not be avoided.

Following the publication of this study, only a few researches attempted to elucidate the association of diabetes with HCC in the next more than ten years; however, over the past more than one decade, more and more researches have been designed and performed to address this relationship<sup>[21,25-27]</sup>. Earlier epidemiologic studies showed inconsistent findings relating to the association of DM with HCC<sup>[21,28-30]</sup> whereas more and more recent studies have identified DM as an established independent risk factor for HCC, especially two prospectively large-scale population-based cohort studies<sup>[31,32]</sup>. In 2008, a review published in the journal of LANCET ranked diabetes as the fourth risk factor for HCC, following cirrhosis, viral hepatitis B and C, and non-alcoholic steatohepatitis<sup>[17]</sup>.

Of the two prospectively large-scale population-based cohort studies<sup>[31,32]</sup>, one was performed in the Sweden, which used the Swedish In-patient Register and included 153852 patients diagnosed with diabetes during the period between 1965 and 1983<sup>[31]</sup>. The patients were followed up through December 31, 1989. The authors identified those incident cases of cancer using the database and excluded those patients who were diagnosed with liver cancers during the first year of follow-up. The results showed that an increased risk of developing primary liver cancers was found in the diabetic patients (standardized incidence ratio, SIR = 4.1; 95%CI: 3.8-4.5). After exclusion of those concomitant diseases which have been associated with HCC, for example hepatitis, cirrhosis, and alcoholism, the persistence of an approximately threefold excess risk was observed<sup>[31]</sup>.

The conclusion from the Swedish study was supported by another followed cohort study conducted in the United States<sup>[32]</sup>, which was performed by doctors in the Department of Veterans Affairs. In this study, the authors also used the computerized records to identify all the patients with a hospital discharge diagnosis of DM in the period from 1985 to 1990, and matched randomly three patients without DM for every diabetic patient. Follow-up of these patients was taken through December 31, 2000. The major strength of this study was the strict inclusion and exclusion criteria and they were pre-determined perfectly on the basis of our current knowledge. The authors decided and used three periods, including: (1) the period dating back to 1980; (2) the period of index hospitalization; and (3) the period

of the first year of follow-up. During these three above-mentioned periods, those patients with all kinds of liver diseases, abnormal liver function tests, alcoholism, or other identified risk factors for HCC, such as HBV and HCV, had been excluded from the study population<sup>[32]</sup>. The authors concluded that among men with diabetes, the risk of HCC was increased, which was not associated with demographic features, viral hepatitis, cirrhosis, and alcoholic liver disease.

The recently published systematic review in this field was designed to evaluate the impact of DM on the risk of HCC among patients with HCV infection<sup>[33]</sup>. This research included seven articles and all of them were conducted in Asian cohorts, including three studies from Taiwan, China, and four from Japan<sup>[34-40]</sup>. Among these studies, six were observational cohorts and six studies were of good quality. The results showed that a significantly increased risk of HCC was associated with DM in five of these seven studies and the effect sizes ranged from HR = 1.73 (95%CI: 1.30-2.30) to RR = 3.52 (95%CI: 1.29-9.24)<sup>[33]</sup>.

## MOLECULAR PATHOLOGY, MOLECULAR EPIDEMIOLOGY AND MPE IN DIABETES

### *Molecular pathology in diabetes*

Pathology is an important constituent part of diagnostics, modern medicine and causal studies of diseases, which focuses upon four research fields of diseases: Etiology (causes), pathogenesis (mechanisms of development and progression), morphologic alterations (structural changes of cells, tissues and organs), and clinical manifestations (consequences of alterations)<sup>[41,42]</sup>. Molecular pathology (MP), whose focus is the examination of molecular signatures, has some similar aspects of practice to other disciplines, such as anatomic pathology, genetics, biochemistry, proteomics, molecular biology, and clinical pathology. Application of modern MP often encompasses three components: (1) exploration and confirmation of predictive molecular biomarkers for development, progression and treatment of diseases; (2) development of genetic and molecular approaches for diagnosis and classification of human diseases; and (3) susceptibility of individuals of different genetic constitution to particular disorders.

Molecular pathological studies in diabetes provide better insight into the etiology. For example type 1 or insulin-dependent diabetes, at least 20 genes have been identified and the dominant susceptibility locus maps to the major histocompatibility complex<sup>[43,44]</sup>. Major areas of MP research include environmental trigger factors, modification of the beta cells, infiltration of the islets by immuno-inflammatory cells, and autoimmune-mediated destruction of the beta cells. For T2DM, since the early genome-wide association studies (GWAS) in 2007, hundreds of genetic loci have been identified. Elucidating the pathology of DM at the molecular level is very important for developing innovative, personalized, and evidence-based treatments<sup>[45,46]</sup>.

From the viewpoint of MP of DM in cancers, disruption of homeostatic glucose metabolism has been significantly associated with the malignant cellular transformation and tumor progression. In addition, the pathophysiology of disrupted glucose-insulin axis pathways of DM has been understood deeply at the subcellular level, thanks for the recent advances in biochemical and molecular technology. They may be useful for better understanding of the malignant cellular transformation, such as HCC.

### **Molecular epidemiology in diabetes**

In the late 20<sup>th</sup> century, with great advancement of biomedical sciences, a number of molecular signatures or biomarkers were identified as predictors of disease initiation, progression, and response to treatment, including diabetes and tumors. Since the identification of these molecular signatures, molecular epidemiology has evolved and been broadly named, which refers to the branch of epidemiology, where investigators examine these signatures in special study populations and its interaction with environmental, lifestyle or dietary factors, to perform the causal studies of diseases with aetiological factors<sup>[6,10]</sup>. Since the 2000s, GWAS has been commonly performed to identify genetic risk factors for diseases and health conditions<sup>[47,48]</sup>.

Molecular epidemiology in diabetes is focused upon the contribution of possible environmental and genetic risk factors, to the distributions and determinants of DM within families and across populations, at the molecular level. For example, a number of molecular epidemiological studies demonstrate that some growth factors, including insulin, growth hormone, insulin-like growth factors and their binding proteins, may be important in the pathophysiological processes of T2DM<sup>[49]</sup>. In addition, many physiological changes have been associated with T2DM, including insulin resistance and hyperinsulinemia, increased estrogen levels, increased inflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$ , and interleukin (IL)-6, as well as altered levels of circulating adipokines<sup>[50]</sup>. It is well known that some of these molecular signatures and physiological changes may contribute to the development of cancers. Therefore, the relationship between DM and cancers, such as HCC, may be built *via* these molecular signatures or biomarkers.

### **MPE in diabetes**

MPE emerges as an integrated approach of molecular pathology and epidemiology, and investigates the relationship between risk factors, molecular signatures, and development and progression of diseases<sup>[10]</sup>. According to the concept and principle of MPE, the MPE approaches can also be used in non-neoplastic diseases, such as DM<sup>[51]</sup>. Although great advancements have been made in molecular pathology and molecular epidemiology, and a lot of molecular signatures have been associated with DM, no MEP studies in DM have been performed and the reason may be deduced that no identified risk factors are found for DM, such as HBV or HCV for HCC.

However, a few MPE studies had been performed when DM was treated as the risk factor for other diseases, such as cancers and coronary artery lesions, before the proposal and/or use of the concept of MPE, and they were conducted usually under the umbrella of molecular epidemiology. For example, one MPE study was designed to determine the relationship between 8-oxoguanine glycosylase (hOGG1) Ser326Cys gene polymorphism and coronary artery lesions in patients with DM<sup>[52]</sup>. In this study, 323 diabetic patients were included and the results showed that hOGG1 Ser326Cys polymorphism was correlated with coronary artery lesions in patients with DM, and Cys/Cys genotype may be associated with the more severity of lesions<sup>[52]</sup>.

## **MOLECULAR PATHOLOGY, MOLECULAR EPIDEMIOLOGY AND MPE IN HCC**

### **Molecular pathology in HCC**

For human cancers, including CRC and HCC<sup>[53-55]</sup>, molecular pathology is commonly used in the diagnosis and classification. Traditional molecular pathology studies are focused upon the molecular characteristics in cancer cells to improve our understanding of tumor cell behavior and carcinogenic processes<sup>[1,6,10]</sup>. However, human cancers are complex multifactorial diseases. Recent studies suggest that cancers should be classified based on salient clinical and pathologic features as well as on molecular fingerprints, which has been named "molecular classification", because of the premise that tumors with similar characteristics share common pathogenic mechanisms and progression patterns, despite each tumor undergoing its own unique neoplastic transformation<sup>[5,6,56]</sup>. Molecular classification is helpful to better understand the pathogenesis of tumors, predict the development and progression of each tumor, and for personalized cancer medicine, optimize the preventive and treatment strategies<sup>[5,6,56]</sup>. For cancer molecular classification, informative biomarkers are needed to be identified to stratify tumors or patients<sup>[57-62]</sup>.

Examples of well-established informative biomarkers include ESR1 (ER- $\alpha$ ), PGR and ERBB2 (HER2) expression in breast cancer<sup>[63-65]</sup>, EGFR mutations in lung cancer<sup>[66,67]</sup>, MSI in colorectal cancer<sup>[68-70]</sup>, TMPRSS2-ERG translocation in prostate cancer<sup>[71]</sup>, and TP53, PIK3CA, BRAF and KRAS mutations, and CpG island methylation in multiple cancers<sup>[72-74]</sup>. Some molecular changes or biomarkers in HCC have also been previously identified. Ojanguren *et al*<sup>[75]</sup> showed that the positive expression of mutant p53 was related to alcohol abuse (42%) and HBV infection (21%). Park *et al*<sup>[76]</sup> found that TNF and IL-6 signaling was correlated with obesity-associated HCC development. In the obese patients, insulin and insulin-like growth factors, TNF- $\alpha$ , IL-1 and IL-6, leptin, adipokines, adiponectin, and plasminogen activator inhibitor-1 are significantly associated with the occurrence and development of some cancers, including HCC<sup>[77]</sup>.

### **Molecular epidemiology in HCC**

HCC is also very complex, for example it occurs in about

1%-7% of cirrhotic patients annually, whereas most of the cirrhotic patients do not progress to HCC during their lifetimes<sup>[78]</sup>. Molecular biomarkers are expected to satisfy this need and resolve the question at the molecular level. To date, molecular epidemiology studies show that a number of molecular risk factors of HCC have been identified, such as numerous genetic polymorphisms reported as host genetic factors<sup>[79]</sup>. Most of HCC-associated single-nucleotide polymorphisms are identified in genes involved in biological pathways, including oxidative stress (GSTT1, GSTM1), cell cycle (MDM2), immune response (IL10, TNF), DNA damage repair (XPC), growth signaling (EGF), and iron metabolism (HFE) in viral hepatitis- or alcohol-related HCC<sup>[80-84]</sup>. Recent GWAS identifies the DEPDC5 locus as the risk loci in viral hepatitis-related HCC<sup>[85]</sup>.

Molecular factors associated with etiological agents, for example HBV and HCV could also influence the risk of HCC. It is well known that a high level of serum HBV DNA is indicative of increased risk of HCC. Some studies have demonstrated that HBV genotype is related to the HCC risk<sup>[86]</sup>. Genomics technology has revealed that HCC should be regarded as a heterogeneous group of diseases, not one single disease entity, because each sub-group HCC has different sets of epigenetic and genetic alterations<sup>[87]</sup>. The heterogeneous molecular features of HCC tumors are associated with the biological behavior, clinical outcome and prognosis<sup>[87-91]</sup>. Molecular classification is recommended to HCC, and previous studies have identified subsets of HCC tumors characterized by TP53 and CTNNB1 activation mutations, progenitor cell-like features, Met activation, Myc activation, and transforming growth factor- $\beta$  activation<sup>[92-94]</sup>. These molecular risk factors of HCC would play important roles in the design and implementation of MPE studies.

### MPE in HCC

Epidemiological studies have showed that DM is an established independent risk factor for HCC<sup>[12,20-23]</sup>; however, how DM affects the development and progression of HCC has not been explained clearly. MPE approaches and studies may be helpful to improve our understanding of the molecular mechanisms of carcinogenesis of HCC. MPE can be used to investigate the relationship between DM and risk of HCC by molecular subtypes. A few MPE studies have been performed for this question, although they were usually under the umbrella of molecular epidemiology. They would be described in the next section in detail. MPE can provide some useful insights for the pathological processes of DM in HCC, although a few challenges must be overcome.

### MPE IN DM AND RISK OF HCC

Currently, based on our knowledge, very few MPE researches are available for DM and risk of HCC<sup>[95-97]</sup>. For these studies, the original design are not for MPE, and the term of "molecular pathological epidemiology" have

not appeared in their articles, but they can be treated as MPE researches, according to the objectives and methods.

One MPE research which was performed in the Japan was designed to determine the relationship between PNPLA3 and JAZF1, and risk of HCC, in patients with non-viral hepatitis and type 2 DM<sup>[95]</sup>. The objective of this research was to identify genetic determinants associated with T2DM patients who have a high risk of developing HCC by genotyping T2DM susceptibility loci and PNPLA3. This study included 389 T2DM patients, including 59 patients with HCC (DM-HCC) and 330 patients without HCC (DM-non-HCC). Those patients who followed these criteria were included: (1) history of T2DM > 10 years; (2) alcohol intake < 60 g/d; and (3) negative for anti-HCV Ab and HBs-Ag. The authors found that the SNP rs738409 located in PNPLA3 was the greatest risk factor associated with HCC in these diabetic patients. Compared to DM-non-HCC patients, DM-HCC patients had the significantly higher frequency of the PNPLA3 G allele (OR = 2.53,  $P = 1.05 \times 10^{-5}$ ). Moreover, among the 115 DM patients homozygous for the PNPLA3 G allele, HCC patients had the significantly higher frequency of the JAZF1 rs864745 G allele (OR = 3.44,  $P = 0.0002$ )<sup>[95]</sup>. They concluded that PNPLA3 and JAZF1 were associated with an increased risk of developing HCC among T2DM patients without viral hepatitis<sup>[95]</sup>.

Another study was designed to evaluate the cytokinome profile, including the serum levels of growth factors, chemokines, cytokines, as well as of other diabetes and cancer biomarkers, in a cohort of patients, including 17 patients with T2DM, 20 patients with chronic hepatitis C infection, 34 patients with HCC, 10 patients with T2DM-HCC, and 20 healthy controls<sup>[96]</sup>. The results demonstrated that: (1) T2DM-HCC patients had the higher levels of IL-2R, sIL-6Ra, IL-16, IL-18, HGF,  $\beta$ -NGF, CXCL1, CXCL12, ADIPOQ, and IFN- $\alpha$  than those with T2DM or HCC; (2) T2DM-HCC patients had the lower level of LEP than those with T2DM or HCC; (3) T2DM-HCC and only HCC patients had the similar levels of CXCL9, PECAM-1, Prolactin, glucagon, sVEGFR-1 and sVEGFR-2; (4) T2DM-HCC patients had the higher levels of CXCL9, PECAM-1, Prolactin, and glucagon than those with only T2DM; and (5) T2DM-HCC patients had the lower levels of sVEGFR-1 and sVEGFR-2 than those with only T2DM<sup>[96]</sup>. The major limitation of this study was the very limited number of included patients; however, these molecular changes could be used to design and perform the MPE researches in DM and risk of HCC in future.

Some molecular pathology researches can also be regarded as MPE studies, for example one study which was conducted in the Second Military Medical University, Shanghai, China<sup>[97]</sup>. The objectives of this study were to determine the effect of p-Ser9-GSK-3 $\beta$  (glycogen synthase kinase-3 $\beta$ ) on the prognosis in HCC patients and to explore the interaction between GSK-3 $\beta$ , T2DM and prognosis of HCC. This research included 178 HCC patients after curative partial hepatectomy and showed that expression of P-Ser9-GSK-3 $\beta$  was significantly

higher in tumor tissues than that in their normal counterparts<sup>[97]</sup>. Moreover, the authors also found that: (1) over-expression of p-Ser9-GSK-3 $\beta$  was associated with T2DM; (2) T2DM and over-expression of p-Ser9-GSK-3 $\beta$  were closely related with each other; and (3) these two variables were independently associated with poor prognosis of HCC<sup>[97]</sup>. Therefore, p-Ser9-GSK-3 $\beta$  may be regarded as the mediator between T2DM and HCC.

One case report which was published in 2015 was also considered to be related to this field<sup>[98]</sup>. This report describes a 23-year-old woman with HCC and type 2 DM; and results of histological and immunohistochemical examination showed that this HCC arose in the background of hepatocyte nuclear factor-1 $\alpha$  mutated hepatocellular adenomas (H-HCA). However, traditionally, we consider that H-HCA have no minimal malignant potential. For the molecular changes and tumor biomarkers of HCC, the authors found that by immunohistochemical tests, CD34 expression in sinusoidal endothelial cells and expression of glutamine synthetase in tumor cells were increased, whereas exon 3 of CTNNB1 and TERT promoter mutations, and nuclear expression of  $\beta$ -catenin were absent in this patients with HCC and DM. Although such cases are rare, they reinforce the potential of H-HCA for HCC, which may be related to DM<sup>[98]</sup>.

Considering that DM is an independent risk factor for HCC, some efforts have been focused on understanding of the molecular mechanisms of DM in the development and progression of HCC, which may be useful for the design and implementation of MPE studies. For example, one mini-review focused on the impact of TNF- $\alpha$  and IL-6 along with epigenetic regulations<sup>[99]</sup>. Two approaches are suggested as followed: (1) the first is about the role of TNF- $\alpha$  and IL-6 as inflammatory mediators, from the point of role of apoptosis and inflammation in HCC; and apoptotic regulators can be used for this purpose, such as Bax (bcl-2-like protein 4 encoded by the BAX gene) and Bcl-2 (B-cell lymphoma 2 protein encoded by *BCL2* gene); and (2) the second is about the possible epigenomic reprogramming, from the point of role of epigenetic modification of DNA in HCC. According to these two approaches, apoptotic and inflammatory markers (Bcl2 and Bax), and DNA methylation, hypomethylation or histone modifications can be used as the candidate molecular biomarkers for the understanding of role of DM in HCC<sup>[99]</sup>.

Another review focused on the influence of insulin resistance and hyperinsulinemia of DM in the pathogenesis of hepatocarcinogenesis, and the author summarized that some molecular pathways were involved, for example phosphatase and tensin homolog/P13K/Akt and MAPK kinase<sup>[100]</sup>. It is well known that different anti-diabetic medications have different influences on the risk of HCC in diabetic patients<sup>[23,100]</sup>. Metformin has been associated with the decreased risk of HCC in patients diagnosed with DM<sup>[23]</sup>. The molecular mechanism is deduced that metformin can activate 5-adenosine monophosphate-activated protein kinase (AMPK) and decrease the expression of protein Livin<sup>[100]</sup>. AMPK can inhibit its downstream target mammalian target of rapamycin, and

then inhibit the growth of human cancer cell lines. Livin has been involved in both cell proliferation and survival. Thiazolidinediones seem to inhibit peroxisome proliferator-activated receptor gamma-independent regulation of nucleophosmin and prevent tumor formation<sup>[100]</sup>.

Although these studies are not enough for understanding of molecular mechanisms of DM in the increased risk of HCC, they and the involved molecular biomarkers can be very useful for future MPE researches. I hope that more and more MPE researches are performed exploring the molecular mechanisms as well as novel biomarkers.

## CONCLUSION

DM is an established independent risk factor for HCC; however, how DM affects the occurrence and development of HCC remains as yet unclearly understood. "MPE" is the branch of epidemiology and pathology, and its basis is the molecular classification of tumors. MPE is a multidisciplinary, interdisciplinary and transdisciplinary study field, and molecular pathology plays a central role in this relatively new field. In MPE, investigators examine the relationship between tumor molecular signatures, endogenous and exogenous factors, and development, progression and prognosis of tumors. I believe that this research field can be very helpful to improve our understanding of the pathogenesis, molecular mechanisms, diagnosis, personalized prevention and treatment for DM and risk of HCC in future.

## REFERENCES

- 1 **Harris TJ**, McCormick F. The molecular pathology of cancer. *Nat Rev Clin Oncol* 2010; **7**: 251-265 [PMID: 20351699 DOI: 10.1038/nrclinonc.2010.41]
- 2 **Menendez KR**, Garcia M, Spatz S, Tablante NL. Molecular epidemiology of infectious laryngotracheitis: a review. *Avian Pathol* 2014; **43**: 108-117 [PMID: 24460399 DOI: 10.1080/03079457.2014.886004]
- 3 **Izzotti A**, Neri M, Vecchio D, Puntoni R. Molecular epidemiology in cancer research (review). *Int J Oncol* 1997; **11**: 1053-1069 [PMID: 21528304]
- 4 **Lloyd C**, Cullinan P. Year in review 2014: basic science and epidemiology. *Thorax* 2015; **70**: 581-584 [PMID: 25977391 DOI: 10.1136/thoraxjnl-2015-207222]
- 5 **Ogino S**, Stampfer M. Lifestyle factors and microsatellite instability in colorectal cancer: the evolving field of molecular pathological epidemiology. *J Natl Cancer Inst* 2010; **102**: 365-367 [PMID: 20208016 DOI: 10.1093/jnci/djq031]
- 6 **Ogino S**, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging trans-disciplinary and interdisciplinary field. *Gut* 2011; **60**: 397-411 [PMID: 21036793 DOI: 10.1136/gut.2010.217182]
- 7 **Ogino S**, Noshio K, Meyerhardt JA, Kirkner GJ, Chan AT, Kawasaki T, Giovannucci EL, Loda M, Fuchs CS. Cohort study of fatty acid synthase expression and patient survival in colon cancer. *J Clin Oncol* 2008; **26**: 5713-5720 [PMID: 18955444 DOI: 10.1200/JCO.2008.18.2675]
- 8 **Morikawa T**, Kuchiba A, Yamauchi M, Meyerhardt JA, Shima K, Noshio K, Chan AT, Giovannucci E, Fuchs CS, Ogino S. Association of CTNNB1 (beta-catenin) alterations, body mass index, and physical activity with survival in patients with colorectal cancer. *JAMA* 2011; **305**: 1685-1694 [PMID: 21521850 DOI: 10.1001/jama.2011.513]



- 9 **Campbell PT**, Jacobs ET, Ulrich CM, Figueiredo JC, Poynter JN, McLaughlin JR, Haile RW, Jacobs EJ, Newcomb PA, Potter JD, Le Marchand L, Green RC, Parfrey P, Younghusband HB, Cotterchio M, Gallinger S, Jenkins MA, Hopper JL, Baron JA, Thibodeau SN, Lindor NM, Limburg PJ, Martinez ME, Colon Cancer Family R. Case-control study of overweight, obesity, and colorectal cancer risk, overall and by tumor microsatellite instability status. *J Natl Cancer Inst* 2010; **102**: 391-400 [PMID: 20208017 DOI: 10.1093/jnci/djq011]
- 10 **Gao C**. Molecular pathological epidemiology: an interdisciplinary field for study of hepatocellular carcinoma. *Austin J Gastroenterol* 2015; **2**: 1040
- 11 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]
- 12 **Zhang H**, Gao C, Fang L, Yao SK. Increased international normalized ratio level in hepatocellular carcinoma patients with diabetes mellitus. *World J Gastroenterol* 2013; **19**: 2395-2403 [PMID: 23613635 DOI: 10.3748/wjg.v19.i15.2395]
- 13 **Madkhali AA**, Fadel ZT, Aljiffry MM, Hassanain MM. Surgical treatment for hepatocellular carcinoma. *Saudi J Gastroenterol* 2015; **21**: 11-17 [PMID: 25672233 DOI: 10.4103/1319-3767.151216]
- 14 **El-Serag HB**, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. *Ann Intern Med* 2003; **139**: 817-823 [PMID: 14623619]
- 15 **Yuen MF**, Hou JL, Chutaputti A. Hepatocellular carcinoma in the Asia pacific region. *J Gastroenterol Hepatol* 2009; **24**: 346-353 [PMID: 19220670 DOI: 10.1111/j.1440-1746.2009.05784.x]
- 16 **Davila JA**, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut* 2005; **54**: 533-539 [PMID: 15753540 DOI: 10.1136/gut.2004.052167]
- 17 **Schuppan D**, Afdhal NH. Liver cirrhosis. *Lancet* 2008; **371**: 838-851 [PMID: 18328931 DOI: 10.1016/S0140-6736(08)60383-9]
- 18 **Hirokawa F**, Hayashi M, Asakuma M, Shimizu T, Inoue Y, Uchiyama K. Risk factors and patterns of early recurrence after curative hepatectomy for hepatocellular carcinoma. *Surg Oncol* 2016; **25**: 24-29 [PMID: 26979637 DOI: 10.1016/j.suronc.2015.12.002]
- 19 **Yang WT**, Wu LW, Tseng TC, Chen CL, Yang HC, Su TH, Wang CC, Kuo SF, Liu CH, Chen PJ, Chen DS, Liu CJ, Kao JH. Hepatitis B Surface Antigen Loss and Hepatocellular Carcinoma Development in Patients With Dual Hepatitis B and C Infection. *Medicine* (Baltimore) 2016; **95**: e2995 [PMID: 26962809 DOI: 10.1097/MD.0000000000002995]
- 20 **Gao C**, Fang L, Zhao HC, Li JT, Yao SK. Potential role of diabetes mellitus in the progression of cirrhosis to hepatocellular carcinoma: a cross-sectional case-control study from Chinese patients with HBV infection. *Hepatobiliary Pancreat Dis Int* 2013; **12**: 385-393 [PMID: 23924496]
- 21 **Gao C**, Yao SK. Diabetes mellitus: a "true" independent risk factor for hepatocellular carcinoma? *Hepatobiliary Pancreat Dis Int* 2009; **8**: 465-473 [PMID: 19822488]
- 22 **Gao C**, Zhao HC, Li JT, Yao SK. Diabetes mellitus and hepatocellular carcinoma: comparison of Chinese patients with and without HBV-related cirrhosis. *World J Gastroenterol* 2010; **16**: 4467-4475 [PMID: 20845516]
- 23 **Zhang H**, Gao C, Fang L, Zhao HC, Yao SK. Metformin and reduced risk of hepatocellular carcinoma in diabetic patients: a meta-analysis. *Scand J Gastroenterol* 2013; **48**: 78-87 [PMID: 23137049 DOI: 10.3109/00365521.2012.719926]
- 24 **Lawson DH**, Gray JM, McKillop C, Clarke J, Lee FD, Patrick RS. Diabetes mellitus and primary hepatocellular carcinoma. *Q J Med* 1986; **61**: 945-955 [PMID: 2819932]
- 25 **Fujino Y**, Mizoue T, Tokui N, Yoshimura T. Prospective study of diabetes mellitus and liver cancer in Japan. *Diabetes Metab Res Rev* 2001; **17**: 374-379 [PMID: 11747142]
- 26 **El-Serag HB**, Richardson PA, Everhart JE. The role of diabetes in hepatocellular carcinoma: a case-control study among United States Veterans. *Am J Gastroenterol* 2001; **96**: 2462-2467 [PMID: 11513191 DOI: 10.1111/j.1572-0241.2001.04054.x]
- 27 **Yu L**, Sloane DA, Guo C, Howell CD. Risk factors for primary hepatocellular carcinoma in black and white Americans in 2000. *Clin Gastroenterol Hepatol* 2006; **4**: 355-360 [PMID: 16527700 DOI: 10.1016/j.cgh.2005.12.022]
- 28 **Kessler II**. Cancer mortality among diabetics. *J Natl Cancer Inst* 1970; **44**: 673-686 [PMID: 11515436]
- 29 **Ragozzino M**, Melton LJ, Chu CP, Palumbo PJ. Subsequent cancer risk in the incidence cohort of Rochester, Minnesota, residents with diabetes mellitus. *J Chronic Dis* 1982; **35**: 13-19 [PMID: 7068798]
- 30 **Lu SN**, Lin TM, Chen CJ, Chen JS, Liaw YF, Chang WY, Hsu ST. A case-control study of primary hepatocellular carcinoma in Taiwan. *Cancer* 1988; **62**: 2051-2055 [PMID: 2844388]
- 31 **Adami HO**, Chow WH, Nyrén O, Berne C, Linet MS, Ekblom A, Wolk A, McLaughlin JK, Fraumeni JF. Excess risk of primary liver cancer in patients with diabetes mellitus. *J Natl Cancer Inst* 1996; **88**: 1472-1477 [PMID: 8841022]
- 32 **El-Serag HB**, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460-468 [PMID: 14762783]
- 33 **Dyal HK**, Aguilar M, Bartos G, Holt EW, Bhuket T, Liu B, Cheung R, Wong RJ. Diabetes Mellitus Increases Risk of Hepatocellular Carcinoma in Chronic Hepatitis C Virus Patients: A Systematic Review. *Dig Dis Sci* 2016; **61**: 636-645 [PMID: 26703125 DOI: 10.1007/s10620-015-3983-3]
- 34 **Arase Y**, Kobayashi M, Suzuki F, Suzuki Y, Kawamura Y, Akuta N, Kobayashi M, Sezaki H, Saito S, Hosaka T, Ikeda K, Kumada H, Kobayashi T. Effect of type 2 diabetes on risk for malignancies includes hepatocellular carcinoma in chronic hepatitis C. *Hepatology* 2013; **57**: 964-973 [PMID: 22991257 DOI: 10.1002/hep.26087]
- 35 **Wang CS**, Yao WJ, Chang TT, Wang ST, Chou P. The impact of type 2 diabetes on the development of hepatocellular carcinoma in different viral hepatitis statuses. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 2054-2060 [PMID: 19549812 DOI: 10.1158/1055-9965.EPI-08-1131]
- 36 **Chen CL**, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, Wang LY, Sun CA, Lu SN, Chen DS, Chen CJ. Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. *Gastroenterology* 2008; **135**: 111-121 [PMID: 18505690 DOI: 10.1053/j.gastro.2008.03.073]
- 37 **Kawamura Y**, Arase Y, Ikeda K, Hirakawa M, Hosaka T, Kobayashi M, Saitoh S, Yatsuji H, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Kumada H. Diabetes enhances hepatocarcinogenesis in noncirrhotic, interferon-treated hepatitis C patients. *Am J Med* 2010; **123**: 951-956.e1 [PMID: 20920698 DOI: 10.1016/j.amjmed.2010.05.013]
- 38 **Ko WH**, Chiu SY, Yang KC, Chen HH. Diabetes, hepatitis virus infection and hepatocellular carcinoma: A case-control study in hepatitis endemic area. *Hepatol Res* 2012; **42**: 774-781 [PMID: 22469194 DOI: 10.1111/j.1872-034X.2012.00979.x]
- 39 **Konishi I**, Hiasa Y, Shigematsu S, Hirooka M, Furukawa S, Abe M, Matsuura B, Michitaka K, Horiike N, Onji M. Diabetes pattern on the 75 g oral glucose tolerance test is a risk factor for hepatocellular carcinoma in patients with hepatitis C virus. *Liver Int* 2009; **29**: 1194-1201 [PMID: 19422477 DOI: 10.1111/j.1478-3231.2009.02043.x]
- 40 **Ohata K**, Hamasaki K, Toriyama K, Matsumoto K, Saeki A, Yanagi K, Abiru S, Nakagawa Y, Shigeno M, Miyazoe S, Ichikawa T, Ishikawa H, Nakao K, Eguchi K. Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Cancer* 2003; **97**: 3036-3043 [PMID: 12784339 DOI: 10.1002/cncr.11427]
- 41 **van den Tweel JG**, Taylor CR. A brief history of pathology: Preface to a forthcoming series that highlights milestones in the evolution of pathology as a discipline. *Virchows Arch* 2010; **457**: 3-10 [PMID: 20499087 DOI: 10.1007/s00428-010-0934-4]
- 42 **Jiang C**, Gu J. History and current state of pathology in China. *Virchows Arch* 2013; **463**: 599-608 [PMID: 23881278 DOI: 10.1007/s00428-013-1449-6]
- 43 **Campbell IL**, Harrison LC. Molecular pathology of type 1 diabetes. *Mol Biol Med* 1990; **7**: 299-309 [PMID: 2233244]

- 44 **Adorini L**, Gregori S, Harrison LC. Understanding autoimmune diabetes: insights from mouse models. *Trends Mol Med* 2002; **8**: 31-38 [PMID: 11796264]
- 45 **Wiltshire S**, Bell JT, Groves CJ, Dina C, Hattersley AT, Frayling TM, Walker M, Hitman GA, Vaxillaire M, Farrall M, Froguel P, McCarthy MI. Epistasis between type 2 diabetes susceptibility Loci on chromosomes 1q21-25 and 10q23-26 in northern Europeans. *Ann Hum Genet* 2006; **70**: 726-737 [PMID: 17044847 DOI: 10.1111/j.1469-1809.2006.00289.x]
- 46 **Lima SM**, Grisi DC, Kogawa EM, Franco OL, Peixoto VC, Gonçalves-Júnior JF, Arruda MP, Rezende TM. Diabetes mellitus and inflammatory pulpal and periapical disease: a review. *Int Endod J* 2013; **46**: 700-709 [PMID: 23442003 DOI: 10.1111/iej.12072]
- 47 **Tenesa A**, Dunlop MG. New insights into the aetiology of colorectal cancer from genome-wide association studies. *Nat Rev Genet* 2009; **10**: 353-358 [PMID: 19434079 DOI: 10.1038/nrg2574]
- 48 **Fletcher O**, Houlston RS. Architecture of inherited susceptibility to common cancer. *Nat Rev Cancer* 2010; **10**: 353-361 [PMID: 20414203 DOI: 10.1038/nrc2840]
- 49 **Sandhu MS**. Insulin-like growth factor-I and risk of type 2 diabetes and coronary heart disease: molecular epidemiology. *Endocr Dev* 2005; **9**: 44-54 [PMID: 15879687 DOI: 10.1159/000085755]
- 50 **Gallagher EJ**, LeRoith D. Epidemiology and molecular mechanisms tying obesity, diabetes, and the metabolic syndrome with cancer. *Diabetes Care* 2013; **36** Suppl 2: S233-S239 [PMID: 23882051 DOI: 10.2337/dcS13-2001]
- 51 **Field AE**, Camargo CA, Ogino S. The merits of subtyping obesity: one size does not fit all. *JAMA* 2013; **310**: 2147-2148 [PMID: 24189835 DOI: 10.1001/jama.2013.281501]
- 52 **Wu ZY**, Wang MH, Qi HM, Wu MH, Ge YZ, Li HT. Relationship between hOGG1 Ser326Cys gene polymorphism and coronary artery lesions in patients with diabetes mellitus. *Int J Clin Exp Med* 2015; **8**: 18629-18637 [PMID: 26770476]
- 53 **Remo A**, Pancione M, Zanella C, Vendraminelli R. Molecular pathology of colorectal carcinoma. A systematic review centred on the new role of the pathologist. *Pathologica* 2012; **104**: 432-441 [PMID: 23547429]
- 54 **Paral J**, Slaninka I, Kalabova H, Hadzi-Nikolov D. Gastrointestinal stromal tumors: review on morphology, molecular pathology, diagnostics, prognosis and treatment options. *Acta Gastroenterol Belg* 2010; **73**: 349-359 [PMID: 21086937]
- 55 **Ray A**, Manjila S, Hdeib AM, Radhakrishnan A, Nock CJ, Cohen ML, Sloan AE. Extracranial metastasis of glioblastoma: Three illustrative cases and current review of the molecular pathology and management strategies. *Mol Clin Oncol* 2015; **3**: 479-486 [PMID: 26137254 DOI: 10.3892/mco.2015.494]
- 56 **Ogino S**, Goel A. Molecular classification and correlates in colorectal cancer. *J Mol Diagn* 2008; **10**: 13-27 [PMID: 18165277 DOI: 10.2353/jmoldx.2008.070082]
- 57 **Baudhuin LM**, Donato LJ, Uphoff TS. How novel molecular diagnostic technologies and biomarkers are revolutionizing genetic testing and patient care. *Expert Rev Mol Diagn* 2012; **12**: 25-37 [PMID: 22133117 DOI: 10.1586/ERM.11.85]
- 58 **Roukos DH**. Novel clinico-genome network modeling for revolutionizing genotype-phenotype-based personalized cancer care. *Expert Rev Mol Diagn* 2010; **10**: 33-48 [PMID: 20014921 DOI: 10.1586/erm.09.69]
- 59 **Metodiev MV**. Biomarkers research in Europe: focus on personalized medicine. *Expert Rev Mol Diagn* 2011; **11**: 689-690 [PMID: 21902529 DOI: 10.1586/erm.11.55]
- 60 **Hamilton SR**. Targeted therapy of cancer: new roles for pathologists in colorectal cancer. *Mod Pathol* 2008; **21** Suppl 2: S23-S30 [PMID: 18437170 DOI: 10.1038/modpathol.2008.14]
- 61 **Gulley ML**, Brazier RM, Halling KC, Hsi ED, Kant JA, Nikiforova MN, Nowak JA, Ogino S, Oliveira A, Polesky HF, Silverman L, Tubbs RR, Van Deerlin VM, Vance GH, Versalovic J. Clinical laboratory reports in molecular pathology. *Arch Pathol Lab Med* 2007; **131**: 852-863 [PMID: 17550311 DOI: 10.1043/1543-2165(2007)131]
- 62 **Tonellato PJ**, Crawford JM, Boguski MS, Saffitz JE. A national agenda for the future of pathology in personalized medicine: report of the proceedings of a meeting at the Banbury Conference Center on genome-era pathology, precision diagnostics, and preemptive care: a stakeholder summit. *Am J Clin Pathol* 2011; **135**: 668-672 [PMID: 21502420 DOI: 10.1309/AJCP9GDNLWB4GACI]
- 63 **Dolle JM**, Daling JR, White E, Brinton LA, Doody DR, Porter PL, Malone KE. Risk factors for triple-negative breast cancer in women under the age of 45 years. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1157-1166 [PMID: 19336554 DOI: 10.1158/1055-9965.EPI-08-1005]
- 64 **Trivers KF**, Lund MJ, Porter PL, Liff JM, Flagg EW, Coates RJ, Eley JW. The epidemiology of triple-negative breast cancer, including race. *Cancer Causes Control* 2009; **20**: 1071-1082 [PMID: 19343511 DOI: 10.1007/s10552-009-9331-1]
- 65 **Pervaiz F**, Rehmani S, Majid S, Anwar H. Evaluation of Hormone Receptor Status (ER/PR/HER2-neu) in Breast Cancer in Pakistan. *J Pak Med Assoc* 2015; **65**: 747-752 [PMID: 26160085]
- 66 **Ichihara E**, Lovly CM. Shades of T790M: Intratumor Heterogeneity in EGFR-Mutant Lung Cancer. *Cancer Discov* 2015; **5**: 694-696 [PMID: 26152920 DOI: 10.1158/2159-8290.CD-15-0616]
- 67 **Li W**, Qu J, Xu Z. Clinical features and mutation status of EGFR, KRAS, BRAF, EML4-ALK and ROS1 between surgical resection samples and non surgical resection samples in lung cancer. *J Thorac Dis* 2015; **7**: 875-880 [PMID: 26101643 DOI: 10.3978/j.issn.2072-1439.2015.04.49]
- 68 **Webber EM**, Kauffman TL, O'Connor E, Goddard KA. Systematic review of the predictive effect of MSI status in colorectal cancer patients undergoing 5FU-based chemotherapy. *BMC Cancer* 2015; **15**: 156 [PMID: 25884995 DOI: 10.1186/s12885-015-1093-4]
- 69 **Yamane LS**, Scapulatempo-Neto C, Alvarenga L, Oliveira CZ, Berardinelli GN, Almodova E, Cunha TR, Fava G, Colaiacovo W, Melani A, Fregnani JH, Reis RM, Guimarães DP. KRAS and BRAF mutations and MSI status in precursor lesions of colorectal cancer detected by colonoscopy. *Oncol Rep* 2014; **32**: 1419-1426 [PMID: 25050586 DOI: 10.3892/or.2014.3338]
- 70 **Genther Williams SM**, Kuznicki AM, Andrade P, Dolinski BM, Elbi C, O'Hagan RC, Toniatti C. Treatment with the PARP inhibitor, niraparib, sensitizes colorectal cancer cell lines to irinotecan regardless of MSI/MSS status. *Cancer Cell Int* 2015; **15**: 14 [PMID: 25685067 DOI: 10.1186/s12935-015-0162-8]
- 71 **Rastogi A**, Tan SH, Mohamed AA, Chen Y, Hu Y, Petrovics G, Sreenath T, Kagan J, Srivastava S, McLeod DG, Sesterhenn IA, Srivastava S, Dobi A, Srinivasan A. Functional antagonism of TMPRSS2-ERG splice variants in prostate cancer. *Genes Cancer* 2014; **5**: 273-284 [PMID: 25221645]
- 72 **Esteller M**. Epigenetics in cancer. *N Engl J Med* 2008; **358**: 1148-1159 [PMID: 18337604 DOI: 10.1056/NEJMr072067]
- 73 **Samuels Y**, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE. High frequency of mutations of the PIK3CA gene in human cancers. *Science* 2004; **304**: 554 [PMID: 15016963 DOI: 10.1126/science.1096502]
- 74 **Wood LD**, Parsons DW, Jones S, Lin J, Sjöblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N, Szabo S, Dezso Z, Ustyanksky V, Nikolskaya T, Nikolsky Y, Karchin R, Wilson PA, Kaminker JS, Zhang Z, Croshaw R, Willis J, Dawson D, Shipitsin M, Willson JK, Sukumar S, Polyak K, Park BH, Pethiyagoda CL, Pant PV, Ballinger DG, Sparks AB, Hartigan J, Smith DR, Suh E, Papadopoulos N, Buckhaults P, Markowitz SD, Parmigiani G, Kinzler KW, Velculescu VE, Vogelstein B. The genomic landscapes of human breast and colorectal cancers. *Science* 2007; **318**: 1108-1113 [PMID: 17932254 DOI: 10.1126/science.1145720]
- 75 **Ojanguren I**, Castellà E, Llatjós M, Ariza A, Navas Palacios JJ. p53 immunoreaction in hepatocellular carcinoma and its relationship to etiologic factors. A fine needle aspiration study. *Acta Cytol* 1996; **40**: 1148-1153 [PMID: 8960021]
- 76 **Park EJ**, Lee JH, Yu GY, He G, Ali SR, Holzer RG, Osterreicher CH, Takahashi H, Karin M. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 2010; **140**: 197-208 [PMID: 20141834 DOI: 10.1016/j.cell.2009.12.052]

- 77 **Jiang N**, Sun R, Sun Q. Leptin signaling molecular actions and drug target in hepatocellular carcinoma. *Drug Des Devel Ther* 2014; **8**: 2295-2302 [PMID: 25484575 DOI: 10.2147/DDDT.S69004]
- 78 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]
- 79 **Yu XJ**, Fang F, Tang CL, Yao L, Yu L, Yu L. dbHCCvar: a comprehensive database of human genetic variations in hepatocellular carcinoma. *Hum Mutat* 2011; **32**: E2308-E2316 [PMID: 21936021 DOI: 10.1002/humu.21595]
- 80 **Wei Y**, Liu F, Li B, Chen X, Ma Y, Yan L, Wen T, Xu M, Wang W, Yang J. Polymorphisms of tumor necrosis factor- $\alpha$  and hepatocellular carcinoma risk: a HuGE systematic review and meta-analysis. *Dig Dis Sci* 2011; **56**: 2227-2236 [PMID: 21336601 DOI: 10.1007/s10620-011-1617-y]
- 81 **Wei YG**, Liu F, Li B, Chen X, Ma Y, Yan LN, Wen TF, Xu MQ, Wang WT, Yang JY. Interleukin-10 gene polymorphisms and hepatocellular carcinoma susceptibility: a meta-analysis. *World J Gastroenterol* 2011; **17**: 3941-3947 [PMID: 22025883 DOI: 10.3748/wjg.v17.i34.3941]
- 82 **Wang B**, Huang G, Wang D, Li A, Xu Z, Dong R, Zhang D, Zhou W. Null genotypes of GSTM1 and GSTT1 contribute to hepatocellular carcinoma risk: evidence from an updated meta-analysis. *J Hepatol* 2010; **53**: 508-518 [PMID: 20561699 DOI: 10.1016/j.jhep.2010.03.026]
- 83 **Tanabe KK**, Lemoine A, Finkelstein DM, Kawasaki H, Fujii T, Chung RT, Lauwers GY, Kulu Y, Muzikansky A, Kuruppu D, Lanuti M, Goodwin JM, Azoulay D, Fuchs BC. Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. *JAMA* 2008; **299**: 53-60 [PMID: 18167406 DOI: 10.1001/jama.2007.65]
- 84 **Jin F**, Qu LS, Shen XZ. Association between C282Y and H63D mutations of the HFE gene with hepatocellular carcinoma in European populations: a meta-analysis. *J Exp Clin Cancer Res* 2010; **29**: 18 [PMID: 20196837 DOI: 10.1186/1756-9966-29-18]
- 85 **Miki D**, Ochi H, Hayes CN, Abe H, Yoshima T, Aikata H, Ikeda K, Kumada H, Toyota T, Morizono T, Tsunoda T, Kubo M, Nakamura Y, Kamatani N, Chayama K. Variation in the DEPDC5 locus is associated with progression to hepatocellular carcinoma in chronic hepatitis C virus carriers. *Nat Genet* 2011; **43**: 797-800 [PMID: 21725309 DOI: 10.1038/ng.876]
- 86 **Yang HI**, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, Wang LY, Lu SN, You SL, Chen DS, Liaw YF, Chen CJ. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 1134-1143 [PMID: 18695135 DOI: 10.1093/jnci/djn243]
- 87 **Kim SM**, Leem SH, Chu IS, Park YY, Kim SC, Kim SB, Park ES, Lim JY, Heo J, Kim YJ, Kim DG, Kaseb A, Park YN, Wang XW, Thorgeirsson SS, Lee JS. Sixty-five gene-based risk score classifier predicts overall survival in hepatocellular carcinoma. *Hepatology* 2012; **55**: 1443-1452 [PMID: 22105560 DOI: 10.1002/hep.24813]
- 88 **Guichard C**, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, Calderaro J, Bioulac-Sage P, Letexier M, Degos F, Clément B, Balabaud C, Chevet E, Laurent A, Couchy G, Letouze E, Calvo F, Zucman-Rossi J. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat Genet* 2012; **44**: 694-698 [PMID: 22561517 DOI: 10.1038/ng.2256]
- 89 **Fujimoto A**, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, Aoki M, Hosono N, Kubo M, Miya F, Arai Y, Takahashi H, Shiraki-hara T, Nagasaki M, Shibuya T, Nakano K, Watanabe-Makino K, Tanaka H, Nakamura H, Kusuda J, Ojima H, Shimada K, Okusaka T, Ueno M, Shigekawa Y, Kawakami Y, Arihiro K, Ohdan H, Gotoh K, Ishikawa O, Ariizumi S, Yamamoto M, Yamada T, Chayama K, Kosuge T, Yamaue H, Kamatani N, Miyano S, Nakagama H, Nakamura Y, Tsunoda T, Shibata T, Nakagawa H. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. *Nat Genet* 2012; **44**: 760-764 [PMID: 22634756 DOI: 10.1038/ng.2291]
- 90 **Hoshida Y**, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009; **69**: 7385-7392 [PMID: 19723656 DOI: 10.1158/0008-5472.CAN-09-1089]
- 91 **Yamashita T**, Forgues M, Wang W, Kim JW, Ye Q, Jia H, Budhu A, Zanetti KA, Chen Y, Qin LX, Tang ZY, Wang XW. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. *Cancer Res* 2008; **68**: 1451-1461 [PMID: 18316609 DOI: 10.1158/0008-5472.CAN-07-6013]
- 92 **Hoshida Y**, Toffanin S, Lachenmayer A, Villanueva A, Minguez B, Llovet JM. Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. *Semin Liver Dis* 2010; **30**: 35-51 [PMID: 20175032 DOI: 10.1055/s-0030-1247131]
- 93 **Villanueva A**, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. *Semin Liver Dis* 2007; **27**: 55-76 [PMID: 17295177 DOI: 10.1055/s-2006-960171]
- 94 **Hoshida Y**, Moeini A, Alsinet C, Kojima K, Villanueva A. Gene signatures in the management of hepatocellular carcinoma. *Semin Oncol* 2012; **39**: 473-485 [PMID: 22846864 DOI: 10.1053/j.seminoncol.2012.05.003]
- 95 **Ueyama M**, Nishida N, Korenaga M, Korenaga K, Kumagai E, Yanai H, Adachi H, Katsuyama H, Moriyama S, Hamasaki H, Sako A, Sugiyama M, Aoki Y, Imamura M, Murata K, Masaki N, Kawaguchi T, Torimura T, Hyogo H, Aikata H, Ito K, Sumida Y, Kanazawa A, Watada H, Okamoto K, Honda K, Kon K, Kanto T, Mizokami M, Watanabe S. The impact of PNPLA3 and JAZF1 on hepatocellular carcinoma in non-viral hepatitis patients with type 2 diabetes mellitus. *J Gastroenterol* 2016; **51**: 370-379 [PMID: 26337813 DOI: 10.1007/s00535-015-1116-6]
- 96 **Capone F**, Guerriero E, Colonna G, Maio P, Mangia A, Marfella R, Paolisso G, Izzo F, Potenza N, Tomeo L, Castello G, Costantini S. The Cytokine Profile in Patients with Hepatocellular Carcinoma and Type 2 Diabetes. *PLoS One* 2015; **10**: e0134594 [PMID: 26226632 DOI: 10.1371/journal.pone.0134594]
- 97 **Qiao G**, Le Y, Li J, Wang L, Shen F. Glycogen synthase kinase-3 $\beta$  is associated with the prognosis of hepatocellular carcinoma and may mediate the influence of type 2 diabetes mellitus on hepatocellular carcinoma. *PLoS One* 2014; **9**: e105624 [PMID: 25157753 DOI: 10.1371/journal.pone.0105624]
- 98 **Stueck AE**, Qu Z, Huang MA, Campreciós G, Ferrell LD, Thung SN. Hepatocellular Carcinoma Arising in an HNF-1 $\alpha$ -Mutated Adenoma in a 23-Year-Old Woman with Maturity-Onset Diabetes of the Young: A Case Report. *Semin Liver Dis* 2015; **35**: 444-449 [PMID: 26676820 DOI: 10.1055/s-0035-1567827]
- 99 **Ali Kamkar MM**, Ahmad R, Alsmadi O, Behbehani K. Insight into the impact of diabetes mellitus on the increased risk of hepatocellular carcinoma: mini-review. *J Diabetes Metab Disord* 2014; **13**: 57 [PMID: 24918094 DOI: 10.1186/2251-6581-13-57]
- 100 **Facciorusso A**. The influence of diabetes in the pathogenesis and the clinical course of hepatocellular carcinoma: recent findings and new perspectives. *Curr Diabetes Rev* 2013; **9**: 382-386 [PMID: 23845075]

P- Reviewer: Haidara MAA S- Editor: Qi Y  
L- Editor: A E- Editor: Li D





## Implication of the intestinal microbiome in complications of cirrhosis

Mamatha Bhat, Bianca M Arendt, Venkat Bhat, Eberhard L Renner, Atul Humar, Johane P Allard

Mamatha Bhat, Bianca M Arendt, Eberhard L Renner, Johane P Allard, Division of Gastroenterology, University Health Network, Toronto M5G 2N2, Canada

Mamatha Bhat, Eberhard L Renner, Atul Humar, Multi-organ Transplant Program, University Health Network, Toronto M5G 2N2, Canada

Mamatha Bhat, Eberhard L Renner, Atul Humar, Johane P Allard, Department of Medicine, University of Toronto, Toronto M5G 2N2, Canada

Venkat Bhat, Department of Psychiatry, McGill University, Montreal H3A 1A1, Canada

**Author contributions:** Bhat M performed the data collection, majority of the writing, prepared the figures and tables; Bhat V helped with data collection; Arendt BM, Renner EL, Humar A and Allard JP provided the input in writing the paper.

**Conflict-of-interest statement:** There is no conflict of interest associated with the senior author or other coauthors contributed their efforts in this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Mamatha Bhat, MD, Department of Medicine, University of Toronto, 585 University Avenue, Toronto M5G 2N2, Canada. [mamatha.bhat@mail.mcgill.ca](mailto:mamatha.bhat@mail.mcgill.ca)  
Telephone: +1-416-3404800  
Fax: +1-416-3404041

Received: March 18, 2016

Peer-review started: March 21, 2016

First decision: April 19, 2016

Revised: May 6, 2016

Accepted: July 29, 2016

Article in press: August 1, 2016

Published online: September 28, 2016

### Abstract

The intestinal microbiome (IM) is altered in patients with cirrhosis, and emerging literature suggests that this impacts on the development of complications. The PubMed database was searched from January 2000 to May 2015 for studies and review articles on the composition, pathophysiologic effects and therapeutic modulation of the IM in cirrhosis. The following combination of relevant text words and MeSH terms were used, namely intestinal microbiome, microbiota, or dysbiosis, and cirrhosis, encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, variceal bleeding, hepatopulmonary syndrome, portopulmonary hypertension and hepatocellular carcinoma. The search results were evaluated for pertinence to the subject of IM and cirrhosis, as well as for quality of study design. The IM in cirrhosis is characterized by a decreased proportion of *Bacteroides* and *Lactobacilli*, and an increased proportion of *Enterobacteriaceae* compared to healthy controls. Except for alcoholic cirrhosis, the composition of the IM in cirrhosis is not affected by the etiology of the liver disease. The percentage of *Enterobacteriaceae* increases with worsening liver disease severity and decompensation and is associated with bacteremia, spontaneous bacterial peritonitis and hepatic encephalopathy. Lactulose, rifaximin and Lactobacillus-containing probiotics have been shown to partially reverse the cirrhosis associated enteric dysbiosis, in conjunction with improvement in encephalopathy. The IM is altered in cirrhosis, and this may contribute to the development of complications associated with end-stage liver disease. Therapies such as lactulose, rifaximin and probiotics may, at least partially, reverse the cirrhosis-associated changes in the IM. This, in turn, may prevent or alleviate the severity of complications.



**Key words:** Encephalopathy; Intestinal microbiome; Cirrhosis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** There has recently been an increasing understanding of the importance of the intestinal microbiome (IM) in the physiology of cirrhosis and its complications. Novel sequencing techniques have enabled a better characterization of the bacteria in the IM of patients with cirrhosis, and how this differs from the microbiome in a healthy individual. Additionally, therapeutics for enteric dysbiosis in patients with cirrhosis have been studied, and have shown promise in reducing the morbidity of complications in cirrhosis. In this review, we will critically review the literature on characterization of the IM in cirrhosis, its role in complications, and the evidence for strategies to address enteric dysbiosis.

Bhat M, Arendt BM, Bhat V, Renner EL, Humar A, Allard JP. Implication of the intestinal microbiome in complications of cirrhosis. *World J Hepatol* 2016; 8(27): 1128-1136 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i27/1128.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i27.1128>

## INTRODUCTION

Emerging literature has demonstrated that the intestinal microbiome (IM) plays an important role in health and disease. The intestine of a healthy adult harbors 100 trillion intestinal bacteria, and at least 500 different species have been identified with novel molecular biology techniques that allow for sequencing of whole genomes of the IM<sup>[1,2]</sup>. The healthy adult IM consists principally of *Bacteroides* and *Firmicutes*, which together comprise over 90% of the bacteria present in the colon<sup>[3]</sup>. The *Bacteroides* are Gram-negative, anaerobic, non-spore-forming bacteria, and especially produce carbohydrate-degrading enzymes, whereas the *Firmicutes* are Gram-positive, anaerobic, spore-forming bacteria, that ferment simple sugars leading to the production of short-chain fatty acids such as butyrate, acetate and propionate<sup>[4]</sup>. The concentrations of bacteria progressively increase from the proximal to the distal digestive tract, from a maximum of 10<sup>3</sup> bacteria/mL in the stomach to 10<sup>12</sup> bacteria/mL in the colon<sup>[5]</sup>. The IM has various functions that affect biochemical, metabolic and physiologic processes both within the intestine and elsewhere in the body<sup>[6]</sup>. These physiologic functions include the digestion of nutrients, with bacterial disaccharidases transforming unabsorbed dietary sugars to short chain fatty acids (SCFA)<sup>[7]</sup>. These SCFA can be used as a source of energy by the human body, as they are absorbed through the colon. In addition, butyrate and acetate can be a source of fuel for the enterocytes<sup>[8]</sup>, affect lipid metabolism<sup>[9]</sup>, and have anti-inflammatory effects<sup>[10]</sup>. Intestinal bacteria

can also produce vitamins such as folate and vitamin K<sup>[11,12]</sup>, which are absorbed into the bloodstream<sup>[12]</sup>. Additionally, the presence of the physiologic IM within the intestinal milieu prevents colonization by pathogenic bacteria and decreases intestinal permeability<sup>[8]</sup>. Crosstalk between bacteria and enterocytes *via* binding sites and Toll-like receptors help distinguish commensal from pathogenic bacteria<sup>[13]</sup>. The microbiota then generate an immune response to pathogenic bacteria, and enable oral tolerance by preventing a reaction to dietary protein antigens. Many endogenous bacteria can produce bacteriocins that hinder replication of pathogenic bacterial species<sup>[14]</sup>. Additionally, commensal microbiota have been shown to promote regulatory T cell function<sup>[15]</sup> and maturation of natural killer T cells<sup>[16]</sup>. Finally, many medications including digoxin, opiates, hormones and various antibiotics are transformed into their active forms through intestinal bacterial metabolism. Bacterial deconjugation of glucuronide, sulfate and cysteine conjugates decreases the polarity of drugs, and enables enterohepatic circulation, reabsorption, and prolonged retention<sup>[17]</sup>. One prime example of a compound whose bacterial metabolism is essential to its activity is sulfasalazine, which is broken down into 5-aminosalicylic acid and sulfapyridine<sup>[18]</sup>. Additionally, the effects of anticancer immunotherapy can be modulated by the intestinal microbiota. The antitumor effects of CTLA4 blockade were shown to be dependent on specific *Bacteroides* species<sup>[19]</sup>.

Bacterial growth and functions may be affected by several physiologic and anatomic conditions in the GI tract such as peristalsis (may inhibit mucosal attachment of ingested bacteria), the presence of gastric acid and bile (toxic effects), the presence of proteolytic enzymes (degradation of bacteria), the intestinal mucus layer (trapping of bacteria), the ileocecal valve preventing retrograde bacterial translocation<sup>[20,21]</sup>, and secretory IgA inhibiting bacterial proliferation<sup>[22]</sup>. Changes in small intestinal and colonic motility, gastric acid secretion, bile flow/composition, and the intestinal innate immune response can impact bacterial composition and lead to overgrowth<sup>[23]</sup>. In addition, external factors such as diet<sup>[24]</sup>, antibiotic use<sup>[25]</sup> and other environmental factors<sup>[26]</sup> affect IM composition.

IM composition can also be affected by disease states and vice versa, as shown in various types of autoimmune, metabolic and malignant conditions including colon and gynecologic cancers<sup>[3,27-30]</sup>. Intestinal microbial dysbiosis, with both qualitative and quantitative changes in bacterial species, has also been associated with the development of obesity<sup>[31]</sup>, diabetes<sup>[32]</sup>, metabolic syndrome<sup>[33]</sup> and inflammatory bowel disease<sup>[34]</sup>. In relation to liver disease, recent studies have reported differences in the IM associated with non-alcoholic fatty liver disease (NAFLD)<sup>[35,36]</sup>, alcoholic liver disease<sup>[37]</sup> and liver cancer<sup>[38]</sup>. The IM composition in cirrhosis has been shown to be associated with the development of complications, particularly spontaneous bacterial peritonitis and encephalopathy. The goal of this review is to highlight the unique composition of the IM in cirrhosis,

**Table 1** Characterization of Intestinal microbiota across spectrum of liver disease severity

Patient population	Changes in IM
Healthy patients	Comprised principally of <i>Bacteroides</i> and <i>Firmicutes</i> (over 90% of IM) <sup>[3]</sup>
Compensated cirrhosis	Higher <i>Enterobacteriaceae</i> , <i>Staphylococcaeae</i> , and <i>Enterococcaceae</i> <sup>[53,55,56]</sup> Decreased <i>Lachnospiraceae</i> , <i>Ruminococcaceae</i> , <i>Clostridiales</i> XIV, <i>Bacteroides</i> , <i>Faecalibacterium prausnitzii</i> and <i>Coprococcus comes</i> <sup>[45,53,55,56]</sup>
Alcoholic cirrhosis	Higher <i>Enterobacteriaceae</i> and endotoxemia compared to other cirrhosis <sup>[46]</sup>
Decompensated cirrhosis	<i>Enterobacteriaceae</i> species correlated with increasing MELD score, <i>Ruminococcaceae</i> species associated with lower MELD scores <sup>[56]</sup>
Overt hepatic encephalopathy	Higher <i>Enterobacteriaceae</i> <sup>[57]</sup>
Hepatorenal syndrome	No established data
Hepatocellular carcinoma	No established data
Therapeutic strategies and effects on IM	
Lactulose	No RCT or prospective studies of microbiome Decreased urea-producing <i>Klebsiella</i> and <i>Proteus</i> species, increased non-urease-producing lactobacilli <sup>[70]</sup>
Rifaximin	Improved cognitive function due to change in microbiome-metabolome correlation networks, particularly <i>Enterobacteriaceae</i>
Probiotics	Decreased risk of endotoxemia, TNF- $\alpha$ <sup>[74]</sup>
Fecal microbiota transplantation	Enteric dysbiosis reduced, relatively decreased proportion of <i>Enterobacteriaceae</i> and <i>Porphyromonadaceae</i> <sup>[74,75]</sup> Case report data <sup>[76]</sup> Resolution of hepatic encephalopathy with healthy IM transfer, however IM not characterized

MELD: Model for end stage liver disease; IM: Intestinal microbiome; RCT: Randomized controlled trial; TNF: Tumor necrosis factor.

its underlying pathophysiology, and its association with clinical manifestations and complications of cirrhosis. Additionally, we will review therapeutic strategies in cirrhosis aimed at restoring a healthy microbiome and at reducing complications.

## RESEARCH AND LITERATURE

The PubMed database was searched from 2000 to May 2015 for studies on the causes, outcomes and modulation of the IM in cirrhosis. The following combination of relevant text words and MeSH terms were used: "IM", microbiome, microbiota, or dysbiosis, and cirrhosis, encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, variceal bleeding, hepatopulmonary syndrome, portopulmonary hypertension and Hepatocellular carcinoma (HCC). Our search included both original and review articles as well as letters to the editor. We (Mamatha Bhat and Venkat Bhat) obtained 369 abstracts, manually searching the abstracts for pertinence to the subject of IM and cirrhosis. This resulted in 46 entries that provided information on the etiology, pathophysiology, characterization of the IM, and management of enteric dysbiosis in cirrhosis. Table 1 summarizes the results of our systematic review.

## ENTERIC DYSBIOSIS IN LIVER DISEASE

Emerging literature suggests that the IM is not only altered in liver disease of various etiologies, but that this dysbiosis may play an etiopathogenetic role. For example, dysbiosis may contribute to NAFLD<sup>[35,39]</sup> by contributing to enhanced hepatic fat accumulation<sup>[39]</sup>. In a cross-sectional study, patients with non-alcoholic steatohepatitis (NASH) had a significantly lower percentage of *Bacteroides* in their stool<sup>[35]</sup> compared to patients with simple steatosis and healthy controls, although a cause-effect relationship

could not be established. Mechanisms engendered by microbial genes, such as an increase in appetite signaling, energy extraction from the diet, and expression of lipogenic genes likely contribute to enhanced hepatic fat accumulation<sup>[31,40]</sup>. In addition, hepatic inflammation and fibrosis in patients with NASH are thought to occur due to bacterial translocation of intestine-derived microbial products (including endotoxin) and activation of Toll-like receptor (TLR) signaling<sup>[41,42]</sup>. This results in stimulation of hepatic stellate cell activity, with subsequent liver fibrogenesis<sup>[43]</sup>. The role of bacterial lipopolysaccharide (endotoxin) in fibrogenesis has been confirmed in mouse models, where TLR4 knockout significantly decreased expression of markers for liver fibrosis such as collagen,  $\alpha$ -smooth muscle actin, procollagen-I, transforming growth factor- $\beta$ 1 and matrix metalloproteinase-2<sup>[44]</sup>. It is thought that enteric dysbiosis in the context of liver disease of any etiology contributes through the above mechanism to liver disease progression.

## CHARACTERIZATION OF IM IN CIRRHOSIS

### Evidence of bacterial overgrowth in cirrhosis

Patients with cirrhosis have both qualitative and quantitative changes in their gut microbiota<sup>[45-47]</sup>. Small intestinal bacterial overgrowth, defined as  $>10^5$  CFU/mL and/or the presence of colonic bacteria in upper jejunal aspirate, is present in 48% to 73% of cirrhotic patients<sup>[48,49]</sup>. Impaired small intestinal motility<sup>[50]</sup>, decreased bile flow<sup>[51]</sup>, and dysregulated secretion of immunoglobulin A<sup>[51]</sup> and antimicrobial molecules<sup>[52]</sup> all contribute to small intestinal bacterial overgrowth in patients with cirrhosis (Figure 1).

### IM composition in cirrhosis

In addition to this increased bacterial burden, taxonomic

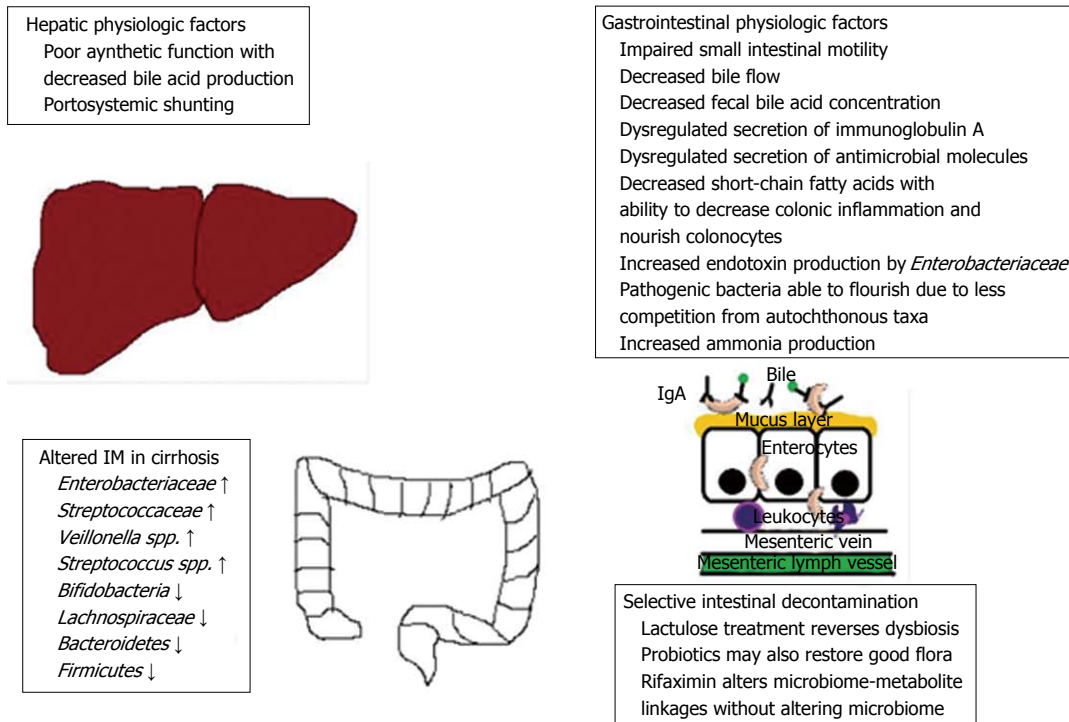


Figure 1 Factors influencing intestinal microbiome composition in cirrhosis. IM: Intestinal microbiome.

differences in the fecal microbial communities have been demonstrated<sup>[51,53-56]</sup>. Patients with cirrhosis commonly have decreased proportions of beneficial, autochthonous taxa, such as *Lachnospiraceae*, *Ruminococcaceae* and *Clostridiales* XIV. There is a relative overgrowth of potentially pathogenic bacteria such as *Enterobacteriaceae*, *Staphylococcaceae*, and *Enterococcaceae*, whose abundance correlates with disease progression and endotoxemia<sup>[53,55,56]</sup>. A recent study comparing the microbiome in cirrhosis vs healthy controls revealed that the beneficial *Bacteroides* genus was significantly decreased in patients with cirrhosis<sup>[24]</sup>. Additional bacterial species that enrich the health and diversity of the microbiome, such as *Faecalibacterium prausnitzii* (anti-inflammatory properties) and *Coprococcus comes* (butyrate production) were found to occupy a relatively lower proportion of the microbiome in cirrhosis<sup>[41]</sup>. Most studies have shown that the etiology of cirrhosis does not affect taxonomic composition, with similar fecal microbial communities across the spectrum of liver disease etiologies<sup>[51,57]</sup>. Recently however, the pattern of dysbiosis has been reported to be slightly different in alcoholic cirrhosis, with higher *Enterobacteriaceae* and a higher proportion of patients with endotoxemia compared to cirrhotic patients of non-alcoholic etiologies. This held true after adjusting for severity of disease (MELD score) and abstinence<sup>[42]</sup>.

#### Mechanisms associated with dysbiosis in cirrhosis

The beneficial, autochthonous taxa of the IM generate SCFA that sustain colonocytes and decrease inflammation, in addition to anti-bacterial peptides that help prevent colonization by pathogenic taxa and reinforce the intestinal barrier<sup>[58]</sup>. The decreased presence of

certain benign bacteria is thought to be due to decreased bile acid production in cirrhosis. This environment allows pathogenic bacteria to thrive and outgrow the "beneficial" species<sup>[6]</sup>. The combination of a decreased, "leaky" intestinal barrier with increased endotoxin production by pathogenic taxa such as the *Enterobacteriaceae* can lead to endotoxemia<sup>[41]</sup>.

#### IM and severity of cirrhosis

The IM composition appears to vary with the severity of cirrhosis and the presence of complications. The increased presence of the *Streptococcaceae* taxon has been correlated with worsening Child-Pugh score, whereas the *Lachnospiraceae* taxon was associated with less severe disease<sup>[53]</sup>. However, the Child-Pugh score includes hepatic encephalopathy as a component, and encephalopathy itself is associated with a distinct IM as described further below. Later studies of the IM in cirrhotics have therefore employed the MELD score, in order to allow for simple correlation of the IM with severity of liver dysfunction. *Enterobacteriaceae* species have been reported to be associated with higher MELD scores, whereas the *Ruminococcaceae* species have been associated with lower MELD scores<sup>[56]</sup>. A study of the IM in patients with advanced liver disease revealed that decreased abundance of *Bifidobacterium* and increased abundance of *Enterococcus* were associated with increasing liver dysfunction<sup>[59]</sup>. The term "cirrhosis dysbiosis ratio" was coined to describe the ratio of autochthonous taxa (taxa that are benign and usually present in the gut such as *Ruminococcaceae*, *Lachnospiraceae*, and *Clostridiales*) to non-autochthonous ones (*Enterobacteriaceae* and *Bacteroidaceae*). This ratio was highest

among healthy individuals as expected, and decreased with worsening MELD score and degree of hepatic decompensation<sup>[57]</sup>. Those with compensated cirrhosis had a cirrhosis-dysbiosis ratio of 0.89, whereas those with decompensated cirrhosis had a ratio of 0.66, and patients hospitalized for cirrhosis related complications had a ratio of 0.32 ( $P < 0.0001$ ). An increase in the relative abundance of pathogenic bacteria was associated with the development of complications such as hepatic encephalopathy. Liver disease stability over months was associated with a stable cirrhosis-dysbiosis ratio<sup>[57]</sup>.

Interestingly, salivary dysbiosis is concomitantly present with enteric dysbiosis, with a relative increased abundance of *Enterobacteriaceae* and decrease in autochthonous species<sup>[60]</sup>. Dysbiosis of the salivary microbiome was particularly pronounced in patients requiring 90-d liver-related hospitalizations. Thus, the salivary microbiome may serve as a substitute for the IM, and would be an easier sample to obtain.

Patients with cirrhosis show changes in both serum and fecal bile acids, which results from decreased liver synthetic function, altered enterohepatic circulation and altered IM composition. Overgrowth of *Enterobacteriaceae* leads to impaired conversion of primary to secondary bile acids<sup>[61]</sup>. This results in a decreased ratio of secondary to primary bile acids, along with a reduced overall fecal bile acid concentration, which correlate with increasing severity of liver disease. These findings are accompanied by a concomitant increase in serum bile acids<sup>[61]</sup>.

## IMPACT OF MICROBIOME ON COMPLICATIONS OF CIRRHOSIS

### IM and sepsis

Complications of end-stage liver disease, such as spontaneous bacterial peritonitis and hepatic encephalopathy, have been linked to pathological bacterial translocation. The translocation of bacteria or their products (such as muramyl-dipeptides, lipopolysaccharides (endotoxin), peptidoglycans and bacterial DNA) from the intestine to the mesenteric lymph nodes is a normal physiological process that bolsters host immunity<sup>[62]</sup>. Pathological bacterial translocation occurs due to an increase in the rate or degree of translocation. This is the case in cirrhosis, given the leaky intestinal barrier and relatively immunodeficient state<sup>[49]</sup>. The bacteria causing SBP are mostly gram-negative bacilli such as *Escherichia coli* and other members of the *Enterobacteriaceae* family (*Proteus*, *Klebsiella* and *Enterobacter*), which are present in higher abundance in the gut microbiota of cirrhotic patients<sup>[51,53,55,56]</sup>. Migration of these bacteria to the peritoneal cavity or systemic circulation results in peritonitis and bacteremia, respectively. Pathological bacterial translocation triggers inflammation and the hyperdynamic circulation of cirrhosis that contributes to portal hypertension. These in turn result in serious systemic infections with up to 38% mortality<sup>[6,63]</sup>. Therefore, translocation of bacteria from an altered IM represents an important determinant of

mortality in cirrhotic patients.

### IM and hepatic encephalopathy

The IM contributes to development of hepatic encephalopathy through ammoniogenesis and an endotoxin-driven inflammatory response. Additional compounds produced by the microbiota, such as mercaptans, phenols, short- and medium-chain fatty acids and benzodiazepine-like compounds, potentially contribute as well<sup>[2]</sup>. In a metagenomic study of the microbiome in cirrhosis, Qin *et al.*<sup>[64]</sup> performed in-depth assessment of functions of the microbiome enriched in liver cirrhosis. In cirrhosis, bacterial genes involved in the assimilation or dissimilation of nitrate to or from ammonia, denitrification, gamma-aminobutyric acid biosynthesis, and amino acid transport were highly represented<sup>[64]</sup>. Additionally, manganese-related transport system modules were enriched in the IM of patients with cirrhosis<sup>[64]</sup>. This may be associated with manganese accumulation within the basal ganglia of cirrhotic patients, which is thought to contribute to hepatic encephalopathy<sup>[65]</sup>. A patient discrimination index was developed based on a group of 15 bacterial species, and it was highly accurate as a biomarker for cirrhosis. In addition to the enteric dysbiosis described above, altered intestinal permeability results in translocation of bacteria and their products, which has an important effect on the progression of cirrhosis<sup>[66]</sup>.

### IM and hepatocellular carcinoma

HCC is another complication of cirrhosis whose development may be influenced by the altered IM in the cirrhotic patient<sup>[67]</sup>, although there is no concrete evidence as yet. It is well known that chronic inflammation can foster the initiation and progression of malignancies. Translocation of intestinal bacteria can lead to hepatic inflammation, with release of key inflammatory mediators such as NF- $\kappa$ B and TLR4. Downregulation of the NF- $\kappa$ B signaling pathway *in vivo* (by ablating the protein that activates this transcription factor) was shown to sequentially induce NAFLD, fibrosis and finally HCC<sup>[68]</sup>. TLR4 and the IM contributed to tumor progression in an HCC mouse model, and have therefore been proposed as chemopreventive targets<sup>[69]</sup>. This study suggests that the IM may have adverse effects on hepatic stellate cell function, activating the release of inflammatory mediators that promote HCC development.

## MODULATION OF IM IN CIRRHOSIS

### Lactulose

The longstanding practice of treating hepatic encephalopathy with lactulose not only decreases ammonia absorption, but also results in modulation of the IM with decreased ammonia production<sup>[70]</sup>. Lactulose acidifies the colonic pH, which renders the environment hostile to the urease-producing *Klebsiella* and *Proteus* species. Conversely, the intestinal lumen becomes friendlier to non-urease-producing lactobacilli and bifidobacteria.



The end-result of these changes in the microbiome is decreased ammonia production<sup>[71]</sup>.

### Antibiotics

Antibiotics such as neomycin, metronidazole and ciprofloxacin have been used in the past to treat hepatic encephalopathy, although the IM was never characterized in this context. More recently, rifaximin has been offered to patients with lactulose-resistant hepatic encephalopathy. In a prospective, open-label study, 20 cirrhotic patients with minimal hepatic encephalopathy were treated with rifaximin 550 mg twice daily for 8 wk<sup>[72]</sup>. The IM, serum metabolome, and cognitive function were assessed before and after rifaximin treatment in all 20 patients. Although a significant improvement in cognitive function and endotoxemia was seen, the composition of the IM was not distinctly different. Rather, there was a shift from pathogenic to beneficial metabolite linkages around pathogenic bacterial species (*Enterobacteriaceae* and *Porphyromonadaceae*). Therefore, although the IM composition itself was not altered, the metabolic profile produced by the pathogenic species was more beneficial. On the other hand, the correlation networks around the autochthonous bacteria (looking at the interactions between the microbiome and metabolome) remained the same. This study therefore illustrated how rifaximin could alter intestinal microbial linkages with metabolites, without any significant effect on microbial composition or abundance *per se*<sup>[72]</sup>.

### Probiotics

The effect of probiotic therapy on the IM in cirrhotic patients has also been studied<sup>[73]</sup>. One appealing benefit of probiotics is their excellent safety profile. A phase I, 8-wk, randomized controlled trial of the probiotic *Lactobacillus* GG in 30 patients with minimal hepatic encephalopathy revealed that it was safe and well-tolerated, while decreasing the risk of endotoxemia and lowering TNF- $\alpha$  in the serum, plasma and liver<sup>[74]</sup>. Enteric dysbiosis was reduced, with a relatively decreased proportion of *Enterobacteriaceae* and *Porphyromonadaceae* (both associated with worse disease in cirrhosis). Conversely, the abundance of autochthonous species like *Lachnospiraceae*, *Ruminococcaceae*, and *Clostridiales* XIV increased. There was no change in the *Lactobacillaceae* abundance, and it was hypothesized that this species either promoted colonization by beneficial microbiota or enhanced intestinal epithelial function and the immune system, thereby displacing pathogens<sup>[74]</sup>. Additionally, changes in metabolites related to amino acid, vitamin and secondary bile acid were found. Cognition however was not improved, although this trial was a phase I study without the statistical power to determine this outcome<sup>[72,74]</sup>.

A second randomized, double-blind, placebo-controlled trial of VSL#3 daily for 6 mo assessed the probiotic's efficacy in preventing recurrent encephalopathy, reducing severity of liver disease and reducing hospitalizations<sup>[75]</sup>. There was a tendency towards decreased episodes of

recurrent encephalopathy (primary outcome), with 34.8% in the probiotic group vs 51.6% in the placebo group ( $P = 0.12$ ). In addition, there was a significantly reduced risk of hospitalization, as well as improved Child-Pugh and MELD scores with daily use of VSL#3.

### Fecal microbiota transplantation

This is a potentially interesting approach to addressing enteric dysbiosis, although the only evidence to date is a single case report where healthy gut microbiota transfer was used to treat hepatic encephalopathy<sup>[76]</sup>. This was recently described in a case report of a patient with Grade 1-2 encephalopathy not responsive to lactulose, and unable to afford rifaximin. Fecal microbiota from a healthy stool donor was transplanted into the patient by colonoscopy and by retention enemas weekly over a 5-wk period. The patient's alertness, as well as his performance on measures of encephalopathy (inhibitory control test and Stroop test) significantly improved and normalized. This case demonstrates that fecal microbiota transplantation is a plausible strategy in treating mild encephalopathy by correcting enteric dysbiosis, although further larger-scale studies are required.

In summary, the IM is significantly altered in cirrhosis, with a decrease in beneficial, autochthonous bacterial species such as *Bacteroides*, and an increase in pathogenic bacteria such as the *Enterobacteriaceae*. Except for alcoholic liver disease, IM composition appears to be similar across etiologies of hepatic cirrhosis. The dysregulated IM likely is associated with and may contribute to the development of complications of end-stage liver disease, including hyperdynamic circulation, portal hypertension, bacteremia, spontaneous bacterial peritonitis, and encephalopathy. The role of the IM in the development of hepatorenal syndrome and HCC is suspected, but not yet elucidated. Treatment with lactulose, antibiotics, and probiotics may be effective in preventing or improving these complications by targeting the enteric dysbiosis.

## REFERENCES

- 1 Petrosino JF, Highlander S, Luna RA, Gibbs RA, Versalovic J. Metagenomic pyrosequencing and microbial identification. *Clin Chem* 2009; **55**: 856-866 [PMID: 19264858 DOI: 10.1373/clinchem.2008.107565]
- 2 Minemura M, Shimizu Y. Gut microbiota and liver diseases. *World J Gastroenterol* 2015; **21**: 1691-1702 [PMID: 25684933 DOI: 10.3748/wjg.v21.i6.1691]
- 3 Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59-65 [PMID: 20203603 DOI: 10.1038/nature08821]
- 4 Fischbach MA, Sonnenburg JL. Eating for two: how metabolism

- establishes interspecies interactions in the gut. *Cell Host Microbe* 2011; **10**: 336-347 [PMID: 22018234 DOI: 10.1016/j.chom.2011.10.002]
- 5 **Vanderhoof JA**. Etiology and pathogenesis of small intestinal bacterial overgrowth. [accessed 2015 Mar]. Available from: URL: <http://www.uptodate.com.proxy3.library.mcgill.ca/contents/etiology-and-pathogenesis-of-small-intestinal-bacterial-overgrowth?source=machineLearning&search=bacterialovergrowth&selectedTitle=3~122&ionRank=5&anchor=H2>
  - 6 **Schnabl B**, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology* 2014; **146**: 1513-1524 [PMID: 24440671 DOI: 10.1053/j.gastro.2014.01.020]
  - 7 **Schwartz A**, Taras D, Schäfer K, Beijer S, Bos NA, Donus C, Hardt PD. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity* (Silver Spring) 2010; **18**: 190-195 [PMID: 19498350 DOI: 10.1038/oby.2009.167]
  - 8 **Donohoe DR**, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, Bultman SJ. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab* 2011; **13**: 517-526 [PMID: 21531334 DOI: 10.1016/j.cmet.2011.02.018]
  - 9 **Awad AB**, Horvath PJ, Andersen MS. Influence of butyrate on lipid metabolism, survival, and differentiation of colon cancer cells. *Nutr Cancer* 1991; **16**: 125-133 [PMID: 1796008]
  - 10 **Maslowski KM**, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM, Mackay CR. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009; **461**: 1282-1286 [PMID: 19865172 DOI: 10.1038/nature08530]
  - 11 **LeBlanc JG**, Milani C, de Giori GS, Sesma F, van Sinderen D, Ventura M. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr Opin Biotechnol* 2013; **24**: 160-168 [PMID: 22940212 DOI: 10.1016/j.copbio.2012.08.005]
  - 12 **Conly JM**, Stein K, Worobetz L, Rutledge-Harding S. The contribution of vitamin K2 (menaquinones) produced by the intestinal microflora to human nutritional requirements for vitamin K. *Am J Gastroenterol* 1994; **89**: 915-923 [PMID: 8198105]
  - 13 **Wells JM**, Rossi O, Meijerink M, van Baarlen P. Epithelial crosstalk at the microbiota-mucosal interface. *Proc Natl Acad Sci USA* 2011; **108** Suppl 1: 4607-4614 [PMID: 20826446 DOI: 10.1073/pnas.1000092107]
  - 14 **Gorbach SL**. Probiotics and gastrointestinal health. *Am J Gastroenterol* 2000; **95**: S2-S4 [PMID: 10634218]
  - 15 **Round JL**, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci USA* 2010; **107**: 12204-12209 [PMID: 20566854 DOI: 10.1073/pnas.0909122107]
  - 16 **Zeissig S**, Blumberg RS. Commensal microbial regulation of natural killer T cells at the frontiers of the mucosal immune system. *FEBS Lett* 2014; **588**: 4188-4194 [PMID: 24983499 DOI: 10.1016/j.febslet.2014.06.042]
  - 17 **Mikov M**. The metabolism of drugs by the gut flora. *Eur J Drug Metab Pharmacokinet* 1994; **19**: 201-207 [PMID: 7867662]
  - 18 **Peppercorn MA**. Sulfasalazine. Pharmacology, clinical use, toxicity, and related new drug development. *Ann Intern Med* 1984; **101**: 377-386 [PMID: 6147110]
  - 19 **Vétizou M**, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP, Poirier-Colame V, Roux A, Becharef S, Formenti S, Golden E, Cording S, Eberl G, Schlitzer A, Ginhoux F, Mani S, Yamazaki T, Jacquilot N, Enot DP, Bérard M, Nigou J, Opolon P, Eggermont A, Woerther PL, Chachaty E, Chaput N, Robert C, Mateus C, Kroemer G, Raoult D, Boneca IG, Carbonnel F, Chamaillard M, Zitvogel L. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 2015; **350**: 1079-1084 [PMID: 26541610 DOI: 10.1126/science.12329]
  - 20 **Phillips SF**, Quigley EM, Kumar D, Kamath PS. Motility of the ileocolonic junction. *Gut* 1988; **29**: 390-406 [PMID: 3281873]
  - 21 **Roland BC**, Ciarleglio MM, Clarke JO, Semler JR, Tomakin E, Mullin GE, Pasricha PJ. Low ileocecal valve pressure is significantly associated with small intestinal bacterial overgrowth (SIBO). *Dig Dis Sci* 2014; **59**: 1269-1277 [PMID: 24795035 DOI: 10.1007/s10620-014-3166-7]
  - 22 **Riordan SM**, McIver CJ, Wakefield D, Duncombe VM, Thomas MC, Bolin TD. Small intestinal mucosal immunity and morphometry in luminal overgrowth of indigenous gut flora. *Am J Gastroenterol* 2001; **96**: 494-500 [PMID: 11232696]
  - 23 **Bures J**, Cyrany J, Kohoutova D, Förstl M, Rejchrt S, Kvetina J, Vorisek V, Kopacova M. Small intestinal bacterial overgrowth syndrome. *World J Gastroenterol* 2010; **16**: 2978-2990 [PMID: 20572300]
  - 24 **Wu GD**, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; **334**: 105-108 [PMID: 21885731 DOI: 10.1126/science.1208344]
  - 25 **Morgun A**, Dzutsev A, Dong X, Greer RL, Sexton DJ, Ravel J, Schuster M, Hsiao W, Matzinger P, Shulzhenko N. Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks. *Gut* 2015; **64**: 1732-1743 [PMID: 25614621 DOI: 10.1136/gutjnl-2014-308820]
  - 26 **Hollister EB**, Gao C, Versalovic J. Compositional and functional features of the gastrointestinal microbiome and their effects on human health. *Gastroenterology* 2014; **146**: 1449-1458 [PMID: 24486050 DOI: 10.1053/j.gastro.2014.01.052]
  - 27 **Clemente JC**, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell* 2012; **148**: 1258-1270 [PMID: 22424233 DOI: 10.1016/j.cell.2012.01.035]
  - 28 **Mima K**, Sukawa Y, Nishihara R, Qian ZR, Yamauchi M, Inamura K, Kim SA, Masuda A, Nowak JA, Noshio K, Kostic AD, Giannakis M, Watanabe H, Bullman S, Milner DA, Harris CC, Giovannucci E, Garraway LA, Freeman GJ, Dranoff G, Chan AT, Garrett WS, Huttenhower C, Fuchs CS, Ogino S. *Fusobacterium nucleatum* and T Cells in Colorectal Carcinoma. *JAMA Oncol* 2015; **1**: 653-661 [PMID: 26181352 DOI: 10.1001/jamaoncol.2015.1377]
  - 29 **Chase D**, Goulder A, Zenhausern F, Monk B, Herbst-Kralovetz M. The vaginal and gastrointestinal microbiomes in gynecologic cancers: a review of applications in etiology, symptoms and treatment. *Gynecol Oncol* 2015; **138**: 190-200 [PMID: 25957158 DOI: 10.1016/j.ygyno.2015.04.036]
  - 30 **Sheflin AM**, Whitney AK, Weir TL. Cancer-promoting effects of microbial dysbiosis. *Curr Oncol Rep* 2014; **16**: 406 [PMID: 25123079 DOI: 10.1007/s11912-014-0406-0]
  - 31 **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031 [PMID: 17183312]
  - 32 **Qin J**, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012; **490**: 55-60 [PMID: 23023125 DOI: 10.1038/nature11450]
  - 33 **Vijay-Kumar M**, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, Sitaraman SV, Knight R, Ley RE, Gewirtz AT. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science* 2010; **328**: 228-231 [PMID: 20203013 DOI: 10.1126/science.1179721]
  - 34 **Garrett WS**, Gallini CA, Yatsunenkov T, Michaud M, DuBois A, Delaney ML, Punit S, Karlsson M, Bry L, Glickman JN, Gordon JI, Onderdonk AB, Glimcher LH. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe* 2010; **8**: 292-300 [PMID: 20833380 DOI: 10.1016/j.chom.2010.08.004]

- 35 **Mouzaki M**, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, McGilvray ID, Allard JP. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* 2013; **58**: 120-127 [PMID: 23401313 DOI: 10.1002/hep.26319]
- 36 **Wieland A**, Frank DN, Harnke B, Bambha K. Systematic review: microbial dysbiosis and nonalcoholic fatty liver disease. *Aliment Pharmacol Ther* 2015; **42**: 1051-1063 [PMID: 26304302 DOI: 10.1111/apt.13376]
- 37 **Hartmann P**, Seebauer CT, Schnabl B. Alcoholic liver disease: the gut microbiome and liver cross talk. *Alcohol Clin Exp Res* 2015; **39**: 763-775 [PMID: 25872593 DOI: 10.1111/acer.12704]
- 38 **Usami M**, Miyoshi M, Kanbara Y, Aoyama M, Sakaki H, Shuno K, Hirata K, Takahashi M, Ueno K, Hamada Y, Tabata S, Asahara T, Nomoto K. Analysis of fecal microbiota, organic acids and plasma lipids in hepatic cancer patients with or without liver cirrhosis. *Clin Nutr* 2013; **32**: 444-451 [PMID: 23068014 DOI: 10.1016/j.clnu.2012.09.010]
- 39 **Abu-Shanab A**, Quigley EM. The role of the gut microbiota in nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 691-701 [PMID: 21045794 DOI: 10.1038/nrgastro.2010.172]
- 40 **Bäckhed F**, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004; **101**: 15718-15723 [PMID: 15505215]
- 41 **Lin RS**, Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, Hsu WC, Huang CC, Wang SS, Lo KJ. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. *J Hepatol* 1995; **22**: 165-172 [PMID: 7790704]
- 42 **Frasinariu OE**, Ceccarelli S, Alisi A, Moraru E, Nobili V. Gut-liver axis and fibrosis in nonalcoholic fatty liver disease: an input for novel therapies. *Dig Liver Dis* 2013; **45**: 543-551 [PMID: 23280158 DOI: 10.1016/j.dld.2012.11.010]
- 43 **Alisi A**, Carsetti R, Nobili V. Pathogen- or damage-associated molecular patterns during nonalcoholic fatty liver disease development. *Hepatology* 2011; **54**: 1500-1502 [PMID: 22045668 DOI: 10.1002/hep.24611]
- 44 **Csak T**, Velayudham A, Hritz I, Petrasek J, Levin I, Lippai D, Catalano D, Mandrekar P, Dolganiuc A, Kurt-Jones E, Szabo G. Deficiency in myeloid differentiation factor-2 and toll-like receptor 4 expression attenuates nonalcoholic steatohepatitis and fibrosis in mice. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G433-G441 [PMID: 21233280 DOI: 10.1152/ajpgi.00163.2009]
- 45 **Gómez-Hurtado I**, Such J, Sanz Y, Francés R. Gut microbiota-related complications in cirrhosis. *World J Gastroenterol* 2014; **20**: 15624-15631 [PMID: 25400446 DOI: 10.3748/wjg.v20.i42.15624]
- 46 **Giannelli V**, Di Gregorio V, Iebba V, Giusto M, Schippa S, Merli M, Thalheimer U. Microbiota and the gut-liver axis: bacterial translocation, inflammation and infection in cirrhosis. *World J Gastroenterol* 2014; **20**: 16795-16810 [PMID: 25492994 DOI: 10.3748/wjg.v20.i45.16795]
- 47 **Macnaughtan J**, Jalan R. Clinical and pathophysiological consequences of alterations in the microbiome in cirrhosis. *Am J Gastroenterol* 2015; **110**: 1399-1410; quiz 1411 [PMID: 26416191 DOI: 10.1038/ajg.2015]
- 48 **Bauer TM**, Steinbrückner B, Brinkmann FE, Ditzel AK, Schwacha H, Aponte JJ, Pelz K, Kist M, Blum HE. Small intestinal bacterial overgrowth in patients with cirrhosis: prevalence and relation with spontaneous bacterial peritonitis. *Am J Gastroenterol* 2001; **96**: 2962-2967 [PMID: 11693333]
- 49 **Wiest R**, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. *J Hepatol* 2014; **60**: 197-209 [PMID: 23993913 DOI: 10.1016/j.jhep.2013.07.044]
- 50 **Chang CS**, Chen GH, Lien HC, Yeh HZ. Small intestine dysmotility and bacterial overgrowth in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 1998; **28**: 1187-1190 [PMID: 9794900]
- 51 **Lu H**, Wu Z, Xu W, Yang J, Chen Y, Li L. Intestinal microbiota was assessed in cirrhotic patients with hepatitis B virus infection. Intestinal microbiota of HBV cirrhotic patients. *Microb Ecol* 2011; **61**: 693-703 [PMID: 21286703 DOI: 10.1007/s00248-010-9801-8]
- 52 **Teltschik Z**, Wiest R, Beisner J, Nuding S, Hofmann C, Schoelmerich J, Bevens CL, Stange EF, Wehkamp J. Intestinal bacterial translocation in rats with cirrhosis is related to compromised Paneth cell antimicrobial host defense. *Hepatology* 2012; **55**: 1154-1163 [PMID: 22095436 DOI: 10.1002/hep.24789]
- 53 **Chen Y**, Yang F, Lu H, Wang B, Chen Y, Lei D, Wang Y, Zhu B, Li L. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* 2011; **54**: 562-572 [PMID: 21574172 DOI: 10.1002/hep.24423]
- 54 **Xu M**, Wang B, Fu Y, Chen Y, Yang F, Lu H, Chen Y, Xu J, Li L. Changes of fecal Bifidobacterium species in adult patients with hepatitis B virus-induced chronic liver disease. *Microb Ecol* 2012; **63**: 304-313 [PMID: 21814872 DOI: 10.1007/s00248-011-9925-5]
- 55 **Bajaj JS**, Hylemon PB, Ridlon JM, Heuman DM, Daita K, White MB, Monteith P, Noble NA, Sikaroodi M, Gillevet PM. Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. *Am J Physiol Gastrointest Liver Physiol* 2012; **303**: G675-G685 [PMID: 22821944 DOI: 10.1152/ajpgi.00152.2012]
- 56 **Bajaj JS**, Ridlon JM, Hylemon PB, Thacker LR, Heuman DM, Smith S, Sikaroodi M, Gillevet PM. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G168-G175 [PMID: 21940902 DOI: 10.1152/ajpgi.00190.2011]
- 57 **Bajaj JS**, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, Noble NA, Unser AB, Daita K, Fisher AR, Sikaroodi M, Gillevet PM. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014; **60**: 940-947 [PMID: 24374295 DOI: 10.1016/j.jhep.2013.12.019]
- 58 **Nava GM**, Stappenbeck TS. Diversity of the autochthonous colonic microbiota. *Gut Microbes* 2011; **2**: 99-104 [PMID: 21694499 DOI: 10.4161/gmic.2.2.15416]
- 59 **Grąt M**, Holówkó W, Wronka KM, Grąt K, Lewandowski Z, Kosińska I, Krasnodębski M, Wasilewicz M, Gałęcka M, Szachta P, Zborowska H, Patkowski W, Krawczyk M. The relevance of intestinal dysbiosis in liver transplant candidates. *Transpl Infect Dis* 2015; **17**: 174-184 [PMID: 25728703 DOI: 10.1111/tid.12352]
- 60 **Bajaj JS**, Betrapally NS, Hylemon PB, Heuman DM, Daita K, White MB, Unser A, Thacker LR, Sanyal AJ, Kang DJ, Sikaroodi M, Gillevet PM. Salivary microbiota reflects changes in gut microbiota in cirrhosis with hepatic encephalopathy. *Hepatology* 2015; **62**: 1260-1271 [PMID: 25820757 DOI: 10.1002/hep.27819]
- 61 **Kakiyama G**, Pandak WM, Gillevet PM, Hylemon PB, Heuman DM, Daita K, Takei H, Muto A, Nittono H, Ridlon JM, White MB, Noble NA, Monteith P, Fuchs M, Thacker LR, Sikaroodi M, Bajaj JS. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. *J Hepatol* 2013; **58**: 949-955 [PMID: 23333527 DOI: 10.1016/j.jhep.2013.01.003]
- 62 **Berg RD**, Garlington AW. Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in a gnotobiotic mouse model. *Infect Immun* 1979; **23**: 403-411 [PMID: 154474]
- 63 **Arvaniti V**, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, Burroughs AK. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* 2010; **139**: 1246-1256, 1256.e1-e5 [PMID: 20558165 DOI: 10.1053/j.gastro.2010.06.019]
- 64 **Qin N**, Yang F, Li A, Prifti E, Chen Y, Shao L, Guo J, Le Chatelier E, Yao J, Wu L, Zhou J, Ni S, Liu L, Pons N, Batto JM, Kennedy SP, Leonard P, Yuan C, Ding W, Chen Y, Hu X, Zheng B, Qian G, Xu W, Ehrlich SD, Zheng S, Li L. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014; **513**: 59-64 [PMID: 25079328 DOI: 10.1038/nature13568]
- 65 **Krieger D**, Krieger S, Jansen O, Gass P, Theilmann L, Lichtnecker H. Manganese and chronic hepatic encephalopathy. *Lancet* 1995; **346**: 270-274 [PMID: 7630246]
- 66 **Wiest R**, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. *Hepatology* 2005; **41**: 422-433 [PMID: 15723320]

- 67 **Michelotti GA**, Machado MV, Diehl AM. NAFLD, NASH and liver cancer. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 656-665 [PMID: 24080776 DOI: 10.1038/nrgastro.2013.183]
- 68 **Luedde T**, Beraza N, Kotsikoris V, van Loo G, Nenci A, De Vos R, Roskams T, Trautwein C, Pasparakis M. Deletion of NEMO/IKKgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. *Cancer Cell* 2007; **11**: 119-132 [PMID: 17292824]
- 69 **Dapito DH**, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, Caviglia JM, Khiabanian H, Adeyemi A, Bataller R, Lefkowitz JH, Bower M, Friedman R, Sartor RB, Rabadan R, Schwabe RF. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 2012; **21**: 504-516 [PMID: 22516259 DOI: 10.1016/j.ccr.2012.02.007]
- 70 **Prasad S**, Dhiman RK, Duseja A, Chawla YK, Sharma A, Agarwal R. Lactulose improves cognitive functions and health-related quality of life in patients with cirrhosis who have minimal hepatic encephalopathy. *Hepatology* 2007; **45**: 549-559 [PMID: 17326150]
- 71 **Riordan SM**, Williams R. Treatment of hepatic encephalopathy. *N Engl J Med* 1997; **337**: 473-479 [PMID: 9250851]
- 72 **Bajaj JS**, Heuman DM, Sanyal AJ, Hylemon PB, Sterling RK, Stravitz RT, Fuchs M, Ridlon JM, Daita K, Monteith P, Noble NA, White MB, Fisher A, Sikaroodi M, Rangwala H, Gillevet PM. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. *PLoS One* 2013; **8**: e60042 [PMID: 23565181 DOI: 10.1371/journal.pone.0060042]
- 73 **Qamar AA**. Probiotics in Nonalcoholic Fatty Liver Disease, Nonalcoholic Steatohepatitis, and Cirrhosis. *J Clin Gastroenterol* 2015; **49** Suppl 1: S28-S32 [PMID: 26447961 DOI: 10.1097/MCG.0000000000000347]
- 74 **Bajaj JS**, Heuman DM, Hylemon PB, Sanyal AJ, Puri P, Sterling RK, Luketic V, Stravitz RT, Siddiqui MS, Fuchs M, Thacker LR, Wade JB, Daita K, Sistrun S, White MB, Noble NA, Thorpe C, Kakiyama G, Pandak WM, Sikaroodi M, Gillevet PM. Randomised clinical trial: Lactobacillus GG modulates gut microbiome, metabolome and endotoxemia in patients with cirrhosis. *Aliment Pharmacol Ther* 2014; **39**: 1113-1125 [PMID: 24628464 DOI: 10.1111/apt.12695]
- 75 **Dhiman RK**, Rana B, Agrawal S, Garg A, Chopra M, Thumburu KK, Khattri A, Malhotra S, Duseja A, Chawla YK. Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: a randomized, controlled trial. *Gastroenterology* 2014; **147**: 1327-1337.e3 [PMID: 25450083 DOI: 10.1053/j.gastro.2014.08.031]
- 76 **Kao D**, Roach B, Park H, Hotte N, Madsen K, Bain V, Tandon P. Fecal microbiota transplantation in the management of hepatic encephalopathy. *Hepatology* 2016; **63**: 339-340 [PMID: 26264779 DOI: 10.1002/hep.28121]

**P- Reviewer:** Dirchwolf M, Elalfy H, Moller S, Sunami Y  
**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Li D





Clinical Trials Study

## Independent effects of diet and exercise training on fat oxidation in non-alcoholic fatty liver disease

Ilaria Croci, Nuala M Byrne, Veronique S Chachay, Andrew P Hills, Andrew D Clouston, Trisha M O'Moore-Sullivan, Johannes B Prins, Graeme A Macdonald, Ingrid J Hickman

Ilaria Croci, Veronique S Chachay, School of Human Movement and Nutrition Sciences, University of Queensland, St. Lucia 4072, Australia

Ilaria Croci, Veronique S Chachay, Ingrid J Hickman, Translational Research Institute, the University of Queensland Diamantina Institute, Woolloongabba 4102, Australia

Nuala M Byrne, Bond Institute of Health and Sport, Bond University, Robina 4229, Australia

Nuala M Byrne, Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove 4059, Australia

Andrew P Hills, School of Health Sciences, University of Tasmania, Launceston 7250, Australia

Andrew P Hills, Trisha M O'Moore-Sullivan, Johannes B Prins, Ingrid J Hickman, Mater Research Institute University of Queensland, Brisbane 4101, Australia

Andrew D Clouston, Graeme A Macdonald, School of Medicine, the University of Queensland, Brisbane 4102, Australia

Graeme A Macdonald, Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Woolloongabba 4102, Australia

Graeme A Macdonald, Translational Research Institute, Woolloongabba 4102, Australia

Ingrid J Hickman, Department of Nutrition and Dietetics, Princess Alexandra Hospital, Woolloongabba 4102, Australia

**Author contributions:** Croci I wrote the paper; Croci I, Byrne NM, Chachay VS, Hills AP, Clouston AD, O'Moore-Sullivan TM, Prins JB, Macdonald GA and Hickman IJ designed the research; Croci I, Chachay VS, O'Moore-Sullivan TM, Macdonald GA and Hickman IJ collected data; Croci I, Hickman IJ and Clouston AD analyzed the data; Hickman IJ obtained funding; all authors approved the manuscript.

Supported by The National Health and Medical Research

Council of Australia; and the Lions Medical Research Foundation.

**Institutional review board statement:** The study was approved by the Human Research Ethics Committees of the Princess Alexandra Hospital and the University of Queensland.

**Clinical trial registration statement:** This registration policy applies to prospective, randomized, controlled trials only.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** The authors have no conflicts of interest to disclose.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Ilaria Croci, PhD, School of Human Movement and Nutrition Sciences, University of Queensland, Blair Drive, St. Lucia 4072, Australia. [ilaria.croci@uqconnect.edu.au](mailto:ilaria.croci@uqconnect.edu.au)  
**Telephone:** +61-7-33656851  
**Fax:** +61-7-33656877

**Received:** April 30, 2016

**Peer-review started:** May 3, 2016

**First decision:** June 17, 2016

**Revised:** July 21, 2016

**Accepted:** August 17, 2016

**Article in press:** August 18, 2016

**Published online:** September 28, 2016

## Abstract

### AIM

To investigate the independent effects of 6-mo of dietary energy restriction or exercise training on whole-body and hepatic fat oxidation of patients with non-alcoholic fatty liver disease (NAFLD).

### METHODS

Participants were randomised into either circuit exercise training (EX;  $n = 13$ ; 3 h/wk without changes in dietary habits), or dietary energy restriction (ER) without changes in structured physical activity (ER;  $n = 8$ ). Respiratory quotient (RQ) and whole-body fat oxidation rates (Fat<sub>ox</sub>) were determined by indirect calorimetry under basal, insulin-stimulated and exercise conditions. Severity of disease and steatosis was determined by liver histology; hepatic Fat<sub>ox</sub> was estimated from plasma  $\beta$ -hydroxybutyrate concentrations; cardiorespiratory fitness was expressed as  $\dot{V}O_{2peak}$ . Complete-case analysis was performed (EX:  $n = 10$ ; ER:  $n = 6$ ).

### RESULTS

Hepatic steatosis and NAFLD activity score decreased with ER but not with EX.  $\beta$ -hydroxybutyrate concentrations increased significantly in response to ER ( $0.08 \pm 0.02$  mmol/L *vs*  $0.12 \pm 0.04$  mmol/L,  $P = 0.03$ ) but remained unchanged in response to EX ( $0.10 \pm 0.03$  mmol/L *vs*  $0.11 \pm 0.07$  mmol/L,  $P = 0.39$ ). Basal RQ decreased ( $P = 0.05$ ) in response to EX, while this change was not significant after ER ( $P = 0.38$ ).  $\dot{V}O_{2peak}$  ( $P < 0.001$ ) and maximal Fat<sub>ox</sub> during aerobic exercise ( $P = 0.03$ ) improved with EX but not with ER ( $P > 0.05$ ). The increase in  $\beta$ -hydroxybutyrate concentrations was correlated with the reduction in hepatic steatosis ( $r = -0.56$ ,  $P = 0.04$ ).

### CONCLUSION

ER and EX lead to specific benefits on fat metabolism of patients with NAFLD. Increased hepatic Fat<sub>ox</sub> in response to ER could be one mechanism through which the ER group achieved reduction in steatosis.

**Key words:** Non-alcoholic steatohepatitis; Steatosis; Fat and carbohydrate oxidation; Exercise; Fitness; Beta-hydroxybutyrate; Ketone bodies; Fatty acid oxidation

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We investigated hepatic fat oxidation and whole-body substrate oxidation under basal, insulin-stimulated and exercise conditions before and after 6 mo of circuit exercise training (EX) or dietary energy restriction (ER) in patients with non-alcoholic fatty liver disease. ER increased  $\beta$ -hydroxybutyrate concentrations (a marker of hepatic fat oxidation) and reduced severity of steatosis, but did not change substrate oxidation rates during acute exercise. EX improved substrate oxidation under basal, insulin-stimulated and exercise conditions, but not  $\beta$ -hydroxybutyrate concentrations and severity of disease. Increase in  $\beta$ -hydroxybutyrate was associated with decrease in hepatic steatosis and this could be one

mechanism through which the ER group achieved reduction in steatosis.

Crocì I, Byrne NM, Chachay VS, Hills AP, Clouston AD, O'Moore-Sullivan TM, Prins JB, Macdonald GA, Hickman IJ. Independent effects of diet and exercise training on fat oxidation in non-alcoholic fatty liver disease. *World J Hepatol* 2016; 8(27): 1137-1148 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i27/1137.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i27.1137>

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in industrialised countries and its prevalence is increasing globally<sup>[1]</sup>. The term NAFLD describes a range of liver damage ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis that occur in the absence of hazardous alcohol consumption. NAFLD is linked with obesity, visceral adiposity, physical inactivity, insulin resistance<sup>[2]</sup>, and genetic predisposition<sup>[3]</sup>. Intrahepatic triglycerides (TGs) (steatosis) accumulate when the sum of *de novo* hepatic fatty acid synthesis rate and hepatic fatty acid uptake rate is greater than those of TG export and hepatic fat oxidation<sup>[4]</sup>. In a recent cross-sectional study we have shown that overweight patients with NAFLD do not adequately adapt fuel oxidation to fuel availability, with reduced fat oxidation rates (Fat<sub>ox</sub>) in resting and fasting conditions, a reduced suppression of Fat<sub>ox</sub> after insulin stimulation and a lower increase in Fat<sub>ox</sub> during exercise compared to lean controls<sup>[5]</sup>. Further, we observed that patients with NAFLD had reduced hepatic Fat<sub>ox</sub>, as measured by plasma  $\beta$ -hydroxybutyrate, when compared to lean controls.

Lifestyle interventions consisting of diet (improved diet quality with or without energy restriction) or diet in conjunction with exercise training are currently the most commonly advocated therapies for NAFLD management<sup>[6-8]</sup>. Limited research has assessed the effect of a lifestyle intervention in NAFLD on whole-body Fat<sub>ox</sub>. Hallsworth *et al*<sup>[9]</sup> showed that 8 wk of resistance training without weight loss did not change substrate oxidation rates in the basal state (resting and fasting) but increased Fat<sub>ox</sub> during aerobic exercise. However, substrate oxidation during exercise was assessed at a single intensity and at the same absolute intensity pre and post intervention (50% of the pre-intervention  $\dot{V}O_{2peak}$ ). Therefore, assessment of maximal rate of Fat<sub>ox</sub> (MFO) and the intensity at which it occurs (Fat<sub>max</sub>) was not possible, and participants likely were assessed at a lower relative intensity post-intervention (due to improved  $\dot{V}O_{2peak}$ ). Gaining a deeper understanding of substrate metabolism during exercise is of interest because the full body metabolic demands are higher and potential alterations not observable at rest may become apparent.

The effect of different treatment options for NAFLD on

hepatic Fat<sub>ox</sub> is also unclear. In response to dietary energy restriction (ER), little information is available. A study in which 18 patients with NAFLD underwent 2 wk of dietary ER reported increased plasma  $\beta$ -hydroxybutyrate concentrations (indicating increased hepatic Fat<sub>ox</sub>), and this was correlated with reduction in steatosis<sup>[10]</sup>. This is in agreement with findings in animal models showing that an increase in hepatic Fat<sub>ox</sub> leads to a reduction in hepatic steatosis<sup>[11,12]</sup>. However, whether a similar response is seen in response to a longer dietary intervention, with the assessment being performed in energy balance (as opposed to energy deficit), needs to be established. Furthermore, the effect of an exercise training program on plasma  $\beta$ -hydroxybutyrate concentrations is unknown<sup>[13]</sup>. Understanding the independent effect of energy restriction and exercise training on whole-body Fat<sub>ox</sub> and hepatic Fat<sub>ox</sub> in patients with NAFLD can contribute to elucidate how these interventions impact on the disease and could lead to more specific guidelines for NAFLD management.

Improvement in cardiorespiratory fitness (CRF) is a key endpoint in exercise training interventions. Cross-sectional evidence shows that lower levels of physical activity and CRF correlate with more severe hepatic injury on histology and greater steatosis<sup>[14-17]</sup>. However, the relationship between change in CRF measured with a graded exercise test and change in steatosis (measured quantitatively) has not been explored longitudinally in NAFLD<sup>[18,19]</sup>. Investigating the associations between changes in markers of CRF, substrate oxidation, and histological, metabolic and biochemical features of NAFLD in response to exercise can help understand the mechanisms through which exercise may benefit features of NAFLD.

This study aimed to investigate changes in hepatic Fat<sub>ox</sub> and in whole-body substrate oxidation rates under basal, insulin-stimulated and exercise conditions, in patients with NAFLD who completed either 6 mo of dietary energy restriction or circuit exercise training. The second aim was to assess whether changes in CRF, whole-body fat and hepatic Fat<sub>ox</sub> were associated with changes in hepatic steatosis.

## MATERIALS AND METHODS

### Participants

Overweight patients with NAFLD (diagnosed on liver biopsy) participated in the study ( $n = 21$ ). Exclusion criteria included: Type 2 diabetes, cirrhosis, decompensated liver disease, presence of other causes of liver disease, and daily ethanol consumption > 20 g in females or > 40 g in males. The study was approved by the local Human Research Ethics Committees (Princess Alexandra Hospital and University of Queensland, Australia). All participants provided informed written consent. The randomised controlled clinical trial was registered with the Australian and New Zealand Clinical Trials Registry (<http://www.anzctr.org.au>). The registration identification number is ACTRN12612001087842.

### General design

Participants were randomised into either a dietary energy restriction intervention (ER;  $n = 8$ ) or an exercise training intervention (EX;  $n = 13$ ). A consort diagram describing the flow of patients through the randomised controlled trial is presented in Figure 1. Outcome measures were assessed prior to randomisation (pre-intervention) and after 6 mo of intervention. At both time-points participants undertook three testing sessions within a 7-d period. Patients had stable body weight for at least 2 wk before the post intervention testing.

During the first testing session, body composition was assessed by dual-energy X-ray absorptiometry. The second session involved a hyperinsulinaemic-euglycaemic clamp with indirect calorimetry measurements to assess substrate oxidation rates under basal and insulin-stimulated conditions. This session also involved clinical assessments, including blood pressure and anthropometry. During the third testing session, indirect calorimetry measurement was performed during a graded exercise test on an ergocycle to determine substrate oxidation rate and CRF (as measured by  $\dot{V}O_{2peak}$ ). The second and third sessions were conducted in the morning after an overnight fast. Both ER and EX groups were instructed not to change exercise and physical activity patterns throughout the intervention and this was monitored with accelerometers at three time points during the intervention.

The primary outcomes of the trial were hepatic steatosis and IR and have been published elsewhere<sup>[20]</sup>. The present manuscript focuses on secondary outcome measures including plasma  $\beta$ -hydroxybutyrate concentrations, and whole-body Fat<sub>ox</sub> under basal, insulin-stimulated and exercise conditions. The flow of participants for the present analysis is presented in the Consort Diagram in Figure 1. Complete-case analysis, including 10 EX and 6 ER participants, was performed.

### Exercise training intervention

EX, as previously detailed<sup>[20]</sup>, involved 3 sessions per week of circuit exercise training during 6 mo without dietary restriction. The aim was to improve CRF, muscle strength and body composition without significant body weight loss. EX was selected based on preliminary research conducted in our laboratory<sup>[21]</sup>.

Training intensity was fixed at 50% of 1-RM for the entire duration of the training program; 1-RM was reassessed monthly to account for strength adaptations. The training volume was progressively increased from one circuit (12 min) in week 1 to five circuits (60 min) in week 11; and then remained constant at five circuits from week 11 until the end of the intervention. Each circuit comprised 12 light resistance exercises covering the major muscle groups. The training program consisted of alternating 30 s exercise intervals and 30 s rest periods. Pneumatic resistance training machines were employed (Ab Hur Oy, Kokkola, Finland). All training sessions were supervised by an exercise physiologist.

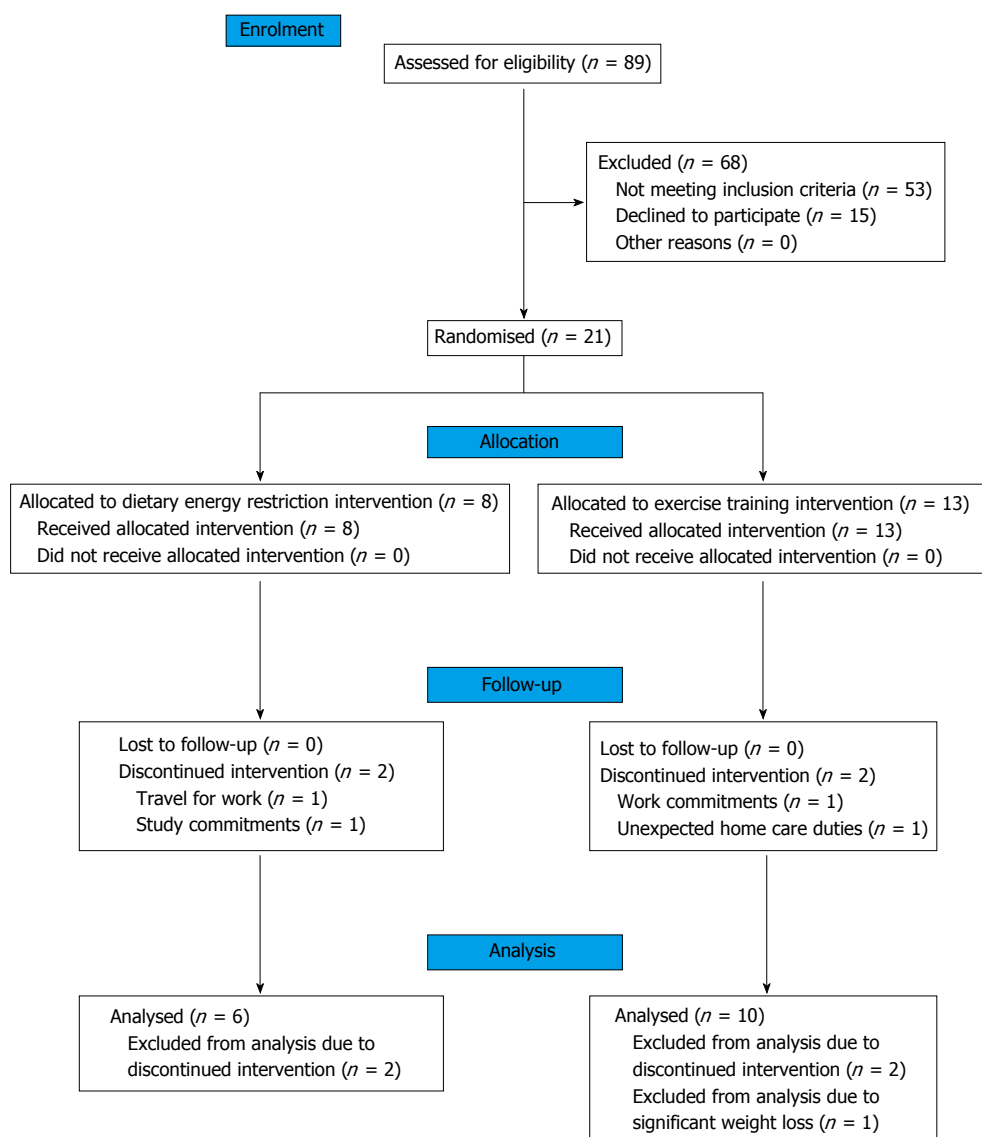


Figure 1 Consort diagram describing the flow of patients through the randomised controlled trial.

### Energy restriction

ER involved a weight loss program under the guidance of a dietitian. Patients attended weekly face-to-face appointments for 16 wk and were provided with an individualised dietary prescription with the aim of 5%-10% of body weight loss within 16 wk. This was followed by an 8-wk period aimed at body weight maintenance, with fortnightly reviews with the dietitian. The target macronutrient composition was 40% carbohydrate, 20% protein and 40% fat (< 10% saturated fat). Recommendations included choosing foods that are low in saturated fats; avoiding micronutrient-poor/energy-dense food options; avoiding added sugar; and aiming for regular meal patterns. Weekly weight and waist measures, and 24-h diet recalls encouraged adherence and self-monitoring.

### Histological analysis of liver biopsy

Liver biopsy specimens were analysed as previously detailed<sup>[5,20]</sup>. The severity of liver injury was determined

with the NAFLD activity score (NAS)<sup>[22]</sup> and the criteria described by Brunt<sup>[23]</sup>. Using conventional histologic criteria<sup>[24]</sup>, a diagnosis of NASH or steatosis alone was made.

### Body composition

Body composition assessments including determination of fat-free mass (FFM) and fat mass by dual-energy X-ray absorptiometry. Subcutaneous abdominal fat and visceral abdominal fat were assessed by computed tomography as previously described<sup>[25]</sup>.

### Insulin sensitivity

Insulin sensitivity was assessed with the hyperinsulinemic-euglycemic clamp technique<sup>[26]</sup>, as we previously detailed<sup>[20]</sup>. Briefly, primed insulin was infused at a rate of 1 mU/kg per minute throughout the procedure (2 h), and a 25% glucose solution was infused at a variable rate to maintain euglycemia<sup>[26]</sup>. The glucose infusion rate in the steady state of the hyperinsulinemic-euglycemic clamp



(M-value) corresponded to the whole-body glucose disposal rate.

### Biochemical analysis

Biochemical analyses were performed as previously described<sup>[5,20]</sup>. Plasma  $\beta$ -hydroxybutyrate concentrations, an index of hepatic ketogenesis<sup>[27-30]</sup>, were measured with an enzymatic assay (Stanbio, Boerne, TX, CV 2.2%).

### Exercise testing

Maximal aerobic power and substrate utilization were assessed with a graded exercise test on an ergocycle. Testing comprised a sub-maximal phase to determine  $\text{Fat}_{\text{ox}}$  and  $\text{CHO}_{\text{ox}}$  at multiple intensities (with workload increments occurring every 5 min), and a maximal phase to assess peak oxygen consumption ( $\dot{V}\text{O}_{2\text{peak}}$ ) (increments every min). The testing protocol adopted has been described in detail in a previous publication<sup>[5]</sup>.

### Indirect calorimetry measurements

Indirect calorimetry measurements (TrueOne 2400 Metabolic Measurement System, Parvo Medics, UT) were conducted in three physiological states (basal, insulin-stimulated and exercise). Whole-body  $\text{Fat}_{\text{ox}}$  and  $\text{CHO}_{\text{ox}}$  were calculated using stoichiometric equations, with the assumption that the urinary nitrogen excretion rate was negligible<sup>[31]</sup>. The methodological approach adopted has been previously described in detail<sup>[5]</sup>.

$\text{Fat}_{\text{ox}}$  rates during exercise were estimated from respiratory gases averaged over the last minute of each exercise stage. Then, the stage at which MFO was achieved was determined, and the corresponding intensity was identified ( $\text{Fat}_{\text{max}}$ )<sup>[32]</sup>.  $\Delta\text{RQ}$  represented the RQ change from basal to hyperinsulinemic state (RQ in the insulin-stimulated condition minus basal RQ).

Testing sessions involving indirect calorimetry measurements were conducted in the morning after a 10-12 h overnight fast and under standardised conditions<sup>[5]</sup>. Standardisation of pre-test conditions was in line with previous studies<sup>[32-40]</sup>.

### Daily physical activity

Daily physical activity was quantified with RT3 accelerometers Activity Monitor, 2003, Stayhealthy, Incorporated, Monrovia, CA, United States) worn for 7 consecutive days at 0, 3 and 6 mo, as previously described<sup>[20]</sup>.

### Statistical analysis

A secondary analysis of outcomes from a larger clinical trial was performed. Independent *t*-tests were used to compare the pre-intervention (baseline) characteristics between groups (ER vs EX). Paired Student *t*-tests were used to compare within group outcome measures pre and post intervention. Wilcoxon matched-pair signed rank test was used if samples were not normally distributed. Correlation analyses were performed using Pearson's correlation coefficient or Spearman's non-parametric rank correlation coefficient. As outlined in the consort dia-

gram (Figure 1), complete-case analysis was performed. Complete-case analysis was deemed more suitable than intention to treat analysis given that the aim of this study was to study mechanisms of benefit of the two interventions. Statistical analysis was performed with SPSS 17.0 (SPSS, Chicago, IL, United States) and Graph Pad Prism 5.0 (GraphPad Software, San Diego, CA, United States). Data are expressed as mean  $\pm$  SD or median and range. For all statistical analyses, the level of significance was set at  $P < 0.05$ . Statistical methods used in this study were reviewed a biostatistician.

## RESULTS

### Characteristics of study groups

Two patients from each arm ( $n = 4$ ) did not complete the study due to time constraints. One participant ( $n = 1$ ) from the EX group was excluded from analysis due to significant weight loss at 6 mo ( $-13.3\%$  body weight, which cannot be achieved with the type and volume of exercise prescribed as part of this exercise intervention). Data analysis (complete-case analysis) was thus performed on 10 participants from the EX and 6 participants from the ER groups (see the Consort Diagram presented in Figure 1). There were no significant differences between pre-intervention patients' characteristics of completers and non-completers. Compliance with both interventions was good. The ER group achieved an average weight loss of  $9.7\% \pm 4.6\%$ , and the EX group attendance to the exercise sessions was greater than 90% with no significant weight loss. As per protocol, usual daily time spent on low, moderate and high intensity physical activity did not change in either group ( $P > 0.05$ ). ER and EX interventions were well tolerated by participants with no adverse events reported.

Characteristics of the EX and ER groups are presented in Table 1. At baseline, the prevalence of NASH was not different between ER and EX groups ( $67\%$  vs  $80\%$ ,  $P = 0.64$ ). Primary results of the randomised controlled trial are reported elsewhere<sup>[20]</sup>. Briefly, in the ER group steatosis and the NAS decreased significantly, while in the EX group neither steatosis nor NAS decreased significantly. Skeletal muscle insulin resistance (M-value) improved significantly in response to EX, while it did not improve in patients from the ER group.

### Substrate oxidation under basal conditions

Total energy expenditure in resting and fasted conditions (basal) did not significantly change in response to both interventions ( $P > 0.05$ ). However, with the EX intervention the relative contribution of fat and CHO to energy expenditure changed: The RQ and the  $\text{CHO}_{\text{ox}}$  decreased (by  $30\%$ ,  $P = 0.02$ ), while  $\text{Fat}_{\text{ox}}$  tended to increase (Table 2). With the ER intervention, the same direction of change as for EX was seen, however statistical significance was not reached. In the whole-group, the pre-post intervention change in basal RQ was not associated with the pre-post intervention changes in steatosis ( $r = 0.05$ ,  $P = 0.88$ ) or

**Table 1** Characteristics of the study groups at baseline (pre-intervention) and after 6 mo of energy restriction or exercise training (post-intervention)

	Energy restriction ( <i>n</i> = 6)		Exercise training ( <i>n</i> = 10)	
	Pre	Post	Pre	Post
Age (yr)	45.5 ± 13.5		51.8 ± 6.7	
Gender (M:F)	3:3		7:3	
BMI (kg/m <sup>2</sup> )	33.5 ± 9.0	30.0 ± 7.0 <sup>a</sup>	31.2 ± 3.2	30.8 ± 3.5
Fat-mass (%)	38 ± 9	35 ± 11 <sup>b</sup>	36 ± 7	33 ± 6 <sup>a</sup>
Fat-free mass (kg)	54.1 ± 12.3	51.3 ± 11.8	63.1 ± 14.3	64.4 ± 14.2 <sup>a</sup>
Waist (cm)	106 ± 16	90 ± 13	110 ± 14	105 ± 13 <sup>a</sup>
Systolic blood pressure (mmHg)	126 ± 13	118 ± 13 <sup>a</sup>	139 ± 19	137 ± 18
Subcutaneous adipose tissue (cm <sup>2</sup> )	358 ± 282	268 ± 202 <sup>b</sup>	322 ± 116	298 ± 117 <sup>a</sup>
Visceral adipose tissue (cm <sup>2</sup> )	202 ± 110	203 ± 56 <sup>b</sup>	182 ± 67	117 ± 36 <sup>a</sup>
Diastolic blood pressure (mmHg)	83 ± 8	75 ± 12	88 ± 11	83 ± 10
Triglycerides (mmol/L)	1.6 ± 0.8	1.1 ± 0.4	2.0 ± 1.3	2.0 ± 0.2
HDL cholesterol (mmol/L)	0.9 ± 0.2	1.0 ± 0.3	1.0 ± 0.2	1.1 ± 0.2 <sup>a</sup>
LDL cholesterol (mmol/L)	3.5 ± 0.8	3.0 ± 0.6	3.2 ± 1.1	3.1 ± 1.0
VLDL cholesterol (mmol/L)	0.7 ± 0.3	0.5 ± 0.2 <sup>b</sup>	0.9 ± 0.6	0.7 ± 0.5
Free fatty acids (mmol/L)	0.59 ± 0.15	0.63 ± 0.23	0.59 ± 0.17	0.62 ± 0.25
Glucose (mmol/L)	5.2 ± 0.3	5.0 ± 0.7	5.5 ± 0.5	5.3 ± 0.4
Insulin (mU/L)	18 ± 18	10 ± 5	24 ± 23	12 ± 10
M-value (mg/kgFFM per minute)	4.2 ± 1.4	5.2 ± 1.5	4.0 ± 0.9	5.2 ± 1.6 <sup>a</sup>
hsCRP (mg/L)	4.9 ± 3.7	2.0 ± 1.6	3.9 ± 3.6	1.5 ± 1.3
Alanine aminotransferase (U/L)	80 ± 65	55 ± 55	54 ± 19	49 ± 28
Aspartate aminotransferase (U/L)	40 ± 22	28 ± 16	38 ± 11	39 ± 22

Complete-case analysis performed. <sup>a</sup>*P* < 0.05, within group difference in response to the intervention; <sup>b</sup>*P* value < 0.10, within group trend in response to the intervention. Pre-intervention there was no difference between energy restriction and exercise groups in any of the parameters presented (*P* > 0.05). M:F: Male: Female; BMI: Body mass index; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; hsCRP: High sensitivity C reactive protein.

NAS (*P* = 0.35).

### β-hydroxybutyrate concentrations

As shown in Figure 2, basal plasma β-hydroxybutyrate concentrations, increased significantly in response to ER (0.08 ± 0.02 mmol/L vs 0.12 ± 0.04 mmol/L, *P* = 0.03) but remained unchanged in response to EX (0.10 ± 0.03 mmol/L vs 0.11 ± 0.07 mmol/L, *P* = 0.39). This result (unchanged β-hydroxybutyrate concentrations in response to EX) was confirmed also when the analysis was performed excluding the outlier (0.09 ± 0.03 mmol/L vs 0.09 ± 0.03 mmol/L, *P* = 0.87) (Figure 2). In the combined cohort including participants from both groups, there was a negative association between pre-post intervention changes in β-hydroxybutyrate and in hepatic steatosis (*r* = -0.56, *P* = 0.04) (Figure 3). This relationship persisted after controlling for changes in body weight (*r* = -0.67, *P* = 0.02) and percentage body weight (*r* = -0.56, *P* = 0.05).

### Substrate oxidation under insulin-stimulated conditions

Hyperinsulinaemic concentrations were reached by both groups at both times points (ER, 79.0 ± 31.5 mU/L vs 80.0 ± 21.5 mU/L; EX, 83.0 ± 0.5 mU/L vs 78.1 ± 18.0 mU/L; all *P* > 0.05). The effect of the two interventions on substrate oxidation in insulin-stimulated conditions is presented in Table 3. Post-intervention, the EX group tended to increase the insulin-stimulated suppression of Fat<sub>ox</sub> compared with pre-intervention (-0.24 ± 0.36 mg/kgFFM per minute vs -0.55 ± 0.35 mg/kgFFM per

minute, *P* = 0.06). The ER group displayed a similar response, however statistical significance was not reached. In the pooled group, the pre-post intervention increase in ΔRQ (change in RQ from the basal to the insulin-stimulated state) was not correlated with the change in the severity of steatosis (*r* = 0.28, *P* = 0.28) or NAS (*P* = 0.31).

### Substrate oxidation during exercise

VO<sub>2peak</sub> and MFO improved significantly (by 18% and 71%, respectively) in response to EX but did not change in the ER group (Table 4 and Figure 4). Fat<sub>max</sub> increased by 72% in response to EX when expressed in absolute terms (45 ± 20 vs 76 ± 46 Watts, *P* = 0.03), whereas it remained unchanged after both interventions when expressed in relative terms (%VO<sub>2peak</sub>). Within the EX group, the increase in VO<sub>2peak</sub> (mL/kgFFM per minute) was correlated with the increase in ΔRQ (*r* = 0.73, *P* = 0.02) and the reduction in systolic blood pressure (*r* = -0.81, *P* = 0.01). The improvement in VO<sub>2peak</sub> was not related with the change in steatosis (*r* = 0.14, *P* = 0.73), NAS (*P* = 0.40) or basal RQ (*r* = -0.18, *P* = 0.62). Similarly, the change in MFO was not related to changes in hepatic steatosis (*r* = 0.03, *P* = 0.91), or changes in NAS (*P* = 0.63).

## DISCUSSION

ER and EX are standard interventions for the management of obesity and related comorbidities, including NAFLD. ER

**Table 2** Resting substrate metabolism pre-intervention and after 6 mo of energy restriction or exercise training (post-intervention) in patients with non-alcoholic fatty liver disease

	Energy restriction ( <i>n</i> = 6)			Exercise ( <i>n</i> = 10)		
	Pre	Post	<i>P</i>	Pre	Post	<i>P</i>
Respiratory quotient	0.82 ± 0.04	0.80 ± 0.04	0.38	0.84 ± 0.06	0.81 ± 0.06	0.05
Fat <sub>ox</sub> (mg/kgFFM per minute)	1.18 ± 0.25	1.46 ± 0.33	0.17	1.15 ± 0.54	1.35 ± 0.48	0.08
CHO <sub>ox</sub> (mg/kgFFM per minute)	2.33 ± 0.69	1.72 ± 0.83	0.19	2.70 ± 1.24	1.90 ± 1.17	0.02

Complete-case analysis performed. Fat<sub>ox</sub>: Fat oxidation rates; CHO<sub>ox</sub>: Carbohydrate oxidation rates; FFM: Fat-free mass.

**Table 3** Change in substrate metabolism from basal (resting and fasting) to insulin-stimulation conditions pre-intervention and after 6 mo of energy restriction or exercise training (post-intervention)

	Energy restriction ( <i>n</i> = 6)			Exercise ( <i>n</i> = 10)		
	Pre	Post	<i>P</i>	Pre	Post	<i>P</i>
Δ Respiratory quotient	0.05 ± 0.05	0.08 ± 0.05	0.58	0.04 ± 0.02	0.07 ± 0.05	0.11
Δ Fat <sub>ox</sub> (mg/kgFFM per minute)	-0.29 ± 0.46	-0.56 ± 0.32	0.31	-0.24 ± 0.36	-0.55 ± 0.35	0.06
Δ CHO <sub>ox</sub> (mg/kgFFM per minute)	0.92 ± 0.98	1.41 ± 0.98	0.46	0.54 ± 0.85	1.02 ± 0.93	0.18

Complete-case analysis performed. Fat<sub>ox</sub>: Fat oxidation rates; CHO<sub>ox</sub>: Carbohydrate oxidation rates; FFM: Fat-free mass; Δ: Change from basal to insulin-stimulated condition.

**Table 4** Maximal aerobic power and substrate oxidation during exercise pre-intervention, and after 6 mo of energy restriction or exercise treatment (post-intervention)

	Energy restriction ( <i>n</i> = 6)			Exercise ( <i>n</i> = 10)		
	Pre	Post	<i>P</i>	Pre	Post	<i>P</i>
VO <sub>2peak</sub> (mL/kg per minute)	20.4 ± 5.1	20.7 ± 6.4	0.73	23.9 ± 6.4	28.3 ± 6.3	< 0.001
VO <sub>2peak</sub> (mL/kgFFM per minute)	32.5 ± 5.0	31.0 ± 5.4	0.31	39.2 ± 8.4	43.6 ± 7.4	0.004
Workload at VO <sub>2peak</sub> (W)	121 ± 53	121 ± 57	0.94	176 ± 78	224 ± 81	< 0.001
MFO (g/min)	0.14 ± 0.13	0.06 ± 0.04	0.17	0.17 ± 0.09	0.29 ± 0.14	0.03
MFO (mg/kgFFM per minute)	2.5 ± 1.7	1.2 ± 0.7	0.18	2.8 ± 1.5	4.4 ± 1.9	0.04
Workload at MFO (W)	44.8 ± 16.5	41.3 ± 13.4	0.43	44.7 ± 19.5	76.3 ± 46.0	0.03
Fat <sub>max</sub> (% VO <sub>2peak</sub> )	48.7 ± 14.7	47.9 ± 8.8	0.62	45.2 ± 12.3	47.0 ± 7.2	0.94

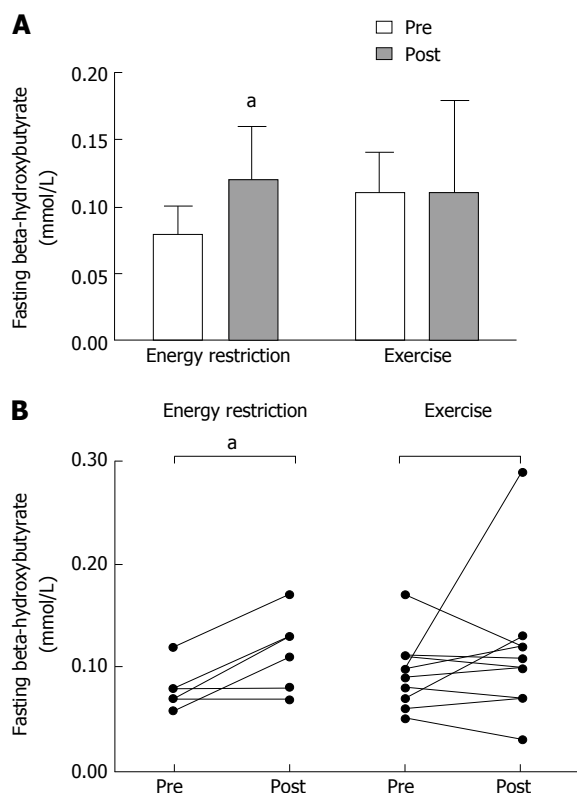
Complete-case analysis performed. VO<sub>2peak</sub>: Peak oxygen uptake; MFO: Maximal fat oxidation; W: Watts; Fat<sub>max</sub>: Exercise intensity eliciting maximal fat oxidation; FFM: Fat-free mass.

induced weight loss, reduced hepatic steatosis, increased β-hydroxybutyrate concentrations (a marker of hepatic Fat<sub>ox</sub>) but did not lead to changes in substrate oxidation rates tested during an acute exercise session. EX lead to improvements in CRF and in substrate oxidation rates under basal, insulin stimulated and exercise conditions. However, this dose of circuit exercise did not lead to improvements in hepatic Fat<sub>ox</sub> or hepatic steatosis. In the combined cohort, the reduction in hepatic steatosis was associated with increased β-hydroxybutyrate concentrations.

A novel finding from this study was that ER and EX interventions had different effects on β-hydroxybutyrate concentrations in patients with NAFLD. In response to ER, the increase in β-hydroxybutyrate (product of the oxidation pathway) was accompanied by the trend for a decrease in the very low-density lipoprotein (product of the esterification pathway), despite no change in free fatty acids concentrations. These are favourable changes given that pre-intervention patients with NAFLD showed lower β-hydroxybutyrate and higher very low-density lipoprotein compared to healthy controls<sup>[5]</sup>. These changes may suggest that the ER intervention lead to

a change in hepatic fatty acid partitioning, with free fatty acids being more directed towards oxidation than towards esterification<sup>[41]</sup>. Increase in hepatic Fat<sub>ox</sub> could be a mechanism through which the ER group achieved reduction in steatosis. Accordingly, it was shown in animal models that interventions that increase hepatic Fat<sub>ox</sub> lead to a reduction in hepatic steatosis<sup>[11,12]</sup>.

In contrast, β-hydroxybutyrate concentrations remained unaltered in response to EX. This observation is valuable because, as highlighted in a recent review, no information is available on the chronic effects of exercise training on β-hydroxybutyrate concentrations<sup>[13]</sup>. Results from the present study do not confirm findings from rodent models, which showed that chronic exercise training increased hepatic Fat<sub>ox</sub><sup>[42]</sup> and that the shift from an active to a sedentary lifestyle reduced hepatic Fat<sub>ox</sub><sup>[43]</sup>. Future studies assessing the effects of different training prescriptions (volume, intensity, frequency, duration) and the optimal type of exercise (aerobic vs circuit vs resistance) on hepatic lipid metabolism are warranted. Inclusion of genetic and molecular parameters in future investigations might provide insights on the mechanisms responsible for the inter-individual variability observed in

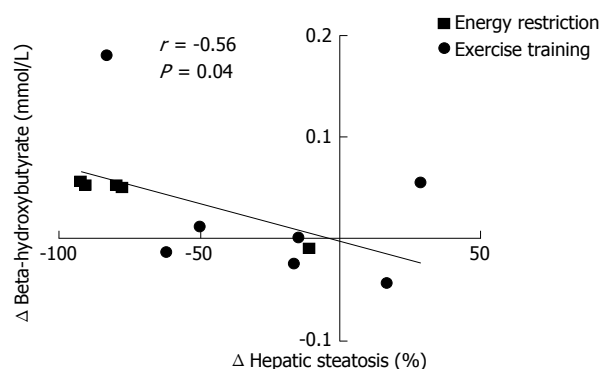


**Figure 2** Basal  $\beta$ -hydroxybutyrate concentrations before and after 6 mo of energy restriction ( $n = 6$ ) or exercise training ( $n = 10$ ). A: Average responses; B: Individual responses. <sup>a</sup> $P < 0.05$  between pre and post treatment.

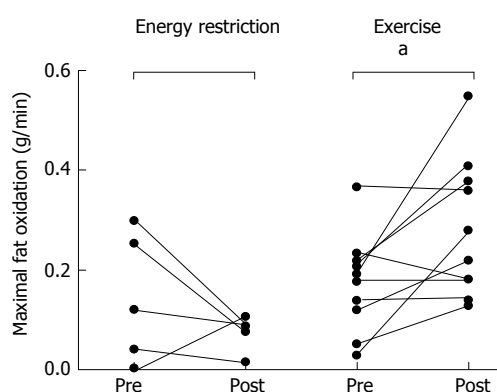
response to the treatments.

The effect of the two interventions on MFO was different: It markedly increased in response to EX, while it remained unchanged in response to ER. The improvement in MFO in the exercise group could be attributable to increased mitochondrial content, increased oxidative capacity and improved transport of free fatty acids across muscle and mitochondrial membranes<sup>[44-47]</sup>. Such changes likely were not achieved in response to ER<sup>[48]</sup>. To our knowledge, this was the first study comparing the effect of two types of lifestyle intervention (*i.e.*, ER and EX) on MFO in patients with NAFLD. It was also the first study to assess  $Fat_{max}$  and MFO in response to circuit exercise training. The improvement observed in MFO was consistent with previous studies conducted in other populations: Higher whole-body  $Fat_{ox}$  during exercise was observed in response to a moderate intensity aerobic training program conducted in obese males<sup>[49]</sup>, and in response to high-intensity aerobic training<sup>[50]</sup> or resistance exercise training<sup>[51]</sup> programs conducted in healthy individuals. Overall, the improvement in MFO in response to EX and lack of change in response to ER are in agreement with findings from a recent cross-sectional study showing that substrate oxidation rates during exercise are correlated with CRF but not with body weight or percentage body fat<sup>[52]</sup>.

Another observation from the present study was that EX improved whole-body substrate oxidation rates in resting and insulin-stimulated conditions (greater



**Figure 3** Relationship between change in  $\beta$ -hydroxybutyrate concentrations and relative change in hepatic steatosis in response to 6 mo of energy restriction or exercise training ( $n = 13$ ). This relationship remained significant after controlling for changes in body weight ( $r = -0.67$ ,  $P = 0.02$ ).



**Figure 4** Maximal fat oxidation before and after six months of energy restriction ( $n = 6$ ) or exercise training ( $n = 10$ ); individual data. <sup>a</sup> $P < 0.05$  between pre and post intervention.

$Fat_{ox}$  in basal conditions and greater increase in  $CHO_{ox}$  in response to insulin stimulation). The increased basal whole-body  $Fat_{ox}$  observed in response to EX treatment is in agreement with studies conducted in obese patients<sup>[53,54]</sup>. On the other hand, no change was observed by the only other study which investigated whole-body fat oxidation in response to exercise training in NAFLD. The different outcome compared to the present study could be explained by the shorter duration of the intervention (8 wk) and the different baseline characteristics of the study population (less severe NAFLD)<sup>[9]</sup>. In response to ER, there appeared to be a change towards a greater proportion of basal energy expenditure derived from  $Fat_{ox}$ , however statistical significance was not achieved due to the small sample size. These results are in line with other dietary interventions involving high-fat diets with carbohydrate restriction<sup>[55-58]</sup>. Increase in whole-body  $Fat_{ox}$  after treatment is of relevance in this patient population because in a recent cross-sectional study<sup>[5]</sup> we showed that whole-body  $Fat_{ox}$  is reduced in patients with NAFLD compared to healthy controls, and that this alteration was associated with the degree of steatosis.

This study comprehensively investigated the independent effects of ER and EX, the cornerstones of lifestyle treatment, on fat and carbohydrate oxidation assessed in



different physiological conditions including basal, insulin stimulation, and exercise. This forms an ideal framework to study changes in whole-body energy homeostasis and elucidate mechanisms of change in response to a therapy. Assessment of severity of liver disease, insulin resistance and body composition were conducted using gold standard techniques. A further strength was that the EX program was supervised by an exercise physiologist and was the longest exercise training intervention performed in NAFLD to date.

The randomised controlled trial was powered for detecting within group changes in primary outcome measures (hepatic steatosis and M-value), meaning that type 2 error for other outcome measures cannot be excluded. However, this did not interfere with the interpretation of key results of the present manuscript (*i.e.*,  $\beta$ -hydroxybutyrate concentrations and  $\text{Fat}_{\text{ox}}$  during exercise) given that statistically significant differences were still observed. The sample size was relatively small but it was comparable to those from similar studies conducted in NAFLD to date<sup>[9,59]</sup>. Further, a very specific population was studied: Patients were non-diabetic with histologically proven NAFLD and a large proportion (> 75%) of those patients had NASH, which represents an important distinction because patients with NASH are more likely to progress to end stage liver disease<sup>[60]</sup>. Finally, it must be acknowledged that  $\beta$ -hydroxybutyrate concentrations, while being a commonly used marker of hepatic  $\text{Fat}_{\text{ox}}$ <sup>[41]</sup>, do not represent a direct measure of hepatic  $\text{Fat}_{\text{ox}}$ . Future studies assessing the effect of lifestyle intervention in NAFLD on rates of hepatic fatty acid uptake, oxidation, and storage using a newly validated method combining <sup>11</sup>C-palmitate imaging by positron emission tomography with compartmental modelling<sup>[61]</sup>, would be of interest. Studies including assessment of redox metabolism and gene expression are also warranted.

Based on the length of intervention and type of exercise training provided, the findings of this study suggest that exercise training should not be proposed as a sole therapy for NAFLD. Guidelines should remain unchanged to recommend a combination of both ER and exercise training given that these interventions provide complementary benefits. EX is particularly beneficial for improving skeletal muscle fat metabolism and CRF, while ER provided greater benefits on hepatic fat metabolism<sup>[6]</sup>. Future research is required to investigate the impact of different doses and types of exercise programs on the severity of disease as well as on hepatic and whole-body substrate metabolism. Dose and type of exercise are likely to be crucial factors impacting on the clinical benefits of an exercise intervention<sup>[62,63]</sup>. To date, the beneficial effects of exercise training on NAFLD have been mostly seen in response to aerobic training<sup>[59,64-69]</sup> or with an aerobic component<sup>[9]</sup>. It is possible that aerobic exercise training has a greater impact on hepatic steatosis and hepatic  $\text{Fat}_{\text{ox}}$  than other training regimes because during aerobic exercise substrate availability is more closely matched with substrate oxidation and

energy deficit is greater than during other training regimes.

In conclusion, this study showed ER and EX, standard care interventions for NAFLD management, have specific and complementary benefits on fat metabolism. ER induced weight loss, increased  $\beta$ -hydroxybutyrate concentrations in basal condition, reduced severity of steatosis and severity of disease, but did not lead to changes in substrate oxidation rates during an acute exercise session. EX without weight loss, led to improvements in substrate oxidation under basal, insulin-stimulated and exercise conditions. However, this dose of circuit exercise training was not sufficient for improvements in  $\beta$ -hydroxybutyrate and severity of liver disease. Increased hepatic  $\text{Fat}_{\text{ox}}$  in response to ER could be one of the mechanisms through which the ER group achieved reduction in steatosis.

## ACKNOWLEDGMENTS

The authors would like to acknowledge the contribution of clinical and laboratory staff including Julianne Wilson, Sue Cruikshank, Fiona Henderson, Stephane Choquette, William Petchey and Stephanie Ipavec-Levasseur.

## COMMENTS

### Background

Lifestyle interventions consisting of diet or diet in conjunction with exercise training are currently the most commonly advocated therapies for non-alcoholic fatty liver disease (NAFLD) management. Limited research has assessed the effect of a lifestyle intervention in NAFLD on whole-body and hepatic fat oxidation in NAFLD.

### Research frontiers

Understanding the independent effect of diet and exercise on whole-body and hepatic fat oxidation in patients with NAFLD can contribute to elucidate how these interventions impact on the disease and could lead to more specific guidelines for NAFLD management. Exercise training as a treatment option to reduce the burden of NAFLD is an emerging field of research.

### Innovations and breakthroughs

This study showed diet and exercise, standard care interventions for NAFLD management, have specific and complementary benefits on fat metabolism. Dietary energy restriction provided greater hepatic benefits, while exercise training provided greater peripheral (whole-body) improvements.

### Applications

Based on the length of intervention and type of exercise program provided (6 mo of circuit exercise training), the findings of this study suggest that exercise training should not be proposed as a sole therapy for NAFLD. Guidelines should continue to recommend a combination of both diet and exercise given that these interventions provide complementary benefits.

### Terminology

$\beta$ -hydroxybutyrate is a ketone body produced uniquely by the liver, therefore plasma concentrations of  $\beta$ -hydroxybutyrate are used as an index of hepatic fat oxidation or hepatic ketogenesis.

### Peer-review

Authors comment adequately the only problem of this study, which is the short number of individuals who completed the study. Results are interesting and the

study is well conducted.

## REFERENCES

- 1 **Corrado RL**, Torres DM, Harrison SA. Review of treatment options for nonalcoholic fatty liver disease. *Med Clin North Am* 2014; **98**: 55-72 [PMID: 24266914 DOI: 10.1016/j.mcna.2013.09.001]
- 2 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923 [PMID: 12668987 DOI: 10.1053/jhep.2003.50161]
- 3 **Daly AK**, Ballestri S, Carulli L, Loria P, Day CP. Genetic determinants of susceptibility and severity in nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2011; **5**: 253-263 [PMID: 21476920 DOI: 10.1586/egh.11.18]
- 4 **Fabbrini E**, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 2010; **51**: 679-689 [PMID: 20041406 DOI: 10.1002/hep.23280]
- 5 **Crocì I**, Byrne NM, Choquette S, Hills AP, Chachay VS, Clouston AD, O'Moore-Sullivan TM, Macdonald GA, Prins JB, Hickman IJ. Whole-body substrate metabolism is associated with disease severity in patients with non-alcoholic fatty liver disease. *Gut* 2013; **62**: 1625-1633 [PMID: 23077135 DOI: 10.1136/gutjnl-2012-302789]
- 6 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Am J Gastroenterol* 2012; **107**: 811-826 [PMID: 22641309 DOI: 10.1038/ajg.2012.128]
- 7 **European Association for the Study of the Liver**. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]
- 8 **Abenavoli L**, Milic N, Peta V, Alfieri F, De Lorenzo A, Bellentani S. Alimentary regimen in non-alcoholic fatty liver disease: Mediterranean diet. *World J Gastroenterol* 2014; **20**: 16831-16840 [PMID: 25492997 DOI: 10.3748/wjg.v20.i45.16831]
- 9 **Hallsworth K**, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, Day CP, Trenell MI. Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. *Gut* 2011; **60**: 1278-1283 [PMID: 21708823 DOI: 10.1136/gut.2011.242073]
- 10 **Browning JD**, Baker JA, Rogers T, Davis J, Satapati S, Burgess SC. Short-term weight loss and hepatic triglyceride reduction: evidence of a metabolic advantage with dietary carbohydrate restriction. *Am J Clin Nutr* 2011; **93**: 1048-1052 [PMID: 21367948 DOI: 10.3945/ajcn.110.007674]
- 11 **Reid BN**, Ables GP, Otlivanchik OA, Schoiswohl G, Zechner R, Blaner WS, Goldberg IJ, Schwabe RF, Chua SC, Huang LS. Hepatic overexpression of hormone-sensitive lipase and adipose triglyceride lipase promotes fatty acid oxidation, stimulates direct release of free fatty acids, and ameliorates steatosis. *J Biol Chem* 2008; **283**: 13087-13099 [PMID: 18337240 DOI: 10.1074/jbc.M800533200]
- 12 **Savage DB**, Choi CS, Samuel VT, Liu ZX, Zhang D, Wang A, Zhang XM, Cline GW, Yu XX, Geisler JG, Bhanot S, Monia BP, Shulman GI. Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *J Clin Invest* 2006; **116**: 817-824 [PMID: 16485039 DOI: 10.1172/JCI27300]
- 13 **Lira FS**, Carnevali LC, Zanchi NE, Santos RV, Lavoie JM, Seelaender M. Exercise intensity modulation of hepatic lipid metabolism. *J Nutr Metab* 2012; **2012**: 809576 [PMID: 22545209 DOI: 10.1155/2012/809576]
- 14 **Church TS**, Kuk JL, Ross R, Priest EL, Biloft E, Blair SN. Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. *Gastroenterology* 2006; **130**: 2023-2030 [PMID: 16762625 DOI: 10.1053/j.gastro.2006.03.019]
- 15 **Nguyen-Duy TB**, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat and liver fat are independent predictors of metabolic risk factors in men. *Am J Physiol Endocrinol Metab* 2003; **284**: E1065-E1071 [PMID: 12554597 DOI: 10.1152/ajpendo.00442.2002]
- 16 **Ross R**, Dagnone D, Jones PJ, Smith H, Paddags A, Hudson R, Janssen I. Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Ann Intern Med* 2000; **133**: 92-103 [PMID: 10896648]
- 17 **Perseghin G**, Lattuada G, De Cobelli F, Ragogna F, Ntali G, Esposito A, Belloni E, Canu T, Terruzzi I, Scifo P, Del Maschio A, Luzi L. Habitual physical activity is associated with intrahepatic fat content in humans. *Diabetes Care* 2007; **30**: 683-688 [PMID: 17327341 DOI: 10.2337/dc06-2032]
- 18 **Yasari S**, Prud'homme D, Tesson F, Jankowski M, Gutkowska J, Levy E, Lavoie JM. Effects of exercise training on molecular markers of lipogenesis and lipid partitioning in fructose-induced liver fat accumulation. *J Nutr Metab* 2012; **2012**: 181687 [PMID: 21860785 DOI: 10.1155/2012/181687]
- 19 **Magkos F**, Lavoie JM, Kantartzis K, Gastaldelli A. Diet and exercise in the treatment of Fatty liver. *J Nutr Metab* 2012; **2012**: 257671 [PMID: 21941637 DOI: 10.1155/2012/257671]
- 20 **Hickman IJ**, Byrne NM, Croci I, Chachay VS, Clouston AD, Hills AP, Bugianesi E, Whitehead JP, AmaliaGastaldelli, Sullivan TMOM, Prins JB, Macdonald GA. A Pilot Randomised Study of the Metabolic and Histological Effects of Exercise in Non-alcoholic Steatohepatitis. *J Diabetes Metab* 2013 [DOI: 10.4172/2155-6156.1000300]
- 21 **Byrne N**, Hills A, Meerkin J, Kennedy D. The site specific effectiveness of circuit-weight training on body composition. Proceedings of the 9th Annual Conference of the European College of Sports Science, Clermont-Ferrand, 2004 3-6 July
- 22 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]
- 23 **Brunt EM**. Nonalcoholic steatohepatitis: definition and pathology. *Semin Liver Dis* 2001; **21**: 3-16 [PMID: 11296695]
- 24 **Brunt EM**, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. *Hepatology* 2011; **53**: 810-820 [PMID: 21319198 DOI: 10.1002/hep.24127]
- 25 **Després JP**, Ross R, Boka G, Almérás N, Lemieux I. Effect of rimonabant on the high-triglyceride/ low-HDL-cholesterol dyslipidemia, intraabdominal adiposity, and liver fat: the ADAGIO-Lipids trial. *Arterioscler Thromb Vasc Biol* 2009; **29**: 416-423 [PMID: 19112166 DOI: 10.1161/ATVBAHA.108.176362]
- 26 **DeFronzo RA**, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; **237**: E214-E223 [PMID: 382871]
- 27 **Nosadini R**, Avogaro A, Trevisan R, Duner E, Marescotti C, Iori E, Cobelli C, Toffolo G. Acetoacetate and 3-hydroxybutyrate kinetics in obese and insulin-dependent diabetic humans. *Am J Physiol* 1985; **248**: R611-R620 [PMID: 3922234]
- 28 **Weiss M**, Keller U, Stauffacher W. Effect of epinephrine and somatostatin-induced insulin deficiency on ketone body kinetics and lipolysis in man. *Diabetes* 1984; **33**: 738-744 [PMID: 6146545 DOI: 10.2337/diabetes.33.8.738]
- 29 **Havel RJ**, Kane JP, Balasse EO, Segel N, Basso LV. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J Clin Invest* 1970; **49**: 2017-2035 [PMID: 5475985 DOI: 10.1172/JCI106422]
- 30 **Robinson AM**, Williamson DH. Physiological roles of ketone

- bodies as substrates and signals in mammalian tissues. *Physiol Rev* 1980; **60**: 143-187 [PMID: 6986618]
- 31 **Frayn KN**. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol Respir Environ Exerc Physiol* 1983; **55**: 628-634 [PMID: 6618956]
  - 32 **Achten J**, Gleeson M, Jeukendrup AE. Determination of the exercise intensity that elicits maximal fat oxidation. *Med Sci Sports Exerc* 2002; **34**: 92-97 [PMID: 11782653 DOI: 10.1097/00005768-200201000-00015]
  - 33 **Achten J**, Jeukendrup AE. Maximal fat oxidation during exercise in trained men. *Int J Sports Med* 2003; **24**: 603-608 [PMID: 14598198 DOI: 10.1055/s-2003-43265]
  - 34 **Achten J**, Jeukendrup AE. Relation between plasma lactate concentration and fat oxidation rates over a wide range of exercise intensities. *Int J Sports Med* 2004; **25**: 32-37 [PMID: 14750010 DOI: 10.1055/s-2003-45231]
  - 35 **Brandou F**, Dumortier M, Garandeau P, Mercier J, Brun JF. Effects of a two-month rehabilitation program on substrate utilization during exercise in obese adolescents. *Diabetes Metab* 2003; **29**: 20-27 [PMID: 12629444]
  - 36 **Tolfrey K**, Jeukendrup AE, Batterham AM. Group- and individual-level coincidence of the 'Fatmax' and lactate accumulation in adolescents. *Eur J Appl Physiol* 2010; **109**: 1145-1153 [PMID: 20376480 DOI: 10.1007/s00421-010-1453-3]
  - 37 **Aucouturier J**, Rance M, Meyer M, Isacco L, Thivel D, Fellmann N, Duclos M, Duché P. Determination of the maximal fat oxidation point in obese children and adolescents: validity of methods to assess maximal aerobic power. *Eur J Appl Physiol* 2009; **105**: 325-331 [PMID: 19002708 DOI: 10.1007/s00421-008-0907-3]
  - 38 **Kang J**, Rashti SL, Tranchina CP, Ratamess NA, Faigenbaum AD, Hoffman JR. Effect of preceding resistance exercise on metabolism during subsequent aerobic session. *Eur J Appl Physiol* 2009; **107**: 43-50 [PMID: 19504118 DOI: 10.1007/s00421-009-1100-z]
  - 39 **Crocì I**, Borrani F, Byrne NM, Wood RE, Hickman IJ, Chenevière X, Malatesta D. Reproducibility of Fatmax and fat oxidation rates during exercise in recreationally trained males. *PLoS One* 2014; **9**: e97930 [PMID: 24886715 DOI: 10.1371/journal.pone.0097930]
  - 40 **Ipavec-Levasseur S**, Crocì I, Choquette S, Byrne NM, Cowin G, O'Moore-Sullivan TM, Prins JB, Hickman IJ. Effect of 1-h moderate-intensity aerobic exercise on intramyocellular lipids in obese men before and after a lifestyle intervention. *Appl Physiol Nutr Metab* 2015; **40**: 1262-1268 [PMID: 26575100 DOI: 10.1139/apnm-2015-0258]
  - 41 **Hodson L**, Frayn KN. Hepatic fatty acid partitioning. *Curr Opin Lipidol* 2011; **22**: 216-224 [PMID: 21494141 DOI: 10.1097/MOL.0b013e3283462e16]
  - 42 **Rector RS**, Thyfault JP, Morris RT, Laye MJ, Borengasser SJ, Booth FW, Ibdah JA. Daily exercise increases hepatic fatty acid oxidation and prevents steatosis in Otsuka Long-Evans Tokushima Fatty rats. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G619-G626 [PMID: 18174272 DOI: 10.1152/ajpgi.00428.2007]
  - 43 **Rector RS**, Thyfault JP, Laye MJ, Morris RT, Borengasser SJ, Uptergrove GM, Chakravarthy MV, Booth FW, Ibdah JA. Cessation of daily exercise dramatically alters precursors of hepatic steatosis in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *J Physiol* 2008; **586**: 4241-4249 [PMID: 18617560 DOI: 10.1113/jphysiol.2008.156745]
  - 44 **Holloszy JO**, Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol Respir Environ Exerc Physiol* 1984; **56**: 831-838 [PMID: 6373687]
  - 45 **Holloway GP**, Bezaire V, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, Bonen A, Spriet LL. Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise. *J Physiol* 2006; **571**: 201-210 [PMID: 16357012 DOI: 10.1113/jphysiol.2005.102178]
  - 46 **Schenk S**, Horowitz JF. Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle increases proportionally with fat oxidation after endurance exercise training. *Am J Physiol Endocrinol Metab* 2006; **291**: E254-E260 [PMID: 16670153 DOI: 10.1152/ajpendo.00051.2006]
  - 47 **Molé PA**, Oscai LB, Holloszy JO. Adaptation of muscle to exercise. Increase in levels of palmitoyl CoA synthetase, carnitine palmitoyltransferase, and palmitoyl CoA dehydrogenase, and in the capacity to oxidize fatty acids. *J Clin Invest* 1971; **50**: 2323-2330 [PMID: 5096516 DOI: 10.1172/JCI106730]
  - 48 **Simoneau JA**, Veerkamp JH, Turcotte LP, Kelley DE. Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. *FASEB J* 1999; **13**: 2051-2060 [PMID: 10544188]
  - 49 **Venables MC**, Jeukendrup AE. Endurance training and obesity: effect on substrate metabolism and insulin sensitivity. *Med Sci Sports Exerc* 2008; **40**: 495-502 [PMID: 18379212 DOI: 10.1249/MSS.0b013e31815f256f]
  - 50 **Talanian JL**, Galloway SD, Heigenhauser GJ, Bonen A, Spriet LL. Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. *J Appl Physiol* (1985) 2007; **102**: 1439-1447 [PMID: 17170203 DOI: 10.1152/japplphysiol.01098.2006]
  - 51 **Goto K**, Ishii N, Sugihara S, Yoshioka T, Takamatsu K. Effects of resistance exercise on lipolysis during subsequent submaximal exercise. *Med Sci Sports Exerc* 2007; **39**: 308-315 [PMID: 17277595 DOI: 10.1249/01.mss.0000246992.33482.cb]
  - 52 **Crocì I**, Hickman IJ, Wood RE, Borrani F, Macdonald GA, Byrne NM. Fat oxidation over a range of exercise intensities: fitness versus fatness. *Appl Physiol Nutr Metab* 2014; **39**: 1352-1359 [PMID: 25356842 DOI: 10.1139/apnm-2014-0144]
  - 53 **Goodpaster BH**, Katsiaras A, Kelley DE. Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. *Diabetes* 2003; **52**: 2191-2197 [PMID: 12941756]
  - 54 **Berggren JR**, Boyle KE, Chapman WH, Houmard JA. Skeletal muscle lipid oxidation and obesity: influence of weight loss and exercise. *Am J Physiol Endocrinol Metab* 2008; **294**: E726-E732 [PMID: 18252891 DOI: 10.1152/ajpendo.00354.2007]
  - 55 **Achten J**, Jeukendrup AE. Optimizing fat oxidation through exercise and diet. *Nutrition* 2004; **20**: 716-727 [PMID: 15212756 DOI: 10.1016/j.nut.2004.04.005]
  - 56 **Burke LM**, Hawley JA. Effects of short-term fat adaptation on metabolism and performance of prolonged exercise. *Med Sci Sports Exerc* 2002; **34**: 1492-1498 [PMID: 12218744 DOI: 10.1097/00005768-200209000-00015]
  - 57 **Helge JW**. Adaptation to a fat-rich diet: effects on endurance performance in humans. *Sports Med* 2000; **30**: 347-357 [PMID: 11103848 DOI: 10.2165/00007256-200030050-00003]
  - 58 **Helge JW**. Long-term fat diet adaptation effects on performance, training capacity, and fat utilization. *Med Sci Sports Exerc* 2002; **34**: 1499-1504 [PMID: 12218745 DOI: 10.1097/00005768-200209000-00016]
  - 59 **Sullivan S**, Kirk EP, Mittendorfer B, Patterson BW, Klein S. Randomized trial of exercise effect on intrahepatic triglyceride content and lipid kinetics in nonalcoholic fatty liver disease. *Hepatology* 2012; **55**: 1738-1745 [PMID: 22213436 DOI: 10.1002/hep.25548]
  - 60 **Brunt EM**, Kleiner DE, Wilson LA, Unalp A, Behling CE, Lavine JE, Neuschwander-Tetri BA. Portal chronic inflammation in nonalcoholic fatty liver disease (NAFLD): a histologic marker of advanced NAFLD-Clinicopathologic correlations from the nonalcoholic steatohepatitis clinical research network. *Hepatology* 2009; **49**: 809-820 [PMID: 19142989 DOI: 10.1002/hep.22724]
  - 61 **Iozzo P**, Bucci M, Roivainen A, Nägren K, Järvisalo MJ, Kiss J, Guiducci L, Fielding B, Naum AG, Borra R, Virtanen K, Savunen T, Salvadori PA, Ferrannini E, Knuuti J, Nuutila P. Fatty acid metabolism in the liver, measured by positron emission tomography, is increased in obese individuals. *Gastroenterology* 2010; **139**: 846-856, 856.e1-6 [PMID: 20685204 DOI: 10.1053/j.gastro.2010.05.039]
  - 62 **Hansen D**, Dendale P, van Loon LJ, Meeusen R. The impact of training modalities on the clinical benefits of exercise intervention in patients with cardiovascular disease risk or type 2 diabetes mellitus. *Sports Med* 2010; **40**: 921-940 [PMID: 20942509 DOI: 10.1007/s00005-010-0001-0]

- 10.2165/11535930-000000000-00000]
- 63 **Ordóñez R**, Carbajo-Pescador S, Mauriz JL, Gonzalez-Gallego J. Understanding nutritional interventions and physical exercise in non-alcoholic fatty liver disease. *Curr Mol Med* 2015; **15**: 3-26 [PMID: 25601465 DOI: 10.2174/1566524015666150114110551]
  - 64 **Johnson NA**, George J. Fitness versus fatness: moving beyond weight loss in nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 370-381 [PMID: 20578153 DOI: 10.1002/hep.23711]
  - 65 **Johnson NA**, Keating SE, George J. Exercise and the liver: implications for therapy in fatty liver disorders. *Semin Liver Dis* 2012; **32**: 65-79 [PMID: 22418889 DOI: 10.1055/s-0032-1306427]
  - 66 **Johnson NA**, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW, George J. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology* 2009; **50**: 1105-1112 [PMID: 19637289 DOI: 10.1002/hep.23129]
  - 67 **Johnson NA**, van Overbeek D, Chapman PG, Thompson MW, Sachinwalla T, George J. Effect of prolonged exercise and pre-exercise dietary manipulation on hepatic triglycerides in trained men. *Eur J Appl Physiol* 2012; **112**: 1817-1825 [PMID: 21915700 DOI: 10.1007/s00421-011-2158-y]
  - 68 **Keating SE**, Hackett DA, George J, Johnson NA. Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 2012; **57**: 157-166 [PMID: 22414768 DOI: 10.1016/j.jhep.2012.02.023]
  - 69 **Keating SE**, Hackett DA, Parker HM, O'Connor HT, Gerofi JA, Sainsbury A, Baker MK, Chuter VH, Caterson ID, George J, Johnson NA. Effect of aerobic exercise training dose on liver fat and visceral adiposity. *J Hepatol* 2015; **63**: 174-182 [PMID: 25863524 DOI: 10.1016/j.jhep.2015.02.022]

**P- Reviewer:** Abenavoli L, Amodio P, Gonzalez-Reimers E, Li GL, Montalto G, Schlegel A

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D





Prospective Study

## Ohio solid organ transplantation consortium criteria for liver transplantation in patients with alcoholic liver disease

Kaveh Hajifathalian, Annette Humberson, Mohamad A Hanouneh, David S Barnes, Zubin Arora, Nizar N Zein, Bijan Egtesad, Dympna Kelly, Ibrahim A Hanouneh

Kaveh Hajifathalian, Mohamad A Hanouneh, Department of Internal Medicine, Cleveland Clinic Foundation, Cleveland, OH 44195, United States

Annette Humberson, Department of Transplant Social Work, Cleveland Clinic Foundation, Cleveland, OH 44195, United States

David S Barnes, Zubin Arora, Nizar N Zein, Ibrahim A Hanouneh, Department of Gastroenterology and Hepatology, Cleveland Clinic Foundation, Cleveland, OH 44195, United States

Bijan Egtesad, Dympna Kelly, Department of General Surgery, Cleveland Clinic Foundation, Cleveland, OH 44195, United States

**Author contributions:** Hajifathalian K analyzed the data; Zein NN, Egtesad B and Hanouneh IA designed the research; Hajifathalian K, Humberson A, Arora Z, Barnes DS and Kelly D performed the research; Hajifathalian K, Hanouneh IA, Humberson A and Barnes DS wrote the paper.

**Institutional review board statement:** This study was reviewed and approved by the Cleveland Clinic Foundation Institutional Review Board.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** The authors claim no conflict of interest to be declared.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Ibrahim A Hanouneh, MD, Department of Gastroenterology and Hepatology, Cleveland Clinic Foundation, 9500 Euclid Avenue, A30, Cleveland, OH 44195, United States. [ibrahim.hanouneh@mngastro.com](mailto:ibrahim.hanouneh@mngastro.com)  
Telephone: +1-216-4441762  
Fax: +1-216-4446302

Received: April 13, 2016

Peer-review started: April 15, 2016

First decision: May 19, 2016

Revised: June 19, 2016

Accepted: July 29, 2016

Article in press: August 1, 2016

Published online: September 28, 2016

### Abstract

#### AIM

To evaluate risk of recidivism on a case-by-case basis.

#### METHODS

From our center's liver transplant program, we selected patients with alcoholic liver disease who were listed for transplant based on Ohio Solid Organ Transplantation Consortium (OSOTC) exception criteria. They were considered to have either a low or medium risk of recidivism, and had at least one or three or more months of abstinence, respectively. They were matched based on gender, age, and Model for End-Stage Liver Disease (MELD) score to controls with alcohol-induced cirrhosis from Organ Procurement and Transplant Network data.

#### RESULTS

Thirty six patients with alcoholic liver disease were approved for listing based on OSOTC exception criteria and were matched to 72 controls. Nineteen patients

(53%) with a median [Inter-quartile range (IQR)] MELD score of 24 (13) received transplant and were followed for a median of 3.4 years. They were matched to 38 controls with a median (IQR) MELD score of 25 (9). At one and five years, cumulative survival rates ( $\pm$  standard error) were  $90\% \pm 7\%$  and  $92\% \pm 5\%$  and  $73\% \pm 12\%$  and  $77\% \pm 8\%$  in patients and controls, respectively (Log-rank test,  $P = 0.837$ ). Four (21%) patients resumed drinking by last follow-up visit.

## CONCLUSION

Compared to traditional criteria for assessment of risk of recidivism, a careful selection process with more flexibility to evaluate eligibility on a case-by-case basis can lead to similar survival rates after transplantation.

**Key words:** Alcohol-induced disorders; Alcoholic liver cirrhosis; Mortality; Survival; Liver transplantation

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** For the first time, we report the rates of liver transplant and survival for patients with alcohol-induced cirrhosis who were deemed eligible for liver transplant and listed based on approval under the Ohio Solid Organ Transplantation Consortium medically urgent exception criteria. These criteria allow patients with low to medium risk of recidivism, to receive a liver transplant after only one to three months of abstinence. We showed that transplant rate and short and long term survival after transplant is comparable between these patients and United States general population of patients with alcohol-induced cirrhosis who received liver transplant.

Hajifathalian K, Humberson A, Hanouneh MA, Barnes DS, Arora Z, Zein NN, Eghtesad B, Kelly D, Hanouneh IA. Ohio solid organ transplantation consortium criteria for liver transplantation in patients with alcoholic liver disease. *World J Hepatol* 2016; 8(27): 1149-1154 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i27/1149.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i27.1149>

## INTRODUCTION

Cirrhosis due to alcoholic liver disease is an important cause of morbidity and mortality both globally and in the United States. Globally, in 2010 cirrhosis due to alcoholic liver disease led to more than 493000 deaths<sup>[1]</sup>. In United States in 2011 liver cirrhosis was responsible for 34860 deaths, 48% of which were related to alcohol consumption<sup>[2]</sup>. Among patients with cirrhosis due to alcoholic liver disease mortality rates vary based on presence or absence of complications of cirrhosis but it is generally high with a one-year mortality ranging from 17% to 64% and five-year mortalities ranging from 58% to 85%<sup>[3]</sup>.

Liver transplantation imparts great survival benefit

to appropriately selected patients with advanced and de-compensated cirrhosis due to alcohol consumption, which is comparable to survival benefit of transplant in other types of chronic liver disease<sup>[4,5]</sup>. The definition of "appropriately selected patients" in this context remains controversial<sup>[5]</sup>, with the most important factor being minimum duration of abstinence. Conventionally, most liver transplant programs in United States require patients to be abstinent for at least 6 mo and participate in an alcohol rehabilitation program to be considered for transplant<sup>[6,7]</sup>; while it is known that delayed referral for transplant and longer waiting times, even for a few months, will significantly decrease the probability of patient's survival in the pre-transplant period<sup>[8]</sup>. There are data suggesting that careful evaluation of patients for transplant on an individual basis instead of using general and inflexible enrollment rules might lead to favorable outcomes in highly selected patients with alcoholic liver disease<sup>[9,10]</sup>. The state of Ohio Solid Organ Transplantation Consortium (OSOTC) provides such a mechanism for case-by-case evaluation based on clinical guidelines for medically urgent patients with cirrhosis due to alcoholic liver disease. Based on factors such as estimated risk of recidivism, severity of their alcohol use history and previous attempts to remain sober, social support, insight into alcohol use, and willingness of the patient to comply with OSOTC regulations, these patients can be approved as an exception and listed for transplant after only one to three months of abstinence.

The aim of this study was to determine the effect of using OSOTC transplant eligibility criteria on patients' survival compared with conventional criteria for assessment of risk of recidivism, in patient with cirrhosis due to alcoholic liver disease.

## MATERIALS AND METHODS

### Study population and data collection

The study protocol was approved by the Cleveland Clinic Institutional Review Board. Since 2009 we selected patients with alcoholic cirrhosis for consideration of liver transplantation based on OSOTC exception criteria. No donor organs were obtained from executed prisoners or other institutionalized persons. As defined below these are medically urgent patients with low to medium risk of recidivism, who were approved for a medically urgent exception to be transplanted either during the time they were completing alcohol treatment, or some completed treatment after their transplant.

Transplant rates were compared between these patients and matched patients with alcoholic cirrhosis from Organ Procurement and Transplant Network (OPTN) data records who had complete data to calculate their Model for End-Stage Liver Disease (MELD) score<sup>[11,12]</sup> at the time of listing. To compare survival after transplant we used the OPTN patients with alcoholic cirrhosis who had complete data to calculate their MELD score at the time of transplant as well as follow-up data on survival after transplant. Patients from OPTN dataset

**Table 1 Ohio Solid Organ Transplantation Consortium medically urgent except criteria**

Ohio Solid Organ Transplantation Consortium Criteria	
Low-risk	1 mo confirmed abstinence, a signed contract and commitment to begin a rehabilitation program and finish it either before or after transplant No previous failure with substance rehabilitation; never been told that substance was affecting health; and good social support
Medium-risk	Three month confirmed abstinence, a signed contract and commitment to begin a rehabilitation program and/or finish it either before or after transplant One or more failures with rehabilitation; and minimal support system
High-risk	Two or more failures to remain abstinent despite medical complication Refusal to sign contract Minimal to poor social support Must complete standard criteria treatment plan, not eligible for an exception
Other barriers	No insight into their alcohol use consequences No recognition that alcohol caused their liver failure Refusal to start treatment No sober support network

were matched to our patients randomly and according to the following predetermined variables: 10-year age category, gender, and MELD score category same as the case patient's category (< 10, 10-19, 20-29, 30-39,  $\geq 40$ )<sup>[13]</sup>. MELD score was calculated as  $(9.57 \times \log \text{creatinine mg/dL}) + (3.78 \times \log \text{bilirubin mg/dL}) + (11.20 \times \log \text{international normalized ratio}) + 6.43$ . All laboratory values which were less than 1 were set to 1 and serum creatinine for patients with values of more than 4 or on dialysis was set to 4 in order to calculate MELD score. The MELD score was truncated at 40 for patients with a MELD score of more than 40 (<http://optn.transplant.hrsa.gov/resources/MeldPeldCalculator.asp?index=98>).

### OSOTC chemical disorder criteria

OSOTC follows standard criteria for patients in need of liver transplant who are diagnosed with substance use disorder at the time of evaluation (Table 1). This includes patients with alcohol-induced cirrhosis who are diagnosed with alcohol use disorder. The standard criteria includes demonstrating abstinence for at least 12 mo before listing, or at least three months of abstinence plus three months of current participation in an active recovery program and negative random toxicology screens prior to listing confirmed by collateral information (<http://www.osotc.org/resources/chemical-dependency-criteria/>). In addition, patients must show insight into substance use and understanding of the effects of substance use on their health.

OSOTC also provides exception criteria for medically urgent patients who have not been abstinent for 12 mo and are too ill to complete the recovery program participation conditions in the standard criteria. These exception criteria apply to patients with MELD score

of more than 22 (calculated or eligible for exception). According to OSOTC exception criteria, and after signing a contract and showing commitment to rehabilitation, patients at low risk of recidivism - defined as no previous failure with substance rehabilitation, never having been told that substance was affecting health, and good social support - can be listed for transplant after one month of abstinence. Patients at medium risk of recidivism - defined as one or more failures with rehabilitation, and minimal support system - can be listed after a minimum of three months of abstinence, and after signing a contract and showing commitment to rehabilitation. All patients' records are reviewed by OSOTC chemical disorder committee representatives and discussed in a committee conference call, in addition to our liver transplant patient selection committee, in order to decide approval or not of these exception criteria. Patients at high risk for recidivism - defined as two or more failures to remain abstinent despite medical complications, refusal to sign a contract, and minimal or poor social support - do not qualify for OSOTC exception criteria.

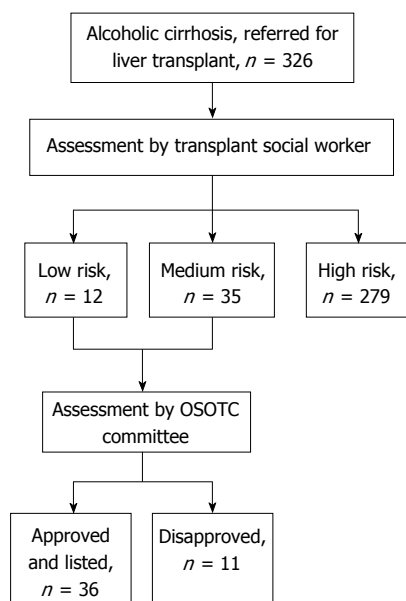
### Statistical analysis

All analysis was done with Stata Data Analysis and Statistical Software (version 11.2 SE, StataCrop LP). Variables are reported as number (percentage) or median (IQR). Survival probabilities are reported as percentage  $\pm$  SE. Categorical variables are compared between patients and controls with  $\chi^2$  test. Waiting time was defined as the period from the day an individual is listed for liver transplant to the day the transplant is done. Waiting time was compared between cases and controls with a Cox proportional hazards model containing patient group as the only independent variable to predict waiting time (*i.e.*, time to liver transplant). Follow-up time after transplant was defined as the period from the day an individual receives a liver transplant until death or the last follow-up visit. Data for patients who remained alive by the end of the follow-up period was censored at the time of last follow-up visit. Survival probabilities were estimated with Kaplan-Meier method and were compared between groups with Log-Rank test. All *P*-values are two-sided.

## RESULTS

### Patients' selection

Between 2009 and 2013, 326 patients with alcoholic liver disease were evaluated for liver transplant at the Cleveland Clinic, of whom 279 (85%) patients were considered high-risk for recidivism or alcohol relapse based on the OSOTC criteria (Figure 1). These high-risk patients underwent the standard chemical dependency requirements defined above before being considered eligible for liver transplant. Forty-seven (15%) patients were considered by our social workers and liver transplant committee at the Cleveland Clinic to be at medium or low-risk for recidivism or alcohol relapse based on the OSOTC criteria, but only 36 (13%) patients were approved by the consortium.



**Figure 1** Selection of patients with alcoholic liver disease for liver transplantation based on Ohio Solid Organ Transplantation Consortium Criteria. OSOTC: Ohio Solid Organ Transplantation Consortium.

### Baseline characteristics

Thirty-six patients with alcoholic cirrhosis were approved for liver transplant at the Cleveland Clinic based on OSOTC exception criteria. They were matched based on age, gender, and MELD score category to a random sample of 72 controls from OPTN database with alcohol-induced cirrhosis that underwent liver transplant following conventionally used criteria of alcohol rehabilitation. Table 2 represents the baseline characteristics of patients and control groups. Sixty four percent of patients and controls were male. At the time of listing five patients had a MELD score of 10-19 (14%), 18 had a MELD score of 20-29 (50%), 12 had a MELD score of 30-39 (33%), and one patient (3%) had a MELD score of 40 or more. These were individually matched to controls with the same MELD score category, leading to a median (IQR) MELD score of 27 (11) among patients and 24 (11) among controls (Table 1). At the time of liver transplant one patient had a MELD score of less than 10 (5%), two had MELD scores of 10-19 (11%), 11 had MELD scores of 20-29 (58%), four had MELD scores of 30-39 (21%), and one patient had a MELD score of 40 or more (5%). Again, these patients were individually matched to controls with the same MELD score category leading to a median (IQR) MELD score of 24 (13) among patients and 25 (9) among controls.

### Liver transplantation

Nineteen out of 36 (53%) patients received a liver transplant and 17 dropped off the transplant list. The most common cause of drop-off transplant list was infection and the vast majority of dropped off patients died ( $n = 15$ , 88%). The transplant drop-off rate was not different for controls of whom 41 (57%) received a transplant ( $P$ -value = 0.681). Patients in the OSOTC group received their liver after a median waiting time of 19 d after listing, and

**Table 2** Characteristics of study patients and controls

	Patients	Controls
At listing		
<i>n</i>	36	72
Age, median (IQR)	58 (14)	60 (11)
Male, <i>n</i> (%)	23 (64)	46 (64)
INR, median (IQR)	1.8 (0.6)	1.9 (0.7)
Total Bilirubin, median (IQR)	8.9 (19.3)	5 (7.7)
Creatinine, median (IQR)	2.1 (2.4)	1.5 (2)
Albumin, median (IQR)	3.2 (0.9)	2.9 (1)
MELD, median (IQR)	27 (11)	24 (11)
At transplant		
<i>n</i>	19	38
Age, median (IQR)	56 (17)	55 (13)
Male, <i>n</i> (%)	13 (68)	26 (68)
INR, median (IQR)	1.6 (0.5)	1.9 (0.8)
Total Bilirubin, median (IQR)	8.3 (12.1)	6 (5.7)
Creatinine, median (IQR)	2.6 (2.9)	1.3 (1.3)
Albumin, median (IQR)	2.9 (0.8)	2.8 (1)
MELD, median (IQR)	24 (13)	25 (9)

IQR: Inter-quartile range; INR: International normalized ratio; MELD: Model for end stage liver disease.

controls received their transplant after a median 21 d of waiting time ( $P$ -value = 0.648). Although the majority of both patients and controls received their transplant in less than 2 mo (Table 3), 10% of controls had to wait more than five months while all patients received their transplants before the five months mark.

### Survival outcome and recidivism

Both patients and controls had a median follow-up of more than three years after transplant (Table 3). One year after transplant 90%  $\pm$  7% of patients were alive compared with 92%  $\pm$  5% of controls. At five years, 73%  $\pm$  12% of patients was still alive compared with 77%  $\pm$  8% of controls (Figure 2). Survival rates after transplant did not differ significantly between patients and controls (log rank test,  $P$ -value = 0.837).

Among 19 patients who received their transplant based on OSOTC medically urgent exception criteria, four patients had resumed drinking by last follow-up visit for a 21% relapse rate after a median follow up of 3.4 years.

## DISCUSSION

For the first time, we report the rates of liver transplant and survival for patients with alcohol-induced cirrhosis who were deemed eligible for liver transplant and listed based on approval under the OSOTC medically urgent except criteria. These criteria allow patients with low to medium risk of recidivism, to receive a liver transplant after only one to three months of abstinence. These patients all committed to begin an alcohol treatment program before or during listing and to finish the program, even if it was after their transplant. We showed that transplant rate and short and long term survival after transplant is comparable between these patients and United States general population of patients with alcohol-induced cirrhosis who received their transplant after being eva-



**Table 3** Follow-up details of listed and transplanted patients and controls

	Patients	Controls	P-value
After listing			
No. listed	36	72	
No. transplanted (%)	19 (53)	41 (57)	0.687 <sup>1</sup>
Waiting time for transplant, d, median (IQR)	19 (7-65)	21 (5-54)	0.648 <sup>2</sup>
After transplant			
No. transplanted	19	38	
Follow-up after transplant, months, median (IQR)	41 (29-58)	37 (14-61)	
1-yr survival, % ± SE	90 ± 7	92 ± 5	0.837 <sup>3</sup>
5-yr survival, % ± SE	73 ± 12	77 ± 8	

<sup>1</sup> $\chi^2$  test; <sup>2</sup>P-value for patient *vs* control time to transplant Cox proportional hazards model; <sup>3</sup>Log-Rank test. IQR: Inter-quartile range; SE: Standard error.

luated for risk of recidivism based on conventionally used criteria. The risk of recidivism in our patients was comparable to previously published rates ranging from 15% to more than 20%<sup>[14-17]</sup>.

Our findings challenge the notion of a defined abstinence period as the only criterion for liver transplant eligibility in patient with alcoholic liver cirrhosis<sup>[18]</sup>. However, the stringency of OSOTC process resulted in our selecting a very small number of patients with alcoholic cirrhosis for liver transplantation. Numerous studies have observed that the enforcement of sobriety period delays listing for transplantation in a significant number of patients with a low probability of alcohol relapse following liver transplant<sup>[17,19-23]</sup>. Indeed, the duration of alcohol abstinence before liver transplant is a poor indicator of relapse of alcoholism following transplantation<sup>[24]</sup>.

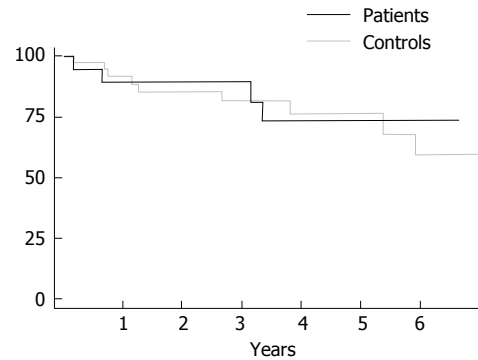
Although our results are encouraging, the study has several limitations. The number of patients included in the study was small. Matched controls may not have been comparable to OSOTC patients in terms of family support, intention to remain abstinent from alcohol, or availability of counseling services at transplant center in the event of alcohol relapse. Future studies will benefit from a control group of patients with alcoholic liver disease undergoing liver transplantation that are matched to OSOTC patients on the basis of social and familial support.

In summary, liver transplantation may be an appropriate rescue option for selected patients with alcoholic liver disease after only one to three months of abstinence. Our results show that OSOTC transplant eligibility criteria provide a valid method to identify these patients who may benefit from liver transplantation with low to medium risk of recidivism.

## COMMENTS

### Background

A minimum of 12 mo of abstinence, or three months of abstinence and participation in an alcohol rehabilitation program, are the standard requirements



No. at risk							
Patients	19	17	16	11	7	4	2
Controls	38	30	25	19	15	9	7

**Figure 2** Kaplan–meier estimates of survival after liver transplant in the 19 study patients and the 38 matched controls.

before patients with alcoholic liver disease are eligible for transplantation in Ohio. Some patients are too ill to participate in a rehab program. The Ohio Solid Organ Transplantation Consortium (OSOTC) has a mechanism to evaluate risk of recidivism on a case-by-case basis.

### Research frontiers

Liver transplantation imparts great survival benefit to appropriately selected patients with advanced and de-compensated cirrhosis due to alcohol consumption, which is comparable to survival benefit of transplant in other types of chronic liver disease.

### Innovations and breakthroughs

The aim of this study was to determine the effect of using OSOTC transplant eligibility criteria on patients' survival compared with conventional criteria for assessment of risk of recidivism, in patient with cirrhosis due to alcoholic liver disease.

### Applications

Patients can be approved as an exception and listed for transplant after only one to three months of abstinence.

### Terminology

They were matched based on gender, age, and MELD score to controls with alcohol-induced cirrhosis from Organ Procurement and Transplant Network data.

### Peer-review

The manuscript "Ohio solid organ transplantation consortium criteria for liver transplantation in patients with alcoholic liver disease" by Hajifathalian *et al* is an interesting paper and the important contribution from OSOTC group supporting an update of the Criteria for Liver Transplantation based on alcohol abstinence duration potentially help to obtain the transplant for larger group of patients with a low probability of alcohol relapse.

## REFERENCES

- 1 **Rehm J**, Samokhvalov AV, Shield KD. Global burden of alcoholic liver diseases. *J Hepatol* 2013; **59**: 160-168 [PMID: 23511777 DOI: 10.1016/j.jhep.2013.03.007]
- 2 **Yoon YH**, Chen CM, Yi HY. Surveillance Report #100: Liver Cirrhosis Mortality in the United States: National, State, and Regional Trends, 2000–2011. Available from: URL: <http://pubs.niaaa.nih.gov/publications/Surveillance100/Cirr11.htm>
- 3 **Jepsen P**, Ott P, Andersen PK, Sørensen HT, Vilstrup H. Clinical course of alcoholic liver cirrhosis: a Danish population-based cohort study. *Hepatology* 2010; **51**: 1675-1682 [PMID: 20186844]

- DOI: 10.1002/hep.23500]
- 4 **Bellamy CO**, DiMartini AM, Ruppert K, Jain A, Dodson F, Torbenson M, Starzl TE, Fung JJ, Demetris AJ. Liver transplantation for alcoholic cirrhosis: long term follow-up and impact of disease recurrence. *Transplantation* 2001; **72**: 619-626 [PMID: 11544420]
  - 5 **Webb K**, Shepherd L, Day E, Masterton G, Neuberger J. Transplantation for alcoholic liver disease: report of a consensus meeting. *Liver Transpl* 2006; **12**: 301-305 [PMID: 16447187 DOI: 10.1002/lt.20681]
  - 6 **Everhart JE**, Beresford TP. Liver transplantation for alcoholic liver disease: a survey of transplantation programs in the United States. *Liver Transpl Surg* 1997; **3**: 220-226 [PMID: 9346743]
  - 7 **Watt KD**, McCashland TM. Transplantation in the alcoholic patient. *Semin Liver Dis* 2004; **24**: 249-255 [PMID: 15349803 DOI: 10.1055/s-2004-832938]
  - 8 **Everhart JE**, Lombardero M, Detre KM, Zetterman RK, Wiesner RH, Lake JR, Hoofnagle JH. Increased waiting time for liver transplantation results in higher mortality. *Transplantation* 1997; **64**: 1300-1306 [PMID: 9371672]
  - 9 **Singal AK**, Bashar H, Anand BS, Jampana SC, Singal V, Kuo YF. Outcomes after liver transplantation for alcoholic hepatitis are similar to alcoholic cirrhosis: exploratory analysis from the UNOS database. *Hepatology* 2012; **55**: 1398-1405 [PMID: 22213344 DOI: 10.1002/hep.25544]
  - 10 **Mathurin P**, Moreno C, Samuel D, Dumortier J, Salleron J, Durand F, Castel H, Duhamel A, Pageaux GP, Leroy V, Dharancy S, Louvet A, Boleslawski E, Lucidi V, Gustot T, Francoz C, Letoublon C, Castaing D, Belghiti J, Donckier V, Pruvot FR, Duclos-Vallée JC. Early liver transplantation for severe alcoholic hepatitis. *N Engl J Med* 2011; **365**: 1790-1800 [PMID: 22070476 DOI: 10.1056/NEJMoa1105703]
  - 11 **Malinchoc M**, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871 [PMID: 10733541 DOI: 10.1053/he.2000.5852]
  - 12 **Wiesner RH**, McDiarmid SV, Kamath PS, Edwards EB, Malinchoc M, Kremers WK, Krom RA, Kim WR. MELD and PELD: application of survival models to liver allocation. *Liver Transpl* 2001; **7**: 567-580 [PMID: 11460223 DOI: 10.1053/jlts.2001.25879]
  - 13 **Wiesner R**, Edwards E, Freeman R, Harper A, Kim R, Kamath P, Kremers W, Lake J, Howard T, Merion RM, Wolfe RA, Krom R. Model for end-stage liver disease (MELD) and allocation of donor livers. *Gastroenterology* 2003; **124**: 91-96 [PMID: 12512033 DOI: 10.1053/gast.2003.50016]
  - 14 **Dumortier J**, Dharancy S, Cannesson A, Lassailly G, Rolland B, Pruvot FR, Boillot O, Faure S, Guillaud O, Rigole-Donnadieu H, Herrero A, Scoazec JY, Mathurin P, Pageaux GP. Recurrent alcoholic cirrhosis in severe alcoholic relapse after liver transplantation: a frequent and serious complication. *Am J Gastroenterol* 2015; **110**: 1160-1166; quiz 1167 [PMID: 26169514 DOI: 10.1038/ajg.2015.204]
  - 15 **Jauhar S**, Talwalkar JA, Schneekloth T, Jowsey S, Wiesner RH, Menon KV. Analysis of factors that predict alcohol relapse following liver transplantation. *Liver Transpl* 2004; **10**: 408-411 [PMID: 15004769 DOI: 10.1002/lt.20086]
  - 16 **Foster PF**, Fabrega F, Karademir S, Sankary HN, Mital D, Williams JW. Prediction of abstinence from ethanol in alcoholic recipients following liver transplantation. *Hepatology* 1997; **25**: 1469-1477 [PMID: 9185770 DOI: 10.1002/hep.510250627]
  - 17 **Miguet M**, Monnet E, Vanlemmens C, Gache P, Messner M, Hruskovsky S, Perarnau JM, Pageaux GP, Duvoux C, Minello A, Hillon P, Bresson-Hadni S, Manton G, Miguet JP. Predictive factors of alcohol relapse after orthotopic liver transplantation for alcoholic liver disease. *Gastroenterol Clin Biol* 2004; **28**: 845-851 [PMID: 15523219]
  - 18 **Lucey MR**, Brown KA, Everson GT, Fung JJ, Gish R, Keeffe EB, Kneteman NM, Lake JR, Martin P, McDiarmid SV, Rakela J, Shiffman ML, So SK, Wiesner RH. Minimal criteria for placement of adults on the liver transplant waiting list: a report of a national conference organized by the American Society of Transplant Physicians and the American Association for the Study of Liver Diseases. *Liver Transpl Surg* 1997; **3**: 628-637 [PMID: 9404965]
  - 19 **Bird GL**, O'Grady JG, Harvey FA, Calne RY, Williams R. Liver transplantation in patients with alcoholic cirrhosis: selection criteria and rates of survival and relapse. *BMJ* 1990; **301**: 15-17 [PMID: 2383700]
  - 20 **Bravata DM**, Olkin I, Barnato AE, Keeffe EB, Owens DK. Employment and alcohol use after liver transplantation for alcoholic and nonalcoholic liver disease: a systematic review. *Liver Transpl* 2001; **7**: 191-203 [PMID: 11244159 DOI: 10.1053/jlts.2001.22326]
  - 21 **Kumar S**, Stauber RE, Gavalier JS, Basista MH, Dindzans VJ, Schade RR, Rabinovitz M, Tarter RE, Gordon R, Starzl TE. Orthotopic liver transplantation for alcoholic liver disease. *Hepatology* 1990; **11**: 159-164 [PMID: 2307394]
  - 22 **Osorio RW**, Ascher NL, Avery M, Bacchetti P, Roberts JP, Lake JR. Predicting recidivism after orthotopic liver transplantation for alcoholic liver disease. *Hepatology* 1994; **20**: 105-110 [PMID: 8020879]
  - 23 **Yates WR**, Martin M, LaBrecque D, Hillebrand D, Voigt M, Pfab D. A model to examine the validity of the 6-month abstinence criterion for liver transplantation. *Alcohol Clin Exp Res* 1998; **22**: 513-517 [PMID: 9581661]
  - 24 **DiMartini A**, Day N, Dew MA, Javed L, Fitzgerald MG, Jain A, Fung JJ, Fontes P. Alcohol consumption patterns and predictors of use following liver transplantation for alcoholic liver disease. *Liver Transpl* 2006; **12**: 813-820 [PMID: 16528710 DOI: 10.1002/lt.20688]

**P- Reviewer:** Bubnov RV, Gatselis NK, Jin B, Penkova-Radicheva MP, Sirin G **S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Li D



## Is MELD score failing patients with liver disease and hepatorenal syndrome?

Lena Sibulesky, Nicolae Leca, Christopher Blosser, Amir A Rahnama-Azar, Renuka Bhattacharya, Jorge Reyes

Lena Sibulesky, Amir A Rahnama-Azar, Jorge Reyes, Department of Surgery, Division of Transplant Surgery, University of Washington, Seattle, WA 98195, United States

Nicolae Leca, Christopher Blosser, Department of Medicine, Division of Nephrology, University of Washington, Seattle, WA 98195, United States

Renuka Bhattacharya, Department of Medicine, Division of Gastroenterology, University of Washington, Seattle, WA 98195, United States

**Author contributions:** Sibulesky L wrote the paper and designed and conducted research; Leca N, Blosser C, Rahnama-Azar AA, Bhattacharya R and Reyes J designed research and reviewed the manuscript.

**Conflict-of-interest statement:** The authors declare no conflicts of interest. No funding was received for this research.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Lena Sibulesky, MD, Assistant Professor, Department of Surgery, Division of Transplant Surgery, University of Washington, 1959 NE Pacific Street, Box 356410, Seattle, WA 98195, United States. [lenasi@uw.edu](mailto:lenasi@uw.edu)  
Telephone: +1-206-5987797  
Fax: +1-206-5984287

Received: May 26, 2016  
Peer-review started: May 26, 2016  
First decision: July 6, 2016  
Revised: July 22, 2016  
Accepted: August 6, 2016  
Article in press: August 8, 2016  
Published online: September 28, 2016

### Abstract

There is a need to reassess the application of MELD and the impact of renal insufficiency with consideration for developing an algorithm with exception points that would lead to timely allocation of livers to patients with hepatorenal syndrome prior to occurrence of permanent renal damage without jeopardizing post-transplant survival.

**Key words:** MELD; Hepatorenal syndrome; Cirrhosis; Graft survival; Liver allocation

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The decompensation of patients with cirrhosis is associated with the development of hepatorenal syndrome (HRS) and renal insufficiency. There are several consequences of a high serum creatinine level in cirrhotic patients, including increased post-liver transplant mortality and increased risk of non-reversal of renal insufficiency/renal failure. We propose a change to the MELD scoring that would lead to timely liver transplantation in patients with HRS.

Sibulesky L, Leca N, Blosser C, Rahnama-Azar AA, Bhattacharya R, Reyes J. Is MELD score failing patients with liver disease and hepatorenal syndrome? *World J Hepatol* 2016; 8(27): 1155-1156  
Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i27/1155.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i27.1155>

### TO THE EDITOR

The decompensation of patients with cirrhosis is associated with the development of complications. This physiology can lead to renal hypoperfusion which contributes to the development of hepatorenal syndrome (HRS) and renal insufficiency<sup>[1,2]</sup>. It is rare to develop HRS with well-compensated liver disease.

There are several consequences of a high serum creatinine level in cirrhotic patients.

Serum creatinine is one of the most important independent predictors of waitlist and post-liver transplant (LT) mortality. While having the same MELD score, patients with higher serum creatinine level have a significantly higher mortality rate<sup>[3]</sup>. Analysis of the Scientific Registry of Transplant Recipients database linked with Centers for Medicare and Medicaid Services' end-stage renal disease (ESRD) data by Sharma *et al*<sup>[4]</sup> demonstrated that post-LT ESRD is associated with higher post-LT mortality (HR = 3.32;  $P < 0.0001$ ).

Serum creatinine prior to liver transplantation is one of the most significant predictors of post-liver transplantation ESRD<sup>[5]</sup>. Wong *et al*<sup>[6]</sup> recently demonstrated that the only predictor of type 1 HRS non-reversal was the duration of pre-transplant dialysis with a 6% increased risk of non-reversal with each additional day of dialysis. Prolonged ischemic physiology may lead to structural renal damage and thus, prevent renal recovery. This has led many to consider combined liver-kidney transplantation (CLKT) for patients whose HRS has lasted longer than 6 wk because the outcomes for patients who receive CLKT seem to be better than those of patients who receive a liver transplant alone<sup>[7,8]</sup>. Since the introduction of MELD score, the number of patients treated with CLKT has increased markedly<sup>[9]</sup>. Almost 1000 kidneys a year are used in a combined transplantation, thus, diminishing the donor pool for patients on the kidney list.

It has also been shown that patients with renal insufficiency have longer hospital and intensive care unit stays and an increased need for dialysis, which likely increases the cost of transplantation. It likely adds to already increased healthcare costs through additional dialysis cases, and increased hospitalization rates secondary to morbidities associated with ESRD<sup>[10]</sup>.

While MELD score is the gold standard for predicting wait list mortality, a notable weakness for liver allocation lies in predicting post transplantation survival, particularly with renal insufficiency<sup>[11,12]</sup>. In addition to MELD, various scoring systems, including Child Pugh score, the risk, injury, failure, loss, end-stage kidney disease criteria, sequential organ failure assessment (SOFA) score, and the Chronic Liver Failure-SOFA score have been designed to predict outcomes in post liver transplant patients<sup>[13]</sup>. Without a timely liver transplant for patients with acute kidney injury, the patient mortality is shifting from the waitlist to the post-transplant period<sup>[14]</sup>. It is time for a conversation within the transplant community to reassess the application of MELD and the impact of renal insufficiency with consideration for developing an algorithm with exception points that would lead to timely allocation of livers to patients with HRS prior to occurrence of permanent renal damage without jeopardizing post-

transplant survival.

## REFERENCES

- 1 **Garcia-Tsao G**, Parikh CR, Viola A. Acute kidney injury in cirrhosis. *Hepatology* 2008; **48**: 2064-2077 [PMID: 19003880 DOI: 10.1002/hep.22605]
- 2 **Angeli P**, Ginès P, Wong F, Bernardi M, Boyer TD, Gerbes A, Moreau R, Jalan R, Sarin SK, Piano S, Moore K, Lee SS, Durand F, Salerno F, Caraceni P, Kim WR, Arroyo V, Garcia-Tsao G. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. *J Hepatol* 2015; **62**: 968-974 [PMID: 25638527 DOI: 10.1016/j.jhep.2014.12.029]
- 3 **Sharma P**, Schaubel DE, Guidinger MK, Merion RM. Effect of pretransplant serum creatinine on the survival benefit of liver transplantation. *Liver Transpl* 2009; **15**: 1808-1813 [PMID: 19938142 DOI: 10.1002/lt.21951]
- 4 **Sharma P**, Schaubel DE, Guidinger MK, Goodrich NP, Ojo AO, Merion RM. Impact of MELD-based allocation on end-stage renal disease after liver transplantation. *Am J Transplant* 2011; **11**: 2372-2378 [PMID: 21883908 DOI: 10.1111/j.1600-6143.2011.03703.x]
- 5 **Bahirwani R**, Reddy KR. Outcomes after liver transplantation: chronic kidney disease. *Liver Transpl* 2009; **15** Suppl 2: S70-S74 [PMID: 19876956 DOI: 10.1002/lt.21900]
- 6 **Wong F**, Leung W, Al Beshir M, Marquez M, Renner EL. Outcomes of patients with cirrhosis and hepatorenal syndrome type 1 treated with liver transplantation. *Liver Transpl* 2015; **21**: 300-307 [PMID: 25422261 DOI: 10.1002/lt.24049]
- 7 **OPTN/UNOS Kidney Transplantation Committee**. Simultaneous Liver Kidney (SLK) allocation policy. 2016. Available from: URL: <https://optn.transplant.hrsa.gov/governance/public-comment/simultaneous-liver-kidney-allocation/>
- 8 **Fong TL**, Khemichian S, Shah T, Hutchinson IV, Cho YW. Combined liver-kidney transplantation is preferable to liver transplant alone for cirrhotic patients with renal failure. *Transplantation* 2012; **94**: 411-416 [PMID: 22805440 DOI: 10.1097/TP.0b013e3182590d6b]
- 9 **Locke JE**, Warren DS, Singer AL, Segev DL, Simpkins CE, Maley WR, Montgomery RA, Danovitch G, Cameron AM. Declining outcomes in simultaneous liver-kidney transplantation in the MELD era: ineffective usage of renal allografts. *Transplantation* 2008; **85**: 935-942 [PMID: 18408571 DOI: 10.1097/TP.0b013e318168476d]
- 10 **Brown RS**, Lombardero M, Lake JR. Outcome of patients with renal insufficiency undergoing liver or liver-kidney transplantation. *Transplantation* 1996; **62**: 1788-1793 [PMID: 8990364 DOI: 10.1097/00007890-199612270-00018]
- 11 **Klein KB**, Stafinski TD, Menon D. Predicting survival after liver transplantation based on pre-transplant MELD score: a systematic review of the literature. *PLoS One* 2013; **8**: e80661 [PMID: 24349010 DOI: 10.1371/journal.pone.0080661]
- 12 **Oberkofler CE**, Dutkowski P, Stocker R, Schuepbach RA, Stover JF, Clavien PA, Béchir M. Model of end stage liver disease (MELD) score greater than 23 predicts length of stay in the ICU but not mortality in liver transplant recipients. *Crit Care* 2010; **14**: R117 [PMID: 20550662 DOI: 10.1186/cc9068]
- 13 **Pan HC**, Jenq CC, Lee WC, Tsai MH, Fan PC, Chang CH, Chang MY, Tian YC, Hung CC, Fang JT, Yang CW, Chen YC. Scoring systems for predicting mortality after liver transplantation. *PLoS One* 2014; **9**: e107138 [PMID: 25216239 DOI: 10.1371/journal.pone.0107138]
- 14 **Weber ML**, Ibrahim HN, Lake JR. Renal dysfunction in liver transplant recipients: evaluation of the critical issues. *Liver Transpl* 2012; **18**: 1290-1301 [PMID: 22847917 DOI: 10.1002/lt.23522]

**P-Reviewer:** Chamuleau RAFM, Gong ZJ, Kabir A, Rostami K, Silva LD, Tomizawa M

**S-Editor:** Ji FF **L-Editor:** A **E-Editor:** Li D







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 October 8; 8(28): 1157-1204





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashiwara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**REVIEW**

- 1157 Cirrhosis and autoimmune liver disease: Current understanding

*Liberal R, Grant CR*

- 1169 Current status of diagnosis and treatment of hepatic echinococcosis

*Mihmanli M, Idiz UO, Kaya C, Demir U, Bostanci O, Omeroglu S, Bozkurt E*

**MINIREVIEWS**

- 1182 Management of refractory ascites in cirrhosis: Are we out of date?

*Annamalai A, Wisdom L, Herada M, Noureddin M, Ayoub W, Sundaram V, Klein A, Nissen N*

**ORIGINAL ARTICLE****Basic Study**

- 1194 DNA methylation of angiotensin II receptor gene in nonalcoholic steatohepatitis-related liver fibrosis

*Asada K, Aihara Y, Takaya H, Noguchi R, Namisaki T, Moriya K, Uejima M, Kitade M, Mashitani T, Takeda K, Kawaratani H, Okura Y, Kaji K, Douhara A, Sawada Y, Nishimura N, Seki K, Mitoro A, Yamao J, Yoshiji H*

**Retrospective Study**

- 1200 Impaired liver function attenuates liver regeneration and hypertrophy after portal vein embolization

*Kageyama Y, Kokudo T, Amikura K, Miyazaki Y, Takahashi A, Sakamoto H*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Qiang Li, MD, PhD, Chief Doctor, Director, Professor, Department of Liver Diseases, Jinan Infectious Disease Hospital, Shandong University, Jinan 250021, Shandong Province, China

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

**I-IV Editorial Board**

**EDITORS FOR THIS ISSUE**

**Responsible Assistant Editor:** *Xiang Li*  
**Responsible Electronic Editor:** *Dan Li*  
**Proofing Editor-in-Chief:** *Lian-Sheng Ma*

**Responsible Science Editor:** *Fang-Fang Ji*  
**Proofing Editorial Office Director:** *Xin-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Department of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL BOARD MEMBERS**  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

**EDITORIAL OFFICE**  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
October 8, 2016

**COPYRIGHT**  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
<http://www.wjgnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esps/>



## Cirrhosis and autoimmune liver disease: Current understanding

Rodrigo Liberal, Charlotte R Grant

Rodrigo Liberal, Charlotte R Grant, Institute of Liver Studies, King's College London School of Medicine at King's College Hospital, London SE 9RS, United Kingdom

**Author contributions:** Liberal R and Grant CR contributed to paper design, literature search, drafting and editing of the manuscript; both authors approved the final version of the manuscript.

**Conflict-of-interest statement:** Authors declare no conflict of interests for this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Rodrigo Liberal, Institute of Liver Studies, King's College London School of Medicine at King's College Hospital, Denmark Hill, London SE5 9RS, United Kingdom. [rodrigo.liberal@kcl.ac.uk](mailto:rodrigo.liberal@kcl.ac.uk)  
Telephone: +44-2032-993397  
Fax: +44-2032-993760

Received: March 3, 2016  
Peer-review started: March 7, 2016  
First decision: April 15, 2016  
Revised: July 22, 2016  
Accepted: August 6, 2016  
Article in press: August 8, 2016  
Published online: October 8, 2016

### Abstract

Primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH) constitute the classic autoimmune liver diseases (AILDs). While

AIH target the hepatocytes, in PBC and PSC the targets of the autoimmune attack are the biliary epithelial cells. Persistent liver injury, associated with chronic AILD, leads to un-resolving inflammation, cell proliferation and the deposition of extracellular matrix proteins by hepatic stellate cells and portal myofibroblasts. Liver cirrhosis, and the resultant loss of normal liver function, inevitably ensues. Patients with cirrhosis have higher risks of morbidity and mortality, and that in the decompensated phase, complications of portal hypertension and/or liver dysfunction lead to rapid deterioration. Accurate diagnosis and monitoring of cirrhosis is, therefore of upmost importance. Liver biopsy is currently the gold standard technique, but highly promising non-invasive methodology is under development. Liver transplantation (LT) is an effective therapeutic option for the management of end-stage liver disease secondary to AIH, PBC and PSC. LT is indicated for AILD patients who have progressed to end-stage chronic liver disease or developed intractable symptoms or hepatic malignancy; in addition, LT may also be indicated for patients presenting with acute liver disease due to AIH who do not respond to steroids.

**Key words:** Hepatic fibrosis; Cirrhosis; Myofibroblasts; Primary biliary cirrhosis; Primary sclerosing cholangitis; Autoimmune hepatitis; Liver transplantation

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In chronic liver disease, including autoimmune liver diseases, perpetual liver injury leads to persistent inflammation, cell proliferation and the deposition of extracellular matrix proteins. If left untreated, this process eventually leads to the development of liver cirrhosis, characterised by the presence of fibrosis and nodular regeneration. Liver biopsy is currently the gold standard technique, but highly promising non-invasive methodology is under development.

Liberal R, Grant CR. Cirrhosis and autoimmune liver disease:

Current understanding. *World J Hepatol* 2016; 8(28): 1157-1168  
Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i28/1157.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i28.1157>

## INTRODUCTION

Liver disorders with probable autoimmune aetiology include autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Each disease complies with, to varying extents, a proposed “multiple hit hypothesis” accounting for autoimmunity development, in which interacting environmental, infectious, genetic, epigenetic and immunological factors account for the loss of tolerance to self-constituents<sup>[1]</sup>. While AIH target the hepatocytes, in PBC and PSC the targets of the autoimmune attack are the biliary epithelial cells. Each of the autoimmune liver diseases (AILDs) is associated with distinct epidemiological and clinical characteristics. However, overlap syndromes, characterised by the coexistence of features of more than one AILD, are increasingly being recognised<sup>[2]</sup>.

## AILDS

### PBC

PBC is a cholestatic autoimmune liver disease characterised by progressive destruction of the small and intermediate-sized bile ducts<sup>[3]</sup>. The histologic picture of PBC involves non-suppurative cholangitis with destruction of the biliary epithelium and portal infiltration of inflammatory cells. PBC also presents with biochemical evidence of cholestasis. PBC has pronounced female preponderance and a strong tendency to present in middle age<sup>[3]</sup>. Epidemiological characteristics of PBC are outlined in Table 1.

High titre positivity for serum anti-mitochondrial autoantibodies (AMAs) is pathognomonic for PBC, being detected in up to 95% of patients<sup>[3-5]</sup>. Moreover, asymptomatic people with AMA-positivity eventually progress to disease development<sup>[6]</sup>. AMAs target lipoylated domains of the 2-oxoacid dehydrogenase complexes, with the immunodominant epitope belonging to the E2 components of the pyruvate dehydrogenase complex<sup>[3,4,7]</sup>. PBC-specific anti-nuclear autoantibodies (ANAs), with a characteristic “multiple nuclear dot” or “nuclear membrane” pattern, are found in 25%-40% of patients<sup>[8]</sup>.

There is mounting evidence that the development of PBC can be accounted for by a proposed “multiple hit” hypothesis for the development of autoimmunity (Figure 1). The molecular mimicry hypothesis postulates that microorganisms with epitopes that are structurally similar to self-components trigger an immune response with interspecies promiscuity. Several potential infectious triggers have been proposed<sup>[9]</sup> including *Escherichia coli*<sup>[10-14]</sup> and *Nosfingobium aromaticivorans*<sup>[15-17]</sup>.

Numerous lines of evidence demonstrate that genetic factors alter susceptibility to PBC development. Female

relatives of patients are at increased risk of developing PBC, and there is a high concordance rate between monozygotic twins<sup>[18]</sup>. Strong genetic associations lying within the MHC, for example HLA-DR8 in Europe and North America, have consistently been reported<sup>[19,20]</sup>. Genome wide association studies (GWAS) have revealed non-MHC gene associations that could be related to abnormal immune activation, including *IL12A*, *IL12RB2*, *STAT-4* and *CTLA-4*<sup>[21-23]</sup>.

Ursodeoxycholic acid (UDCA) is the standard treatment for PBC, improving both biochemical and histological indicators of disease activity and elongating transplant-free survival time in a significant proportion of patients<sup>[24,25]</sup>.

### PSC

PSC is a chronic inflammatory disease of the biliary epithelium, characterised by progressive bile duct destruction. The small, medium and large bile ducts are affected by obliterative concentric fibrosis which leads to the development of biliary strictures<sup>[26]</sup>. In contrast to the other AILDs, PSC affects males more commonly than females<sup>[27]</sup>. The median age of onset is approximately 41 years of age<sup>[27]</sup> (Table 1).

The most common biochemical abnormality in PSC patients is elevated serum alkaline phosphatase (AP)<sup>[28]</sup>. The most reliable diagnostic tool is cholangiography, which enables visualisation of characteristic multifocal strictures within the intra- and extra-hepatic bile ducts<sup>[29]</sup>. Concomitant inflammatory bowel disease (IBD), most frequently ulcerative colitis, is found in up to 80% of patients<sup>[28,30]</sup>.

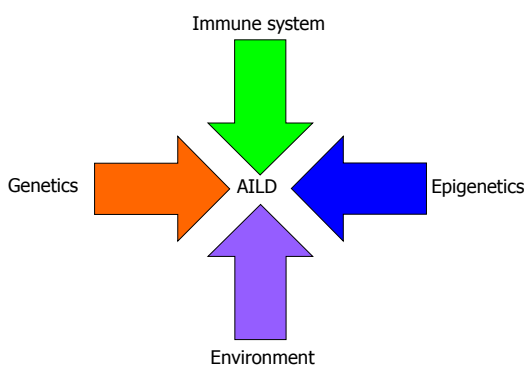
As with the other AILDs, the aetiology of PSC remains unknown but it is likely to follow the proposed multiple hit hypothesis (Figure 1), resulting from interplay between numerous genetic and environmental factors. The strong link with IBD has led to the emergence of the gut/lymphocyte homing hypothesis, which postulates that memory lymphocytes primed in the gut-associated lymphoid tissue, and therefore expressing the gut-homing integrin  $\alpha 4\beta 7$  and the chemokine receptor CCR9, migrate from the gastrointestinal tract to the liver<sup>[31,32]</sup>. Importantly, the ligand for  $\alpha 4\beta 7$ , MAdCAM-1, and the cognate chemokine for CCR9, CCL25, both usually restricted to the gut<sup>[32,33]</sup>, are aberrantly expressed in the portal vein endothelium and sinusoidal endothelium respectively in PSC patients. Moreover, approximately 20% of liver-infiltrating T cells express  $\alpha 4\beta 7$  and CCR9, and have an effector memory phenotype<sup>[34,35]</sup>. The “leaky gut hypothesis”, on the other hand, involves direct translocation of intestinal flora *via* the portal vein<sup>[28]</sup>. Although direct evidence of this phenomenon is lacking<sup>[36]</sup>, future studies investigating the influence of the gut microbiota on PSC development/progression are warranted.

Similarly to the other AILDs, the strongest PSC genetic associations lie within *HLA* gene. In GWAS, the strongest association signals have been found near *HLA-B*<sup>[37-39]</sup>. There are, however, also believed to be *HLA* class II

**Table 1** Epidemiological characteristics associated with the three autoimmune liver diseases

	PBC	PSC	AIH
Female/male ratio	10/1	1/2	4/1
Average age at presentation	50	41	Childhood/adolescence and approximately 40
Incidence	0.33-5.8/100000	0-1.3/100000	0.08-3/100000
Prevalence	1.91-40.2/100000	0-16.2/100000	11.6-35.9/100000
Risk within family	1 <sup>st</sup> degree relative incidence 4%-6%	Unknown	Unknown
Concordance in monozygotic twins	60%	Only case reports	Only case reports
Note	AMA positivity	Frequent association with IBD Increased risk of hepatobiliary/colorectal malignancies	Positivity for ANA and/or SMA (AIH type-1) or anti-LKM-1 (AIH type-2)

AIH: Autoimmune hepatitis; AMA: Anti-mitochondrial autoantibodies; ANA: Anti-nuclear autoantibody; IBD: Inflammatory bowel disease; LKM: Liver kidney microsomal; PBC: Primary biliary cirrhosis; PSC: Primary sclerosing cholangitis; SMA: Smooth muscle autoantibody.



**Figure 1** “Multiple hit hypothesis” accounting for the development of autoimmune disease. Interplay between immunological, genetic, epigenetic and environmental factors is thought to account for the loss of tolerance to self constituents in AILD. AILD: Autoimmune liver disease.

susceptibility genes contributing to the association signal found within in this region<sup>[38,39]</sup>. Non HLA associations identified by GWAS include *BCL2L1*, which encodes the pro-apoptotic protein *BIM*, *TNFRSF14* and *IL2RA*<sup>[37-39]</sup>.

### AIH

AIH is a progressive inflammatory disease which, in contrast to the two cholestatic AILDs, targets the hepatocytes themselves. AIH has marked female predilection. AIH can present at all ages, but the two peak ages of incidence are in childhood or adolescence and at around 40 years of age<sup>[40]</sup> (Table 1). Trademark biochemical/serological characteristics of AIH are elevated aminotransferase levels, positivity for autoantibodies and increased IgG. A histological picture of interface hepatitis is typical of AIH. Autoantibody positivity is an important clinical feature of AIH, facilitating diagnosis and enabling distinction between two types of the disease. Patients seropositive for ANA and/or anti-smooth muscle autoantibodies (SMA) have AIH type-1 whereas those presenting with positivity for anti-liver kidney type-1 autoantibody (anti-LKM-1) or anti-liver cytosol type-1 (anti-LC-1) have AIH type-2<sup>[41,42]</sup>.

Although AIH aetiology remains to be elucidated, available evidence is strongly suggestive of interplay between genetic and environmental factors (Figure 1).

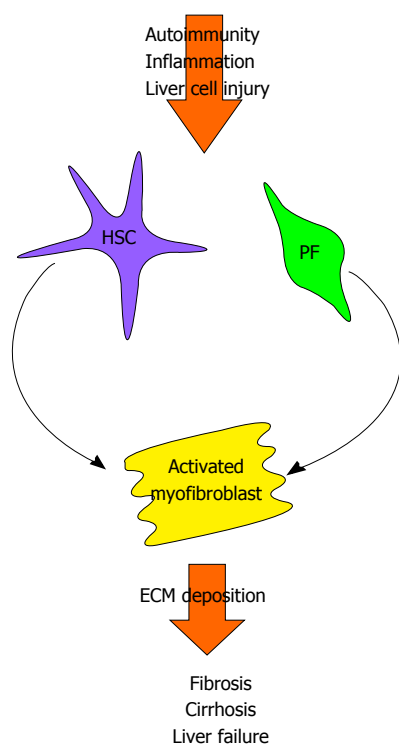
The observation that the hepatitis C virus shares high sequence homology with the auto-antigenic target of anti-LKM-1 autoantibodies, cytochrome P450-2D6, has led to the suggestion that molecular mimicry could trigger AIH development in a genetically predisposed host<sup>[43,44]</sup>. Other potential triggers for AIH include the hepatitis B virus, cytomegalovirus and the herpes simplex virus<sup>[43]</sup>.

Genetic associations affecting susceptibility to disease development, response to therapy and prognosis have been reported<sup>[45]</sup>. The most significant genetic associations lie within the MHC, at the HLA-DRB1 locus. Susceptibility to AIH type-1 is linked to alleles encoding the HLA-DR3 and DR4 molecules<sup>[46]</sup>, while AIH-2 susceptibility and severity have been linked to alleles encoding the HLA-DR3 and DR7 molecules<sup>[47]</sup>. Susceptibility to AIH has also been linked to polymorphisms in genes located outside the MHC, including *CTLA-4*<sup>[48]</sup>, *TNF-α*<sup>[49]</sup> and *Fas*<sup>[50]</sup>.

With the standard treatment regimen for AIH - prednisolone, with or without the addition of azathioprine - up to 80% of AIH patients are able to reach remission<sup>[51]</sup>.

### Overlap syndromes

It is not uncommon for patients to present with features characteristic of AIH and either PSC or PBC. Because standardised and validated diagnostic criteria are lacking, these “overlap syndromes” remain ill defined. PBC/AIH overlap is present in some 10% of AIH or PBC patients<sup>[52,53]</sup>, and the most commonly used method for diagnosis is the presence of two of the following features of AIH in conjunction with two of the following features of PBC. The AIH features are: (1) ALT at least 5 times the upper limit of normal (ULN); (2) SMA positivity or IgG level of at least 2 times ULN; and (3) liver histology showing moderate or severe periportal or periseptal inflammation. The PBC criteria are: (1) AP at least twice ULN or gamma glutamyl transferase above 5 times ULN; (2) AMA positivity; and (3) bile duct lesions on liver biopsy<sup>[52,54,55]</sup>. AIH/PSC overlap is now believed to represent a significant proportion of patients with AILD<sup>[56,57]</sup>. The characteristics of AIH/PSC overlap are the classical features of AIH-1 - positivity for ANA and/or SMA, high IgG levels and interface hepatitis on biopsy - in addition to biochemical evidence of cholestasis, frequent



**Figure 2 Development of fibrosis and cirrhosis in autoimmune liver disease.** Persistent autoimmune-mediated inflammation and liver cell injury leads to the activation and differentiation of quiescent hepatic stellate cells (HSC) and portal fibroblasts (PF) into activated myofibroblasts. These proliferative, pro-inflammatory and pro-fibrogenic myofibroblasts increase collagen synthesis and deposit extracellular matrix proteins (ECM), leading to the development of fibrous scar tissue. Cirrhosis, characterised by significant fibrosis and nodular regeneration, eventually ensues, with the resultant loss of liver function and eventually liver failure.

occurrence of IBD, histological features consistent with PSC<sup>[58]</sup>. Cholangiographic evidence of intrahepatic or extrahepatic PSC also supports this diagnosis<sup>[59]</sup>.

## FIBROSIS: KEY PLAYERS

In chronic liver disease, including AILD, perpetual liver injury leads to persistent inflammation, cell proliferation and the deposition of extracellular matrix proteins. If left untreated, this process eventually leads to the development of liver cirrhosis, characterised by nodular regeneration diffuse nodular regeneration surrounded by fibrotic septa with consequent extinction of the parenchyma, together leading to distortion of hepatic vascular architecture<sup>[60]</sup>. Loss of normal liver function inevitably ensues (Figure 2)<sup>[61]</sup>.

Hepatic stellate cells, found in the space of Dissé, have long been believed to be the main contributors to liver fibrosis. Liver damage induces hepatic stellate cells to differentiate into proliferative and contractile myofibroblasts, with a pro-inflammatory and fibrogenic phenotype<sup>[61-63]</sup>. Portal fibroblasts, located in the connective tissue of the portal triad are another source of myofibroblasts<sup>[64]</sup>. These are of particular importance in the context of the cholestatic AILDs. Liver damage leads to the myofibroblastic differentiation of quiescent

portal fibroblasts<sup>[64]</sup>, a process which can be enhanced in these conditions by the cholangiocytes themselves. When cholangiocytes become “reactive”, they proliferate and express co-stimulatory molecules, chemokines and pro-fibrogenic molecules, therefore further promoting fibrogenesis<sup>[65-70]</sup>. Bile acids, elevated as a consequence of cholestasis, could also perpetuate fibrogenesis indirectly by damaging hepatocytes<sup>[71]</sup>, or by directly targeting myofibroblasts<sup>[72]</sup>.

It has also been suggested that hepatic myofibroblasts could arise from hepatocytes or cholangiocytes *via* epithelial-mesenchymal transition, whereby polarised epithelial cells undergo phenotypic transformation in response to microenvironmental cues<sup>[73]</sup>. There are reports that hepatic epithelial cells can acquire some of the phenotypic characteristics of myofibroblastic cells *in vitro*. Co-expression of epithelial and fibroblastic cell markers has also been described in human tissue sections<sup>[74,75]</sup>. However, partly because these cell “markers” inadequately define both the epithelial and fibroblastic populations, conclusive evidence of epithelial-mesenchymal transition has been hard to come by. Furthermore, lineage tracing studies, using Cre/lox recombination, have failed to find evidence of liver epithelial cell-mesenchymal transition in murine models of bile-duct ligation or hepatitis induced by carbon tetrachloride (CCl<sub>4</sub>) or 3,5-diethoxycarbonyl-1,4-dihydrocollidine<sup>[76,77]</sup>.

## NATURAL HISTORY OF CIRRHOSIS

It is well known that, compared with pre-cirrhotic patients, patients with cirrhosis have higher risks of morbidity and mortality<sup>[78]</sup>. Cirrhosis can be divided into a compensated phase, free of symptoms, and a decompensated phase, in which complications of portal hypertension and/or liver dysfunction lead to rapid deterioration. The two stages can be considered separate clinical entities according to the AASLD and EASL guidelines<sup>[79]</sup>. Median survival time in the compensated phase is over 12 years, whereas survival in the decompensated phase drops to approximately 2 years. The decompensated phase is defined by the development of jaundice, ascites, variceal haemorrhage or encephalopathy<sup>[80,81]</sup>. The compensated stage has been further divided into stage 1, consisting of patients lacking varices, and stage 2, characterised by the presence of varices in the absence or variceal bleeding. The decompensated stage has been split into stage 3, associated with ascites and a lack of variceal haemorrhage, and stage 4, comprising patients with variceal haemorrhage (with or without ascites). One year mortality rates of 1%, 3%, 20% and 57% respectively have been reported<sup>[82,83]</sup>. In a recent study, however, Zipprich *et al.*<sup>[84]</sup> (2012) failed to replicate entirely these reported values, finding that stage 3 and stage 4 patients had one year survival rates of approximately 20% and 18% respectively. The authors of this study cite recent advances in variceal haemorrhage therapy<sup>[85]</sup> as a potential reason for this discrepancy and proposed modifications to the system of stratification. The newly defined



stage 3 consists of patients with variceal haemorrhage but without ascites, while stage 4 is characterised by the presence of ascites (with or without variceal bleeding)<sup>[84]</sup>.

The risk of progression from compensated to decompensated cirrhosis is approximately 31% in the first year of diagnosis and 5%-7% thereafter<sup>[86]</sup>. Because of the striking reduction in survival time in the decompensated state, it is important to identify patients at greatest risk of cirrhosis progression. Newly developed non-invasive techniques for fibrosis/cirrhosis assessment are currently being tested.

The Child-Pugh, and more recently developed Model of End-Stage Liver Disease (MELD) Scores are the most widely used methods by which prognosis is assessed in the context of end-stage liver disease. The Child-Pugh Score incorporates values between 1 and 3 for each of the following criteria: Degree of encephalopathy, presence of ascites, serum bilirubin and albumin levels and international normalised ratio (INR). The MELD score encompasses bilirubin, INR and creatinine levels<sup>[87]</sup>. MELD was initially developed for predicting survival following transhepatic portosystemic shunt, but is now used to accurately predict survival in the context of cirrhosis<sup>[88]</sup>, list patients for transplant and allocate organs.

## DIAGNOSING CIRRHOSIS

Liver biopsy is still the most accurate and widely used method by which cirrhosis can be diagnosed and staged. There are, however, notable disadvantages to this method of examination, including cost, risk of bleeding, and sampling error<sup>[89]</sup>. Non-invasive tests for both diagnosis and assessment of fibrosis/cirrhosis progression are becoming increasingly sought. Proposed tests include those using the results of routine liver-function examinations, such as the AST-to-platelet ratio index, as well as examinations to measure liver stiffness; FibroTest and transient elastography (TE; FibroScan)<sup>[90,91]</sup>. There are promising indications that non-invasive methods could be used in the context of AILD. In PBC, liver stiffness tests show high performance in diagnosing significant fibrosis, severe fibrosis and cirrhosis. Progression of liver stiffness has also been used as an accurate measure of overall prognosis in PBC<sup>[92-94]</sup>. The addition of serological markers to the liver stiffness score does not appear to improve test outcome<sup>[93]</sup>. In a study also involving both PBC and PSC patients, liver stiffness was also shown to correlate with progression of fibrosis and histological scores<sup>[95]</sup>. Using a cohort of 404 patients with varied liver diseases, including PBC, PSC and AIH, Malik *et al*<sup>[96]</sup> (2010) found that liver stiffness scores accurately identified patients with compensated cirrhosis. Although highly promising, these results, particularly in the context of AIH, need to be confirmed in larger cohorts of patients.

## CIRRHOSIS IN AILDS

### *Cirrhosis in PBC*

PBC progresses through a number of stages: Preclinical,

asymptomatic, symptomatic, and liver failure. The pre-clinical phase is symptom-free and is associated with AMA positivity in the absence of biochemical indications of liver disease<sup>[6,97]</sup>. Biochemical abnormalities eventually appear after a median time of 5.6 years (range, 1-20 years)<sup>[6]</sup>, but this phase is not yet associated with the presence of symptoms. When symptoms eventually develop, they are most commonly fatigue and pruritus, and later varices, oedema or ascites.

Liver failure is characterised by the accelerated development of jaundice, and is associated with poor prognosis<sup>[98]</sup>. Mean survival for patients with a bilirubin of 2.0 mg/dL is 4 years, while for those with bilirubin of 6.0 mg/dL is only 2 years<sup>[98]</sup>. PBC prognosis has dramatically improved in the last 20 years thanks to earlier diagnosis and the introduction of UDCA as the mainstay of treatment<sup>[99,100]</sup>.

UDCA slows fibrosis progression and delays cirrhosis development<sup>[101]</sup>. In clinical trials, UDCA treatment of PBC patients decreased the development of oesophageal varices and prolonged survival<sup>[102-106]</sup>. Cirrhosis does, however, still develop in UDCA-treated PBC patients<sup>[107]</sup>. Indeed, the development of cirrhosis under UDCA treatment is an independent predictor of negative outcome<sup>[101,107]</sup>.

Histologically, PBC can be divided according to the presence of fibrosis/cirrhosis into four stages<sup>[108,109]</sup>. Stage one is characterised by portal inflammatory cell infiltrate, which, in stage two, invades the liver parenchyma. In stage three, bridging fibrosis, in which fibrotic septa extend from and link the portal tracts, can be seen. Stage four is characterised by progression to cirrhosis<sup>[109]</sup>. The development of cirrhosis does not occur uniformly throughout the liver, thus features of all four stages can occur simultaneously in a single biopsy specimen. Histological staging should depend upon the most advanced histological features<sup>[25]</sup>.

Histological stages can predict survival of PBC patients<sup>[110]</sup>. In untreated PBC patients, the median time to the development of extensive fibrosis is 2 years. The probability of remaining in early stages after 4 years is 29%, whereas development of cirrhosis occurs in 50% of patients originally demonstrating histological evidence of interface hepatitis without fibrosis<sup>[111]</sup>. In two studies the proportion of patients developing liver failure during a follow-up time of 5 years was found to be 15%<sup>[112]</sup> and 25%<sup>[113]</sup>. The development of oesophageal varices, and the associated impact on survival, has been examined in a prospective study over the course of 5.6 years, which included 256 patients<sup>[114]</sup>. Twenty-eight percent of patients were cirrhotic. Nearly one-third of patients developed oesophageal varices, after which the 3-year survival was 59%. Survival after the first bleeding episode was 46%<sup>[114]</sup>.

The introduction of UDCA as first line treatment for PBC patients has changed the natural history of the disease<sup>[25,100,115,116]</sup>. Indeed, the number of PBC patients requiring liver transplantation (LT) decreased by 20% in between 1996 and 2006<sup>[115]</sup>. Additionally, PBC has fallen in the ranking of the most common indications for LT

from the first to the sixth place LT over a period of 20 years<sup>[25]</sup>.

Several papers have also assessed the impact of UDCA therapy on the progression rate of cirrhosis in PBC patients. Corpechot *et al.*<sup>[107]</sup> examined progression to cirrhosis in 183 UDCA-treated PBC patients. In this study, 21% of patients developed cirrhosis during follow-up. The incidence of cirrhosis in patients followed up from stages 1, 2 and 3 was 4%, 12% and 59% respectively and the median length of times to cirrhosis development was 25, 20 and 4 years respectively. Albumin and bilirubin levels, and the histological severity of interface hepatitis were independently associated with progression to cirrhosis; cirrhosis was most likely to develop in patients with serum bilirubin over 17  $\mu\text{mol/L}$ , serum albumin below 38 g/L and in patients with moderate to severe interface hepatitis<sup>[107]</sup>. The impact of UDCA treatment oesophageal varices development has been examined in a 4-year prospective study including patients who received UDCA vs patients who received placebo. In the UDCA arm, the risk of varices development was 16%, while for those in the placebo group was 58%<sup>[103]</sup>.

### Cirrhosis in PSC

Typical symptoms of PSC, occurring in a variable number of patients include pruritus, abdominal pain, malaise, weight loss, and episodes of fever and chills<sup>[117]</sup>. About 50% of PSC patients will present symptomatically<sup>[118,119]</sup>. Similarly to PBC, PSC progresses through four histological stages<sup>[120]</sup>. In stage 1, which is known as the portal stage, changes are restricted to the portal tracts with features of mild hepatitis and cholangitis. Stage 2, known as the periportal stage, is characterised by extension of the lesion to include periportal fibrosis and occasionally interphase hepatitis. In this phase, the portal tracts are often notably enlarged. By stage 3, the septal stage, bridging fibrous septa have developed and the bile ducts have begun to degenerate and disappear. Stage 4 is characterised by cirrhosis<sup>[120]</sup>. The rate of progression through these stages has been investigated. Of PSC patients in the periportal stage, 42%, 66% and 93% progressed over 1, 2 and 5 years respectively. Of patients in the septal stage, 14%, 25% and 52% progressed over 1, 2 and 5 years respectively. In 15% of total observations, regression of histologic stage could be observed, highlighting the problem of sample variability when serial liver biopsies are used during the period of follow-up<sup>[121]</sup>.

PSC can present at later stages of disease development, with complications of cirrhosis and portal hypertension<sup>[122]</sup>. Similarly to other causes of cirrhosis, portal hypertension gradually develops in cirrhotic PSC patients<sup>[119]</sup>. In one study, 36% of 283 newly diagnosed PSC patients had varices<sup>[123]</sup>.

### Cirrhosis in AIH

In a cohort of over 450 AIH patients, 30% had evidence of cirrhosis at diagnosis, with a further 10% developing cirrhosis during a median follow-up time of 7.2 years.

The presence of cirrhosis at diagnosis correlated with negative outcome (LT or death)<sup>[124]</sup>. In another study, including 126 AIH patients, Feld *et al.*<sup>[125]</sup> (2005) reported that 33% of patients had histological evidence of cirrhosis at diagnosis. With the exception of platelet count, which was lower in patients with cirrhosis, laboratory parameters, patient demographics and AIH scores did not differ between cirrhotic and non-cirrhotic patients. A similar frequency of patients from each group were symptomatic at diagnosis and an equivalent proportion had good response to treatment<sup>[125]</sup>. Importantly, similar response to treatment has also been reported elsewhere<sup>[126]</sup>. Feld *et al.*<sup>[125]</sup> (2005) also found, however, that the presence of cirrhosis significantly increased risk of progression to LT or death. Consistent with the above studies, Verma *et al.*<sup>[127]</sup> (2004) reported that 28% of AIH patients were cirrhotic at diagnosis. In this study, a further 20% of patients developed cirrhosis during 52 mo of follow-up. Again, cirrhosis was an independent predictor of poor outcome in this cohort<sup>[127]</sup>. On the other hand, studies in the adult<sup>[126,128]</sup> and paediatric<sup>[129]</sup> settings, of comparable size and methodology to those described above, have not found associations between the presence of cirrhosis at diagnosis and the likelihood of poor outcome.

In one study, patients diagnosed between the ages of 21 and 60 years of age were more likely to present with cirrhosis than those outside of this range. Male patients were also more likely to have cirrhosis compared to their female counterparts. Low serum albumin concentrations, prolonged INR and low platelet count were all more frequently associated with the cirrhotic group of AIH patients<sup>[130]</sup>.

There are indications that cirrhosis is more common among AIH type-1 patients compared to patients with type-2 AIH. In a paediatric study, 69% of ANA/SMA positive patients had evidence of "definite cirrhosis" on initial biopsy, whereas only 38% of patients positive for anti-LKM-1 were cirrhotic. On follow-up these values increased to 74% and 44% respectively<sup>[131]</sup>.

## LT IN AILDS

LT is indicated for AILD patients who have progressed to end-stage chronic liver disease or developed intractable symptoms or hepatocellular carcinoma (HCC)<sup>[132,133]</sup>; in addition, LT may also be indicated for patients presenting with acute liver disease due to AIH who do not respond to steroids<sup>[134]</sup>. In total, AILDs accounts for almost one fourth of LTs performed in the United States and in Europe<sup>[135]</sup>.

### LT for PBC

The indications for LT in PBC are, for the most part, identical to those in patients with end-stage chronic liver disease of other aetiology<sup>[100,132]</sup>. The majority of transplants occur due to end-stage chronic liver disease when the MELD score is higher than 16<sup>[136]</sup>. Other indications for LT include HCC, portopulmonary hypertension or hepato-pulmonary syndrome<sup>[137]</sup>. Other than this, few

PBC patients with non-cirrhotic portal hypertension associated with obliterative portal venopathy or nodular regenerative hyperplasia will benefit from transplant<sup>[138]</sup>. Finally, even when liver function is sufficient<sup>[139]</sup>, LT may be indicated if intractable symptoms, most notable refractory pruritus, are present<sup>[137,140]</sup>.

The immunosuppressive regimen most commonly used following LT is a combination of corticosteroids, which are withdrawn over a period of three months, a calcineurin inhibitor (CNI) and mycophenolate mofetil or azathioprine. This regimen has a very successful outcome<sup>[136]</sup>; with 1, 3 and 5 year patient survivals of 94%, 91% and 82% respectively, and graft survivals of 85%, 83% and 75% respectively<sup>[141]</sup>. Analysis of the UNOS database showed that PBC living donor transplant recipients had estimated 1, 3 and 5 year patient survivals of 93%, 90% and 86% and deceased donor transplant recipients had estimated survivals of 90%, 87% and 85% respectively. Estimated graft survivals at 1, 3 and 5 years for living donor LT was 86%, 81% and 77% respectively, and for deceased donor LT was 85%, 83% and 81% respectively<sup>[142]</sup>.

### LT for PSC

Similarly to PBC, and other liver diseases associated with cirrhosis, LT is indicated in PSC patients with end-stage liver disease (*i.e.*, with a MELD score above 16)<sup>[143,144]</sup>. HCC can occur in PSC patients with cirrhosis, and in this context, LT prioritisation follows the same rule as that for other cirrhotic patients with HCC<sup>[137,145]</sup>. In PSC, LT may also be indicated in patients with intractable pruritus or those with recurrent bacterial cholangitis, and limited stage cholangiocarcinoma<sup>[118,122,143]</sup>.

LT for PSC usually has good outcome<sup>[139]</sup>. In one report, the 1, 2 and 5 year actuarial patient survivals for LT for PSC were 90%, 86% and 85%, and graft survivals were 82%, 77% and 72% respectively<sup>[146]</sup>. In a study from the Mayo Clinic comprising 150 transplanted PSC cases, similar patient survival at 1, 2, 5 and 10 years of 94%, 92%, 86% and 70%, and graft survival of 83%, 83%, 79% and 61% was reported<sup>[147]</sup>.

### LT for AIH

Overall, AIH accounts for some 3% of paediatric and up to 6% of adult LTs<sup>[40]</sup>. The natural course of AIH is understood mostly thanks to the last placebo-controlled trials published 4 decades ago<sup>[148-150]</sup>. These reports demonstrated that, without treatment, AIH patients have poor survival with 40% of deaths within 6 mo from diagnosis. With treatment, 10-year survival rate of AIH patients is over 80%<sup>[125,151]</sup>.

LT is indicated for AIH patients presenting with acute liver failure who do not respond to steroids, for those patients with advanced cirrhosis and for those with HCC<sup>[136,152]</sup>.

The immunosuppressive strategy most commonly adopted consists in the combination of prednisolone and a CNI<sup>[153]</sup>, leading to excellent outcome with 5 and 10 year patient survivals of 90% and 75%<sup>[51]</sup>, and 1 and 5 year graft survivals of 84% and 75%<sup>[51,154]</sup>.

## REFERENCES

- Bogdanos DP**, Gershwin ME. What is new in primary biliary cirrhosis? *Dig Dis* 2012; **30** Suppl 1: 20-31 [PMID: 23075865 DOI: 10.1159/000341118]
- Boberg KM**, Chapman RW, Hirschfield GM, Lohse AW, Manns MP, Schrupf E. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. *J Hepatol* 2011; **54**: 374-385 [PMID: 21067838 DOI: 10.1016/j.jhep.2010.09.002]
- Kaplan MM**, Gershwin ME. Primary biliary cirrhosis. *N Engl J Med* 2005; **353**: 1261-1273 [PMID: 16177252 DOI: 10.1056/NEJMra043898]
- Bogdanos DP**, Baum H, Vergani D. Antimitochondrial and other autoantibodies. *Clin Liver Dis* 2003; **7**: 759-777 [PMID: 14594130 DOI: 10.1016/S1089-3261(03)00104-1]
- Bogdanos DP**, Komorowski L. Disease-specific autoantibodies in primary biliary cirrhosis. *Clin Chim Acta* 2011; **412**: 502-512 [PMID: 21185272 DOI: 10.1016/j.cca.2010.12.019]
- Metcalfe JV**, Mitchison HC, Palmer JM, Jones DE, Bassendine MF, James OF. Natural history of early primary biliary cirrhosis. *Lancet* 1996; **348**: 1399-1402 [PMID: 8937278 DOI: 10.1016/S0140-6736(96)04410-8]
- Van de Water J**, Gershwin ME, Leung P, Ansari A, Coppel RL. The autoepitope of the 74-kD mitochondrial autoantigen of primary biliary cirrhosis corresponds to the functional site of dihydrolipoamide acetyltransferase. *J Exp Med* 1988; **167**: 1791-1799 [PMID: 2455013 DOI: 10.1084/jem.167.6.1791]
- Hirschfield GM**, Gershwin ME. The immunobiology and pathophysiology of primary biliary cirrhosis. *Annu Rev Pathol* 2013; **8**: 303-330 [PMID: 23347352 DOI: 10.1146/annurev-pathol-020712-164014]
- Smyk DS**, Rigopoulou EI, Bogdanos DP. Potential Roles for Infectious Agents in the Pathophysiology of Primary Biliary Cirrhosis: What's New? *Curr Infect Dis Rep* 2013; **15**: 14-24 [PMID: 23188623 DOI: 10.1007/s11908-012-0304-2]
- Bogdanos DP**, Baum H, Grasso A, Okamoto M, Butler P, Ma Y, Rigopoulou E, Montalto P, Davies ET, Burroughs AK, Vergani D. Microbial mimics are major targets of crossreactivity with human pyruvate dehydrogenase in primary biliary cirrhosis. *J Hepatol* 2004; **40**: 31-39 [PMID: 14672611 DOI: 10.1016/S0168-8278(03)00501-4]
- Burroughs AK**, Rosenstein IJ, Epstein O, Hamilton-Miller JM, Brumfitt W, Sherlock S. Bacteriuria and primary biliary cirrhosis. *Gut* 1984; **25**: 133-137 [PMID: 6363217 DOI: 10.1136/gut.25.2.133]
- Shigematsu H**, Shimoda S, Nakamura M, Matsushita S, Nishimura Y, Sakamoto N, Ichiki Y, Niho Y, Gershwin ME, Ishibashi H. Fine specificity of T cells reactive to human PDC-E2 163-176 peptide, the immunodominant autoantigen in primary biliary cirrhosis: implications for molecular mimicry and cross-recognition among mitochondrial autoantigens. *Hepatology* 2000; **32**: 901-909 [PMID: 11050037 DOI: 10.1053/jhep.2000.18714]
- Shimoda S**, Van de Water J, Ansari A, Nakamura M, Ishibashi H, Coppel RL, Lake J, Keeffe EB, Roche TE, Gershwin ME. Identification and precursor frequency analysis of a common T cell epitope motif in mitochondrial autoantigens in primary biliary cirrhosis. *J Clin Invest* 1998; **102**: 1831-1840 [PMID: 9819369 DOI: 10.1172/JCI4213]
- Shimoda S**, Nakamura M, Shigematsu H, Tanimoto H, Gushima T, Gershwin ME, Ishibashi H. Mimicry peptides of human PDC-E2 163-176 peptide, the immunodominant T-cell epitope of primary biliary cirrhosis. *Hepatology* 2000; **31**: 1212-1216 [PMID: 10827144 DOI: 10.1053/jhep.2000.8090]
- Mohammed JP**, Fusakio ME, Rainbow DB, Moule C, Fraser HI, Clark J, Todd JA, Peterson LB, Savage PB, Wills-Karp M, Ridgway WM, Wicker LS, Mattner J. Identification of Cd101 as a susceptibility gene for *Novosphingobium aromaticivorans*-induced liver autoimmunity. *J Immunol* 2011; **187**: 337-349 [PMID: 21613619 DOI: 10.4049/jimmunol.1003525]
- Olafsson S**, Gudjonsson H, Selmi C, Amano K, Invernizzi P, Podda



- M, Gershwin ME. Antimitochondrial antibodies and reactivity to N. aromaticivorans proteins in Icelandic patients with primary biliary cirrhosis and their relatives. *Am J Gastroenterol* 2004; **99**: 2143-2146 [PMID: 15554994 DOI: 10.1111/j.1572-0241.2004.40397.x]
- 17 Selmi C, Balkwill DL, Invernizzi P, Ansari AA, Coppel RL, Podda M, Leung PS, Kenny TP, Van De Water J, Nantz MH, Kurth MJ, Gershwin ME. Patients with primary biliary cirrhosis react against a ubiquitous xenobiotic-metabolizing bacterium. *Hepatology* 2003; **38**: 1250-1257 [PMID: 14578864 DOI: 10.1053/jhep.2003.50446]
- 18 Selmi C, Mayo MJ, Bach N, Ishibashi H, Invernizzi P, Gish RG, Gordon SC, Wright HI, Zweiban B, Podda M, Gershwin ME. Primary biliary cirrhosis in monozygotic and dizygotic twins: genetics, epigenetics, and environment. *Gastroenterology* 2004; **127**: 485-492 [PMID: 15300581 DOI: 10.1053/j.gastro.2004.05.005]
- 19 Donaldson PT, Baragiotta A, Heneghan MA, Floreani A, Venturi C, Underhill JA, Jones DE, James OF, Bassendine MF. HLA class II alleles, genotypes, haplotypes, and amino acids in primary biliary cirrhosis: a large-scale study. *Hepatology* 2006; **44**: 667-674 [PMID: 16941709 DOI: 10.1002/hep.21316]
- 20 Invernizzi P, Selmi C, Poli F, Frison S, Floreani A, Alvaro D, Almasio P, Rosina F, Marziani M, Fabris L, Muratori L, Qi L, Seldin MF, Gershwin ME, Podda M. Human leukocyte antigen polymorphisms in Italian primary biliary cirrhosis: a multicenter study of 664 patients and 1992 healthy controls. *Hepatology* 2008; **48**: 1906-1912 [PMID: 19003916 DOI: 10.1002/hep.22567]
- 21 Hirschfield GM, Liu X, Xu C, Lu Y, Xie G, Lu Y, Gu X, Walker EJ, Jing K, Juran BD, Mason AL, Myers RP, Peltekian KM, Ghent CN, Coltescu C, Atkinson EJ, Heathcote EJ, Lazaridis KN, Amos CI, Siminovich KA. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *N Engl J Med* 2009; **360**: 2544-2555 [PMID: 19458352 DOI: 10.1056/NEJMoa0810440]
- 22 Liu X, Invernizzi P, Lu Y, Kosoy R, Lu Y, Bianchi I, Podda M, Xu C, Xie G, Macciardi F, Selmi C, Lupoli S, Shigeta R, Ransom M, Lleo A, Lee AT, Mason AL, Myers RP, Peltekian KM, Ghent CN, Bernuzzi F, Zuin M, Rosina F, Borghesio E, Floreani A, Lazzari R, Niro G, Andriulli A, Muratori L, Muratori P, Almasio PL, Andreone P, Margotti M, Brunetto M, Coco B, Alvaro D, Bragazzi MC, Marra F, Pisano A, Rigamonti C, Colombo M, Marziani M, Benedetti A, Fabris L, Strazzabosco M, Portincasa P, Palmieri VO, Tiribelli C, Croce L, Bruno S, Rossi S, Vinci M, Prisco C, Mattalia A, Toniutto P, Picciotto A, Galli A, Ferrari C, Colombo S, Casella G, Morini L, Caporaso N, Colli A, Spinzi G, Montanari R, Gregersen PK, Heathcote EJ, Hirschfield GM, Siminovich KA, Amos CI, Gershwin ME, Seldin MF. Genome-wide meta-analyses identify three loci associated with primary biliary cirrhosis. *Nat Genet* 2010; **42**: 658-660 [PMID: 20639880 DOI: 10.1038/ng.627]
- 23 Mells GF, Floyd JA, Morley KI, Cordell HJ, Franklin CS, Shin SY, Heneghan MA, Neuberger JM, Donaldson PT, Day DB, Ducker SJ, Muriithi AW, Wheat EF, Hammond CJ, Dawwas MF, Jones DE, Peltonen L, Alexander GJ, Sandford RN, Anderson CA. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. *Nat Genet* 2011; **43**: 329-332 [PMID: 21399635 DOI: 10.1038/ng.789]
- 24 Lindor K. Ursodeoxycholic acid for the treatment of primary biliary cirrhosis. *N Engl J Med* 2007; **357**: 1524-1529 [PMID: 17928600 DOI: 10.1056/NEJMct074694]
- 25 Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ. Primary biliary cirrhosis. *Hepatology* 2009; **50**: 291-308 [PMID: 19554543 DOI: 10.1002/hep.22906]
- 26 Portmann B, Zen Y. Inflammatory disease of the bile ducts-cholangiopathies: liver biopsy challenge and clinicopathological correlation. *Histopathology* 2012; **60**: 236-248 [PMID: 21668470 DOI: 10.1111/j.1365-2559.2011.03853.x]
- 27 Molodecky NA, Kareemi H, Parab R, Barkema HW, Quan H, Myers RP, Kaplan GG. Incidence of primary sclerosing cholangitis: a systematic review and meta-analysis. *Hepatology* 2011; **53**: 1590-1599 [PMID: 21351115 DOI: 10.1002/hep.24247]
- 28 Hirschfield GM, Karlsen TH, Lindor KD, Adams DH. Primary sclerosing cholangitis. *Lancet* 2013; **382**: 1587-1599 [PMID: 23810223 DOI: 10.1016/S0140-6736(13)60096-3]
- 29 MacCarty RL, LaRusso NF, Wiesner RH, Ludwig J. Primary sclerosing cholangitis: findings on cholangiography and pancreatography. *Radiology* 1983; **149**: 39-44 [PMID: 6412283 DOI: 10.1148/radiology.149.1.6412283]
- 30 Boonstra K, van Erpecum KJ, van Nieuwkerk KM, Drenth JP, Poen AC, Witteman BJ, Tuynman HA, Beuers U, Ponsioen CY. Primary sclerosing cholangitis is associated with a distinct phenotype of inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 2270-2276 [PMID: 22407885 DOI: 10.1002/ibd.22938]
- 31 Johansson-Lindbom B, Svensson M, Pabst O, Palmqvist C, Marquez G, Förster R, Agace WW. Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. *J Exp Med* 2005; **202**: 1063-1073 [PMID: 16216890 DOI: 10.1084/jem.20051100]
- 32 Trivedi PJ, Adams DH. Mucosal immunity in liver autoimmunity: a comprehensive review. *J Autoimmun* 2013; **46**: 97-111 [PMID: 23891169 DOI: 10.1016/j.jaut.2013.06.013]
- 33 Briskin M, Winsor-Hines D, Shyjan A, Cochran N, Bloom S, Wilson J, McEvoy LM, Butcher EC, Kassam N, Mackay CR, Newman W, Ringler DJ. Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue. *Am J Pathol* 1997; **151**: 97-110 [PMID: 9212736]
- 34 Grant AJ, Lalor PF, Hübscher SG, Briskin M, Adams DH. MAdCAM-1 expressed in chronic inflammatory liver disease supports mucosal lymphocyte adhesion to hepatic endothelium (MAdCAM-1 in chronic inflammatory liver disease). *Hepatology* 2001; **33**: 1065-1072 [PMID: 11343233 DOI: 10.1053/jhep.2001.24231]
- 35 Eksteen B, Grant AJ, Miles A, Curbishley SM, Lalor PF, Hübscher SG, Briskin M, Salmon M, Adams DH. Hepatic endothelial CCL25 mediates the recruitment of CCR9+ gut-homing lymphocytes to the liver in primary sclerosing cholangitis. *J Exp Med* 2004; **200**: 1511-1517 [PMID: 15557349 DOI: 10.1084/jem.20041035]
- 36 Pollheimer MJ, Halilbasic E, Fickert P, Trauner M. Pathogenesis of primary sclerosing cholangitis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 727-739 [PMID: 22117638 DOI: 10.1016/j.bpg.2011.10.009]
- 37 Karlsen TH, Franke A, Melum E, Kaser A, Hov JR, Balschun T, Lie BA, Bergquist A, Schramm C, Weismüller TJ, Gotthardt D, Rust C, Philipp EE, Fritz T, Henckaerts L, Weersma RK, Stokkers P, Ponsioen CY, Wijmenga C, Sterneck M, Nothnagel M, Hampe J, Teufel A, Runz H, Rosenstiel P, Stiehl A, Vermeire S, Beuers U, Manns MP, Schrumpf E, Boberg KM, Schreiber S. Genome-wide association analysis in primary sclerosing cholangitis. *Gastroenterology* 2010; **138**: 1102-1111 [PMID: 19944697 DOI: 10.1053/j.gastro.2009.11.046]
- 38 Melum E, Franke A, Schramm C, Weismüller TJ, Gotthardt DN, Offner FA, Juran BD, Laerdahl JK, Labi V, Björnsson E, Weersma RK, Henckaerts L, Teufel A, Rust C, Ellinghaus E, Balschun T, Boberg KM, Ellinghaus D, Bergquist A, Sauer P, Ryu E, Hov JR, Wedemeyer J, Lindkvist B, Wittig M, Porte RJ, Holm K, Gieger C, Wichmann HE, Stokkers P, Ponsioen CY, Runz H, Stiehl A, Wijmenga C, Sterneck M, Vermeire S, Beuers U, Villunger A, Schrumpf E, Lazaridis KN, Manns MP, Schreiber S, Karlsen TH. Genome-wide association analysis in primary sclerosing cholangitis identifies two non-HLA susceptibility loci. *Nat Genet* 2011; **43**: 17-19 [PMID: 21151127 DOI: 10.1038/ng.728]
- 39 Liu JZ, Hov JR, Folseraas T, Ellinghaus E, Rushbrook SM, Doncheva NT, Andreassen OA, Weersma RK, Weismüller TJ, Eksteen B, Invernizzi P, Hirschfield GM, Gotthardt DN, Pares A, Ellinghaus D, Shah T, Juran BD, Milkiewicz P, Rust C, Schramm C, Müller T, Srivastava B, Dalekos G, Nöthen MM, Herms S, Winkelmann J, Mitrovic M, Braun F, Ponsioen CY, Croucher PJ, Sterneck M, Teufel A, Mason AL, Saarela J, Leppä V, Dorfman R, Alvaro D, Floreani A, Onengut-Gumuscu S, Rich SS, Thompson WK, Schork AJ, Næss S, Thomsen I, Mayr G, König IR, Hveem K, Cleynen I, Gutierrez-Achury J, Ricaño-Ponce I, van Heel D, Björnsson E, Sandford RN, Durie PR, Melum E, Vatn MH,



- Silverberg MS, Duerr RH, Padyukov L, Brand S, Sans M, Anness V, Achkar JP, Boberg KM, Marschall HU, Chazouillères O, Bowlus CL, Wijmenga C, Schrupp E, Vermeire S, Albrecht M, Rioux JD, Alexander G, Bergquist A, Cho J, Schreiber S, Manns MP, Färkkilä M, Dale AM, Chapman RW, Lazaridis KN, Franke A, Anderson CA, Karlsen TH. Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis. *Nat Genet* 2013; **45**: 670-675 [PMID: 23603763 DOI: 10.1038/ng.2616]
- 40 Manns MP, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, Vierling JM. Diagnosis and management of autoimmune hepatitis. *Hepatology* 2010; **51**: 2193-2213 [PMID: 20513004 DOI: 10.1002/hep.23584]
- 41 Vergani D, Alvarez F, Bianchi FB, Cancado EL, Mackay IR, Manns MP, Nishioka M, Penner E. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. *J Hepatol* 2004; **41**: 677-683 [PMID: 15464251 DOI: 10.1016/j.jhep.2004.08.002]
- 42 Mieli-Vergani G, Vergani D. Paediatric autoimmune liver disease. *Arch Dis Child* 2013; **98**: 1012-1017 [PMID: 24001955 DOI: 10.1136/archdischild-2013-303848]
- 43 Bogdanos DP, Choudhuri K, Vergani D. Molecular mimicry and autoimmune liver disease: virtuous intentions, malign consequences. *Liver* 2001; **21**: 225-232 [PMID: 11454184 DOI: 10.1034/j.1600-0676.2001.021004225.x]
- 44 Kerkar N, Choudhuri K, Ma Y, Mahmoud A, Bogdanos DP, Muratori L, Bianchi F, Williams R, Mieli-Vergani G, Vergani D. Cytochrome P4502D6(193-212): a new immunodominant epitope and target of virus/self cross-reactivity in liver kidney microsomal autoantibody type 1-positive liver disease. *J Immunol* 2003; **170**: 1481-1489 [PMID: 12538711 DOI: 10.4049/jimmunol.170.3.1481]
- 45 Donaldson PT. Genetics in autoimmune hepatitis. *Semin Liver Dis* 2002; **22**: 353-364 [PMID: 12447707 DOI: 10.1055/s-2002-35705]
- 46 Donaldson PT, Doherty DG, Hayllar KM, McFarlane IG, Johnson PJ, Williams R. Susceptibility to autoimmune chronic active hepatitis: human leukocyte antigens DR4 and A1-B8-DR3 are independent risk factors. *Hepatology* 1991; **13**: 701-706 [PMID: 2010165 DOI: 10.1002/hep.1840130415]
- 47 Djilali-Saiah I, Fakhfakh A, Louafi H, Caillat-Zucman S, Debray D, Alvarez F. HLA class II influences humoral autoimmunity in patients with type 2 autoimmune hepatitis. *J Hepatol* 2006; **45**: 844-850 [PMID: 17050030 DOI: 10.1016/j.jhep.2006.07.034]
- 48 Agarwal K, Czaja AJ, Jones DE, Donaldson PT. Cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphisms and susceptibility to type 1 autoimmune hepatitis. *Hepatology* 2000; **31**: 49-53 [PMID: 10613727 DOI: 10.1002/hep.510310110]
- 49 Cookson S, Constantini PK, Clare M, Underhill JA, Bernal W, Czaja AJ, Donaldson PT. Frequency and nature of cytokine gene polymorphisms in type 1 autoimmune hepatitis. *Hepatology* 1999; **30**: 851-856 [PMID: 10498633 DOI: 10.1002/hep.510300412]
- 50 Agarwal K, Czaja AJ, Donaldson PT. A functional Fas promoter polymorphism is associated with a severe phenotype in type 1 autoimmune hepatitis characterized by early development of cirrhosis. *Tissue Antigens* 2007; **69**: 227-235 [PMID: 17493146 DOI: 10.1111/j.1399-0039.2006.00794.x]
- 51 Krawitt EL. Autoimmune hepatitis. *N Engl J Med* 2006; **354**: 54-66 [PMID: 16394302 DOI: 10.1056/NEJMra050408]
- 52 Chazouillères O, Wendum D, Serfaty L, Montembault S, Rosmorduc O, Poupon R. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy. *Hepatology* 1998; **28**: 296-301 [PMID: 9695990 DOI: 10.1002/hep.510280203]
- 53 Klöppel G, Seifert G, Lindner H, Dammermann R, Sack HJ, Berg PA. Histopathological features in mixed types of chronic aggressive hepatitis and primary biliary cirrhosis. Correlations of liver histology with mitochondrial antibodies of different specificity. *Virchows Arch A Pathol Anat Histol* 1977; **373**: 143-160 [PMID: 139750 DOI: 10.1007/BF00432159]
- 54 European Association for the Study of the L. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. *J Hepatol* 2009; **51**: 237-267 [PMID: 19501929 DOI: 10.1016/j.jhep.2009.04.009]
- 55 Liberal R, Grant CR, Mieli-Vergani G, Vergani D. Autoimmune hepatitis: a comprehensive review. *J Autoimmun* 2013; **41**: 126-139 [PMID: 23218932 DOI: 10.1016/j.jaut.2012.11.002]
- 56 Gohlke F, Lohse AW, Dienes HP, Löhr H, Märker-Hermann E, Gerken G, Meyer zum Büschenfelde KH. Evidence for an overlap syndrome of autoimmune hepatitis and primary sclerosing cholangitis. *J Hepatol* 1996; **24**: 699-705 [PMID: 8835745 DOI: 10.1016/S0168-8278(96)80266-2]
- 57 el-Shabrawi M, Wilkinson ML, Portmann B, Mieli-Vergani G, Chong SK, Williams R, Mowat AP. Primary sclerosing cholangitis in childhood. *Gastroenterology* 1987; **92**: 1226-1235 [PMID: 3493939]
- 58 Floreani A, Rizzotto ER, Ferrara F, Carderi I, Caroli D, Blasone L, Baldo V. Clinical course and outcome of autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome. *Am J Gastroenterol* 2005; **100**: 1516-1522 [PMID: 15984974 DOI: 10.1111/j.1572-0241.2005.41841.x]
- 59 Kaya M, Angulo P, Lindor KD. Overlap of autoimmune hepatitis and primary sclerosing cholangitis: an evaluation of a modified scoring system. *J Hepatol* 2000; **33**: 537-542 [PMID: 11059857 DOI: 10.1034/j.1600-0641.2000.033004537.x]
- 60 Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet* 2014; **383**: 1749-1761 [PMID: 24480518 DOI: 10.1016/S0140-6736(14)60121-5]
- 61 Penz-Österreicher M, Österreicher CH, Trauner M. Fibrosis in autoimmune and cholestatic liver disease. *Best Pract Res Clin Gastroenterol* 2011; **25**: 245-258 [PMID: 21497742 DOI: 10.1016/j.bpg.2011.02.001]
- 62 Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI24282]
- 63 Magness ST, Bataller R, Yang L, Brenner DA. A dual reporter gene transgenic mouse demonstrates heterogeneity in hepatic fibrogenic cell populations. *Hepatology* 2004; **40**: 1151-1159 [PMID: 15389867 DOI: 10.1002/hep.20427]
- 64 Dranoff JA, Wells RG. Portal fibroblasts: Underappreciated mediators of biliary fibrosis. *Hepatology* 2010; **51**: 1438-1444 [PMID: 20209607 DOI: 10.1002/hep.23405]
- 65 Alvaro D, Metalli VD, Alpini G, Onori P, Franchitto A, Barbaro B, Glaser SS, Francis H, Cantafora A, Blotta I, Attili AF, Gaudio E. The intrahepatic biliary epithelium is a target of the growth hormone/insulin-like growth factor 1 axis. *J Hepatol* 2005; **43**: 875-883 [PMID: 16083987 DOI: 10.1016/j.jhep.2005.04.011]
- 66 Cramer T, Schuppan D, Bauer M, Pfander D, Neuhaus P, Herbst H. Hepatocyte growth factor and c-Met expression in rat and human liver fibrosis. *Liver Int* 2004; **24**: 335-344 [PMID: 15287857 DOI: 10.1111/j.1478-3231.2004.0926.x]
- 67 Fabris L, Strazzabosco M, Crosby HA, Ballardini G, Hubscher SG, Kelly DA, Neuberger JM, Strain AJ, Joplin R. Characterization and isolation of ductular cells coexpressing neural cell adhesion molecule and Bcl-2 from primary cholangiopathies and ductal plate malformations. *Am J Pathol* 2000; **156**: 1599-1612 [PMID: 10793072 DOI: 10.1016/S0002-9440(10)65032-8]
- 68 Grappone C, Pinzani M, Parola M, Pellegrini G, Caligiuri A, DeFranco R, Marra F, Herbst H, Alpini G, Milani S. Expression of platelet-derived growth factor in newly formed cholangiocytes during experimental biliary fibrosis in rats. *J Hepatol* 1999; **31**: 100-109 [PMID: 10424289 DOI: 10.1016/S0168-8278(99)80169-X]
- 69 Luo B, Tang L, Wang Z, Zhang J, Ling Y, Feng W, Sun JZ, Stockard CR, Frost AR, Chen YF, Grizzle WE, Fallon MB. Cholangiocyte endothelin 1 and transforming growth factor beta1 production in rat experimental hepatopulmonary syndrome. *Gastroenterology* 2005; **129**: 682-695 [PMID: 16083721 DOI: 10.1016/j.gastro.2005.05.050]
- 70 Rockey DC, Fouassier L, Chung JJ, Carayon A, Vallee P, Rey C, Housset C. Cellular localization of endothelin-1 and increased production in liver injury in the rat: potential for autocrine and paracrine effects on stellate cells. *Hepatology* 1998; **27**: 472-480

- [PMID: 9462646 DOI: 10.1002/hep.510270222]
- 71 **Hofmann AF**, Hagey LR. Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. *Cell Mol Life Sci* 2008; **65**: 2461-2483 [PMID: 18488143 DOI: 10.1007/s00018-008-7568-6]
  - 72 **Svegliati-Baroni G**, Ridolfi F, Hannivoort R, Saccomanno S, Homan M, De Minicis S, Jansen PL, Candelaresi C, Benedetti A, Moshage H. Bile acids induce hepatic stellate cell proliferation via activation of the epidermal growth factor receptor. *Gastroenterology* 2005; **128**: 1042-1055 [PMID: 15825085 DOI: 10.1053/j.gastro.2005.01.007]
  - 73 **Choi SS**, Diehl AM. Epithelial-to-mesenchymal transitions in the liver. *Hepatology* 2009; **50**: 2007-2013 [PMID: 19824076 DOI: 10.1002/hep.23196]
  - 74 **Díaz R**, Kim JW, Hui JJ, Li Z, Swain GP, Fong KS, Csizsar K, Russo PA, Rand EB, Furth EE, Wells RG. Evidence for the epithelial to mesenchymal transition in biliary atresia fibrosis. *Hum Pathol* 2008; **39**: 102-115 [PMID: 17900655 DOI: 10.1016/j.humpath.2007.05.021]
  - 75 **Robertson H**, Kirby JA, Yip WW, Jones DE, Burt AD. Biliary epithelial-mesenchymal transition in posttransplantation recurrence of primary biliary cirrhosis. *Hepatology* 2007; **45**: 977-981 [PMID: 17393507 DOI: 10.1002/hep.21624]
  - 76 **Chu AS**, Diaz R, Hui JJ, Yanger K, Zong Y, Alpini G, Stanger BZ, Wells RG. Lineage tracing demonstrates no evidence of cholangiocyte epithelial-to-mesenchymal transition in murine models of hepatic fibrosis. *Hepatology* 2011; **53**: 1685-1695 [PMID: 21520179 DOI: 10.1002/hep.24206]
  - 77 **Taura K**, Miura K, Iwaisako K, Osterreicher CH, Kodama Y, Penz-Osterreicher M, Brenner DA. Hepatocytes do not undergo epithelial-mesenchymal transition in liver fibrosis in mice. *Hepatology* 2010; **51**: 1027-1036 [PMID: 20052656 DOI: 10.1002/hep.23368]
  - 78 **Udell JA**, Wang CS, Tinmouth J, FitzGerald JM, Ayas NT, Simel DL, Schulzer M, Mak E, Yoshida EM. Does this patient with liver disease have cirrhosis? *JAMA* 2012; **307**: 832-842 [PMID: 22357834 DOI: 10.1001/jama.2012.186]
  - 79 **Garcia-Tsao G**, Bosch J, Groszmann RJ. Portal hypertension and variceal bleeding--unresolved issues. Summary of an American Association for the study of liver diseases and European Association for the study of the liver single-topic conference. *Hepatology* 2008; **47**: 1764-1772 [PMID: 18435460 DOI: 10.1002/hep.22273]
  - 80 **Ginés P**, Quintero E, Arroyo V, Terés J, Bruguera M, Rimola A, Caballería J, Rodés J, Rozman C. Compensated cirrhosis: natural history and prognostic factors. *Hepatology* 1987; **7**: 122-128 [PMID: 3804191 DOI: 10.1002/hep.1840070124]
  - 81 **Saunders JB**, Walters JR, Davies AP, Paton A. A 20-year prospective study of cirrhosis. *Br Med J (Clin Res Ed)* 1981; **282**: 263-266 [PMID: 6779978 DOI: 10.1136/bmj.282.6260.263]
  - 82 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]
  - 83 **de Franchis R**. Evolving consensus in portal hypertension. Report of the Baveno IV consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2005; **43**: 167-176 [PMID: 15925423 DOI: 10.1016/j.jhep.2005.05.009]
  - 84 **Zipprich A**, Garcia-Tsao G, Rogowski S, Fleig WE, Seufferlein T, Dollinger MM. Prognostic indicators of survival in patients with compensated and decompensated cirrhosis. *Liver Int* 2012; **32**: 1407-1414 [PMID: 22679906 DOI: 10.1111/j.1478-3231.2012.02830.x]
  - 85 **Carbonell N**, Pauwels A, Serfaty L, Fourdan O, Lévy VG, Poupon R. Improved survival after variceal bleeding in patients with cirrhosis over the past two decades. *Hepatology* 2004; **40**: 652-659 [PMID: 15349904 DOI: 10.1002/hep.20339]
  - 86 **Fleming KM**, Aithal GP, Card TR, West J. The rate of decompensation and clinical progression of disease in people with cirrhosis: a cohort study. *Aliment Pharmacol Ther* 2010; **32**: 1343-1350 [PMID: 21050236 DOI: 10.1111/j.1365-2036.2010.04473.x]
  - 87 **Wendon J**, Bernal W, Willars C, Auzinger G. Critical care and cirrhosis: outcome and benefit. *Curr Opin Crit Care* 2011; **17**: 533-537 [PMID: 21844797 DOI: 10.1097/MCC.0b013e32834ab06f]
  - 88 **Kamath PS**, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: 11172350 DOI: 10.1053/jhep.2001.22172]
  - 89 **Bianchi L**. Liver biopsy in elevated liver functions tests? An old question revisited. *J Hepatol* 2001; **35**: 290-294 [PMID: 11580154]
  - 90 **Castera L**, Pinzani M. Non-invasive assessment of liver fibrosis: are we ready? *Lancet* 2010; **375**: 1419-1420 [PMID: 20417845 DOI: 10.1016/S0140-6736(09)62195-4]
  - 91 **Pinzani M**, Vizzutti F, Arena U, Marra F. Technology Insight: noninvasive assessment of liver fibrosis by biochemical scores and elastography. *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 95-106 [PMID: 18253138 DOI: 10.1038/ncpgasthep1025]
  - 92 **Corpechot C**, Carrat F, Poujol-Robert A, Gaouar F, Wendum D, Chazouillères O, Poupon R. Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis. *Hepatology* 2012; **56**: 198-208 [PMID: 22271046 DOI: 10.1002/hep.25599]
  - 93 **Floreani A**, Cazzagon N, Martinez D, Cavalletto L, Baldo V, Chemello L. Performance and utility of transient elastography and noninvasive markers of liver fibrosis in primary biliary cirrhosis. *Dig Liver Dis* 2011; **43**: 887-892 [PMID: 21783442 DOI: 10.1016/j.dld.2011.06.011]
  - 94 **Friedrich-Rust M**, Müller C, Winckler A, Kriener S, Herrmann E, Holtmeier J, Poynard T, Vogl TJ, Zeuzem S, Hammerstingl R, Sarrazin C. Assessment of liver fibrosis and steatosis in PBC with FibroScan, MRI, MR-spectroscopy, and serum markers. *J Clin Gastroenterol* 2010; **44**: 58-65 [PMID: 19581812 DOI: 10.1097/MCG.0b013e3181a84b8d]
  - 95 **Corpechot C**, El Naggar A, Poujol-Robert A, Ziol M, Wendum D, Chazouillères O, de Lédinghen V, Dhumeaux D, Marcellin P, Beaugrand M, Poupon R. Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. *Hepatology* 2006; **43**: 1118-1124 [PMID: 16628644 DOI: 10.1002/hep.21151]
  - 96 **Malik R**, Lai M, Sadiq A, Farnan R, Mehta S, Nasser I, Challies T, Schuppan D, Afdhal N. Comparison of transient elastography, serum markers and clinical signs for the diagnosis of compensated cirrhosis. *J Gastroenterol Hepatol* 2010; **25**: 1562-1568 [PMID: 20796156 DOI: 10.1111/j.1440-1746.2010.06371.x]
  - 97 **Mayo MJ**. Natural history of primary biliary cirrhosis. *Clin Liver Dis* 2008; **12**: 277-288 [PMID: 18456180 DOI: 10.1016/j.cld.2008.02.012]
  - 98 **Shapiro JM**, Smith H, Schaffner F. Serum bilirubin: a prognostic factor in primary biliary cirrhosis. *Gut* 1979; **20**: 137-140 [PMID: 428825]
  - 99 **Prince MI**, James OF. The epidemiology of primary biliary cirrhosis. *Clin Liver Dis* 2003; **7**: 795-819 [PMID: 14594132]
  - 100 **Selmi C**, Bowlus CL, Gershwin ME, Coppel RL. Primary biliary cirrhosis. *Lancet* 2011; **377**: 1600-1609 [PMID: 21529926 DOI: 10.1016/S0140-6736(10)61965-4]
  - 101 **Corpechot C**, Carrat F, Bahr A, Chrétien Y, Poupon RE, Poupon R. The effect of ursodeoxycholic acid therapy on the natural course of primary biliary cirrhosis. *Gastroenterology* 2005; **128**: 297-303 [PMID: 15685541]
  - 102 **Poupon RE**, Lindor KD, Parés A, Chazouillères O, Poupon R, Heathcote EJ. Combined analysis of the effect of treatment with ursodeoxycholic acid on histologic progression in primary biliary cirrhosis. *J Hepatol* 2003; **39**: 12-16 [PMID: 12821038]
  - 103 **Lindor KD**, Jorgensen RA, Therneau TM, Malinchoc M, Dickson ER. Ursodeoxycholic acid delays the onset of esophageal varices in primary biliary cirrhosis. *Mayo Clin Proc* 1997; **72**: 1137-1140 [PMID: 9413293 DOI: 10.1016/S0025-6196(11)63676-8]
  - 104 **Lindor KD**, Therneau TM, Jorgensen RA, Malinchoc M, Dickson ER. Effects of ursodeoxycholic acid on survival in patients with primary biliary cirrhosis. *Gastroenterology* 1996; **110**: 1515-1518 [PMID: 8613058]
  - 105 **Poupon RE**, Balkau B, Eschwège E, Poupon R. A multicenter,

- controlled trial of ursodiol for the treatment of primary biliary cirrhosis. UDCA-PBC Study Group. *N Engl J Med* 1991; **324**: 1548-1554 [PMID: 1674105 DOI: 10.1056/NEJM199105303242204]
- 106 **Poupon RE**, Poupon R, Balkau B. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. *N Engl J Med* 1994; **330**: 1342-1347 [PMID: 8152446 DOI: 10.1056/NEJM199405123301903]
- 107 **Corpechot C**, Carrat F, Poupon R, Poupon RE. Primary biliary cirrhosis: incidence and predictive factors of cirrhosis development in ursodiol-treated patients. *Gastroenterology* 2002; **122**: 652-658 [PMID: 11874998]
- 108 **Ludwig J**, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). *Virchows Arch A Pathol Anat Histol* 1978; **379**: 103-112 [PMID: 150690 DOI: 10.1007/BF00432479]
- 109 **Scheuer PJ**. Primary biliary cirrhosis: diagnosis, pathology and pathogenesis. *Postgrad Med J* 1983; **59** Suppl 4: 106-115 [PMID: 6359113]
- 110 **Roll J**, Boyer JL, Barry D, Klatskin G. The prognostic importance of clinical and histologic features in asymptomatic and symptomatic primary biliary cirrhosis. *N Engl J Med* 1983; **308**: 1-7 [PMID: 6847917 DOI: 10.1056/NEJM198301063080101]
- 111 **Locke GR**, Therneau TM, Ludwig J, Dickson ER, Lindor KD. Time course of histological progression in primary biliary cirrhosis. *Hepatology* 1996; **23**: 52-56 [PMID: 8550048 DOI: 10.1002/hep.510230108]
- 112 **Prince M**, Chetwynd A, Newman W, Metcalf JV, James OF. Survival and symptom progression in a geographically based cohort of patients with primary biliary cirrhosis: follow-up for up to 28 years. *Gastroenterology* 2002; **123**: 1044-1051 [PMID: 12360466]
- 113 **Christensen E**, Neuberger J, Crowe J, Portmann B, Williams R, Altman DG, Popper H, Doniach D, Ranek L, Tygstrup N. Azathioprine and prognosis in primary biliary cirrhosis. *Gastroenterology* 1986; **90**: 508-509 [PMID: 3510149 DOI: 10.1016/0016-5085(86)90972-8]
- 114 **Gores GJ**, Wiesner RH, Dickson ER, Zinsmeister AR, Jorgensen RA, Langworthy A. Prospective evaluation of esophageal varices in primary biliary cirrhosis: development, natural history, and influence on survival. *Gastroenterology* 1989; **96**: 1552-1559 [PMID: 2785470]
- 115 **Lee J**, Belanger A, Doucette JT, Stanca C, Friedman S, Bach N. Transplantation trends in primary biliary cirrhosis. *Clin Gastroenterol Hepatol* 2007; **5**: 1313-1315 [PMID: 17900996 DOI: 10.1016/j.cgh.2007.07.015]
- 116 **Gong Y**, Huang Z, Christensen E, Glud C. Ursodeoxycholic acid for patients with primary biliary cirrhosis: an updated systematic review and meta-analysis of randomized clinical trials using Bayesian approach as sensitivity analyses. *Am J Gastroenterol* 2007; **102**: 1799-1807 [PMID: 17459023 DOI: 10.1111/j.1572-0241.2007.01235.x]
- 117 **Broomé U**, Olsson R, Lööf L, Bodemar G, Hultcrantz R, Danielsson A, Prytz H, Sandberg-Gertzén H, Wallerstedt S, Lindberg G. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. *Gut* 1996; **38**: 610-615 [PMID: 8707097 DOI: 10.1136/gut.38.4.610]
- 118 **Jenner RG**, Townsend MJ, Jackson I, Sun K, Bouwman RD, Young RA, Glimcher LH, Lord GM. The transcription factors T-bet and GATA-3 control alternative pathways of T-cell differentiation through a shared set of target genes. *Proc Natl Acad Sci USA* 2009; **106**: 17876-17881 [PMID: 19805038 DOI: 10.1073/pnas.0909357106]
- 119 **Mendes FD**, Lindor KD. Primary sclerosing cholangitis. *Clin Liver Dis* 2004; **8**: 195-211 [PMID: 15062201 DOI: 10.1016/S1089-3261(03)00127-2]
- 120 **Ludwig J**. Surgical pathology of the syndrome of primary sclerosing cholangitis. *Am J Surg Pathol* 1989; **13** Suppl 1: 43-49 [PMID: 2699167]
- 121 **Angulo P**, Larson DR, Therneau TM, LaRusso NF, Batts KP, Lindor KD. Time course of histological progression in primary sclerosing cholangitis. *Am J Gastroenterol* 1999; **94**: 3310-3313 [PMID: 10566735 DOI: 10.1111/j.1572-0241.1999.01543.x]
- 122 **Chapman R**, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ. Diagnosis and management of primary sclerosing cholangitis. *Hepatology* 2010; **51**: 660-678 [PMID: 20101749 DOI: 10.1002/hep.23294]
- 123 **Zein CO**, Lindor KD, Angulo P. Prevalence and predictors of esophageal varices in patients with primary sclerosing cholangitis. *Hepatology* 2004; **39**: 204-210 [PMID: 14752839 DOI: 10.1002/hep.20029]
- 124 **Werner M**, Prytz H, Ohlsson B, Almer S, Björnsson E, Bergquist A, Wallerstedt S, Sandberg-Gertzén H, Hultcrantz R, Sangfelt P, Weiland O, Danielsson A. Epidemiology and the initial presentation of autoimmune hepatitis in Sweden: a nationwide study. *Scand J Gastroenterol* 2008; **43**: 1232-1240 [PMID: 18609163 DOI: 10.1080/00365520802130183]
- 125 **Feld JJ**, Dinh H, Arenovich T, Marcus VA, Wanless IR, Heathcote EJ. Autoimmune hepatitis: effect of symptoms and cirrhosis on natural history and outcome. *Hepatology* 2005; **42**: 53-62 [PMID: 15954109 DOI: 10.1002/hep.20732]
- 126 **Ngu JH**, Bechly K, Chapman BA, Burt MJ, Barclay ML, Gearry RB, Stedman CA. Population-based epidemiology study of autoimmune hepatitis: a disease of older women? *J Gastroenterol Hepatol* 2010; **25**: 1681-1686 [PMID: 20880179 DOI: 10.1111/j.1440-1746.2010.06384.x]
- 127 **Verma S**, Gunuwan B, Mendler M, Govindarajan S, Redeker A. Factors predicting relapse and poor outcome in type I autoimmune hepatitis: role of cirrhosis development, patterns of transaminases during remission and plasma cell activity in the liver biopsy. *Am J Gastroenterol* 2004; **99**: 1510-1516 [PMID: 15307869 DOI: 10.1111/j.1572-0241.2004.30457.x]
- 128 **Roberts SK**, Therneau TM, Czaja AJ. Prognosis of histological cirrhosis in type I autoimmune hepatitis. *Gastroenterology* 1996; **110**: 848-857 [PMID: 8608895]
- 129 **Radhakrishnan KR**, Alkhouiri N, Worley S, Arrigain S, Hupertz V, Kay M, Yerian L, Wyllie R, Feldstein AE. Autoimmune hepatitis in children—impact of cirrhosis at presentation on natural history and long-term outcome. *Dig Liver Dis* 2010; **42**: 724-728 [PMID: 20163994 DOI: 10.1016/j.dld.2010.01.002]
- 130 **Ngu JH**, Gearry RB, Frampton CM, Stedman CA. Predictors of poor outcome in patients with autoimmune hepatitis: a population-based study. *Hepatology* 2013; **57**: 2399-2406 [PMID: 23359353 DOI: 10.1002/hep.26290]
- 131 **Gregorio GV**, Portmann B, Reid F, Donaldson PT, Doherty DG, McCartney M, Mowat AP, Vergani D, Mieli-Vergani G. Autoimmune hepatitis in childhood: a 20-year experience. *Hepatology* 1997; **25**: 541-547 [PMID: 9049195 DOI: 10.1002/hep.510250308]
- 132 **Ahmed A**, Keeffe EB. Current indications and contraindications for liver transplantation. *Clin Liver Dis* 2007; **11**: 227-247 [PMID: 17606204 DOI: 10.1016/j.cld.2007.04.008]
- 133 **Liberal R**, Zen Y, Mieli-Vergani G, Vergani D. Liver transplantation and autoimmune liver diseases. *Liver Transpl* 2013; **19**: 1065-1077 [PMID: 23873751 DOI: 10.1002/lt.23704]
- 134 **Kessler WR**, Cummings OW, Eckert G, Chalasani N, Lumeng L, Kwo PY. Fulminant hepatic failure as the initial presentation of acute autoimmune hepatitis. *Clin Gastroenterol Hepatol* 2004; **2**: 625-631 [PMID: 15224287]
- 135 **Ilyas JA**, O'Mahony CA, Vierling JM. Liver transplantation in autoimmune liver diseases. *Best Pract Res Clin Gastroenterol* 2011; **25**: 765-782 [PMID: 22117641 DOI: 10.1016/j.bpg.2011.09.008]
- 136 **Mottershead M**, Neuberger J. Transplantation in autoimmune liver diseases. *World J Gastroenterol* 2008; **14**: 3388-3395 [PMID: 18528936 DOI: 10.3748/wjg.14.3388]
- 137 **Freeman RB**, Gish RG, Harper A, Davis GL, Vierling J, Lieblein L, Klintmalm G, Blazek J, Hunter R, Punch J. Model for end-stage liver disease (MELD) exception guidelines: results and recommendations from the MELD Exception Study Group and Conference (MESSAGE) for the approval of patients who need liver transplantation with diseases not considered by the standard MELD formula. *Liver Transpl* 2006; **12**: S128-S136 [PMID:



- 17123284 DOI: 10.1002/lt.20979]
- 138 **Abraham SC**, Kamath PS, Eghtesad B, Demetris AJ, Krasinskas AM. Liver transplantation in precirrhotic biliary tract disease: Portal hypertension is frequently associated with nodular regenerative hyperplasia and obliterative portal venopathy. *Am J Surg Pathol* 2006; **30**: 1454-1461 [PMID: 17063088 DOI: 10.1097/01.pas.0000213286.65907.ea]
  - 139 **Milkiewicz P**, Wunsch E, Elias E. Liver transplantation in chronic cholestatic conditions. *Front Biosci* (Landmark Ed) 2012; **17**: 959-969 [PMID: 22201783 DOI: 10.2741/3966]
  - 140 **Neuberger J**. Liver transplantation for primary biliary cirrhosis: indications and risk of recurrence. *J Hepatol* 2003; **39**: 142-148 [PMID: 12873808]
  - 141 **Garcia CE**, Garcia RF, Gunson B, Christensen E, Neuberger J, McMaster P, Mirza DF. Analysis of marginal donor parameters in liver transplantation for primary biliary cirrhosis. *Exp Clin Transplant* 2004; **2**: 183-188 [PMID: 15859926]
  - 142 **Kashyap R**, Safadjou S, Chen R, Mantry P, Sharma R, Patil V, Maloo M, Ryan C, Marroquin C, Barry C, Ramaraju G, Maliakkal B, Orloff M. Living donor and deceased donor liver transplantation for autoimmune and cholestatic liver diseases--an analysis of the UNOS database. *J Gastrointest Surg* 2010; **14**: 1362-1369 [PMID: 20617395 DOI: 10.1007/s11605-010-1256-1]
  - 143 **Wiesner RH**. Liver transplantation for primary sclerosing cholangitis: timing, outcome, impact of inflammatory bowel disease and recurrence of disease. *Best Pract Res Clin Gastroenterol* 2001; **15**: 667-680 [PMID: 11492975 DOI: 10.1053/bega.2001.0212]
  - 144 **Freeman RB**, Edwards EB, Harper AM. Waiting list removal rates among patients with chronic and malignant liver diseases. *Am J Transplant* 2006; **6**: 1416-1421 [PMID: 16686765 DOI: 10.1111/j.1600-6143.2006.01321.x]
  - 145 **Leidenius M**, Höckersted K, Broomé U, Ericzon BG, Friman S, Olausson M, Schrumpf E. Hepatobiliary carcinoma in primary sclerosing cholangitis: a case control study. *J Hepatol* 2001; **34**: 792-798 [PMID: 11451160]
  - 146 **Goss JA**, Shackleton CR, Farmer DG, Arnaout WS, Seu P, Markowitz JS, Martin P, Stribling RJ, Goldstein LI, Busuttil RW. Orthotopic liver transplantation for primary sclerosing cholangitis. A 12-year single center experience. *Ann Surg* 1997; **225**: 472-481; discussion 481-483 [PMID: 9193175]
  - 147 **Graziadei IW**, Wiesner RH, Marotta PJ, Porayko MK, Hay JE, Charlton MR, Poterucha JJ, Rosen CB, Gores GJ, LaRusso NF, Krom RA. Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis. *Hepatology* 1999; **30**: 1121-1127 [PMID: 10534330 DOI: 10.1002/hep.510300501]
  - 148 **Cook GC**, Mulligan R, Sherlock S. Controlled prospective trial of corticosteroid therapy in active chronic hepatitis. *Q J Med* 1971; **40**: 159-185 [PMID: 4933363 DOI: 10.1093/oxfordjournals.qjmed.a067264]
  - 149 **Soloway RD**, Summerskill WH, Baggenstoss AH, Geall MG, Gitnick GL, Elveback IR, Schoenfield LJ. Clinical, biochemical, and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. *Gastroenterology* 1972; **63**: 820-833 [PMID: 4538724]
  - 150 **Murray-Lyon IM**, Stern RB, Williams R. Controlled trial of prednisone and azathioprine in active chronic hepatitis. *Lancet* 1973; **1**: 735-737 [PMID: 4121073 DOI: 10.1016/S0140-6736(73)92125-9]
  - 151 **Kogan J**, Safadi R, Ashur Y, Shouval D, Ilan Y. Prognosis of symptomatic versus asymptomatic autoimmune hepatitis: a study of 68 patients. *J Clin Gastroenterol* 2002; **35**: 75-81 [PMID: 12080231]
  - 152 **Reich DJ**, Fiel I, Guarrera JV, Emre S, Guy SR, Schwartz ME, Miller CM, Sheiner PA. Liver transplantation for autoimmune hepatitis. *Hepatology* 2000; **32**: 693-700 [PMID: 11003612 DOI: 10.1053/jhep.2000.16666]
  - 153 **Liberal R**, Longhi MS, Grant CR, Mieli-Vergani G, Vergani D. Autoimmune hepatitis after liver transplantation. *Clin Gastroenterol Hepatol* 2012; **10**: 346-353 [PMID: 22056300 DOI: 10.1016/j.cgh.2011.10.028]
  - 154 **Futagawa Y**, Terasaki PI. An analysis of the OPTN/UNOS Liver Transplant Registry. *Clin Transpl* 2004; 315-329 [PMID: 16704160]

**P- Reviewer:** Gonzalez-Reimers E, Vij M, Weng HL **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Li D





## Current status of diagnosis and treatment of hepatic echinococcosis

Memmet Mihmanli, Ufuk Oguz Idiz, Cemal Kaya, Uygur Demir, Ozgur Bostanci, Sinan Omeroglu, Emre Bozkurt

Memmet Mihmanli, Ufuk Oguz Idiz, Cemal Kaya, Uygur Demir, Ozgur Bostanci, Sinan Omeroglu, Emre Bozkurt, Department of General Surgery, Sisli Hamidiye Etfal Training and Research Hospital, 34371 Istanbul, Turkey

**Author contributions:** Mihmanli M and Idiz UO contributed equally to this work, generated the figures and wrote the manuscript; Kaya C, Demir U, Bostanci O, Omeroglu S and Bozkurt E contributed to the writing of the manuscript; Mihmanli M designed the aim of the editorial and wrote the manuscript.

**Conflict-of-interest statement:** The authors declare no conflict of interests for this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Memmet Mihmanli, MD, Professor, Department of General Surgery, Sisli Hamidiye Etfal Training and Research Hospital, Halaskargazi Caddesi, 34371 Istanbul, Turkey. [mmihmanli@yahoo.com](mailto:mmihmanli@yahoo.com)  
 Telephone: +90-53-22853159

Received: April 27, 2016  
 Peer-review started: April 28, 2016  
 First decision: June 16, 2016  
 Revised: June 21, 2016  
 Accepted: July 11, 2016  
 Article in press: July 13, 2016  
 Published online: October 8, 2016

### Abstract

*Echinococcus granulosus* (*E. granulosus*) and *Echino-*

*coccus multilocularis* (*E. multilocularis*) infections are the most common parasitic diseases that affect the liver. The disease course is typically slow and the patients tend to remain asymptomatic for many years. Often the diagnosis is incidental. Right upper quadrant abdominal pain, hepatitis, cholangitis, and anaphylaxis due to dissemination of the cyst are the main presenting symptoms. Ultrasonography is important in diagnosis. The World Health Organization classification, based on ultrasonographic findings, is used for staging of the disease and treatment selection. In addition to the imaging methods, immunological investigations are used to support the diagnosis. The available treatment options for *E. granulosus* infection include open surgery, percutaneous interventions, and pharmacotherapy. Aggressive surgery is the first-choice treatment for *E. multilocularis* infection, while pharmacotherapy is used as an adjunct to surgery. Due to a paucity of clinical studies, empirical evidence on the treatment of *E. granulosus* and *E. multilocularis* infections is largely lacking; there are no prominent and widely accepted clinical algorithms yet. In this article, we review the diagnosis and treatment of *E. granulosus* and *E. multilocularis* infections in the light of recent evidence.

**Key words:** *Echinococcus granulosus*; *Echinococcus multilocularis*; Liver; Ultrasonography; Albendazole

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** *Echinococcus granulosus* and *Echinococcus multilocularis* infections are the most common parasitic diseases of the liver. They could be asymptomatic for many years. Most of the asymptomatic patients are diagnosed incidentally. Ultrasonography is important in diagnosis. There is no standardized and widely accepted treatment approach.

Mihmanli M, Idiz UO, Kaya C, Demir U, Bostanci O, Omeroglu

S, Bozkurt E. Current status of diagnosis and treatment of hepatic echinococcosis. *World J Hepatol* 2016; 8(28): 1169-1181 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i28/1169.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i28.1169>

## INTRODUCTION

*Cystic echinococcus* (CE) is a parasitic illness, caused by infection with *Echinococcus granulosus* (*E. granulosus*) in its larval stage<sup>[1]</sup>. Although the disease occurs worldwide, it is endemic in Africa, South America, and Eurasia<sup>[2-4]</sup>. The liver is the most commonly affected organ; however, the lungs, spleen, kidney, brain, and breasts may be involved<sup>[5]</sup>. Mortality from CE is usually due to the development of complications and is reported to be 2%-4%<sup>[6,7]</sup>. The disease course is typically slow and most CE patients remain asymptomatic for several years. In addition, due to non-specific symptoms, the diagnosis is often incidental<sup>[8]</sup>. Hepatic alveolar echinococcus (AE) referring to the intrahepatic growth of the larvae of *Echinococcus multilocularis* (*E. multilocularis*) is a rare yet serious disease. When the epidemiology of AE is analyzed, it is striking that the disease is encountered in the northern hemisphere only<sup>[9]</sup>.

Complications of the echinococcal disease include allergic reactions to the dissemination of cyst contents due to spontaneous, traumatic or iatrogenic rupture, secondary infection, and cholangitis<sup>[3,10-12]</sup>. While most CE patients have a single cyst, 20%-40% tend to harbor multiple cysts<sup>[13]</sup>.

Although a wide range of treatment methods have been identified (medical, percutaneous, monitoring, and surgical), a standardized treatment protocol has yet to be defined.

In this article, we present an update on the diagnosis and treatment of the CE and AE diseases in the liver in the light of emanating evidence.

## E. GRANULOSUS INFECTION

### Life cycle

*E. granulosus* is a small sized tapeworm with 10 different genotypes. The definitive host of this parasite is the dog and other members of canids; the intermediate hosts include members of the ungulates such as sheep, goat, and pigs. The adult parasites localize in the liver of the definitive host; eggs are excreted *via* the stool of the host. Upon oral ingestion of the eggs by the intermediate host, the eggs hatch within the stomach and intestine. Oncosphere larvae emerge and cling onto the small intestine by its hooks. Subsequently, the oncosphere larvae migrate to organs such as the liver and lungs through the blood and lymph vessels. Humans are accidental hosts and not essential to the life-cycle of *Echinococcus*. Infection occurs after the oral ingestion of eggs. The eggs grow inside the host organs and form a cyst (hydatid cyst). Hydatid cysts are round in shape and

are usually filled with a clear fluid. The inner part of the cyst features a germinating membrane while the outer part features a laminated layer. In time, the parasite matures and evokes a granulomatous inflammatory reaction which leads to walling off of the cyst by fibrous tissue. In time, budding (germination) occurs from the germinative membrane and blisters are formed (Figure 1). The protoscolexes, which occur inside the organ that the definitive host consumed, open up and *Echinococcus* matures into adult from clinging onto the intestine of the definitive host, thus completing the cycle<sup>[14-17]</sup> (Figure 2).

### Clinical presentation

Most patients have an asymptomatic disease course. The most important reason for this is the slow growth rate of the cysts (1-5 mm per year). Therefore, symptoms usually develop in adulthood<sup>[13,14,18]</sup>. The most common presenting symptoms are discomfort in the right upper quadrant of abdomen and loss of appetite. Other symptoms may include pain caused by an increase in the size of the cyst, anaphylactic reaction<sup>[11]</sup> induced by the rupture of the cyst, hepatitis, and cholangitis due to biliary obstruction caused by the daughter vesicles<sup>[19]</sup>, secondary infection of the cyst, embolism<sup>[14]</sup>, and subphrenic or intracystic abscess<sup>[13]</sup>. In 90% of the patients, the cysts open into the biliary tract, which causes the complications listed above<sup>[20]</sup>. In approximately 10% of cases, intraperitoneal rupture of the cyst induces anaphylaxis. In addition, secondary CE may develop due to the rupture of the cyst, and this may lead to a much larger mass developing over a relatively short period<sup>[13]</sup>. Patients are usually diagnosed incidentally during radiological examination conducted for complaints unrelated to CE. During physical examination, hepatomegaly, palpable mass in one right upper quadrant, and abdominal distension may be encountered as well.

For patients who develop hepatitis, colic pain, portal hypertension, acidity, pressure in inferior vena cava, and Budd-Chiari syndrome, liver hemangioma, liver cysts, adenoma, liver abscess, hepatocellular cancer, liver metastasis, and in addition, liver *Echinococcus* should be taken into account during the differential diagnosis of the masses that are found in the liver<sup>[21,22]</sup>.

### Diagnosis

Most of CE patients at the asymptomatic early stage are diagnosed incidentally. Diagnosis relies on imaging and immunological tests. Ultrasonography is a convenient tool for diagnosis that indicates the location, number, and size of the cysts with relative ease<sup>[2,3,13,18,23,24]</sup>.

However, small-sized cysts may not be detected by ultrasonography. The criteria for classification of liver cysts on ultrasonography, which were first developed by Gharbi in 1981, were improved by the World Health Organization (WHO) in 2001 (WHO-IWGE)<sup>[25,26]</sup> (Tables 1 and 2). The WHO classification includes cysts of unknown origin and includes modified subtypes of the Types 2 and 3 cysts<sup>[14]</sup>. There are three categories of cysts: Active,

**Table 1 The Gharbi classification of hydatid cysts**

Type	Characteristics
I	Unilocular cyst, wall and internal echogenicities
II	Cyst with detached membrane (water-lily sign)
III	Multivesicular, multiseptated cyst, daughter cyst (honeycomb pattern)
IV	Hererogeneous cyst, no daughter vesicles
V	Cyst with partially or completely calcified wall

**Table 2 The World Health Organization classification of hydatid cysts**

WHO stage	Characteristics	Activity
CE1	Unilocular, anechoic cyst with double line sign	Active
CE2	Multiseptated "rosette-like" "honeycomb pattern" cyst	Active
CE3a	Cyst with detached membrane (water-lily sign)	Transitional
CE3b	Daughter cysts in solid matrix	Transitional
CE4	Hererogeneous cyst, no daughter vesicles	Inactive
CE5	Solid matrix with calcified wall	Inactive

WHO: World Health Organization.

transitional, and inactive<sup>[27]</sup>. Types 1 and 2 cysts are considered "active" while Type 3 cysts are considered "transitional". Types 4 and 5 cysts are categorized as "inactive"<sup>[27]</sup>. However, this classification has changed with the long term results of the medical and percutaneous treatment and the usage of the high-field magnetic resonance spectroscopy. Type 3 cysts, which are considered transitional, are further divided into two sub-groups: CE3a (separated endocysts) and CE3b (solid type containing daughter vesicle)<sup>[7,28]</sup>. Some studies have suggested that CE3a cysts are inactive while CE3b cysts are active<sup>[14,29]</sup>. Ultrasonography may also be used for monitoring of the lesion. For patients who have received treatment, post-treatment follow-up examinations every 3-6 mo until stabilization of the cyst, and annual examinations thereafter, are recommended. In general, a period of 5 years without recurrence is considered sufficient<sup>[30]</sup>. Magnetic resonance imaging (MRI) and computer tomography (CT) may be required in some cases, where ultrasonography fails to provide a definitive diagnosis. These include obese patients, patients with subdiaphragmatic cyst or secondary infection of cysts, complicated cases such as biliary fistula, cases with extra-abdominal spread, and patients who have a common disease. CT and MRI are particularly useful for pre-operative and follow-up examinations. Use of MRI for diagnosis and follow-up examination is known to be superior to CT<sup>[28,31,32]</sup>.

There are no workups amongst the routine blood workups that may be used specifically for CE. Hyperbilirubinemia and increased levels of alkaline phosphatase and gamma glutamyl transferase may indicate opening of the cyst into the biliary tract<sup>[15,30,33]</sup>. Although EC is

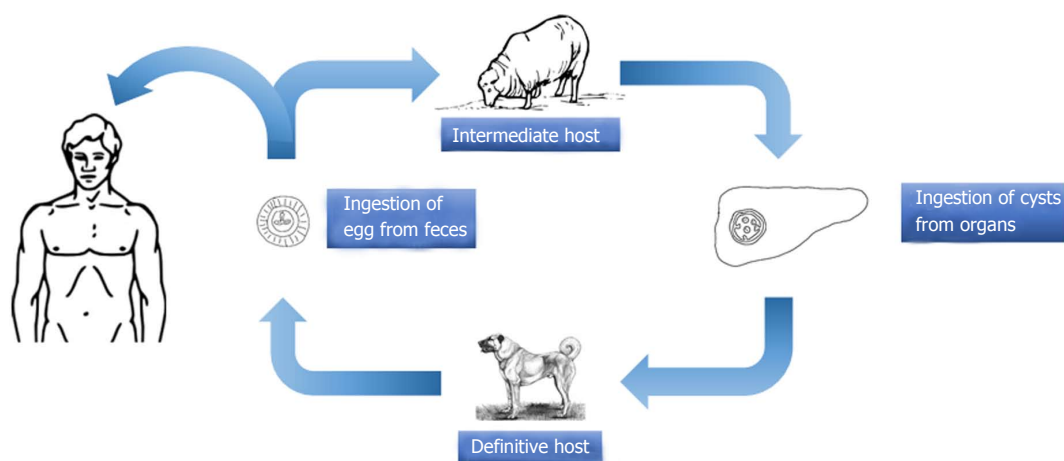
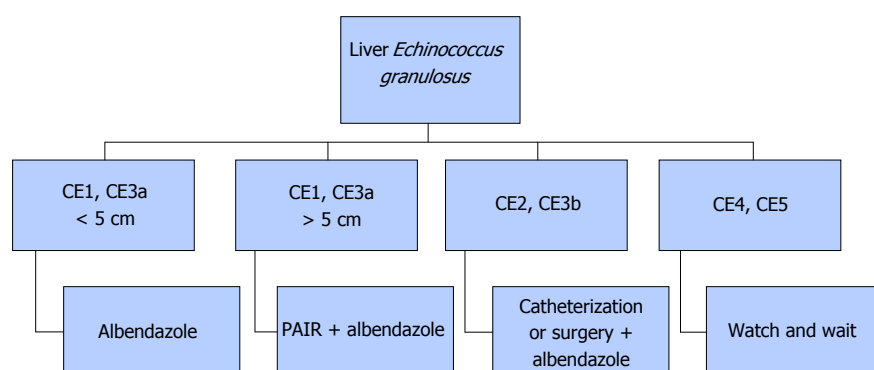
**Figure 1 Daughter vesicles of *Echinococcus granulosus*.**

a parasitic infection, eosinophilia may not be always present. Serologic diagnostic methods are used to support the radiological diagnosis and for follow-up assessment. The immunological response to the disease tends to vary from one individual to another. Rugged and intact cysts tend to show minimal immune response, while leaking or ruptured cysts tend to evoke a strong immune response<sup>[2,34,35]</sup>.

The indirect hemagglutination (IHA) is usually non-specific and is of value in tandem with other investigations such as enzyme-linked immunosorbent assay (ELISA) and immunoblotting<sup>[36]</sup>. Concomitant use of IHA and ELISA is associated with diagnostic sensitivity rates up to 85%-96%<sup>[37-41]</sup>. Immunoblotting is generally used to confirm the diagnosis in cases where IHA and ELISA findings are not definitive<sup>[14]</sup>. *E. granulosus* antigen B and antigen 5 (Ag5) are the most specific antigens used for immunological diagnosis<sup>[2,35]</sup>. However, these immunological methods often show cross reactivity with other parasitic antigens or with non-parasitic diseases such as malignancy or liver cirrhosis<sup>[15,42-45]</sup>. Sensitivity of the serological tests tends to vary with the location, stage, and size of the cyst<sup>[11]</sup>.

While seronegativity is observed in 20% of patients with CE, those with multiple cysts are usually seropositive. Rate of seronegativity is relatively higher in patients with CE1, CE4, and CE5 cyst types as compared to those with CE2 and CE3 types. Moreover, seropositive patients may continue to remain so for more than 10 years despite treatment<sup>[14,46-48]</sup>. This may lead to unnecessary treatment and an increase in costs.

Percutaneous fine needle aspiration (FNA) biopsy under ultrasound guidance is used in suspected cases with equivocal radiological and serological test results. Observing the protoscolexes and cyst membranes, or Echinococcal antigen or DNA in aspirated fluid confirms the diagnosis<sup>[49]</sup>. Percutaneous procedure requires meticulous care due to the associated risk of anaphylaxis; informed consent of the patient should be obtained prior to the procedure<sup>[50]</sup>. Anaphylaxis risk of FNA is 2.5%<sup>[51]</sup>. In order to prevent secondary CE, pretreatment with albendazole for 4 d prior to the biopsy and continuation of treatment for one month after the biopsy are recommended<sup>[20,52]</sup>.

Figure 2 Life cycle of *Echinococcus granulosus*.Figure 3 Treatment algorithm for *Echinococcus granulosus* infection. PAIR: Puncture, aspiration, injection of a scolecidal agent, and reaspiration.

### Treatment and management of *E. granulosus* infection

The treatment options for CE included surgery, percutaneous treatment, medical pharmacotherapy, and monitoring<sup>[10]</sup>. In the literature, there is no randomized clinical study that compares the treatment methods with each other. Therefore, there is no standardized and widely accepted treatment approach for CE either<sup>[14]</sup>. The treatment planning is done according to the WHO diagnostic classification. In case CE1 and CE3a cysts are < 5 cm in diameter, albendazole alone may suffice, while for cysts exceeding 5 cm in size, the puncture, aspiration, injection of a scolecidal agent, and reaspiration (PAIR) treatment in tandem with albendazole is preferred. Types CE2 and CE3b cysts are treated by catheterization or surgery. For types CE4 and CE5 inactive cysts, monitoring is often sufficient<sup>[10]</sup> (Figure 3).

**Medical treatment:** Exclusive medical pharmacotherapy is used in special cases where surgical or percutaneous treatment (such as elderly patients, cases with high comorbidity, patients who opt out of surgical and percutaneous treatment, and inoperable cases) is not suitable, or as an adjunct to surgical and percutaneous treatment.

Ever since benzimidazoles became available for use in 1970s, therapeutic efficacy of albendazole and mebendazole for larval stage of *E. granulosus* has been

proved<sup>[14]</sup>. At present, albendazole is the most commonly used drug in the treatment of *E. granulosus* infection<sup>[53]</sup>. The dose of albendazole is 10-15 mg/kg per day and the treatment usually lasts for 3-6 mo. Efficacy of mebendazole is comparable to that of albendazole, but requires higher doses for a longer period of time, due to its poor absorption<sup>[53-55]</sup>. The dose of mebendazole is 40-50 mg/kg per day for the patients who can not use albendazole.

With benzimidazoles, the duration of treatment is 3-6 mo without interruption for CE1, CE3a cysts that are < 5 cm<sup>[10,56]</sup>. Studies have demonstrated that 28.5%-58% of patients who undergo medical treatment are cured, and that cure rates do not increase with the increase in the duration of treatment<sup>[54,57-61]</sup>.

According to the recommendations of WHO, the medical treatment should be initiated 4-30 d prior to the surgical operation and continued for at least 1 mo thereafter for albendazole, and at least 3 mo for mebendazole. Medical pharmacotherapy is also indicated in patients with spontaneous or traumatic ruptured of cysts. In these cases, too, albendazole should be used for at least 1 mo or mebendazole for 3 mo<sup>[62-64]</sup>.

In a large study (929 cysts) of the effectiveness of medical therapy in late stages, albendazole therapy was associated with a significantly higher incidence of degenerative changes than that with mebendazole the-



rapy (82.2% vs 56.1%;  $P < 0.001$ ). However, the relapse rates were comparable between the two groups<sup>[65]</sup>.

Headache, nausea, neutropenia, hair loss, and hepatotoxicity are the most commonly reported side effects of albendazole and mebendazole. Monthly monitoring of leukocyte counts and liver function tests is recommended in patients who experience significant side effects. Contraindications to medical treatment include liver failure, pregnancy, and bone marrow suppression<sup>[13]</sup>.

Praziquantel has protoscolicidal activity and can be used for treatment of CE, either as a standalone therapy or in combination with albendazole. A study suggested higher efficacy of the combination of praziquantel plus albendazole<sup>[66]</sup>. More studies on the efficacy of praziquantel are required.

**Percutaneous treatment:** The percutaneous treatment methods defined in the 1980s for liver CE continue to be popular today<sup>[67-70]</sup>. These are classified under two main categories. The first and more popular one is the PAIR method<sup>[71]</sup>. This method is based on the destruction of the germinal membrane by use of a scolical agent. However, PAIR is not a suitable method for cysts that contain daughter vesicles and for multi-vesicular cysts that have a higher solid content<sup>[7,69,72]</sup>.

Secondary percutaneous treatment modalities include catheterization of the cyst with a broad tube to remove the solid contents of the cyst as well as the daughter vesicles. Several catheterization methods such as percutaneous evacuation, a modified catheterization technique, and dilatable multi-function trocar have been described<sup>[73-75]</sup>. This treatment method can be used for treatment of Types CE2 and CE3a cysts and for post-PAIR relapsing cysts<sup>[76]</sup>.

A review of percutaneous CE treatment ( $n = 5.943$ ) revealed a 0.03% incidence of lethal anaphylaxis and 1.7% incidence of allergic reactions<sup>[49]</sup>. Using albendazole starting from 4 h prior to the percutaneous treatment until 30 d after the percutaneous treatment is convenient<sup>[10]</sup>.

The PAIR treatment is a less invasive method than surgery. In selected patients (CE1 and CE3b) success rates of up to 97% have been reported; the reported mortality and morbidity rates have varied from 0%-1% to 8.5%-32%<sup>[77-80]</sup>. In a study of ethanol plus PAIR treatment ( $n = 231$ ), only one case of relapse was reported<sup>[80]</sup>. Eleven percent to thirteen percent of patients undergoing PAIR tend to develop fever and rash; however, the risk of anaphylaxis is quite low<sup>[77,81]</sup>.

PAIR treatment is not recommended for the cysts which are containing materials that can not be absorbed, cysts which carry the risk of spread into the abdominal cavity, cysts that have already opened into the peritoneal cavity or biliary tract, and inactive and calcified cysts<sup>[7]</sup>.

The relation of the cyst with the biliary tract should be examined prior to administration of scolical agent. Although no cases of scolical agent-related cholangitis after PAIR procedure have been reported, several such

cases have been reported after surgical procedure<sup>[82-84]</sup>. The commonly used scolical agents used during PAIR are hypertonic saline and ethanol<sup>[14]</sup>. Successful intra-cystic application of albendazole and mebendazole solutions as scolical agents during PAIR has been reported in sheep<sup>[85]</sup>.

The reported success rate of percutaneous treatment plus albendazole in non-complicated cysts is similar to that of surgery but has the advantage of a shorter duration of hospital stay<sup>[86]</sup>. In a retrospective comparison of conservative surgery and PAIR, the incidence of biliary fistula and residual cavity relapse was considerably lower with the latter<sup>[87]</sup>.

**Surgical treatment:** While surgical treatment was once the most commonly used treatment modality, it is currently, to a large extent, reserved for complicated cysts (such as cysts that develop biliary fistula or perforated cysts) or is applied to the cysts that contain daughter cysts (CE2, CE3b). In addition, it is a suitable treatment option for superficial cysts that are smaller than 10 cm or are at high risk of rupture and for cases not suitable for percutaneous treatment<sup>[7,10,53,88]</sup>. The surgical treatment options include open surgery and laparoscopic surgery<sup>[5,89,90]</sup>. Open surgical options include radical and conservative surgery. Radical surgery refers to the removal of the cyst along with the pericystic membrane (Figure 4) and may also include liver resection if indicated. Conservative surgery includes removal of the cyst contents only, while the pericystic membrane is retained (Figure 5). Omentoplasty, external drainage, or obliteration of the residual cavity by imbricating sutures from within (capitonnage) is used for drainage from the residual cavity. The complication rates of the surgical treatment options vary between 3%-25%, while the recurrence rates vary between 2% and 40%<sup>[89,91-93]</sup>. The complication and recurrence rates tend to differ based on the location and size of the cyst, as well as the experience of the surgeon and the selected treatment method.

It is not clear which one of the given treatment options is the safest and the most effective. However, recurrence and complication rates tend to be higher with conservative surgery as compared to those with radical surgery<sup>[94]</sup>. Many retrospective studies have revealed similar results<sup>[93,95]</sup>.

The recurrences usually occur due to failure of complete removal of the endocysts and/or their dissemination during the surgery. For this reason, special attention should be paid to prevent spread during the operation<sup>[96,97]</sup>. Of note, spread during the surgery may also lead to other complications such as anaphylaxis.

The most common complication of liver EC is the infection and the contact with the biliary tract. The contact of the cyst with the biliary tracts is encountered in 3%-7% of all cases<sup>[98]</sup>. A relationship between cyst size and its contact with the biliary tract has been reported. In cases where the radius of the cyst is  $> 7.5$  cm, the sensitivity



Figure 4 An example of pericyclectomy material.

of the contact of the cyst with the biliary tract is reported to be 73% while its specificity is indicated to be 79%<sup>[99]</sup>. Prior to intraoperative administration of drugs in the cyst, the relation of the cyst with the biliary tract should be ascertained as protoscolicidal agents are known to induce sclerosis, cholangitis, and pancreatitis.

In case of preoperative evidence of opening of the cyst into the biliary tract, sphincterotomy by endoscopic retrograde cholangiopancreatography (ERCP) prior to surgery decreases the risk of postoperative external fistula from 11.1% to 7.6%<sup>[100]</sup>. When the relation of the cyst with the biliary tract is noticed during the surgery, presence of a cystic component within the biliary branches or within the common biliary duct should be checked. For this, intraoperative cholangiography is often required. In addition, the width of the biliary tract would be in normal range if there is no cystic component within the biliary branches or within the common biliary duct. The biliary tracts, which can be clearly seen through the cyst, should be sutured. In case there is a cystic component inside the biliary tract, the biliary tract would be widened. In such cases, removal of the cystic components within the biliary branches and applying T-tube or choledochoduodenostomy is recommended<sup>[101,102]</sup>. In addition, postoperative bilioma or high flow biliary fistula requires ERCP and sphincterotomy along with nasobiliary drainage or biliary stenting<sup>[103,104]</sup>.

The most commonly used protoscolicidal agent during the surgery is 20% hypertonic saline. The hypertonic saline should be in contact with the germinal membrane for at least 15 min. Albendazole, ivermectin, and praziquantel can also be used as protoscolicidal agents<sup>[105,106]</sup>. In a recently conducted *ex vivo* research, use of selenium nano-particles (250-500 µg/mL) as a protoscolicidal agent for 10-20 min showed good results<sup>[107]</sup>.

Intraoperative dissemination of the mass in the peritoneum should be rinsed with hypertonic saline. Postoperative albendazole for 3-6 mo plus praziquantel for 7 d is recommended<sup>[108]</sup>.

In a retrospective review of conservative surgery methods ( $n = 304$ ), use of external drainage was associated with a statistically significant increase in com-

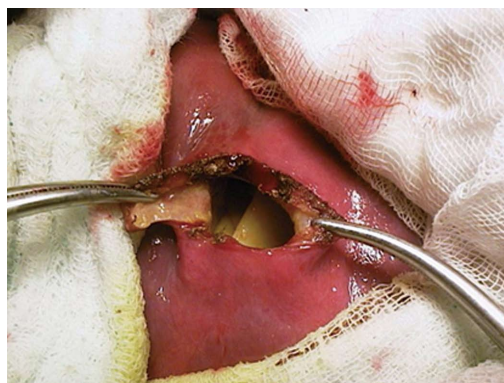


Figure 5 An example of conservative surgery.

plication rates as compared to patients who received omentoplasty or capitonnage<sup>[109]</sup>. In another randomized clinical trial and one retrospective study, patients who received omentoplasty in addition to the conservative surgery showed fewer complications as compared to patients with external drainage<sup>[5,110]</sup>.

The first laparoscopic surgery for CE was reported in 1992<sup>[111]</sup>. While the laparoscopic surgery offers some advantages such as shorter duration of hospital stay, lesser postoperative pain, and lower infection rates, it is applicable only to selected cases. Further laparoscopic procedures are associated with an increased risk of intraoperative dissemination of the cyst contents due to the increased pressure inside the mass<sup>[5,88]</sup>. No studies comparing open surgery with laparoscopic surgery were retrieved on the literature search. Appropriate patient selection is critical to the success of laparoscopic surgery. Deep-seated cysts in the hepatic parenchyma, posterior cysts close to the vena cava, multiple cysts ( $> 3$ ), and cysts with calcified walls are unsuitable for laparoscopic surgery<sup>[88,112-114]</sup>.

**Monitoring:** Some studies suggest that inactive cysts, such as CE4 and CE5, require no treatment<sup>[7,49,76]</sup>. However, more studies in this regard are required.

## E. MULTILOCULARIS INFECTION

### Life cycle

*E. multilocularis* is a small cestode. The definitive hosts of the sylvatic cycle are feral carnivores, and the definitive hosts of the synanthropic cycle are domestic cats and dogs. The fully grown parasites within the small intestine of the definitive host excrete their eggs with the feces of the definitive host. Upon ingestion of the eggs by intermediate hosts such as small rodents, echinococcal metacestodes form alveolar structures with multiple vesicles of different sizes within the liver. Humans get infected after oral ingestion of eggs<sup>[3,17]</sup>. Each vesicle has a structure, similar to the cysts of *E. granulosus*<sup>[115]</sup>. Potential complications include the formation of pseudocysts due to fluid accumulation or central necrosis. Small cysts usually do not contain liquid within them and

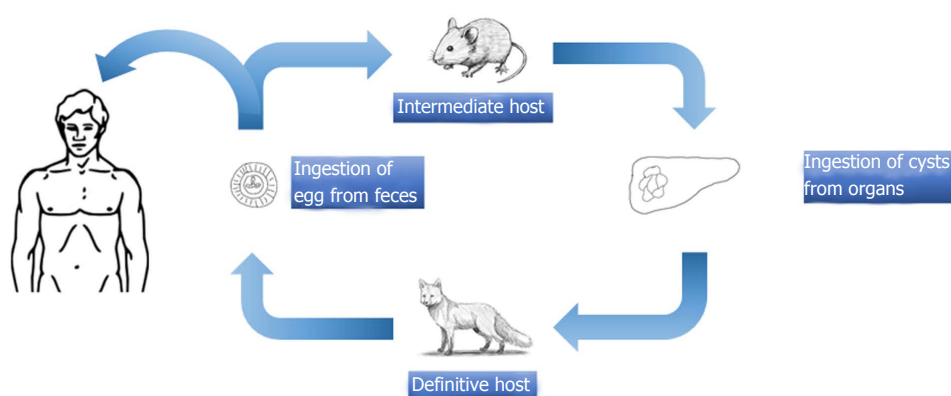


Figure 6 Life circle of *Echinococcus multilocularis*.

are semisolid in structure<sup>[16]</sup> (Figure 6).

### Clinical symptoms of *E. multilocularis* infections

The latent period for infection in which the patients are asymptomatic lasts around 5-15 years and is rather longer compared to the CE. In general, the AE is set into the right lobe of the liver and its size may vary from a few millimeters to 20 cm<sup>[11,13]</sup>. The AE may spread locally or metastasize to the brain, bones, and lungs *via* blood<sup>[115]</sup>. Extrahepatic manifestations are rare in primary disease<sup>[11]</sup>. The typical presenting symptoms include fatigue, weight loss, abdominal pain, and signs of hepatitis or hepatomegaly. Up to one-third of patients suffer from hepatitis and abdominal pain<sup>[115-117]</sup>. The prognosis for untreated cases or cases with incomplete treatment is grim; liver failure, splenomegaly, portal hypertension, and acidity may occur in advanced stages. The life expectancy may extend up to 20 years with treatment<sup>[118]</sup>.

### Diagnosis of *E. multilocularis* infection

The radiological imaging methods are the main methods of diagnosis of AE and the serologic examinations are used to support the diagnosis<sup>[3,4,10,119]</sup>. Ultrasonography is the diagnostic method of choice. On ultrasonography, a pseudotumoral mass with hypo and hyperechoic areas together that contain irregular, limited, and dispersed calcifications is diagnostic<sup>[120,121]</sup>. Doppler ultrasonography may be useful for imaging of biliary tracts and vascular infiltrations. Although CT renders the anatomical details in a better manner, MRI is considered the best method to determine invasion of the contiguous structures<sup>[120-122]</sup>. Percutaneous cholangiography is an important method for diagnosis in order to view the relation between the alveolar lesions and the biliary tracts. In addition cranial and thoracic imaging should be required to rule out extra-hepatic involvement in AE patients<sup>[120]</sup>. Despite the fact that the fluorodeoxyglucose positron emission tomography can be used for diagnosis, negative results do not necessarily mean that the parasite is active<sup>[123]</sup>. The WHO classification developed for *Echinococcus* is based on the imaging methods and aims to establish standardization in the diagnosis and treatment

of the disease<sup>[3,10,124]</sup>. WHO-IWGE PNM classification system resembles the TNM classification used for the tumors<sup>[3,124]</sup>. P indicates the size and location of the parasite within the liver, N indicates the adjunct organ involvement while M indicates distant metastasis (Table 3).

The immunological diagnostic methods are helpful for diagnosis as well as for monitoring the effectiveness of the treatment<sup>[125,126]</sup>. The serological investigations for AE (ELISA or IHA test) are more specific than the ones used for the diagnosis of CE (antigens Em2 and Em II/3-10 are highly specific to AE)<sup>[127]</sup>. However, EM2-ELISA may remain positive for many years even in the treated cases as the EM2 antigen is present in inactive lesions. The most active component of AE is the protoscolex that has EM16 and EM18 antigens. The activity of the lesion can be obtained by using those antigens in immunoblot tests<sup>[128]</sup>. In addition, EM18 is helpful for distinction between AE and CE<sup>[2]</sup>. In some studies, AE patients had high levels of IgG1 and IgG4 antibodies and their IgG4 antibody levels decreased after treatment. Therefore, an increase in IgG4 levels may be a surrogate marker of reactivation of the parasite<sup>[129-132]</sup>. Demonstration of alveolar vesicles in the samples extracted by percutaneous needle biopsy in suspected cases helps confirm the diagnosis. Although PCR imaging of the *E. multilocularis* DNA in the liver biopsy samples has high positive predictive value, negative results do not necessarily rule out the presence of an active parasite<sup>[10]</sup>. There are several studies evaluating the serologic agents best suited for post-treatment follow-up<sup>[133,134]</sup>.

### Treatment and management of *E. multilocularis* infection

AE is comparatively difficult to treat than CE. The main treatment modalities are medical pharmacotherapy and surgery (Figure 7).

Surgical treatment is the primary method for AE; radical resection is often required for hepatic lesions. Conservative and palliative surgery is not recommended since they offer no advantage over medical pharmacotherapy<sup>[135,136]</sup>. Treatment is based on pre-operative assessment and the disease stage as per the WHO-IWGE PNM classification<sup>[124]</sup>. Liver transplantation is an option for patients with advanced stage liver failure,

**Table 3** PNM classification of *Echinococcus multilocularis*<sup>[146]</sup>

P	Hepatic localization of the metacestode
Px	Primary lesion unable to be assessed
P0	No detectable hepatic lesion
P1	Peripheral lesion without biliary or proximal vascular involvement
P2	Central lesions with biliary or proximal vascular involvement of one lobe
P3	Central lesions with biliary or proximal vascular involvement of both lobes or two hepatic veins or both
P4	Any lesion with extension along the portal vein, inferior vena cava or hepatic arteries
N	Extra-hepatic involvement of neighbouring organs
Nx	Not evaluable
N0	No regional involvement
N1	Involvement of neighboring organs or tissues
M	Absence or presence of distant metastasis
Mx	Not completely assessed
M0	No metastasis on chest radiograph and computer tomography brain scan
M1	Metastasis present

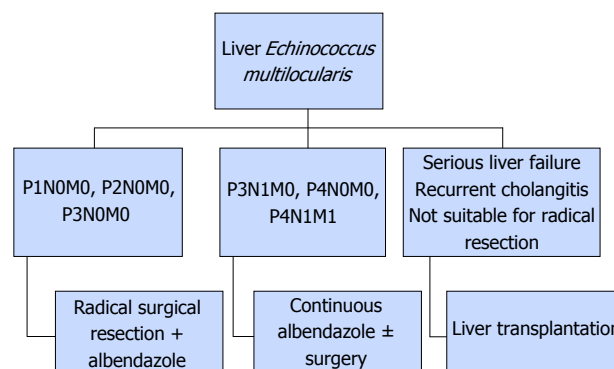
patients that have recurrent cholangitis, and patients unsuitable for radical surgery. Extrahepatic spread of AE during surgery is particularly hazardous in liver transplant recipients, due to drug-induced immunosuppression<sup>[10]</sup>. These patients are at risk of relapse<sup>[137]</sup>.

Although there is no information regarding the effectiveness of pre-operative pharmacotherapy, it is generally used for liver transplant recipients. Postoperative albendazole is recommended in all patients for at least 2 years<sup>[30,137]</sup>. Although there are alternative drugs such as mebendazole, praziquantel, and amphotericin, none is as effective as albendazole<sup>[138,139]</sup>. In a recently conducted study, it was revealed that nitazoxanide has no effect on the treatment of AE<sup>[140]</sup>.

Optimal duration of albendazole treatment in patients not treated by surgery is not clear. However, cases have been documented where albendazole was continuously used for up to 20 years without any complications<sup>[10]</sup>. The use of albendazole in patients who do not undergo surgical treatment increases the 15-year survival from 0% to 53%-80%<sup>[141-145]</sup>. Interventions such as endoscopic sclerosis of the varicose veins of the esophagus and stent implantation may be required during treatment<sup>[53]</sup>.

## CONCLUSION

*E. granulosus* and *E. multilocularis* infections are the most common parasitic diseases that involve the liver. Due to the typical slow growth, these often present in adulthood. Their symptoms include right upper quadrant abdominal pain, chlorosis, cholangitis, and anaphylaxis due to cyst rupture. AE is one of the most fatal helminthic infections. Ultrasonography plays a special role in diagnosis. WHO classification is used for staging and treatment selection. Immunological diagnostic methods are used to support the diagnosis. Cysts smaller than 5 cm (WHO stages CE1 and CE3a) are treated with albendazole only, while PAIR plus albendazole therapy is recommended for cysts > 5 cm.

**Figure 7** Treatment algorithm for *Echinococcus multilocularis* infection.

PAIR treatment for patients with CE2 and CE3b cysts is associated with frequent relapses. Therefore, broad tube percutaneous treatment should be considered in these cases. During open surgery and percutaneous treatment, all necessary efforts should be made to prevent dissemination of cyst contents; albendazole should be used at least for 4 d prior to such procedures and for 1 mo after the procedures. For AE, despite the fact that albendazole is not used preoperatively, postoperative treatment for 2 years is recommended. For CE, radical surgery is reported to be more effective than conservative surgery. For AE, the radical treatment option is also recommended as palliative surgery offers no advantages over medical treatment. Despite the fact that the general templates regarding the treatment seem clear, the lack of randomized clinical studies that compare the treatment options leads to failure in the selection of treatment.

## REFERENCES

- 1 Li H, Song T, Shao Y, Aili T, Ahan A, Wen H. Comparative Evaluation of Liposomal Albendazole and Tablet-Albendazole Against Hepatic Cystic Echinococcosis: A Non-Randomized Clinical Trial. *Medicine* (Baltimore) 2016; **95**: e2237 [PMID: 26825878 DOI: 10.1097/MD.0000000000002237]
- 2 Zhang W, McManus DP. Recent advances in the immunology and diagnosis of echinococcosis. *FEMS Immunol Med Microbiol* 2006; **47**: 24-41 [PMID: 16706785 DOI: 10.1111/j.1574-695X.2006.00060.x]
- 3 Pawlowski ZS, Eckert DA, Vuitton DA, Ammann RW, Kern P, Craig PS, Dar K.F, De Rosa F, Filice C, Gottstein B, Grimm F, Macpherson C.N.L, Sato N, Todorov T, Uchino J, von Sinner W, Wen H. Echinococcosis in humans: clinical aspects, diagnosis and treatment. In: Eckert J, Gemmell MA, Meslin F-X, Pawlowski ZS. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris, World Organisation for Animal Health, 2001: 20-72
- 4 Eckert J, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev* 2004; **17**: 107-135 [PMID: 14726458 DOI: 10.1128/CMR.17.1.107-135.2004]
- 5 Dziri C, Haouet K, Fingerhut A. Treatment of hydatid cyst of the liver: where is the evidence? *World J Surg* 2004; **28**: 731-736 [PMID: 15457348 DOI: 10.1007/s00268-004-7516-z]
- 6 Belhassen-García M, Romero-Alegria A, Velasco-Tirado V, Alonso-Sardón M, Lopez-Bernus A, Alvela-Suarez L, del Villar LP, Carpio-Perez A, Galindo-Perez I, Cordero-Sanchez M, Pardo-



- Lledias J. Study of hydatidosis-attributed mortality in endemic area. *PLoS One* 2014; **9**: e91342 [PMID: 24632824 DOI: 10.1371/journal.pone.0091342]
- 7 **Junghanss T**, da Silva AM, Horton J, Chiodini PL, Brunetti E. Clinical management of cystic echinococcosis: state of the art, problems, and perspectives. *Am J Trop Med Hyg* 2008; **79**: 301-311 [PMID: 18784219]
  - 8 **Manzano-Román R**, Sánchez-Ovejero C, Hernández-González A, Casulli A, Siles-Lucas M. Serological Diagnosis and Follow-Up of Human Cystic Echinococcosis: A New Hope for the Future? *Biomed Res Int* 2015; **2015**: 428205 [PMID: 26504805 DOI: 10.1155/2015/428205]
  - 9 **Liu W**, Delabrousse É, Blagosklonov O, Wang J, Zeng H, Jiang Y, Wang J, Qin Y, Vuitton DA, Wen H. Innovation in hepatic alveolar echinococcosis imaging: best use of old tools, and necessary evaluation of new ones. *Parasite* 2014; **21**: 74 [PMID: 25531446 DOI: 10.1051/parasite/2014072]
  - 10 **Brunetti E**, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* 2010; **114**: 1-16 [PMID: 19931502 DOI: 10.1016/j.actatropica.2009.11.001]
  - 11 **McManus DP**, Gray DJ, Zhang W, Yang Y. Diagnosis, treatment, and management of echinococcosis. *BMJ* 2012; **344**: e3866 [PMID: 22689886 DOI: 10.1136/bmj.e3866]
  - 12 **Torgerson PR**, Keller K, Magnotta M, Ragland N. The global burden of alveolar echinococcosis. *PLoS Negl Trop Dis* 2010; **4**: e722 [PMID: 20582310 DOI: 10.1371/journal.pntd.0000722]
  - 13 **Nunnari G**, Pinzone MR, Gruttadauria S, Celesia BM, Madeddu G, Malaguarnera G, Pavone P, Cappellani A, Cacopardo B. Hepatic echinococcosis: clinical and therapeutic aspects. *World J Gastroenterol* 2012; **18**: 1448-1458 [PMID: 22509076 DOI: 10.3748/wjg.v18.i13.1448]
  - 14 **Rinaldi F**, Brunetti E, Neumayr A, Maestri M, Goblirsch S, Tamarozzi F. Cystic echinococcosis of the liver: A primer for hepatologists. *World J Hepatol* 2014; **6**: 293-305 [PMID: 24868323 DOI: 10.4254/wjh.v6.i5.293]
  - 15 **Eckert JGM**, Meslin FX, Pawlowski ZS, editors. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Health Organization for Animal Health, 2001
  - 16 **Thompson RC**, McManus DP. Aetiology: parasites and lifecycles. In: Eckert J, Gemmell M, Meslin FX, Pawlowski Z. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Organisation for Animal Health, 2001: 1-19
  - 17 **Thompson RC**. Biology and systematics of Echinococcus. In: Thompson RCA, Lymbery AJ. The biology of Echinococcus and hydatid disease. Wallingford: CAB International, 1995: 1-50
  - 18 **Moro P**, Schantz PM. Echinococcosis: a review. *Int J Infect Dis* 2009; **13**: 125-133 [PMID: 18938096 DOI: 10.1016/j.ijid.2008.03.037]
  - 19 **Atli M**, Kama NA, Yuksek YN, Doganay M, Gozalan U, Kologlu M, Daglar G. Intrahepatic rupture of a hepatic hydatid cyst: associated clinical factors and proper management. *Arch Surg* 2001; **136**: 1249-1255 [PMID: 11695968 DOI: 10.1001/archsurg.136.11.1249]
  - 20 **Pedrosa I**, Saiz A, Arrazola J, Ferreirós J, Pedrosa CS. Hydatid disease: radiologic and pathologic features and complications. *Radiographics* 2000; **20**: 795-817 [PMID: 10835129 DOI: 10.1148/radiographics.20.3.g00ma06795]
  - 21 **Cattaneo F**, Graffeo M, Brunetti E. Extrahepatic textiloma long misdiagnosed as calcified echinococcal cyst. *Cas Rep Gastrointest Med* 2013; **2013**: 261685 [PMID: 23533840]
  - 22 **Polat P**, Kantarci M, Alper F, Suma S, Koruyucu MB, Okur A. Hydatid disease from head to toe. *Radiographics* 2003; **23**: 475-494; quiz 536-537 [PMID: 12640161 DOI: 10.1148/rg.232025704]
  - 23 **Cohen H**, Paolillo E, Bonifacino R, Botta B, Parada L, Cabrera P, Snowden K, Gasser R, Tessier R, Dibarboure L, Wen H, Allan JC, Soto de Alfaro H, Rogan MT, Craig PS. Human cystic echinococcosis in a Uruguayan community: a sonographic, serologic, and epidemiologic study. *Am J Trop Med Hyg* 1998; **59**: 620-627 [PMID: 9790441]
  - 24 **Shambesh MA**, Craig PS, Macpherson CN, Rogan MT, Gusbi AM, Echtuish EF. An extensive ultrasound and serologic study to investigate the prevalence of human cystic echinococcosis in northern Libya. *Am J Trop Med Hyg* 1999; **60**: 462-468 [PMID: 10466978]
  - 25 **Gharbi HA**, Hassine W, Brauner MW, Dupuch K. Ultrasound examination of the hydatid liver. *Radiology* 1981; **139**: 459-463 [PMID: 7220891 DOI: 10.1148/radiology.139.2.7220891]
  - 26 **WHO Informal Working Group**. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. *Acta Trop* 2003; **85**: 253-261 [PMID: 12606104 DOI: 10.1016/S0001-706X(02)00223-1]
  - 27 **Grisolia A**, Troia G, Mariani G, Brunetti E, Filice C. A simple sonographic scoring system combined with routine serology is useful in differentiating parasitic from non-parasitic cysts of the liver. *J Ultrasound* 2009; **12**: 75-79 [PMID: 23396670 DOI: 10.1016/j.jus.2009.02.004]
  - 28 **Hosch W**, Junghanss T, Stojkovic M, Brunetti E, Heye T, Kauffmann GW, Hull WE. Metabolic viability assessment of cystic echinococcosis using high-field 1H MRS of cyst contents. *NMR Biomed* 2008; **21**: 734-754 [PMID: 18384178 DOI: 10.1002/nbm.1252]
  - 29 **Kabaalioglu A**, Ceken K, Alimoglu E, Apaydin A. Percutaneous imaging-guided treatment of hydatid liver cysts: do long-term results make it a first choice? *Eur J Radiol* 2006; **59**: 65-73 [PMID: 16513311 DOI: 10.1016/j.ejrad.2006.01.014]
  - 30 **Brunetti E**, White AC. Cestode infestations: hydatid disease and cysticercosis. *Infect Dis Clin North Am* 2012; **26**: 421-435 [PMID: 22632647 DOI: 10.1016/j.idc.2012.02.001]
  - 31 **Stojkovic M**, Rosenberger K, Kauczor HU, Junghanss T, Hosch W. Diagnosing and staging of cystic echinococcosis: how do CT and MRI perform in comparison to ultrasound? *PLoS Negl Trop Dis* 2012; **6**: e1880 [PMID: 23145199 DOI: 10.1371/journal.pntd.0001880]
  - 32 **Hosch W**, Stojkovic M, Jänisch T, Heye T, Werner J, Friess H, Kauffmann GW, Junghanss T. MR imaging for diagnosing cystobiliary fistulas in cystic echinococcosis. *Eur J Radiol* 2008; **66**: 262-267 [PMID: 17888605 DOI: 10.1016/j.ejrad.2007.08.002]
  - 33 **Filippou D**, Tselepis D, Filippou G, Papadopoulos V. Advances in liver echinococcosis: diagnosis and treatment. *Clin Gastroenterol Hepatol* 2007; **5**: 152-159 [PMID: 17157079 DOI: 10.1016/j.cgh.2006.08.017]
  - 34 **Gottstein B**. Molecular and immunological diagnosis of echinococcosis. *Clin Microbiol Rev* 1992; **5**: 248-261 [PMID: 1498767 DOI: 10.1128/CMR.5.3.248]
  - 35 **Ito A**. Serologic and molecular diagnosis of zoonotic larval cestode infections. *Parasitol Int* 2002; **51**: 221-235 [PMID: 12243777 DOI: 10.1016/S1383-5769(02)00036-3]
  - 36 **Craig PS**, Rogan MT, Campos-Ponce M. Echinococcosis: disease, detection and transmission. *Parasitology* 2003; **127** Suppl: S5-S20 [PMID: 15027602 DOI: 10.1017/S0031182003004451]
  - 37 **Ortona E**, Siracusano A, Castro A, Rigano R, Mühlischlegel F, Ioppolo S, Notargiacomo S, Frosch M. Use of a monoclonal antibody against the antigen B of Echinococcus granulosus for purification and detection of antigen B. *Appl Parasitol* 1995; **36**: 220-225 [PMID: 8541895]
  - 38 **Lorenzo C**, Ferreira HB, Monteiro KM, Rosenzvit M, Kamenetzky L, García HH, Vasquez Y, Naquira C, Sánchez E, Lorca M, Contreras M, Last JA, González-Sapienza GG. Comparative analysis of the diagnostic performance of six major Echinococcus granulosus antigens assessed in a double-blind, randomized multicenter study. *J Clin Microbiol* 2005; **43**: 2764-2770 [PMID: 15956395 DOI: 10.1128/JCM.43.6.2764-2770.2005]
  - 39 **Ortona E**, Riganò R, Buttari B, Delunardo F, Ioppolo S, Margutti P, Profumo E, Teggi A, Vaccari S, Siracusano A. An update on immunodiagnosis of cystic echinococcosis. *Acta Trop* 2003; **85**: 165-171 [PMID: 12606093 DOI: 10.1016/S0001-706X(02)00225-5]
  - 40 **Ito A**, Craig PS. Immunodiagnostic and molecular approaches for the detection of taeniid cestode infections. *Trends Parasitol*

- 2003; **19**: 377-381 [PMID: 12957509 DOI: 10.1016/S1471-4922(03)00200-9]
- 41 **Zhang W**, Li J, McManus DP. Concepts in immunology and diagnosis of hydatid disease. *Clin Microbiol Rev* 2003; **16**: 18-36 [PMID: 12525423 DOI: 10.1128/CMR.16.1.18-36.2003]
- 42 **Ortona E**, Riganò R, Margutti P, Notargiacomo S, Ioppolo S, Vaccari S, Barca S, Buttari B, Profumo E, Teggi A, Siracusano A. Native and recombinant antigens in the immunodiagnosis of human cystic echinococcosis. *Parasite Immunol* 2000; **22**: 553-559 [PMID: 11116435 DOI: 10.1046/j.1365-3024.2000.00336.x]
- 43 **Liu D**, Rickard MD, Lightowers MW. Assessment of monoclonal antibodies to Echinococcus granulosus antigen 5 and antigen B for detection of human hydatid circulating antigens. *Parasitology* 1993; **106** (Pt 1): 75-81 [PMID: 8479805 DOI: 10.1017/S0031182000074849]
- 44 **Carmena D**, Benito A, Eraso E. Antigens for the immunodiagnosis of Echinococcus granulosus infection: An update. *Acta Trop* 2006; **98**: 74-86 [PMID: 16527225 DOI: 10.1016/j.actatropica.2006.02.002]
- 45 **Poretti D**, Felleisen E, Grimm F, Pfister M, Teuscher F, Zuercher C, Reichen J, Gottstein B. Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. *Am J Trop Med Hyg* 1999; **60**: 193-198 [PMID: 10072135]
- 46 **Moro PL**, Gilman RH, Verastegui M, Bern C, Silva B, Bonilla JJ. Human hydatidosis in the central Andes of Peru: evolution of the disease over 3 years. *Clin Infect Dis* 1999; **29**: 807-812 [PMID: 10589894 DOI: 10.1086/520440]
- 47 **Cirenei A**, Bertoldi I. Evolution of surgery for liver hydatidosis from 1950 to today: analysis of a personal experience. *World J Surg* 2001; **25**: 87-92 [PMID: 11213161 DOI: 10.1007/s002680020368]
- 48 **Perdomo R**, Alvarez C, Monti J, Ferreira C, Chiesa A, Carbó A, Alvez R, Grauert R, Stern D, Carmona C, Yarzabal L. Principles of the surgical approach in human liver cystic echinococcosis. *Acta Trop* 1997; **64**: 109-122 [PMID: 9095292 DOI: 10.1016/S0001-706X(96)00641-9]
- 49 **Brunetti E**, Junghanss T. Update on cystic hydatid disease. *Curr Opin Infect Dis* 2009; **22**: 497-502 [PMID: 19633552 DOI: 10.1097/QCO.0b013e328330331c]
- 50 **Neumayr A**, Troia G, de Bernardis C, Tamarozzi F, Goblrirsch S, Piccoli L, Hatz C, Filice C, Brunetti E. Justified concern or exaggerated fear: the risk of anaphylaxis in percutaneous treatment of cystic echinococcosis-a systematic literature review. *PLoS Negl Trop Dis* 2011; **5**: e1154 [PMID: 21695106 DOI: 10.1371/journal.pntd.0001154]
- 51 **von Sinner WN**, Nyman R, Linjawi T, Ali AM. Fine needle aspiration biopsy of hydatid cysts. *Acta Radiol* 1995; **36**: 168-172 [PMID: 7710798 DOI: 10.3109/02841859509173372]
- 52 **Hira PR**, Shweiki H, Lindberg LG, Shaheen Y, Francis I, Leven H, Behbehani K. Diagnosis of cystic hydatid disease: role of aspiration cytology. *Lancet* 1988; **2**: 655-657 [PMID: 2458514 DOI: 10.1016/S0140-6736(88)90470-9]
- 53 Guidelines for treatment of cystic and alveolar echinococcosis in humans. WHO Informal Working Group on Echinococcosis. *Bull World Health Organ* 1996; **74**: 231-242 [PMID: 8789923]
- 54 **Senyüz OF**, Yeşildag E, Celayir S. Albendazole therapy in the treatment of hydatid liver disease. *Surg Today* 2001; **31**: 487-491 [PMID: 11428598 DOI: 10.1007/s005950170106]
- 55 **Nahmias J**, Goldsmith R, Soibelman M, el-On J. Three- to 7-year follow-up after albendazole treatment of 68 patients with cystic echinococcosis (hydatid disease). *Ann Trop Med Parasitol* 1994; **88**: 295-304 [PMID: 7944675]
- 56 **Vutova K**, Mechkov G, Vachkov P, Petkov R, Georgiev P, Handjiev S, Ivanov A, Todorov T. Effect of mebendazole on human cystic echinococcosis: the role of dosage and treatment duration. *Ann Trop Med Parasitol* 1999; **93**: 357-365 [PMID: 10656037 DOI: 10.1080/00034989958357]
- 57 **Stojkovic M**, Zwahlen M, Teggi A, Vutova K, Cretu CM, Virdone R, Nicolaidou P, Cobanoglu N, Junghanss T. Treatment response of cystic echinococcosis to benzimidazoles: a systematic review. *PLoS Negl Trop Dis* 2009; **3**: e524 [PMID: 19787039 DOI: 10.1371/journal.pntd.0000524]
- 58 **Teggi A**, Lastilla MG, De Rosa F. Therapy of human hydatid disease with mebendazole and albendazole. *Antimicrob Agents Chemother* 1993; **37**: 1679-1684 [PMID: 8215283 DOI: 10.1128/AAC.37.8.1679]
- 59 **Erzurumlu K**, Hökelek M, Gönlösen L, Tas K, Amanvermez R. The effect of albendazole on the prevention of secondary hydatidosis. *Hepatogastroenterology* 2000; **47**: 247-250 [PMID: 10690616]
- 60 **Aktan AO**, Yalin R. Preoperative albendazole treatment for liver hydatid disease decreases the viability of the cyst. *Eur J Gastroenterol Hepatol* 1996; **8**: 877-879 [PMID: 8889454]
- 61 **Arif SH**, Shams-ul-Bari NA, Zargar SA, Wani MA, Tabassum R, Hussain Z, Baba AA, Lone RA. Albendazole as an adjuvant to the standard surgical management of hydatid cyst liver. *Int J Surg* 2008; **6**: 448-451 [PMID: 18819855 DOI: 10.1016/j.ijssu.2008.08.003]
- 62 **Manterola C**, Mansilla JA, Fonseca F. Preoperative albendazole and scolices viability in patients with hepatic echinococcosis. *World J Surg* 2005; **29**: 750-753 [PMID: 15880282 DOI: 10.1007/s00268-005-7691-6]
- 63 **Gil-Grande LA**, Rodriguez-Caabeiro F, Prieto JG, Sánchez-Ruano JJ, Brasa C, Aguilar L, García-Hoz F, Casado N, Bárcena R, Alvarez AI. Randomised controlled trial of efficacy of albendazole in intra-abdominal hydatid disease. *Lancet* 1993; **342**: 1269-1272 [PMID: 7901585 DOI: 10.1016/0140-6736(93)92361-V]
- 64 **Bildik N**, Cevik A, Altintaş M, Ekinçi H, Canberk M, Gülmen M. Efficacy of preoperative albendazole use according to months in hydatid cyst of the liver. *J Clin Gastroenterol* 2007; **41**: 312-316 [PMID: 17426473 DOI: 10.1097/01.mcg.0000225572.50514.e6]
- 65 **Franchi C**, Di Vico B, Teggi A. Long-term evaluation of patients with hydatidosis treated with benzimidazole carbamates. *Clin Infect Dis* 1999; **29**: 304-309 [PMID: 10476732 DOI: 10.1086/520205]
- 66 **Cobo F**, Yarnoz C, Sesma B, Fraile P, Aizcorbe M, Trujillo R, Diaz-de-Liaño A, Ciga MA. Albendazole plus praziquantel versus albendazole alone as a pre-operative treatment in intra-abdominal hydatidosis caused by Echinococcus granulosus. *Trop Med Int Health* 1998; **3**: 462-466 [PMID: 9657508 DOI: 10.1046/j.1365-3156.1998.00257.x]
- 67 **Filice C**, Pirola F, Brunetti E, Dughetti S, Strosselli M, Foglieni CS. A new therapeutic approach for hydatid liver cysts. Aspiration and alcohol injection under sonographic guidance. *Gastroenterology* 1990; **98**: 1366-1368 [PMID: 2182372]
- 68 **Ben Amor N**, Gargouri M, Gharbi HA, Golvan YJ, Ayachi K, Kchouk H. [Trial therapy of inoperable abdominal hydatid cysts by puncture]. *Ann Parasitol Hum Comp* 1986; **61**: 689-692 [PMID: 3566087]
- 69 **Mueller PR**, Dawson SL, Ferrucci JT, Nardi GL. Hepatic echinococcal cyst: successful percutaneous drainage. *Radiology* 1985; **155**: 627-628 [PMID: 3890001 DOI: 10.1148/radiology.155.3.3890001]
- 70 **Gargouri M**, Ben Amor N, Ben Chehida F, Hammou A, Gharbi HA, Ben Cheikh M, Kchouk H, Ayachi K, Golvan JY. Percutaneous treatment of hydatid cysts (Echinococcus granulosus). *Cardiovasc Intervent Radiol* 1990; **13**: 169-173 [PMID: 2121344 DOI: 10.1007/BF02575469]
- 71 **World Health Organization**. PAIR: Puncture, Aspiration, Injection, Re- Aspiration. An option for the treatment of Cystic echinococcosis. WHO/CDS/CSR/APH/2001.6 Geneva, 2003: 1-4
- 72 **Nasseri Moghaddam S**, Abrishami A, Malekzadeh R. Percutaneous needle aspiration, injection, and reaspiration with or without benzimidazole coverage for uncomplicated hepatic hydatid cysts. *Cochrane Database Syst Rev* 2006; **2**: CD003623 [PMID: 16625588 DOI: 10.1002/14651858.cd003623.pub2]
- 73 **Akhan O**, Gumus B, Akinci D, Karcaaltincaba M, Ozmen M. Diagnosis and percutaneous treatment of soft-tissue hydatid cysts. *Cardiovasc Intervent Radiol* 2007; **30**: 419-425 [PMID: 17295079 DOI: 10.1007/s00270-006-0153-1]
- 74 **Schipper HG**, Laméris JS, van Delden OM, Rauws EA, Kager PA. Percutaneous evacuation (PEVAC) of multivesicular echinococcal cysts with or without cystobiliary fistulas which contain non-drainable material: first results of a modified PAIR method. *Gut* 2002; **50**: 718-723 [PMID: 11950823 DOI: 10.1136/gut.50.5.718]

- 75 **Vuitton DA**, Wang XZ, Feng SL, Chen JS, Shou LY, Li SF, Ke TQ. PAIR-derived US-guided techniques for the treatment of cystic echinococcosis: a Chinese experience (e-letter). *Gut* 2002
- 76 **Brunetti E**, Garcia HH, Junghanss T. Cystic echinococcosis: chronic, complex, and still neglected. *PLoS Negl Trop Dis* 2011; **5**: e1146 [PMID: 21814584 DOI: 10.1371/journal.pntd.0001146]
- 77 **Ustünsöz B**, Akhan O, Kamiloğlu MA, Somuncu I, Uğurel MS, Cetiner S. Percutaneous treatment of hydatid cysts of the liver: long-term results. *AJR Am J Roentgenol* 1999; **172**: 91-96 [PMID: 9888746 DOI: 10.2214/ajr.172.1.9888746]
- 78 **Giorgio A**, de Stefano G, Esposito V, Liorre G, Di Sarno A, Giorgio V, Sangiovanni V, Iannece MD, Mariniello N. Long-term results of percutaneous treatment of hydatid liver cysts: a single center 17 years experience. *Infection* 2008; **36**: 256-261 [PMID: 18473119 DOI: 10.1007/s15010-007-7103-y]
- 79 **Salama H**, Farid Abdel-Wahab M, Strickland GT. Diagnosis and treatment of hepatic hydatid cysts with the aid of echo-guided percutaneous cyst puncture. *Clin Infect Dis* 1995; **21**: 1372-1376 [PMID: 8749617 DOI: 10.1093/clinids/21.6.1372]
- 80 **Filice C**, Brunetti E. Use of PAIR in human cystic echinococcosis. *Acta Trop* 1997; **64**: 95-107 [PMID: 9095291 DOI: 10.1016/S0001-706X(96)00642-0]
- 81 **Men S**, Hekimoğlu B, Yücesoy C, Arda IS, Baran I. Percutaneous treatment of hepatic hydatid cysts: an alternative to surgery. *AJR Am J Roentgenol* 1999; **172**: 83-89 [PMID: 9888745 DOI: 10.2214/ajr.172.1.9888745]
- 82 **Castellano G**, Moreno-Sanchez D, Gutierrez J, Moreno-Gonzalez E, Colina F, Solis-Herruzo JA. Caustic sclerosing cholangitis. Report of four cases and a cumulative review of the literature. *Hepato-gastroenterology* 1994; **41**: 458-470 [PMID: 7851856]
- 83 **Belghiti J**, Benhamou JP, Houry S, Grenier P, Huguier M, Fékété F. Caustic sclerosing cholangitis. A complication of the surgical treatment of hydatid disease of the liver. *Arch Surg* 1986; **121**: 1162-1165 [PMID: 3767649 DOI: 10.1001/archsurg.1986.01400100070014]
- 84 **Taranto D**, Beneduce F, Vitale LM, Loguercio C, Del Vecchio Blanco C. Chemical sclerosing cholangitis after injection of scolicedal solution. *Ital J Gastroenterol* 1995; **27**: 78-79 [PMID: 7579597]
- 85 **Paksoy Y**, Odev K, Sahin M, Dik B, Ergül R, Arslan A. Percutaneous sonographically guided treatment of hydatid cysts in sheep: direct injection of mebendazole and albendazole. *J Ultrasound Med* 2003; **22**: 797-803 [PMID: 12901407]
- 86 **Khuroo MS**, Wani NA, Javid G, Khan BA, Yattoo GN, Shah AH, Jeelani SG. Percutaneous drainage compared with surgery for hepatic hydatid cysts. *N Engl J Med* 1997; **337**: 881-887 [PMID: 9302302 DOI: 10.1056/NEJM199709253371303]
- 87 **Gupta N**, Javed A, Puri S, Jain S, Singh S, Agarwal AK. Hepatic hydatid: PAIR, drain or resect? *J Gastrointest Surg* 2011; **15**: 1829-1836 [PMID: 21826545 DOI: 10.1007/s11605-011-1649-9]
- 88 **Derveniz C**, Delis S, Avgerinos C, Madariaga J, Milicevic M. Changing concepts in the management of liver hydatid disease. *J Gastrointest Surg* 2005; **9**: 869-877 [PMID: 15985246 DOI: 10.1016/j.gassur.2004.10.016]
- 89 **Gollackner B**, Längle F, Auer H, Maier A, Mittlböck M, Agstner I, Karner J, Langer F, Aspöck H, Loidolt H, Rockenschaub S, Steininger R. Radical surgical therapy of abdominal cystic hydatid disease: factors of recurrence. *World J Surg* 2000; **24**: 717-721 [PMID: 10773125 DOI: 10.1007/s002689910115]
- 90 **El Malki HO**, El Mejdoubi Y, Souadka A, Mohsine R, Ifrine L, Abouqal R, Belkouchi A. Predictive factors of deep abdominal complications after operation for hydatid cyst of the liver: 15 years of experience with 672 patients. *J Am Coll Surg* 2008; **206**: 629-637 [PMID: 18387467 DOI: 10.1016/j.jamcollsurg.2007.11.012]
- 91 **Buttenschoen K**, Carli Buttenschoen D. Echinococcus granulosus infection: the challenge of surgical treatment. *Langenbecks Arch Surg* 2003; **388**: 218-230 [PMID: 12845535 DOI: 10.1007/s00423-003-0397-z]
- 92 **Daradkeh S**, El-Muhtaseb H, Farah G, Sroujeh AS, Abu-Khalaf M. Predictors of morbidity and mortality in the surgical management of hydatid cyst of the liver. *Langenbecks Arch Surg* 2007; **392**: 35-39 [PMID: 17021792 DOI: 10.1007/s00423-006-0064-2]
- 93 **Aydin U**, Yazici P, Onen Z, Ozsoy M, Zeytinlu M, Kiliç M, Coker A. The optimal treatment of hydatid cyst of the liver: radical surgery with a significant reduced risk of recurrence. *Turk J Gastroenterol* 2008; **19**: 33-39 [PMID: 18386238]
- 94 **Yüksel O**, Akyürek N, Sahin T, Salman B, Azili C, Bostanci H. Efficacy of radical surgery in preventing early local recurrence and cavity-related complications in hydatid liver disease. *J Gastrointest Surg* 2008; **12**: 483-489 [PMID: 17917786 DOI: 10.1007/s11605-007-0301-1]
- 95 **Tagliacozzo S**, Miccini M, Amore Bonapasta S, Gregori M, Tocchi A. Surgical treatment of hydatid disease of the liver: 25 years of experience. *Am J Surg* 2011; **201**: 797-804 [PMID: 20832053 DOI: 10.1016/j.amjsurg.2010.02.011]
- 96 **Kapan M**, Kapan S, Goksoy E, Perek S, Kol E. Postoperative recurrence in hepatic hydatid disease. *J Gastrointest Surg* 2006; **10**: 734-739 [PMID: 16713547 DOI: 10.1016/j.gassur.2005.10.013]
- 97 **Lissandrin R**, Agliata S, Brunetti E. Secondary peritoneal echinococcosis causing massive bilateral hydronephrosis and renal failure. *Int J Infect Dis* 2013; **17**: e141-e142 [PMID: 23218548 DOI: 10.1016/j.ijid.2012.11.008]
- 98 **Kumar R**, Reddy SN, Thulker S. Intrabiliary rupture of hydatid cyst: diagnosis with MRI and hepatobiliary isotope study. *Br J Radiol* 2002; **75**: 271-274 [PMID: 11932222 DOI: 10.1259/bjr.75.891.750271]
- 99 **Kilic M**, Yoldas O, Koc M, Keskek M, Karakose N, Ertan T, Gocmen E, Tez M. Can biliary-cyst communication be predicted before surgery for hepatic hydatid disease: does size matter? *Am J Surg* 2008; **196**: 732-735 [PMID: 18513700 DOI: 10.1016/j.amjsurg.2007.07.034]
- 100 **Galati G**, Sterpetti AV, Caputo M, Adduci M, Lucandri G, Brozzetti S, Bolognese A, Cavallaro A. Endoscopic retrograde cholangiography for intrabiliary rupture of hydatid cyst. *Am J Surg* 2006; **191**: 206-210 [PMID: 16442947 DOI: 10.1016/j.amjsurg.2005.09.014]
- 101 **Bedirli A**, Sakrak O, Sozuer EM, Kerek M, Ince O. Surgical management of spontaneous intrabiliary rupture of hydatid liver cysts. *Surg Today* 2002; **32**: 594-597 [PMID: 12111515 DOI: 10.1007/s005950200107]
- 102 **Erzurumlu K**, Dervisoglu A, Polat C, Senyurek G, Yetim I, Hokelek M. Intrabiliary rupture: an algorithm in the treatment of controversial complication of hepatic hydatidosis. *World J Gastroenterol* 2005; **11**: 2472-2476 [PMID: 15832420 DOI: 10.3748/wjg.v11.i16.2472]
- 103 **Agarwal S**, Sikora SS, Kumar A, Saxena R, Kapoor VK. Bile leaks following surgery for hepatic hydatid disease. *Indian J Gastroenterol* 2005; **24**: 55-58 [PMID: 15879650]
- 104 **Chowbey PK**, Shah S, Khullar R, Sharma A, Soni V, Bajjal M, Vashistha A, Dhir A. Minimal access surgery for hydatid cyst disease: laparoscopic, thoracoscopic, and retroperitoneoscopic approach. *J Laparoendosc Adv Surg Tech A* 2003; **13**: 159-165 [PMID: 12855097 DOI: 10.1089/109264203766207672]
- 105 **Paksoy Y**, Odev K, Sahin M, Arslan A, Koç O. Percutaneous treatment of liver hydatid cysts: comparison of direct injection of albendazole and hypertonic saline solution. *AJR Am J Roentgenol* 2005; **185**: 727-734 [PMID: 16120926 DOI: 10.2214/ajr.185.3.01850727]
- 106 **Dziri C**, Haouet K, Fingerhut A, Zaouche A. Management of cystic echinococcosis complications and dissemination: where is the evidence? *World J Surg* 2009; **33**: 1266-1273 [PMID: 19350321 DOI: 10.1007/s00268-009-9982-9]
- 107 **Mahmoudvand H**, Fasihi Harandi M, Shakibaie M, Aflatoonian MR, ZiaAli N, Makki MS, Jahanbakhsh S. Scolicedal effects of biogenic selenium nanoparticles against protoscolices of hydatid cysts. *Int J Surg* 2014; **12**: 399-403 [PMID: 24686032 DOI: 10.1016/j.ijsu.2014.03.017]
- 108 **Taylor DH**, Morris DL. Combination chemotherapy is more effective in postspillage prophylaxis for hydatid disease than either albendazole or praziquantel alone. *Br J Surg* 1989; **76**: 954 [PMID: 2804596 DOI: 10.1002/bjs.1800760927]
- 109 **Balik AA**, Başoğlu M, Celebi F, Oren D, Polat KY, Atamanalp



- SS, Akçay MN. Surgical treatment of hydatid disease of the liver: review of 304 cases. *Arch Surg* 1999; **134**: 166-169 [PMID: 10025457 DOI: 10.1001/archsurg.134.2.166]
- 110 **Utkan NZ**, Cantürk NZ, Gönüllü N, Yıldırım C, Dülger M. Surgical experience of hydatid disease of the liver: omentoplasty or cap-  
itonnage versus tube drainage. *Hepatogastroenterology* 2001; **48**: 203-207 [PMID: 11268966]
- 111 **Katkhouda N**, Fabiani P, Benizri E, Mouiel J. Laser resection of a liver hydatid cyst under videolaparoscopy. *Br J Surg* 1992; **79**: 560-561 [PMID: 1535261 DOI: 10.1002/bjs.1800790628]
- 112 **Bickel A**, Daud G, Urbach D, Lefler E, Barasch EF, Eitan A. Laparoscopic approach to hydatid liver cysts. Is it logical? Physical, experimental, and practical aspects. *Surg Endosc* 1998; **12**: 1073-1077 [PMID: 9685545 DOI: 10.1007/s004649900783]
- 113 **Baskaran V**, Patnaik PK. Feasibility and safety of laparoscopic management of hydatid disease of the liver. *JSLs* 2004; **8**: 359-363 [PMID: 15554281]
- 114 **Seven R**, Berber E, Mercan S, Eminoglu L, Budak D. Laparoscopic treatment of hepatic hydatid cysts. *Surgery* 2000; **128**: 36-40 [PMID: 10876183 DOI: 10.1067/msy.2000.107062]
- 115 **Eckert J**. Alveolar echinococcosis (*Echinococcus multilocularis*) and other forms of echinococcosis (*Echinococcus oligarthrus* and *Echinococcus vogeli*). In: Palmer SR, Soulsby EJJ, Simpson DIH. Oxford Textbook of Zoonoses: Biology, Clinical Practice, and Public Health Control. Oxford: Oxford University Press, 1998: 689-716
- 116 **Sato N**, Aoki S, Matsushita M, Uchino J. Clinical features. In: Uchino J, Sato N. Alveolar echinococcosis of the liver. Sapporo: Hokkaido University School of Medicine, 1993: 63-68
- 117 **Ammann RW**, Eckert J. Cestodes. *Echinococcus*. *Gastroenterol Clin North Am* 1996; **25**: 655-689 [PMID: 8863045 DOI: 10.1016/S0889-8553(05)70268-5]
- 118 **Torgerson PR**, Schweiger A, Deplazes P, Pohar M, Reichen J, Ammann RW, Tarr PE, Halkik N, Müllhaupt B. Alveolar echinococcosis: from a deadly disease to a well-controlled infection. Relative survival and economic analysis in Switzerland over the last 35 years. *J Hepatol* 2008; **49**: 72-77 [PMID: 18485517 DOI: 10.1016/j.jhep.2008.03.023]
- 119 **McManus DP**, Zhang W, Li J, Bartley PB. Echinococcosis. *Lancet* 2003; **362**: 1295-1304 [PMID: 14575976]
- 120 **Bresson-Hadni S**, Delabrousse E, Blagosklonov O, Bartholomot B, Koch S, Miguet JP, Manton GA, Vuitton DA. Imaging aspects and non-surgical interventional treatment in human alveolar echinococcosis. *Parasitol Int* 2006; **55** Suppl: S267-S272 [PMID: 16403670]
- 121 **Bartholomot G**, Vuitton DA, Harraga S, Shi DZ, Giraudoux P, Barnish G, Wang YH, MacPherson CN, Craig PS. Combined ultrasound and serologic screening for hepatic alveolar echinococcosis in central China. *Am J Trop Med Hyg* 2002; **66**: 23-29 [PMID: 12135263]
- 122 **Reuter S**, Nüssle K, Kolokythas O, Haug U, Rieber A, Kern P, Kratzer W. Alveolar liver echinococcosis: a comparative study of three imaging techniques. *Infection* 2001; **29**: 119-125 [PMID: 11440381 DOI: 10.1007/s15010-001-1081-2]
- 123 **Stumpe KD**, Renner-Schneiter EC, Kuenzle AK, Grimm F, Kadry Z, Clavien PA, Deplazes P, von Schulthess GK, Muellhaupt B, Ammann RW, Renner EL. F-18-fluorodeoxyglucose (FDG) positron-emission tomography of *Echinococcus multilocularis* liver lesions: prospective evaluation of its value for diagnosis and follow-up during benzimidazole therapy. *Infection* 2007; **35**: 11-18 [PMID: 17297583 DOI: 10.1007/s15010-007-6133-9]
- 124 **Kern P**, Wen H, Sato N, Vuitton DA, Gruener B, Shao Y, Delabrousse E, Kratzer W, Bresson-Hadni S. WHO classification of alveolar echinococcosis: principles and application. *Parasitol Int* 2006; **55** Suppl: S283-S287 [PMID: 16343985 DOI: 10.1016/j.parint.2005.11.041]
- 125 **Ma L**, Ito A, Liu YH, Wang XG, Yao YQ, Yu DG, Chen YT. Alveolar echinococcosis: Em2plus-ELISA and Em18-western blots for follow-up after treatment with albendazole. *Trans R Soc Trop Med Hyg* 1997; **91**: 476-478 [PMID: 9373660 DOI: 10.1016/S0035-9203(97)90291-1]
- 126 **Scheuring UJ**, Seitz HM, Wellmann A, Hartlapp JH, Tappe D, Brehm K, Spengler U, Sauerbruch T, Rockstroh JK. Long-term benzimidazole treatment of alveolar echinococcosis with hematogenic subcutaneous and bone dissemination. *Med Microbiol Immunol* 2003; **192**: 193-195 [PMID: 12684758]
- 127 **Gottstein B**, Jacquier P, Bresson-Hadni S, Eckert J. Improved primary immunodiagnosis of alveolar echinococcosis in humans by an enzyme-linked immunosorbent assay using the Em2plus antigen. *J Clin Microbiol* 1993; **31**: 373-376 [PMID: 8432825]
- 128 **Ito A**, Schantz PM, Wilson JF. Em18, a new serodiagnostic marker for differentiation of active and inactive cases of alveolar hydatid disease. *Am J Trop Med Hyg* 1995; **52**: 41-44 [PMID: 7531957]
- 129 **Wen H**, Bresson-Hadni S, Vuitton DA, Lenys D, Yang BM, Ding ZX, Craig PS. Analysis of immunoglobulin G subclass in the serum antibody responses of alveolar echinococcosis patients after surgical treatment and chemotherapy as an aid to assessing the outcome. *Trans R Soc Trop Med Hyg* 1995; **89**: 692-697 [PMID: 8594699 DOI: 10.1016/0035-9203(95)90449-2]
- 130 **Wen H**, Craig PS, Ito A, Vuitton DA, Bresson-Hadni S, Allan JC, Rogan MT, Paollilo E, Shambesh M. Immunoblot evaluation of IgG and IgG-subclass antibody responses for immunodiagnosis of human alveolar echinococcosis. *Ann Trop Med Parasitol* 1995; **89**: 485-495 [PMID: 7495362]
- 131 **Dreweck CM**, Lüder CG, Soboslay PT, Kern P. Subclass-specific serological reactivity and IgG4-specific antigen recognition in human echinococcosis. *Trop Med Int Health* 1997; **2**: 779-787 [PMID: 9294548 DOI: 10.1046/j.1365-3156.1997.d01-385.x]
- 132 **Wen H**, Craig PS. Immunoglobulin G subclass responses in human cystic and alveolar echinococcosis. *Am J Trop Med Hyg* 1994; **51**: 741-748 [PMID: 7810806]
- 133 **Ben Nouir N**, Gianinazzi C, Gorgii M, Müller N, Nouri A, Babba H, Gottstein B. Isolation and molecular characterization of recombinant *Echinococcus granulosus* P29 protein (recP29) and its assessment for the post-surgical serological follow-up of human cystic echinococcosis in young patients. *Trans R Soc Trop Med Hyg* 2009; **103**: 355-364 [PMID: 19027129 DOI: 10.1016/j.trstmh.2008.09.020]
- 134 **Ben Nouir N**, Nuñez S, Gianinazzi C, Gorgii M, Müller N, Nouri A, Babba H, Gottstein B. Assessment of *Echinococcus granulosus* somatic protoscolex antigens for serological follow-up of young patients surgically treated for cystic echinococcosis. *J Clin Microbiol* 2008; **46**: 1631-1640 [PMID: 18367566 DOI: 10.1128/JCM.01689-07]
- 135 **Buttenschoen K**, Carli Buttenschoen D, Gruener B, Kern P, Beger HG, Henne-Bruns D, Reuter S. Long-term experience on surgical treatment of alveolar echinococcosis. *Langenbecks Arch Surg* 2009; **394**: 689-698 [PMID: 18651165]
- 136 **Kadry Z**, Renner EC, Bachmann LM, Attigah N, Renner EL, Ammann RW, Clavien PA. Evaluation of treatment and long-term follow-up in patients with hepatic alveolar echinococcosis. *Br J Surg* 2005; **92**: 1110-1116 [PMID: 16044412 DOI: 10.1002/bjs.4998]
- 137 **Reuter S**, Jensen B, Buttenschoen K, Kratzer W, Kern P. Benzimidazoles in the treatment of alveolar echinococcosis: a comparative study and review of the literature. *J Antimicrob Chemother* 2000; **46**: 451-456 [PMID: 10980173 DOI: 10.1093/jac/46.3.451]
- 138 **Reuter S**, Buck A, Grebe O, Nüssle-Kügele K, Kern P, Manfras BJ. Salvage treatment with amphotericin B in progressive human alveolar echinococcosis. *Antimicrob Agents Chemother* 2003; **47**: 3586-3591 [PMID: 14576122 DOI: 10.1128/AAC.47.11.3586-3591.2003]
- 139 **Stettler M**, Fink R, Walker M, Gottstein B, Geary TG, Rossignol JF, Hemphill A. In vitro parasitocidal effect of Nitazoxanide against *Echinococcus multilocularis* metacestodes. *Antimicrob Agents Chemother* 2003; **47**: 467-474 [PMID: 12543645 DOI: 10.1128/AAC.47.2.467-474.2003]
- 140 **Kern P**, Abboud P, Kern W, Stich A, Bresson-Hadni S, Guerin B, Buttenschoen K, Gruener B, Reuter S, Hemphill A. Critical appraisal of nitazoxanide for the treatment of alveolar



- echinococcosis. *Am J Trop Med Hyg* 2008; **79**: 119
- 141 **Ammann RW**, Hirsbrunner R, Cotting J, Steiger U, Jacquier P, Eckert J. Recurrence rate after discontinuation of long-term mebendazole therapy in alveolar echinococcosis (preliminary results). *Am J Trop Med Hyg* 1990; **43**: 506-515 [PMID: 2240375]
  - 142 **Wilson JF**, Rausch RL, McMahon BJ, Schantz PM. Parasitocidal effect of chemotherapy in alveolar hydatid disease: review of experience with mebendazole and albendazole in Alaskan Eskimos. *Clin Infect Dis* 1992; **15**: 234-249 [PMID: 1520758 DOI: 10.1093/clinids/15.2.234]
  - 143 **Ammann RW**, Fleiner-Hoffmann A, Grimm F, Eckert J. Long-term mebendazole therapy may be parasitocidal in alveolar echinococcosis. *J Hepatol* 1998; **29**: 994-998 [PMID: 9875648 DOI: 10.1016/S0168-8278(98)80129-3]
  - 144 **Ishizu H**, Uchino J, Sato N, Aoki S, Suzuki K, Kuribayashi H. Effect of albendazole on recurrent and residual alveolar echinococcosis of the liver after surgery. *Hepatology* 1997; **25**: 528-531 [PMID: 9049192 DOI: 10.1002/hep.510250305]
  - 145 **Ammann RW**, Ilitsch N, Marincek B, Freiburghaus AU. Effect of chemotherapy on the larval mass and the long-term course of alveolar echinococcosis. Swiss Echinococcosis Study Group. *Hepatology* 1994; **19**: 735-742 [PMID: 8119701 DOI: 10.1002/hep.1840190328]
  - 146 **Krige J**, Bornman J.C, Belghiti J. Hydatid disease of the liver. In: Belghiti J, Büchler MW, Chapman WC, D'Angelica MI, DeMatteo RP, Hann LE. *Blumgart's Surgery of the Liver, Biliary Tract and Pancreas*. Philadelphia: Elsevier Saunders, 2012: 1050 [DOI: 10.1016/B978-1-4377-1454-8.00068-0]

**P- Reviewer:** Grattagliano I, Reshetnyak VI, Tanaka N  
**S- Editor:** Ji FF **L- Editor:** Wang TQ **E- Editor:** Li D



## Management of refractory ascites in cirrhosis: Are we out of date?

Alagappan Annamalai, Lauren Wisdom, Megan Herada, Mazen Nouredin, Walid Ayoub, Vinay Sundaram, Andrew Klein, Nicholas Nissen

Alagappan Annamalai, Lauren Wisdom, Megan Herada, Mazen Nouredin, Walid Ayoub, Vinay Sundaram, Andrew Klein, Nicholas Nissen, Comprehensive Transplant Center, Cedars Sinai Medical Center, Los Angeles, CA 90048, United States

**Author contributions:** All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

**Conflict-of-interest statement:** No potential conflicts of interest. No financial support.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Alagappan Annamalai, Comprehensive Transplant Center, Cedars Sinai Medical Center, 8900 Beverly Blvd, 2<sup>nd</sup> fl. Suite 262, Los Angeles, CA 90048, United States. [alagappan.annamalai@cshs.org](mailto:alagappan.annamalai@cshs.org)  
 Telephone: +1-310-4232975

Received: March 11, 2016  
 Peer-review started: March 14, 2016  
 First decision: April 20, 2016  
 Revised: July 22, 2016  
 Accepted: August 6, 2016  
 Article in press: August 8, 2016  
 Published online: October 8, 2016

### Abstract

Cirrhosis is a major cause of morbidity and mortality

worldwide with liver transplantations as it only possible cure. In the face of a significant organ shortage many patients die waiting. A major complication of cirrhosis is the development of portal hypertension and ascites. The management of ascites has barely evolved over the last hundred years and includes only a few milestones in our treatment approach, but has overall significantly improved patient morbidity and survival. Our mainstay to ascites management includes changes in diet, diuretics, shunt procedures, and large volume paracentesis. The understanding of the pathophysiology of cirrhosis and portal hypertension has significantly improved in the last couple of decades but the changes in ascites management have not seemed to mirror this newer knowledge. We herein review the history of ascites management and discuss some its current limitations.

**Key words:** Portal hypertension; Cirrhosis; Ascites; Transhepatic portosystemic shunts; Paracentesis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Few randomized control studies have been performed in the management of refractory ascites, of which all were performed either in the pre-model for end-stage liver disease (MELD) era or done in patients with low MELD scores. As such, most of the management guidelines have significant limitations in its utility for patients admitted to the hospital with significant hemodynamic dysfunction and other complications of cirrhosis. Our objective is to review the origins of our current management of refractory ascites and its limitations.

Annamalai A, Wisdom L, Herada M, Nouredin M, Ayoub W, Sundaram V, Klein A, Nissen N. Management of refractory ascites in cirrhosis: Are we out of date? *World J Hepatol* 2016; 8(28): 1182-1193 Available from: URL: <http://www.wjgnet.com>

## INTRODUCTION

Ascites is the most common complication of liver cirrhosis, affecting over half of all cirrhotic patients within ten years of their cirrhosis diagnosis. The onset of ascites marks a critical point in the progression of liver disease, indicating a 50% mortality rate within 2-5 years<sup>[1]</sup>. Ascites is typically well managed with strict adherence to a low sodium diet and diuretic therapy<sup>[2]</sup>. However, in 10% of cirrhotic patients with ascites, maximal diuretic therapy is not effective<sup>[3]</sup>. In these patients with refractory tense ascites, repeated large-volume paracentesis (LVP) becomes the mainstay of chronic management.

LVP for treatment of refractory ascites is fast and effective. However, the removal of large fluid volumes may result in impaired circulatory function up to 6 d after paracentesis<sup>[4]</sup>. This complication, termed paracentesis induced circulatory dysfunction (PICD), is associated with a disruption in the renin-angiotensin axis and results in a hyperdynamic state<sup>[4]</sup>. Defined as an increase in the plasma renin activity by more than 50% of the pre-treatment value to a level of > 4 ng/mL per hour on the 6<sup>th</sup> day after paracentesis, PICD is clinically silent and not spontaneously reversible<sup>[5]</sup>. The occurrence of PICD is associated with a rapid recurrence of ascites, renal failure, and a significant decrease in the probability of survival.

Over the last three decades, only a few prospective studies with limited sample sizes and several large retrospective studies have examined PICD. Therefore, there continues to be a lack of understanding of PICD pathophysiology and management. The purpose of this review is to highlight the evidence supporting current guidelines for the management of patients with refractory tense ascites requiring repeated paracentesis.

## HISTORY OF MANAGEMENT OF TENSE REFRACTORY ASCITES IN CIRRHOTIC PATIENTS

### *The role of paracentesis in the management of ascites*

Paracentesis was first described for the management of tense ascites in the first half of the twentieth century. In the 1950's, however, paracentesis lost favor due to data associating ascitic fluid removal with complications such as hypotension, hyponatremia, acute kidney injury, and hepatic encephalopathy (HE)<sup>[6]</sup>. Two studies, one in 1967, by Knauer *et al*<sup>[7]</sup> and one by Guazzi *et al*<sup>[8]</sup> in 1975, reexamined the value of paracentesis, showing that removing between 1 and 5 L of fluid improved cardiac output (CO). They theorized that small volume removal improved CO by decreasing intra abdominal pressure, increasing venous drainage of the lower extremities, and increasing negative thoracic pressure. Several studies

have since been performed in order to understand the pathophysiology and management of refractory ascites (Table 1).

In 1985, Quintero *et al*<sup>[9]</sup> found that paracentesis with albumin replacement adversely affected hemodynamics, renal function, hospital readmission, and mortality when compared with diuretic therapy in patients treated for tense ascites. Later that same year, Kao *et al*<sup>[10]</sup> studied the effects of paracentesis on circulating blood volume and suggested that paracentesis was a safe therapy in the management of tense ascites secondary to chronic liver disease. This study provided a foundation for current paracentesis guidelines in the setting of cirrhosis in which the authors "arbitrarily selected a volume of 5 L," claiming 5 L of fluid removal to be "large enough to adequately decompress the distended abdomen while affording the patient a reasonable length of time before re-accumulation of ascites becomes a serious problem again". The 18 patient study with strict inclusion/exclusion criteria concluded that no untoward symptoms or findings were caused by 5 L paracentesis, specifically stating that no patients were found to have symptomatic orthostatic hypotension, hyponatremia, worsening renal function, acute renal failure, or HE relatable to paracentesis. The authors did note that all patients had pitting edema, which partially improved soon after paracentesis. They concluded that the absence of clinically significant effects from LVP in their patient cohort could partially be explained by the mobilization of peripheral edema replenishing the plasma volume as it rapidly equilibrated to the loss of ascetic fluid. Thus, the authors did not recommend that their findings be applied to patients without peripheral edema.

In 1987, Salerno *et al*<sup>[11]</sup> investigated the role of paracentesis as a therapy for ascites when compared with traditional diuretic therapy. The study included 41 patients randomized into 2 groups who either received LVP and intravenous (IV) albumin infusions of 20-60 g after each paracentesis or were treated with diuretics and did not receive paracentesis. Salerno concluded that LVP can be performed safely and successfully with equivalent outcomes to diuretics alone. Additionally, Salerno *et al*<sup>[11]</sup> included patients without pitting edema in their study, administering albumin to replace 60%-80% of the protein lost in paracentesis. The authors also found that LVP decreased hospital length of stay without additional risk.

In 1988, Ginès *et al*<sup>[12]</sup> demonstrated that paracentesis followed by IV administration of albumin decreased the risks of renal impairment, hyponatremia, and mortality by preventing systemic hemodynamic alterations. Their study included 105 patients randomized into 2 groups; Group A ( $n = 52$ ) underwent LVP followed by IV albumin infusion of 40 g and Group B ( $n = 53$ ) underwent LVP (4-6 L/d) only. Serious complications were observed in 9 (17%) patients in Group A and 16 (30%) patients in Group B. Hyponatremia and renal impairment were significantly more frequent in Group B, affecting 11 (21%) patients in Group B compared with 1 (2%) patient in Group A. These findings indicated that,

**Table 1 Studies evaluating large-volume paracentesis with albumin infusion and diuretic therapy in hospitalized patients with cirrhosis and refractory ascites**

Ref.	Study design	Results	Conclusions/comments
Quintero <i>et al</i> <sup>[9]</sup> , 1985	Total <i>n</i> : 72 Group 1: LVP and albumin - <i>n</i> of 38 Group 2: Diuretic therapy - <i>n</i> of 34	LVP with albumin had worse outcomes than diuretic therapy with adverse effects on hemodynamics, renal function, readmission, mortality	Diuretic therapy is better than LVP
Kao <i>et al</i> <sup>[10]</sup> , 1985	Total <i>n</i> : 18 underwent LVP of exactly 5 L Exclusion criteria: Cardiac disease chronic renal disease active intestinal bleed encephalopathy 500 mg/d Na and 1 L/d fluid restriction Diuretic discontinued 3 d prior	No untoward effects LVP of 5 L No symptomatic hypotension or hyponatremia No worsening or acute renal failure No encephalopathy Improved pitting edema	LVP is safe in patients with peripheral edema due to mobilization of fluid to intravascular space
Salerno <i>et al</i> <sup>[11]</sup> , 1987	Total <i>n</i> : 41 patients randomized into 2 groups Group A: Paracentesis + IV albumin: 20 patients Group B: Paracentesis + diuretics: 21 patients Exclusion criteria: Urinary sodium excretion rate > 20 mEq/d on a sodium-restricted diet and without diuretics Presence of cancer, encephalopathy, active gastrointestinal bleeding, renal failure, diabetes, infection, or primary cardiac disorders Hemoglobin < 9 g/dL Total bilirubin > 6 mg/dL Aminotransferases > 200 U/L Serum urea > 60 mg/dL Serum creatinine > 1.5 mg/dL	Deaths: Group A: 2/20 Group B: 3/21 Complications (encephalopathy, renal failure, and gastrointestinal bleeding): Group A: 3/20 patients Group B: 4/21 patients Group A: Satisfactory mobilization for ascites for 19/20 patients 4/20 patients did not reaccumulate ascites while 15/20 patients did reaccumulate ascites Group B: Resolution of ascites in 19/21 patients Diuretic treatment was unsuccessful for 2/21 Group B patients who were receiving the highest doses of diuretic therapy Group A: Mean body weight significantly reduced at all times after paracentesis, slight decrease in heart rate and urine osmolality (day 10). Increase noted in PAC (days 5 and 10) and urine flow rates (days 5, 10, and 15). Increased urine flow rates in 14 patients who also had significantly lower baseline urine excretions than the other 5 responsive Group A patients In the 19/21 responsive Group B patients, significant body weight reductions observed on days 10 and 15. Mean blood pressure and heart rate did not change. Significant increases noted in urine flow rate, sodium and potassium excretion, plasma albumin and potassium concentrations. Significant decrease in urine osmolality	LVP is faster and equally effective alternative to diuretic therapy and suggested that LVP might be used to decrease hospital length of stay without additional risk
Ginès <i>et al</i> <sup>[12]</sup> , 1988	105 patients randomized into 2 groups Group A: Paracentesis + IV albumin: 52 patients Group B: paracentesis without fluid replacement: 53 patients Exclusion criteria: Similar to study by Salerno <sup>[10]</sup>	Died in hospital: Group A: 2/52 Group B: 2/53 Deaths at 1 yr: Group A: 20/52 Group B: 16/53 Complications of hyponatremia, renal impairment, encephalopathy, gastrointestinal hemorrhage, and severe infection: Group A 9/52 Group B 16/53 Group A: Significant increase in serum albumin, GFR, free water clearance Group B: No change in serum albumin, significant increase in BUN, PRA, PAC, significant decrease in serum sodium PRA significant increase at 48 h and 5 d post LVP Group B 23/24 and 9/24 respectively Group A had none Readmission: Group A 29/52 Group B 36/53 Renal impairment: Group A: None Group B: 11/53	These findings indicated that, aside from systemic hemodynamics, there are likely multiple factors, such as renal production of vasodilators or ADH antagonists, which contribute to the development of renal failure



Ginès <i>et al</i> <sup>[5]</sup> , 1996	289 patients randomized into 3 groups	Deaths:	PICD found to not be spontaneously reversible and persists during follow-up
	Group A: Paracentesis + IV albumin: 97 patients	Group A 2/97	PICD associated with faster reaccumulation of ascites and impaired prognosis
	Group B: Paracentesis + Dextran 70: 93 patients	Group B 4/93	
	Group C: Paracentesis + Polygeline: 99 patients	Group C 6/99	
	Exclusion criteria: Similar to study by Salerno <sup>[10]</sup>	PICD (based on 280 patients who developed dysfunction and had PRA measured at baseline and 6 d after the procedure):	The authors suggest that albumin is more effective than dextran 70 or polygeline at preventing postparacentesis circulatory dysfunction and is the volume expander of choice for cirrhotics who undergo paracentesis with > 5 L of ascites removed
		Total 85/289	The authors discussed the pathophysiology of PICD, theorizing that PICD was most likely secondary to variable changes in neurohormonal responses, which accelerate the disease and lead to decreased long-term survival. They felt that PICD was unlikely due to a more advanced disease state, as patients with and without PICD did not differ in their degree of liver, renal, or hemodynamic function after paracentesis
		PRA > 50% increase (at 2 d after LVP) if PICD occurred: 47/85	
		PICD associated with shorter survival	
		Complications of hyponatremia, renal impairment, hepatic encephalopathy, gastrointestinal bleeding, bacterial infection	
		Group A: 28/97 patients, 30 complications	
		Group B: 28/93 patients, 43 complications	
		Group C: 30/99 patients, 39 complications	
		Incidence of death with PICD: 5/85	
		Incidence of death without PICD: 6/195	

LVP: Large-volume paracentesis; IV: Intravenous; PICD: Paracentesis induced circulatory dysfunction; ADH: Antidiuretic hormone.

aside from systemic hemodynamics, there are likely multiple factors, such as renal production of vasodilators or antidiuretic hormone (ADH) antagonists, which contribute to the development of renal failure.

In 1988, Pinto *et al*<sup>[13]</sup> and Gentile *et al*<sup>[14]</sup> both independently studied the hemodynamic and hormonal impacts of LVP of exactly 5 L in 12 non-edematous cirrhotic patients. Both studies concluded that LVP of 5 L could be safely performed without significant changes in plasma volumes, PRA, or vasopressin. They did, however, note a significant decrease in diastolic pressure and a significant increase in aldosterone, which corresponded with reduced urinary sodium excretion.

In 1990, Panos *et al*<sup>[15]</sup> confirmed an earlier finding of Simon *et al*<sup>[16]</sup> in 1987 that, up to 3 h after LVP, CO increased, right atrial pressure decreased, and pulmonary capillary wedge pressure (PCWP) remained the same. After 3 h post-LVP, right atrial pressure, PCWP, and CO all decreased significantly. These findings indicated that, although paracentesis initially results in hemodynamic improvement, a relative hypovolemia occurs hours after paracentesis.

Two studies in 1990 and two in 1991 evaluated the effect of various IV infusions to prevent hypovolemia after LVP<sup>[17]</sup>. The studies included comparisons between albumin, dextran-70, dextran-40, hemaccel, and saline<sup>[18]</sup>. They concluded that dextran-70, albumin, and hemaccel were all equally effective in preventing renal and electrolyte complications, while dextran-40 was ineffective. A third study by Cabrera *et al*<sup>[19]</sup> in 1990 found that IV saline prevented hypovolemia with no changes in PRA or aldosterone.

Albumin was effective in preventing hypovolemic complications, however, it was a costly product. To investigate possible alternatives, Planas *et al*<sup>[18]</sup> conducted a

randomized trial comparing the efficacy of three different plasma expanders for preventing, PICD. PICD was defined as an increase in PRA of more than 50% of the pretreatment value to a level of > 4 ng/mL per hour on the 6<sup>th</sup> day after paracentesis. This pretreatment value was determined by the upper value of PRA found in 36 healthy subjects studied on a 50-mmol/d sodium diet and was arbitrarily chosen to represent physiologically relevant activation of the renin-angiotensin system. In the study of Planas *et al*<sup>[18]</sup>, patients were randomized to receive one of the three infusion types: Albumin, dextran-70, or polygeline. Eighty-five patients developed PICD, with a significantly greater frequency when treated with dextran-70 (34.4%) and polygeline (37.8%) than when treated with albumin (18.5%). Additionally, they found a significantly higher 6-mo mortality rate in patients who develop PICD. They further concluded that PICD was predictive in fluid removal > 5 L with the use of dextran-70 or polygeline. This trend did not appear in patients receiving > 5 L of fluid removal followed by albumin infusion. The authors discussed the pathophysiology of PICD, theorizing that PICD was most likely secondary to variable changes in neurohormonal responses, which accelerate the disease and lead to decreased long-term survival. They felt that PICD was unlikely due to a more advanced disease state, as patients with and without PICD did not differ in their degree of liver, renal, or hemodynamic function after paracentesis.

The following year, in 1997, Ruiz-del-Arbol *et al*<sup>[20]</sup> demonstrated an inverse correlation between PRA and systemic vascular resistance (SVR) associated with PICD. Out of the 37 patients who underwent LVP (mean > 7 L) followed by a dextran-70 infusion, 10 (27%) developed PICD. More specifically, they found that despite the normalization of PRA, aldosterone, and norepinephrine

by the 6<sup>th</sup> day after paracentesis, cardiopulmonary pressures and SVR remained lower than baseline. The authors believed that LVP is an inciting event that leads to an accentuation of the vasodilatory response already present in cirrhotic patients. This exaggerated vasodilatory response then causes an increase in PRA to compensate for increases in SVR. In addition, utilizing a transjugular intrahepatic venous catheter they found that the hepatic venous pressure gradient did not change in patients without PICD but increased significantly, secondary to PRA, if PICD occurred. They theorized that this was also likely due to endogenous vasoactivation.

In 1998, Vila *et al*<sup>[21]</sup> confirmed these conclusions and also found that if effective hypovolemia did not develop, there were no significant changes in CO, CVP, or SVR and there was a significant reduction in PRA at the 1 and 3 h period after paracentesis. In contrast, if effective hypovolemia did develop, there were significant reductions in CO, CVP and SVR, no change in PRA or aldosterone level, and an increase in CO. This paradoxical finding was believed to be due to physiological responses secondary to abrupt falls in intraabdominal pressure after paracentesis procedures.

In a pilot study in 2002, Moreau *et al*<sup>[22]</sup> compared the effect of terlipressin and albumin on arterial blood volume in 20 cirrhotic patients who underwent paracentesis. Assuming that PICD is predominantly caused by exacerbation of an already dilated arterial system, the authors theorized that terlipressin, a vasoconstrictor, may prevent PICD more effectively than albumin. After paracentesis, 10 patients received albumin and the other 10 received terlipressin. They found that both treatments had the same beneficial effect of preventing arterial vasodilation. The authors favored the use of terlipressin, arguing for cheaper cost.

In 2003, Sola-Vera *et al*<sup>[23]</sup> compared PICD in 37 patients receiving albumin and 35 patients receiving saline infusion after LVP. They found that patients who received saline had a significant increase in PRA and PAC on the 6<sup>th</sup> day after paracentesis, which contradicted data published by Cabrera *et al*<sup>[19]</sup> in 1990. Only 11% of patients developed PICD after albumin infusion compared to 33% after saline infusion. If < 6 L was removed, the PICD was similarly low in both groups (6.7% in albumin group vs 5.6% in saline group). Additionally, they found that nitric oxide (NO) was elevated in the saline group and likely contributed to the pathogenesis of PICD.

The prevention of PICD using albumin infusion was compared to the use of midodrine post-paracentesis in a study by Appenrodt *et al*<sup>[24]</sup> in 2008. They performed a blinded study in 24 patients with tense ascites and included patients with similar comorbidities as prior studies. Additionally, since this study was conducted after the inception of MELD scoring in 2002, they reported a mean MELD of 11 in both the midodrine and albumin groups. Midodrine was given immediately after paracentesis at a dose of 12.5 mg orally every 8 h for 2 d. In the midodrine group, they found a large, but

insignificant, increase in the PRA level on day 6 after paracentesis. They concluded that the use of midodrine was less effective than albumin in preventing PICD.

In 2010, Nasr *et al*<sup>[25]</sup> evaluated the risk factors for PICD. The study included 45 patients with cirrhosis and used similar inclusion criteria as the prior studies mentioned. The patients received either albumin or dextran-70 post-paracentesis and the volume removed ranged from 8 to 18 L. They evaluated several demographic, clinical and laboratory factors, and found, based upon logistic regression analysis, that only the use of dextran-70 and younger age were independent predictors of PICD.

A multicenter trial including 26 patients was published in 2011 by Fimiani *et al*<sup>[26]</sup>. This trial evaluated the impact of a combination of diuretics, albumin, and terlipressin in treating tense ascites. The study examined several clinical factors after paracentesis, including ascites recurrence, body weight, abdominal circumference, and urinary sodium excretion. The combination of changes in these factors was given a grade of severity and a degree of response. Based upon these definitions, they concluded that combination treatment decreased the need for repeated LVP, improved urinary sodium, reduced abdominal circumference, and decreased the severity of ascites.

In the same year, Alessandria *et al*<sup>[27]</sup> compared the efficacy of different volumes of post-paracentesis albumin infusion, comparing the incidence of PICD between patients who received 4 g of albumin per liter of fluid removed and patients who received 8 g of albumin per liter of fluid removed. They found the same incidence of PICD, hyponatremia, and renal failure in both groups and concluded that half the standard dose of albumin is as effective and safe as the full standard dose in patients undergoing paracentesis.

In 2013, Carl *et al*<sup>[28]</sup> performed a small trial including 10 patients with the purpose of studying the relationship between inflammation and PICD after LVP. They looked at several factors over a 24-h period, including blood pressure, BUN, creatinine (Cr), PRA, aldosterone, angiotensin II, asymmetrical dimethylarginine (ADMA), norepinephrine, CD14, interleukin-6, tumor necrosis factor- $\alpha$ , and monocyte chemoattractant protein-1 (MCP-1). Both MCP-1 and CD14 increased concurrently while blood pressure decreased in the 24 h after LVP. These results suggested that the inflammatory cascade may be involved in the genesis and severity of PICD.

### **The role of transhepatic portosystemic shunts in the management of ascites**

Until 1996, large volume paracentesis was the standard therapy for refractory tense ascites. Although this was proven to be an effective treatment approach, it did not address the underlying issue of portal hypertension. After LVP, ascites would quickly re-accumulate and require repeated paracentesis. On the other hand, a transhepatic portosystemic shunts (TIPS) has the potential to mitigate portal hypertension by diverting portal blood flow from the liver directly into the systemic

venous circulation *via* an intrahepatic shunt. Several studies have been conducted comparing TIPS to LVP<sup>[29]</sup> (Table 2).

In 1996, Lebrech *et al.*<sup>[30]</sup> compared the effect of TIPS and LVP in 25 cirrhotic patients with refractory ascites who were randomized to TIPS or repeat LVP. The authors concluded that intrahepatic shunts were selectively effective in patients with Childs-Pugh class B, although they did not improve survival, and actually decreased survival in class C patients compared to LVP. They believed that the prominent factor is ascites management were dependent on both neurohormonal factors which control natriuresis and the hepatic sinusoidal pressures.

In 2000, Rössle *et al.*<sup>[31]</sup> conducted a similar randomized study in 60 patients comparing TIPS to LVP. Fifteen of the 29 TIPS patients died while 23 of the 31 LVP patients died at 1 year. Although 10 patients required rescue shunt treatment, no deaths or long-term illnesses occurred secondary to the shunting procedure. In comparison with LVP, the creation of a transjugular intrahepatic portosystemic shunt can improve the chance of survival without liver transplantation in patients with refractory or recurrent ascites.

In 2002, Ginès *et al.*<sup>[32]</sup> published a study comparing survival rates and associated healthcare costs between patients receiving TIPS and patients receiving paracentesis with albumin replacement. Seventy cirrhotic patients with refractory ascites were selected for the study and randomly assigned to either undergo TIPS ( $n = 35$ ) or repeat LVP ( $n = 35$ ) with albumin infusions. MELD scores were not used, as this study was conducted prior to the start of MELD scoring. They concluded that TIPS lowers the rate of ascites recurrence and the risk of developing hepatorenal syndrome, but does not improve survival and has increased occurrence of encephalopathy and higher cost than LVP.

In 2003, Sanyal *et al.*<sup>[33]</sup> also compared TIPS to LVP in 109 patients with refractory ascites. The LVP group consisted of 57 patients who received low sodium diets, diuretics, and LVP. The TIPS group consisted of 52 patients who received TIPS in addition to the same low sodium diets and diuretics as the LVP group. In the first year following randomization, they found that 22 (42%) TIPS patients and 48 (84%) LVP patients required repeat LVP's for recurrent tense ascites. The average rate of paracentesis per patient in the first year was 1.69 for TIPS patients and 6.11 per year for LVP patients. Mortality was 21 (40%) in the TIPS group and 21 (37%) in the LVP group. Sixteen (31%) TIPS patients and 17 (30%) LVP patients received liver transplants.

In 2004, Salerno *et al.*<sup>[34]</sup> randomized 65 cirrhotic patients with refractory ascites into 2 groups. Thirty-two patients received TIPS and 33 patients received LVP. Mean baseline MELD was  $11.1 \pm 0.8$  in the TIPS group and  $11.1 \pm 0.9$  in the LVP group. The Cox proportional hazard model indicated that the treatment assigned and MELD scores were independent predictors of mortality. In 2007, Salerno *et al.*<sup>[35]</sup> published a meta-analysis based

upon individual patient data on outcomes of TIPS for refractory ascites. The study included all published data from randomized control trials with available patient data. This excluded the study by Lebrech *et al.*<sup>[30]</sup>, which was the only study to show a negative effect of TIPS on survival. Salerno *et al.*<sup>[35]</sup> concluded: (1) TIPS improves transplant-free survival compared to LVP; (2) patient survival is independently associated with age, bilirubin levels, and serum sodium concentrations; (3) the risk of ascites recurrence is decreased with TIPS; (4) the probability of HE after TIPS is increased; and (5) patients with low arterial pressure, high MELD score, and low portosystemic pressure gradient after TIPS have the greatest probability of experiencing post-TIPS HE.

## **PATHOPHYSIOLOGY OF PICD**

Over the last three decades, as LVP has become more widely accepted as the standard first line approach in treating refractory tense ascites, we have gained further insight into the pathophysiology of PICD. Portal hypertension is a major sequel of cirrhosis and occurs secondary to increases in intrahepatic resistance to portal blood flow<sup>[36]</sup>. The deposition of collagen in the hepatic acinus of the cirrhotic patient leads to narrowing of the sinusoidal lumen, compression of the venules due to regenerative nodules, the development of fibrosis, and portal inflammation<sup>[1]</sup>. Each of these sequelae contribute to liver stiffness, which resists the inflow of portal blood<sup>[37]</sup>. In addition to these structural changes, there are several neuro-hormonal factors that alter the contractile tone of intrahepatic endothelial cells<sup>[38]</sup>. Shear stress and bacterial translocation occurs, leading to endothelial dysfunction in the pre-sinusoidal areas. This causes the release of NO and the increased production of COX-derived prostanoids<sup>[2]</sup>. The combination of portal blood flow resistance due to cirrhosis and increased arterial inflow from splanchnic vasodilation leads to portal hypertension. Portal hypertension is maintained by the opening of portal-systemic collaterals as well as the generation of new vessels *via* angiogenesis. Splanchnic vasodilation is mediated by several substances, including glucagon, prostacyclin, intestinal vasoactive peptide, histamine, substance P, estrogens, cholecystokinin, ammonia, endotoxins, adenosine, biliary acids, NO, alpha-calcitonin gene-related peptide, vascular endothelial growth factor, adenomedullin, carbon monoxide, and endogenous cannabinoids<sup>[39]</sup>.

There is a complex and relatively poorly understood interaction between these mediators in controlling blood flow. Recently, it has been suggested that NO plays a prominent role. However, several *in vitro* studies have demonstrated variable changes in compensatory factors when NO is inhibited or promoted, suggesting that its control is not the only important factor. In response to the release of vasodilators in the splanchnic system, there is a release of vasoconstrictors. Due to the high levels of NO and CO, these vasoconstrictors have a blunted effect on splanchnic circulation and mostly affect the kidneys

**Table 2 Randomized control studies evaluating transhepatic portosystemic shunts vs paracentesis in patients with cirrhosis and refractory ascites**

Ref.	Study design	Results	Conclusions/comments
Lebrec <i>et al</i> <sup>[30]</sup> , 1996	Total of 25 13 TIPS 12 LVP Excluded: Age > 70 Severe diseases other than liver Pulmonary hypertension Hepatocellular carcinoma Hepatic encephalopathy Sepsis/spontaneous bacterial peritonitis Severe alcoholic hepatitis Portal/hepatic vein obstruction/ thrombosis Obstruction of biliary tract or hepatic artery Plasma creatinine > 150 mmol/L	Deaths: TIPS - 9/13 LVP - 4/12 3/13 TIPS unsuccessful, of the remaining 10/13 TIPS patients: 8 required a second shunt and 2 required 3 shunts 1/12 LVP patients received liver transplant Survival at 2 yr with "intention to treat" analysis 29% $\pm$ 13% for TIPS and 60% $\pm$ 16% for LVP Survival at 2 yr with "per protocol" analysis was 38% $\pm$ 16% for TIPS and 70% $\pm$ 15% for LVP	The authors concluded that intrahepatic shunts were selectively effective in patients with Childs-Pugh class B, although they did not improve survival, and actually decreased survival in class C patients compared to LVP. They believed that the prominent factor is ascites management were dependent on both neurohormonal factors which control natriuresis and the hepatic sinusoidal pressures
Rössle <i>et al</i> <sup>[31]</sup> , 2000	Total of 60 patients Randomized to 2 groups: TIPS 29/60 LVP 31/60 Excluded: Hepatic encephalopathy > Grade 2 Serum bilirubin > 5 mg/dL Serum creatinine > 3 mg/dL Portal-vein thrombosis Hepatic hydrothorax Advanced cancer Continual ascites after paracentesis or multiple paracentesis within 1 wk	Deaths: TIPS - 15/29 LVP - 23/31 13/29 patients had shunt insufficiency, 11/29 underwent reestablishment of the shunt after 10 $\pm$ 16 mo and 5 of these patients required a second reestablishment 1/29 TIPS patients received liver transplant 2/31 LVP patients received liver transplant These patients were alive 60 mo following transplant Of the patients assigned to paracentesis in whom this procedure was unsuccessful, 10 received a transjugular shunt a mean of 5.5 $\pm$ 4 mo after randomization; 4 had a response to this rescue treatment Estimated probability of survival without transplant: TIPS: 69% and 58% at 1 and 2 yr; LVP: 52% and 32% at 1 and 2 yr In a multivariate analysis, treatment with transjugular shunting was independently associated with survival without the need for transplantation ( $P = 0.02$ ) At three mo, 61% of the patients in the shunt group and 18% of those in the paracentesis group had no ascites ( $P = 0.006$ ) Age > 60 yr, female sex, bilirubin > 3 mg/dL, and serum sodium < 125 mmol/L significantly decreased survival in the TIPS group	In comparison with large-volume paracentesis, the creation of a transjugular intrahepatic portosystemic shunt can improve the chance of survival without liver transplantation in patients with refractory or recurrent ascites
Ginès <i>et al</i> <sup>[32]</sup> , 2002	Total of 70 patients randomized into 2 groups TIPS: 35 LVP + Albumin (8 g/L ascites removed): 35 Primary endpoint: Survival without liver transplantation Secondary endpoints: Complications of cirrhosis and cost Excluded: < 18/> 75 years old Serum bilirubin > 10 mg/dL Prothrombin time < 40% Platelet count < 40000/mm <sup>3</sup> Serum creatinine > 3 mg/dL Hepatocellular carcinoma Complete portal vein thrombosis Cardiac/respiratory failure Organic renal failure Bacterial infection Hormonal measurements (plasma renin	Deaths: TIPS 20/35 LVP 18/35 Transplanted: TIPS 7/35 LVP 7/35 1 TIPS patient required repeat LVP's 3 LVP patients required TIPS placement Ascites recurrence: TIPS - 17 patients developed 60 episodes of ascites (30 episodes attributed to 1 patient who experienced a total occlusion of their shunt), LVP - 29 patients developed 341 episodes of ascites Median time of the first recurrence of ascites: TIPS - 171 d LVP - 20 d 13 TIPS patients experienced shunt dysfunction	They concluded that TIPS lowers the rate of ascites recurrence and the risk of developing hepatorenal syndrome, but does not improve survival and has increased occurrence of encephalopathy and higher cost than LVP



	activity, aldosterone, norepinephrine, and atrial natriuretic peptide) were measured at 1 wk, 1 mo and 6 mo in 18 TIPS patients and 23 LVP patients	Total costs for TIPS patients (calculated separately in United States dollars on intention-to-treat basis from Spanish and then United States hospitals that participated in the study) demonstrated that total costs and costs per patient were greater in the TIPS group TIPS \$693460, or \$19813 per patient. LVP patients were \$341760, or \$9765 per patient	
Sanyal <i>et al</i> <sup>[33]</sup> , 2003	109 patients with refractory ascites were randomized into 2 groups 52 patients received TIPS with medical therapy (low sodium diets, diuretics, and LVP) 57 patients received medical therapy without TIPS Excluded: Similar criteria to prior studies All patients placed on low Na diets and diuretics All patients placed on low Na diets and diuretics Diuretics stopped 5 d prior to LVP Albumin infusion followed LVP at 6-8 g/L removed TIPS patients received shunts Some patients from both groups received repeat LVP's plus Albumin for tense, symptomatic ascites with weight gain > 10 pounds	Deaths: TIPS - 21/52 LVP 21/57 Failed Treatments: TIPS 3/52 unsuccessful LVP 2/57 patients required TIPS Failed treatments in the first year after randomization requiring repeat LVP for tense ascites: TIPS - 22/52 LVP 48/57 Average rate of LVP per patient in the first year after randomization: for TIPS - 1.69 LVP - 6.11 Transplants: TIPS 16/52 LVP 17/57	Although TIPS plus medical therapy is superior to medical therapy alone for the control of ascites, it does not improve survival, affect hospitalization rates, or improve quality of life
Salerno <i>et al</i> <sup>[34]</sup> , 2004	66 patients randomized into 2 groups TIPS group: 33 LVP + Albumin group: 33 Excluded: Similar criteria to prior studies Diuretic doses continued throughout the study and doses adjusted for each patient's clinical needs All patients on low Na diets (80 mg/d) TIPS placed LVP patients received Albumin replacements at 8 g/L ascites removed Patients discharged and followed at 1, 3 and 6 mo, then every 3-6 mo or as clinically necessary Mean follow up time was 18.2 ± 2.3 mo	Deaths: TIPS - 13/33 LVP - 20/33 Failed treatments: TIPS - 3/33 Initial LVP - 0/33 reported Estimated probability of survival at 1 yr: TIPS - 77% LVP - 52% Estimated probability of survival at 2 yr: TIPS 59% LVP 29% Transplanted: TIPS 4/33 LVP 4/33 Cox proportional hazard model indicated that treatment assigned and MELD scores were independent predictors of mortality Failure of treatment noted in 7/33 TIPS patients: 2 patients received LeVeen Shunts and 5 LVP's Failure of treatment noted in 19/33 LVP patients: 1 received a LeVeen Shunt, 11 received TIPS, and 7 elected to continue with LVP treatment	Treatment failure was more frequent in patients assigned to paracentesis, whereas severe episodes of hepatic encephalopathy occurred more frequently in patients assigned to TIPS The number and duration of re-hospitalizations were similar in the two groups Compared to large-volume paracentesis plus albumin, TIPS improves survival without liver transplantation in patients with refractory ascites

LVP: Large-volume paracentesis; TIPS: Transhepatic portosystemic shunts; MELD: Model for end-stage liver disease.

and the brain<sup>[39]</sup>.

Splanchnic vasodilation leads to an abnormally increased distribution of blood into the mesenteric circulation. Over time, there is an exaggerated disequilibrium of blood supply between the central and non-central volumes, characterized by a decrease in the central (heart, lungs, and brain) blood volume and an increase in the non-central (splanchnic) blood volume. These shifts in blood volume are not clinically significant in the early stages of cirrhosis but become more relevant

as the disease worsens. With the development of non-central vasodilation and pooling of blood in the mesenteric circulation, there is an initial compensatory increase in CO and a decrease in MAP and SVR. With the activation of baroreceptors, this is accentuated over time, causing further increases in CO and heart rate. As the sympathetic nervous system, renin-angiotensin-aldosterone system, arginine-vasopressin, and endothelin responses heighten, renal vascular resistance increases. This increase causes vasoconstriction and decreased

renal blood flow leading to sodium and water retention. Over time, as more blood volume sequestration occurs in the splanchnic system, the compensatory mechanisms are unable to sustain blood flow, leading to tissue hypoxemia and end-organ damage. This cascade of pathophysiological responses to portal hypertension is termed hyperdynamic circulatory syndrome and is generally characterized by an increase in CO and heart rate and a decrease in SVR and MAP<sup>[36]</sup>.

Most patients who require LVP to manage refractory ascites exhibit hyperdynamic physiology, with increased CO and heart rate and decreased MAP. Generally after paracentesis, there is an immediate and significant decrease in intraabdominal pressure. This leads to initial hemodynamic improvement, increasing CO as venous return and negative thoracic pressures improve. In general if less than 5 L of fluid is removed, there appears to be no ill effects of paracentesis. If > 5 L, or an "LVP", is performed, relative hypovolemia develops hours after the procedure<sup>[40]</sup>. This causes a series of complex neuro-hormonal responses that are not well understood. It appears that within 1 h after LVP, there is an increase in cardiac index and an associated decrease in SVR. There are discrepant findings in the literature regarding the pathophysiological cause of the decrease in SVR. However, it may be related to improved CO alone or changes in both the renin-angiotensin system and the sympathetic nervous system. The exact neurohormonal changes, sequence of events, progression over time, and impact on the cardiovascular and renal systems are also not clear. Overall, the initial improvement in hemodynamics after paracentesis is followed by a relative hypovolemia. This leads to circulatory dysfunction demonstrated by increased PRA, ADH, and aldosterone levels and decreased MAP and SVR. This constellation of events, termed PICD, is most commonly associated with hyponatremia and renal insufficiency<sup>[5]</sup>.

## SUMMARY AND CURRENT CLINICAL PRACTICE GUIDELINES ON MANAGEMENT OF REFRACTORY ASCITES

Refractory ascites is defined as fluid overload that is unresponsive to high-dose diuretics (spironolactone 400 mg/d and furosemide 160 mg/d) and sodium-restrictive diets, recurring rapidly after therapeutic paracentesis<sup>[36]</sup>. Diuretic therapy is considered to have failed when there is minimal or no weight loss coupled with poor urinary sodium restriction (< 78 mmol/d) or when there are clinical complications of encephalopathy, serum Cr > 2.0 mg/dL, serum sodium < 120 mmol/L, or serum potassium > 6.0 mmol/L. Initial failure of diuretic therapy should be treated medically (fluid restriction, sodium restriction, and diuretic therapy), followed by serial LVP while awaiting liver transplant. If LVP is not feasible, TIPS or surgical peritoneovenous shunting is recommended<sup>[1,41]</sup>.

The American Association for the Study of Liver Disease (AASLD), the European Association for the Study of Liver Disease, and International Ascites Club have written review articles and recommended summary guidelines for the management of ascites secondary to portal hypertension in cirrhotic patients. The most recent AASLD practice guideline update, published in 2012 by Runyon, made several recommendations for treating cirrhotic patients diagnosed with refractory ascites. The guidelines stated that: (1) beta blockers should be discontinued or not initiated due to risks of complications of systemic hypotension and evidence of decreased survival (Class III, Level B); (2) angiotensin converting enzyme inhibitors should be avoided due to complications of hypotension (Class III, Level B); (3) in patients with hypotension, randomized trials have shown that oral midodrine (7.5 mg TID) improves urinary volume, urine sodium, MAP, and survival theoretically due to its ability to improve blood pressure and convert patients from diuretic-resistant to diuretic-sensitive (Class II a, Level B); (4) after discontinuation of beta blockers and administration of midodrine, refractory ascites should be treated with serial LVP (Class I, Level C); (5) following a single paracentesis of < 4-5 L, albumin infusion may not be required to prevent PICD (Class I, Level C); (6) LVP (> 5 L), requires albumin infusion of 6-8 g/L of fluid removed to improve survival (Class II a, Level A); (7) TIPS should be considered in patients who meet criteria as described in above mentioned randomized trials but is considered a second line therapy after LVP (Class I, Level A); and (8) peritoneovenous shunting should be performed if patients are not candidates for paracentesis, TIPS, or transplant (Class II b, Level A). These are the current management guidelines to which most transplant centers in North America adhere.

## ISSUES AND CONTROVERSIES

In our review of the literature regarding the management of refractory ascites, there are several major issues. The first liver transplant was performed in 1963 but it did not become a practical therapy for patients with end-stage liver disease until the 1980's when the use of cyclosporine for preventing organ rejection allowed long-term patient survival. Research efforts in cirrhosis have since intensified, but the pathophysiology of the complications of cirrhosis remain incompletely understood. As such, research has tended to compartmentalized each of the various complications. While many complex diseases are evaluated using this method of scientific research, cirrhosis may require a more holistic approach since cirrhosis occurs affects essentially every organ system in the body during its progression.

Our current understanding of ascites and its management seems to be based, in large measure, on evidence and observations derived from research performed decades ago. Furthermore, the evidence is based on a focused perspective rather than a global one and does not take into account the dynamic and evolving

systemic nature of cirrhosis.

Large volume paracentesis is defined as a volume of > 5 L. This amount of fluid removal is somewhat arbitrary, originally coined in 1987 by Kao *et al.*<sup>[10]</sup> based upon a description of the volume required to “flatten the abdomen”. Since then, LVP of > 5 L has been used universally as the gold standard when considering fluid replacement. We could not find a single study that examined the impact of variations in paracentesis volume on neuro-hormonal changes in equivalent patients. Hence, we would challenge the validity of defining a 5 L paracentesis as what constitutes a “large volume”.

In addition, a paracentesis volume of > 5 L is considered the amount above which PICD occurs. Before 1986, there were few studies that analyzed patients with paracentesis of < 5 L. In the studies published since 1986, which evaluate the impact of fluid replacement, neuro-hormonal responses, and effects of medications on PICD, the mean volumes of paracentesis were always > 5 L. Thus, it is unclear how the conclusion that a paracentesis of > 5 L causes PICD can be made when no significantly sized group of similar patients with < 5 L fluid removal have been compared. It is likely that the occurrence of physiologically significant changes after paracentesis are dependent upon a multitude of factors and not only on this “minimum” amount of 5 L of removal.

Patient volume status, fluid responses, medication doses, and many other physiological effects are based upon patient sex, height, weight, muscle mass, renal function, or body mass index (BMI). Along the same lines, one would assume that the effect, responses, and management of fluid shifts in cirrhotic patients undergoing paracentesis should be affected similarly. The accepted management guidelines for refractory ascites requiring paracentesis does not incorporate any of these principles and is instead based only on a removal volume of > 5 L. Although never studied, it is more likely that physiological responses after paracentesis in cirrhotic patients have a graded effect based upon variables such as milliliter of fluid removed per kilogram body weight, BMI, muscle mass, and sex.

Additionally, the definition of PICD as “an increase in the plasma renin activity by more than 50% of the pretreatment value to a level of > 4 ng/mL per hour on the 6<sup>th</sup> day after paracentesis” appears to have been arbitrarily created based upon the mean PRA levels of 36 healthy subjects. Studies conducted based upon this definition showed that PICD is associated with decreased 6-mo survival. It can be safely concluded that there is survival disadvantage when untoward effects of paracentesis occur, but it is not exactly clear what the “cut-off” values of PRA should be. Another approach may be to linearly determine the effect of changes in PRA on mortality and hence determine what correctly defines LVP. Because PICD has been associated with hyponatremia and renal insufficiency, there may be some utility in proving end organ damage. However, it is not clear how this can be

achieved in cirrhotic patients who already have significant multi-organ compromise. One crude method would be to assess mixed venous oxygenation or lactate levels at different time points after paracentesis.

Our current management guidelines for refractory ascites and PICD are based upon physiological effects of LVP determined in studies conducted before the inception of MELD scoring in 2002. Although individual patient data is not available, based upon the patient characteristics published in each manuscript, the mean MELD scores of the groups of patients included in these studies appears to be < 15. In our current era, the mean MELD at the time of transplant ranges from 23-35 depending on the UNOS Region. Given this difference in disease severity, the effects of paracentesis established in previous studies may not be applicable in patients with more advanced cirrhosis. There is no published data comparing the effects of similar volumes of paracentesis with more progressive cirrhosis or higher MELD scores.

Furthermore, all of these studies had very strict inclusion criteria, excluding patients with common cirrhosis complications, such as HE, active gastrointestinal bleeding, renal failure, diabetes, infection, cardiac disorders, hemoglobin < 9 g/dL; total bilirubin lower than 6-10 mg/dL; and serum creatinine < 1.5-3 mg/dL, or platelet count > 40000. As cirrhosis progresses, most patients develop these complications and begin to exhibit hyperdynamic physiology. These patients often have refractory ascites and require more frequent paracentesis. However, the exact same paracentesis guidelines are applied in these patients with decompensated cirrhosis as in patients with a MELD < 15. It is likely that patients with advanced cirrhosis lack the pathophysiological reserve to compensate for paracentesis-induced fluid shifts. It is therefore imperative that we continue to examine the evolving hemodynamic and neurohormonal responses in this sicker group of patients and adjust the way we manage paracentesis and PICD.

## CONCLUSION

Paracentesis is a mainstay for the treatment of refractory ascites in patients with cirrhosis. There is clear evidence that there is a decrease in survival in patients who undergo paracentesis and develop circulatory dysfunction. Our current guidelines for the management of patients requiring paracentesis are founded on a few studies from several decades ago, which include only patients with well-compensated cirrhosis. Moreover, current guidelines are based on definitions of LVP and PICD created arbitrarily and without a significant amount of comparative evidence. Yet, we continue to apply these guidelines to all cirrhotic patients with ascites, regardless of patient demographics, co-morbidities, or degree of disease decompensation. A more acute and discriminating understanding of the acute neuro-hormonal, hemodynamic, and end organ effects of fluid shifts and how these factors impact patients with more

decompensated cirrhosis is needed.

## REFERENCES

- Arroyo V, García-Martínez R, Salvatella X. Human serum albumin, systemic inflammation, and cirrhosis. *J Hepatol* 2014; **61**: 396-407 [PMID: 24751830 DOI: 10.1016/j.jhep.2014.04.012]
- Hu LS, George J, Wang JH. Current concepts on the role of nitric oxide in portal hypertension. *World J Gastroenterol* 2013; **19**: 1707-1717 [PMID: 23555159 DOI: 10.3748/wjg.v19.i11.1707]
- Runyon BA, Montano AA, Akriviadis EA, Antillon MR, Irving MA, McHutchison JG. The serum-ascites albumin gradient is superior to the exudate-transudate concept in the differential diagnosis of ascites. *Ann Intern Med* 1992; **117**: 215-220 [PMID: 1616215]
- Ginès P, Arroyo V. Paracentesis in the management of cirrhotic ascites. *J Hepatol* 1993; **17** Suppl 2: S14-S18 [PMID: 8491965 DOI: 10.1016/S0168-8278(05)80449-0]
- Ginès A, Fernández-Esparrach G, Monescillo A, Vila C, Domènech E, Abecasis R, Angeli P, Ruiz-Del-Arbol L, Planas R, Solà R, Ginès P, Terg R, Inglada L, Vaqué P, Salerno F, Vargas V, Clemente G, Quer JC, Jiménez W, Arroyo V, Rodés J. Randomized trial comparing albumin, dextran 70, and polygeline in cirrhotic patients with ascites treated by paracentesis. *Gastroenterology* 1996; **111**: 1002-1010 [PMID: 8831595 DOI: 10.1016/S0016-5085(96)70068-9]
- Patek AJ, Mankin H. The effects of intravenous injection of concentrated human serum albumin upon blood plasma, ascites and renal functions in three patients with cirrhosis of the liver. *J Clin Invest* 1948; **27**: 135-144 [PMID: 18897635 DOI: 10.1172/JCI101916]
- Knauer CM, Lowe HM. Hemodynamics in the cirrhotic patient during paracentesis. *N Engl J Med* 1967; **276**: 491-496 [PMID: 6018279 DOI: 10.1056/nejm196703022760903]
- Guazzi M, Polese A, Magrini F, Fiorentini C, Olivari MT. Negative influences of ascites on the cardiac function of cirrhotic patients. *Am J Med* 1975; **59**: 165-170 [PMID: 1155476]
- Quintero E, Ginès P, Arroyo V, Rimola A, Bory F, Planas R, Viver J, Cabrera J, Rodés J. Paracentesis versus diuretics in the treatment of cirrhotics with tense ascites. *Lancet* 1985; **1**: 611-612 [PMID: 2857949 DOI: 10.1016/S0140-6736(85)92147-6]
- Kao HW, Rakov NE, Savage E, Reynolds TB. The effect of large volume paracentesis on plasma volume--a cause of hypovolemia? *Hepatology* 1985; **5**: 403-407 [PMID: 3888808 DOI: 10.1002/hep.1840050310]
- Salerno F, Badalamenti S, Incerti P, Tempini S, Restelli B, Bruno S, Bellati G, Roffi L. Repeated paracentesis and i.v. albumin infusion to treat 'tense' ascites in cirrhotic patients. A safe alternative therapy. *J Hepatol* 1987; **5**: 102-108 [PMID: 3655306]
- Ginès P, Titó L, Arroyo V, Planas R, Panés J, Viver J, Torres M, Humbert P, Rimola A, Llach J. Randomized comparative study of therapeutic paracentesis with and without intravenous albumin in cirrhosis. *Gastroenterology* 1988; **94**: 1493-1502 [PMID: 3360270 DOI: 10.1016/0016-5085(88)90691-9]
- Pinto PC, Amerian J, Reynolds TB. Large-volume paracentesis in nonedematous patients with tense ascites: its effect on intravascular volume. *Hepatology* 1988; **8**: 207-210 [PMID: 3356400 DOI: 10.1002/hep.1840080202]
- Gentile S, Angelico M, Bologna E, Capocaccia L. Clinical, biochemical, and hormonal changes after a single, large-volume paracentesis in cirrhosis with ascites. *Am J Gastroenterol* 1989; **84**: 279-284 [PMID: 2645767]
- Panos MZ, Moore K, Vlavianos P, Chambers JB, Anderson JV, Gimson AE, Slater JD, Rees LH, Westaby D, Williams R. Single, total paracentesis for tense ascites: sequential hemodynamic changes and right atrial size. *Hepatology* 1990; **11**: 662-667 [PMID: 2139430 DOI: 10.1002/hep.1840110420]
- Simon DM, McCain JR, Bonkovsky HL, Wells JO, Hartle DK, Galambos JT. Effects of therapeutic paracentesis on systemic and hepatic hemodynamics and on renal and hormonal function. *Hepatology* 1987; **7**: 423-429 [PMID: 3570154 DOI: 10.1002/hep.1840070302]
- Salerno F, Badalamenti S, Moser P, Lorenzano E, Incerti P, Dioguardi N. Atrial natriuretic factor in cirrhotic patients with tense ascites. Effect of large-volume paracentesis. *Gastroenterology* 1990; **98**: 1063-1070 [PMID: 2138104 DOI: 10.1016/0016-5085(90)90034-X]
- Planas R, Ginès P, Arroyo V, Llach J, Panés J, Vargas V, Salmerón JM, Ginès A, Toledo C, Rimola A. Dextran-70 versus albumin as plasma expanders in cirrhotic patients with tense ascites treated with total paracentesis. Results of a randomized study. *Gastroenterology* 1990; **99**: 1736-1744 [PMID: 1699835]
- Cabrera J, Inglada L, Quintero E, Jimenez W, Losada A, Mayor J, Guerra C. Large-volume paracentesis and intravenous saline: effects on the renin-angiotensin system. *Hepatology* 1991; **14**: 1025-1028 [PMID: 1959849 DOI: 10.1002/hep.1840140613]
- Ruiz-del-Arbol L, Monescillo A, Jimenez W, Garcia-Plaza A, Arroyo V, Rodés J. Paracentesis-induced circulatory dysfunction: mechanism and effect on hepatic hemodynamics in cirrhosis. *Gastroenterology* 1997; **113**: 579-586 [PMID: 9247479 DOI: 10.1053/gast.1997.v113.pm9247479]
- Vila MC, Solà R, Molina L, Andreu M, Coll S, Gana J, Marquez J, Palà J, Bory F, Pons S, Szescielinski L, Jimenez W. Hemodynamic changes in patients developing effective hypovolemia after total paracentesis. *J Hepatol* 1998; **28**: 639-645 [PMID: 9566833 DOI: 10.1016/S0168-8278(98)80288-2]
- Moreau R, Asselah T, Condat B, de Kerguenec C, Pessione F, Bernard B, Poynard T, Binn M, Grangé JD, Valla D, Lebre C. Comparison of the effect of terlipressin and albumin on arterial blood volume in patients with cirrhosis and tense ascites treated by paracentesis: a randomised pilot study. *Gut* 2002; **50**: 90-94 [PMID: 11772973 DOI: 10.1136/gut.50.1.90]
- Sola-Vera J, Miñana J, Ricart E, Planella M, González B, Torras X, Rodríguez J, Such J, Pascual S, Soriano G, Pérez-Mateo M, Guarner C. Randomized trial comparing albumin and saline in the prevention of paracentesis-induced circulatory dysfunction in cirrhotic patients with ascites. *Hepatology* 2003; **37**: 1147-1153 [PMID: 12717396 DOI: 10.1053/jhep.2003.50169]
- Appenrodt B, Wolf A, Grünhage F, Trebicka J, Schepke M, Rabe C, Lammert F, Sauerbruch T, Heller J. Prevention of paracentesis-induced circulatory dysfunction: midodrine vs albumin. A randomized pilot study. *Liver Int* 2008; **28**: 1019-1025 [PMID: 18410283 DOI: 10.1111/j.1478-3231.2008.01734.x]
- Nasr G, Hassan A, Ahmed S, Serwah A. Predictors of large volume paracentesis induced circulatory dysfunction in patients with massive hepatic ascites. *J Cardiovasc Dis Res* 2010; **1**: 136-144 [PMID: 21187868 DOI: 10.4103/0975-3583.70914]
- Fimiani B, Guardia DD, Puoti C, D'Adamo G, Cioffi O, Pagano A, Tagliamonte MR, Izzi A. The use of terlipressin in cirrhotic patients with refractory ascites and normal renal function: a multicentric study. *Eur J Intern Med* 2011; **22**: 587-590 [PMID: 22075285 DOI: 10.1016/j.ejim.2011.06.013]
- Alessandria C, Elia C, Mezzabotta L, Risso A, Andrealli A, Spandre M, Morgando A, Marzano A, Rizzetto M. Prevention of paracentesis-induced circulatory dysfunction in cirrhosis: standard vs half albumin doses. A prospective, randomized, unblinded pilot study. *Dig Liver Dis* 2011; **43**: 881-886 [PMID: 21741331 DOI: 10.1016/j.dld.2011.06.001]
- Carl DE, Ghosh S, Cheng J, Gehr TW, Stravitz RT, Sanyal A. Post-paracentesis circulatory derangements are related to monocyte activation. *Liver Int* 2014; **34**: 1001-1007 [PMID: 24373155 DOI: 10.1111/liv.12450]
- Bai M, Qi XS, Yang ZP, Yang M, Fan DM, Han GH. TIPS improves liver transplantation-free survival in cirrhotic patients with refractory ascites: an updated meta-analysis. *World J Gastroenterol* 2014; **20**: 2704-2714 [PMID: 24627607 DOI: 10.3748/wjg.v20.i10.2704]
- Lebre C, Giuly N, Hadengue A, Vilgrain V, Moreau R, Poynard T, Gadano A, Lassen C, Benhamou JP, Erlinger S. Transjugular



- intrahepatic portosystemic shunts: comparison with paracentesis in patients with cirrhosis and refractory ascites: a randomized trial. French Group of Clinicians and a Group of Biologists. *J Hepatol* 1996; **25**: 135-144 [PMID: 8878773 DOI: 10.1016/S0168-8278(96)80065-1]
- 31 **Rössle M**, Ochs A, Güllberg V, Siegerstetter V, Holl J, Deibert P, Olschewski M, Reiser M, Gerbes AL. A comparison of paracentesis and transjugular intrahepatic portosystemic shunting in patients with ascites. *N Engl J Med* 2000; **342**: 1701-1707 [PMID: 10841872 DOI: 10.1056/nejm200006083422303]
  - 32 **Ginès P**, Uriz J, Calahorra B, Garcia-Tsao G, Kamath PS, Del Arbol LR, Planas R, Bosch J, Arroyo V, Rodés J. Transjugular intrahepatic portosystemic shunting versus paracentesis plus albumin for refractory ascites in cirrhosis. *Gastroenterology* 2002; **123**: 1839-1847 [PMID: 12454841 DOI: 10.1053/gast.2002.37073]
  - 33 **Sanyal AJ**, Genning C, Reddy KR, Wong F, Kowdley KV, Benner K, McCashland T. The North American Study for the Treatment of Refractory Ascites. *Gastroenterology* 2003; **124**: 634-641 [PMID: 12612902 DOI: 10.1053/gast.2003.50088]
  - 34 **Salerno F**, Merli M, Riggio O, Cazzaniga M, Valeriano V, Pozzi M, Nicolini A, Salvatori F. Randomized controlled study of TIPS versus paracentesis plus albumin in cirrhosis with severe ascites. *Hepatology* 2004; **40**: 629-635 [PMID: 15349901 DOI: 10.1002/hep.20364]
  - 35 **Salerno F**, Cammà C, Enea M, Rössle M, Wong F. Transjugular intrahepatic portosystemic shunt for refractory ascites: a meta-analysis of individual patient data. *Gastroenterology* 2007; **133**: 825-834 [PMID: 17678653 DOI: 10.1053/j.gastro.2007.06.020]
  - 36 **Kim MY**, Baik SK. [Hyperdynamic circulation in patients with liver cirrhosis and portal hypertension]. *Korean J Gastroenterol* 2009; **54**: 143-148 [PMID: 19844149 DOI: 10.4166/kjg.2009.54.3.143]
  - 37 **Runyon BA**. Introduction to the revised American Association for the Study of Liver Diseases Practice Guideline management of adult patients with ascites due to cirrhosis 2012. *Hepatology* 2013; **57**: 1651-1653 [PMID: 23463403 DOI: 10.1002/hep.26359]
  - 38 **Gatta A**, Bolognesi M, Merkel C. Vasoactive factors and hemodynamic mechanisms in the pathophysiology of portal hypertension in cirrhosis. *Mol Aspects Med* 2008; **29**: 119-129 [PMID: 18036654 DOI: 10.1016/j.mam.2007.09.006]
  - 39 **Víteček J**, Lojek A, Valacchi G, Kubala L. Arginine-based inhibitors of nitric oxide synthase: therapeutic potential and challenges. *Mediators Inflamm* 2012; **2012**: 318087 [PMID: 22988346 DOI: 10.1155/2012/318087]
  - 40 **Cabrera J**, Falcón L, Gorriç E, Pardo MD, Granados R, Quinones A, Maynar M. Abdominal decompression plays a major role in early postparacentesis haemodynamic changes in cirrhotic patients with tense ascites. *Gut* 2001; **48**: 384-389 [PMID: 11171830 DOI: 10.1136/gut.48.3.384]
  - 41 **European Association for the Study of the Liver**, Ginès P, Angeli P, Lenz K, Möller S, Moore K, Moreau R, Merkel C, Ring-Larsen H, Bernardi M. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]

**P- Reviewer:** Li YY, Ratnasari N, Sato T **S- Editor:** Kong JX

**L- Editor:** A **E- Editor:** Li D



## Basic Study

# DNA methylation of angiotensin II receptor gene in nonalcoholic steatohepatitis-related liver fibrosis

Kiyoshi Asada, Yosuke Aihara, Hiroaki Takaya, Ryuichi Noguchi, Tadashi Namisaki, Kei Moriya, Masakazu Uejima, Mitsuteru Kitade, Tsuyoshi Mashitani, Kosuke Takeda, Hideto Kawaratani, Yasushi Okura, Kosuke Kaji, Akitoshi Douhara, Yasuhiko Sawada, Norihisa Nishimura, Kenichiro Seki, Akira Mitoro, Junichi Yamao, Hitoshi Yoshiji

Kiyoshi Asada, Yosuke Aihara, Hiroaki Takaya, Ryuichi Noguchi, Tadashi Namisaki, Kei Moriya, Masakazu Uejima, Mitsuteru Kitade, Tsuyoshi Mashitani, Kosuke Takeda, Hideto Kawaratani, Yasushi Okura, Kosuke Kaji, Akitoshi Douhara, Yasuhiko Sawada, Norihisa Nishimura, Kenichiro Seki, Akira Mitoro, Junichi Yamao, Hitoshi Yoshiji, Third Department of Internal Medicine, Nara Medical University, Kashihara, Nara 634-8521, Japan

**Author contributions:** Asada K performed the majority of experiments and analyzed the data; Aihara Y and Takaya H performed the molecular investigations; Noguchi R, Namisaki T, Moriya K, Uejima M, Kitade M, Mashitani T, Takeda K, Kawaratani H, Okura Y, Kaji K, Douhara A, Sawada Y, Nishimura N and Seki K participated in treatment of animals; Mitoro A, Yamao J and Yoshiji H designed and coordinated the research; Asada K, Kaji K and Yoshiji H wrote the paper.

**Institutional review board statement:** As for the file of Institutional review board statement, it is not applicable to my study because no human subjects were analyzed in my study.

**Institutional animal care and use committee statement:** This study was approved by the animal experimental ethical committee at the Nara Medical University (No. 9354).

**Conflict-of-interest statement:** The authors declare that they have no conflicts of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Kiyoshi Asada, MD, PhD, Research Scientist, Third Department of Internal Medicine, Nara Medical University, 840 Shijocho, Kashihara, Nara 634-8521, Japan. [kasada@naramed-u.ac.jp](mailto:kasada@naramed-u.ac.jp)  
Telephone: +81-744-223051  
Fax: +81-744-247122

Received: May 20, 2016

Peer-review started: May 21, 2016

First decision: July 4, 2016

Revised: July 8, 2016

Accepted: August 27, 2016

Article in press: August 29, 2016

Published online: October 8, 2016

## Abstract

### AIM

To clarify whether *Agtr1a* methylation is involved in the development of nonalcoholic steatohepatitis (NASH)-related liver fibrosis in adult rats.

### METHODS

A choline-deficient amino acid (CDAA) diet model was employed for methylation analysis of NASH-related liver fibrosis. *Agtr1a* methylation levels were measured in the livers of CDAA- and control choline-sufficient amino acid (CSAA)-fed rats for 8 and 12 wk using quantitative methylation-specific PCR. Hepatic stellate cells (HSCs) were isolated by collagenase digestion of the liver, followed by centrifugation of the crude cell suspension through a density gradient. *Agtr1a* methylation and its gene expression were also analyzed during the activation of HSCs.

## RESULTS

The mean levels of *Agtr1a* methylation in the livers of CDAA-fed rats (11.5% and 18.6% at 8 and 12 wk, respectively) tended to be higher ( $P = 0.06$  and  $0.09$ , respectively) than those in the livers of CSAA-fed rats (2.1% and 5.3% at 8 and 12 wk, respectively). *Agtr1a* was not methylated at all in quiescent HSCs, but was clearly methylated in activated HSCs (13.8%,  $P < 0.01$ ). Interestingly, although *Agtr1a* was hypermethylated, the *Agtr1a* mRNA level increased up to 2.2-fold ( $P < 0.05$ ) in activated HSCs compared with that in quiescent HSCs, suggesting that *Agtr1a* methylation did not silence its expression but instead had the potential to upregulate its expression. These findings indicate that *Agtr1a* methylation and its upregulation of gene expression are associated with the development of NASH-related liver fibrosis.

## CONCLUSION

This is the first study to show that DNA methylation is potentially involved in the regulation of a renin-angiotensin system-related gene expression during liver fibrosis.

**Key words:** Epigenetics; DNA methylation; Angiotensin II receptor; Liver fibrosis; Nonalcoholic steatohepatitis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We report the first study to show that *Agtr1a* methylation occurred during the development of nonalcoholic steatohepatitis-related liver fibrosis. Interestingly, *Agtr1a* gene expression was upregulated during liver fibrosis, although *Agtr1a* was methylated. This study demonstrates for the first time that renin-angiotensin system-related gene expression is regulated by DNA methylation during liver fibrosis. This finding raises expectations about the therapeutic application of demethylating agents for the treatment of liver fibrosis.

Asada K, Aihara Y, Takaya H, Noguchi R, Namisaki T, Moriya K, Uejima M, Kitade M, Mashitani T, Takeda K, Kawaratani H, Okura Y, Kaji K, Douhara A, Sawada Y, Nishimura N, Seki K, Mito A, Yamao J, Yoshiji H. DNA methylation of angiotensin II receptor gene in nonalcoholic steatohepatitis-related liver fibrosis. *World J Hepatol* 2016; 8(28): 1194-1199 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i28/1194.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i28.1194>

## INTRODUCTION

Liver fibrosis is a characteristic feature of chronic liver disease regardless of the etiology. Cirrhosis is the terminal condition of chronic liver diseases, and hepatic failure due to liver cirrhosis is caused by progressive fibrosis that ultimately results in nodular regeneration with loss of function<sup>[1-3]</sup>. Considering that hepatocellular carcinoma (HCC) also develops from liver fibrosis, it is necessary to investigate the molecular mechanisms

underlying liver fibrosis development to reduce the morbidity and mortality of chronic liver disease.

The renin-angiotensin system (RAS) is continually activated in patients with chronic liver diseases, such as cirrhosis<sup>[4]</sup>. Angiotensin II (AT-II), an octapeptide produced mainly via the enzymatic cleavage of angiotensin I by angiotensin I-converting enzyme, reportedly plays an important role in chronic liver disease progression. AT-II activates a series of signal transduction pathways in activated hepatic stellate cells (HSCs) by binding to the AT-II type 1 receptor (AT1-R)<sup>[5]</sup>. We previously reported that AT1-R blockers significantly attenuate experimental liver fibrosis development with the suppression of activated HSC proliferation<sup>[6-8]</sup>. However, the molecular mechanisms regulating RAS-related gene expression remain unelucidated.

Epigenetic alterations, including DNA methylation, are involved in the progression of liver fibrosis and HCC in human and animal studies<sup>[9-11]</sup>. Recently, Chen *et al.*<sup>[12]</sup> reported that RAS-related genes, especially *Agtr1a* encoding rat AT1-R, are methylated in rats born to mothers fed a methyl donor-deficient diet during gestation and lactation. They showed that *Agtr1a* methylation can be a surrogate marker to predict susceptibility in developing nonalcoholic fatty liver disease (NAFLD) later in life. However, it is unclear whether *Agtr1a* methylation is associated with the development of nonalcoholic steatohepatitis (NASH)-related liver fibrosis.

Here we employed choline-deficient amino acid (CDAA)-fed rats to evaluate the importance of *Agtr1a* methylation in the development of NASH-related liver fibrosis. Our results demonstrate that *Agtr1a* methylation is potentially associated with liver fibrosis development and HSC activation.

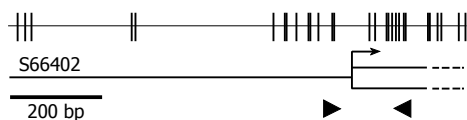
## MATERIALS AND METHODS

### Animal model of liver disease

Six-week-old male Fisher 344 rats (CLEA Japan, Inc., Osaka, Japan) were housed in a room under a controlled temperature and a 12/12-h light-dark cycle. The animals were divided into the following four experimental groups: (1) choline-sufficient amino acid diet (CSAA) for 8 wk ( $n = 4$ ); (2) CSAA for 12 wk ( $n = 11$ ); (3) CDAA for 8 wk ( $n = 10$ ); and (4) CDAA for 12 wk ( $n = 12$ ). Initially, sample sizes for group (1)-(5) were 5, 12, 10, and 12, respectively, but two animals (one for CSAA-diet for 8 wk and the other for CSAA-diet for 12 wk) were dropped out because of entry in another experiment. All animal procedures were performed in accordance with standard protocols and following the standard recommendations for the appropriate care and use of laboratory animals. This study was approved by the animal experiment ethical committee at the Nara Medical University (protocol number: 9354).

### Isolation and activation of HSCs

HSCs were isolated by the collagenase digestion of the liver of a 6-week-old male Fisher 344 rat using a



**Figure 1** *Agtr1a* genomic structure. Each vertical tick on the top line shows an individual CpG site. GenBank accession number is listed at the left end on the bottom line. Open box shows exon 1, and dashed lines show the ambiguous boundary region of exon 1. Quantitative real-time methylation-specific PCR was performed in the region marked with closed arrowheads.

perfusion system, followed by the centrifugation of the crude cell suspension through a density gradient, as described previously<sup>[13]</sup>. Genomic DNA and total RNA were isolated from freshly isolated HSCs in a quiescent state. Thereafter, HSCs were activated in a culture on a plastic dish for 5 d.

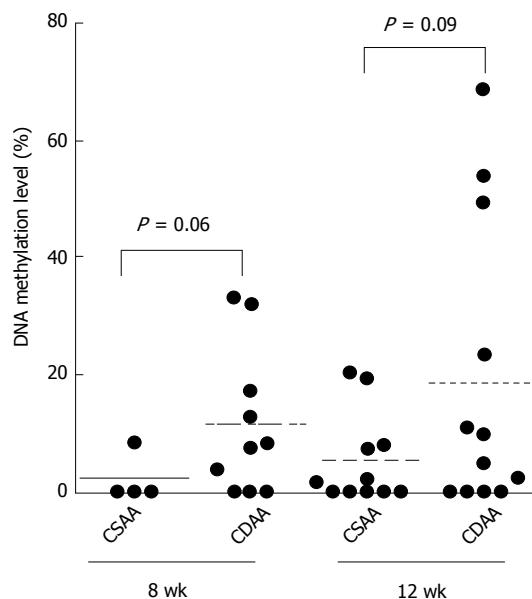
#### Genomic DNA isolation, sodium bisulfite modification, and quantitative real-time methylation-specific PCR

Genomic DNA was isolated using a DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany). Fully methylated control DNA was prepared by methylating genomic DNA with SssI methylase (New England Biolabs, Beverly, MA), and completely unmethylated control DNA was purchased from EpigenDx (Hopkinton, MA). Bisulfite modification was performed using an EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA). *Agtr1a* genomic structure is illustrated in Figure 1. An aliquot of 1  $\mu$ L was used for quantitative real-time methylation-specific PCR (qMSP) with primers specific to a methylated sequence of *Agtr1a* (forward 5'-GGT TGG AAT TTG TAG AGT AGC GAC-3', reverse 5'-CAA CGC TAA TAC CGA CCT CG-3') and to a B2 repeat sequence, regardless of the methylation status, as demonstrated in a previous report<sup>[14]</sup>.

qMSP was performed by real-time PCR using a Power SYBR® Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA) and a StepOnePlus™ Real-Time PCR® (Thermo Fisher Scientific, Waltham, MA). The methylation level was calculated as the methylation percentage obtained as follows: {[number of DNA molecules methylated at a target CpG island (CGI) in a sample]/(number of B2 repeats in the sample)}/[(number of DNA molecules methylated at the target CGI in completely methylated control DNA)/(number of B2 repeats in the completely methylated control DNA)]  $\times$  100, as described previously<sup>[15]</sup>.

#### Quantitative real-time reverse transcription PCR

Total RNA was extracted using an RNeasy® Mini Kit (Qiagen, Hilden, Germany). cDNA was synthesized from 1  $\mu$ g of total RNA using a High Capacity RNA to cDNA Master Mix (Thermo Fisher Scientific, Waltham, MA). *Agtr1a* mRNA level was measured by quantitative PCR using the StepOnePlus™ Real-Time PCR® (Thermo Fisher Scientific, Waltham, MA). Primer sequences for *Agtr1a* and for *Ppia* were reported previously<sup>[14,16]</sup>. The number of *Agtr1a* cDNA molecules was normalized to that of *Ppia* cDNA molecules.



**Figure 2** Levels of *Agtr1a* methylation in the livers of control choline-sufficient amino acid - and choline-deficient amino acid - fed rats. The livers of choline-deficient amino acid (CDAA) - fed rats show higher *Agtr1a* methylation than that shown by the livers of choline-sufficient amino acid (CSAA) - fed rats at 8 (mean, 11.5% and 2.1%,  $P = 0.06$ ) and 12 wk (mean, 18.6% and 5.3%,  $P = 0.09$ ), respectively.

#### Statistical analysis

The difference in mean methylation levels was analyzed using Welch's *t*-test. The results were considered significant with a  $P$  value of  $< 0.05$ .

## RESULTS

#### *Agtr1a* methylation in the livers of CDAA-fed rats and activated HSCs

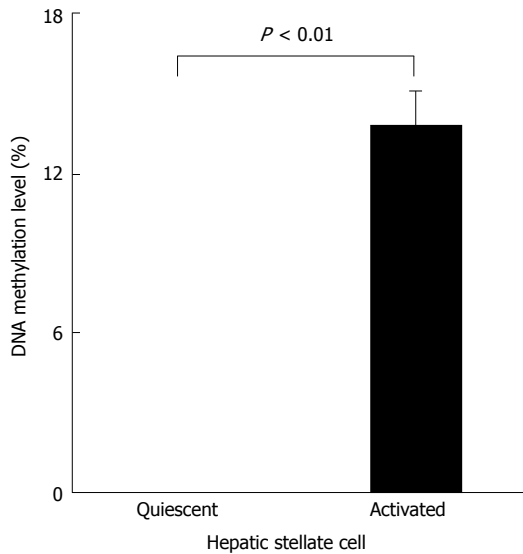
To evaluate the status of *Agtr1a* methylation in the whole liver, we performed qMSP using the liver samples of CSAA- and CDAA-fed rats after the two feeding periods, 8 and 12 wk. The mean levels of *Agtr1a* methylation in the livers of CDAA-fed rats were 11.5% and 18.6% at 8 and 12 wk, respectively, whereas those in the livers of CSAA-fed rats were 2.1% and 5.3% at 8 and 12 wk, respectively. These findings suggested that the levels of *Agtr1a* methylation in the livers of CDAA-fed rats tended to be higher than those in the livers of CSAA-fed rats at 8 and 12 wk ( $P = 0.06$  and  $0.09$ , respectively; Figure 2).

Next, we evaluated the level of *Agtr1a* methylation during HSC activation *in vitro*. We found that *Agtr1a* methylation was not detected at all in quiescent HSCs, but was clearly observed in activated HSCs (13.8%,  $P < 0.01$ ; Figure 3). Taken together with the *in vivo* results, our findings indicate that *Agtr1a* is hypermethylated in accordance with the development of NASH-related liver fibrosis.

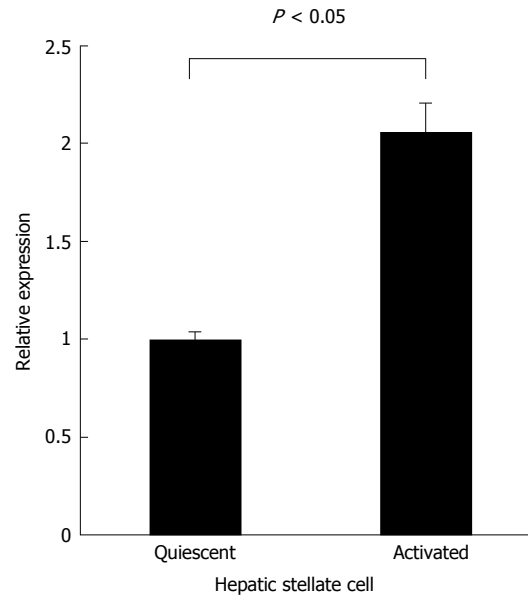
#### *Agtr1a* expression in activated HSCs, and its association with methylation

To address the contribution of *Agtr1a* methylation to its





**Figure 3 Levels of *Agtr1a* methylation in the quiescent and activated hepatic stellate cells.** *Agtr1a* is not methylated at all in quiescent hepatic stellate cells (HSCs) but hypermethylated (13.8%,  $P < 0.01$ ) in activated HSCs. Data are presented as the mean  $\pm$  SE.



**Figure 4 Relative *Agtr1a* expression normalized to *Ppia* in quiescent and activated hepatic stellate cells.** Activated hepatic stellate cells (HSCs) show 2.2-fold higher ( $P < 0.05$ ) *Agtr1a* expression than that shown by quiescent HSCs. Data are presented as the mean  $\pm$  SE.

gene expression, we performed quantitative real-time reverse transcription PCR using quiescent and activated HSCs. *Agtr1a* expression was observed in quiescent HSCs in which *Agtr1a* was unmethylated. Unexpectedly, *Agtr1a* expression increased up to 2.2-fold ( $P < 0.05$ ) in the activated HSCs compared with that in quiescent HSCs, although *Agtr1a* was methylated (Figure 4). Interestingly, in contrast to the general relationship between promoter CGIs and gene expression, *Agtr1a* methylation did not silence its expression but instead had the potential to upregulate its expression.

## DISCUSSION

In this study, we found that *Agtr1a* methylation occurred during the development of NASH-related liver fibrosis. *Agtr1a*, which encodes rat AT1-R, the receptor for AT-II, is an important factor in liver fibrosis development<sup>[17,18]</sup>. Our previous reports demonstrated that both *AT-II* and *AT1-R* gene expressions were upregulated during fibrosis development in rat liver, and the blockage of AT-II/AT1-R signaling could attenuate liver fibrosis<sup>[6-8]</sup>. Considering that *Agtr1a* methylation upregulates its gene expression, *Agtr1a* demethylation can suppress liver fibrosis.

*Agtr1a* methylation was first demonstrated in the liver of rats born to mothers fed a methyl donor-deficient diet during gestation and lactation, and it was reported that rat pups with *Agtr1a* methylation have a high risk of developing NAFLD<sup>[12]</sup>. Epigenetics derived from mother-pup interaction is a prominent research field, and epigenetic susceptibility to phenotypes and diseases, such as yellow coat color, stress response, and breast cancer in offspring, has been identified<sup>[19-21]</sup>. However, few studies have focused on whether these epigenetic changes responsible for susceptibility to particular diseases occur when the diseases actually develop in adults. Here we

found that *Agtr1a* methylation, associated with susceptibility to NAFLD in pups, occurs in liver fibrosis development in adult NASH model rats.

As an experimental NASH model, we employed the CDAA model in this study. In the CDAA model, liver fibrosis develops at 8 wk and severely progresses at 12 wk<sup>[22,23]</sup>. This model has an advantage of histological progression of liver fibrosis, which is very similar to human NASH. However, there are critical disadvantages of this model. For examples, obesity, glucose intolerance, and insulin resistance, which are common features in human NASH, are not observed in this model. It remains to be elucidated whether *Agtr1a* methylation is induced in other experimental NASH models.

In CDAA model, *Agtr1a* methylation in the livers of CDAA-fed rats tended to be higher than that in the livers of CSAA-fed rats, but it was not statistically significant. We consider that methylation levels are highly variable in each diet group and the difference between CDAA- and CSAA-fed rats appears to be small. This variability depends on individual differences in rats and tissue heterogeneity in each sample, but both of them are hardly avoided. On the other hand, in HSC analysis, *Agtr1a* methylation and upregulation was clearly observed. Even in the CDAA model, it would be better to isolate HSC from the livers of CDAA-fed rats to obtain clear methylation changes.

*Agtr1a* hypermethylation was associated with *Agtr1a* upregulation. As for the promoter CGI, hypermethylation is generally considered to be strongly associated with gene silencing<sup>[24]</sup>. On the other hand, in the case of a CGI at the gene body, hypermethylation occasionally contributes to overexpression<sup>[25]</sup>. In the *Agtr1a* gene, 5'-CGI was not located at the promoter region but was just downstream of the transcription initiation site (Figure 1),

which might contribute to gene overexpression. It is hoped that the mechanism by which gene body methylation induces overexpression can be demonstrated.

In conclusion, this study demonstrates for the first time that RAS-related gene expression is regulated by DNA methylation during liver fibrosis. This finding raises expectations about the therapeutic application of demethylating agents for the treatment of liver fibrosis.

## COMMENTS

### Background

The renin-angiotensin system (RAS) plays a crucial role in the development of liver fibrosis. Among the RAS-related genes, the methylation of *Agtr1a*, the rat Angiotensin II type 1 receptor gene, is a potential risk marker for the development of nonalcoholic fatty liver disease in rat pups. However, it remains to be elucidated whether *Agtr1a* methylation occurs in liver fibrosis development in adult rats with nonalcoholic steatohepatitis (NASH).

### Research frontiers

Epigenetics derived from mother-pup interaction is a prominent research field. However, few studies have focused on whether these epigenetic changes responsible for susceptibility to particular diseases occur when the diseases actually develop in adults.

### Innovations and breakthroughs

This study demonstrates for the first time that the expression of *Agtr1a*, a RAS-related gene, is regulated by DNA methylation during liver fibrosis.

### Applications

The authors finding raises expectations about the therapeutic application of demethylating agents for the treatment of liver fibrosis.

### Terminology

Epigenetics refers to heritable marks regulating tissue-specific gene expression without changes in the DNA sequence. Prominent epigenetic marks consist of DNA methylation and histone modifications. Aberrant epigenetic changes are involved in various diseases, including cancer.

### Peer-review

This manuscript addresses the role of DNA methylation of angiotensin II receptor in fibrosis development in a rat model of NASH. The study is original and well designed.

## REFERENCES

- 1 Albanis E, Friedman SL. Hepatic fibrosis. Pathogenesis and principles of therapy. *Clin Liver Dis* 2001; **5**: 315-334, v-vi [PMID: 11385966 DOI: 10.1016/S1089-3261(05)70168-9]
- 2 Olaso E, Friedman SL. Molecular regulation of hepatic fibrogenesis. *J Hepatol* 1998; **29**: 836-847 [PMID: 9833926 DOI: 10.1016/S0168-8278(98)80269-9]
- 3 Friedman SL. Liver fibrosis -- from bench to bedside. *J Hepatol* 2003; **38** Suppl 1: S38-S53 [PMID: 12591185 DOI: 10.1016/S0168-8278(02)00429-4]
- 4 Helmy A, Jalan R, Newby DE, Hayes PC, Webb DJ. Role of angiotensin II in regulation of basal and sympathetically stimulated vascular tone in early and advanced cirrhosis. *Gastroenterology* 2000; **118**: 565-572 [PMID: 10702208 DOI: 10.1016/S0016-5085(00)70263-0]
- 5 Bataller R, Ginès P, Nicolás JM, Görbig MN, Garcia-Ramallo E, Gasull X, Bosch J, Arroyo V, Rodés J. Angiotensin II induces contraction and proliferation of human hepatic stellate cells. *Gastroenterology* 2000; **118**: 1149-1156 [PMID: 10833490]
- 6 Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Nakatani T, Tsujinoue H, Fukui H. Angiotensin-II type 1 receptor interaction is a major regulator for liver fibrosis development in rats. *Hepatology* 2001; **34**: 745-750 [PMID: 11584371 DOI: 10.1053/jhep.2001.28231]
- 7 Yoshiji H, Kuriyama S, Fukui H. Blockade of renin-angiotensin system in antifibrotic therapy. *J Gastroenterol Hepatol* 2007; **22** Suppl 1: S93-S95 [PMID: 17567477 DOI: 10.1111/j.1440-1746.2006.04663.x]
- 8 Yoshiji H, Noguchi R, Ikenaka Y, Namisaki T, Kitade M, Kaji K, Shirai Y, Yoshii J, Yanase K, Yamazaki M, Tsujimoto T, Kawaratani H, Akahane T, Aihara Y, Fukui H. Losartan, an angiotensin-II type 1 receptor blocker, attenuates the liver fibrosis development of non-alcoholic steatohepatitis in the rat. *BMC Res Notes* 2009; **2**: 70 [PMID: 19416517 DOI: 10.1186/1756-0500-2-70]
- 9 Kondo Y, Kanai Y, Sakamoto M, Mizokami M, Ueda R, Hirohashi S. Genetic instability and aberrant DNA methylation in chronic hepatitis and cirrhosis--A comprehensive study of loss of heterozygosity and microsatellite instability at 39 loci and DNA hypermethylation on 8 CpG islands in microdissected specimens from patients with hepatocellular carcinoma. *Hepatology* 2000; **32**: 970-979 [PMID: 11050047 DOI: 10.1053/jhep.2000.19797]
- 10 Arai E, Ushijima S, Gotoh M, Ojima H, Kosuge T, Hosoda F, Shibata T, Kondo T, Yokoi S, Imoto I, Inazawa J, Hirohashi S, Kanai Y. Genome-wide DNA methylation profiles in liver tissue at the precancerous stage and in hepatocellular carcinoma. *Int J Cancer* 2009; **125**: 2854-2862 [PMID: 19569176 DOI: 10.1002/ijc.24708]
- 11 Asada K, Kotake Y, Asada R, Saunders D, Broyles RH, Towner RA, Fukui H, Floyd RA. LINE-1 hypomethylation in a choline-deficiency-induced liver cancer in rats: dependence on feeding period. *J Biomed Biotechnol* 2006; **2006**: 17142 [PMID: 16877811 DOI: 10.1155/JBB/2006/17142]
- 12 Chen G, Broséus J, Hergalant S, Donnat A, Chevalier C, Bolaños-Jiménez F, Guéant JL, Houlgatte R. Identification of master genes involved in liver key functions through transcriptomics and epigenomics of methyl donor deficiency in rat: relevance to nonalcoholic liver disease. *Mol Nutr Food Res* 2015; **59**: 293-302 [PMID: 25380481 DOI: 10.1002/mnfr.201400483]
- 13 Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Nakatani T, Tsujinoue H, Yanase K, Namisaki T, Imazu H, Fukui H. Tissue inhibitor of metalloproteinases-1 attenuates spontaneous liver fibrosis resolution in the transgenic mouse. *Hepatology* 2002; **36**: 850-860 [PMID: 12297832 DOI: 10.1053/jhep.2002.35625]
- 14 Hattori N, Okochi-Takada E, Kikuyama M, Wakabayashi M, Yamashita S, Ushijima T. Methylation silencing of angiotensin-like 4 in rat and human mammary carcinomas. *Cancer Sci* 2011; **102**: 1337-1343 [PMID: 21489049 DOI: 10.1111/j.1349-7006.2011.01955.x]
- 15 Niwa T, Tsukamoto T, Toyoda T, Mori A, Tanaka H, Maekita T, Ichinose M, Tatematsu M, Ushijima T. Inflammatory processes triggered by *Helicobacter pylori* infection cause aberrant DNA methylation in gastric epithelial cells. *Cancer Res* 2010; **70**: 1430-1440 [PMID: 20124475 DOI: 10.1158/0008-5472.CAN-09-2755]
- 16 Vaswani K, Chan HW, Verma P, Dekker Nitert M, Peiris HN, Wood-Bradley RJ, Armitage JA, Rice GE, Mitchell MD. The rat placental renin-angiotensin system - a gestational gene expression study. *Reprod Biol Endocrinol* 2015; **13**: 89 [PMID: 26260700 DOI: 10.1186/s12958-015-0088-y]
- 17 Granzow M, Schierwagen R, Klein S, Kowallick B, Huss S, Linhart M, Mazar IG, Görtzen J, Vogt A, Schildberg FA, Gonzalez-Carmona MA, Wojtalla A, Krämer B, Nattermann J, Siegmund SV, Werner N, Fürst DO, Laleman W, Knolle P, Shah VH, Sauerbruch T, Trebicka J. Angiotensin-II type 1 receptor-mediated Janus kinase 2 activation induces liver fibrosis. *Hepatology* 2014; **60**: 334-348 [PMID: 24619965 DOI: 10.1002/hep.27117]
- 18 Rong X, Li Y, Ebihara K, Zhao M, Naowaboot J, Kusakabe T, Kuwahara K, Murray M, Nakao K. Angiotensin II type 1 receptor-independent beneficial effects of telmisartan on dietary-induced obesity, insulin resistance and fatty liver in mice. *Diabetologia* 2010; **53**: 1727-1731 [PMID: 20390403 DOI: 10.1007/s00125-010-

- 1744-6]
- 19 **Waterland RA**, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 2003; **23**: 5293-5300 [PMID: 12861015]
  - 20 **Weaver IC**, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci* 2004; **7**: 847-854 [PMID: 15220929 DOI: 10.1038/nn1276]
  - 21 **Govindarajah V**, Leung YK, Ying J, Gear R, Bornschein RL, Medvedovic M, Ho SM. In utero exposure of rats to high-fat diets perturbs gene expression profiles and cancer susceptibility of prepubertal mammary glands. *J Nutr Biochem* 2016; **29**: 73-82 [PMID: 26895667 DOI: 10.1016/j.jnutbio.2015.11.003]
  - 22 **Douhara A**, Moriya K, Yoshiji H, Noguchi R, Namisaki T, Kitade M, Kaji K, Aihara Y, Nishimura N, Takeda K, Okura Y, Kawaratani H, Fukui H. Reduction of endotoxin attenuates liver fibrosis through suppression of hepatic stellate cell activation and remission of intestinal permeability in a rat non-alcoholic steatohepatitis model. *Mol Med Rep* 2015; **11**: 1693-1700 [PMID: 25421042 DOI: 10.3892/mmr.2014.2995]
  - 23 **Aihara Y**, Yoshiji H, Noguchi R, Kaji K, Namisaki T, Shirai Y, Douhara A, Moriya K, Kawaratani H, Fukui H. Direct renin inhibitor, aliskiren, attenuates the progression of non-alcoholic steatohepatitis in the rat model. *Hepatol Res* 2013; **43**: 1241-1250 [PMID: 23448275 DOI: 10.1111/hepr.12081]
  - 24 **Deaton AM**, Bird A. CpG islands and the regulation of transcription. *Genes Dev* 2011; **25**: 1010-1022 [PMID: 21576262 DOI: 10.1101/gad.2037511]
  - 25 **Kulis M**, Queirós AC, Beekman R, Martín-Subero JI. Intragenic DNA methylation in transcriptional regulation, normal differentiation and cancer. *Biochim Biophys Acta* 2013; **1829**: 1161-1174 [PMID: 23938249 DOI: 10.1016/j.bbagr.2013.08.001]

**P- Reviewer:** Bridle KR, Hamidi C, Safer AM **S- Editor:** Qi Y

**L- Editor:** A **E- Editor:** Li D



## Retrospective Study

# Impaired liver function attenuates liver regeneration and hypertrophy after portal vein embolization

Yumiko Kageyama, Takashi Kokudo, Katsumi Amikura, Yoshihiro Miyazaki, Amane Takahashi, Hirohiko Sakamoto

Yumiko Kageyama, Takashi Kokudo, Katsumi Amikura, Yoshihiro Miyazaki, Amane Takahashi, Hirohiko Sakamoto, Division of Gastroenterological Surgery, Saitama Cancer Center, Kita-adachi gun, Saitama Prefecture 362-0806, Japan

**Author contributions:** Kageyama Y designed and performed the research and wrote the paper; Kokudo T contributed to the analysis and supervised the report; Amikura K designed the research and supervised the report; Miyazaki Y, Takahashi A and Sakamoto H supervised the report.

**Institutional review board statement:** This study was reviewed and approved by the Ethics Committee of the Saitama Cancer Center.

**Informed consent statement:** Patients were not required to give informed consent because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

**Conflict-of-interest statement:** We have no financial relationships to disclose.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Takashi Kokudo, MD, Division of Gastroenterological Surgery, Saitama Cancer Center, 780 Komuro, Ina, Kita-adachi gun, Saitama Prefecture 362-0806, Japan. kokudo-ty@umin.ac.jp  
 Telephone: +81-48-7221111  
 Fax: +81-48-7221129

Received: May 15, 2016

Peer-review started: May 17, 2016

First decision: June 14, 2016

Revised: June 26, 2016

Accepted: August 15, 2016

Article in press: August 16, 2016

Published online: October 8, 2016

## Abstract

### AIM

To clarify the clinical factors associated with liver regeneration after major hepatectomy and the hypertrophic rate after portal vein embolization (PVE).

### METHODS

A total of 63 patients who underwent major hepatectomy and 13 patients who underwent PVE in a tertiary care hospital between January 2012 and August 2015 were included in the analysis. We calculated the remnant liver volume following hepatectomy using contrast-enhanced computed tomography (CT) performed before and approximately 3-6 mo after hepatectomy. Furthermore, we calculated the liver volume using CT performed 2-4 wk after PVE. Preoperative patient characteristics and laboratory data were analyzed to identify factors affecting postoperative liver regeneration or hypertrophy rate following PVE.

### RESULTS

The remnant liver volume/total liver volume ratio negatively correlated with the liver regeneration rate after hepatectomy ( $\rho = -0.850, P < 0.001$ ). The regeneration rate was significantly lower in patients with an indocyanine green retention rate at 15 min (ICG-R15) of  $\geq 20\%$  in the right hepatectomy group but not in the left hepatectomy group. The hypertrophic rate after PVE positively correlated with the regeneration rate after hepatectomy ( $\rho = 0.648, P = 0.017$ ). In addition, the hypertrophic rate after PVE was significantly lower in



patients with an ICG-R15  $\geq 20\%$  and a serum total bilirubin  $\geq 1.5$  mg/dL.

### CONCLUSION

The regeneration rate after major hepatectomy correlated with hypertrophic rate after PVE. Both of them were attenuated in the presence of impaired liver function.

**Key words:** Regeneration after hepatectomy; Major hepatectomy; Portal vein embolization; Clinical factors; Hypertrophy

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Little is known about the clinical factors associated with liver regeneration after major hepatectomy. In the present study, the liver regeneration rate after major hepatectomy correlated with the remnant liver volume and hypertrophic rate after portal vein embolization. The regeneration rate after major hepatectomy and hypertrophic rate after portal vein embolization were attenuated in the presence of impaired liver function.

Kageyama Y, Kokudo T, Amikura K, Miyazaki Y, Takahashi A, Sakamoto H. Impaired liver function attenuates liver regeneration and hypertrophy after portal vein embolization. *World J Hepatol* 2016; 8(28): 1200-1204 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i28/1200.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i28.1200>

## INTRODUCTION

Curative resection is the most effective treatment for liver cancer<sup>[1]</sup>. Although resection-related mortality and morbidity have substantially decreased in recent years, the postoperative mortality rate remain to be as high as 1%-5%<sup>[2-7]</sup>. The capacity of hepatic regeneration after hepatectomy and the hypertrophic rate after portal vein embolization (PVE) are important for allowing surgeons to determine the appropriate extent of resection<sup>[8-11]</sup>. Better regeneration after hepatectomy and liver hypertrophy after PVE may prevent posthepatectomy complications, including hepatic failure<sup>[12,13]</sup>. Little is known about preoperative clinical factors influencing postoperative liver regeneration.

The aim of this study was to clarify the relationship between preoperative clinical factors and the regenerative capacity of the remnant liver after hepatectomy. Furthermore, we examined the relationship between the regeneration rate after hepatectomy and hypertrophic rate after PVE and clinical factors that affect the hypertrophic rate after PVE.

## MATERIALS AND METHODS

### Liver volume analysis

A total of 63 patients who underwent major hepatectomy

in the Division of Gastroenterological Surgery, Saitama Cancer Center, between January 2012 and August 2015 were included in the analysis. The liver volume was measured using enhanced computed tomography (CT) images taken before and approximately 3-6 mo after hepatectomy<sup>[14]</sup>. For volumetric analysis, a three-dimensional image analysis software was used (SYNAPSE VINCENT; Fuji Medical Systems, Tokyo, Japan). The regeneration rate was calculated as follows: [(liver volume after hepatectomy/estimated remnant liver volume before hepatectomy)  $\times 100$ ] - 100 (%). The indications for PVE were determined by the balance between the indocyanine green fractional disappearance rate (ICG-K) and the volumetric ratio of the future remnant liver volume. PVE was performed in patients whose values were estimated as follows: (ICG-K)  $\times$  (remnant liver volume/total liver volume)  $< 0.05$ <sup>[15]</sup>. The liver volume after PVE was calculated using enhanced CT images taken 2-4 wk after PVE. The hypertrophic rate after PVE was estimated as follows: [(remnant liver volume after PVE/remnant liver volume before PVE)  $\times 100$ ] - 100 (%). Preoperative patient characteristics and laboratory data, including platelet count, total bilirubin, and indocyanine green retention rate at 15 min (ICG-R15), were analyzed to identify factors affecting postoperative liver regeneration. For the measurement of ICG-R15, Indocyanine green (Diagnogreen, Daiichi-Sankyo, Tokyo, Japan) was administered at dose of 0.5 mg/kg by the antecubital vein of the opposite arm. Then, venous peripheral blood samples were collected every 5 min for 15 min to measure the ICG absorbance. ICG-K and ICG-R15 were calculated by fitting the serum disappearance curve by a single-exponential decay equation.

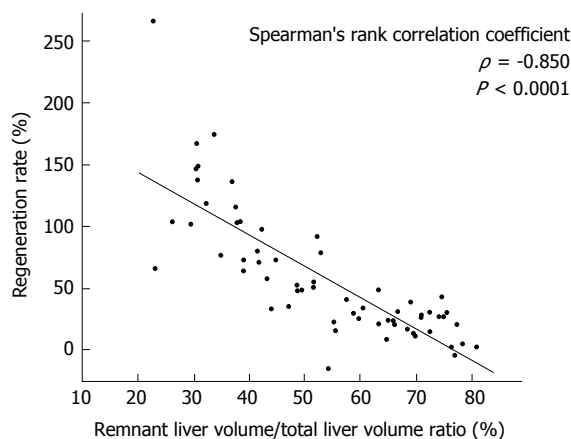
### Statistical analysis

Statistical analysis was performed using the JMP 11 software (SAS Institute, Inc., Cary, NC). Categorical variables were analyzed using the Wilcoxon rank sum test. Correlations between two parameters were examined by calculating the Spearman's rank correlation coefficient. A 2-tailed *P* value of  $< 0.05$  was considered statistically significant.

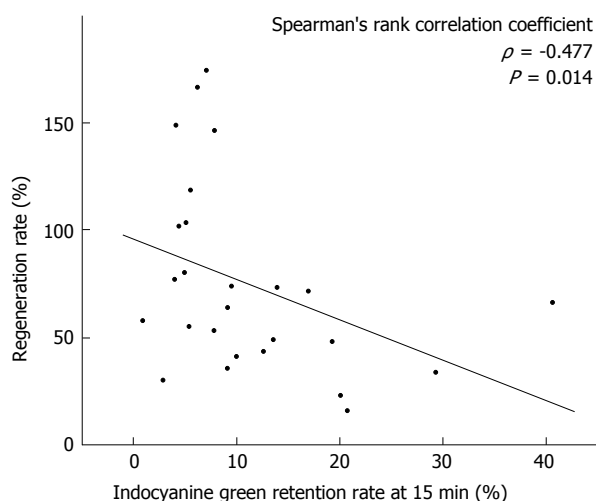
## RESULTS

### Liver regeneration after hepatectomy

Among the 63 patients, 42 were men and 21 were women, with a mean age of 68.1 years (range: 45-89 years). The diseases indicating the need for hepatectomy were metastatic liver carcinoma ( $n = 31$ ), intrahepatic cholangiocarcinoma ( $n = 14$ ), hilar cholangiocarcinoma ( $n = 10$ ), hepatocellular carcinoma ( $n = 4$ ), gallbladder carcinoma ( $n = 2$ ), hemangioma ( $n = 1$ ), and neuroendocrine tumor ( $n = 1$ ). A total of 22 patients had background liver diseases, including chronic viral hepatitis ( $n = 6$ ), alcoholic hepatitis ( $n = 1$ ), and obstructive jaundice ( $n = 15$ ). Preoperative chemotherapy within 6 mo was performed in 18 patients and 13 patients underwent preoperative PVE. The operative procedures



**Figure 1** Relationship between the remnant liver volume/total liver volume ratio and the liver regeneration rate after hepatectomy ( $n = 63$ ).

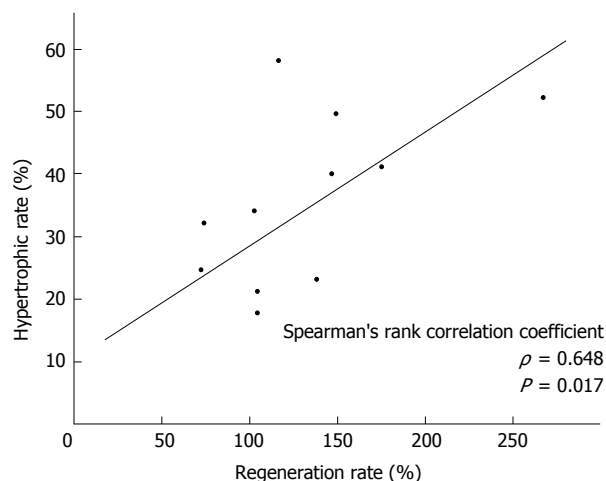


**Figure 2** Relationship between indocyanine green retention rate at 15 min and liver regeneration rate in patients who underwent right hepatectomy or extended right hepatectomy ( $n = 13$ ).

performed in the 63 patients included right hepatectomy or extended right hepatectomy ( $n = 26$ ), left hepatectomy or extended left hepatectomy ( $n = 32$ ), right trisegmentectomy ( $n = 3$ ), and left trisegmentectomy ( $n = 2$ ). The median remnant liver volume/total liver volume ratios after right hepatectomy and extended right hepatectomy, left hepatectomy and extended left hepatectomy, right trisegmentectomy, and left trisegmentectomy were 42.5%, 68.4%, 26.2% and 40.3%, respectively. Their median regeneration rates were 65.6%, 25.7%, 138.1% and 101.2%, respectively. The remnant liver volume/total liver volume ratio negatively correlated with the regeneration rate after hepatectomy ( $\rho = -0.850$ ,  $P < 0.001$ ; Figure 1).

#### Factors associated with liver regeneration

Because the liver regeneration rates were significantly different between the patients who underwent right hepatectomy or extended right hepatectomy (right hepatectomy group) and left hepatectomy or extended left hepatectomy (left hepatectomy group), we analyzed these



**Figure 3** Relationship between liver regeneration rate after major hepatectomy and hypertrophic rate after portal vein embolization ( $n = 13$ ).

two groups separately. In the right hepatectomy group, regeneration rate was significantly lower in patients with an ICG-R15 of  $\geq 20\%$ . It was not associated with platelet count, total bilirubin, diabetes mellitus, viral hepatitis, obstructive jaundice, or preoperative chemotherapy. The ICG-R15 value negatively correlated with liver regeneration rate in the right hepatectomy group ( $\rho = -0.477$ ,  $P = 0.014$ ; Figure 2). In the left hepatectomy group, no factor was associated with the regeneration rate (Table 1). In the 13 patients who underwent preoperative PVE, the median hypertrophic rate was 32.2% (range: 2.7%-58.3%). The hypertrophic rate positively correlated with the regeneration rate after hepatectomy ( $\rho = 0.648$ ,  $P = 0.017$ ; Figure 3). The hypertrophic rate was significantly lower in patients with an ICG-R15 of  $\geq 20\%$  and total bilirubin of  $\geq 1.5$  mg/dL (Table 2).

## DISCUSSION

Our study demonstrated that the liver regeneration rate was significantly lower in patients with an ICG-R15 of  $\geq 20\%$  in the right hepatectomy group, but not in the left hepatectomy group. The hypertrophic rate after PVE positively correlated with the regeneration rate after hepatectomy. In addition, the hypertrophic rate after PVE was significantly lower in patients with an ICG-R15 of  $\geq 20\%$  and a serum total bilirubin of  $\geq 1.5$  mg/dL.

Although several studies reported factors affecting liver regeneration after hepatectomy, the factors vary among studies. Yamanaka *et al.*<sup>[16]</sup> reported that the extent of resection and impaired liver function were associated with the liver regeneration, whereas Ogata *et al.*<sup>[17]</sup> reported that serum hyaluronan was a predictor of liver regeneration in patients with hepatocellular carcinoma. In living-donor liver transplantation, remnant liver volume<sup>[18]</sup>, sex<sup>[19]</sup>, and age<sup>[20]</sup> have been reported to be associated with liver regeneration. Aoki *et al.*<sup>[14]</sup> reported that sex and alanine aminotransferase values were associated with liver regeneration in the early phase, and the final regeneration rate was associated with the ratio of re-

**Table 1 Patient characteristics and liver regeneration rate after hepatectomy in 26 patients who underwent right hepatectomy or extended right hepatectomy vs 32 patients who underwent left hepatectomy or extended left hepatectomy**

	Right hepatectomy			Left hepatectomy		
	<i>n</i>	Regeneration rate (%) <sup>1</sup>	<i>P</i>	<i>n</i>	Regeneration rate (%) <sup>1</sup>	<i>P</i>
Age (mean)	45-83 (69)		<i>P</i> = 0.891	46-89 (69)		<i>P</i> = 0.321
Sex (male/female)	18/8	65.6/69.2	<i>P</i> = 0.355	20/12	25.7/25.9	<i>P</i> = 0.969
Background liver disease (yes/no)	6/20	51.0/70.2	<i>P</i> = 0.248	14/18	24.0/25.7	<i>P</i> = 0.621
Platelet count (/mm <sup>3</sup> ) ≥ 100	24	69.3	<i>P</i> = 0.178	30	24.7	<i>P</i> = 0.586
< 100	2	41.6		2	28.8	
Total bilirubin (mg/dL) ≥ 1.5	1	71.8	<i>P</i> = 0.842	4	28.2	<i>P</i> = 0.724
< 1.5	25	64.4		27	24.9	
ICG-R15 (%) ≥ 20	4	28.5	<i>P</i> < 0.05	4	28.2	<i>P</i> = 0.724
< 20	22	72.7		27	24.9	
Diabetes mellitus (yes/no)	4/22	63.7/65.6	<i>P</i> = 0.570	4/28	37.8/24.7	<i>P</i> = 0.459
Preoperative chemotherapy (yes/no)	10/16	60.0/74.5	<i>P</i> = 0.317	8/24	29.7/24.1	<i>P</i> = 0.361

<sup>1</sup>Median. ICG-R15: Indocyanine green retention rate at 15 min.**Table 2 Patient characteristics and hypertrophic rate in 13 patients who underwent portal vein embolization**

	<i>n</i>	Hypertrophic rate (%) <sup>1</sup>	<i>P</i>
Age (mean)	50-80 (65)		<i>P</i> = 0.845
Sex (male/female)	10/3	30.5/40.1	<i>P</i> = 0.612
Background liver disease (yes/no)	6/7	23.1/40.1	<i>P</i> = 0.087
Platelet count (/mm <sup>3</sup> ) ≥ 100	12	30.5	<i>P</i> = 0.593
< 100	1	40.1	
Total bilirubin (mg/dL) ≥ 1.5	4	19.7	<i>P</i> < 0.05
< 1.5	9	40.0	
ICG-R15 (%) ≥ 20	2	12.0	<i>P</i> < 0.05
< 20	11	34.2	
Diabetes mellitus (yes/no)	3/10	32.2/31.4	<i>P</i> = 1.000
Preoperative chemotherapy (yes/no)	3/10	40.1/26.8	<i>P</i> = 0.237

<sup>1</sup>Median. ICG-R15: Indocyanine green retention rate at 15 min.

sected liver volume. In our study, the remnant liver volume in the right hepatectomy group was significantly larger than that in the left hepatectomy group, and together with previous reports, the regeneration rate was highly affected by remnant liver volume/total liver volume ratio. Therefore, left and right hepatectomy should be separately considered when analyzing liver regeneration.

The regeneration rate was significantly lower in patients with a higher ICG-R15 in the right hepatectomy group, whereas no variables related to liver regeneration were identified in the left hepatectomy group. These results also confirmed that liver regeneration after right and left hepatectomy should be separately considered.

Our study demonstrated the correlation between the hypertrophic rate after PVE and liver regeneration rate after hepatectomy. The hypertrophic rate positively correlated with the regeneration rate, and regeneration rate after major hepatectomy and hypertrophic rate after PVE were attenuated in the presence of impaired liver function.

In conclusion, the regeneration rate after major hepatectomy correlated with the remnant liver volume and hypertrophic rate after PVE. The regeneration rate after right hepatectomy and hypertrophic rate after PVE were

attenuated in the presence of impaired liver function.

## COMMENTS

### Background

Although resection-related mortality and morbidity have substantially decreased in recent years, the postoperative mortality rate has remained as high as 1%-5%. Portal vein embolization (PVE) is proposed to induce hypertrophy of the anticipated liver remnant to reduce such complications. The capacity of hepatic regeneration after hepatectomy and the hypertrophic rate after PVE are important for allowing surgeons to determine the appropriate extent of resection. Better regeneration after hepatectomy and liver hypertrophy after PVE may prevent posthepatectomy complications, including hepatic failure.

### Research frontiers

Little is known about preoperative clinical factors influencing postoperative liver regeneration and liver hypertrophy after PVE.

### Innovations and breakthroughs

In this study, the relationship between preoperative clinical factors and the regenerative capacity of the remnant liver after hepatectomy were clarified. Furthermore, the authors examined the relationship between the regeneration rate after hepatectomy and hypertrophic rate after PVE and clinical factors that affect the hypertrophic rate after PVE.

### Applications

This study suggests that the regeneration rate after major hepatectomy correlated with the remnant liver volume and hypertrophic rate after PVE, and the

regeneration rate after right hepatectomy and hypertrophic rate after PVE were attenuated in the presence of impaired liver function.

### Terminology

PVE: A procedure in the preoperative treatment of patients selected for major hepatic resection. PVE is performed via either the percutaneous transhepatic or the transileocolic route and is usually reserved for patients whose future liver remnants are too small to allow resection.

### Peer-review

The manuscript is an interesting one. The authors, using 63 patients who underwent major hepatectomy and 13 patients who underwent portal vein embolization, calculated regeneration rate correlated with the remnant liver volume. In conclusion, they found that the regeneration rate after right hepatectomy and the hypertrophic rate after PVE were attenuated in the presence of impaired liver function. It is a well-written and presented manuscript.

## REFERENCES

- 1 Venook AP. Treatment of hepatocellular carcinoma: too many options? *J Clin Oncol* 1994; **12**: 1323-1334 [PMID: 8201395]
- 2 Wei AC, Tung-Ping Poon R, Fan ST, Wong J. Risk factors for perioperative morbidity and mortality after extended hepatectomy for hepatocellular carcinoma. *Br J Surg* 2003; **90**: 33-41 [PMID: 12520572 DOI: 10.1002/bjs.4018]
- 3 Belghiti J, Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O. Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. *J Am Coll Surg* 2000; **191**: 38-46 [PMID: 10898182 DOI: 10.1016/S1072-7515(00)00261-1]
- 4 Gomez D, Malik HZ, Bonney GK, Wong V, Toogood GJ, Lodge JP, Prasad KR. Steatosis predicts postoperative morbidity following hepatic resection for colorectal metastasis. *Br J Surg* 2007; **94**: 1395-1402 [PMID: 17607707 DOI: 10.1002/bjs.5820]
- 5 Kaneko K, Shirai Y, Wakai T, Yokoyama N, Akazawa K, Hatakeyama K. Low preoperative platelet counts predict a high mortality after partial hepatectomy in patients with hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 5888-5892 [PMID: 16270404 DOI: 10.3748/wjg.v11.i37.5888]
- 6 Mullen JT, Ribero D, Reddy SK, Donadon M, Zorzi D, Gautam S, Abdalla EK, Curley SA, Capussotti L, Clary BM, Vauthey JN. Hepatic insufficiency and mortality in 1,059 noncirrhotic patients undergoing major hepatectomy. *J Am Coll Surg* 2007; **204**: 854-862 [PMID: 17481498 DOI: 10.1016/j.jamcollsurg.2006.12.032]
- 7 Balzan S, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D, Durand F. The "50-50 criteria" on postoperative day5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**: 824-828, discussion 828-829 [PMID: 16327492 DOI: 10.1097/01.sla.0000189131.90876.9e]
- 8 Okamoto E, Kyo A, Yamanaka N, Tanaka N, Kuwata K. Prediction of the safe limits of hepatectomy by combined volumetric and functional measurements in patients with impaired hepatic function. *Surgery* 1984; **95**: 586-592 [PMID: 6324403]
- 9 Yamanaka N, Okamoto E, Kuwata K, Tanaka N. A multiple regression equation for prediction of posthepatectomy liver failure. *Ann Surg* 1984; **200**: 658-663 [PMID: 6486915 DOI: 10.1097/00006558-198411000-00018]
- 10 Shimada M, Matsumata T, Maeda T, Itasaka H, Suehiro T, Sugimachi K. Hepatic regeneration following right lobectomy: estimation of regenerative capacity. *Surg Today* 1994; **24**: 44-48 [PMID: 8054774 DOI: 10.1007/BF01676884]
- 11 Kubota K, Makuuchi M, Kusaka K, Kobayashi T, Miki K, Hasegawa K, Harihara Y, Takayama T. Measurement of liver volume and hepatic functional reserve as a guide to decision-making in resectional surgery for hepatic tumors. *Hepatology* 1997; **26**: 1176-1181 [PMID: 9362359]
- 12 Aoki T, Imamura H, Hasegawa K, Matsukura A, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Sequential preoperative arterial and portal venous embolization in patients with hepatocellular carcinoma. *Arch Surg* 2004; **139**: 766-774 [PMID: 15249411 DOI: 10.1001/archsurg.139.7.766]
- 13 Di Stefano DR, de Baere T, Denys A, Hakime A, Gorin G, Gillet M, Saric J, Trillaud H, Petit P, Bartoli JM, Elias D, Delperro JR. Preoperative percutaneous portal vein embolization: evaluation of adverse events in 188 patients. *Radiology* 2005; **234**: 625-630 [PMID: 15591428 DOI: 10.1148/radiol.2342031996]
- 14 Aoki T, Imamura H, Matsuyama Y, Kishi Y, Kobayashi T, Sugawara Y, Makuuchi M, Kokudo N. Convergence process of volumetric liver regeneration after living-donor hepatectomy. *J Gastrointest Surg* 2011; **15**: 1594-1601 [PMID: 21710329 DOI: 10.1007/s11605-011-1590-y]
- 15 Okochi O, Kaneko T, Sugimoto H, Inoue S, Takeda S, Nakao A. ICG pulse spectrophotometry for perioperative liver function in hepatectomy. *J Surg Res* 2002; **103**: 109-113 [PMID: 11855925 DOI: 10.1006/jsre.2001.6328]
- 16 Yamanaka N, Okamoto E, Kawamura E, Kato T, Oriyama T, Fujimoto J, Furukawa K, Tanaka T, Tomoda F, Tanaka W. Dynamics of normal and injured human liver regeneration after hepatectomy as assessed on the basis of computed tomography and liver function. *Hepatology* 1993; **18**: 79-85 [PMID: 8392029 DOI: 10.1002/hep.1840180114]
- 17 Ogata T, Okuda K, Ueno T, Saito N, Aoyagi S. Serum hyaluronan as a predictor of hepatic regeneration after hepatectomy in humans. *Eur J Clin Invest* 1999; **29**: 780-785 [PMID: 10469166 DOI: 10.1046/j.1365-2362.1999.00513.x]
- 18 Marcos A, Fisher RA, Ham JM, Shiffman ML, Sanyal AJ, Luketic VA, Sterling RK, Fulcher AS, Posner MP. Liver regeneration and function in donor and recipient after right lobe adult to adult living donor liver transplantation. *Transplantation* 2000; **69**: 1375-1379 [PMID: 10798757 DOI: 10.1097/00007890-200004150-00028]
- 19 Pomfret EA, Pomposelli JJ, Gordon FD, Erbay N, Lyn Price L, Lewis WD, Jenkins RL. Liver regeneration and surgical outcome in donors of right-lobe liver grafts. *Transplantation* 2003; **76**: 5-10 [PMID: 12865779 DOI: 10.1097/01.TP.0000079064.08263.8E]
- 20 Yokoi H, Isaji S, Yamagiwa K, Tabata M, Sakurai H, Usui M, Mizuno S, Uemoto S. Donor outcome and liver regeneration after right-lobe graft donation. *Transpl Int* 2005; **18**: 915-922 [PMID: 16008740 DOI: 10.1111/j.1432-2277.2005.00158.x]

P- Reviewer: Chiu KW, Qin JM, Sazci A, Zhang X S- Editor: Qiu S  
L- Editor: A E- Editor: Li D







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 October 18; 8(29): 1205-1250





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verducci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**MINIREVIEWS**

- 1205** Nutritional evaluation in cirrhosis: Emphasis on the phase angle

*Fernandes SA, de Mattos AA, Tovo CV, Marroni CA*

**ORIGINAL ARTICLE****Basic Study**

- 1212** Potential role of killer immunoglobulin receptor genes among individuals vaccinated against hepatitis B virus in Lebanon

*Melhem NM, Mahfouz RA, Kreidieh K, Abdul-Khalik R, El-Khatib R, Talhouk R, Musharrafieh U, Hamadeh G*

- 1222** Lycopene modulates cellular proliferation, glycolysis and hepatic ultrastructure during hepatocellular carcinoma

*Gupta P, Bhatia N, Bansal MP, Koul A*

**Case Control Study**

- 1234** Polymorphisms of folate metabolism genes in patients with cirrhosis and hepatocellular carcinoma

*Peres NP, Galbiatti-Dias ALS, Castanhole-Nunes MMU, da Silva RF, Pavarino ÉC, Goloni-Bertollo EM, Ruiz-Cintra MT*

**CASE REPORT**

- 1244** Hepatitis C and double-hit B cell lymphoma successfully treated by antiviral therapy

*Galati G, Rampa L, Vespasiani-Gentilucci U, Marino M, Pisani F, Cota C, Guidi A, Picardi A*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Dr. Francisco Igor Macedo, MD, Department of General Surgery, Providence Hospital and Medical Centers, Michigan State University College of Human Medicine, Southfield, MI 48075, United States

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL BOARD MEMBERS**  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

**EDITORIAL OFFICE**  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
October 18, 2016

**COPYRIGHT**  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
<http://www.wjgnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esps/>



## Nutritional evaluation in cirrhosis: Emphasis on the phase angle

Sabrina Alves Fernandes, Angelo Alves de Mattos, Cristiane Valle Tovo, Claudio Augusto Marroni

Sabrina Alves Fernandes, Angelo Alves de Mattos, Cristiane Valle Tovo, Claudio Augusto Marroni, Postgraduate Program at Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS 90420-060, Brazil

Sabrina Alves Fernandes, Post Graduation Program in Bioscience and Rehabilitation and the Post Graduation Program in Rehabilitation and Inclusion, Methodist University - IPA, Porto Alegre, RS 90420-060, Brazil

**Author contributions:** Fernandes SA and Tovo CV performed the data collection; all the authors wrote the paper and approved the final version.

**Conflict-of-interest statement:** Authors declare no conflict of interests for this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Sabrina Alves Fernandes, PhD, Postgraduate Program at Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Rua Professor Duplan 72/01, Porto Alegre, RS 90420-060, Brazil. [sabrinaaferrandes@gmail.com](mailto:sabrinaaferrandes@gmail.com)  
Telephone: +55-51-33038795  
Fax: +55-51-33038795

Received: March 11, 2016  
Peer-review started: March 14, 2016  
First decision: April 20, 2016  
Revised: August 2, 2016  
Accepted: August 17, 2016  
Article in press: August 18, 2016  
Published online: October 18, 2016

### Abstract

Protein-calorie malnutrition (PCM) is a common condition in cirrhotic patients, leading to a worse prognosis, complications, poor quality of life and lower survival rates. Among ways of assessing nutritional status, there are anthropometric methods such as the evaluation of the triceps skinfold, the arm circumference, the arm muscle circumference and the body mass index, and non-anthropometric methods such as the subjective global assessment, the handgrip strength of non-dominant hand, and the bioelectrical impedance analysis (BIA). PCM is frequently under-diagnosed in clinical settings in patients with cirrhosis due to the limitations of nutritional evaluation methods in this population. BIA is a useful method, but cannot be indicated in patients with abnormal body composition. In these situations, the phase angle (PA) has been used, and can become an important tool in assessing nutritional status in any situation. The PA is superior to anthropometric methods and might be considered as a nutritional indicator in cirrhosis. The early characterization of the nutritional status in patients with cirrhosis means an early nutritional intervention, with a positive impact on patients' overall prognosis. Among the usually accepted methods for nutritional diagnosis, the PA provides information in a quick and objective manner.

**Key words:** Malnutrition; Bioelectrical impedance; Phase angle; Sarcopenia; Nutrition

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Malnutrition in cirrhotic patients is a common clinical condition, but there is currently no nutritional diagnosis method defined as the gold standard. Presently, the only nutritional indicator compatible with the clinical condition through the Child-Pugh score in cirrhosis is the phase angle (PA). The PA has been

a reliable method and is free of influences regarding changes in body composition of cirrhotic patients at an advanced stage. The PA measured by bioelectrical impedance analysis promises to be a significant parameter for early nutritional intervention in patients with chronic liver disease.

Fernandes SA, de Mattos AA, Tovo CV, Marroni CA. Nutritional evaluation in cirrhosis: Emphasis on the phase angle. *World J Hepatol* 2016; 8(29): 1205-1211 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i29/1205.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i29.1205>

## INTRODUCTION

Many factors favor the development of protein-calorie malnutrition (PCM) in cirrhosis, a common condition that leads to serious repercussions regarding the general state and clinical course of patients<sup>[1]</sup> and that presents a worse prognosis, complications, poor quality of life and lower survival rates<sup>[2,3]</sup>. However, malnutrition is often underdiagnosed in this situation<sup>[4]</sup>. It is difficult to evaluate the nutritional status of patients with cirrhosis, as there are particularities due to the clinical condition that make it hard to precisely inform the real nutritional status and its consequent prognosis<sup>[5]</sup>.

Malnutrition can be directly related to a poor survival rate in patients with cirrhosis, and its improvement is a strong indicator of quality of life, especially for those who are on the waiting list for liver transplantation<sup>[6]</sup>. Early detection of malnutrition in cirrhotic patients is of great clinical relevance and interferes positively in patient recovery<sup>[7,8]</sup>.

The new European Society of Clinical Nutrition and Metabolism (ESPEN) consensus<sup>[9]</sup>, recommends that subjects at risk of malnutrition be identified by validated screening tools, and they advocate two options for the diagnosis of malnutrition: The body mass index (BMI, kg/m<sup>2</sup>) lower than 18.5 to characterize malnutrition, and the combined finding of unintentional weight loss and either reduced BMI or a low fat-free mass index (FFMI), or both. Weight loss could be either greater than 10% of habitual weight regardless of time, or greater than 5% over 3 mo. Low FFMI is characterized as lower than 15 or lower than 17 kg/m<sup>2</sup> in females or males, respectively. However, many other tools, such as anthropometric and non-anthropometric methods as well as laboratory tests may be used, classifying the degrees of malnutrition as mild, moderate and severe in different ways - although none of these other tools are widely recognized as a gold standard, and thus must be considered together. Various nutritional parameters has been used to assess the nutritional status such as anthropometry parameters [mid arm circumference, triceps skinfold thickness (TSF)], hand grip, serum albumin level, creatinine height index, and total lymphocyte count<sup>[8-10]</sup>. Recently electrical bioimpedance has been proposed for body composition

analysis of patients with chronic liver disease<sup>[8,11-13]</sup>. In view of paucity of data on prevalence of malnutrition and its relationship with morbidity and mortality in patients with liver cirrhosis as well as the absence of a gold standard method for nutritional evaluation in these patients, we conducted this study to determine the prevalence of malnutrition by various methods and its clinical importance in cirrhotic patients according the severity of disease.

Considering the scarcity of data in the evaluation of malnutrition in patients with liver cirrhosis as well as the absence of a gold standard for nutritional evaluation in these patients, the present review was performed, critically addressing the following points: Malnutrition in cirrhosis; sarcopenia; nutritional assessment in cirrhosis; bioelectrical impedance analysis and the phase angle<sup>[10]</sup>.

## MALNUTRITION IN CIRRHOSIS

Malnutrition is one of the most frequent complications in cirrhotic patients<sup>[11]</sup>. However, its frequency in cirrhosis is highly variable, and may affect between 20% of the patients with compensated cirrhosis and more than 60% of these patients with severe hepatic dysfunction<sup>[12,13]</sup>.

Alberino *et al*<sup>[6]</sup> studied 212 hospitalized patients with liver cirrhosis that were followed for 2 years or until death. The severely and moderately malnourished patients had lower survival rates than normal and over nourished patients, and severe depletion of muscle mass and body fat was found to be an independent predictor of survival. This data suggests that malnutrition is an independent predictor of survival in patients with liver cirrhosis. Additionally, a nationwide analysis of the prevalence of PCM in patients with cirrhosis and portal hypertension (PHTN) and its mortality was conducted in the United States<sup>[13]</sup>. There were 114703 admissions with cirrhosis and PHTN between 1998 and 2005, and the prevalence of PCM was higher among patients with cirrhosis and PHTN compared with general medical inpatients; this prevalence was also associated with higher in-hospital mortality and resource utilization. The authors concluded that PCM may be an indicator of disease severity and should be routinely assessed on admission.

Besides the metabolic changes observed in cirrhosis, there are factors that can contribute to increased malnutrition in this population. Factors such as anorexia and early satiety, triggered by changes in endogenous leptin, mineral deficiencies and reduction in gastric expandability favor a negative energetic balance, with an imbalance between ingestion and energy intake and expenditure, and PCM may develop as a result<sup>[14]</sup>. The zinc and magnesium deficiencies that may be often seen in the population of patients with cirrhosis contribute to the development of dysgeusia, which aggravates the intake capacity<sup>[15,16]</sup>.

The clinical complications that can occur in decompensated cirrhosis - such as gastrointestinal bleeding,

hepatic encephalopathy (HE) and ascites - can further accentuate the PCM situation<sup>[14]</sup>, alongside the diet offered to these patients, which is restrictive in most cases. Thus, although for a short period of time, a hypoproteic diet may be eventually implemented, especially in cases of HE grades III and IV<sup>[4]</sup>. It is worth highlighting that the protein restriction has been banned in HE in order to prevent the worsening of PCM, and a diet with 1 to 1.5 g of protein per kilogram of weight is suggested<sup>[4]</sup>.

The recommended low-sodium diet in the treatment of patients with ascites and peripheral edema makes the food intake even more difficult and significantly decreases the daily calorie intake, thus stimulating PCM. A low-sodium diet with a daily intake of 2 g of salt is recommended<sup>[17]</sup>.

It is known that skeletal muscles contribute in the proper metabolic functioning of macro and micro-nutrients, favoring body homeostasis. In individuals with cirrhosis, there is a significant loss and dysfunction of such musculature, often characterizing sarcopenia<sup>[18,19]</sup>. This state generates systemic and inflammatory changes associated with the PCM, negatively impacting the patient's clinical status<sup>[20]</sup>.

The classical study by Merli *et al.*<sup>[21]</sup> prospectively evaluated a total of 1053 cirrhotic patients to determine whether malnutrition is a risk factor for mortality in cirrhosis. They found that the cumulative survival was lower in patients with a reduction in muscle mass in Child-Pugh classes A and B.

Montano-Loza *et al.*<sup>[22]</sup> studied 112 cirrhotic patients consecutively evaluated for liver transplantation, and observed that sarcopenia occurred in up to 40% of the patients and was related to the worsening of clinical conditions represented by biochemical and clinical parameters; moreover, by multivariate Cox analysis, the Child-Pugh (HR = 1.85;  $P = 0.04$ ), the model for end-stage liver disease (MELD) scores (HR = 1.08;  $P = 0.001$ ) and sarcopenia (HR = 2.21;  $P = 0.008$ ) were independently associated with mortality. The median survival time for patients with sarcopenia was  $19 \pm 6$  mo, compared with  $34 \pm 11$  mo among non-sarcopenic patients ( $P = 0.005$ ). Sarcopenia can be considered an indicator of risk of infection in cirrhotic individuals, directly reflecting a decline in immune function, worsening the quality of life and decreasing survival.

## SARCOPENIA

Malnutrition in cirrhosis is closely related to the development of sarcopenia, which will be one of the most common complications related to survival in this population of patients. Nevertheless, there is a lack of an optimal index for sarcopenia and of a consensus definition for sarcopenia in patients with cirrhosis in whom ascites and edema may interfere with body composition analysis<sup>[23]</sup>.

Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal muscle mass

and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death<sup>[24,25]</sup>. The European Working Group on Sarcopenia in Older People recommends using the presence of both low muscle mass and low muscle function (strength or performance) for the diagnosis of sarcopenia<sup>[26]</sup>.

Montano-Loza *et al.*<sup>[19]</sup> evaluated a population of 248 cirrhotic patients enlisted for liver transplantation and identified sarcopenia in 45% of patients; sarcopenia was associated with a longer period of hospitalization and higher risk of bacterial infection after transplantation.

Similarly, Tandon *et al.*<sup>[5]</sup> evaluated 142 patients with cirrhosis listed for liver transplantation, and found that 41% were sarcopenic. Male gender, the BMI, and Child-Pugh class C cirrhosis (but not the MELD score) were independent predictors of sarcopenia, which was an independent predictor of mortality after adjustments for age and MELD scores. The authors concluded that sarcopenia is associated with increased waiting-list mortality and is poorly predicted by subjective nutritional assessment tools such as BMI and subjective global assessment (SGA). The objective assessment of sarcopenia holds promise for prognostication in this patient population.

## NUTRITIONAL ASSESSMENT IN CIRRHOSIS

Among the different ways of assessing nutritional status, there are anthropometric methods such as determining the TSF, the arm circumference (AC), the arm muscle circumference (AMC) and the BMI, as well as non-anthropometric methods such as SGA, handgrip strength (HS) of non-dominant hand, the adductor pollicis muscle thickness (APMT) and the phase angle (PA) by bioelectrical impedance analysis (BIA).

Classical anthropometry assesses the measurement of body size and its proportions. The results obtained are compared with the points of reference previously described<sup>[27]</sup>.

Cirrhotic individuals present significant changes regarding body weight by hydric retention, making BMI an inadequate method for nutritional diagnosis<sup>[28]</sup>. Such distortion was observed in the study performed by Gottschall *et al.*<sup>[29]</sup>, in which 61.8% of patients were classified as overweight, while other techniques, such as SGA or HS, found malnutrition in 38% and 85.7% in the same population of patients, respectively.

The TSF measurement indirectly estimates fat mass by measuring the thickness of two layers of skin and the adjacent subcutaneous fat. This is a good assessment method, although some studies have found a low prevalence of malnutrition in cirrhosis when comparing this method to others<sup>[30-32]</sup>.

Abbott *et al.*<sup>[33]</sup> and Alberino *et al.*<sup>[6]</sup> described, in their studies, that 54% of the evaluated cirrhotic patients were malnourished when utilizing AC and AMC, supporting the findings of Merli *et al.*<sup>[21]</sup>, which suggest

AMC as an accurate indicator of malnutrition in patients in the early stages of cirrhosis. On the other hand, a study performed in our center by Fernandes *et al.*<sup>[34]</sup> showed that AC and AMC are not sensible parameters for the nutritional diagnosis.

As a general rule, the anthropometric parameters may be affected when there is hydric retention; the results are observer-dependent and can be conflicting, becoming inadequate in the nutritional assessment of patients with cirrhosis.

When considering the non-anthropometric methods, SGA is a method of interest that uses easily reproducible parameters such as the clinical history and physical conditions of the individual, focusing on the nutritional aspects and offering a score that provides the nutritional diagnosis<sup>[35]</sup>. However, this method shows limitations, especially when the patient has some difficulty to understanding or even HE, as patients will not report their nutritional history adequately<sup>[36]</sup>.

Figueiredo *et al.*<sup>[37]</sup> observed that SGA has a sensitivity of only 22% in cirrhotic individuals and underestimates their nutritional status in 57%, while overestimating it in 6%<sup>[38]</sup>. On the other side, Ritter and Gazzola<sup>[38]</sup> established SGA as a good option for the nutritional assessment of patients with liver disease.

Although some authors<sup>[36,39,40]</sup> have suggested that SGA might be useful to assess the nutritional status evolution of cirrhotic patients who are liver transplant candidates, these studies have detected malnutrition in only 25% of cases with this method.

The HS assessment through dynamometry refers to the measurement of muscle strength and of pressure distribution<sup>[41]</sup>, classifying the nutritional status of individuals by gender and age. In dynamometry, there is the assumption that in PCM there is a decrease in muscle mass, hindering one's functional capacity<sup>[42]</sup>. Studies with cirrhotic patients have shown the superiority of HS assessment when compared to SGA in diagnosing malnutrition; HS is considered a low-cost and simple method that is not influenced by the presence of hydric retention<sup>[42]</sup>. Curiously, in different studies, HS - while proving to be a good method in assessing nutritional risk - does not present a correlation between malnutrition and the staging of liver disease through the Child-Pugh score, although it is considered that liver disease patients, when classified as Child-Pugh C, are malnourished *per se*<sup>[41]</sup>.

The APMT has been suggested as a promising marker of muscle mass<sup>[43,44]</sup>. The adductor pollicis muscle is the only muscle that allows direct thickness assessment, as it is anatomically well defined and flat in shape<sup>[45]</sup>. However, few have looked into it as a marker of nutritional status<sup>[46]</sup>.

## BIOELECTRICAL IMPEDANCE ANALYSIS

The BIA is a method for assessing body composition that has shown good results regarding the nutritional state, as it shows fat mass, lean mass and basal metabolic rate, in addition to total body water in

healthy subjects<sup>[47]</sup>. The distribution of body fat has an important influence in the severity of certain diseases, such as in cardiovascular disease and depending on the type of fat mass distribution, may pose a higher risk of developing tumors<sup>[48]</sup>. Thus, in addition to providing a nutritional status assessment, BIA can also be a good prognosis method that is characterized as a practical, quick, non-invasive and low-cost method<sup>[48,49]</sup>.

In the clinical nutritional assessment of a cirrhotic patient, it is possible to perform compartmentalized body assessment through BIA not only in the classical model that is normally used (fat mass and fat-free mass), but also in a quantitative manner, obtaining cellular distribution and providing information on body composition<sup>[50]</sup>.

In the past, there were restrictions on the use of BIA for individuals with abnormal body composition; that is, amputations, electrolyte disorders (edema and ascites), obesity, dystrophies and pregnancy, because the BIA assumes that the human body resembles a cylinder of constant hydration and invariably lean mass<sup>[47,51]</sup>.

Some tissues with high water and electrolyte composition - such as cerebrospinal fluid, blood or muscles - are high electrical conductors. On the other hand, fatty tissues or bones are highly resistant to electric current<sup>[47]</sup>. The conductivity of biological tissues is virtually ionic, meaning that electric charges are transferred by the ionization of the salts, bases or acids in body fluid. Thus, organic conductivity is directly proportional to the quantity of body fluid volume. Therefore, if the patient is in a state of overhydration, the amount of lean body mass is overestimated, modifying the result of the body assessment, which is one of the limitations of this method<sup>[47]</sup>.

For the assessment of nutritional state by BIA, there are monofrequencial or multifrequencial portable equipment, differing on the options of the amperage of the electric current to allow greater sensitivity of the examination. The patient remains in dorsal decubitus position, with hands and legs parallel to the body. One electrode is placed on the dorsal hand, at the middle finger level, and one in the wrist joint, both on the right side. Another pair of electrodes is placed on the dorsal foot, at the middle toe level, and in the ankle joint, also on the right side. The electrical current enables measuring resistance and reactance and obtaining the PA value.

## THE PHASE ANGLE

In view of the limitations of BIA, the clinically established bioelectrical impedance parameter is the PA. The PA was originally described by Baumgartner *et al.*<sup>[51]</sup> for the diagnosis of metabolic disorders. The data is obtained through BIA and is directly calculated through the arc tangent formula ( $X_c/R$ ). The tissues' capacitance ( $X_c$ ) is related to cellularity, cell-size and integrity of cellular membrane. The resistance ( $R$ ) is dependent on the hydration state of the tissues. The ratio of components results in a geometric graphic, where the ratio of  $R$  and



Xc results in an angle called the PA.

BIA is represented by the vector Z, which is a combination of the perpendicular vectors R and Xc. The vector Z has a module M, and the horizontal axis defines the PA<sup>[52]</sup>.

The PA reflects the cellular vitality and integrity, where normal values (according to gender and age) indicate preserved cellular activity<sup>[34,53,54]</sup>, being highly predictive of clinical progression in a number of diseases<sup>[55]</sup>.

It has been suggested that the PA can become an important tool in assessing nutritional status in any situation, being superior to anthropometric and biochemical methods<sup>[44]</sup>.

There are reference values according to age and gender<sup>[8]</sup>, and some authors prefer to establish cutoff points according to the disease being studied<sup>[47]</sup>.

The PA has also been studied as a prognostic marker in different clinical situations, such as tumors, acquired immunodeficiency syndrome, and heart and liver diseases<sup>[54]</sup>.

In a review, Llamas *et al.*<sup>[55]</sup> concluded that the PA may be sufficient to monitor the nutritional status of an individual. In a population-based study, they observed a higher PA in men than in women, except in individuals over 70 years of age. When stratified by age and gender, the values tend to increase as BMI increases in values of up to 35 kg/m<sup>2</sup>; however, there is a decrease in PA in groups with BMI above 35 kg/m<sup>2</sup><sup>[55]</sup>.

There are few studies evaluating the PA in cirrhotic patients.

Selberg *et al.*<sup>[56]</sup>, in a prospective study of 305 patients with cirrhosis, correlated the PA with muscle mass, muscle strength, and survival rates. They observed that patients with a PA equal to or lower than 5.4 degrees showed lower survival rates than those with PA values above 6.6 degrees. In those with PA under 4.4 degrees, survival was even (and significantly) lower. Variables such as total body potassium, anthropometric measurements and BIA were evaluated separately; however, only the PA proved to be an isolated predictor of survival. The authors concluded that the PA appears to be superior to conventional methods in the clinical assessment of patients with cirrhosis.

In a retrospective study, Pirlich *et al.*<sup>[53]</sup> evaluated the cellular mass composition of 41 cirrhotic patients (20 with ascites and 21 without) through BIA, which was considered the reference method. The study shows that the PA is a tool that is able to detect body cellular mass and to identify its decrease in cirrhotic patients. The PA offers reliable PCM estimates even in patients with large amount of ascites, proving to be superior to commonly used techniques.

In a cohort that assessed 66 cirrhotic patients stratified by their clinical condition through the Child-Pugh score and followed-up during a 17-mo period, the established PA for this population was 5.18 degrees. Patients with values below this angle were considered

to have poor prognosis and shorter survival rates. It is worth highlighting that as the patients' clinical situation worsened, the PA decreased, showing a prognostic value<sup>[57]</sup>.

Corroborating these findings, we assessed the nutritional status of 129 cirrhotic patients through different methods and demonstrated that the only method that is able to correlate malnutrition with the staging of liver disease, evaluated through the Child-Pugh classification, was the PA. We set the PA cutoff point as 5.4 degrees, and patients with values below this discriminatory level showed a worse prognosis. We should point out the discrepancies between the results of different evaluation methods (anthropometry, HGS and BIA) used to diagnose PCM, once the diagnosis for malnutrition may vary from 5.4% to 69.3% in the same population, depending on the assessment method employed<sup>[34]</sup>. The PA evaluated through the BIA presented a sensitivity and specificity of 68.9%-70.0% and 49.2%-56%, respectively, when compared to the HGS<sup>[34]</sup>.

Later, another study performed in our center evaluated 195 cirrhotic patients, reinforcing the idea that the PA is a good prognostic marker when compared to other methods, as it is the only one that correlates with the real clinical condition of the patient<sup>[58]</sup>.

Recently, Ruiz-Margáin *et al.*<sup>[59]</sup> assessed 249 compensated cirrhotic patients in a prospective cohort study with a 48-mo follow-up period. The PA cutoff point for malnutrition was lower than or equal to 4.9 degrees. This study also concluded that the PA is a good prognostic marker, associating the PCM with mortality rate.

A cohort study conducted in our center evaluated 32 cirrhotic patients enlisted for liver transplantation<sup>[36]</sup>. The patients were interviewed and evaluated on the day of or on the day before the transplant, and 1, 6, and 12 mo after surgery. The assessment of nutritional status was performed applying diagnostic procedures in sequence: Anthropometry, HS, APMT and PA. Methods that better demonstrated the real prevalence of malnourished patients before transplantation were PA (25%), AMC (21.9%) and AC (18.8%). The percentage of malnourished patients was significantly higher after 1 mo of transplantation when compared to the percentage in 6 mo and 1 year after transplantation. It was suggested that the PA could be widely used with this population, since the results are consistent, reliable and reproducible.

Wagner *et al.*<sup>[60]</sup> evaluated nutritional methods that informed the nutritional status of 71 post-transplantation patients. Patients were divided into 3 groups according to time since transplantation: 5 years, between 5 and 10 years, and over 10 years. They used the PA cutoff point as below 5 degrees in order to diagnose malnutrition. The PCM diagnosis was made in 81.2%, 31.6% and 31.7% in each group, respectively ( $P = 0.008$ ). In this study, the PA showed a higher prevalence of malnutrition among the population of patients in the first years after liver transplantation.

## CONCLUSION

The cirrhotic patient is malnourished *per se*, regardless of etiology and the severity of the disease. The early characterization of the nutritional status in patients with cirrhosis means an early nutritional intervention, with a positive impact on patients' overall prognosis. Compared to the usually accepted methods for nutritional diagnosis, the PA obtained through BIA is the only appropriate method to evaluate the nutritional status of cirrhotic, providing safe information in a quick and objective manner as a prognostic index.

## REFERENCES

- 1 **Carvalho L**, Parise ER. Evaluation of nutritional status of nonhospitalized patients with liver cirrhosis. *Arq Gastroenterol* 2006; **43**: 269-274 [PMID: 17406753 DOI: 10.1590/S0004-28032006000400005]
- 2 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]
- 3 **Maharshi S**, Sharma BC, Srivastava S. Malnutrition in cirrhosis increases morbidity and mortality. *J Gastroenterol Hepatol* 2015; **30**: 1507-1513 [PMID: 25974421 DOI: 10.1111/jgh.12999]
- 4 **Vilstrup H**, Amodio P, Bajaj J, Cordoba J, Ferenci P, Mullen KD, Weissenborn K, Wong P. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. *Hepatology* 2014; **60**: 715-735 [PMID: 25042402 DOI: 10.1002/hep.27210]
- 5 **Tandon P**, Ney M, Irwin I, Ma MM, Gramlich L, Bain VG, Esfandiari N, Baracos V, Montano-Loza AJ, Myers RP. Severe muscle depletion in patients on the liver transplant wait list: its prevalence and independent prognostic value. *Liver Transpl* 2012; **18**: 1209-1216 [PMID: 22740290 DOI: 10.1002/lt.23495]
- 6 **Alberino F**, Gatta A, Amodio P, Merkel C, Di Pascoli L, Boffo G, Caregaro L. Nutrition and survival in patients with liver cirrhosis. *Nutrition* 2001; **17**: 445-450 [PMID: 11399401 DOI: 10.1016/S0899-9007(01)00521-4]
- 7 **Barbosa-Silva MC**, Barros AJ. Bioelectrical impedance analysis in clinical practice: a new perspective on its use beyond body composition equations. *Curr Opin Clin Nutr Metab Care* 2005; **8**: 311-317 [PMID: 15809535]
- 8 **Barbosa-Silva MC**, Barros AJ, Wang J, Heymsfield SB, Pierson RN. Bioelectrical impedance analysis: population reference values for phase angle by age and sex. *Am J Clin Nutr* 2005; **82**: 49-52 [PMID: 16002799]
- 9 **Cederholm T**, Bosaeus I, Barazzoni R, Bauer J, Van Gossum A, Klek S, Muscaritoli M, Nyulasi I, Ockenga J, Schneider SM, de van der Schueren MA, Singer P. Diagnostic criteria for malnutrition - An ESPEN Consensus Statement. *Clin Nutr* 2015; **34**: 335-340 [PMID: 25799486 DOI: 10.1016/j.clnu.2015.03.001]
- 10 **Periyalwar P**, Dasarathy S. Malnutrition in cirrhosis: contribution and consequences of sarcopenia on metabolic and clinical responses. *Clin Liver Dis* 2012; **16**: 95-131 [PMID: 22321468 DOI: 10.1016/j.cld.2011.12.009]
- 11 **Lochs H**, Plauth M. Liver cirrhosis: rationale and modalities for nutritional support--the European Society of Parenteral and Enteral Nutrition consensus and beyond. *Curr Opin Clin Nutr Metab Care* 1999; **2**: 345-349 [PMID: 10453318]
- 12 **Ma Z**, Zhang Y, Huet PM, Lee SS. Differential effects of jaundice and cirrhosis on beta-adrenoceptor signaling in three rat models of cirrhotic cardiomyopathy. *J Hepatol* 1999; **30**: 485-491 [PMID: 10190733]
- 13 **Sam J**, Nguyen GC. Protein-calorie malnutrition as a prognostic indicator of mortality among patients hospitalized with cirrhosis and portal hypertension. *Liver Int* 2009; **29**: 1396-1402 [PMID: 19602136 DOI: 10.1111/j.1478-3231.2009.02077.x]
- 14 **Mesejo A**, Juan M, Serrano A. [Liver cirrhosis and encephalopathy: clinical and metabolic consequences and nutritional support]. *Nutr Hosp* 2008; **23** Suppl 2: 8-18 [PMID: 18714406]
- 15 **Merli M**, Riggio O, Romiti A, Ariosto F, Mango L, Pinto G, Savioli M, Capocaccia L. Basal energy production rate and substrate use in stable cirrhotic patients. *Hepatology* 1990; **12**: 106-112 [PMID: 2373471 DOI: 10.1002/hep.1840120117]
- 16 **Port GZ**, Oliveira K, Soldera J, Tovo CV. Biochemical nutritional profile of liver cirrhosis patients with hepatocellular carcinoma. *Arq Gastroenterol* 2014; **51**: 10-15 [PMID: 24760057 DOI: 10.1590/S0004-28032014000100003]
- 17 **Lenz K**, Buder R, Kapun L, Voglmayr M. Treatment and management of ascites and hepatorenal syndrome: an update. *Therap Adv Gastroenterol* 2015; **8**: 83-100 [PMID: 25729433 DOI: 10.1177/1756283X14564673]
- 18 **Dasarathy S**. Consilience in sarcopenia of cirrhosis. *J Cachexia Sarcopenia Muscle* 2012; **3**: 225-237 [PMID: 22648736 DOI: 10.1007/s13539-012-0069-3]
- 19 **Montano-Loza AJ**, Meza-Junco J, Baracos VE, Prado CM, Ma M, Meeberg G, Beaumont C, Tandon P, Esfandiari N, Sawyer MB, Kneteman N. Severe muscle depletion predicts postoperative length of stay but is not associated with survival after liver transplantation. *Liver Transpl* 2014; **20**: 640-648 [PMID: 24678005 DOI: 10.1002/lt.23863]
- 20 **Montano-Loza AJ**. Clinical relevance of sarcopenia in patients with cirrhosis. *World J Gastroenterol* 2014; **20**: 8061-8071 [PMID: 25009378 DOI: 10.3748/wjg.v20.i25.8061]
- 21 **Merli M**, Riggio O, Dally L. Does malnutrition affect survival in cirrhosis? PINC (Policentrica Italiana Nutrizione Cirrosi). *Hepatology* 1996; **23**: 1041-1046 [PMID: 8621131 DOI: 10.1002/hep.510230516]
- 22 **Montano-Loza AJ**, Meza-Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, Sawyer MB. Muscle wasting is associated with mortality in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2012; **10**: 166-173, 173.e1 [PMID: 21893129 DOI: 10.1016/j.cgh.2011.08.028]
- 23 **Kim HY**, Jang JW. Sarcopenia in the prognosis of cirrhosis: Going beyond the MELD score. *World J Gastroenterol* 2015; **21**: 7637-7647 [PMID: 26167066 DOI: 10.3748/wjg.v21.i25.7637]
- 24 **Delmonico MJ**, Harris TB, Lee JS, Visser M, Nevitt M, Kritchevsky SB, Tylavsky FA, Newman AB. Alternative definitions of sarcopenia, lower extremity performance, and functional impairment with aging in older men and women. *J Am Geriatr Soc* 2007; **55**: 769-774 [PMID: 17493199 DOI: 10.1111/j.1532-5415.2007.01140.x]
- 25 **Goodpaster BH**, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, Simonsick EM, Tylavsky FA, Visser M, Newman AB. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. *J Gerontol A Biol Sci Med Sci* 2006; **61**: 1059-1064 [PMID: 17077199 DOI: 10.1093/gerona/61.10.1059]
- 26 **Cruz-Jentoft AJ**, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, Topinková E, Vandewoude M, Zamboni M. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* 2010; **39**: 412-423 [PMID: 20392703 DOI: 10.1093/ageing/afq034]
- 27 **Frisancho AR**. New norms of upper limb fat and muscle areas for assessment of nutritional status. *Am J Clin Nutr* 1981; **34**: 2540-2545 [PMID: 6975564]
- 28 **McCullough AJ**. Malnutrition in liver disease. *Liver Transpl* 2000; **6**: S85-S96 [PMID: 10915197 DOI: 10.1002/lt.500060516]
- 29 **Gottschall CB**, Alvares-da-Silva MR, Camargo AC, Burtett RM, da Silveira TR. [Nutritional assessment in patients with cirrhosis: the use of indirect calorimetry]. *Arq Gastroenterol* 2004; **41**: 220-224 [PMID: 15806264 DOI: 10.1590/S0004-28032004000400004]
- 30 **Finger TE**, Danilova V, Barrows J, Bartel DL, Vigers AJ, Stone L, Hellekant G, Kinnamon SC. ATP signaling is crucial for

- communication from taste buds to gustatory nerves. *Science* 2005; **310**: 1495-1499 [PMID: 16322458 DOI: 10.1126/science.1118435]
- 31 **Plauth M**, Merli M, Kondrup J, Weimann A, Ferenci P, Müller MJ. ESPEN guidelines for nutrition in liver disease and transplantation. *Clin Nutr* 1997; **16**: 43-55 [PMID: 16844569 DOI: 10.1016/S0261-5614(97)80022-2]
  - 32 **Tajika M**, Kato M, Mohri H, Miwa Y, Kato T, Ohnishi H, Moriawaki H. Prognostic value of energy metabolism in patients with viral liver cirrhosis. *Nutrition* 2002; **18**: 229-234 [PMID: 11882395 DOI: 10.1016/S0899-9007(01)00754-7]
  - 33 **Abbott WJ**, Thomson A, Steadman C, Gattton ML, Bothwell C, Kerlin P, Wall DR, Lynch SV. Child-Pugh class, nutritional indicators and early liver transplant outcomes. *Hepatogastroenterology* 2001; **48**: 823-827 [PMID: 11462932]
  - 34 **Fernandes SA**, Bassani L, Nunes FF, Aydos ME, Alves AV, Marroni CA. Nutritional assessment in patients with cirrhosis. *Arq Gastroenterol* 2012; **49**: 19-27 [PMID: 22481682 DOI: 10.1590/S0004-28032012000100005]
  - 35 **Detsky AS**, Baker JP, Mendelson RA, Wolman SL, Wesson DE, Jeejeebhoy KN. Evaluating the accuracy of nutritional assessment techniques applied to hospitalized patients: methodology and comparisons. *JPEN J Parenter Enteral Nutr* 1984; **8**: 153-159 [PMID: 6538911 DOI: 10.1177/0148607184008002153]
  - 36 **Aidos MED**, Fernandes SA, Nunes FF, Bassani L, Leonhardt LR, Harter DL, Pivato B, Miranda D, Marroni CA. One-year follow-up of the nutritional status of patients undergoing liver transplantation. *Nutr Hosp* 2016; **33**: 8-13
  - 37 **Figueiredo FA**, Perez RM, Freitas MM, Kondo M. Comparison of three methods of nutritional assessment in liver cirrhosis: subjective global assessment, traditional nutritional parameters, and body composition analysis. *J Gastroenterol* 2006; **41**: 476-482 [PMID: 16799890 DOI: 10.1007/s00535-006-1794-1]
  - 38 **Ritter L**, Gazzola J. [Nutritional evaluation of the cirrhotic patient: an objective, subjective or multicompartamental approach?]. *Arq Gastroenterol* 2006; **43**: 66-70 [PMID: 16699622 DOI: 10.1590/S0004-28032006000100016]
  - 39 **Hasse J**, Strong S, Gorman MA, Liepa G. Subjective global assessment: alternative nutrition-assessment technique for liver-transplant candidates. *Nutrition* 1993; **9**: 339-343 [PMID: 8400590]
  - 40 Nutritional status in cirrhosis. Italian Multicentre Cooperative Project on Nutrition in Liver Cirrhosis. *J Hepatol* 1994; **21**: 317-325 [PMID: 7836699]
  - 41 **Álvares-da-Silva MR**, Silveira TRD. Hand-grip strength or muscle mass in cirrhotic patients: who is the best? *Nutrition* 2006; **22**: 218-219 [DOI: 10.1016/j.nut.2005.06.001]
  - 42 **Álvares-da-Silva MR**, Gottschall CA, Pruineli RD, Pinto RD, Waechter FL, Cardoso F, Sampaio JA, Smith MM, Francisconi CFM, Pereira-Lima LM. Nutritional evaluation in liver transplantation [abstract]. *Hepatology* 1998; **28**: 746(A)
  - 43 **Oliveira DR**, Frangella VS. [Adductor pollicis muscle and hand grip strength: potential methods of nutritional assessment in outpatients with stroke]. *Einstein (Sao Paulo)* 2010; **8**: 467-472 [PMID: 26760331 DOI: 10.1590/S1679-45082010AO1763]
  - 44 **Bragnolo R**, Caporossi FS, Dock-Nascimento DB, de Aguiar-Nascimento JE. [Adductor pollicis muscle thickness: a fast and reliable method for nutritional assessment in surgical patients]. *Rev Col Bras Cir* 2009; **36**: 371-376 [PMID: 20069147 DOI: 10.1590/S0100-69912009000500003]
  - 45 **Lameu EB**, Gerude MF, Corrêa RC, Lima KA. Adductor pollicis muscle: a new anthropometric parameter. *Rev Hosp Clin Fac Med Sao Paulo* 2004; **59**: 57-62 [PMID: 15122418 DOI: 10.1590/S0041-87812004000200002]
  - 46 **Pereira RA**, Caetano AL, Cuppari L, Kamimura MA. Adductor pollicis muscle thickness as a predictor of handgrip strength in hemodialysis patients. *J Bras Nefrol* 2013; **35**: 177-184 [PMID: 24100736 DOI: 10.5935/0101-2800.20130029]
  - 47 **Kyle UG**, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gómez JM, Heitmann BL, Kent-Smith L, Melchior JC, Pirlich M, Scharfetter H, Schols AM, Pichard C. Bioelectrical impedance analysis--part I: review of principles and methods. *Clin Nutr* 2004; **23**: 1226-1243 [PMID: 15380917 DOI: 10.1016/j.clnu.2004.06.004]
  - 48 **Paiva SI**, Borges LR, Halpern-Silveira D, Assunção MC, Barros AJ, Gonzalez MC. Standardized phase angle from bioelectrical impedance analysis as prognostic factor for survival in patients with cancer. *Support Care Cancer* 2010; **19**: 187-192 [PMID: 20039074 DOI: 10.1007/s00520-009-0798-9]
  - 49 **Romeiro FG**, Augusti L. Nutritional assessment in cirrhotic patients with hepatic encephalopathy. *World J Hepatol* 2015; **7**: 2940-2954 [PMID: 26730273 DOI: 10.4254/wjh.v7.i30.2940]
  - 50 **Ellis KJ**. Human body composition: in vivo methods. *Physiol Rev* 2000; **80**: 649-680 [PMID: 10747204]
  - 51 **Baumgartner RN**, Heymsfield SB, Lichtman S, Wang J, Pierson RN. Body composition in elderly people: effect of criterion estimates on predictive equations. *Am J Clin Nutr* 1991; **53**: 1345-1353 [PMID: 2035461]
  - 52 **Máttar JA**. Application of total body bioimpedance to the critically ill patient. Brazilian Group for Bioimpedance Study. *New Horiz* 1996; **4**: 493-503 [PMID: 8968982]
  - 53 **Pirlich M**, Schütz T, Spachos T, Ertl S, Weiss ML, Lochs H, Plauth M. Bioelectrical impedance analysis is a useful bedside technique to assess malnutrition in cirrhotic patients with and without ascites. *Hepatology* 2000; **32**: 1208-1215 [PMID: 11093726 DOI: 10.1053/jhep.2000.20524]
  - 54 **Norman K**, Stobäus N, Pirlich M, Bosy-Westphal A. Bioelectrical phase angle and impedance vector analysis--clinical relevance and applicability of impedance parameters. *Clin Nutr* 2012; **31**: 854-861 [PMID: 22698802 DOI: 10.1016/j.clnu.2012.05.008]
  - 55 **Llames L**, Baldomero V, Iglesias ML, Rodot LP. [Values of the phase angle by bioelectrical impedance; nutritional status and prognostic value]. *Nutr Hosp* 2013; **28**: 286-295 [PMID: 23822677 DOI: 10.3305/nh.2013.28.2.6306]
  - 56 **Selberg O**, Selberg D. Norms and correlates of bioimpedance phase angle in healthy human subjects, hospitalized patients, and patients with liver cirrhosis. *Eur J Appl Physiol* 2002; **86**: 509-516 [PMID: 11944099 DOI: 10.1007/s00421-001-0570-4]
  - 57 **Peres WA**, Lento DF, Baluz K, Ramalho A. Phase angle as a nutritional evaluation tool in all stages of chronic liver disease. *Nutr Hosp* 2012; **27**: 2072-2078 [PMID: 23588459 DOI: 10.3305/nh.2012.27.6.6015]
  - 58 **Fernandes SA**, Gonzalez MC, Bassani L, Miranda D, Pivatto B, Harter DL, Marroni CA. Is the phase angle, a prognostic indicator for nutritional status in cirrhotic patients? *J Antivir Antiretrovir* 2013; **S3**: 1-4 [DOI: 10.4172/jaa.S3-004]
  - 59 **Ruiz-Margáin A**, Macías-Rodríguez RU, Duarte-Rojo A, Ríos-Torres SL, Espinosa-Cuevas Á, Torre A. Malnutrition assessed through phase angle and its relation to prognosis in patients with compensated liver cirrhosis: a prospective cohort study. *Dig Liver Dis* 2015; **47**: 309-314 [PMID: 25618555 DOI: 10.1016/j.dld.2014.12.015]
  - 60 **Wagner D**, Adunka C, Kniepeiss D, Jakoby E, Schaffellner S, Kandlbauer M, Fahrleitner-Pammer A, Roller RE, Kornprat P, Müller H, Iberer F, Tscheliessnigg KH. Serum albumin, subjective global assessment, body mass index and the bioimpedance analysis in the assessment of malnutrition in patients up to 15 years after liver transplantation. *Clin Transplant* 2011; **25**: E396-E400 [PMID: 21457329 DOI: 10.1111/j.1399-0012.2011.01442.x]

P- Reviewer: Celikbilek M, He JY, Xu R S- Editor: Kong JX

L- Editor: A E- Editor: Li D



Basic Study

## Potential role of killer immunoglobulin receptor genes among individuals vaccinated against hepatitis B virus in Lebanon

Nada M Melhem, Rami A Mahfouz, Khalil Kreidieh, Rabab Abdul-Khalik, Rolla El-Khatib, Reem Talhouk, Umayya Musharrafieh, Ghassan Hamadeh

Nada M Melhem, Khalil Kreidieh, Rolla El-Khatib, Medical Laboratory Sciences Program, Faculty of Health Sciences, American University of Beirut, Beirut 1107-2020, Lebanon

Rami A Mahfouz, Rabab Abdul-Khalik, Department of Pathology and Laboratory Medicine, Faculty of Medicine, American University of Beirut, Beirut 1107-2020, Lebanon

Reem Talhouk, Department of Health Management and Policy, Faculty of Health Sciences, American University of Beirut, Beirut 1107-2020, Lebanon

Umayya Musharrafieh, Ghassan Hamadeh, Department of Family Medicine, Faculty of Medicine, American University of Beirut, Beirut 1107-2020, Lebanon

**Author contributions:** Melhem NM designed the study, performed the analysis and wrote the paper; Mahfouz RA contributed to the analysis; Kreidieh K and Abdul-Khalik R performed the experiments; El-Khatib R, Talhouk R, Musharrafieh U and Hamadeh G helped in the recruitment of participants.

**Supported by** The University Review Board at the American University of Beirut, No. A88507; and the Lebanese National Council for Scientific Research, No. A522185.

**Institutional review board statement:** The study was reviewed and approved by the Institutional Review Board (IRB) of the American University of Beirut.

**Conflict-of-interest statement:** The authors declare no conflict of interest.

**Data sharing statement:** Not applicable.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this

work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Dr. Nada M Melhem, PhD, Associate Professor of Infectious Diseases and Microbiology, Medical Laboratory Sciences Program, Faculty of Health Sciences, American University of Beirut, 11-0236 Riad El Solh, Beirut 1107-2020, Lebanon. [melhemn@aub.edu.lb](mailto:melhemn@aub.edu.lb)

**Telephone:** +961-1-350000-4699

**Fax:** +961-1-744470

**Received:** February 16, 2016

**Peer-review started:** February 18, 2016

**First decision:** March 30, 2016

**Revised:** April 13, 2016

**Accepted:** July 11, 2016

**Article in press:** July 13, 2016

**Published online:** October 18, 2016

### Abstract

#### AIM

To explore the role of killer immunoglobulin receptor (*KIR*) genes in responsiveness or non-responsiveness to vaccination against hepatitis B virus.

#### METHODS

We recruited 101 voluntary participants between March 2010 and December 2011. Sera samples from vaccinated and non-vaccinated participants were tested for the presence of anti-HBs antibodies as a measure of protection against hepatitis B, hepatitis B surface antigen and hepatitis B core antibody as indicators of



infection by enzyme-linked immunosorbent assay. *KIR* gene frequencies were determined by polymerase chain reaction.

## RESULTS

Sera samples from 99 participants were tested for the levels of anti-HBs as an indicator of protection ( $\geq 10$  mIU/mL) following vaccination as defined by the World Health Organization international reference standard. Among the vaccinated participants, 47% (35/74) had anti-HBs titers above 100 mIU/mL, 22% (16/74) had anti-HBs ranging between 10-100 mIU/mL, and 20% (15/74) had values of less than 10 mIU/mL. We report the lack of significant association between the number of vaccine dosages and the titer of antibodies among our vaccinated participants. The inhibitory KIR2DL1, KIR2DL4, KIR3DL1, KIR3DL2, and KIR3DL3 were detected in more than 95%, whereas KIR2DL2, KIR2DL3, KIR2DL5 (KIR2DL5A and KIR2DL5B) were expressed in 56%, 84% and 42% (25% and 29%) of participants, respectively. The observed frequency of the activating *KIR* genes ranged between 35% and 55% except for KIR2DS4, detected in 95% of the study participants (40.6% 2DS4\*001/002; 82.2% 2DS4\*003/007). KIR2DP1 pseudogene was detected in 99% of our participants, whereas KIR3DP1\*001/02/04 and KIR3DP1\*003 had frequencies of 17% and 100%, respectively. No association between the frequency of *KIR* genes and anti-HBs antibodies was detected. When we compared the frequency of *KIR* genes between vaccinated individuals with protective antibodies titers and those who lost their protective antibody levels, we did not detect a significant difference. KIR2DL5B was significantly different among different groups of vaccinated participants (group I > 100 mIU/mL, group II 10-100 mIU/mL, group III < 10 mIU/mL and group IV with undetectable levels of protective antibodies).

## CONCLUSION

To our knowledge, this is the first study screening for the possible role of *KIR* genes among individuals vaccinated against hepatitis B virus (HBV). Our results can be used to design larger studies to better understand the role of *KIR* genes in protection against or susceptibility to HBV post vaccination.

**Key words:** Hepatitis B virus; Killer immunoglobulin receptors; Hepatitis B vaccine; Lebanon; Natural killer cells

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Currently, there are no data supporting the use of booster doses of hepatitis B vaccine among immuno-competent individuals responding to a complete primary vaccination regimen. Importantly, 5%-10% of healthy adults do not generate protective levels of antibodies and are hence considered non-responders. This study aims to explore the role of killer immunoglobulin receptor genes in responsiveness or non-responsiveness

to vaccination against hepatitis B virus.

Melhem NM, Mahfouz RA, Kreidieh K, Abdul-Khalik R, El-Khatib R, Talhouk R, Musharrafieh U, Hamadeh G. Potential role of killer immunoglobulin receptor genes among individuals vaccinated against hepatitis B virus in Lebanon. *World J Hepatol* 2016; 8(29): 1212-1221 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i29/1212.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i29.1212>

## INTRODUCTION

Infection with hepatitis B virus (HBV) results in a spectrum of clinical outcomes ranging from acute hepatitis to end-stage liver disease and hepatocellular carcinoma<sup>[1]</sup> with an estimated lifetime risk of 25%-40%<sup>[2]</sup>. Booster studies suggest that memory begins to decline 15 years following vaccination among adolescents vaccinated in infancy<sup>[3-6]</sup>. Other studies suggest the persistence of immune memory for 20 years or longer<sup>[7-9]</sup>. Currently, there are no data supporting the use of booster doses of hepatitis B vaccine among immuno-competent individuals responding to a complete primary vaccination regimen (3 doses). Importantly, 5%-10% of healthy adults do not generate protective levels of antibodies and are hence considered non-responders<sup>[10]</sup>. Consequently, long-term protection is still debatable<sup>[11]</sup> and not linked to genetic factors.

Natural killer (NK) cells are known to induce antiviral and antitumor immunity via production of pro-inflammatory cytokines and lysis of infected or transformed cells<sup>[12]</sup>. Killer immunoglobulin receptor (*KIR*) genes encode receptors expressed on NK cells. Based on the gene content, two groups of *KIR* haplotypes are known in humans: A and B. Haplotype A encodes inhibitory receptors and consists of nine genes (3DL3, 2DL3, 2DP1, 2DL1, 3DP1, 2DL4, 3DL1, one activating (2DS4), 3DL2, and 2DL5) whereas haplotype B carries a variety of gene combinations and encodes more activating receptors as compared to haplotype A (3DL3, 2DS2, 2DL2, 2DL5B (inhibitory) 2DS3, 2DP1, 2DL1, 3DP1, 2DL4, 3DS1, 2DL5A (inhibitory), 2DS5, 2DS1, and 3DL2)<sup>[13]</sup>.

KIR3DS1- and KIR3DL1-expressing NK cells were reported to expand in acute and chronic human immunodeficiency virus (HIV)-1 infection, respectively<sup>[14]</sup>. Similarly, reports suggest that KIR2DL2 and/or KIR2DL3 along with their ligand human leukocyte antigen (HLA)-C1 are associated with severe influenza infection; in addition, the frequency of KIR3DS1, KIR2DS5 and KIR2DL5 was also related to the severity of the disease<sup>[15]</sup>. KIR2DS2 and KIR2DS3 were found to be associated with susceptibility to chronic hepatitis B infection, whereas KIR2DS1, KIR3DS1 and KIR2DL5 may act as protective genes leading to viral clearance among the Chinese Han population<sup>[16]</sup>. A difference between the frequency of

different *KIR* haplotypes among chronically infected individuals and those spontaneously recovering from HBV infection was also demonstrated in this population<sup>[17]</sup>. Recently, the rates of KIR2DL3 and 3DS1 were reported to be higher in healthy Turkish individuals as compared to patients with chronic HBV and those with spontaneous remission<sup>[18]</sup>; authors suggested the possible role of these genes in protection against HBV infection. In addition, genetic factors have been reported to play a role in the regulation of post-vaccine immune responses<sup>[19]</sup>. This was observed with antibody responses to a number of vaccine antigens including hepatitis B.

It is clear that the interaction between KIRs and their corresponding HLA ligands is implicated in differential responses to HIV, hepatitis C virus (HCV) and HBV as well as other disease conditions<sup>[20-23]</sup>. Immune responses, like many biological responses, are characterized by a wide range of variation between individuals. This has been described following natural infection or in response to vaccination<sup>[24]</sup>. HLA, cytokines, toll-like receptors and related gene variants have been associated with a variety of immune responses following vaccinations. Recently, single-nucleotide polymorphism associations were described to be involved in innate and adaptive immune response regulation following measles and rubella vaccinations<sup>[25,26]</sup>. Similarly, a difference in gene expression was also reported between high and low responders to smallpox vaccine<sup>[27]</sup>. The fact that antibody response to hepatitis B vaccine is non-protective in up to 10% of individuals<sup>[10]</sup>, and that a genetic basis to non-responsiveness is reported<sup>[19,24]</sup>, prompted us to explore the role of *KIR* genes in response to hepatitis B vaccine in a cohort of healthy vaccinated Lebanese adults.

## MATERIALS AND METHODS

### Study participants and samples

Human subject approval was obtained for this study from the institutional review board of the American University of Beirut and all the methods were carried out in accordance with the approved ethical guidelines. A written informed consent was signed by the study subjects before participation in the study. A data collection form was administered to the study participants ( $\geq 18$  years old) to collect demographic information, data related to exposure and risk behavior information. One hundred and one subjects were recruited during the time period March 2010–December 2011. Subjects were excluded if they had a prior or current history of HCV, HIV-1, renal disease or cancer. Children or adolescents of HBV carrier mothers and vaccinated in infancy were not included in the study. Blood was drawn from the study participants and peripheral blood mononuclear cells<sup>[28]</sup> and sera were collected and stored in liquid nitrogen and at  $-80^{\circ}\text{C}$ , respectively. DNA was extracted from whole blood of the study participants using the QIAamp DNA Blood Midikit (Qiagen, Germany), as per manufacturer's

instructions. The integrity of the purified DNA was checked by gel electrophoresis and storage was at  $-20^{\circ}\text{C}$ .

### Enzyme-linked immunosorbent assay

While we recruited 101 voluntary participants, sera samples of 99 HBV vaccinated and non-vaccinated study participants were tested in duplicate for the presence of anti-HBs antibodies as a measure of protection against hepatitis B, hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (anti-HBc) as indicators of infection. We did not have enough sera to use in the analysis for two of our study participants. The Monolisa HBsAg ULTRA, anti-HBs PLUS and anti-HBc PLUS assays (BIO-RAD, France) were used as per manufacturer's instructions, respectively. Anti-HBs antibodies were measured in mIU/mL and levels  $\geq 10$  mIU/mL were indicative of post-vaccination protection<sup>[29,30]</sup>.

### KIR genotyping

The polymerase chain reaction (PCR)-based *KIR* genotyping SSP Kit (Invitrogen, Brown Deer, WI, United States) was used to detect the presence and absence of *KIR* genes, as per manufacturer's instructions. Briefly, 25  $\mu\text{L}$  of DNA was used along with the primer sets to amplify the alleles described by the World Health Organization (WHO) international nomenclature committee (<http://www.ebi.ac.uk/ipd/kir/>). All amplifications were performed using PX2 thermocycler (ThermoHybrid, United Kingdom) programmed with a 1-min denaturation step at  $95^{\circ}\text{C}$ , followed by 30 cycles of  $94^{\circ}\text{C}$  for 20 s,  $63^{\circ}\text{C}$  for 20 s, and  $72^{\circ}\text{C}$  for 90 s and finally  $4^{\circ}\text{C}$  in the thermal cycler. PCR products were gel-purified and visualized under UV transillumination (Sigma, California, United States)<sup>[31]</sup>. The presence and absence of the following gene loci and variants were tested: 2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B, 2DS1, 2DS2, 2DS3, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, and 3DP1. The variants of the KIR3DP1 pseudogene, KIR3DP\*001/002/004 and KIR3DP1\*003 were also detected in addition to KIR2DS4 variants: 2DS4\*001/002 and 2DS4\*003/007. The frequency of *KIR* was calculated by direct count of the observed phenotype and referred to as observed frequency (OF). In addition, the estimated *KIR* gene frequency (KF) for the putative loci was calculated using the following formula:  $\text{KF} = 1 - \sqrt{1 - \text{OF}}$  based on the assumption of Hardy-Weinberg equilibrium<sup>[32]</sup>. The frequencies of haplotype A and B were calculated using the following formula: haplotype A =  $(2n_{AA} + n_{AB})/2n$  and haplotype B =  $(2n_{BB} + n_{AB})/2n$ , where  $n_{AA}$ ,  $n_{AB}$  and  $n_{BB}$  are the numbers of individuals with haplotype group AA, AB and BB, respectively and  $n$  is the total number of individuals<sup>[33]</sup>.

### Statistical analysis

SPSS 19 was used for statistical analyses. We compared vaccinated and non-vaccinated subjects for each of the *KIR* polymorphisms using  $\chi^2$  and Fisher-exact test (FET) and reported the odds ratio and 95%CI. Similar analyses

**Table 1** Demographics and characteristics of participants

	<i>n</i>	%
Gender ( <i>n</i> = 101)		
Male	39	38.6
Female	62	61.4
Age (yr) ( <i>n</i> = 95)		
19-29	38	40.0
30-39	19	20.0
40-49	17	17.9
50-59	11	11.6
60-69	6	6.3
70-79	2	2.1
80-89	2	2.1
Education ( <i>n</i> = 99)		
Illiterate	3	3.0
Primary School Education	3	3.0
Secondary School Graduate	9	9.1
High School Education	10	10.1
University Undergraduate Level	50	50.5
Others	24	24.2
Occupation ( <i>n</i> = 99)		
Student	15	15.2
Employed	74	74.7
Unemployed	7	7.1
Retired	3	3

were conducted for the comparison of protected and non-protected subjects within the vaccinated group. We also examined the relationship between genotypes and the presence or absence of *KIR* genes and the levels of anti-HBs and *KIR* genes among the vaccinated subjects using  $\chi^2$  and FET; for these comparisons, post-hoc tests were conducted only if the omnibus test was significant. We corrected for multiple comparisons for post-hoc tests using Bonferroni correction.

## RESULTS

### Characteristics of study participants

One hundred and one subjects were recruited between March 2010 and December 2011; 39% of the study participants were males and 61% were females. The majority of our study participants were 19-29 years old (40%). Table 1 summarizes the characteristics of the study participants. The majority (75%) held a university undergraduate degree or higher and was employed. During recruitment and when participants were asked about their vaccine status, 50% of our voluntary participants self-reported that they were vaccinated against HBV whereas 25% thought they were not vaccinated and 26% did not know their vaccine status.

In an attempt to confirm the vaccination status of our study participants, sera samples from 99 participants were tested for the levels of anti-HBs as an indicator of protection ( $\geq 10$  mIU/mL) as defined by the WHO international reference standard<sup>[30,34]</sup>. This is especially due to the lack of documented dosages of hepatitis B vaccine for many of the study participants as well as lack of knowledge of the vaccination status of many of the

study participants. In the subsequent analyses, data on these 99 voluntary subjects are reported; participants with anti-HBs antibodies  $\geq 10$  mIU/mL will be considered vaccinated against hepatitis B. We also tested the sera samples for anti-HBc as a marker of previous infection and for HBsAg, a marker associated with recent exposure to HBV. All our participants were negative for HBsAg. Among the voluntary participants, 74/99 (75%) were vaccinated against hepatitis B as judged by the detection of anti-HBs titers; whereas 25% (25/99) were classified as non-vaccinated against hepatitis B. Among the vaccinated participants, 47% (35/74) had anti-HBs titers above 100 mIU/mL, 22% (16/74) had anti-HBs ranging between 10-100 mIU/mL, and 20% (15/74) had values of less than 10 mIU/mL. The time of vaccination (when available) ranged between the years 1999 and 2011, with some participants receiving 2 doses and others receiving 3 or more doses. When we tested for an association between the age groups of our study participants (19-29, 30-39, 40-49, 50-59, 60-69, 70-79 and 80-89) and the concentration of anti-HBs antibodies among vaccinated subjects, a *P* value of 0.047 was detected (FET). Nine percent (7/74) of the vaccinated participants had undetectable levels of protective antibodies. Five out of 7 (71%) of the former group were health-care workers; the latter are expected to be continuously monitored for protection against HBV due to the nature of their work. Importantly, anti-HBc was positive in 3% (3/99) of our study participants presenting with anti-HBs levels ranging between 110-1000 mIU/mL. This is associated with protection as a result of natural infection. One of these participants is a vaccinated female nurse, whereas the other 2 are non-vaccinated and are not in the health care profession. None of the anti-HBc positive participants were HBsAg positive.

### KIR genotypes and genes frequencies

We next determined the *KIR* genotypes among the study participants: 44%, 40% and 16% were carriers of AA, AB and BB genotypes, respectively, with a 1.77 A to B ratio. The genotype was classified as B if any of the following genes was detected: 2DL2, 2DL5, 3DS1, 2DS1, 2DS2, 2DS3 and 2DS5. If none of these was detected, the genotype was considered as AA. Similarly, if none of the A haplotypes was detected, the genotype was classified as BB; 77%, 75% and 69% of the AA, AB and BB carriers were vaccinated, respectively. There was no significant difference between the expression of AA, AB and BB among vaccinated and non-vaccinated participants (FET, *P* = 0.784). Similarly, we did not detect any significant difference when we compared the frequencies of *KIR* genotypes among the vaccinated with anti-HBs levels less than 10, 10-100 and above 100 mIU/mL. We did not detect any difference between the frequencies of *KIR* genotypes among vaccinated participants with protective and non-protective levels of anti-HBs (FET, *P* = 0.865).

**Table 2** The observed and estimated killer immunoglobulin receptor gene frequencies in the study participants

	Inhibitory <i>KIR</i>								Non-inhibitory <i>KIR</i>						Pseudogene		
	2DL1	2DL2	2DL3	2DL4	2DL5	3DL1	3DL2	3DL3	2DS1	2DS2	2DS3	2DS4	2DS5	3DS1	2DP1	3DP*001/002/004	3DP1*003
OF	99	56	84	100	42	96	100	100	40.6	55.4	46.5	95	34.7	41.6	99	17	100
KLF	0.9	0.34	0.6	1	0.24	0.8	1	1	0.23	0.33	0.27	0.78	0.19	0.24	0.9	1	1

2DL5A, 24.8%; 2DL5B, 28.7%; 2DS4\*001/\*002, 40.6%; 2DS4\*003/007, 82.2%. KIR: Killer immunoglobulin receptor; OF: Observed frequency calculated by direct counting; KLF: Gene frequency calculated using the formula  $1 - \sqrt[3]{1 - \text{OF}}$ .

**Table 3** Killer immunoglobulin receptor gene frequencies among study participants vaccinated against hepatitis B virus (protected and non-protected by hepatitis B vaccine) *n* (%)

<i>KIR</i> genes	Anti-HBsAg < 10 mIU/mL ( <i>n</i> = 22)	Anti-HBsAg ≥ 10 mIU/mL ( <i>n</i> = 52)	Test <sup>1</sup>	OR (95%CI)	<i>P</i> value
2DL2	14 (63.60)	27 (51.90)	0.858	0.62 (0.22-1.72)	0.446
2DL3	18 (81.80)	45 (86.40)	FET	1.43 (0.37-5.48)	0.723
2DL5A	8 (36.40)	13 (25.00)	0.982	0.58 (0.20-1.70)	0.4
2DL5B	5 (22.70)	19 (36.50)	1.346	1.97 (0.62-6.16)	0.289
2DS1	11 (50.00)	19 (36.50)	1.162	0.58 (0.21-1.58)	0.31
2DS2	14 (63.60)	26 (50.00)	1.157	0.57 (0.21-1.59)	0.318
2DS3	9 (40.90)	23 (44.20)	0.069	1.15 (0.42-3.15)	0.804
2DS4*001/002	8 (36.40)	20 (38.40)	0.029	1.09 (0.39-3.072)	1.00
2DS4*003/007	19 (86.40)	41 (78.85)	FET	0.59 (0.15-2.36)	0.534
2DS5	10 (45.50)	17 (32.60)	1.087	0.58 (0.21-1.62)	0.428
3DS1	11 (50.00)	21 (40.40)	0.582	0.68 (0.25-1.85)	0.608
3DP*001/002/004	6 (27.30)	9 (17.30)	FET	0.56 (0.17-1.82)	0.355

<sup>1</sup>The statistical test performed to compare the frequencies of *KIR* gene expression among vaccinated and non-vaccinated study participants where by FET refers to Fisher's exact test and the rest of the values represent the Pearson  $\chi^2$  value. We compared the frequencies of *KIR* genes with enough variability between study participants that were vaccinated against hepatitis B virus. The vaccinated participants were divided for this analysis into 2 groups depending on the level of anti-HBsAg. Anti-HBsAg < 10 mIU/mL, not protected against hepatitis B virus infection; anti-HBsAg ≥ 10 mIU/mL, protected against hepatitis B virus infection as a result of vaccination. KIR: Killer immunoglobulin receptor; FET: Fisher-exact test; HBsAg: Hepatitis B surface antigen. OR: Odds ratio.

We divided the *KIR* genes expressed in our study participants into inhibitory, non-inhibitory (or activating) and those encoding inhibitory and activating signals, as previously described<sup>[13]</sup>. The inhibitory KIR2DL1, KIR2DL4, KIR3DL1, KIR3DL2, and KIR3DL were detected in more than 95%, whereas KIR2DL2, KIR2DL3, KIR2DL5 (KIR2DL5A and KIR2DL5B) were expressed in 56%, 84% and 42% (25% and 29%) of participants, respectively (Table 2). The OF of the activating *KIR* genes ranged between 35% and 55% except for KIR2DS4, detected in 95% of the study participants (40.6% 2DS4\*001/002; 82.2% 2DS4\*003/007). KIR2DP1 pseudogene was detected in 99% of our participants, whereas KIR3DP\*001/02/04 and KIR3DP1\*003 had frequencies of 17% and 100%, respectively. The corresponding estimated frequencies of each *KIR* gene followed the same trend or order as the OF data.

For this analysis and thereafter, we studied the genes with enough variability, particularly 2DL2, 2DL3, 2DL5 (2DL5A, 2DL5B), 2DS1, 2DS2, 2DS3, 2DS4 (and its variants), 2DS5, 3DS1 and the pseudogene 3DP1\*001/002/004 (Table 2). We report the lack of significant difference in the frequency of *KIR* genes among vaccinated and non-vaccinated participants (Table 3). Moreover, there was no significant difference in the frequency of these genes among participants protected against HBV and those that are not protected

against HBV, as judged by their level of anti-HBs antibodies (Table 4).

We performed similar analyses to determine the relationship between AA, AB, BB genotypes and the expression of *KIR* genes showing enough variability (Table 5). 2DL2 ( $\chi^2$ , *P* = 0.00), 2DL3 ( $\chi^2$ , *P* = 0.00), 2DS2 ( $\chi^2$ , *P* = 0.00), 2DS3 ( $\chi^2$ , *P* = 0.00), 2DL5B FET (*P* = 0.00), and 3DP1001/002/004 (FET, *P* = 0.006) were found to be significantly different between genotypes but not 2DS1 ( $\chi^2$ , *P* = 0.749), 2DL5A (FET, *P* = 0.782), 2DS4\*001/002 ( $\chi^2$ , *P* = 0.621), 2DS4\*003/007 (FET, *P* = 0.392) and 3DS1 ( $\chi^2$ , *P* = 0.948). For those genes showing significant differences among the AA, AB and BB genotypes, we computed post-hoc comparisons. These differences still hold significance between subgroups except for 2DL5B, 2DS2, 2DS3 and 3DP1\*001/002/004 between genotypes AB and BB.

#### ***KIR* gene frequencies among vaccinated and non-vaccinated participants**

To evaluate the role of *KIR* genes in protection against or susceptibility to HBV, we next compared the frequency of *KIR* genes among HBV-vaccinated and non-vaccinated participants (as judged by the level of anti-HBs antibodies). Depending on their anti-HBs levels that we tested for in this study, participants vaccinated against hepatitis B were divided into 4 groups: Group I >



**Table 4** Killer immunoglobulin receptor gene frequencies among study participants vaccinated against hepatitis B virus as compared to non-vaccinated subjects *n* (%)

KIR genes	Vaccinated ( <i>n</i> = 74)	Non-vaccinated ( <i>n</i> = 25)	Test <sup>1</sup>	OR (95%CI)	<i>P</i> value
2DL2	41 (55.40)	15 (60.00)	0.161	0.83 (0.33-2.082)	0.436
2DL3	63 (85.10)	20 (80.00)	0.364	1.43 (0.44-4.62)	0.374
2DL5A	21 (28.40)	4 (16.00)	1.517	2.08 (0.64-6.79)	0.168
2DL5B	24 (32.40)	5 (20.00)	1.395	1.92 (0.64-5.73)	0.178
2DS1	30 (40.50)	10 (40.00)	0.002	1.02 (0.41-2.58)	0.577
2DS2	40 (54.10)	15 (60.00)	0.268	0.78 (0.31-1.97)	0.39
2DS3	32 (43.20)	14 (56.00)	1.223	0.60 (0.24-1.49)	0.191
2DS4*001/002	28 (37.80)	11 (44.00)	0.297	0.77 (0.31-1.94)	0.376
2DS4*003/007	60 (81.10)	22 (88.00)	FET	0.58 (0.15-2.23)	0.324
2DS5	27 (36.50)	7 (28.00)	0.597	1.47 (0.55-3.99)	0.302
3DS1	32 (43.20)	9 (36.00)	0.246	1.35 (0.53-3.46)	0.401
3DP*001/002/004	15 (20.30)	2 (8.00)	FET	2.92 (1.62-13.80)	0.134

We compared the frequencies of *KIR* genes with enough variability between study participants that were vaccinated against hepatitis B virus and those that were not. <sup>1</sup>The statistical test performed to compare the frequencies of *KIR* gene expression among vaccinated and non-vaccinated study participants whereby FET refers to Fisher's exact test and the rest of the values represent the Pearson  $\chi^2$  value. KIR: Killer immunoglobulin receptor; FET: Fisher-exact test; OR: Odds ratio.

**Table 5** The relationship between AA, AB and BB genotypes and the expression of killer immunoglobulin receptor genes among the study participants *n* (%)

KIR genes	AA ( <i>n</i> = 44)	AB ( <i>n</i> = 41)	BB ( <i>n</i> = 16)	Test <sup>1</sup>	<i>P</i> value
2DL2	0 (0.00)	41 (100.00)	16 (100.00)	101	0.00 <sup>a</sup>
2DL3	44 (100.00)	41 (100.00)	0 (0.00)	101	0.00 <sup>a</sup>
2DL5A	10 (22.70)	10 (22.70)	5 (31.25)	FET	0.782
2DL5B	1 (2.27)	20 (24.39)	8 (50.00)	FET	0.00 <sup>a</sup>
2DS1	16 (36.30)	18 (43.90)	7 (43.75)	0.579	0.749
2DS2	0 (0.00)	40 (97.50)	16 (100.00)	97.051	0.00 <sup>a</sup>
2DS3	3 (6.80)	30 (73.17)	14 (87.50)	50.38	0.00 <sup>a</sup>
2DS4*001/002	16 (36.40)	19 (37.50)	6 (37.50)	0.952	0.621
2DS4*003/007	38 (86.40)	31 (75.60)	14 (87.50)	FET	0.392
2DS5	14 (31.80)	16 (39.00)	5 (31.30)	0.584	0.747
3DS1	18 (40.90)	18 (43.90)	6 (40.00)	0.107	0.948
3DP*001/002/004	2 (4.50)	10 (24.40)	5 (31.30)	FET	0.0006 <sup>a</sup>

<sup>1</sup>The statistical test performed to determine the relationship between AA, AB and BB genotypes and the expression of *KIR* genes among vaccinated and non-vaccinated study participants. FET refers to the Fisher's exact test and the rest of the values represent the Pearson  $\chi^2$  value. <sup>a</sup>*P* < 0.05, significant. KIR: Killer immunoglobulin receptor; FET: Fisher-exact test.

100 mIU/mL, group II 10-100 mIU/mL, group III < 10 mIU/mL and group IV with undetectable levels of protective antibodies. The frequency of only KIR2DL5B was significantly different among these categories (FET, *P* = 0.0263) (Table 6). When we performed post-hoc comparisons between these groups, we detected no significant difference in the expression of KIR2DL5B. These groups of vaccinated participants were also similar in relation to the expression of AA, AB and BB genotypes (FET, *P* = 0.669).

We identified 3 participants testing positive for anti-HBc with anti-HBs levels higher than 100 mIU/mL. Two out of three of these participants were non-vaccinated and thus we believe they are protected as a result of natural infection. The third participant was vaccinated against HBV. Two out of three (66.7%) of these participants carry the AB genotype and one participant is AA positive. The three participants expressed 2DL1,

2DL3, 3DL1, 3DL2, 3DL3 (inhibitory), activating genes (2DS3, 2DS4\*001/002 variant) and both inhibitory and activating genes (2DL4, 2DP1 and 3DP1\*003). These subjects did not express 2DL5A or 3DP1\*001/002/004. We did not test for the presence of HBV DNA among these participants. We did not find any significant relationship between the genotype of these participants and the expression of *KIR* genes, or between the *KIR* genes and the susceptibility to natural or breakthrough infection.

## DISCUSSION

The administration of hepatitis B vaccine in infancy is 95% effective and correlates with long-term protection<sup>[8,35,36]</sup>. However, vaccine failure has been reported in 5% of hepatitis B-vaccinated persons; moreover, breakthrough infection has also been reported following vaccination with hepatitis B vaccine<sup>[37]</sup>. The increase

**Table 6** Killer immunoglobulin receptor gene expression and levels of anti-HBs among the vaccinated study participants *n* (%)

<i>KIR</i> genes	Group I <sup>1</sup> ( <i>n</i> = 36)	Group II <sup>2</sup> ( <i>n</i> = 16)	Group III <sup>3</sup> ( <i>n</i> = 15)	Group IV <sup>4</sup> ( <i>n</i> = 7)	Test <sup>5</sup>	<i>P</i> value
2DL2	17 (47.20)	10 (62.50)	11 (73.30)	3 (42.90)	FET	0.362
2DL3	31 (86.10)	14 (87.50)	12 (80.00)	6 (85.70)	FET	0.824
2DL5A	9 (25.00)	4 (25.00)	4 (26.70)	4 (57.10)	FET	0.987
2DL5B	9 (25.00)	10 (62.50)	4 (26.70)	1 (14.30)	FET	0.023 <sup>6</sup>
2DS1	11 (30.60)	8 (50.00)	7 (46.70)	4 (57.10)	FET	0.530
2DS2	16 (44.40)	10 (62.50)	11 (73.30)	3 (42.90)	FET	0.270
2DS3	12 (33.30)	11 (68.80)	8 (53.30)	1 (14.30)	5.01	0.060
2DS4*001/002	15 (41.70)	5 (31.30)	6 (40.00)	2 (28.60)	0.568	0.920
2DS4*003/007	28 (77.80)	13 (81.30)	13 (86.70)	6 (85.70)	FET	0.860
2DS5	11 (30.60)	6 (37.50)	6 (40.00)	3 (42.90)	0.846	0.850
3DS1	14 (38.90)	7 (43.80)	7 (46.70)	6 (85.70)	FET	0.960
2DL4	36 (100.00)	16 (100.00)	15 (100.00)	4 (57.10)	0.258	0.970
3DP*001/002/004	8 (22.20)	1 (6.30)	4 (26.70)	1 (14.30)	FET	0.420

<sup>1</sup>Group I: Vaccinated participants and anti-HBs titers > 100 mIU/mL; <sup>2</sup>Group II: Vaccinated participants and anti-HBs titers 10-100 mIU/mL; <sup>3</sup>Group III: Vaccinated participants and anti-HBs titers 0.1-9.99 mIU/mL; <sup>4</sup>Group IV: Vaccinated participants and anti-HBs titers = 0 mIU/mL; <sup>5</sup>The statistical test performed to compare the frequencies of *KIR* gene expression among the vaccinated study participants whereby FET refers to Fisher's exact test and the rest of the values represent the Pearson  $\chi^2$  value; <sup>6</sup>Significant difference of KIR2DL5B expression among vaccinated study participants ( $P < 0.05$ ). KIR: Killer immunoglobulin receptor; FET: Fisher-exact test.

in circulating NK cells, major players in the innate immune system and regulators of the virus-specific T cell responses through their cross-talk with dendritic cells and T cells<sup>[38-40]</sup>, was suggested to contribute to HBV viral control<sup>[41]</sup>. The impact of genetic regulation on immune responses following vaccinations has been previously reported<sup>[19,24]</sup>. This evidence prompted us to explore the potential role of KIR following hepatitis B vaccination. In Lebanon, hepatitis B vaccine is offered as part of the immunization program early in childhood as per the WHO guidelines. In this study, 69% of the vaccinated participants retained more than 10 mIU/mL of anti-HBs antibodies and hence are immune to HBV infection; whereas 30% are susceptible to the latter due to either undetectable levels of antibodies or levels below 10 mIU/mL. We do not have data on the time of vaccination of these participants to reflect on the duration of the retention or the loss of the immune response post-vaccination. We report the lack of significant association between the number of vaccine dosages (when vaccine dosage is available) and the titer of antibodies among vaccinated participants. Recent reports show that multiple immunizations against hepatitis B are inefficient at mounting antibody responses<sup>[42]</sup>, while others suggest that immunization against hepatitis in infancy is associated with a seroprotective response to a challenge dose of vaccine with extended duration of protection through adolescent years<sup>[43]</sup>. We cannot suggest similar trends from our results due to the lack of data on the time of vaccination, the age at vaccination, as well as the number of dosages administered for many participants.

Anti-HBc antibodies, indicators of HBV infection, were detected in 3 participants (3%) in the absence of HBsAg, with one being a nurse suspected of being exposed to HBV at the work place. This might suggest a "breakthrough" infection occurring following vaccination against hepatitis B; this is suggested since health care

workers are regularly monitored for protective levels of anti-HBs antibodies. However, due to the lack of data on the timing of vaccination and/or infection of this participant, we cannot confirm whether exposure to HBV has occurred before or after vaccination. The other 2 participants are non-vaccinated and are protected with high levels of anti-HBs antibodies as a result of natural infection. We do not have data pertaining to the time of infection following vaccination; moreover, we did not perform HBV DNA testing. Health care workers with undetectable anti-HBsAg levels detected in our study are clearly susceptible to HBV infection and consequently in need of booster vaccination to induce an anamnestic response in order to prevent acute disease and carrier state.

While hepatitis B vaccine booster doses are not currently recommended following vaccination, a better understanding of the correlates of long-term immunity is needed. This is critical especially since several studies show that vaccines with anti-HBs levels of 10-99 mIU/mL achieved following primary vaccination are less likely to produce an anamnestic response following a booster HBV vaccine as compared to those with anti-HBs  $\geq 100$  mIU/mL<sup>[35,44]</sup>. NK cells play a major role in the innate immune system as first line of defense and in the regulation of the virus-specific T cell responses through their cross-talk with dendritic cells and T cells<sup>[38-40]</sup>. Moreover, NK cells are suggested to contribute to HBV control<sup>[41]</sup>. Our data show that genotypes with 11 *KIR* genes were most prevalent, with AA genotype being more frequent among the study participants. The inhibitory *KIR* genes were more frequent among our study participants than the activating genes, which is in agreement with a finding associated with A haplotype being present in higher numbers in inhibitory *KIR* genes<sup>[39]</sup>.

KIR2DL4, KIR3DL2, KIR3DL3 and KIR3DP1\*003 were present in every participant. This is expected since these

are framework genes. The frequency of KIR2DL5B is the only significantly different gene among the vaccinated participants with different anti-HBs antibodies titer. The role of KIR2DL5, expressed at frequencies ranging between 26% and 86% in all human populations, is not completely understood<sup>[45]</sup>. The ligand of KIR2DL5 is also still unknown.

A number of limitations exist, and these include the lack of data on the time of vaccination and corresponding age at time of vaccination of the study participants, and more importantly, the small sample size. Our sample size is powered to detect medium to large effect sizes when some of the effect sizes for group differences are small. However, medium to large effect sizes are those where group differences have more clinical significance, which we are powered to detect. Consequently, the clear impact that *KIR* genes have on susceptibility to acquiring hepatitis B or protection against the infection cannot be addressed in these small groups.

To our knowledge, this is the first study screening for the possible role of *KIR* genes among individuals vaccinated against HBV. While studies have shown the association between gene variants and immune responses to a variety of vaccines, little is known about the strength and the sustainability of antibody responses following vaccination against HBV in relation to expression of *KIR* genes. Our results are useful to design larger studies to better elucidate the role of KIR in susceptibility or long-term protection against HBV as well as other diseases.

## ACKNOWLEDGMENTS

We thank all our voluntary participants and Mr. Doudar for his help in recruitment of volunteers, as well as Ms. Sarah Shamra for technical assistance in the lab.

## COMMENTS

### Background

Killer immunoglobulin receptor (*KIR*) genes encode receptors expressed on the surface of natural killer cells. The literature has described the relationship between KIRs and differential responses in many disease conditions, specifically human immunodeficiency virus and hepatitis C virus. The authors thought to explore the role of *KIR* genes in response to hepatitis B vaccine in a cohort of Lebanese adults.

### Research frontiers

This study aims at elucidating the possible role of genetic factors such as KIRs in the regulation of post-vaccine immune responses specifically following hepatitis B vaccine.

### Applications

While the sample size is powered to detect medium to large effect sizes, the impact that KIR and HLA have on the susceptibility to acquiring hepatitis B virus (HBV) or protection against the infection cannot be addressed in our sample. Nevertheless, our results are useful to design larger studies to better elucidate the role of KIR in susceptibility or long-term protection against HBV and other diseases.

### Terminology

Two groups of *KIR* haplotypes are known in humans: A and B. Haplotype A

encodes inhibitory receptors and consists of nine genes [3DL3, 2DL3, 2DP1, 2DL1, 3DP1, 2DL4, 3DL1, one activating (2DS4), 3DL2 and 2DL5]. Haplotype B carries a variety of gene combinations and encodes more activating receptors as compared to haplotype A [3DL3, 2DS2, 2DL2, 2DL5B (inhibitory), 2DS3, 2DP1, 2DL1, 3DP1, 2DL4, 3DS1, 2DL5A (inhibitory), 2DS5, 2DS1, and 3DL2].

## Peer-review

This is an interesting study aiming to screen for a possible role of *KIR* gene expression and antibody response following hepatitis B vaccination. The major point of this manuscript is that there is no significant association between the frequency of *KIR* genes and anti-HBs antibodies detected. Although it is a negative result, it could be an indicant for understanding the role of *KIR* loci in response to HB vaccine.

## REFERENCES

- 1 **Rehermann B**, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* 2005; **5**: 215-229 [PMID: 15738952]
- 2 **Liaw YF**, Chu CM. Hepatitis B virus infection. *Lancet* 2009; **373**: 582-592 [PMID: 19217993 DOI: 10.1016/S0140-6736(09)60207-5]
- 3 **Lu CY**, Ni YH, Chiang BL, Chen PJ, Chang MH, Chang LY, Su IJ, Kuo HS, Huang LM, Chen DS, Lee CY. Humoral and cellular immune responses to a hepatitis B vaccine booster 15-18 years after neonatal immunization. *J Infect Dis* 2008; **197**: 1419-1426 [PMID: 18444799 DOI: 10.1086/587695]
- 4 **Hammitt LL**, Hennessy TW, Fiore AE, Zanis C, Hummel KB, Dunaway E, Bulkow L, McMahon BJ. Hepatitis B immunity in children vaccinated with recombinant hepatitis B vaccine beginning at birth: a follow-up study at 15 years. *Vaccine* 2007; **25**: 6958-6964 [PMID: 17714836]
- 5 **Bialek SR**, Bower WA, Novak R, Helgenberger L, Auerbach SB, Williams IT, Bell BP. Persistence of protection against hepatitis B virus infection among adolescents vaccinated with recombinant hepatitis B vaccine beginning at birth: a 15-year follow-up study. *Pediatr Infect Dis J* 2008; **27**: 881-885 [PMID: 18756185 DOI: 10.1097/INF.0b013e31817702ba]
- 6 **Chaves SS**, Fischer G, Groeger J, Patel PR, Thompson ND, Teshale EH, Stevenson K, Yano VM, Armstrong GL, Samandari T, Kamili S, Drobeniuc J, Hu DJ. Persistence of long-term immunity to hepatitis B among adolescents immunized at birth. *Vaccine* 2012; **30**: 1644-1649 [PMID: 22245310 DOI: 10.1016/j.vaccine.2011.12.106]
- 7 **But DY**, Lai CL, Lim WL, Fung J, Wong DK, Yuen MF. Twenty-two years follow-up of a prospective randomized trial of hepatitis B vaccines without booster dose in children: final report. *Vaccine* 2008; **26**: 6587-6591 [PMID: 18835318 DOI: 10.1016/j.vaccine.2008.09.034]
- 8 **Poovorawan Y**, Chongsrisawat V, Theamboonlers A, Bock HL, Leyssen M, Jacquet JM. Persistence of antibodies and immune memory to hepatitis B vaccine 20 years after infant vaccination in Thailand. *Vaccine* 2010; **28**: 730-736 [PMID: 19892043 DOI: 10.1016/j.vaccine.2009.10.074]
- 9 **McMahon BJ**, Dentinger CM, Bruden D, Zanis C, Peters H, Hurlburt D, Bulkow L, Fiore AE, Bell BP, Hennessy TW. Antibody levels and protection after hepatitis B vaccine: results of a 22-year follow-up study and response to a booster dose. *J Infect Dis* 2009; **200**: 1390-1396 [PMID: 19785526 DOI: 10.1086/606119]
- 10 **Lavanchy D**. Viral hepatitis: global goals for vaccination. *J Clin Virol* 2012; **55**: 296-302 [PMID: 22999800 DOI: 10.1016/j.jcv.2012.08.022]
- 11 **Schillie SF**, Murphy TV. Seroprotection after recombinant hepatitis B vaccination among newborn infants: a review. *Vaccine* 2013; **31**: 2506-2516 [PMID: 23257713 DOI: 10.1016/j.vaccine.2012.12.012]
- 12 **Terme M**, Ullrich E, Delahaye NF, Chaput N, Zitvogel L. Natural killer cell-directed therapies: moving from unexpected results to successful strategies. *Nat Immunol* 2008; **9**: 486-494 [PMID: 18425105 DOI: 10.1038/ni1580]
- 13 **Bashirova AA**, Thomas R, Carrington M. HLA/KIR restraint of HIV: surviving the fittest. *Annu Rev Immunol* 2011; **29**: 295-317

- [PMID: 21219175 DOI: 10.1146/annurev-immunol-031210-101332]
- 14 **Alter G**, Rihn S, Walter K, Nolting A, Martin M, Rosenberg ES, Miller JS, Carrington M, Altfeld M. HLA class I subtype-dependent expansion of KIR3DS1+ and KIR3DL1+ NK cells during acute human immunodeficiency virus type 1 infection. *J Virol* 2009; **83**: 6798-6805 [PMID: 19386717 DOI: 10.1128/JVI.00256-09]
- 15 **Jost S**, Altfeld M. Control of human viral infections by natural killer cells. *Annu Rev Immunol* 2013; **31**: 163-194 [PMID: 23298212 DOI: 10.1146/annurev-immunol-032712-100001]
- 16 **Zhi-ming L**, Yu-lian J, Zhao-lei F, Chun-xiao W, Zhen-fang D, Bing-chang Z, Yue-ran Z. Polymorphisms of killer cell immunoglobulin-like receptor gene: possible association with susceptibility to or clearance of hepatitis B virus infection in Chinese Han population. *Croat Med J* 2007; **48**: 800-806 [PMID: 18074414]
- 17 **Lu Z**, Zhang B, Chen S, Gai Z, Feng Z, Liu X, Liu Y, Wen X, Li L, Jiao Y, Ma C, Shao S, Cui X, Chen G, Li J, Zhao Y. Association of KIR genotypes and haplotypes with susceptibility to chronic hepatitis B virus infection in Chinese Han population. *Cell Mol Immunol* 2008; **5**: 457-463 [PMID: 19118512 DOI: 10.1038/cmi.2008.57]
- 18 **Kibar F**, Goruroglu Ozturk O, Ulu A, Erken E, Inal S, Dinkci S, Kurtaran B, Tasova Y, Aksu HS, Yaman A. Role of KIR genes and genotypes in susceptibility to or protection against hepatitis B virus infection in a Turkish cohort. *Med Sci Monit* 2014; **20**: 28-34 [PMID: 24407110 DOI: 10.12659/MSM.889893]
- 19 **Newport MJ**, Goetghebuer T, Weiss HA, Whittle H, Siegrist CA, Marchant A. Genetic regulation of immune responses to vaccines in early life. *Genes Immun* 2004; **5**: 122-129 [PMID: 14737096 DOI: 10.1038/sj.gene.6364051]
- 20 **Hirayasu K**, Ohashi J, Kashiwase K, Hananantachai H, Naka I, Ogawa A, Takanashi M, Satake M, Nakajima K, Parham P, Arase H, Tokunaga K, Patarapotikul J, Yabe T. Significant association of KIR2DL3-HLA-C1 combination with cerebral malaria and implications for co-evolution of KIR and HLA. *PLoS Pathog* 2012; **8**: e1002565 [PMID: 22412373 DOI: 10.1371/journal.ppat.1002565]
- 21 **Boyton RJ**, Altmann DM. Natural killer cells, killer immunoglobulin-like receptors and human leucocyte antigen class I in disease. *Clin Exp Immunol* 2007; **149**: 1-8 [PMID: 17521317 DOI: 10.1111/j.1365-2249.2007.03424.x]
- 22 **Carrington M**, Wang S, Martin MP, Gao X, Schiffman M, Cheng J, Herrero R, Rodriguez AC, Kurman R, Mortel R, Schwartz P, Glass A, Hildesheim A. Hierarchy of resistance to cervical neoplasia mediated by combinations of killer immunoglobulin-like receptor and human leukocyte antigen loci. *J Exp Med* 2005; **201**: 1069-1075 [PMID: 15809352 DOI: 10.1084/jem.20042158]
- 23 **Jobim MR**, Jobim M, Salim PH, Portela P, Jobim LF, Leistner-Segal S, Bittelbrunn AC, Menke CH, Biazús JV, Roesler R, Schwartzmann G. Analysis of KIR gene frequencies and HLA class I genotypes in breast cancer and control group. *Hum Immunol* 2013; **74**: 1130-1133 [PMID: 23792055 DOI: 10.1016/j.humimm.2013.06.021]
- 24 **Newport MJ**. The genetic regulation of infant immune responses to vaccination. *Front Immunol* 2015; **6**: 18 [PMID: 25699041 DOI: 10.3389/fimmu.2015.00018]
- 25 **Ovsyannikova IG**, Salk HM, Larrabee BR, Pankratz VS, Poland GA. Single-nucleotide polymorphism associations in common with immune responses to measles and rubella vaccines. *Immunogenetics* 2014; **66**: 663-669 [PMID: 25139337 DOI: 10.1007/s00251-014-0796-z]
- 26 **Haralambieva IH**, Oberg AL, Ovsyannikova IG, Kennedy RB, Grill DE, Middha S, Bot BM, Wang VW, Smith DI, Jacobson RM, Poland GA. Genome-wide characterization of transcriptional patterns in high and low antibody responders to rubella vaccination. *PLoS One* 2013; **8**: e62149 [PMID: 23658707 DOI: 10.1371/journal.pone.0062149]
- 27 **Kennedy RB**, Oberg AL, Ovsyannikova IG, Haralambieva IH, Grill D, Poland GA. Transcriptomic profiles of high and low antibody responders to smallpox vaccine. *Genes Immun* 2013; **14**: 277-285 [PMID: 23594957 DOI: 10.1038/gene.2013.14]
- 28 **Melhem NM**, Liu XD, Boczkowski D, Gilboa E, Barratt-Boyes SM. Robust CD4+ and CD8+ T cell responses to SIV using mRNA-transfected DC expressing autologous viral Ag. *Eur J Immunol* 2007; **37**: 2164-2173 [PMID: 17615585 DOI: 10.1002/eji.200636782]
- 29 **U.S. Public Health Service**. Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis. *MMWR Recomm Rep* 2001; **50**: 1-52 [PMID: 11442229]
- 30 **WHO**. Hepatitis B: Surveillance and Control. Available from: URL: <http://www.who.int/csr/disease/hepatitis/whodocscsrlyo20022/en/index4.html>
- 31 **Mahfouz R**, Rayes R, Mahfouz Z, Bazarbachi A, Zaatar G. Distribution of killer cell immunoglobulin-like receptors genotypes in the Lebanese population. *Tissue Antigens* 2006; **68**: 66-71 [PMID: 16774542 DOI: 10.1111/j.1399-0039.2006.00605.x]
- 32 **Denis L**, Sivula J, Gourraud PA, Kerdudou N, Chout R, Ricard C, Moisan JP, Gagne K, Partanen J, Bignon JD. Genetic diversity of KIR natural killer cell markers in populations from France, Guadeloupe, Finland, Senegal and Réunion. *Tissue Antigens* 2005; **66**: 267-276 [PMID: 16185321 DOI: 10.1111/j.1399-0039.2005.00473.x]
- 33 **Zhen J**, Wang D, He L, Zou H, Xu Y, Gao S, Yang B, Deng Z. Genetic profile of KIR and HLA in southern Chinese Han population. *Hum Immunol* 2014; **75**: 59-64 [PMID: 24055695 DOI: 10.1016/j.humimm.2013.09.006]
- 34 **Schillie S**, Murphy TV, Sawyer M, Ly K, Hughes E, Jiles R, de Perio MA, Reilly M, Byrd K, Ward JW. CDC guidance for evaluating health-care personnel for hepatitis B virus protection and for administering postexposure management. *MMWR Recomm Rep* 2013; **62**: 1-19 [PMID: 24352112]
- 35 **van der Sande MA**, Waight P, Mendy M, Rayco-Solon P, Hutt P, Fulford T, Doherty C, McConkey SJ, Jeffries D, Hall AJ, Whittle HC. Long-term protection against carriage of hepatitis B virus after infant vaccination. *J Infect Dis* 2006; **193**: 1528-1535 [PMID: 16652281 DOI: 10.1086/503433]
- 36 **van der Sande MA**, Waight PA, Mendy M, Zaman S, Kaye S, Sam O, Kahn A, Jeffries D, Akum AA, Hall AJ, Bah E, McConkey SJ, Hainaut P, Whittle HC. Long-term protection against HBV chronic carriage of Gambian adolescents vaccinated in infancy and immune response in HBV booster trial in adolescence. *PLoS One* 2007; **2**: e753 [PMID: 17710152 DOI: 10.1371/journal.pone.0000753]
- 37 **Zuckerman JN**. Protective efficacy, immunotherapeutic potential, and safety of hepatitis B vaccines. *J Med Virol* 2006; **78**: 169-177 [PMID: 16372285 DOI: 10.1002/jmv.20524]
- 38 **Zingoni A**, Sornasse T, Cocks BG, Tanaka Y, Santoni A, Lanier LL. Cross-talk between activated human NK cells and CD4+ T cells via OX40-OX40 ligand interactions. *J Immunol* 2004; **173**: 3716-3724 [PMID: 15356117 DOI: 10.4049/jimmunol.173.6.3716]
- 39 **Lanier LL**. NK cell recognition. *Annu Rev Immunol* 2005; **23**: 225-274 [PMID: 15771571]
- 40 **Vivier E**, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol* 2008; **9**: 503-510 [PMID: 18425107 DOI: 10.1038/ni1582]
- 41 **Maini MK**, Peppas D. NK cells: a double-edged sword in chronic hepatitis B virus infection. *Front Immunol* 2013; **4**: 57 [PMID: 23459859 DOI: 10.3389/fimmu.2013.00057]
- 42 **Zaffina S**, Marcellini V, Santoro AP, Scarsella M, Camisa V, Vinci MR, Musolino AM, Nicolosi L, Rosado MM, Carsetti R. Repeated vaccinations do not improve specific immune defenses against Hepatitis B in non-responder health care workers. *Vaccine* 2014; **32**: 6902-6910 [PMID: 25444815 DOI: 10.1016/j.vaccine.2014.10.066]
- 43 **Middleman AB**, Baker CJ, Kozinetz CA, Kamili S, Nguyen C, Hu DJ, Spradling PR. Duration of protection after infant hepatitis B vaccination series. *Pediatrics* 2014; **133**: e1500-e1507 [PMID: 24843060 DOI: 10.1542/peds.2013-2940]
- 44 **Su FH**, Cheng SH, Li CY, Chen JD, Hsiao CY, Chien CC, Yang YC, Hung HH, Chu FY. Hepatitis B seroprevalence and anamnestic response amongst Taiwanese young adults with full vaccination in



infancy, 20 years subsequent to national hepatitis B vaccination.  
*Vaccine* 2007; **25**: 8085-8090 [PMID: 17920732]

45 **Cisneros E**, Moraru M, Gómez-Lozano N, López-Botet M, Vilches

C. KIR2DL5: An Orphan Inhibitory Receptor Displaying Complex  
Patterns of Polymorphism and Expression. *Front Immunol* 2012; **3**:  
289 [PMID: 23060877 DOI: 10.3389/fimmu.2012.00289]

**P- Reviewer:** Qu D, Saad K **S- Editor:** Gong ZM

**L- Editor:** Logan S **E- Editor:** Li D



## Basic Study

# Lycopene modulates cellular proliferation, glycolysis and hepatic ultrastructure during hepatocellular carcinoma

Prachi Gupta, Nisha Bhatia, Mohinder Pal Bansal, Ashwani Koul

Prachi Gupta, Nisha Bhatia, Mohinder Pal Bansal, Ashwani Koul, Department of Biophysics, Basic Medical Sciences Block II, Panjab University, Chandigarh 160014, India

Author contributions: All authors contributed to the manuscript.

Supported by University Grant Commission, New Delhi, No. 2060930310.

**Institutional review board statement:** The study was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University, Chandigarh (India) and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals (IAEC/284-295 at Sr. No. 48).

**Institutional animal care and use committee statement:** All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Panjab University, Chandigarh, India [IACUC protocol number: (IAEC/284-295 at Sr. No. 48)].

**Conflict-of-interest statement:** The authors declare that there are no conflicts of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Ashwani Koul, Professor, Department of Biophysics, Basic Medical Sciences Block II, Panjab University, South Campus, Sec-25, Chandigarh 160014, India. [drashwanikoul@yahoo.co.in](mailto:drashwanikoul@yahoo.co.in)  
Telephone: +91-172-2534119

Received: May 4, 2016

Peer-review started: May 5, 2016

First decision: June 6, 2016

Revised: June 20, 2016

Accepted: July 20, 2016

Article in press: July 22, 2016

Published online: October 18, 2016

## Abstract

### AIM

To investigate the effect of lycopene extracted from tomatoes (LycT) on ultrastructure, glycolytic enzymes, cell proliferation markers and hypoxia during N-Nitrosodiethylamine (NDEA)-induced hepatocarcinogenesis.

### METHODS

Female BALB/c mice were randomly divided into four groups: The Control, NDEA (200 mg NDEA/kg b.w. given i.p.), LycT (5 mg/kg b.w. given orally on alternate days) and LycT + NDEA group. The mRNA and protein expression of various cell proliferation markers (PCNA, Cyclin D1, and p21) were assessed by reverse transcription-polymerase chain reaction and enzyme linked immunosorbent assay, respectively. The ultrastructure of hepatic tissue was analyzed using scanning and transmission electron microscopy. The enzymatic activity of glycolytic enzymes was estimated using standardized protocols, while glucose-6-phosphate dehydrogenase activity level was estimated using a kit obtained from Reckon Diagnostic P. Ltd. (India).

### RESULTS

Uncontrolled proliferation in the liver of NDEA ( $P \leq 0.001$ ) mice was evident from the high expression of cell-proliferation associated genes (PCNA, Cyclin D1, and p21) when compared to control and LycT mice. In addition, enhanced activities of hexokinase, phosphoglucose isomerase, aldolase, glucose-6-phosphate

dehydrogenase and hypoxia-inducible factor-1 $\alpha$  were observed in NDEA mice as compared to control ( $P \leq 0.001$ ) and LycT ( $P \leq 0.001$ ) mice. The alterations in hepatic ultrastructure observed in the NDEA group correlated with the changes in the above parameters. LycT pre-treatment in NDEA-challenged mice ameliorated the investigated pathways disrupted by NDEA treatment. Moreover, hepatic electron micrographs from the LycT + NDEA group showed increased macrophages, apoptotic bodies and well-differentiated hepatocellular carcinoma (HCC) in comparison to undifferentiated HCC as observed in the NDEA treated group.

## CONCLUSION

This study demonstrates that dietary supplementation with LycT has a multidimensional role in preventing HCC development.

**Key words:** Hepatocellular carcinoma; Ultrastructure; Hypoxia; Cell proliferation; Lycopene; Glycolysis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The present study was designed to evaluate the chemopreventive role of lycopene extracted from tomatoes (LycT) against N-Nitrosodiethylamine-induced hepatocellular carcinoma (HCC). The findings suggested the mechanism underlying LycT-mediated chemoprevention of HCC.

Gupta P, Bhatia N, Bansal MP, Koul A. Lycopene modulates cellular proliferation, glycolysis and hepatic ultrastructure during hepatocellular carcinoma. *World J Hepatol* 2016; 8(29): 1222-1233 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i29/1222.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i29.1222>

## INTRODUCTION

The continuous rising trend in cancer worldwide necessitates potential action against this deadly disease. Cancer is a complex disease and requires attention in multiple directions to prevent its development. Nearly two-thirds of all cancer cases are linked to inadequate components in the diet, environmental exposure to pollutants and occupational exposure to toxic materials<sup>[1,2]</sup>. Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and is the second most common cause of cancer-related death<sup>[3]</sup>. N-Nitrosodiethylamine (NDEA) is a known potent environmental hepatic carcinogen and has been used as an initiator in several hepatic cancer models<sup>[4,5]</sup>. Besides direct exogenous exposure, humans are also exposed to endogenously produced nitrosamines<sup>[6]</sup>. NDEA is metabolized in the liver to its active ethyl radical metabolite and various other reactive metabolites, which are highly reactive towards DNA, proteins and lipids, thus exhibiting sequential cellular and molecular alterations leading to its hepatocarcinogenic effect<sup>[4,7-9]</sup>.

Natural and experimental chemical hepatocarcino-

genesis is accompanied by altered cellular redox status, altered cytochemical pathways, altered molecular phenomena, chromosomal instability and altered physiological environment in cells. Of the various carcinogenic insults, uncontrolled proliferation, dysregulated carbohydrate metabolism and hypoxia play very crucial roles in HCC development. Aerobic glycolysis and diversion to a biosynthetic pathway, *i.e.*, the pentose phosphate pathway are the metabolic hallmarks of carcinogenesis<sup>[10]</sup>. In recent years, interest in these pathways has been renewed in the tumor microenvironment which has a profound effect on core tumor metabolism. Hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) is involved in many compensatory pathways such as angiogenesis, glucose metabolism, survival and tumor development<sup>[11]</sup>. Although there have been advances in therapeutic approaches a complete cure is still unavailable. Dietary multi-targeted agents have attracted the attention of cancer biologists as these agents may provide a solution to this complex problem by targeting multiple targets simultaneously<sup>[12]</sup>. A large body of evidence has revealed an association between phytochemicals and a reduced risk of developing chronic diseases<sup>[13,14]</sup>.

Lycopene, a polyunsaturated hydrocarbon imparting red colour to various fruits is a nutritionally important carotenoid exhibiting beneficial health effects by virtue of its antioxidant activity with minimal side effects<sup>[15]</sup>. A large number of studies have shown an association between lycopene and a reduced risk of developing chronic diseases such as cancer, diabetes, cardiovascular disorders and degenerative diseases<sup>[15-18]</sup>. In addition to its antioxidant property, lycopene is known to modulate other non-oxidative pathways such as regulation of gap junction communication, the hormonal system, the immune system and the metabolic pathways of xenobiotics<sup>[16,19]</sup>. Phytochemicals also tend to be more effective for long-standing health problems that do not respond well to synthetic medicines<sup>[20]</sup>.

In our previous studies we developed a standardized detailed protocol for lycopene extracted from tomatoes (LycT), its characterization and its beneficial effect in inhibiting NDEA-induced HCC development in terms of histopathological observations, tumor statistics, apoptosis, antioxidative capacity and toxicity<sup>[21-23]</sup>. However, further studies are warranted to determine the modulating effect of lycopene on dysregulated glucose metabolism and hypoxia, as these processes play critical roles in cancer. Thus, the present study was designed to explore the influence of LycT on various glycolytic and non-glycolytic enzymes, the expression of HIF-1 $\alpha$  and potent cell proliferation-associated genes, while preventing NDEA-induced HCC. Moreover, an attempt was made to demonstrate the impact of an imbalance between energy production and metabolic demands on the gross morphology and ultrastructure of hepatocytes in HCC.

## MATERIALS AND METHODS

### Chemicals

Azino-bis(ethylbenzthiazoline sulfonic acid) (ABTS),

diaminobenzidine, ethidium bromide, TRI-reagent and NDEA were obtained from Sigma Chemicals (St. Louis, MO, United States). Primary and secondary antibodies were obtained from Santa Cruz Biotechnology, CA, United States. Invitrogen superscript (III) one step reverse transcription-polymerase chain reaction (RT-PCR) was purchased and used for RT-PCR analysis. Other chemicals were purchased from local reputable companies including Sisco Research Laboratory (P) Ltd. Detailed information regarding extraction and characterization of LycT has been reported previously (Gupta *et al.*<sup>[21]</sup>).

### **Animal model and experimental conditions**

The animal protocol was designed to minimize pain or discomfort to the animals. All animals were acclimatized to laboratory conditions, *i.e.*, temperature of 21 °C ± 1 °C and humidity of 50%-60% for one week prior to the various treatments. All the mice were provided with drinking water and a standard animal pellet diet *ad libitum*. Female BALB/c mice (25-30 g) were randomly divided into four groups (*n* = 7 per group). Animals in Group I (Control) received 0.1 mL olive oil (vehicle) orally throughout the experiment. Group II (NDEA) animals received a cumulative dose of 200 mg NDEA/kg body weight (b.w.) given intraperitoneally in 8 wk as described previously<sup>[5]</sup>. Group III (LycT) mice received LycT orally at a dose of 5 mg/kg b.w. thrice a week for 24 wk. Group IV (LycT + NDEA) animals received NDEA in the same manner as Group III and were also given LycT at a dose of 5 mg/kg b.w. thrice a week for 24 wk. LycT administration was commenced two weeks prior to NDEA treatment. Animals were sacrificed after the 24<sup>th</sup> week to evaluate the modulatory effect of LycT in NDEA-induced hepatocarcinogenesis. The experimental study was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University, Chandigarh (India) and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals (IAEC/284-295 at Sr. No. 48).

### **Scanning and transmission electron microscopy**

After 24 wk, liver tissues from animals in the different groups were immediately fixed in 2% para-formaldehyde and 2.5% glutaraldehyde prepared in 100 mmol/L phosphate buffer (pH 7.4) for 6 h at 4 °C. Critical point drying, trimming of the tissue and gold coating were carried out and the tissues were viewed under a LEO 435 VP scanning electron microscope. Secondary fixation, treatment with a mixture of propylene oxide and epoxy resin (1:1), embedding in freshly prepared epoxy resin and ultrathin sections mounted on colloid on-carbon coated grids were carried out and examined with a Philips CM-10 transmission electron microscope.

### **Estimation of the activities of glycolytic enzymes, glucose-6-phosphate dehydrogenase and glycogen content**

The specific activity of hexokinase, phosphoglucoisomerase (PGI) and aldolase was estimated according

to the reported standard protocols<sup>[24-26]</sup>. Glucose-6-phosphate dehydrogenase (G6PD) activity level was estimated in a tissue homogenate using an ENZOPAK G6PD kit obtained from Reckon Diagnostic P. Ltd. (India). The activity of G6PD in the samples was further calculated using 6.22 mmol/L per centimeter as the extinction coefficient of NADPH at 340 nm. The glycogen content in liver was estimated using the protocol described by Seifter *et al.*<sup>[27]</sup>. The amount of glycogen in the aliquot was determined using a glucose standard.

### **mRNA expression analysis**

Total RNA isolation from liver tissue was carried out using TRI-reagent. For RT-PCR analysis, primers for *HIF-1 $\alpha$* , *PCNA*, *p21* and *Cyclin D1* were searched from the database "Gene Runner" and were synthesized by Sigma-Aldrich (United States). The lengths of the primers chosen were approximately 20bp (Table 1). RT-PCR was performed according to the described protocol of the Superscript (III) one step RT-PCR kit. The DNA bands were visualized in agarose gel using an ultraviolet transilluminator and photographed on Gel Doc. Densitometric analysis of the bands was performed using Image J software (National Institute of Health, United States).

### **Quantitation of protein expression**

**Sample preparation:** The animals were fasted overnight before liver dissection. Mice were euthanized by cervical dislocation under light ether anesthesia. Liver perfusion was carried out with 0.9% NaCl and the liver was carefully removed and placed in a Petri plate containing ice-cold saline. The tissue was homogenized in ice-cold 100 mmol/L potassium phosphate buffer (pH 7.4) containing 150 mmol/L KCl in an ice-chamber to obtain 25% homogenate (w/v) using a mechanically driven Teflon fitted Potter Elvehjem homogenizer. The homogenate (25%) was then subjected to centrifugation at 10000 rpm for 30 min at 4 °C for preparation of the post-mitochondrial fraction.

**ELISA:** Post mitochondrial fractions obtained from the hepatic tissue of different groups were quantitated for protein concentration by the method of Lowry *et al.*<sup>[28]</sup>. Two point five microgram protein was loaded onto an ELISA strip containing carbonate buffer. Further, protein expression of *HIF-1 $\alpha$* , *PCNA*, *p21* and *Cyclin D1* were analyzed according to the standard protocol of ELISA using specific primary antibodies and enzyme conjugated secondary antibodies. ABTS in citrate buffer was added along with hydrogen peroxide for color generation. The color thus obtained was quantified at 405 nm.

### **Statistical analysis**

The statistical methods used in this study were reviewed by Dr. Neha Arora Chugh, Department of Biophysics, Panjab University, Chandigarh. Data were expressed as mean ± SD. The results were subjected to analysis of variance (one-way ANOVA) followed by the post



**Table 1** List of primer pairs used

Gene	Strand	Primer
<i>HIF-1α</i>	Sense	5'-GGT/CAG/ATG/ATC/AGA/GTC/C-3'
	Antisense	5'-TGC/TTG/GTG/CTG/ATT/TGTG/A-3'
<i>PCNA</i>	Sense	5'-GAT/GTG/GAG/CAA/CTT/GGA/AT-3'
	Antisense	5'-AGC/TCT/CCA/ACT/TGC/AGA/AAA-3'
<i>p21</i>	Sense	5'-CCG/TGG/ACA/GTG/AGC/AGT/TG-3'
	Antisense	5'-TGG/GCA/CTT/CAG/GGT/TTT/CT-3'
<i>Cyclin D1</i>	Sense	5'-CAC/AAC/GCA/CTT/TCT/TTC/CA-3'
	Antisense	5'-GAC/CAG/CCT/CTT/CCT/CCA/C-3'
<i>β-actin</i>	Sense	5'-ATC/CGT/AAA/GAC/CTC/TAT/GC-3'
	Antisense	5'-AAC/GCA/GCT/CAG/TAA/CAG/TC-3'

hoc test for statistical significance using SPSS (version 14.0) software.  $P \leq 0.05$  was considered statistically significant.

## RESULTS

Scanning electron microscopy (SEM) of the control and LycT groups revealed normal hepatic surface morphology with polyhedral hepatocytes radially arranged around central veins in cords separated by sinusoids (Figure 1A-C). Bile canaliculi were observed on the apical surface of hepatocytes. A few red blood cells were also visible in the sinusoids. However, serious and irreversible alterations in liver architecture were observed in the NDEA and LycT + NDEA groups. Smoothing or rounding of the hepatocytes with hyperplastic tumor along with clumps of hepatocytes with intercellular surfaces covered with numerous microvillus projections were visible (Figure 1D-E). The discernible nodules were of irregular shape and size. Necrotic tumor nodules and uncontrolled cell density revealed the presence of undifferentiated HCC in the NDEA group. In contrast, the surface morphology of liver tissue from the LycT + NDEA group revealed well differentiated HCC characterized by stromal invasion and a trabecular pattern of two to three cells thick plates of hepatocytes (Figure 1H). High cell density with pleomorphism of tumor cells was also evident (Figure 1F). Apoptotic bodies were observed indicating a high rate of apoptosis (Figure 1G). Thus, LycT pre-treatment of NDEA-challenged mice significantly reduced the severity caused by NDEA. Table 2 shows the results of a quantitative comparison of hepatic tissues from the NDEA and LycT + NDEA groups using SEM.

Transmission electron microscopy (TEM) of the control and LycT groups revealed normal hepatic ultrastructural architecture (Figure 2A-C). At low magnification, hexagonal hepatocytes radially arranged around blood vessels with an intact cell membrane, clear and granulated cytoplasm comprising oval-shaped mitochondria, rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum were observed. A smooth, rounded and prominent nucleus with intact double layered nuclear membrane, a darkly stained single and prominent nucleolus along with uniformly distributed chromatin in the nucleoplasm

**Table 2** Comparative analysis of the N-Nitrosodiethylamine and lycopene extracted from tomatoes + N-Nitrosodiethylamine group by scanning electron microscopy

Groups/parameters	NDEA	LycT + NDEA
Cell density	+++	+
Rounding of hepatocytes	+++	+
Trabecular structures	-	+++
Necrotic tumor nodules	+++	+
Apoptotic bodies	-	+++
Type of HCC	Undifferentiated	Well-differentiated

Where “+++” indicates that more than 70%-90% of mice in a group showed this feature; “+” indicates that < 70% of mice showed this feature; “-” indicates that the < 20% of mice showed this feature. LycT: Lycopene extracted from tomatoes; NDEA: N-Nitrosodiethylamine; HCC: Hepatocellular carcinoma.

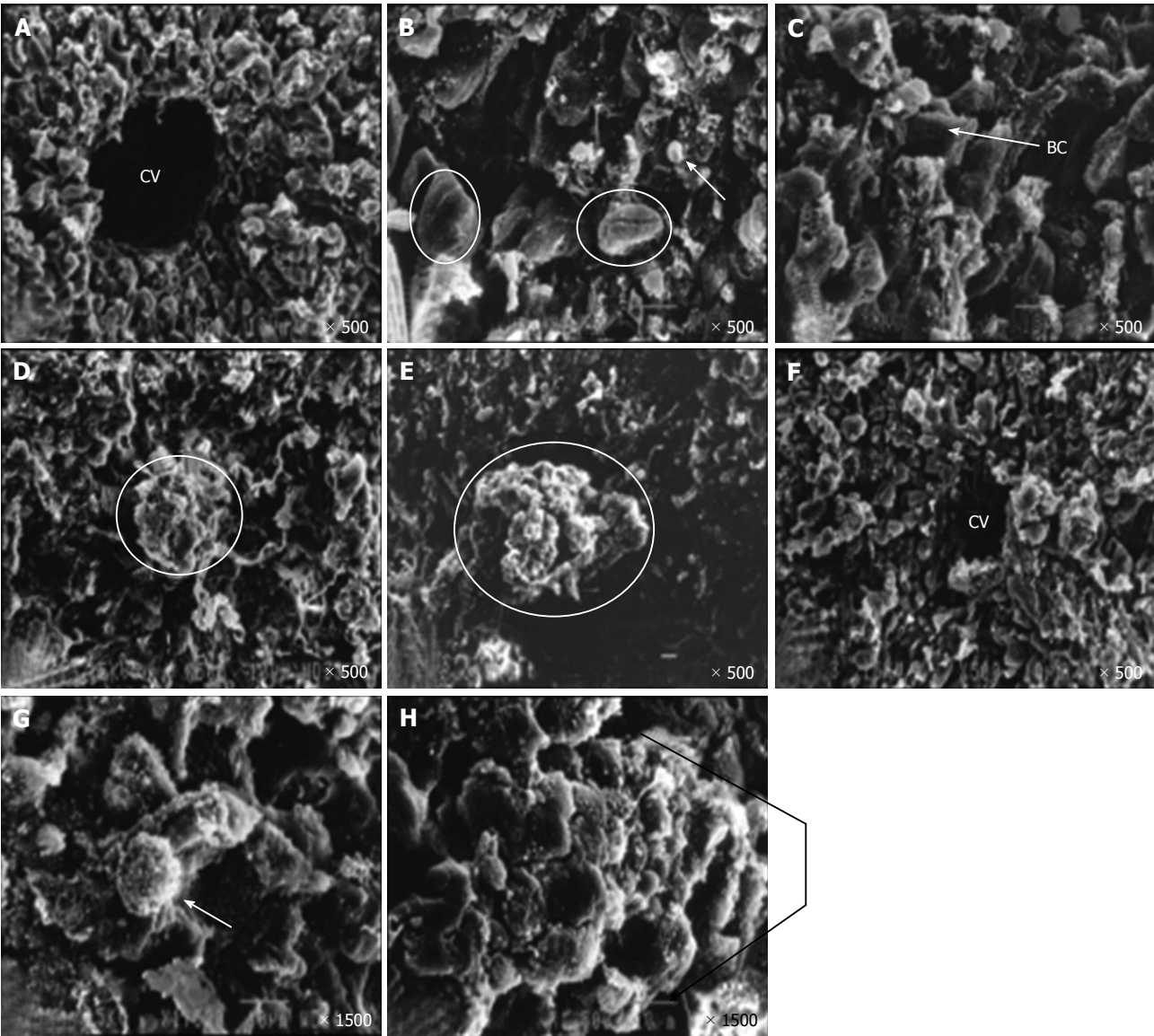
were also visible. Irregular non-membrane bound, faintly stained granules of hepatocellular glycogen were also observed. The presence of lipid granules as darkly stained spots and a few bi-nucleated hepatocytes were found in the LycT group in addition to the above features. Several irreversible alterations in the nucleus of liver cells from the NDEA group were observed (Figure 2D-E). A prominent large and irregular nucleus with interrupted nuclear membrane, multiple prominent nuclei and pseudo-inclusions were observed in liver sections from the NDEA group with an increased nuclear/cytoplasmic ratio. Karyotin (reticular material) deposition along the nuclear membrane was the striking difference when compared with normal nuclei. Loss of organization of cytoplasmic components such as pleomorphic mitochondria varying in shape and size, dilated cisternae of RER associated with mitochondria and decreased lysosomes were observed in tumor cells from the NDEA group. Variable light and dark granules of fat and glycogen deposits were also observed. The liver cells from the LycT + NDEA group showed mild damage to the nuclear membrane with fewer karyotin deposits (Figure 2F). Hepatocytes were evident with prominent nucleoli, pleomorphic mitochondria and a higher number of lysosomes. Moreover, liver sections showed many macrophages in sinusoids and apoptotic cells (Figure 2G-H). Table 3 shows the results of a quantitative comparison of hepatic tissues from the NDEA and LycT + NDEA groups using TEM.

A significant increase in the activities of liver hexokinase, PGI and aldolase in the NDEA and LycT + NDEA groups was evident when compared to the control and LycT groups. However, LycT pre-treatment significantly lowered the activity of hexokinase and PGI in the LycT + NDEA group when compared to the NDEA group. No significant change in aldolase level was observed in the LycT + NDEA group when compared to the NDEA group (Figure 3A-C). Moreover, NDEA treatment caused a significant increase in liver G6PD activity in the NDEA group when compared to the control and LycT groups. Moreover, a significant increase was also observed in the levels of liver G6PD in the LycT + NDEA group when compared to the control group. However, G6PD level

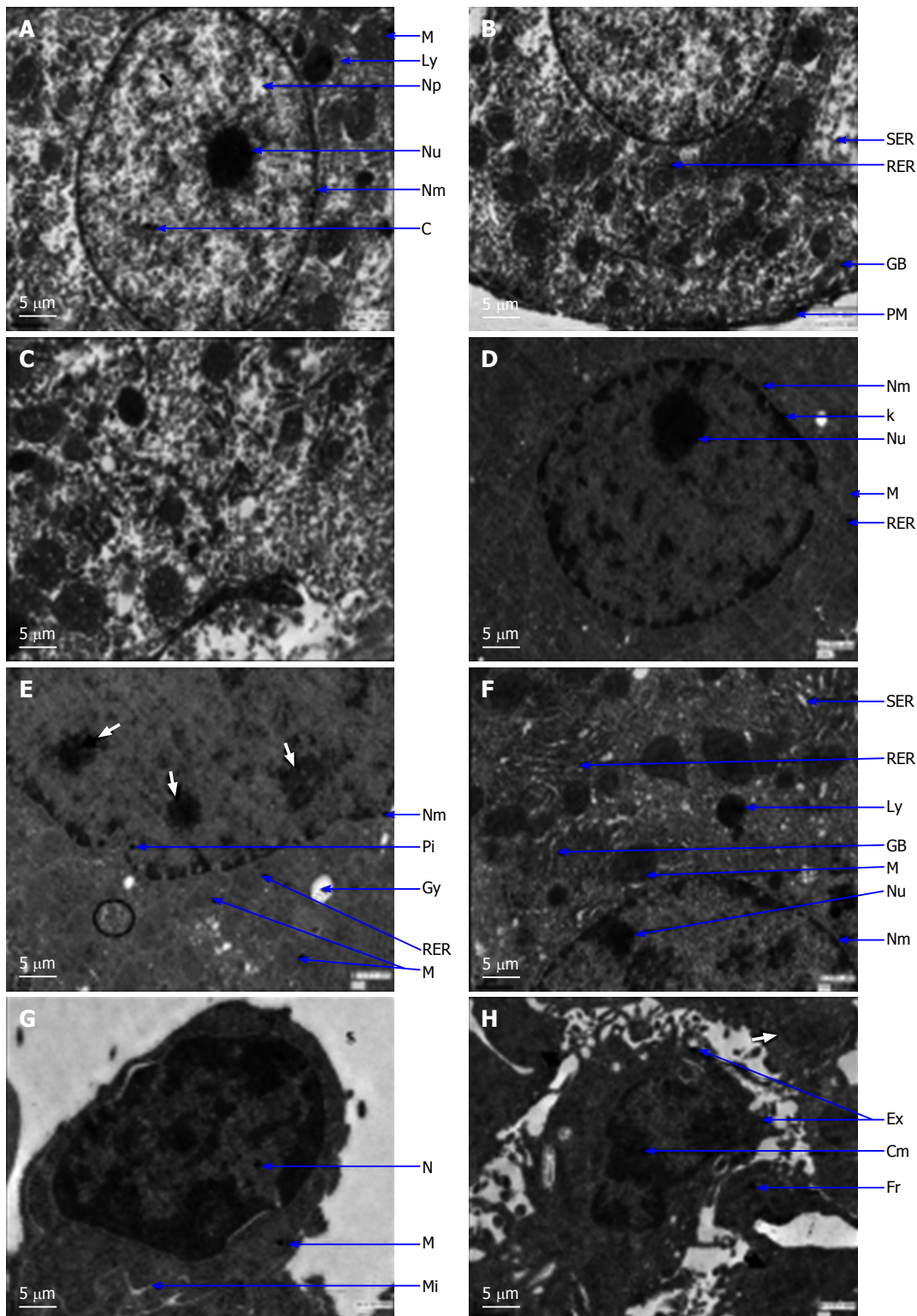
**Table 3** Comparative analysis of the N-Nitrosodiethylamine and lycopene extracted from tomatoes + N-Nitrosodiethylamine group by transmission electron microscopy

Groups/parameters	NDEA	LycT + NDEA
Hepatocytes	Rounded, smaller in size	Polygonal but rounded edges
Nucleus	Large and irregular shape	Oval shaped
Nuclear membrane	Not uniform and pseudoinclusions	Not uniform
Nucleoli	Large, irregular, multiple	One-two
Nuclear/cytoplasmic ratio	High	Low
Karyotin (reticular material)	Deposition along nuclear membrane	Fewer karyotin deposits
Cytoplasm	Loss of organization, dense	Organized
Mitochondria	Pleomorphic with increased density	Pleomorphic but number less than that in the NDEA group
Lysosomes	Few in number	High in number
Fat and glycogen globules	Variable	High
Macrophages	Very few	Many
Apoptotic bodies	No	Clearly visible in the section

LycT: Lycopene extracted from tomatoes; NDEA: N-Nitrosodiethylamine.



**Figure 1** Scanning electron micrographs of liver tissue at  $\times 500$  and  $\times 1500$ . A and B: Control group, illustrating the central vein (CV), hexagonal hepatocytes (encircled) and biconcave RBCs in sinusoids (arrowed); C: LycT group illustrating hexagonal hepatocytes with bile canaliculi (BC) on their surface (arrowed); D and E: NDEA group illustrating tumor nodules (encircled) with abnormal cell proliferation and disturbed ultrastructure; F-H: LycT + NDEA group respectively illustrating high cell density, CV, apoptotic bodies (arrowed) along with various alterations and two-three cell plate thickening indicating well-differentiated hepatocellular carcinoma, respectively. LycT: Lycopene extracted from tomatoes; NDEA: N-Nitrosodiethylamine; RBCs: Red blood cells.



**Figure 2 Transmission electron micrographs (× 2550) of liver tissue.** A and B: Control group illustrating round nucleus (N), nuclear membrane (Nm), nucleolus (Nu), and chromatin (C) and nucleoplasm (Np). Clear cytoplasm with mitochondria (M), dark bodies as lysosomes (Ly), smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), and Golgi bodies (GB) surrounded by plasma membrane (PM) were observed; C: LycT group illustrating similar characteristics to those in the control liver micrograph; D and E: NDEA group illustrating deformed N with disrupted and convoluted Nm, darkly masses in the nucleolus (white arrows), deposition of karyotin (k) and pseudo-inclusions (Pi). Cytoplasm was also found to be compactly packed with multiple pleomorphic M and RER. Percentage of other organelles was found to be lower, and variable light and dark granules of fat (encircled) and glycogen (Gy) deposits were also observed; F-H: LycT + NDEA group illustrating perforated nuclear membrane. Cytoplasm contained was occupied with pleomorphic mitochondria, with a however higher percentage of organelles such as GB, Ly, RER and higher glycogen deposits. Macrophages in the sinusoid (S) were characterized by a large nucleus to cytoplasmic ratio, abundant mitochondria and microvilli (Mi). Apoptotic body characterized by cell shrinkage and condensation of nuclear chromatin into delineated masses (Cm), forming extensions (Ex), and crowded with closely packed cellular organelles (white arrow) and fragments of nucleus (Fr). LycT: Lycopene extracted from tomatoes; NDEA: N-Nitrosodiethylamine.



**Table 4** Effect of N-Nitrosodiethylamine and/or lycopene extracted from tomatoes on protein expression in mice hepatic tissue using ELISA

Protein (absorbance at 405 nm)	Control	NDEA	LycT	LycT + NDEA
PCNA	0.31 ± 0.01	0.47 ± 0.01 <sup>a</sup>	0.31 ± 0.01 <sup>b</sup>	0.39 ± 0.01 <sup>a,b,c</sup>
Cyclin D1	0.25 ± 0.02	0.38 ± 0.02 <sup>a</sup>	0.24 ± 0.02 <sup>b</sup>	0.36 ± 0.01 <sup>a,c</sup>
p21	0.40 ± 0.02	0.33 ± 0.02 <sup>e</sup>	0.39 ± 0.02 <sup>f</sup>	0.37 ± 0.01 <sup>g</sup>
HIF-1α	0.22 ± 0.03	0.41 ± 0.01 <sup>a</sup>	0.23 ± 0.03 <sup>b</sup>	0.36 ± 0.04 <sup>a,c</sup>

<sup>a</sup>*P* ≤ 0.05, compared to the NDEA group; <sup>b,c,e</sup>*P* ≤ 0.001, compared to the control group, NDEA group and LycT group, respectively; <sup>f,g</sup>*P* ≤ 0.01, compared to the control group and NDEA group, respectively. LycT: Lycopene extracted from tomatoes; NDEA: N-Nitrosodiethylamine; PCNA: Proliferating cell nuclear antigen; p21: Cyclin-dependent kinase inhibitor 1A; HIF-1α: Hypoxia inducible factor-1α.

following LycT pre-treatment in NDEA-challenged mice was observed to be significantly lower than that in the NDEA group. No significant change was observed in the level of G6PD in the LycT group when compared to the control group (Figure 3D). A significant decrease in liver glycogen level was observed in the NDEA group when compared to the control and LycT groups. LycT pre-treatment in NDEA-challenged mice caused a significant increase in the levels of tissue glycogen when compared to the NDEA group. However, a significant decrease in tissue glycogen level was observed when compared to the control and LycT groups (Figure 3E). No significant change was observed in the activities of these enzymes and the level of glycogen in the LycT group when compared to the control group.

Figure 4 shows the mRNA expression of various genes involved in proliferation during HCC in the different treatment groups. Densitometric analysis of *HIF-1α* expression revealed a significant increase in the NDEA and LycT + NDEA groups when compared to the control and LycT groups (Figure 4). A significant increase in the expression of *PCNA* and *Cyclin D1* was observed in the NDEA group when compared to the control and LycT groups. The LycT + NDEA group showed a significant decrease in mRNA expression of *PCNA* and *Cyclin D1* when compared to the NDEA group. A significant increase in the expression of *Cyclin D1* was observed in the LycT + NDEA group when compared to the control and LycT groups. Densitometric analysis of *p21* expression revealed a significant decrease in the NDEA group when compared to the control and LycT groups. A significant increase in the expression of *p21* was observed in the LycT + NDEA group when compared to the control, NDEA and LycT groups. No change in the expression of these genes was observed when the LycT group was compared to the control group.

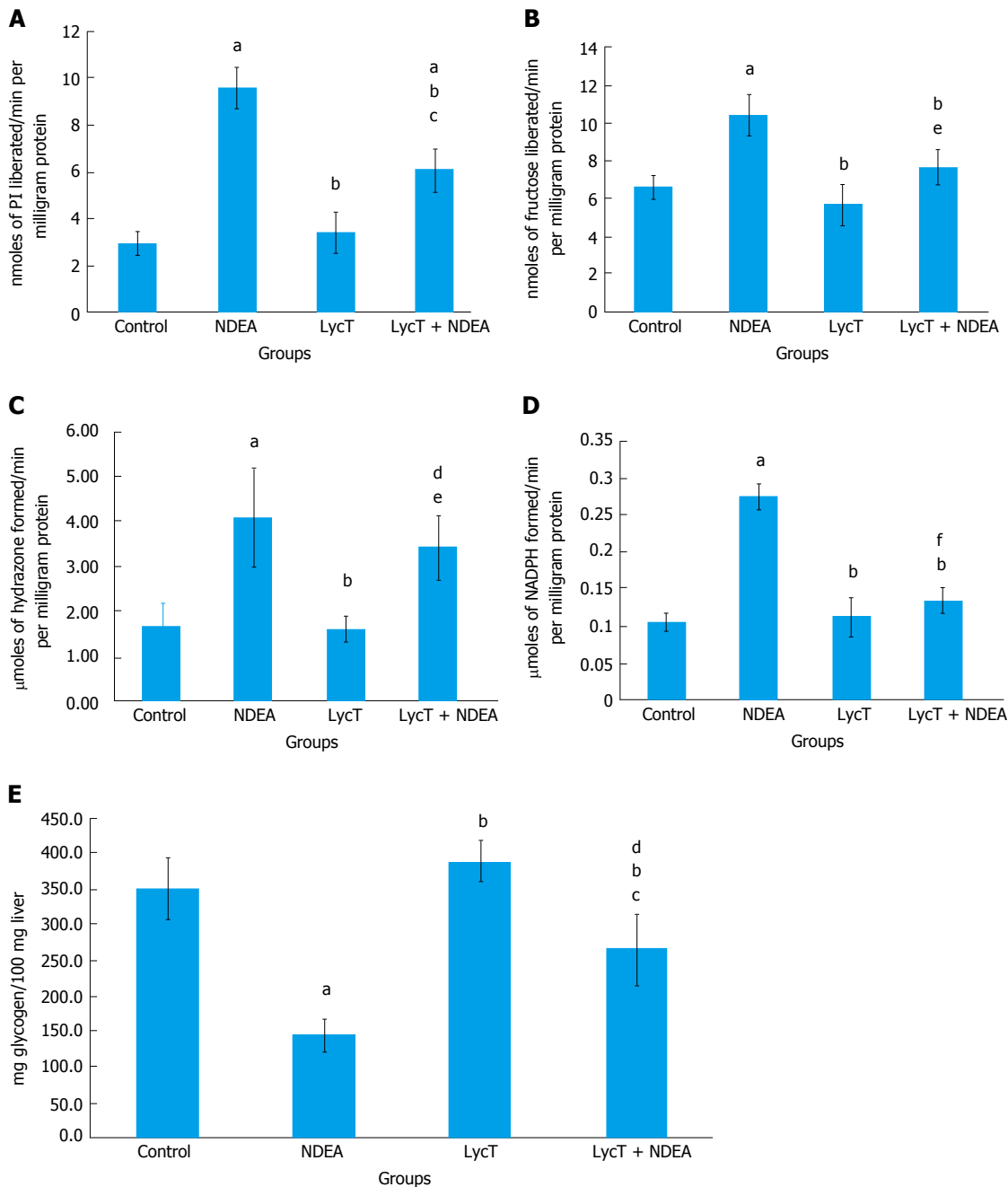
Table 4 shows the protein expression of various genes related to proliferation during HCC in the different treatment groups. The expression of *HIF-1α* was significantly increased in the NDEA and LycT + NDEA groups when compared to the control and LycT groups. Significantly enhanced expression of *PCNA* and *Cyclin D1* was attributed to significantly higher absorbance at 405 nm in the NDEA group when compared to the control and LycT groups (Table 4). A significantly lower absorbance at 405 nm was observed for *PCNA* expression following

LycT administration in NDEA-challenged mice when compared to the NDEA group. However, significantly enhanced expression of *PCNA* and *Cyclin D1* was observed in the LycT + NDEA group when compared to the control and LycT groups. Protein expression of *p21* was analyzed using ELISA in all treatment groups (Table 4). A significantly lower absorbance at 405 nm was found in the NDEA group when compared to the control and LycT groups. Significantly increased expression of *p21* was observed following LycT pre-treatment in NDEA-challenged mice when compared to the NDEA group. No significant change in the expression of *HIF-1α*, *PCNA*, *Cyclin D1*, and *p21* was observed between the LycT and control groups.

## DISCUSSION

Previously, we observed that LycT yielded lycopene phyto-complex (LycT) which delayed and reduced the severity of NDEA-induced HCC as indicated by histopathology, tumor statistics and antioxidant defence system analysis<sup>[21]</sup>. The presence of a myriad number of compounds in the extract has been reported to enhance the medicinal properties of active components through synergistic effects<sup>[29,30]</sup>. The therapeutic activity of a medicinal plant is not due to a single component or a few components. However, one substance is so dependent on the presence of another substance that the plant or part of the plant when used in its entirety often yields better results than any single component if used in isolation. Lycopene extraction following basic solvent separation has been proved to be a better agent as there is substantial evidence to show that synergism further enhances its activity and efficacy<sup>[31]</sup>. Lycopene phyto-complex has also shown high efficacy in triggering apoptosis in addition to its anti-oxidative property<sup>[31]</sup>. However, the study would be incomplete if the effects of LycT on other hallmark pathways of HCC development were not demonstrated. One of the essential and necessary alterations for the development of almost all cancers is the induction of aerobic glycolysis (Warburg effect). Recently, scientists have linked sustained aerobic glycolysis to oncogenic mutations leading to abnormal cell proliferation and apoptosis<sup>[32]</sup>. Limited literature is available regarding the irreversible alterations in hepatic architecture during HCC development and their



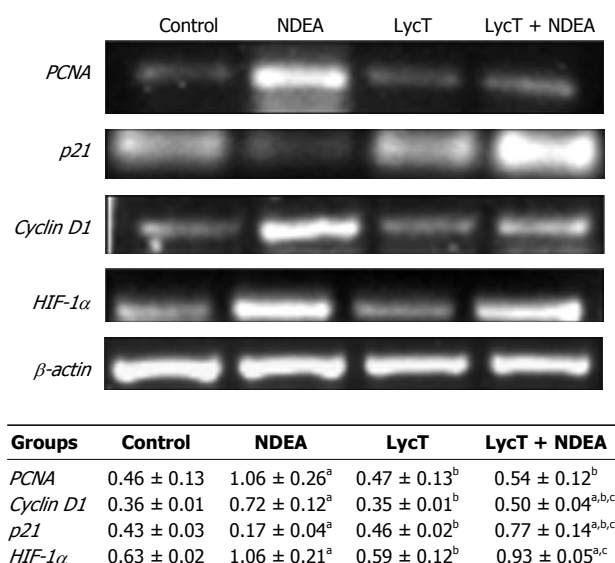


**Figure 3** Effect of lycopene extracted from tomatoes and/or N-Nitrosodiethylamine. A: Hexokinase activity (nmols of pseudo-inclusions liberated/min per milligram protein); B: Phosphoglucosomerase (nmols of fructose liberated/min per milligram protein); C: Aldolase (μmols of hydrazone formed/min per milligram protein); D: G6PD (μmols of NADPH formed/min per milligram protein); E: Glycogen (mg glucose/100 mg liver). <sup>a</sup>*P* ≤ 0.05, compared to the control group; <sup>b,c,d</sup>*P* ≤ 0.001, compared to the control group, NDEA group and LycT group, respectively; <sup>e,f</sup>*P* ≤ 0.01, compared to the control group and LycT group, respectively. LycT: Lycopene extracted from tomatoes; NDEA: N-Nitrosodiethylamine.

association with other dysregulated carcinogenic insults. With this in mind, the present study was designed to provide an insight into the alterations in ultrastructure, cell proliferation and aerobic glycolysis in NDEA-induced HCC and the effects of LycT on NDEA-induced HCC.

Several irreversible distortions using SEM and TEM were clearly observed and indicated the transformation of well-differentiated HCC to undifferentiated HCC in the NDEA group. Rapidly dividing tumor cells attaining a round contour during crowding of the cells has been reported in the literature<sup>[33]</sup>. Gross changes in nuclear mor-

phology, epigenetic regulation, chromatin packing and overall nuclear architecture can be related to alterations in the molecular machinery<sup>[34]</sup>. However, LycT pre-treatment in NDEA-challenged mice resulted in reduced severity as depicted in micrographs. Increased lysosomal bodies and the presence of apoptotic bodies in hepatic tissue from the LycT + NDEA treated group revealed a high apoptotic rate and thus confirms the observations and strengthened our reported data. Aerobic glycolysis arises as a compensatory mechanism due to altered respiration in tumor cells to fulfil the ATP requirements during



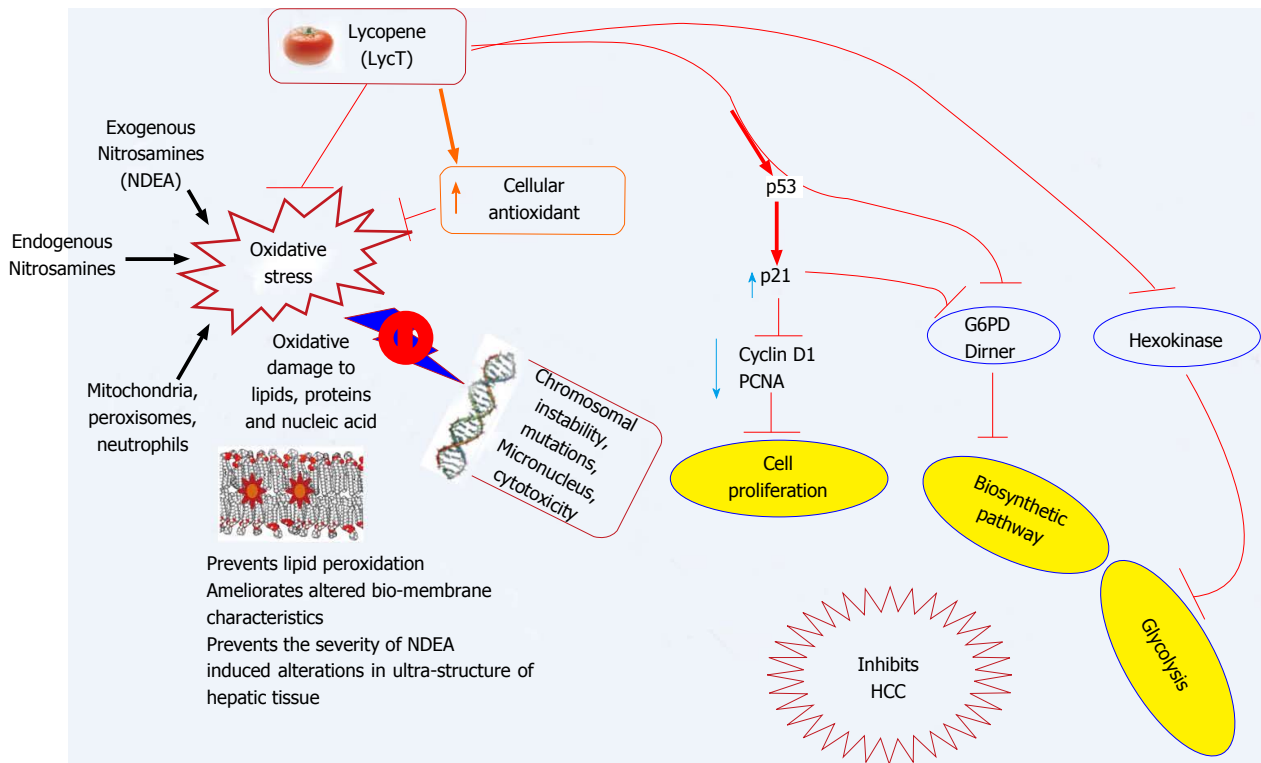
**Figure 4** Effect of lycopene extracted from tomatoes on mRNA expression of *PCNA*, *Cyclin D1*, *p21* and *HIF-1α* during N-Nitrosodiethylamine-induced hepatocarcinogenesis in mice. <sup>a,b,c</sup>*P* ≤ 0.001, compared to the N-Nitrosodiethylamine group, LycT group and control group, respectively. LycT: Lycopene extracted from tomatoes; NDEA: N-Nitrosodiethylamine.

abnormal cell proliferation<sup>[35]</sup>. Similarly, enhanced hepatic hexokinase, PGI and aldolase activities in the NDEA group can be attributed to the high cell density observed. Such observations are in accordance with the available literature where enhanced glycolytic enzymes have been linked with chemically induced HCC<sup>[36]</sup>. Elevated levels of PGI have recently emerged as an excellent response to cancer and PGI level is used as a marker of metastatic growth in patients<sup>[37]</sup>. Moreover, significant reductions in the activities of glycolytic enzymes following LycT pre-treatment in NDEA-challenged mice were inversely related to HCC development. Histopathological and ultrastructural observations revealed well-differentiated HCC in the LycT + NDEA group, whereas poorly to undifferentiated HCC was observed in the NDEA group<sup>[21]</sup>. These structural observations could be correlated with the observed modulation of the glycolytic pathway. Various studies based on <sup>18</sup>F-FDG uptake on PET scans in different HCCs revealed that well differentiated HCC showed lower <sup>18</sup>F-FDG uptake in comparison with poorly differentiated HCC<sup>[38]</sup>.

Ectopic expression of G6PD promotes the survival of tumor cells by maintaining both extracellular pH and redox potential<sup>[39]</sup>. Moreover, loss of p53 or mutated p53 has been linked with enhanced glucose consumption *via* increased activity of G6PD<sup>[40]</sup>. In the current study, LycT pre-treatment in NDEA-challenged mice resulted in significantly low expression of G6PD indicating the inhibitory role of lycopene in HCC by affecting metabolic pathways. Although there is limited research on the role of lycopene in regulating G6PD, some researchers have demonstrated the inhibitory effect of phytochemicals by regulating the activity of G6PD<sup>[41]</sup>. Decreased expression of Bcl-2 and enhanced p53 expression in

the LycT + NDEA group may be responsible for reduced G6PD activity. In the current study, liver glycogen content in HCC was found to be decreased in the NDEA group. However, the reasons for the lack of glycogen accumulation were not fully explored. The transformation of liver cells to tumor cells causes a loss of glucose production *via* gluconeogenesis. According to the literature, overproduction of the molecule, microRNA-23a, is responsible for inhibiting gluconeogenesis<sup>[42]</sup>. Glycogen metabolism then acts as an alternate energy source, enabling growth of the cell under metabolic stress. A significant increase in the level of glycogen content was observed in the LycT + NDEA group when compared to the NDEA group. The current observations indicate that lycopene may interfere with glycogen conversion to glucose or might be due to less severe early HCC followed by lower ATP requirement. Such observations are in accordance with the previously reported effect of lycopene in CCL<sub>4</sub>-challenged mice<sup>[43]</sup>.

These observations in the NDEA group clearly point to a high rate of cell proliferation, which was further evident from increased *PCNA* and *Cyclin D1* expression. Enhanced proliferation attributed to increased expression of *PCNA* and *Cyclin D1* has been reported in the literature<sup>[44]</sup>. Decreased expression of *p21* in the NDEA group could be correlated with enhanced expression of *Cyclin D1* as *p21* is known to inhibit the activity of cyclin-CDKs complexes<sup>[45]</sup>. *p21* is an important downstream mediator of p53 and its anti-proliferative property plays an important role in preventing tumor development. In our previous study it was observed that reduced p53 expression in the NDEA group was correlated with evasion of apoptosis<sup>[22]</sup>. Moreover, the LycT + NDEA group showed significantly enhanced expression of *p21* and reduced expression of *PCNA* when compared to the NDEA group indicating the anti-proliferative activity of LycT. Although it is difficult to comment on how lycopene or its metabolites inhibit HCC, the literature shows that treatment with lycopene or its metabolite increased *p21* expression and hence aided in preventing NDEA-induced cancer<sup>[46]</sup>. This observation is also supported by the current observation of increased cell density as shown by SEM and the hepatic glycolysis rate. Moreover, enhanced mRNA and protein expression of *HIF-1α* clearly indicated the existence of hypoxic conditions in NDEA-treated liver tissue. Various research groups have observed that the expression of *HIF-1* modulates apoptosis in HCC<sup>[11]</sup>. Reports have demonstrated that hypoxia enhances VEGF expression and decreases the ratio of Bax/Bcl-2, thus blocking apoptosis<sup>[47]</sup>. Pre-treatment with LycT in NDEA-challenged mice resulted in a significant reduction in the expression of *HIF-1α* at week 24 when compared to the NDEA group. Many reports have demonstrated similar observations where lycopene had an inhibitory response on HIF-1 in both *in vivo* and *in vitro* studies. Upadhyay *et al.*<sup>[48]</sup> performed a comparative study of different antioxidants in order to assess their cancer preventive activity through the inhibition of HIF-1 activity. According to the results, HIF-1α operated in the presence of free radicals and antioxidants with maximum scavenging



**Figure 5 Overall mechanism of lycopene extracted from tomatoes mediated chemoprevention.** LycT: Lycopene extracted from tomatoes; NDEA: N-Nitrosodiethylamine; HCC: Hepatocellular carcinoma; G6PD: Glucose-6-phosphate dehydrogenase.

efficiency for ROS cause inhibition of HIF-1 $\alpha$ <sup>[49]</sup>. The literature also supports the consumption of tomatoes and lycopene mostly inhibited the expression of HIF-1 $\alpha$  during prostate carcinogenesis<sup>[50]</sup>. Such reports strengthen our current observations, that the delay in HCC development may be attributed to the anti-proliferative effect of lycopene. In summary, our report demonstrates the potential of lycopene as a multi-targeted approach against chemically induced HCC.

Data from the present study and previously published studies show that LycT has beneficial effects against NDEA-induced HCC. Electron micrographs (SEM and TEM) of liver biopsies from the different treatment groups provided a picture of 3-D *in vivo* tissue modulations and thus served as an efficient and accurate tool for demonstrating carcinogenesis and the efficacy of chemopreventive agents along with histopathological observations. Moreover, aerobic glycolysis is also a potential target for determining the chemopreventive efficacy of lycopene and other phytochemicals in preventing carcinogenesis. As shown in the present study, LycT pre-treatment ameliorated disturbed metabolism, however, further studies are warranted to understand the in-depth pharmacokinetics and pharmacodynamics related to lycopene in experimental models of cancer. Finally, an attempt was made to represent diagrammatically the anti-carcinogenic effect of lycopene based on the current study and previous publications (Figure 5).

## ACKNOWLEDGMENTS

Financial assistance provided by the University Grant

Commission, New Delhi, (India) to carry out the present research is gratefully acknowledged.

## COMMENTS

### Background

Cancer is a complex disease and requires attention in multiple directions to prevent its development. Among the various carcinogenic insults, uncontrolled proliferation, dysregulated carbohydrate metabolism and hypoxia play very crucial roles in the development of hepatocellular carcinoma (HCC). Although there have been advances in therapeutic approaches a complete cure is still unavailable. Dietary multi-targeted agents have attracted the attention of cancer biologists as these agents may provide a solution to this complex problem by targeting multiple targets simultaneously. Lycopene, a polyunsaturated hydrocarbon imparting red colour to various fruits, is a nutritionally important carotenoid exhibiting beneficial health effects due to its antioxidant activity with minimal side effects. In addition to its antioxidant property, lycopene is known to modulate other non-oxidative pathways such as regulation of gap junction communication, the hormonal system, immune system and metabolic pathways of xenobiotics. Previously, we demonstrated that lycopene from tomatoes is a potent agent for inhibiting HCC development in terms of histopathological observations, tumor statistics, apoptosis, antioxidative capacity and toxicity. However, studies are warranted to determine the modulating effect of lycopene on dysregulated glucose metabolism and hypoxia, as these processes play critical roles in cancer. Thus, in the current study, the influence of lycopene extracted from tomatoes on various glycolytic and non-glycolytic enzymes, the expression of hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) and potent cell proliferation-associated genes while preventing N-Nitrosodiethylamine (NDEA)-induced HCC was investigated. Moreover, an attempt was made to demonstrate the impact of an imbalance between energy production and metabolic demands on the gross morphology and ultrastructure of hepatocytes in HCC.

### Research frontiers

Important areas related to the current study include: (1) carcinogenesis: Incidence rate, statistics, prognosis, consequences, mortality, molecular and biochemical markers, altered cellular pathways and therapeutic limitations;

and (2) chemoprevention: Natural agents, multifaceted approach, lycopene a colored pigment and its antioxidative and anti-carcinogenic potential.

### Innovations and breakthroughs

Despite significant research efforts, cancer is considered an incurable disease due to its high incidence rate, poor prognosis, high mortality rate, multifactorial causative agents, and side effects associated with chemotherapeutics. As natural agents are safer and have a multifaceted approach they are considered an alternative therapy. Lycopene is known to be a potent antioxidant and phytoagent with a protective effect against chronic diseases such as cancer. However, the detailed mechanism underlying its anti-carcinogenic effects is unclear. The basic requirement in the present investigation was to design a study that could demonstrate the action of lycopene at various stages to cover the maximum number of carcinogenic bioprocesses. The present study is part of this research design, in which hepatic tissue from different groups, *i.e.*, control, LycT, NDEA and LycT + NDEA groups was studied at different levels including structural markers, electro-physical markers, morphological markers, biochemical markers, and molecular markers that are known to be involved in the development of cancer. This type of study provides innovation and achievement for futuristic analyses of natural agents in disease models.

### Applications

The outcomes of the current study provide deeper insight into the mechanistic targets of lycopene which shows ameliorating effects. Lycopene administration directly or indirectly stimulated various molecular targets such as p53, which have been correlated with decreased G6PD activity or biosynthetic pathways that play important roles during tumor proliferation. The protective effect of lycopene can be studied at the ultrastructural level by scanning and transmission electron microscopy (SEM and TEM). A detailed explanation regarding hepatic SEM and TEM during carcinogenesis is also information which is ambiguous in the literature.

### Peer-review

This manuscript entitled "Lycopene modulates cellular proliferation, glycolysis and hepatic ultrastructure during hepatocellular carcinoma" investigated the effect of lycopene on ultra-structure, glycolytic enzymes, cell proliferation markers and hypoxia during NDEA induced hepatocarcinogenesis.

## REFERENCES

- Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. *Pharm Res* 2008; **25**: 2097-2116 [PMID: 18626751 DOI: 10.1007/s11095-008-9661-9]
- Theodoratou E, Farrington SM, Tenesa A, McNeill G, Cetnarskyj R, Korakakis E, Din FV, Porteous ME, Dunlop MG, Campbell H. Associations between dietary and lifestyle risk factors and colorectal cancer in the Scottish population. *Eur J Cancer Prev* 2014; **23**: 8-17 [PMID: 23820601 DOI: 10.1097/CEJ.0b013e3283639fb8]
- Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]
- Inami K, Ishikawa S, Mochizuki M. Activation mechanism of N-Nitrosodialkylamines as environmental mutagens and its application to antitumor research. *Gene Environ* 2009; **31**: 97-104 [DOI: 10.3123/jemsge.31.97]
- Bharati S, Rishi P, Koul A. Azadirachta indica exhibits chemopreventive action against hepatic cancer: Studies on associated histopathological and ultrastructural changes. *Microsc Res Tech* 2012; **75**: 586-595 [PMID: 21998015 DOI: 10.1002/jemt.21095]
- Crews C. The determination of N-nitrosamines in food. *Qual Assur Saf Crop* 2010; **2**: 2-12 [DOI: 10.1111/j.1757-837X.2010.00049.x]
- Heindryckx F, Colle I, Van Vlierberghe H. Experimental mouse models for hepatocellular carcinoma research. *Int J Exp Pathol* 2009; **90**: 367-386 [PMID: 19659896 DOI: 10.1111/j.1365-2613.2009.00656.x]
- Aiub CA, Gadermaier G, Ferreira F, Felzenszwalb I, Eckl P, Pinto LFR. N-Nitrosodiethylamine cytochrome P450 induction and cytotoxicity evaluation in primary cultures of rat hepatocytes. *Am J Mol Biol* 2011; **1**: 70-78 [DOI: 10.4236/ajmb.2011.12009]
- Aiub CA, Gadermaier G, Silva IO, Felzenszwalb I, Pinto LF, Ferreira F, Eckl P. N-nitrosodiethylamine genotoxicity evaluation: a cytochrome P450 induction study in rat hepatocytes. *Genet Mol Res* 2011; **10**: 2340-2348 [PMID: 22002127 DOI: 10.4238/2011]
- Ortega AD, Sánchez-Aragó M, Giner-Sánchez D, Sánchez-Cenizo L, Willers I, Cuezva JM. Glucose avidity of carcinomas. *Cancer Lett* 2009; **276**: 125-135 [PMID: 18790562 DOI: 10.1016/j.canlet.2008.08.007]
- Semenza GL. Hypoxia and cancer. *Cancer Metastasis Rev* 2007; **26**: 223-224 [PMID: 17404692]
- Wu X, Patterson S, Hawk E. Chemoprevention—history and general principles. *Best Pract Res Clin Gastroenterol* 2011; **25**: 445-459 [PMID: 22122762 DOI: 10.1016/j.bpg.2011.10.012]
- Mehta RG, Murillo G, Naithani R, Peng X. Cancer chemoprevention by natural products: how far have we come? *Pharm Res* 2010; **27**: 950-961 [PMID: 20238150 DOI: 10.1007/s11095-010-0085-y]
- Singh S, Singh PP, Roberts AR, Sanchez W. Chemopreventive strategies in hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 45-54 [PMID: 23938452 DOI: 10.1038/nrgastro.2013.143]
- Seren S, Lieberman R, Bayraktar UD, Heath E, Sahin K, Andic F, Kucuk O. Lycopene in cancer prevention and treatment. *Am J Ther* 2008; **15**: 66-81 [PMID: 18223356 DOI: 10.1097/MJT.0b013e31804c7120]
- Heber D, Lu QY. Overview of mechanisms of action of lycopene. *Exp Biol Med* (Maywood) 2002; **227**: 920-923 [PMID: 12424335]
- Coyne T, Ibiebele TI, Baade PD, Dobson A, McClintock C, Dunn S, Leonard D, Shaw J. Diabetes mellitus and serum carotenoids: findings of a population-based study in Queensland, Australia. *Am J Clin Nutr* 2005; **82**: 685-693 [PMID: 16155284]
- Mariani S, Lionetto L, Cavallari M, Tubaro A, Rasio D, De Nunzio C, Hong GM, Borro M, Simmaco M. Low prostate concentration of lycopene is associated with development of prostate cancer in patients with high-grade prostatic intraepithelial neoplasia. *Int J Mol Sci* 2014; **15**: 1433-1440 [PMID: 24451130 DOI: 10.3390/ijms15011433]
- Liu CL, Huang YS, Hosokawa M, Miyashita K, Hu ML. Inhibition of proliferation of a hepatoma cell line by fucoxanthin in relation to cell cycle arrest and enhanced gap junctional intercellular communication. *Chem Biol Interact* 2009; **182**: 165-172 [PMID: 19737546 DOI: 10.1016/j.cbi.2009.08.017]
- Goyal PK. Cancer chemoprevention by natural products: current & future prospects. *J Integr Oncol* 2012; **1**: e101 [DOI: 10.4172/jio.1000e101]
- Gupta P, Bansal MP, Koul A. Spectroscopic characterization of lycopene extract from *Lycopersicon esculentum* (Tomato) and its evaluation as a chemopreventive agent against experimental hepatocarcinogenesis in mice. *Phytother Res* 2013; **27**: 448-456 [PMID: 22628278 DOI: 10.1002/ptr.4741]
- Gupta P, Bansal MP, Koul A. Evaluating the effect of lycopene from *Lycopersicon esculentum* on apoptosis during NDEA induced hepatocarcinogenesis. *Biochem Biophys Res Commun* 2013; **434**: 479-485 [PMID: 23583393 DOI: 10.1016/j.bbrc.2013.03.099]
- Gupta P, Bansal MP, Koul A. Lycopene modulates initiation of N-nitrosodiethylamine induced hepatocarcinogenesis: studies on chromosomal abnormalities, membrane fluidity and antioxidant defense system. *Chem Biol Interact* 2013; **206**: 364-374 [PMID: 24144777 DOI: 10.1016/j.cbi.2013.10.010]
- Crane RK, Sols A. Animal tissue hexokinases: (soluble and particulate forms). *Meth Enzymol* 1955; **1**: 277-286 [DOI: 10.1016/0076-6879(55)01037-9]
- Jagannathan V, Singh K, Damodaran M. Carbohydrate metabolism in citric acid fermentation: Purification and properties of aldolase from *Aspergillus niger*. *Biochem J* 1956; **63**: 94-105 [PMID: 13315254]



- 26 **Horrocks JE**, Ward J, King J. A routine method for the determination of phosphoglucose isomerase activity in body fluid. *J Clin Pathol* 1963; **16**: 248-251 [PMID: 13954993]
- 27 **Seifter S**, Dayton S. The estimation of glycogen with the anthrone reagent. *Arch Biochem* 1950; **25**: 191-200 [PMID: 15401229]
- 28 **Lowry OH**, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-275
- 29 **Stahl W**, Sies H. Bioactivity and protective effects of natural carotenoids. *Biochim Biophys Acta* 2005; **1740**: 101-107 [PMID: 15949675]
- 30 **Chandra S**, Sah K, Bagewadi A, Keluskar V, Shetty A, Ammanagi R, Naik Z. Additive and synergistic effect of phytochemicals in prevention of oral cancer. *Eur J of Gen Pract* 2012; **1**: 142-147 [DOI: 10.4103/2278-9626.105354]
- 31 **Shi J**, Qu Q, Kakuda Y, Yeung D, Jiang Y. Stability and synergistic effect of antioxidative properties of lycopene and other active components. *Crit Rev Food Sci Nutr* 2004; **44**: 559-573 [PMID: 15969328]
- 32 **Cairns RA**, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* 2011; **11**: 85-95 [PMID: 21258394 DOI: 10.1038/nrc2981]
- 33 **Paxton JR**, Bolger BS, Armour A, Symonds RP, Mao JH, Burnett RA. Apoptosis in cervical squamous carcinoma: predictive value for survival following radiotherapy. *J Clin Pathol* 2000; **53**: 197-200 [PMID: 10823138]
- 34 **Di Micco R**, Sulli G, Dobrev M, Lontos M, Botrugno OA, Gargiulo G, dal Zuffo R, Matti V, d'Ario G, Montani E, Mercurio C, Hahn WC, Gorgoulis V, Minucci S, d'Adda di Fagagna F. Interplay between oncogene-induced DNA damage response and heterochromatin in senescence and cancer. *Nat Cell Biol* 2011; **13**: 292-302 [PMID: 21336312 DOI: 10.1038/ncb2170]
- 35 **Warburg O**. On respiratory impairment in cancer cells. *Science* 1956; **124**: 269-270 [PMID: 13351639]
- 36 **Langeswaran K**, Revathy R, Kumar SG, Vijayaprakash S, Balasubramanian MP. Kaempferol ameliorates aflatoxin B1 (AFB1) induced hepatocellular carcinoma through modifying metabolizing enzymes membrane bound ATPases and mitochondrial TCA cycle enzymes. *Asian Pac J Trop Biomed* 2012; **2**: S1653-S1659 [DOI: 10.1016/S2221-1691(12)60471-7]
- 37 **Tsutsumi S**, Fukasawa T, Yamauchi H, Kato T, Kigure W, Morita H, Asao T, Kuwano H. Phosphoglucose isomerase enhances colorectal cancer metastasis. *Int J Oncol* 2009; **35**: 1117-1121 [PMID: 19787266]
- 38 **Lee JD**, Yang WI, Park YN, Kim KS, Choi JS, Yun M, Ko D, Kim TS, Cho AE, Kim HM, Han KH, Im SS, Ahn YH, Choi CW, Park JH. Different glucose uptake and glycolytic mechanisms between hepatocellular carcinoma and intrahepatic mass-forming cholangiocarcinoma with increased (18)F-FDG uptake. *J Nucl Med* 2005; **46**: 1753-1759 [PMID: 16204727]
- 39 **Kobayashi M**, Fujita I, Itagaki S, Hirano T, Iseki K. Transport mechanism for L-lactic acid in human myocytes using human prototypic embryonal rhabdomyosarcoma cell line (RD cells). *Biol Pharm Bull* 2005; **28**: 1197-1201 [PMID: 15997097]
- 40 **Jiang P**, Du W, Wang X, Mancuso A, Gao X, Wu M, Yang X. p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase. *Nat Cell Biol* 2011; **13**: 310-316 [PMID: 21336310 DOI: 10.1038/ncb2172]
- 41 **Edderkaoui M**, Hui H, Li G, Xu J, Lee WN, Go VLW, Pandolfi SJ. Phytochemicals inhibit proliferation and promote death through NADPH oxidase and G6PD. *Cancer Res* 2010; **70** (Suppl 8) Abstract Nr 1895 [DOI: 10.1158/1538-7445.AM10-1895]
- 42 **Wang XD**. Lycopene metabolism and its biological significance. *Am J Clin Nutr* 2012; **96**: 1214S-1222S [PMID: 23053559 DOI: 10.3945/ajcn.111.032359]
- 43 **Ebeid HM**, Gibriel AA, Al-Sayed HM, Elbehairy SA, Motawe EH. Hepatoprotective and antioxidant effects of wheat, carrot, and mango as nutraceutical agents against CCl4-induced hepatocellular toxicity. *J Am Coll Nutr* 2015; **34**: 228-231 [PMID: 25648457 DOI: 10.1080/07315724.2014.887486]
- 44 **Stoimenov I**, Helleday T. PCNA on the crossroad of cancer. *Biochem Soc Trans* 2009; **37**: 605-613 [PMID: 19442257 DOI: 10.1042/BST0370605]
- 45 **Perkins ND**. Not just a CDK inhibitor: regulation of transcription by p21(WAF1/CIP1/SDI1). *Cell Cycle* 2002; **1**: 39-41 [PMID: 12429907]
- 46 **Ip BC**, Hu KQ, Liu C, Smith DE, Obin MS, Ausman LM, Wang XD. Lycopene metabolite, apo-10'-lycopenoic acid, inhibits diethylnitrosamine-initiated, high fat diet-promoted hepatic inflammation and tumorigenesis in mice. *Cancer Prev Res (Phila)* 2013; **6**: 1304-1316 [PMID: 24085778 DOI: 10.1158/1940-6207.CAPR-13-0178]
- 47 **Baek JH**, Jang JE, Kang CM, Chung HY, Kim ND, Kim KW. Hypoxia-induced VEGF enhances tumor survivability via suppression of serum deprivation-induced apoptosis. *Oncogene* 2000; **19**: 4621-4631 [PMID: 11030151]
- 48 **Upadhyay J**, Kesharwani R.K, Misra K. Comparative study of antioxidants as cancer preventives through inhibition of HIF-1 alpha activity. *Bioinformation* 2009; **4**: 233-236 [PMID: 20975915]
- 49 **Burlaka AP**, Sidorik EP, Ganusevich II, Osinsky SP. Effects of radical oxygen species and NO: formation of intracellular hypoxia and activation of matrix metalloproteinases in tumor tissues. *Exp Oncol* 2006; **28**: 49-53 [PMID: 16614708]
- 50 **Thomas-Ahner JM**, Wan L, Tan HL, Moran NE, Elsen AC, Pearl DK, Erdman JW, Clinton SK. Tomato carotenoids and testosterone modulate mRNA and miRNA profiles during prostate carcinogenesis. *Cancer Res* 2013; **73** (8 Supplement): Abstract Nr. 3701 [DOI: 10.1158/1538-7445.AM2013-3701]

P- Reviewer: Camacho J, Xu MY S- Editor: Qi Y

L- Editor: Webster JR E- Editor: Li D



Case Control Study

## Polymorphisms of folate metabolism genes in patients with cirrhosis and hepatocellular carcinoma

Nathália Perpétua Peres, Ana Lívia Silva Galbiatti-Dias, Márcia Maria Urbanin Castanhole-Nunes, Renato Ferreira da Silva, Érika Cristina Pavarino, Eny Maria Goloni-Bertollo, Mariangela Torreglosa Ruiz-Cintra

Nathália Perpétua Peres, Ana Lívia Silva Galbiatti-Dias, Márcia Maria Urbanin Castanhole-Nunes, Érika Cristina Pavarino, Eny Maria Goloni-Bertollo, Mariangela Torreglosa Ruiz-Cintra, Genetics and Molecular Biology Research Unit, UPGEM, São José do Rio Preto 15090-000, Brazil

Nathália Perpétua Peres, Ana Lívia Silva Galbiatti-Dias, Márcia Maria Urbanin Castanhole-Nunes, Renato Ferreira da Silva, Érika Cristina Pavarino, Eny Maria Goloni-Bertollo, São José do Rio Preto Medical School, FAMERP and Medical School Foundation, FUNFARME, São José do Rio Preto 15090-000, Brazil

Renato Ferreira da Silva, Mariangela Torreglosa Ruiz-Cintra, Study Group of Liver Tumors, GETF, Hospital de Base, São José do Rio Preto 15090-000, Brazil

Mariangela Torreglosa Ruiz-Cintra, Federal University of the Triângulo Mineiro, UFTM, Uberaba 38064-200, Brazil

**Author contributions:** All the authors contribute to the manuscript.

**Institutional review board statement:** The research project CAAE 20465713.1.0000.5415 under the responsibility of Mariangela Ruiz Torreglosa Cintra titled "Polymorphisms of folate metabolism genes in patients with cirrhosis and hepatocellular carcinoma" is in accordance with the resolution of the CNS 466/12 and was approved by that Ethics Research Committee.

**Informed consent statement:** Approved by that Ethics Research Committee.

**Conflict-of-interest statement:** The authors declare no conflicts of interest regarding this manuscript.

**Data sharing statement:** Patient data and full dataset are available with open access from the corresponding author at [analiviagalbiattidias@gmail.com](mailto:analiviagalbiattidias@gmail.com). All Participants gave informed consent for data sharing. For more questions, please contact [eny.goloni@famerp.br](mailto:eny.goloni@famerp.br).

**Open-Access:** This article is an open-access article which was

selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Eny Maria Goloni-Bertollo, MD, São José do Rio Preto Medical School, FAMERP and Medical School Foundation, FUNFARME, Av. Brigadeiro Faria Lima, 5416, São José do Rio Preto 15090-000, Brazil. [eny.goloni@famerp.br](mailto:eny.goloni@famerp.br)  
 Telephone: +55-17-32015720

**Received:** May 13, 2016

**Peer-review started:** May 16, 2016

**First decision:** June 14, 2016

**Revised:** June 22, 2016

**Accepted:** August 15, 2016

**Article in press:** August 16, 2016

**Published online:** October 18, 2016

### Abstract

#### AIM

To evaluate the association of the risk factors and polymorphisms in *MTHFR C677T*, *MTHFR A1298C*, *MTR A2756G* and *MTRR A66G* genes.

#### METHODS

Patients with cirrhosis ( $n = 116$ ), hepatocellular carcinoma (HCC) ( $n = 71$ ) and controls ( $n = 356$ ) were included. Polymerase chain reaction followed by enzymatic digestion and allelic discrimination technique real-time PCR techniques were used for analysis. MINITAB-14.0

and SNPstats were utilized for statistical analysis.

## RESULTS

Showed that age  $\geq 46$  years (OR = 10.31; 95%CI: 5.66-18.76;  $P < 0.001$ ) and smoking (OR = 0.47; 95%CI: 0.28-0.78;  $P = 0.003$ ) were associated with cirrhosis. Age  $\geq 46$  years (OR = 16.36; 95%CI: 6.68-40.05;  $P < 0.001$ ) and alcohol habit (OR = 2.01; 95%CI: 1.03-3.89;  $P = 0.039$ ) were associated with HCC. *MTHFR A1298C* in codominant model (OR = 3.37; 95%CI: 1.52-7.50;  $P = 0.014$ ), recessive model (OR = 3.04; 95%CI: 1.43-6.47;  $P = 0.0051$ ) and additive model (OR = 1.71; 95%CI: 1.16-2.52;  $P = 0.0072$ ) was associated with HCC, as well as *MTR A2756G* in the additive model (OR = 1.68; 95%CI: 1.01-2.77;  $P = 0.047$ ), and *MTRR A66G* in the codominant model (OR = 3.26; 95%CI: 1.54-6.87;  $P < 0.001$ ), dominant model (OR = 2.55; 95%CI: 1.24-5.25;  $P = 0.007$ ) and overdominant model (OR = 3.05; 95%CI: 1.66-5.62;  $P < 0.001$ ). *MTR A2756G* in the additive model (OR = 1.54; 95%CI: 1.02-2.33;  $P = 0.042$ ) and smokers who presented at least one polymorphic allele for *MTRR A66G* (OR = 1.71; 95%CI: 0.77-3.82;  $P = 0.0051$ ) showed increased risk for cirrhosis. There was no association between clinical parameters and polymorphisms.

## CONCLUSION

Age  $\geq 46$  years, alcohol habit and *MTR A2756G*, *MTHFR A1298C* and *MTRR A66G* polymorphisms are associated with an increased risk of HCC development; age  $\geq 46$  years, tobacco habit and the *MTR A2756G* polymorphism are associated with cirrhosis.

**Key words:** Polymorphism; Folate metabolism; Liver cirrhosis; Hepatocellular carcinoma

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Our study is relevant because we can get better understanding on the mechanisms involved in the development of hepatocellular and Cirrhosis Carcinoma and folate metabolism. It is already known that polymorphisms cause DNA hypomethylation, which cause abnormal changes in gene expression inactivating suppressor genes tumor. In this study we have found some positive associations which was possible to understand the carcinogenesis of this tumor and offer new possibilities for diagnosis. Throughout these results it is possible to achieve better quality of life in early treatments.

Peres NP, Galbiatti-Dias ALS, Castanhole-Nunes MMU, da Silva RF, Pavarino EC, Goloni-Bertollo EM, Ruiz-Cintra MT. Polymorphisms of folate metabolism genes in patients with cirrhosis and hepatocellular carcinoma. *World J Hepatol* 2016; 8(29): 1234-1243 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i29/1234.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i29.1234>

## INTRODUCTION

Liver cancer is the second most common cause of death from cancer worldwide. Hepatocellular carcinoma (HCC) is considered the major form of primary liver cancer and is responsible for 70%-85% of all liver cancers<sup>[1]</sup>. Each year, more than half a million people are diagnosed with HCC. According to the most recent data, 782000 new cases per hundred thousand inhabitants have been diagnosed, with 745000 deaths resulting from this disease. HCC is the fifth most common cancer in men (554000 cases, 7.5% of all cases) and the ninth most common cancer in women (228000, 3.4% of all cases)<sup>[2]</sup>.

The major risk factor for HCC development, present in 90% of HCC patients, is liver cirrhosis, which is characterized by diffuse fibrosis, progressive and irreversible, with the presence of nodules delimited by fibrous septa<sup>[1,3]</sup>. There are other risk factors such as hepatitis B and C virus infection, liver disease derived from alcohol consumption, exposure to toxins such as aflatoxins and smoking, non-alcoholic fatty liver, obesity and diabetes<sup>[4,5]</sup>.

Cancer is a multifactorial disease that results from complex interactions between genetic and environmental factors<sup>[6]</sup>. Some studies have been conducted using genetic polymorphisms involved in folate metabolism in various types of cancers<sup>[7-11]</sup> because folate metabolism is essential for DNA synthesis and alterations in folate levels are associated with changes in DNA synthesis, methylation and repair, promoting genomic instability that contributes to the process of carcinogenesis<sup>[12]</sup>.

Several enzymes, including methylenetetrahydrofolate reductase enzyme (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR), are involved in folate metabolism<sup>[13]</sup>. Methylenetetrahydrofolate reductase (MTHFR) is a key regulatory enzyme in folate metabolism, and MTHFR can catalyze 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the predominant circulating form of folate. There are two common functional polymorphisms identified in the *MTHFR* gene, the *MTHFR C677T* polymorphism and *MTHFR A1298C* polymorphism<sup>[14]</sup>.

Moreover, 5-methyl-tetrahydrofolate donates one methyl group for homocysteine remethylation to methionine. The remethylation of this reaction is catalysed by the enzyme methionine synthase (MTR), which requires vitamin B12 as a cofactor. The enzyme methionine synthase reductase (MTRR) is responsible for maintaining the active state of the MTR enzyme. Polymorphisms *MTR A2756G* and *MTRR A66G* may cause decreased activity of the enzyme, leading to increased plasma homocysteine and DNA hypomethylation, which causes changes in gene expression, inactivating tumour suppressor genes and activate oncogenesis<sup>[15-19]</sup>.

Studies have confirmed that the genetic polymorphisms involved in folate metabolism may contribute to the development of HCC<sup>[16,19]</sup>. Therefore, the present

study was aimed to evaluate the association of risk factors and polymorphisms in the genes *MTHFR* C677T, *MTHFR* A1298C, *MTR* A2756G and *MTRR* A66G involved in folate metabolism with cirrhosis and HCC development in a case control study and to investigate the association of the polymorphisms with the clinical parameters of the disease in patients with cirrhosis and HCC.

## MATERIALS AND METHODS

### Ethical statement

Patients at the Liver, Intestine and Pancreas Transplant Unit of a university hospital in the northwest of the state of São Paulo, Brazil were included in the study after the diagnosis of liver cirrhosis and HCC, while the control group consisted of healthy individuals without cancer diagnosis. Individuals with a cancer family history were excluded from the control group. Informed consent was obtained from all subjects in this study, and the research protocol was approved by the Research Ethics Committee of FAMERP (CAAE: 20465713.1.0000.5415).

### Patients

In this case-control study, 543 subjects (116 patients with liver cirrhosis, 71 patients with HCC and 356 healthy individuals) were included regardless of sex and age, from 2013 to 2015. Patients with cirrhosis were included because it is known as a well-established risk factor in 90% of patients with HCC<sup>[1,3]</sup>.

The sample calculation was performed according to the reports of Kwak *et al.*<sup>[17]</sup> and Chang *et al.*<sup>[19]</sup> which presented a similar sample calculation. Furthermore, no study has evaluated polymorphisms in folate metabolism in HCC and cirrhosis development in the Brazilian population.

The diagnosis of HCC was based on the criteria of the American Association for the Study of Liver Diseases published in 2012<sup>[20]</sup>. Liver biopsy was performed when the diagnosis was not possible by imaging methods, and the diagnosis of cirrhosis was made by clinical, laboratory, ultrasound and histopathological examinations when possible.

The variables analysed in this study were age, gender, exposure to risk factors (smoking and alcohol habit) and the presence of the *MTHFR* A1298C, *MTHFR* C677T, *MTR* A2756G and *MTRR* A66G polymorphisms. We considered smokers to be those who consumed at least 100 cigarettes during their lifetime and alcohol consumers to be those who drink more than 4 drinks weekly, corresponding 30 mL of liquor, 102 mL of wine, and 340 mL of beer<sup>[21]</sup>.

Patients diagnosed with HCC were also classified according to the Barcelona Clinic Liver Cancer (BCLC) classification, which is a staging system that serves mainly for therapeutic guidance, in which the patient is ranked into five stages and includes other classifications. This classification uses variables related to tumour

stage, the functional state of the liver, physical condition, and symptoms related to cancer. Patients with stage 0, very early HCC and with only a minor 2-cm tumour were nominated for liver resection. Patients with early HCC phase A with up to 3-cm nodules were eligible for curative therapies (resection, liver transplantation or percutaneous treatments). Patients in phase B with intermediate HCC and this multinodular underwent chemoembolization. Patients in advanced stage C presenting portal invasion and metastases received new agents such as sorafenib, which is a palliative treatment, and patients in stage D with end-stage disease received symptomatic treatment<sup>[22]</sup>.

### Methods

Genomic DNA was extracted from peripheral blood leukocytes of the cases and controls according to Miller *et al.*<sup>[23]</sup> and was amplified by multiplex PCR-RFLP to identify the *MTHFR* C677T (rs1801133) and *MTHFR* A1298C (rs1801131), *MTR* A2756G (rs1805087) polymorphisms. The amplification product was subjected to digestion by the restriction enzymes Hinf I, Mbo II, and Hae III, respectively. Electrophoresis was performed in 2.5% agarose gels at 110 volts for 100 min. Allelic discrimination via the Real-Time PCR - SNP Genotyping Assay (Applied Biosystems) was used to identify the *MTRR* A66G (rs1801394) polymorphism, using primers and probes specific for each allele available by the manufacturer (*MTRR* A66G: C\_3068176\_10)<sup>[7]</sup>.

Genotyping confirmation was accomplished in 10% random samples of each group, and 100% concordance was observed.

### Statistical analysis

Hardy-Weinberg equilibrium (HWE) was performed using  $\chi^2$  test. The multiple regression logistic test by the Minitab program - Version 14.0 was used to determine the effects of variables. The model evaluated the following variables: Age (reference: < 46 years; median), smoking habits (reference: No smokers), alcohol habit (reference: Non-consumers) and gender (reference: Female). The polymorphisms were used to adjust the analysis.

The multiple logistic regression model adjusted for age, gender, smoking and alcohol habits was also used to assess the association between polymorphisms and the development of cirrhosis and HCC using the SNPStats program. The effect of the polymorphisms was evaluated in the following models: (1) codominant (heterozygous vs homozygous wild type and polymorphic homozygous vs homozygous wild type); (2) dominant (heterozygous more polymorphic homozygous vs homozygous wild type); (3) recessive (polymorphic homozygous vs homozygous wild type more heterozygous); (4) overdominant (wild homozygous vs heterozygous more polymorphic homozygote); and (5) additive (weight polymorphic homozygote vs heterozygote 2 more homozygous wild-type).

The SNPStats program was also used to assess the



**Table 1 Relationship between risk factors and hepatocellular carcinoma and liver cirrhosis development**

Variables	Controls <i>n</i> (%)	Cirrhosis <i>n</i> (%)	<sup>1</sup> OR (95%CI)	<i>P</i> -value	HCC <i>n</i> (%)	OR (95%CI)	<sup>2</sup> <i>P</i>
Age							
< 46 anos	238 (67)	22 (19)	10.31 (5.66-18.76)	<i>P</i> < 0.001	8 (11)	16.36 (6.68-40.05)	<i>P</i> < 0.001
≥ 46 anos	118 (33)	94 (81)			63 (89)		
Genre							
Female	95 (26.7)	30 (25.9)	0.96 (0.54-1.72)	<i>P</i> = 0.893	19 (26.8)	0.59 (0.29-1.22)	<i>P</i> = 0.154
Male	261 (73.3)	86 (74.1)			52 (73.2)		
Alcoholic habit							
Not	191 (54)	54 (46.6)	1.55 (0.91-2.63)	<i>P</i> = 0.106	27 (38)	2.01 (1.03-3.89)	<i>P</i> = 0.039
Yes	165 (46)	62 (53.4)			44 (62)		
Smoking habit							
Nonsmokers	199 (56)	72 (62)	0.47 (0.28-0.78)	<i>P</i> = 0.003	31 (43.7)	0.9 (0.48-1.67)	<i>P</i> = 0.734
Smokers	157 (44)	44 (38)			40 (56.3)		

<sup>1</sup>Odds ratio (OR) adjusted for age, genre, alcohol consumption, smoking habits and polymorphisms; <sup>2</sup>*P* values significant at *P* ≤ 0.05. HCC: Hepatocellular carcinoma.

potential interaction between the polymorphisms with variables associated with cirrhosis and HCC development (tobacco and alcohol habits) through multiple logistic regression.

The *MTHFR* haplotypes were inferred using the Haploview 4.2 statistical program, which creates population frequency estimates of the haplotypes.

The association between the clinical parameters and polymorphisms with HCC development were also analysed by multiple logistic regression. The patients were subjected to classification and BCLC staging divided into five stages (0, A, B, C and D). The variables alpha fetoprotein dose values, hepatitis B and C, diabetes mellitus and death were utilized in the adjustment of the analysis. The models included BCLC classification (reference: 0, A), alpha fetoprotein (reference: < 500 ng/mL), hepatitis B (reference: No), hepatitis C virus (reference: No), diabetes (reference: Absence), death (reference: No) and the studied polymorphisms (reference: Wild-type genotype).

The Kaplan-Meier method was applied to evaluate the survival rate by considering the period between the disease diagnosis and death to be the end point.

The results were presented as ORs and 95%CIs. The level of significance was set at 5% (*P* = 0.05).

## RESULTS

The results for HWE were similar to those expected in both the case and control groups, respectively, for the *MTHFR* C677T ( $\chi^2$  = 0.8940, *P* = 0.3444 and  $\chi^2$  = 3.1218, *P* = 0.0772), *MTR* A2756G ( $\chi^2$  = 1.1554, *P* = 0.2824 and  $\chi^2$  = 1.1929, *P* = 0.2748) and *MTRR* A66G polymorphisms ( $\chi^2$  = 3, 2227, *P* = 0.0726 and  $\chi^2$  = 0.0530, *P* = 0.8018). However, the *MTHFR* A1298C polymorphism showed no equilibrium ( $\chi^2$  = 8.0244, *P* = 0.0046 and  $\chi^2$  = 8.6427, *P* = 0.0033) for patients with HCC and/or cirrhosis and controls.

Table 1 shows the results for multiple logistic regression analysis between patients with liver cirrhosis and control subjects to determine the effects of variables. Age

≥ 46 years (OR = 10.31; 95%CI: 5.66-18.76; *P* < 0.001) and smoking habit were associated with the disease (OR = 0.47; 95%CI: 0.28-0.78; *P* = 0.003), and the analysis of patients with HCC and control subjects showed that age ≥ 46 years (OR = 16.36; 95%CI: 6.68-40.05; *P* < 0.001) and alcohol habit (OR = 2.01; 95%CI: 1.03-3.89; *P* = 0.039) were associated with the disease.

Table 2 shows the association of the *MTHFR* C677T, *MTHFR* A1298C, *MTR* A2756G and *MTRR* A66G polymorphisms with HCC, adjusted for gender, age, smoking and alcohol habit according to the heritage models. The *MTHFR* A1298C polymorphism in the codominant model (OR = 3.37; 95%CI: 1.52-7.50; *P* = 0.014), recessive model (OR = 3.04; 95%CI: 1.43-6.47; *P* = 0.0051) and additive model (OR = 1.71; 95%CI: 1.16-2.52; *P* = 0.0072), the *MTR* A2756G polymorphism in the additive model (OR = 1.68; 95%CI: 1.01-2.77; *P* = 0.047), the *MTRR* A66G polymorphism in the codominant model (OR = 3.26; 95%CI: 1.54-6.87; *P* < 0.001), dominant model (OR = 2.55; 95%CI: 1.24-5.25; *P* = 0.007) and overdominant model (OR = 3.05; 95%CI: 1.66-5.62; *P* < 0.001) were associated with an increased risk of HCC development.

Table 3 shows the association of the *MTHFR* C677T, *MTHFR* A1298C, *MTR* A2756G and *MTRR* A66G polymorphisms with liver cirrhosis, adjusted for gender, age, smoking and alcohol habit according to the heritage models. The *MTR* A2756G polymorphism was associated with an increased risk of liver cirrhosis in the additive model (OR = 1.54; 95%CI: 1.02-2.33; *P* = 0.042).

Regarding the potential interaction among the polymorphisms with variables associated with the diseases, there was no interaction among the *MTHFR* C677T, *MTHFR* A1298C, *MTR* A2756G and *MTRR* A66G polymorphisms and smoking habit or alcohol habit regarding the risk of HCC (Table 4). However, smokers who presented with the heterozygous genotype (AG) or polymorphic homozygote genotype (GG) for the *MTRR* gene (OR = 1.71; 95%CI: 0.77-3.82; *P* = 0.0051) was associated with liver cirrhosis (Table 5).

The haplotype analysis showed a higher frequency

**Table 2** Association of *MTHFR C677T*, *MTHFR A1298C*, *MTR A2756G* and *MTRR A66G* polymorphisms with hepatocellular carcinoma, adjusted for gender, age, smoking and alcohol consumption

Model	Genotype	Control <i>n</i> (%)	Case <i>n</i> (%)	<sup>1</sup> OR (95%CI)	<sup>2</sup> P-value	Genotype	Control <i>n</i> (%)	Case <i>n</i> (%)	<sup>1</sup> OR (95%CI)	<sup>2</sup> P-value
Codominant	<i>MTHFR C677T</i> C/C	149 (41.9)	28 (39.4)	1	0.91	<i>MTHFR A1298C</i> A/A	205 (57.6)	32 (45.1)	1	0.014
	C/T	174 (48.9)	36 (50.7)	0.93 (0.51-1.68)		A/C	116 (32.6)	24 (33.8)	1.29 (0.69-2.42)	
	T/T	33 (9.3)	7 (9.9)	1.13 (0.41-3.09)		C/C	35 (9.8)	15 (21.1)	3.37 (1.52-7.50)	
Dominant	C/C	149 (41.9)	28 (39.4)	1	0.88	A/A	205 (57.6)	32 (45.1)	1	0.06
Recessive	C/T-T/T	207 (58.1)	43 (60.6)	0.96 (0.54-1.70)		A/C-C/C	151 (42.4)	39 (54.9)	1.71 (0.98-2.99)	
	C/C-C/T	323 (90.7)	64 (90.1)	1		A/A-A/C	321 (90.2)	56 (78.9)	1	
Overdominant	T/T	33 (9.3)	7 (9.9)	1.18 (0.46-3.05)	0.73	C/C	35 (9.8)	15 (21.1)	3.04 (1.43-6.47)	0.0051
	C/C-T/T	182 (51.1)	35 (49.3)	1	0.73	A/A-C/C	240 (67.4)	47 (66.2)	1	0.98
Additive	C/T	174 (48.9)	36 (50.7)	0.91 (0.52-1.59)		A/C	116 (32.6)	24 (33.8)	0.99 (0.55-1.78)	
	---	---	---	1.01 (0.64-1.58)	0.97	---	---	---	1.71 (1.16-2.52)	0.007
Codominant	<i>MTR A2756G</i> A/A	263 (73.9)	46 (64.8)	1	0.13	<i>MTRR A66G</i> A/A	105 (29.5)	12 (16.9)	1	< 0.001
	A/G	83 (23.3)	21 (29.6)	1.58 (0.84-2.98)		A/G	179 (50.3)	50 (70.4)	3.26 (1.54-6.87)	
	G/G	10 (2.8)	4 (5.6)	3.29 (0.81-13.30)		G/G	72 (20.2)	9 (12.7)	1.16 (0.44-3.11)	
Dominant	A/A	263 (73.9)	46 (64.8)	1	0.078	A/A	105 (29.5)	12 (16.9)	1	0.0072
Recessive	A/G-G/G	93 (26.1)	25 (35.2)	1.73 (0.95-3.15)		A/G-G/G	251 (70.5)	59 (83.1)	2.55 (1.24-5.25)	
	A/A-A/G	346 (97.2)	67 (94.4)	1		A/A-A/G	284 (79.8)	62 (87.3)	1	
Overdominant	G/G	10 (2.8)	4 (5.6)	2.91 (0.73-11.59)	0.15	G/G	72 (20.2)	9 (12.7)	0.51 (0.23-1.12)	0.077
	A/A-G/G	273 (76.7)	50 (70.4)	1	0.22	A/A-G/G	177 (49.7)	21 (29.6)	1	< 0.001
Additive	A/G	83 (23.3)	21 (29.6)	1.49 (0.80-2.79)		A/G	179 (50.3)	50 (70.4)	3.05 (1.66-5.62)	
	---	---	---	1.68 (1.01-2.77)	0.047	---	---	---	1.16 (0.77-1.73)	0.48

<sup>1</sup>Odds ratio (OR) adjusted for age, gender and alcohol consumption and smoking habits; <sup>2</sup>P values significant at  $P \leq 0.05$ .**Table 3** Association of *MTHFR C677T*, *MTHFR A1298C*, *MTR A2756G* and *MTRR A66G* polymorphisms with Liver Cirrhosis, adjusted for gender, age, smoking and alcohol consumption

Model	Genotype	Control <i>n</i> (%)	Case <i>n</i> (%)	<sup>1</sup> OR (95%CI)	<sup>2</sup> P-value	Genotype	Control <i>n</i> (%)	Case <i>n</i> (%)	<sup>1</sup> OR (95%CI)	<sup>2</sup> P-value
Codominant	<i>MTHFR C677T</i> C/C	149 (41.9)	48 (41.4)	1	0.56	<i>MTHFR A1298C</i> A/A	205 (57.6)	57 (49.1)	1	0.21
	C/T	174 (48.9)	55 (47.4)	0.90 (0.56-1.45)		A/C	116 (32.6)	43 (37.1)	1.27 (0.78-2.07)	
	T/T	33 (9.3)	13 (11.2)	1.37 (0.64-2.95)		C/C	35 (9.8)	16 (13.8)	1.85 (0.92-3.71)	
Dominant	C/C	149 (41.9)	48 (41.4)	1	0.89	A/A	205 (57.6)	57 (49.1)	1	0.14
Recessive	C/T-T/T	207 (58.1)	68 (58.6)	0.97 (0.62-1.52)		A/C-C/C	151 (42.4)	59 (50.9)	1.40 (0.90-2.18)	
	C/C-C/T	323 (90.7)	103 (88.8)	1		A/A-A/C	321 (90.2)	100 (86.2)	1	
Overdominant	T/T	33 (9.3)	13 (11.2)	1.45 (0.71-2.99)	0.32	C/C	35 (9.8)	16 (13.8)	1.68 (0.86-3.29)	0.14
	C/C-T/T	182 (51.1)	61 (52.6)	1	0.47	A/A-C/C	240 (67.4)	73 (62.9)	1	0.58
Additive	C/T	174 (48.9)	55 (47.4)	0.85 (0.54-1.33)		A/C	116 (32.6)	43 (37.1)	1.14 (0.72-1.82)	
	---	---	---	1.07 (0.75-1.51)	0.72	---	---	---	1.33 (0.97-1.83)	0.079
Codominant	<i>MTR A2756G</i> A/A	263 (73.9)	79 (68.1)	1	0.13	<i>MTRR A66G</i> A/A	105 (29.5)	37 (31.9)	1	0.95
	A/G	83 (23.3)	32 (27.6)	1.52 (0.91-2.53)		A/G	179 (50.3)	55 (47.4)	0.94 (0.56-1.56)	
	G/G	10 (2.8)	05 (4.3)	2.47 (0.75-8.12)		G/G	72 (20.2)	24 (20.7)	0.91 (0.49-1.71)	
Dominant	A/A	263 (73.9)	79 (68.1)	1	0.06	A/A	105 (29.5)	37 (31.9)	1	0.77
Recessive	A/G-G/G	93 (26.1)	37 (31.9)	1.60 (0.98-2.61)		A/G-G/G	251 (70.5)	79 (68.1)	0.93 (0.58-1.50)	
	A/A-A/G	346 (97.2)	111 (95.7)	1		A/A-A/G	284 (79.8)	92 (79.3)	1	
Overdominant	G/G	10 (2.8)	5 (4.3)	2.19 (0.68-7.09)	0.21	G/G	72 (20.2)	24 (20.7)	0.95 (0.55-1.64)	0.86
	A/A-G/G	273 (76.7)	84 (72.4)	1	0.15	A/A-G/G	177 (49.7)	61 (52.6)	1	0.9
Additive	A/G	83 (23.3)	32 (27.6)	1.46 (0.88-2.41)		A/G	179 (50.3)	55 (47.4)	0.97 (0.62-1.52)	
	---	---	---	1.54 (1.02-2.33)	0.042	---	---	---	0.95 (0.70-1.30)	0.77

<sup>1</sup>Odds ratio (OR) adjusted for age, gender and alcohol consumption and smoking habits; <sup>2</sup>P values significant at  $P \leq 0.05$ .

(40.6%) of the AC haplotype observed in both groups (Case group: 0.403, Control group: 0.407;  $\chi^2 = 0.01$ ,  $P = 0.9194$ ). The haplotype frequencies of AT (Case group: 0.283, Control group: 0.312;  $\chi^2 = 0.843$ ,  $P = 0.3584$ ),

haplotype frequencies of CC (Case group: 0.269, Control group: 0.247;  $\chi^2 = 0.573$ ,  $P = 0.4491$ ), and haplotype frequencies of CT (Case group: 0.045, Control group: 0.035;  $\chi^2 = 0.569$ ,  $P = 0.4505$ ) did not show significant

**Table 4** Interaction between *MTHFR C677T*, *MTHFR A1298C*, *MTR A2756G* and *MTRR A66G* polymorphisms and smoking habits or alcohol drinking on the risk of hepatocellular carcinoma

	Smoking habits						<sup>2</sup> <i>p</i> interaction	Alcoholic habit						<sup>2</sup> <i>p</i> interaction
	No smoker			Smoker				Non-alcoholic			Alcoholic			
	Case	Control	<sup>1</sup> OR (95%CI)	Case	Control	<sup>1</sup> OR (95%CI)		Case	Control	<sup>1</sup> OR (95%CI)	Case	Control	<sup>1</sup> OR (95%CI)	
<i>MTR A2756G</i>														
A/A	22	142	1.00	24	121	1.00	0.81	17	137	1.00	29	126	1.00	0.43
A/G-G/G	10	57	1.59 (0.65-3.87)	15	36	1.85 (0.81-4.21)		11	53	2.29 (0.91-5.72)	14	40	1.40 (0.63-3.12)	
<i>MTR R66G</i>														
A/A	4	55	1.00	8	50	1.00	0.7	3	53	1.00	9	52	1.00	0.34
A/G-G/G	28	144	3.04 (0.95-9.71)	31	107	2.27 (0.90-5.72)		25	137	4.16 (1.11-15.55)	34	114	1.98 (0.83-4.75)	
<i>MTHFR C677T</i>														
C/C	15	85	1.00	13	64	1.00	0.55	14	85	1.00	14	64	1.00	0.75
C/T-T/T	17	114	0.80 (0.35-1.81)	26	93	1.13 (0.51-2.52)		14	105	0.86 (0.36-2.06)	29	102	1.04 (0.48-2.22)	
<i>MTHFR A1298C</i>														
A/A	13	119	1.00	19	85	1.00	0.61	11	113	1.00	21	91	1.00	0.56
A/C-C/C	19	80	2.00 (0.88-4.56)	20	72	1.49 (0.69-3.20)		17	77	2.10 (0.86-5.10)	22	75	1.49 (0.72-3.07)	

<sup>1</sup>Odds ratio (OR) adjusted for age, gender and alcohol consumption and smoking habits; <sup>2</sup>P values significant at  $P \leq 0.05$ .

**Table 5** Interaction between *MTHFR C677T*, *MTHFR A1298C*, *MTR A2756G* and *MTRR A66G* polymorphisms and smoking habits or alcohol drinking on the risk of liver cirrhosis

	Smoking habits							<sup>2</sup> <i>p</i> interaction	Alcoholic habit						<sup>2</sup> <i>p</i> interaction
	No smoker			Smoker			Non-alcoholic			Alcoholic					
	Case	Control	<sup>1</sup> OR (95%CI)	Case	Control	OR* (95%CI)	Case		Control	<sup>1</sup> OR (95%CI)	Case	Control	<sup>1</sup> OR (95%CI)		
<i>MTR</i> A2756G															
A/A	50	142	1.00	29	121	1.00	0.39	36	137	1.00	43	126	1.00	0.73	
A/G-G/G	21	57	1.35 (0.72-2.53)	16	36	2.07 (0.97-4.41)		17	53	1.46 (0.72-2.97)	20	40	1.74 (0.89-3.38)		
<i>MTRR</i> A66G															
A/A	26	55	1.00	11	50	1.00	0.051	14	53	1.00	23	52	1.00	0.42	
A/G-G/G	45	144	0.63 (0.35-1.17)	34	107	1.71 (0.77-3.82)		39	137	1.17 (0.56-2.45)	40	114	0.78 (0.42-1.48)		
<i>MTHFR</i> C677T															
C/C	31	85	1.00	17	64	1.00	0.96	24	85	1.00	24	64	1.00	0.76	
C/T-T/T	40	114	0.96 (0.54-1.71)	28	93	0.98 (0.48-2.02)		29	105	1.05 (0.54-2.02)	39	102	0.91 (0.49-1.69)		
<i>MTHFR</i> A1298C															
A/A	33	120	1.00	24	85	1.00	0.32	23	113	1.00	34	92	1.00	0.56	
A/C-C/C	38	79	1.68 (0.95-3.00)	21	72	1.06 (0.53-2.13)		30	77	1.83 (0.95-3.54)	29	74	1.11 (0.61-2.03)		

<sup>1</sup>Odds ratio (OR) adjusted for age, gender and alcohol consumption and smoking habits; <sup>2</sup>P values significant at  $P \leq 0.05$ .

results.

There was no association in the multiple logistic regression analysis of the analysed clinical parameters and polymorphisms in patients with HCC stratified into tumours in stages 0 and A, and tumours in stages B, C and D, according to the BCLC criteria (Table 6).

The Kaplan-Meier survival curves for genotype showed no association of polymorphisms and overall survival with HCC development. No polymorphism was associated (*MTHFR C677T*,  $P = 0.5483$ ; *MTHFR A1298C*,  $P = 0.3861$ ; *MTR A2756G*,  $P = 0.6765$ ; *MTRR A66G*,  $P = 0.3840$ )

with overall survival.

## DISCUSSION

The results showed that age  $\geq 46$  years and alcohol habit were associated with an increased risk of HCC development, similar to the results of Fassio *et al.*<sup>[24]</sup>, Varela *et al.*<sup>[25]</sup>, Munaka *et al.*<sup>[26]</sup>, Carrilho *et al.*<sup>[3]</sup>, Donato *et al.*<sup>[27]</sup>, Hamed *et al.*<sup>[28]</sup> and Mittal *et al.*<sup>[1]</sup>.

Brazilian publications have reported that the mean age of the HCC patients is 54.6 years<sup>[3]</sup>; in Latin

**Table 6** Regression analysis of data from multiple logistic analyzed clinical parameters and polymorphisms in patients with hepatocellular carcinoma tumors divided into stages 0 and tumor in stages A and B, C and D according barcelona clinic liver cancer criteria

Variables	Stage 0 e A Pacientes n (%)	Estage B, C e D Pacientes n (%)	OR (95%CI) <sup>1</sup>	P
Alpha fetoprotein				
> 500 ng/mL	22 (84.6)	21 (46.7)	Reference	Reference
< 500 ng/mL	4 (15.4)	24 (53.3)	2.66 (0.55-12.72)	0.22
Hepatitis B virus				
Absence	25 (96.2)	38 (84.4)	Reference	Reference
Presence	1 (3.85)	7 (15.6)	4.06 (0.34-48.29)	0.27
Hepatitis C virus				
Absence	12 (46.2)	22 (48.9)	Reference	Reference
Presence	14 (53.8)	23 (41.1)	1.43 (0.35-5.78)	0.61
Steatohepatitis				
Absence	26 (100)	42 (93.3)	Reference	Reference
Presence	00 (00)	3 (6.7)	<sup>3</sup>	0.99
Diabetes				
Absence	18 (69.2)	32 (71.1)	Reference	Reference
Presence	8 (30.8)	13 (28.9)	0.25 (0.03-1.63)	0.15
Death				
No	24 (92.3)	17 (37.8)	Reference	Reference
Yes	2 (7.7)	28 (62.2)	25.3 (3.67-174.38)	0.001 <sup>2</sup>
MTHFR A1298C				
AA	10 (38.5)	22 (48.9)	Reference	Reference
AC/CC	16 (61.5)	23 (51.1)	0.93 (0.22-3.86)	0.92
MTHFR C677T				
CC	11 (42.3)	17 (37.8)	Reference	Reference
CT/TT	15 (57.7)	28 (62.2)	1.65 (0.34-7.97)	0.53
MTR A2756G				
AA	18 (69.2)	28 (62.2)	Reference	Reference
AG/GG	8 (30.8)	17 (37.8)	0.78 (0.18-3.43)	0.75
MTRRA66G				
AA	4 (15.4)	8 (17.8)	Reference	Reference
AG/GG	22 (84.6)	37 (82.2)	1.09 (0.16-7.30)	0.93

<sup>1</sup>OR: Odds ratio, CI: Confidence interval; <sup>2</sup>Statistically significant at  $P \leq 0.05$ ; <sup>3</sup>Could not calculate due to numerical proximity.

America, the average age in one published study was 64 years<sup>[24]</sup>; in another multicentre study in Spain, the reported average age was 65.6 years<sup>[25]</sup>, and Mittal *et al.*<sup>[1]</sup> concluded that a more recent increase in the incidence of HCC in the United States population was seen in Hispanics and blacks between the ages of 45 and 65 years, results that are similar to ours.

Regarding the result that alcohol consumption was also significant and more frequent in patients with HCC, a prospective case-control study from Japan has observed that heavy alcohol drinkers had a five-fold increase in the risk of HCC compared with non-drinkers<sup>[26]</sup>. Donato *et al.*<sup>[27]</sup> 2002 in Italy, with a sample size of 464 cases and 824 controls individuals, found a positive relationship between alcohol consumption and HCC. The latter findings were confirmed in review of Hamed *et al.*<sup>[28]</sup>.

Evidence of a positive association between heavy alcohol drinking and liver cancer is derived mainly from case-control studies. The increased risk of those drinking 6 or more drinks per day compared with non-drinkers was 22%. Alcohol was the only cause present in 14% of cases in the 2010 Brazilian study by Carrilho *et al.*<sup>[3]</sup>. Thus, the significance indexes are increased because

drinking alcohol causes poor absorption of vitamin B complex, changing the folate metabolism and causing oxidative damage and breaks in the DNA strands<sup>[29]</sup>. In our study we found the association between alcohol and HCC development, this may be due the fact described above.

Regarding cirrhosis, we found that age  $\geq 46$  years and smoking habit was associated with risk of cirrhosis. As previously mentioned, 85%-90% of primary liver cirrhosis causes cancer, and multiple nonviral factors that are concerned with the development of liver cancer include iron overload syndromes, alcohol use, tobacco, oral contraceptives, aflatoxin, and pesticide exposure, which is prevalent in the developing world<sup>[28]</sup>. Regarding the tobacco habit, the data showed that 38% of individuals with cirrhosis had a tobacco habit. In addition to the liver, which is the target of chemical compounds in tobacco that can progress to cirrhosis, it was also observed in the literature that the development of diseases related to the progression to cirrhosis occurs more frequently in older individuals<sup>[30]</sup>.

In addition to the association that we found between tobacco habit and cirrhosis development, there can be a relationship of tobacco with the dysfunction of genes as



well as enzymes involved in the detoxification of nicotine, consequences that generate various types of liver-related diseases such as fibrosis, alcoholic hepatitis, cirrhosis and HCC<sup>[31]</sup>. Although alcohol habit is a well-established risk factor for cirrhosis development<sup>[32]</sup>, our study did not find this association. However, 53.4% of cirrhosis patients in the present study were alcohol consumers.

In relation to the genetic characteristics, the present study was the first to be performed in a Brazilian population with HCC and cirrhosis and revealed that the *MTR* A2756G, *MTHFR* A1298C and *MTRR* A66G polymorphisms were associated with an increased risk of HCC development, results similar to the studies of Kwak *et al.*<sup>[17]</sup> and Yu *et al.*<sup>[33]</sup>.

Chang *et al.*<sup>[19]</sup>, with a sample of 204 patients with liver cancer and 415 controls found an association between *MTR* A2756G and increased risk for the disease, as well the meta-analysis performed by Yu *et al.*<sup>[33]</sup> that reported a significantly higher association between the genotype and 2756GG cancer risk in Asian populations.

There are studies involving other cancers that have found a positive association with at least one polymorphic allele 2756G and an increased risk of the development of disease. For example, the Hosseini *et al.*<sup>[8]</sup> that evaluated 592 individuals in Iran found an association between in *MTR* GG genotype and breast cancer; Galbiatti *et al.*<sup>[7]</sup> also concluded that *MTR* A2756G polymorphism is involved in the risk of head and neck cancer; de Lima *et al.*<sup>[9]</sup> suggested an association between the *MTR* A2756G polymorphism and retinoblastoma susceptibility in a northeast population of Brazil, Ouerhani *et al.*<sup>[10]</sup> found that *MTR* A2756G affecting bladder cancer risk.

Regarding the *MTRR* A66G polymorphism, Kwak *et al.*<sup>[17]</sup> studied 96 patients and 201 controls and observed an association between the polymorphism and an increased risk of HCC, a finding that has also been found in other types of cancer; the Wu *et al.*<sup>[11]</sup> study demonstrated a positive relationship with the *MTRR* A66G polymorphism and breast cancer, and a meta-analysis performed by Zhou *et al.*<sup>[34]</sup> also found an association between this polymorphism and colorectal cancer, which is in agreement with our study. However, the study of Zhang *et al.*<sup>[35]</sup> did not find an association of this polymorphism with HCC development.

We also found an association between the *MTHFR* A1298C polymorphism and an increased risk of HCC development. Two meta-analyses reported an association of this polymorphism with a decreased risk of HCC, demonstrating a protective effect<sup>[36,37]</sup>. However, Liang *et al.*<sup>[38]</sup> meta-analysis of a total of seven studies showed that the homozygote genotype CC of the *MTHFR* rs1801131 polymorphism was significantly associated with a decreased risk of liver cancer (for CC vs AA: OR = 0.65, 95%CI: 0.47-0.89, *P* = 0.007; for CC vs AA + AC: OR = 0.65, 95%CI: 0.48-0.89, *P* = 0.006), similar our study.

Our results for cirrhosis and polymorphisms showed an association between *MTR* A2756G and an increased

risk of the disease. There are no studies in the literature that have evaluated the association between the *MTR* A2756G polymorphism and cirrhosis development. The present study is the first to investigate the *MTR* A2756G polymorphism and cirrhosis development, and the association that was found can be related to alteration of the *MTR* enzyme that occurs due to the presence of the *MTR* A2756G polymorphism. The alteration of the *MTR* enzyme causes elevation in the homocysteine levels and DNA hypomethylation, leading to chromosomal instability, mutations and the overexpression of proto-oncogenes that can be associated with the development of several types of diseases, including cirrhosis. However, more studies in different populations of individuals with cirrhosis are needed<sup>[39]</sup>.

Regarding the potential interaction among the polymorphisms with variables associated with the diseases, we found that smoking in those with the heterozygous genotype (AG) or polymorphic homozygote genotype (GG) for *MTRR* gene was associated with liver cirrhosis. There are no studies that have investigated this interaction, however, tobacco habit can be related to cirrhosis because the chemical compounds can modify the liver and lead to cirrhosis<sup>[39]</sup> independently of the *MTRR* A66G polymorphism.

Regarding BCLC classification, we did not find an association with the polymorphisms evaluated. Our data showed that 7% of patients in stage 0, 29.6% in stage A, 22.5% in stage B, 31% in stage C and 9.8% in stage D. The study of Varela *et al.*<sup>[25]</sup> reported that 49.8% of 705 cases were in the initial stage (A), 19.8% in the intermediate stage (B), 18.8% in the advanced stage (C) and 11.6% in the terminal phase (D). Additionally, the study of Raphe *et al.*<sup>[40]</sup> reported that 32.7% were in stage A, 22% in stage B, 30.4% in stage C, 14% in stage D. Current published data show that patients who are in stage A are asymptomatic and have preserved liver function have a 5-year survival of 50%-75%. Patients who are in stage B have a median survival of 20 mo; those who are already in the C and D stages have severe liver dysfunction and extrahepatic metastases reach an 11-mo survival, and only 10% of patients in the D stage survive more than a year with an average survival of 3-4 mo<sup>[26]</sup>.

In conclusion, age  $\geq$  46 years, alcohol habit and the *MTR* A2756G, *MTHFR* A1298C and *MTRR* A66G polymorphisms are associated with an increased risk of HCC development; age  $\geq$  46 years, tobacco habit and the *MTR* A2756G polymorphism are associated with cirrhosis development. There is an interaction between the *MTRR* A66G polymorphism and tobacco consumers with liver cirrhosis. The present study can collaborate to establish the etiologic factors related to HCC and cirrhosis development and to contribute to strategies related to health care.

## ACKNOWLEDGMENTS

We greatly appreciate the Faculdade de Medicina de

São José do Rio Preto, FAMERP and Medical School Foundation, FUNFARME for their institutional support and UPGEM-Genetics and Molecular Biology Research Unit.

## COMMENTS

### Background

Age  $\geq 46$  years, alcohol habit and the *MTR* A2756G, *MTHFR* A1298C and *MTRR* A66G polymorphisms are associated with an increased risk of hepatocellular carcinoma (HCC) development; age  $\geq 46$  years, tobacco habit and the *MTR* A2756G polymorphism are associated with cirrhosis development.

### Research frontiers

It is already known that polymorphisms cause DNA hypomethylation, which cause abnormal changes in gene expression inactivating suppressor genes tumor.

### Innovations and breakthroughs

The authors confirm the literature data that report a positive association between the presence of polymorphisms and consumption of alcohol and tobacco to the development in an cirrhosis and later HCC.

### Applications

These results may offer new possibilities of diagnosis with early initiation of treatment reflecting the improved quality of life.

### Peer-review

This is a good descriptive study in which the authors analysed patients with cirrhosis, HCC and healthy individuals in the 2013-2015 period, the authors evaluated the association of the risk factors and polymorphisms in *MTHFR* C677T, *MTHFR* A1298C, *MTR* A2756G and *MTRR* A66G genes involved in folate metabolism.

## REFERENCES

- Mittal S, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. *J Clin Gastroenterol* 2013; **47** Suppl: S2-S6 [PMID: 23632345 DOI: 10.1097/MCG.0b013e3182872f29]
- Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]
- Carrilho FJ, Kikuchi L, Branco F, Goncalves CS, Mattos AA. Clinical and epidemiological aspects of hepatocellular carcinoma in Brazil. *Clinics (Sao Paulo)* 2010; **65**: 1285-1290 [PMID: 21340216 DOI: 10.1590/S1807-59322010001200010]
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
- Bosetti C, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. *Best Pract Res Clin Gastroenterol* 2014; **28**: 753-770 [PMID: 25260306 DOI: 10.1016/j.bpg.2014.08.007]
- Pharoah PD, Dunning AM, Ponder BA, Easton DF. Association studies for finding cancer-susceptibility genetic variants. *Nat Rev Cancer* 2004; **4**: 850-860 [PMID: 15516958 DOI: 10.1038/nrc1476]
- Galbiatti AL, da Silva LM, Ruiz-Cintra MT, Raposo LS, Maníglia JV, Pavarino EC, Goloni-Bertollo EM. Association between 11 genetic polymorphisms in folate-metabolising genes and head and neck cancer risk. *Eur J Cancer* 2012; **48**: 1525-1531 [PMID: 22051736 DOI: 10.1016/j.ejca.2011.09.025]
- Hosseini M. Role of polymorphism of methyltetrahydrofolate-homocysteine methyltransferase (*MTR*) A2756G and breast cancer risk. *Pol J Pathol* 2013; **64**: 191-195 [PMID: 24166605 DOI: 10.5114/pjp.2013.38138]
- de Lima EL, da Silva VC, da Silva HD, Bezerra AM, de Moraes VL, de Moraes AL, Cruz RV, Barros MH, Hassan R, de Freitas AC, Muniz MT. *MTR* polymorphic variant A2756G and retinoblastoma risk in Brazilian children. *Pediatr Blood Cancer* 2010; **54**: 904-908 [PMID: 20310006 DOI: 10.1002/pbc.22472]
- Ouerhani S, Rouissi K, Marrakchi R, Ben Slama MR, Sfaki M, Chebil M, ElGaaied AB. Combined effect of NAT2, *MTR* and *MTHFR* genotypes and tobacco on bladder cancer susceptibility in Tunisian population. *Cancer Detect Prev* 2009; **32**: 395-402 [PMID: 19588544 DOI: 10.1016/j.canep.2009.04.005]
- Wu X, Zou T, Cao N, Ni J, Xu W, Zhou T, Wang X. Plasma homocysteine levels and genetic polymorphisms in folate metabolism are associated with breast cancer risk in Chinese women. *Hered Cancer Clin Pract* 2014; **12**: 2 [PMID: 24559276 DOI: 10.1186/1897-4287-12-2]
- Duthie SJ. Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. *J Inher Metab Dis* 2011; **34**: 101-109 [PMID: 20544289 DOI: 10.1007/s10545-010-9128-0]
- Taflin H, Wettergren Y, Odin E, Carlsson G, Derwinger K. Folate Levels and Polymorphisms in the Genes *MTHFR*, *MTR*, and *TS* in Colorectal Cancer. *Clin Med Insights Oncol* 2014; **8**: 15-20 [PMID: 24596472 DOI: 10.4137/CMO.S12701]
- Föding M, Hörl WH, Sunder-Plassmann G. Molecular biology of 5,10-methylenetetrahydrofolate reductase. *J Nephrol* 2000; **13**: 20-33 [PMID: 10720211]
- Jiang-Hua Q, De-Chuang J, Zhen-Duo L, Shu-de C, Zhenzhen L. Association of methylenetetrahydrofolate reductase and methionine synthase polymorphisms with breast cancer risk and interaction with folate, vitamin B6, and vitamin B12 intakes. *Tumour Biol* 2014; **35**: 11895-11901 [PMID: 25217320 DOI: 10.1007/s13277-014-2456-1]
- Mu LN, Cao W, Zhang ZF, Cai L, Jiang QW, You NC, Goldstein BY, Wei GR, Chen CW, Lu QY, Zhou XF, Ding BG, Chang J, Yu SZ. Methylenetetrahydrofolate reductase (*MTHFR*) C677T and A1298C polymorphisms and the risk of primary hepatocellular carcinoma (HCC) in a Chinese population. *Cancer Causes Control* 2007; **18**: 665-675 [PMID: 17503006 DOI: 10.1007/s10552-007-9012-x]
- Kwak SY, Kim UK, Cho HJ, Lee HK, Kim HJ, Kim NK, Hwang SG. Methylenetetrahydrofolate reductase (*MTHFR*) and methionine synthase reductase (*MTRR*) gene polymorphisms as risk factors for hepatocellular carcinoma in a Korean population. *Anticancer Res* 2008; **28**: 2807-2811 [PMID: 19035314]
- Sun H, Han B, Zhai H, Cheng X, Ma K. Significant association between *MTHFR* C677T polymorphism and hepatocellular carcinoma risk: a meta-analysis. *Tumour Biol* 2014; **35**: 189-193 [PMID: 24132589 DOI: 10.1007/s13277-013-1023-5]
- Chang SC, Chang PY, Butler B, Goldstein BY, Mu L, Cai L, You NC, Baecker A, Yu SZ, Heber D, Lu QY, Li L, Greenland S, Zhang ZF. Single nucleotide polymorphisms of one-carbon metabolism and cancers of the esophagus, stomach, and liver in a Chinese population. *PLoS One* 2014; **9**: e109235 [PMID: 25337902 DOI: 10.1371/journal.pone.0109235]
- Méndez-Sánchez N, Ridruejo E, Alves de Mattos A, Chávez-Tapia NC, Zapata R, Paraná R, Mastai R, Strauss E, Guevara-Casallas LG, Daruich J, Gadano A, Parise ER, Uribe M, Aguilar-Olivos NE, Dagher L, Ferraz-Neto BH, Valdés-Sánchez M, Sánchez-Avila JF. Latin American Association for the Study of the Liver (LAASL) clinical practice guidelines: management of hepatocellular carcinoma. *Ann Hepatol* 2014; **13** Suppl 1: S4-S40 [PMID: 24998696]
- Carpenter CL, Morgenstern H, London SJ. Alcoholic beverage consumption and lung cancer risk among residents of Los Angeles County. *J Nutr* 1998; **128**: 694-700 [PMID: 9521630]
- European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215 [PMID: 3344216]
- Fassio E, Díaz S, Santa C, Reig ME, Martínez Artola Y, Alves de

- Mattos A, Míguez C, Galizzi J, Zapata R, Ridruejo E, de Souza FC, Hernández N, Pinchuk L. Etiology of hepatocellular carcinoma in Latin America: a prospective, multicenter, international study. *Ann Hepatol* 2010; **9**: 63-69 [PMID: 20332549]
- 25 **Varela M**, Reig M, de la Mata M, Matilla A, Bustamante J, Pascual S, Turnes J, Aracil C, Del Val A, Pascasio JM, Rodríguez M, Bruix J. [Treatment approach of hepatocellular carcinoma in Spain. Analysis of 705 patients from 62 centers]. *Med Clin (Barc)* 2010; **134**: 569-576 [PMID: 20036398 DOI: 10.1016/j.medcli.2009.10.042]
- 26 **Munaka M**, Kohshi K, Kawamoto T, Takasawa S, Nagata N, Itoh H, Oda S, Katoh T. Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2003; **129**: 355-360 [PMID: 12759747 DOI: 10.1007/s00432-003-0439-5]
- 27 **Donato F**, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, Decarli A, Trevisi P, Ribero ML, Martelli C, Porru S, Nardi G. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. *Am J Epidemiol* 2002; **155**: 323-331 [PMID: 11836196 DOI: 10.1093/aje/155.4.323]
- 28 **Hamed MA**, Ali SA. Non-viral factors contributing to hepatocellular carcinoma. *World J Hepatol* 2013; **5**: 311-322 [PMID: 23805355 DOI: 10.4254/wjh.v5.i6.311]
- 29 **Sellers TA**, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM, Olson JE, Therneau TM, Folsom AR. Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. *Epidemiology* 2001; **12**: 420-428 [PMID: 11416780]
- 30 **Clark JM**. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; **40** Suppl 1: S5-S10 [PMID: 16540768 DOI: 10.1097/01.mcg.0000168638.84840.ft]
- 31 **Su CH**, Lin Y, Cai L. Genetic factors, viral infection, other factors and liver cancer: an update on current progress. *Asian Pac J Cancer Prev* 2013; **14**: 4953-4960 [PMID: 24175758]
- 32 **Méndez-Sánchez N**, Aguilar-Ramírez JR, Reyes A, Dehesa M, Juárez A, Castañeda B, Sánchez-Avila F, Poo JL, Guevara González L, Lizardi J, Valdovinos MA, Uribe M, Contreras AM, Tirado P, Aguirre J, Rivera-Benítez C, Santiago-Santiago R, Bosques-Padilla F, Muñoz L, Guerrero A, Ramos M, Rodríguez-Hernández H, Jacobo-Karam J. Etiology of liver cirrhosis in Mexico. *Ann Hepatol* 2004; **3**: 30-33 [PMID: 15118577]
- 33 **Yu K**, Zhang J, Zhang J, Dou C, Gu S, Xie Y, Mao Y, Ji C. Methionine synthase A2756G polymorphism and cancer risk: a meta-analysis. *Eur J Hum Genet* 2010; **18**: 370-378 [PMID: 19826453 DOI: 10.1038/ejhg.2009.131]
- 34 **Zhou D**, Mei Q, Luo H, Tang B, Yu P. The polymorphisms in methylenetetrahydrofolate reductase, methionine synthase, methionine synthase reductase, and the risk of colorectal cancer. *Int J Biol Sci* 2012; **8**: 819-830 [PMID: 22719222 DOI: 10.7150/ijbs.4462]
- 35 **Zhang H**, Liu C, Han YC, Ma Z, Zhang H, Ma Y, Liu X. Genetic variations in the one-carbon metabolism pathway genes and susceptibility to hepatocellular carcinoma risk: a case-control study. *Tumour Biol* 2015; **36**: 997-1002 [PMID: 25318605 DOI: 10.1007/s13277-014-2725-z]
- 36 **Qin X**, Peng Q, Chen Z, Deng Y, Huang S, Xu J, Li H, Li S, Zhao J. The association between MTHFR gene polymorphisms and hepatocellular carcinoma risk: a meta-analysis. *PLoS One* 2013; **8**: e56070 [PMID: 23457501 DOI: 10.1371/journal.pone.0056070]
- 37 **Qi X**, Sun X, Xu J, Wang Z, Zhang J, Peng Z. Associations between methylenetetrahydrofolate reductase polymorphisms and hepatocellular carcinoma risk in Chinese population. *Tumour Biol* 2014; **35**: 1757-1762 [PMID: 24385382 DOI: 10.1007/s13277-013-1529-x]
- 38 **Liang TJ**, Liu H, Zhao XQ, Tan YR, Jing K, Qin CY. Quantitative assessment of the association between MTHFR rs1801131 polymorphism and risk of liver cancer. *Tumour Biol* 2014; **35**: 339-343 [PMID: 24014085 DOI: 10.1007/s13277-013-1046-y]
- 39 **Simon S**. Study: Smoking Dramatically Increases Liver Cancer Risk. Liver Cancer, American Cancer Society 2011. [Access on: November 15, 2015]. Available from: URL: <http://www.cancer.org/cancer/news/study-smoking-dramatically-increases-liver-cancer-risk>
- 40 **Raphe R**, Duca WJ, Arroyo PCJ, Silva RC, Silva RF. Hepatocellular Carcinoma: Risk Factors, Diagnosis, Staging and Treatment in a Referral Centre. *Journal of Cancer Therapy* 2013; **4**: 384-393 [DOI: 10.4236/jct.2013.42A046]

**P- Reviewer:** Lesmana CRA, Sargsyants N, Sirin G    **S- Editor:** Qiu S  
**L- Editor:** A    **E- Editor:** Li D



## Hepatitis C and double-hit B cell lymphoma successfully treated by antiviral therapy

Giovanni Galati, Lorenzo Rampa, Umberto Vespasiani-Gentilucci, Mirella Marino, Francesco Pisani, Carlo Cota, Alessandro Guidi, Antonio Picardi

Giovanni Galati, Lorenzo Rampa, Umberto Vespasiani-Gentilucci, Alessandro Guidi, Antonio Picardi, Unit of Internal Medicine and Hepatology, Department of Medicine, Università Campus Bio-Medico di Roma, 00128 Rome, Italy

Mirella Marino, Department of Pathology, Regina Elena National Cancer Institute, 00144 Rome, Italy

Francesco Pisani, Hematology and Stem Cell Transplant Unit, Regina Elena National Cancer Institute, 00144 Rome, Italy

Carlo Cota, Dermopathology Unit, San Gallicano Dermatological Institute, IRCCS, 00144 Rome, Italy

**Author contributions:** All the authors contributed equally to the conception and design of the article.

**Institutional review board statement:** This case report was exempt from the Institutional Review Board standards at University of Campus Bio Medico of Rome.

**Informed consent statement:** The patient involved in this clinical case gave her written informed consent authorizing use and disclosure of her protected health information.

**Conflict-of-interest statement:** All the authors have no conflicts of interests to declare.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Giovanni Galati, MD, Unit of Internal Medicine and Hepatology, Department of Medicine, Università Campus Bio-Medico di Roma, via Álvaro del Portillo 21, 00128 Rome, Italy. [g.galati@unicampus.it](mailto:g.galati@unicampus.it)

Telephone: +39-062-25411446

Fax: +39-062-25411944

Received: March 24, 2016

Peer-review started: March 24, 2016

First decision: June 12, 2016

Revised: June 25, 2016

Accepted: August 15, 2016

Article in press: August 16, 2016

Published online: October 18, 2016

### Abstract

B cells lymphoma is one of the most challenging extra-hepatic manifestations of hepatitis C virus (HCV). Recently, a new kind of B-cell lymphoma, named double-hit B (DHL), was characterized with an aggressive clinical course whereas a potential association with HCV was not investigated. The new antiviral direct agents (DAAs) against HCV are effective and curative in the majority of HCV infections. We report the first case, to our knowledge, of DHL and HCV-infection successfully treated by new DAAs. According to our experience, a DHL must be suspected in case of HCV-related lymphoma, and an early diagnosis could direct towards a different hematological management because a worse prognosis might be expected. A possible effect of DAAs on DHL regression should be investigated, but eradicating HCV would avoid life-threatening reactivation of viral hepatitis during pharmacological immunosuppression in onco-hematological diseases.

**Key words:** Hepatitis C; Lymphoma; Direct antiviral agents; Double hit lymphoma; Chronic hepatitis C

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** B cells lymphoma is one of the most chall-



enging extra-hepatic manifestations of hepatitis C virus. Recently, a new kind of B-cell lymphoma, named Double-hit B, was characterized with an aggressive clinical course. This is the first case described in literature of double hit lymphoma with co-existing chronic hepatitis C, successfully treated by new direct antiviral therapies. This case suggests a potential favorable effect of the new antiviral therapies on double hit lymphoma regression.

Galati G, Rampa L, Vespasiani-Gentilucci U, Marino M, Pisani F, Cota C, Guidi A, Picardi A. Hepatitis C and double-hit B cell lymphoma successfully treated by antiviral therapy. *World J Hepatol* 2016; 8(29): 1244-1250 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i29/1244.htm> DOI: <http://dx.doi.org/10.4254/wjgh.v8.i29.1244>

## INTRODUCTION

Hepatitis C virus (HCV) is a major global public health problem, although the newest and most effective antiviral therapies (AVTs), free from severe side effects related to interferon (IFN), are spreading in developed countries. This could lead in the next future to a lower prevalence of chronic hepatitis C (CHC) and of its extra-hepatic manifestations. Among these, the hematologic manifestations are the most challenging. Indeed, HCV could induce B cell proliferation and cause mixed cryoglobulinaemia and non-Hodgkin lymphoma (NHL)<sup>[1]</sup>. The typical HCV-related lymphomas are marginal zone lymphoma (MZL) and diffuse large B cell lymphoma (DLBCL)<sup>[2]</sup>. The first could benefit from AVTs, whereas the second group needs more aggressive chemotherapies and the only AVTs seem to be ineffective. More recently, a new kind of lymphoma named double-hit B cell (DHL) was described, characterized by chromosomal rearrangements, specifically of *Myc* oncogene and either B cell lymphoma 2 and B cell lymphoma 6 oncogenes (*Bcl2-Bcl6*) or gene for Cyclin D1 (*Ccnd1*)<sup>[3,4]</sup>. DHL represents about 5% of all cases of DLBCL and affected patients generally have an aggressive clinical course with poor prognosis, despite combination chemotherapy, with a median overall survival of less than 1-2 years. To date, due to the limited literature concerning this type of lymphoma, the specific treatment is still not clear. A potential correlation between HCV and DHL has never been investigated.

We describe the first case, to our knowledge, of a patient affected by DHL and CHC who underwent a successful antiviral treatment with IFN-free therapy (ombitasvir/paritaprevir/ritonavir and dasabuvir).

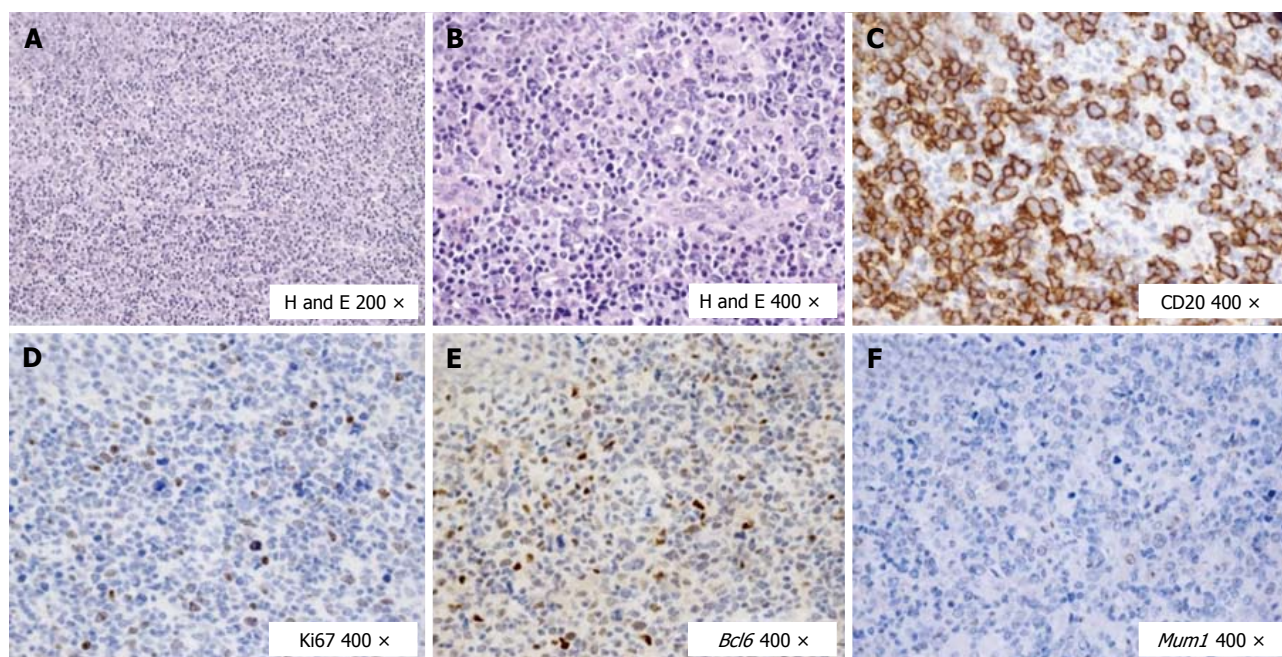
## CASE REPORT

The patient was a 39-year-old Caucasian woman, who tested HCV positive in 2013 during a routine medical evaluation. She had not history of blood transfusions or

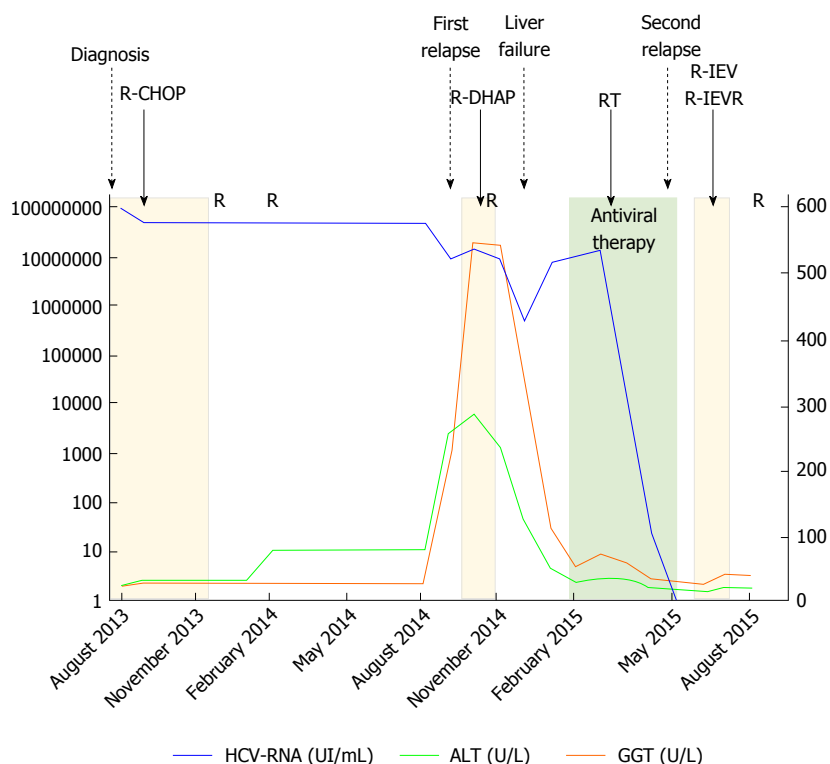
other risk factors for hepatitis virus transmission. The CHC was sustained by genotype 1b virus, with low necro-inflammatory activity and mild liver fibrosis according to non-invasive evaluation performed by Fibroscan® (4.5 kPa), whereas HCV showed a high replication rate (4500000 UI/mL, Cobas AmpliPrep/Cobas TaqMan®-Roche, Rotkreuz, Switzerland). Ultrasound scans of the liver did not demonstrate signs of fibrosis or spleen enlargement secondary to portal hypertension. Of interest, the patient reported a previous episode of major depression occurred in 2006 and, since then, she had been taking venlafazine 75 mg/d.

In July 2013, due to the enlargement of neck lymph nodes, the patient underwent a hematological evaluation and a lymph node biopsy, and she was finally diagnosed with DLBCL (CD20+, CD30-, *Bcl2*+, Ki67 50%, *Bcl6* + 25%, negativity for CD10 and *Mum1*), with involvement of nodes on both sides of the diaphragm, bone marrow and spleen (Figure 1). Treatment with cyclophosphamide, doxorubicin, vincristine, prednisolone plus Rituximab (R) (*R-CHOP* regimen) plus prophylactic intrathecal injection of methotrexate and prednisone, was started. In December 2013, *i.e.*, after six courses of *R-CHOP*, the patient showed a complete remission of lymphoma according both to Contrast Tomography plus Positron Emission Tomography scans and to bone marrow biopsy. Two additional doses of R were prescribed and, at that time, blood tests showed normal levels of transaminases, while HCV replication did not show any substantial changes (Figure 2). In the following months, the patient was referred to our Liver Unit and she was evaluated for AVT. However, since "all-oral" AVTs for CHC was at that time unavailable in Italy, the patient was evaluated for IFN-based AVT, but she presented relative and absolute contraindications to IFN use, such as a previous episode of major depression, mild anemia, leucopenia (*i.e.*, Haemoglobin 10.7 g/dL, White cells counts 1.980 cells/mm<sup>3</sup>), as well as to ribavirin (RBV) use (anemia). We therefore asked our case to be evaluated for the expanded access program (EAP) which was ongoing with Paritaprevir (PTV), a NS3/4A protease inhibitor co-formulated with NS5A inhibitor Ombitasvir (OBV) and the pharmacokinetic enhancer ritonavir (r), plus non-nucleoside polymerase inhibitor Dasabuvir (DSV). Since lymphoma is a possible extra-hepatic manifestation of HCV, the pharmaceutical industry committee accepted our patient for participation to the EAP. Unfortunately, in October 2014, the patient experienced the first relapse of lymphoma at the right maxillary sinus (Figure 3), whereas bilateral bone biopsies were negative. Since chemotherapy was urgent, cisplatin, cytarabin, dexamethasone plus R (*R-DHAP* regimen) were started. After the second cycle of *R-DHAP*, liver tests worsened, with a rapid evolution to liver failure, *i.e.*, jaundice and ascites (Figure 2). She was admitted to our Unit and the liver decompensation was successfully treated with diuretics and anti-oxidant infusion (glutathione 2 g/d until recovery).

From February 2015 to March 2015 she underwent local radiotherapy on the right maxillary sinus (30 Gray).



**Figure 1** Histological and immunohistochemical features of the diffuse large B cell lymphoma at the primary diagnosis. A: In the lymph node a polymorphic lymphoid population is seen; B: Large lymphoid cells prevailed; C: These large cells were B CD20+; D: The proliferation rate marked by Ki67 was around 20% with respect to the overall population, but about 40% if compared with the blastic B cell population; E: The large B cells expressed mainly the germinal center marker *Bcl6*; F: *Mum1* was not found expressed at the primary diagnosis. DLBCL: Diffuse large B cell lymphoma; H and E: Hematoxylin and eosin; CD: Clusters of differentiation; Ki67: Cellular marker for proliferation; *Bcl6*: B cell lymphoma 6; *Mum1*: Multiple myeloma oncogene 1.

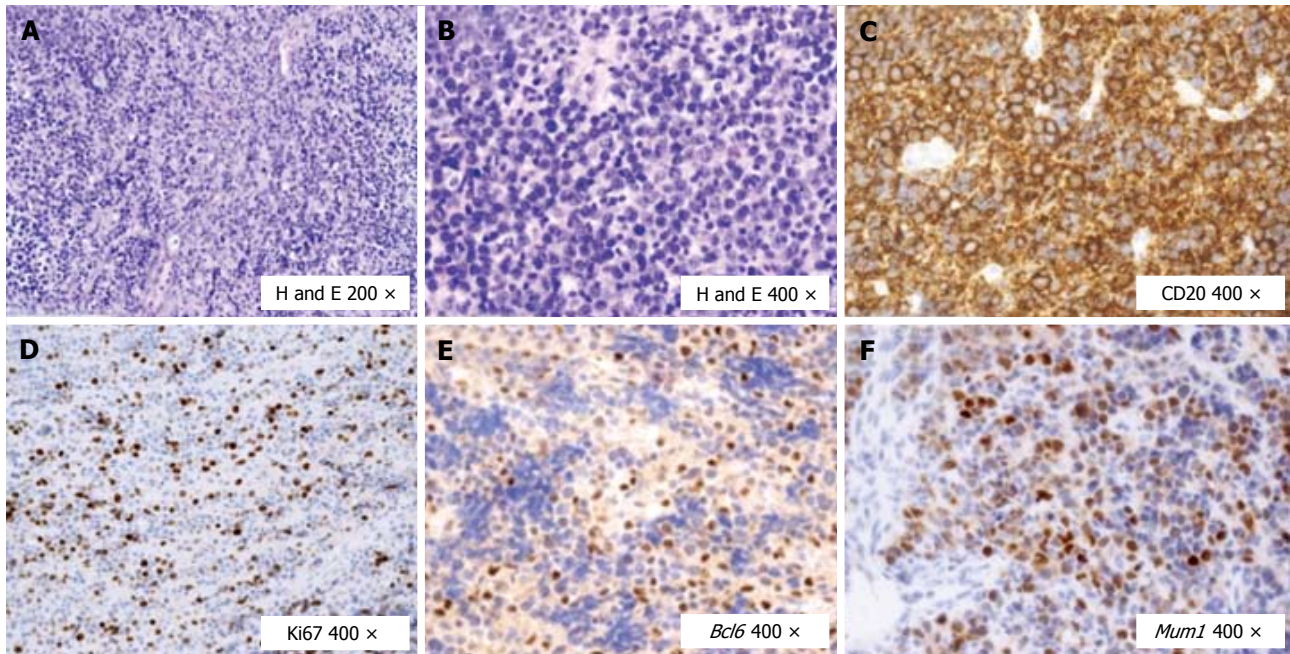


**Figure 2** This figure shows the trends of gamma-glutamyl transpeptidase, alanine amino-transferase and hepatitis C viremia - RNA during chemotherapies and antiviral therapy. R: Rituximab; R-CHOP: Cyclophosphamide, doxorubicin, vincristine, prednisolone plus Rituximab; R-DHAP: Cisplatin, cytarabine, dexamethasone plus Rituximab; R-IEV: Ifosfamide, etoposide, epirubicin plus Rituximab; GGT: Gamma-glutamyl transpeptidase; ALT: Alanine amino-transferase; HCV: Hepatitis C viremia.

After the liver tests returned to normal levels, she started the triple DAAs regimen in the EAP, for a duration of

12 wk (Figure 2). The HCV-RNA showed a fast drop since after two weeks of AVT (42 UI/mL), while viremia





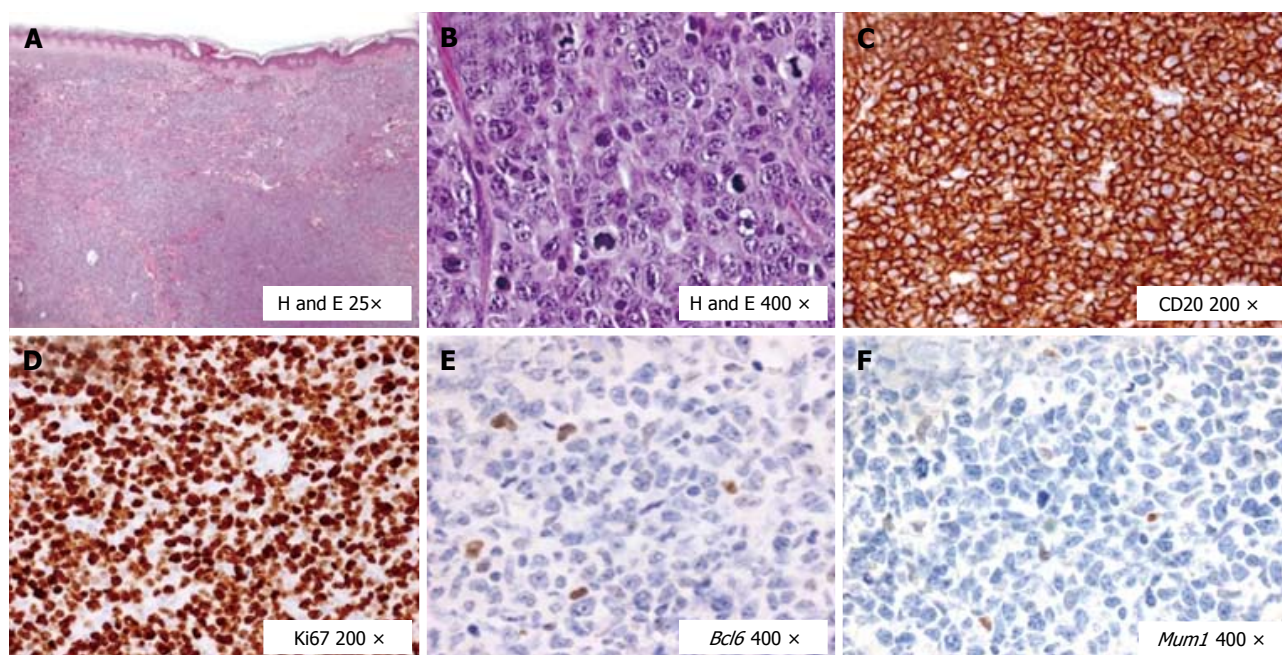
**Figure 3** Histological and immunohistochemical features of the diffuse large B cell lymphoma at the first relapse (mucosal). A: In the mucosal relapse a polymorphic lymphoid population was found; B: The rate of large lymphoid cells increased up to 80%; C: These large cells were B CD20+; D: The proliferation rate marked by Ki67 was around 50%-60% with respect to the blastic B cell population; E: The large B cells expressed the germinal center marker *Bcl6* (about 50%-60%); F: *Mum1* was found to be expressed now by about 70% of the cells. DLBCL: Diffuse large B cell lymphoma; H and E: Hematoxylin and eosin; CD: Clusters of differentiation; Ki67: Cellular marker for proliferation; *Bcl6*: B Cell lymphoma 6; *Mum1*: Multiple myeloma oncogene 1.

became undetectable (limit of quantification according to our virological test was 15 UI/mL) after 8 wk of therapy, and persisted undetectable both at the end of AVT and 24 wk after. However, in May 2015, *i.e.*, after 4 wk from the end of AVT, the patient experienced a new relapse of lymphoma (Figure 4). Due to the appearance of multiple cutaneous nodules on the middle third of the left arm, on the posterior region of the chest wall and on the right and left flanks, a biopsy of one of them was indicated, showing a dense lymphoid infiltrate with atypical growth pattern and cells of large size (CD20+, CD79a+, CD5+, Bcl2+, CD3-, CD30-, *Bcl6*-, *Mum1*-, Ki67 80%). A new PET scan showed multiple pathological uptake of fluorodeoxyglucose in inguinal regions and at the level of the subcutaneous nodules, while the bone marrow biopsy did not show any infiltration. Consistent with the history of two previous relapses and to the high expression of *Bcl2*, a histological reassessment was required on both the lymphoid tissue available at diagnosis and that from the biopsy of the subcutaneous nodule. An analysis with fluorescence *in situ* hybridization for the detection of lymphoma-associated chromosomal abnormalities was performed (Vysis LSI *Bcl2* break apart probe, Vysis LSI *Myc* break apart probe), showing additive copies of *Bcl2* (18q21) in 82% and rearrangement of *Myc* in 89% of the nuclei analyzed (8q24), respectively. Therefore, the final diagnosis was that of DHL, a more aggressive subtype of DBCL. In July 2015, the patient was firstly prescribed a cycle of ifosfamide, etoposide, epirubicin plus R (*R-IEV* regimen), followed by stem cell collection for a possible autologous stem cell transplantation;

subsequently, she underwent a second cycle of *R/IEV/R* (regimen with an additive administration of R), which was burdened by infective complications requiring hospitalization and prolonged antibiotic therapies. At the last hepatological follow-up visit in November 2015, *i.e.*, 24 wk after the end of AVT, HCV-RNA was permanently undetectable despite the immunosuppressive therapy. Afterwards, the patient was evaluated for an allogeneic stem cells transplantation, which was made in another Hospital in January 2016, and she is free from lymphoma recurrence according to recent clinical and radiological evaluations.

## DISCUSSION

The role of HCV in pathogenesis of NHL is well established. The regression of HCV-associated indolent lymphomas after a successful AVT adds the demonstration that there is a relationship between HCV and lymphomagenesis, but there are no data about the response of DHL to AVTs. Because HCV is not integrated into the host genome, there should be indirect mechanisms to induce malignancy ("hit and run theory"). The main hypothesis links the antigenic stimulation caused by CHC to the chronic proliferation of B cells, to produce firstly a polyclonal and after a monoclonal expansion of these cells resulting, in conjunction with further occurrence of additional genetic mutations, in an overt NHL<sup>[5]</sup>. Notably, several years have to pass in order to accumulate mutational changes, whereas a genetic predisposition or additional mutagenic effects in genes *Bcl2*, *Bcl6* or *Cnd1*



**Figure 4** Histological and immunohistochemical features of the diffuse large B cell lymphoma in the second relapse (cutaneous). A: In the cutaneous relapse a rather monomorphic lymphoid population was found; B: The rate of large lymphoid cells increased up to 100%; C: These large cells were B CD20+; D: The proliferation rate marked by Ki67 was around 95% in the blastic B cell population; E: The large B cells expressed poorly the germinal center marker *Bcl6*; F: *Mum1* was found to be poorly expressed by the large B cells. DLBCL: Diffuse large B cell lymphoma; H and E: Hematoxylin and eosin; CD: Clusters of differentiation; Ki67: Cellular marker for proliferation; *Bcl6*: B Cell lymphoma 6; *Mum1*: Multiple myeloma oncogene 1.

could explain the DHL mutation occurrence. Indeed, HCV could induce a mutator phenotype, which involves enhanced mutations of many somatic genes. In the management of HCV-related DLBCL, anthracycline-based chemotherapy, usually *CHOP*, associated with R (chimeric anti-CD20 monoclonal antibody) is the standard of care<sup>[6]</sup>. Unlike indolent B-cell lymphomas<sup>[7]</sup>, AVT does not play a significant role in HCV-positive DLBCL. Sequential immune-chemotherapy followed by ATV has been used in two studies with promising results leading to improved clinical outcome and prolonged disease free survival<sup>[8,9]</sup>. On the other hand, a French study reported a higher overall survival and progression free survival in patients treated firstly by AVT, followed in some cases by chemotherapy<sup>[10]</sup>. All these studies are developed in the "IFN era", both alone or in combination with RBV and, more recently, with protease inhibitors like telaprevir and boceprevir. An IFN-based immunomodulatory and anti-angiogenetic mechanism could be supposed, although IFN-based AVTs cannot be started in most cases, because of contraindications and heavy side effects.

A few reports demonstrated lymphoma regression after HCV clearance with new DAAs. Rossotti *et al.*<sup>[11]</sup> reported a rapid virological and hematological response with the combination of a NS3/NS4A inhibitor (faldaprevir) and a non-nucleoside NS5B inhibitor (deleobuvir) in a patient with HCV associated splenic marginal zone lymphoma. In another report, Sultanik *et al.*<sup>[12]</sup> showed complete regression of MZL after 12 wk of therapy with sofosbuvir (an NS5B RNA-dependent RNA polymerase inhibitor) and RBV. In a case series from France of five

patients with HCV-related lymphoma, two DLBCL were successfully treated with sofosbuvir and daclatasvir (an NS5A protease inhibitor), with complete hematological response after 6 mo<sup>[13]</sup>. There are no reported cases of DHL and CHC treated by new DAAs.

According to clinical data, we could exclude an advanced liver fibrosis in our patient and we estimated a minimal risk of liver decompensation. Therefore, the patient was firstly successfully treated with *R-CHOP*, but after the first hematological relapse, a new immunosuppressive therapy with *R-DHAP* schedule caused an overt liver failure. R has been described to enhance viral replication due to the immune system imbalance, but the rate of severe hepatic complications remains low, with some exceptions, such as in presence of hepatitis B virus or HIV infection, cirrhosis or hepatocellular carcinoma<sup>[7,14]</sup>. There are controversial studies about the impairment of liver function, which could be caused both by a toxicity of immune-chemotherapy treatment and by HCV reactivation. Indeed, it is not clear if the anecdotal episodes of liver failure in this setting are due to higher HCV replication and enhanced necro-inflammatory activity, or to a direct chemo-toxicity. Chemotherapy-induced HCV reactivation in DLBCL is rarely reported, whereas there are no data about HCV and DHL<sup>[15-17]</sup>. In our case the liver failure may have been caused by an add-on effect of HCV reactivation and liver chemo-toxicity, as well suggested by a higher increase of Gamma-Glutamyl Transpeptidase, marker of liver toxicity, instead of a pure elevation of Alanine Aminotransferase, a possible expression of an inflammatory response



HCV-mediated. Interestingly, during the liver failure HCV-RNA slightly dropped, so we can suppose that the event was caused by immune re-activation rather than by HCV direct effect on the necro-inflammatory activity. Following the second immune-chemotherapy and after recovering from the acute liver failure, the patient started antiviral therapy with second generation DAAs: OBV/PTV/r and DSV. This multi-targeted 3-DAAs regimen in combination with or without RBV is approved in many countries to treat HCV genotype 1-4 infection. Approval for the treatment of HCV genotype 1 patients with compensated cirrhosis was based on the evidence of a phase 3 trial of 380 patients in which OBV/PTV/r and DSV plus RBV achieved SVR rates at post-treatment week 12 (SVR12) of 91.8% and 95.9% after 12 or 24 wk of therapy, respectively<sup>[18]</sup>. In the past, the AVTs for HCV were conceived with IFN-based regimens, so an immunomodulatory effect due to interactions with both the adaptive and innate immune response of the host, further than an anti-inflammatory and antiviral effect by inhibiting the synthesis of various cytokines, were possibly responsible for the remission of HCV-related lymphomas. In this context, at the moment, there are no definitive data about IFN free therapies, and it remains to be solved whether IFN is crucial for its direct antitumor and anti-proliferative effect against NHL further than for its antiviral effect.

In conclusion, this is the first case, to our knowledge, of DHL and CHC successfully treated by new DAAs. According to our experience, DHL must be suspected in case of HCV-related lymphoma, and an early diagnosis could direct towards a different hematological management, because a poor prognosis should be expected. Moreover, DAAs triple regimen with OBV/PTV/r and DSV achieves a rapid virological response, and the result is sustained during immunosuppressive therapy, without evidence of HCV reactivation. Finally, we can suggest that in cases of aggressive HCV-related lymphoma, it is mandatory to treat the HCV infection with the new IFN-free regimens at least after the first chemotherapy cycle. This choice could avoid a liver failure in case of re-treatment with hepatotoxic drugs, and it allows a better and safer management of hematological disease. A potential favorable effect of the AVTs on DHL regression should be investigated.

## ACKNOWLEDGMENTS

We thank Cristina Madaudo and Lucia Lo Scalzo for preparing figures and graphics.

## COMMENTS

### Case characteristics

A 39-year-old Caucasian woman presented with neck lymph nodes enlargement and hepatitis C virus (HCV)-infection.

### Clinical diagnosis

Painless swelling in the right side of the neck with increased thickness.

### Differential diagnosis

Inflammatory process of the throat, metastatic cancer of head-neck sites, lymphoproliferative disease.

### Laboratory diagnosis

All blood tests were within normal limits except for high HCV-viremia.

### Imaging diagnosis

Computed tomography showed multiple lymph nodes on both sides of the diaphragm.

### Pathological diagnosis

Double-hit B cell lymphoma.

### Treatments

Chemotherapy, radiotherapy and antiviral therapy for HCV.

### Related reports

Double-hit B cell lymphoma is a rare entity with no standardized chemotherapeutic strategies and its association with HCV-infection is not ever been investigated. There are no reports about the efficacy of antiviral therapy for HCV plus chemotherapies, in order to cure this pathology in presence of HCV.

### Terms explanation

HCV could induce B cell proliferation and cause non-Hodgkin lymphoma. The typical HCV-related lymphomas are marginal zone lymphoma and diffuse large B cell lymphoma. Double-hit B cell (DHL) is a new kind of lymphoma with more aggressive outcome, and it is characterized by chromosomal rearrangements, specifically of *Myc* oncogene and either B cell lymphoma 2 and B cell lymphoma 6 oncogenes or gene for Cyclin D1.

### Experiences and lessons

DHL must be suspected in case of HCV-related lymphoma, and an early diagnosis could direct towards a different hematological management, because a poor prognosis should be expected. A potential favorable effect of new antiviral therapies on DHL regression should be investigated.

### Peer-review

This manuscript has reported the first case of DHL and CHC successfully treated by new DAA.

## REFERENCES

- 1 Negri E, Little D, Boiocchi M, La Vecchia C, Franceschi S. B-cell non-Hodgkin's lymphoma and hepatitis C virus infection: a systematic review. *Int J Cancer* 2004; **111**: 1-8 [PMID: 15185336 DOI: 10.1002/ijc.20205]
- 2 Pellicelli AM, Marignani M, Zoli V, Romano M, Morrone A, Nosotti L, Barbaro G, Picardi A, Gentilucci UV, Remotti D, D' Ambrosio C, Furlan C, Mecenate F, Mazzoni E, Majolino I, Villani R, Andreoli A, Barbarini G. Hepatitis C virus-related B cell subtypes in non Hodgkin's lymphoma. *World J Hepatol* 2011; **3**: 278-284 [PMID: 22125661 DOI: 10.4254/wjh.v3.i11.278]
- 3 Yoshida M, Ichikawa A, Miyoshi H, Kiyasu J, Kimura Y, Arakawa F, Niino D, Ohshima K. Clinicopathological features of double-hit B-cell lymphomas with MYC and BCL2, BCL6 or CCND1 rearrangements. *Pathol Int* 2015; **65**: 519-527 [PMID: 26224092 DOI: 10.1111/pin.12335]
- 4 Aukema SM, Siebert R, Schuurin E, van Imhoff GW, Kluin-Nelemans HC, Boerma EJ, Kluin PM. Double-hit B-cell lymphomas. *Blood* 2011; **117**: 2319-2331 [PMID: 21119107 DOI: 10.1182/blood-2010-09-297879]
- 5 Weng WK, Levy S. Hepatitis C virus (HCV) and lymphomagenesis. *Leuk Lymphoma* 2003; **44**: 1113-1120 [PMID: 12916862 DOI: 10.1080/1042819031000076972]

- 6 **Coiffier B.** Standard treatment of advanced-stage diffuse large B-cell lymphoma. *Semin Hematol* 2006; **43**: 213-220 [PMID: 17027655 DOI: 10.1053/j.seminhematol.2006.07.004]
- 7 **Arcaïni L,** Merli M, Passamonti F, Bruno R, Brusamolino E, Sacchi P, Rattotti S, Orlandi E, Rumi E, Ferretti V, Rizzi S, Meli E, Pascutto C, Paulli M, Lazzarino M. Impact of treatment-related liver toxicity on the outcome of HCV-positive non-Hodgkin's lymphomas. *Am J Hematol* 2010; **85**: 46-50 [PMID: 19957347 DOI: 10.1002/ajh.21564]
- 8 **La Mura V,** De Renzo A, Perna F, D'Agostino D, Masarone M, Romano M, Bruno S, Torella R, Persico M. Antiviral therapy after complete response to chemotherapy could be efficacious in HCV-positive non-Hodgkin's lymphoma. *J Hepatol* 2008; **49**: 557-563 [PMID: 18678434 DOI: 10.1016/j.jhep.2008.06.025]
- 9 **Musto P,** Dell'Olio M, La Sala A, Mantuano S, Cascavilla N. Diffuse B-large cell lymphomas (DLCL) with hepatitis-C virus (HCV) infection: clinical outcome and preliminary results of a pilot study combining R-CHOP with antiviral therapy. *Blood* 2005; **106**: 688a
- 10 **Michot JM,** Canioni D, Driss H, Alric L, Cacoub P, Suarez F, Sibon D, Thieblemont C, Dupuis J, Terrier B, Feray C, Tilly H, Pol S, Leblond V, Settegrana C, Rabiega P, Barthe Y, Hendel-Chavez H, Nguyen-Khac F, Merle-Béral H, Berger F, Molina T, Charlotte F, Carrat F, Davi F, Hermine O, Besson C. Antiviral therapy is associated with a better survival in patients with hepatitis C virus and B-cell non-Hodgkin lymphomas, ANRS HC-13 lympho-C study. *Am J Hematol* 2015; **90**: 197-203 [PMID: 25417909 DOI: 10.1002/ajh.23889]
- 11 **Rossotti R,** Travi G, Pazzi A, Baiguera C, Morra E, Puoti M. Rapid clearance of HCV-related splenic marginal zone lymphoma under an interferon-free, NS3/NS4A inhibitor-based treatment. A case report. *J Hepatol* 2015; **62**: 234-237 [PMID: 25285757 DOI: 10.1016/j.jhep.2014.09.031]
- 12 **Sultanik P,** Klotz C, Brault P, Pol S, Mallet V. Regression of an HCV-associated disseminated marginal zone lymphoma under IFN-free antiviral treatment. *Blood* 2015; **125**: 2446-2447 [PMID: 25858892 DOI: 10.1182/blood-2014-12-618652]
- 13 **Carrier P,** Jaccard A, Jacques J, Tabouret T, Debette-Gratien M, Abraham J, Mesturoux L, Marquet P, Alain S, Sautereau D, Essig M, Loustaud-Ratti V. HCV-associated B-cell non-Hodgkin lymphomas and new direct antiviral agents. *Liver Int* 2015; **35**: 2222-2227 [PMID: 26104059 DOI: 10.1111/liv.12897]
- 14 **Visco C,** Finotto S. Hepatitis C virus and diffuse large B-cell lymphoma: Pathogenesis, behavior and treatment. *World J Gastroenterol* 2014; **20**: 11054-11061 [PMID: 25170194 DOI: 10.3748/wjg.v20.i32.11054]
- 15 **Hsieh CY,** Huang HH, Lin CY, Chung LW, Liao YM, Bai LY, Chiu CF. Rituximab-induced hepatitis C virus reactivation after spontaneous remission in diffuse large B-cell lymphoma. *J Clin Oncol* 2008; **26**: 2584-2586 [PMID: 18487576 DOI: 10.1200/JCO.2007.15.4807]
- 16 **Ennishi D,** Maeda Y, Niitsu N, Kojima M, Izutsu K, Takizawa J, Kusumoto S, Okamoto M, Yokoyama M, Takamatsu Y, Sunami K, Miyata A, Murayama K, Sakai A, Matsumoto M, Shinagawa K, Takaki A, Matsuo K, Kinoshita T, Tanimoto M. Hepatic toxicity and prognosis in hepatitis C virus-infected patients with diffuse large B-cell lymphoma treated with rituximab-containing chemotherapy regimens: a Japanese multicenter analysis. *Blood* 2010; **116**: 5119-5125 [PMID: 20823454 DOI: 10.1182/blood-2010-06-289231]
- 17 **Foran JM.** Hepatitis C in the rituximab era. *Blood* 2010; **116**: 5081-5082 [PMID: 21148335 DOI: 10.1182/blood-2010-09-307827]
- 18 **Feld JJ,** Moreno C, Trinh R, Tam E, Bourgeois S, Horsmans Y, Elkhatab M, Bernstein DE, Younes Z, Reindollar RW, Larsen L, Fu B, Howieson K, Polepally AR, Pangerl A, Shulman NS, Poordad F. Sustained virologic response of 100% in HCV genotype 1b patients with cirrhosis receiving ombitasvir/paritaprevir/r and dasabuvir for 12weeks. *J Hepatol* 2016; **64**: 301-307 [PMID: 26476290 DOI: 10.1016/j.jhep.2015.10.005]

**P- Reviewer:** Einberg AP, El-Shabrawi MH, Ji FP **S- Editor:** Qiu S  
**L- Editor:** A **E- Editor:** Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 October 28; 8(30): 1251-1294







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**Stephen DH Malnick, *Rehovot***Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**Kamal E Bani-Hani, *Zarqa***Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**Angela Peltec, *Chishinev***Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**CA Asabamaka Onyekwere, *Lagos***Pakistan**Bikha Ram Devrajani, *Jamshoro***Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**Jacek Zielinski, *Gdansk***Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**Reem Al Olaby, *Doha***Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**Ser Yee Lee, *Singapore***South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



**REVIEW**

- 1251** Insights for hepatitis C virus related hepatocellular carcinoma genetic biomarkers: Early diagnosis and therapeutic intervention

*Ezzat WM, Amr KS*

**ORIGINAL ARTICLE****Case Control Study**

- 1262** Frontal assessment battery: A tool for screening minimal hepatic encephalopathy?

*de Souza KZ, Zago-Gomes MP*

**Retrospective Study**

- 1269** Retrospective study of the associations between hepatitis C virus infection and metabolic factors

*Yair-Sabag S, Nussinson E, Ben-Assuli O, Shibli F, Shahbari A, Zelber-Sagi S*

**Prospective Study**

- 1279** Reversibility of minimal hepatic encephalopathy following liver transplantation in Egyptian cirrhotic patients

*Osman MA, Sayed MM, Mansour KA, Saleh SA, Ibrahim WA, Abdelhakam SM, Bahaa M, Yousry WA, Elbaz HS, Mikhail RN, Hassan AM, Elsayed EH, Mahmoud DA*

- 1287** Regulatory and activated effector T cells in chronic hepatitis C virus: Relation to autoimmunity

*Fouad H, El Raziky M, Hassan EM, Aziz GMA, Darweesh SK, Sayed AR*

ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Nikolaos G Symeonidis, MD, MSc, PhD, Surgeon, Second Propedeutical Department of Surgery, Aristotle University of Thessaloniki, Medical School, Hippokratio General Hospital, 54642 Thessaloniki, Greece

AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

INDEXING/ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

FLYLEAF

I-IV Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL BOARD MEMBERS  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

EDITORIAL OFFICE  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
October 28, 2016

COPYRIGHT  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS  
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION  
<http://www.wjgnet.com/esps/>

## Insights for hepatitis C virus related hepatocellular carcinoma genetic biomarkers: Early diagnosis and therapeutic intervention

Wafaa M Ezzat, Khalda Sayed Amr

Wafaa M Ezzat, Department of Internal Medicine, National Research Center, Cairo 12311, Egypt

Khalda Sayed Amr, Department of Medical Molecular Genetics, National Research Center, Cairo 12311, Egypt

**Author contributions:** Both authors equally in collecting data and writing the article.

**Conflict-of-interest statement:** Authors declare no conflict of interests for this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Wafaa M Ezzat, MD, Professor of Gastroenterology and Hepatology, Department of Internal Medicine, National Research Center, Elbohosst, Dokki, Cairo 12311, Egypt. [wafaa\\_3t@yahoo.com](mailto:wafaa_3t@yahoo.com)  
Telephone: +20-10-06063558

Received: March 28, 2016

Peer-review started: March 31, 2016

First decision: June 12, 2016

Revised: July 25, 2016

Accepted: September 6, 2016

Article in press: September 8, 2016

Published online: October 28, 2016

on top of hepatitis C virus (HCV). Here we will try to discuss the role genetic and epigenetic factors in pathogenesis of hepatocellular carcinoma. Understanding the role of these factors will help in discovering the mystery of liver carcinogenesis on top of chronic HCV infection. Moreover, use of the studied molecular factors will provide the hepatologists with tailored diagnostic promising biomarkers and flatten the way for establishment of emerging molecular treatment based on exploring the molecular subscription of this aggressive liver cancer.

**Key words:** Hepatitis C virus; Hepatocellular carcinoma; Genetic; Epigenetic; Diagnosis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** It was evident that pathogenesis of hepatocellular carcinoma (HCC) among cases with hepatitis C virus (HCV) infection results from interaction between viral factors and host factors. The host factors include genetic and immunologic factors. Identifying the emerging genetic factors which are contributing in pathogenesis of liver cancer is considered as revolution in research fields of genetics and oncology. Detection of early promising diagnostic biomarkers and development of specific therapy for HCV related HCC is the hope of most researchers in the related fields.

Ezzat WM, Amr KS. Insights for hepatitis C virus related hepatocellular carcinoma genetic biomarkers: Early diagnosis and therapeutic intervention. *World J Hepatol* 2016; 8(30): 1251-1261 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i30/1251.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i30.1251>

### Abstract

The current review explores the role of emerging molecular contributing factors in liver carcinogenesis

### INTRODUCTION

Primary liver cancer is an increasing malignant disease,

being one of the most important causes of cancer deaths all over the world<sup>[1]</sup>. The journey from hepatitis C virus (HCV) infection to hepatocellular carcinoma (HCC) development takes 20-40 years while in some people it may take few years. This variable progression may refer to host factors that interfere; accelerate; delay or even stop HCC development.

Liver fibrosis is the corner stone in the process of hepatic carcinogenesis through course of chronic HCV infection. In cases with liver cirrhosis, the newly discovered cases with HCC are 1%-7% per year, although HCC does not usually develop in livers with early stages of fibrosis<sup>[2,3]</sup>. Recently, emerging efficient antivirals for chronic HCV infection as sofosbuvir is used to decrease the opportunity of liver carcinogenesis<sup>[4]</sup>. Surprisingly, completely cured cases could not guarantee the avoidance of liver cancer development, particularly cases with late stages of liver fibrosis<sup>[5,6]</sup>.

Underlying genetic mechanisms of HCC caused by HCV have not been fully understood. Clinical evaluations indicate that the main task of HCV in liver cancer is to make a cirrhotic tissue background for liver carcinogenesis<sup>[7]</sup>.

Hepatitis B virus can integrate into genetic material of hepatocytes leading to mutation and liver carcinogenesis. The situation in cases of chronic HCV infection is different; hepatocarcinogenesis develop due to direct effects of viral particles or through indirect way which is initiation of chronic hepatitis, liver fibrosis and cirrhosis<sup>[8]</sup>.

Scientists usually face a big challenge to explore the exact underlying mechanism for HCV related liver fibrosis and hepatocarcinogenesis due to the shortage of the ideal animal model for chronic HCV infection. HCV infection is restricted to human and chimpanzees. In a trial to do the researches on tissues which closely resemble that of human, some scientists used treated and modified animal models as HCV transgenic mice and immunocompetent humanized mice and they succeeded to detect known sides of chronic HCV infection natural history. We are still in need of an ideal animal model that can illustrate the chronic HCV infection and its complications as liver tumorigenesis<sup>[5,8]</sup>.

In the current review we explore some of the underlying molecular contributors for liver cancer development in cases with chronic HCV infection. These molecular players may act as promising early detectors or even an emerging therapeutic target for HCC tailored therapy.

## ONCOGENIC EFFECTS OF HCV PROTEINS

Development of HCC on top of HCV occurs due to contribution of viral and host factors. HCV can induce HCC through direct effects of its protein or through indirect way. The indirect way occurs as inflammation of liver tissue and/or its complication as cirrhosis which form the background for HCC in most of HCV - HCC patients. Hoshida *et al*<sup>[9]</sup> described HCV as a single-strand RNA

virus in the Flaviviridae family that encodes structural (core, E1, E2) and non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B). The virus is established by a nucleocapsid containing viral genome; core protein and envelope glycoproteins E1 and E2. HCV infection induces the expression of the nucleocapsid core protein by infected cell. This core protein centralizes in the cytoplasm, lipid droplets, endoplasmic reticulum/Golgi apparatus, nuclei and mitochondria this expression is assumed to alter several cellular functions.

## ONCOGENIC EFFECTS OF CORE PROTEIN

Previous studies concluded that HCV core protein can cause apoptosis, signal transduction, share in oxidative stress by producing reactive oxygen species (ROS), affect metabolism of lipid, activate transcription, and modulate immunity and transformation<sup>[10,11]</sup>. Many scientists have reported frequent mutations in the core gene of HCV among subjects with liver cancer<sup>[12,13]</sup>.

Some scientists suggested that p53 and p73 are tumor suppressor proteins can be affected by HCV core protein<sup>[14,15]</sup>. HCV core protein binds with p73 to inhibit p73  $\alpha$ -dependent cell growth arrest in a p53 - dose dependent manner. This was supported by findings of Alisi *et al*<sup>[16]</sup>. Furthermore, study of Yamanaka *et al*<sup>[17]</sup> suggested that core can also modify the major target of p53 which is known as the cyclin dependent inhibitor p21WAF1 and could control functions of cyclin/cyclin-dependent kinase complexes included in cycle of cell and control and carcinogenesis. This was agreed by study of Kwun *et al*<sup>[18]</sup>. Moreover, other researchers found that signaling pathways such as Raf/MAPK<sup>[19]</sup>, Wnt/ $\beta$ -catenin, 41 and TGF- $\beta$  can be stimulated by HCV core protein<sup>[20,21]</sup>. The inclusion of different pathways is known to be activated in HCC and may help in progression of cirrhosis process; induce mutation for one or more oncogenes or tumor suppressor genes<sup>[22]</sup>.

## ONCOGENIC EFFECTS OF NS3 PROTEIN

HCV NS3 protein acts as an early oncogenic player on hepatocytes<sup>[23,24]</sup>. It inhibits the activity of both p53 and p21WAF1 promoter<sup>[25,26]</sup>. Meanwhile, NS3 protein promotes cell growth, DNA-binding functions of the reproduction agents, AP-1 and ATF-2 and JNK activation<sup>[27]</sup>. It was found that HCV NS3 can activate AP-1 and NF- $\kappa$ B to increase production of TNF- $\alpha$  which has a role in liver carcinogenesis<sup>[28]</sup>.

## ONCOGENIC EFFECTS OF NS5A PROTEIN

Hassan *et al*<sup>[27,28]</sup> proved that NS5A is necessary for replication of the virus and is present in the cytoplasm of infected hepatocytes in conjunction with endoplasmic reticulum. NS5A shares in many function of the cell as transcription, transformation, signal transduction, ROS production and apoptosis. Interestingly, wild-type NS5A gene was up regulated among HCC patients with liver



cirrhosis as background Compared with those who did not develop HCC, taken together, irregular data with regard to the function of core and NS5A proteins on hepatocytes signaling pathways, transcriptional activation, apoptosis and lipid metabolism oxidative stress propose a varied role for HCV proteins in the pathogenesis of chronic hepatitis due to HCV infection, liver fibrosis that results in liver tumorigenesis<sup>[28]</sup>.

## INFLAMMATION-RELATED LIVER CARCINOGENESIS

The indirect way for hepatocarcinogenesis during HCV infection is inflammation of hepatocytes, persistence of chronic hepatitis, liver fibrosis and cirrhosis ending to malignancy transformation. In liver cancer, close to 80% of patients develop malignancy on top of chronic hepatitis. However, the underlying genetic changes for HCC development are not yet fully understood. Continuous formation of regenerative nodules in liver cirrhosis shares in malignant transformation. Previous study reported activation of toll like receptor 4 which promotes the effect of translocation of intestinal microbiota to the liver in late stages of liver carcinogenesis<sup>[29]</sup>. In this study, Dapito *et al.*<sup>[29]</sup> used animal model (*i.e.*, TLR4 genetic inactivation, gut sterilization and long-term treatment with low doses of lipopolysaccharide (LPS)), in which chronic liver injury was modeled using diethyl nitrosamine and carbon tetrachloride.

The researchers proved that the NF- $\kappa$ B pathway is stimulated through identification of TLRs for microbial ligands, like LPS and pathogen-related molecular manner. As a result, the secretion of inflammatory molecules, such as TNF- $\alpha$  and cytokines is stimulated. These molecules regulate the function of liver cells particularly stellate cells which act as the maestro for liver fibrosis process, a step that forego liver cancer growth<sup>[30,31]</sup>. The findings of this study support that of other studies who concluded that the main predisposing factors for HCC development among cases with chronic HCV infection is late liver fibrosis and cirrhosis<sup>[2,3,7]</sup>.

## GENETIC CHANGES DURING HCV RELATED LIVER CARCINOGENESIS

Moeini *et al.*<sup>[31]</sup> suggested that human cancer diseases have been hallmarked by the acquisition of cancer cells to six capabilities: (1) growth signals self-adequacy; (2) loss of sensitivity to anti-growth signals; (3) escaping from apoptosis; (4) unlimited possibility for replication; (5) continuous formation of new blood vessels for the tumor; and (6) metastasis<sup>[32]</sup>. A growing line of evidence has shown that aberrant expression of miRNAs is included in different cancer diseases through deregulating target genes, collectively leading the cell to acquire the six capabilities. miRNAs can act as oncogenes, tumor suppressors, or both and this depends on the targeted genes<sup>[33]</sup>.

Changes in Genetic and epigenetic represent host factors for HCC pathogenesis in late stages of HCV infection. Several signaling Mediators are contributing in liver carcinogenesis, involving some control cell differentiation (Hedgehog, WNT, and Notch), signaling for growth factor (*e.g.*, HGF, IGF, PDGF, EGF, FGF,) and angiogenesis (VEGF). Intracellular modules as AKT/MTOR and RAS could share in pathogenesis of HCV related HCC. Other genetic causes are contributing to stimulate erratic pathway activation. These include mutations, chromosomal abnormalities, and epigenetic mechanisms<sup>[34]</sup>.

Heterogeneity and complexity of carcinogenesis has altered the way we believe concerned with induction, pathogenesis, diagnosis, progression and management of cancer. Although the great advance in exploring of cancer biology, the most of emerging therapies for malignancy do not achieve efficient success, which points to failure of conventional therapeutic interventions. The corner stone in applying of an emerging effective treatment against malignancy is the establishment of efficient clinical trials. Invasive surgical procedures and liver transplantation, the important procedures for HCC therapy, are considered to be the most curative options for treatment of cases of liver cancer. But the frequent recurrent HCC and metastasis after surgical approach is the main hurdle in HCC treatment. Applying effective curative therapeutic procedures to late stages of HCV-HCC disease usually faces big challenge. So that, detection of HCC as early as possible is the corner stone in raising the survival rate and improving the prognosis for cases with this aggressive disease. A main attempt to promote novel treatments should involve the implementation of genetic identification to describe tumors and supply exact foretelling as possible therapeutic targets during the process of liver carcinogenesis and an overall improvement in targeted therapies.

Insights of genetic profiling implying the development of HCC on top of HCV are obscure. The molecular mechanisms include up regulation of oncogenes, inhibition of malignancy oppressor genes, up regulation of growth agents<sup>[35]</sup>, stimulation of telomerase and DNA mismatch repair error may share in the development of liver carcinogenesis<sup>[20,36,37]</sup>. In this context, over expression or down expression of the studied genes which are related to cell cycle progression, growth, disease creation, and reaction to surrounding stimulants cooperate leading to this sophisticated process.

The genomic alterations in malignancy performs a constitutional signature which could involve the control through transcriptional pattern which in turn reflect on a quantitatively gene expression levels<sup>[38,39]</sup>.

Moinzadeh *et al.*<sup>[40]</sup> reported that the implementation of high technologies analysis are so paramount important to improve exploring of genomic alterations in the situation of its relation to pathogenesis of HCC; with the preface of copy number variation (CNV) notion in addition to single nucleotide polymorphisms (SNP), and with the amended mapping of such CNVs throughout the

whole genome of cases vs healthy subjects. In the same concept, Zhao *et al.*<sup>[41]</sup> proved that CNVs as chromosomal SNPs that are several megabases in size, is ending with the size range of CNVs proportionate with the great progress in bioinformatics. The identification of these polymorphisms, either at small (SNPs or mutations) or large CNVs scale as well as regions contains loss of heterozygosity (LOH) blocks may have a role in cancer formation.

## EPIGENETIC ALTERATIONS IN HCV RELATED HCC

Epigenetics refers to all stable alterations in gene expression with no underlying modifications in the genetic sequence itself<sup>[42]</sup>. Epigenetic and genetic mechanisms have a role in silencing of key cellular genes leading to destabilization of the genome and in turn resulting in carcinogenic transformation in human cancers, including HCC<sup>[43]</sup>. Contribution of different epigenetic factors, including genomic DNA methylation, histone modifications, and miRNA regulation, contribute to HCC dissemination, invasion, and metastasis. The reversal of deregulated epigenetic changes has emerged as a potential strategy for the treatment of HCC and is of paramount important in preclinical and clinical development<sup>[44]</sup>. However, obtaining a highly-specific potent epigenetic markers may provide an opportunity for targeting inflammation-epigenome cross-talk in HCC and needs employment of fast screening methods, such as high-throughput screening to navigate efficiently and discovering epigenetic targets<sup>[45]</sup>.

Administration of classical antiviral agents, INF administration with epigenetic drugs (such as DNMT inhibitors or HDAC inhibitors) could confirm an efficient counteracting between cytokines and epigenome changes<sup>[46]</sup>. It was reported that HCV core protein could increase the expression of mRNA and protein values of DNMT1 and DNMT3b, which in turn leads to epigenetic alteration of liver cells of patients with in HCV cells infection<sup>[47]</sup>.

Furthermore, the induction of HCV proteins or the infection of HCC cells with HCV cell culture (HCVcc) resulted in suppression of histone H4 methylation/acetylation and histone H2AX phosphorylation, with significantly altered expression of genes essential for HCC development, indicating that HCV-induced overexpression of PP2Ac involved in pathogenesis of HCC through deregulation of epigenetic histone modifications<sup>[48]</sup>. HCV infection may up regulate histone deacetylation activity through affecting hepcidin expression, a key suppressor of iron availability<sup>[49]</sup>. The induced HCV oxidative stress leads to suppression of hepcidin expression by increased histone deacetylase function.

Other epigenetic changes during HCV induced liver carcinogenesis is deregulation of a class of short, non-coding RNAs [microRNA (miRNA)] that play important roles in gene expression regulation. One miRNA can target several genes through mRNA, this function put

miRNAs in the top not only of diagnostic markers but some of them became a target for personalized therapy. They act as genetic signature for many diseases including HCC with different stages, supporting the potential use of miRNAs in HCC patient stratification of diagnosis and prognosis. Several studies suggested that miRNAs play an important role in carcinogenesis, either as oncogenes or tumor suppressors<sup>[50]</sup>.

Interestingly, miRNAs have been found to be differentially expressed in liver cancer, they are activated to share in pathogenesis of HCV related HCC. Moreover, some miRNAs could be related to different stages of liver carcinogenesis, supporting the possible use of miRNAs in HCC patient correspondence to diagnosis and prognosis. Some of these HCC-associated miRNAs have been validated in independent cohort studies. This confirms the ability of paving the way to develop HCC diagnosis, evaluation of risk exposure, and patient danger accordance with the eventual aim of tailored treatment.

Several previous studies have identified miRNAs expression in pathogenesis of liver cancer on top of chronic HCV infection. miR-21, miR-17, miR-222, miR-224, miR-221, are usually increased in liver cancer<sup>[51,52]</sup> while miR-200, let-7, miR-29, miR-123, miR-122, miR-199a, miR-199b, are decreased<sup>[53,54]</sup>, miR-199 is consistently down-regulated in HCC<sup>[55]</sup>. Since miR-199a/b-3p suppresses HCC in part by preventing the p21-stimulated kinase 4/Raf/MEK/ERL pathway, down-regulation of miR-199a/b is related to bad prognosis and low survival rate<sup>[56]</sup>. On the other hand, miR-224 has been found to be increased in liver cancer<sup>[57]</sup> and was reported to be related to malignancy aggression, deteriorated liver function, and poor prognosis<sup>[58]</sup>.

## BIOMARKERS FOR HCC EARLY DETECTION

Cancer diagnostics based on measuring biomarkers in tissue samples has already in the past decade provided revolutionary advances in diagnosis, prognosis, and therapy selection. A major drawback of the tissue-based approach centers on the need for invasive surgical procedures in sample collection, which in a great many instances preclude following the progression or regression of disease during therapy.

In recent years, an impressive number of cancer biomarker researchers have turned their attention to the demonstration of markers present in biological fluid or blood based biomarkers have also significantly impacted approach of "molecular pharmacogenomics and therapeutics"<sup>[59,60]</sup>. Deep understanding of pathogenic evolution of cancer has improved considerably through Launching of molecular diagnostics in the marketplace and involves expertise in managing resources and navigating a competitive environment. Rising healthcare costs have led to innovative solutions which include molecular testing matched with targeted therapies, point-of-care testing to provide rapid results for improved

patient outcomes, and non-invasive testing options<sup>[60]</sup>.

Screening for HCC among Patients with chronic HCV infection should be done by conventional abdominal ultrasonography; serum  $\alpha$ -fetoprotein (AFP); protein induced vitamin K absence-II and abdominal computed tomography scan. Other serum markers could be used as AFP-L3 (a glycosylated form of AFP); Des gamma carboxyprothrombin and Golgi membrane protein 73, Dickkopf-1<sup>[61]</sup>, and squamous cell carcinoma antigen<sup>[62]</sup> have increased the chance for early HCC detection. We face challenge in diagnosis of small tumors or in well-to-moderately differentiated HCC as serum markers are rarely elevated. Thus, development of sensitive and specific diagnostic biomarkers became an urgent need. It was found that use of autoantibody to tumor-associated antigens (TAA) as a diagnostic biomarker for early detection as indicators of disease prognosis has been explored. Hong *et al.*<sup>[63]</sup> investigated the serum autoantibodies to TAA, and detect that centromere protein F, and hot shock protein were new promising early detectors for HCC. Anti-TAA antibodies might reflect molecular events associated with tumorigenesis.

### AFP

The first serologic assay for diagnosis and clinical follow-up of patients with liver cancer was AFP which has been the conventional tumor biomarker for HCC for many years. Serum AFP levels are often increased in HCC, but this is not always the case. AFP levels may be elevated initially in the early stages of HCC and then drop or even normalize before increasing again as disease progression occurs<sup>[64]</sup>. Total AFP can be divided into three different glycoforms, AFP-L1, AFP-L2, and AFP-L3-based on their binding capability to lectin Lens culinaris agglutinin. High percentage of AFP-L3 has been shown to be associated with poor differentiation and biologically malignant characteristics, worse liver function, and larger tumor mass<sup>[65]</sup>.

### mRNAs circulating biomarker

The advantage of circulating nucleic acids in plasma offers another avenue for noninvasive monitoring of a variety of physiological and pathologic conditions<sup>[66,67]</sup>. Numerous applications based on the detection of circulating cell-free nucleic acids in human plasma have been reported for the management of malignancies. Cell-free plasma RNA detection methods offer an opportunity for the development of pathology-related markers<sup>[68,69]</sup>. From cell free mRNAs HCC biomarkers are the AFP mRNA, gamma-glutamyl transferase mRNA, insulin-like growth factor II, and Albumin mRNA.

Now, accumulating studies have addressed that biomarkers are validated components of tumor pathogenesis. Different biomarkers that better predict patients who are at higher risk of recurrence and shown poorer prognosis would help guide the alternative treatment<sup>[70-83]</sup>. Despite the investigation of curative or palliative treatments, prognosis is still poor due to underlying liver diseases and the unique biology of HCC.

**RNAi biomarker:** Derives its attractiveness as a therapeutic tool from several factors. Its principle based on its extreme specificity, the ease of siRNA synthesis, low cost of production and chemical stability makes RNAi an attractive candidate for therapeutic use<sup>[77]</sup>. RNAi, with its simplicity of design and specificity, is being investigated for its potential for cancer therapy. RNAi is advantageous in this case, in the sense that it can be used to target a large number of genes involved in different pathways. Genes involved in cancer can be classified into oncogenes, tumor suppressor genes, and tumor promoting genes (growth and angiogenesis) among others. RNAi can be used to silence oncogenes, tumor promoting genes and/or genes that negatively regulate tumor suppressor genes. Cancer-specific genes that are mutated are ideal targets for siRNA therapy as they can be efficiently targeted without affecting the wild type form of the gene<sup>[77]</sup>. *In vitro* studies using siRNAs directed toward mutated cancer-specific genes have shown extreme specificity towards the mutated form of the gene, whereas silencing of the wild type did not occur<sup>[78]</sup>. Several studies demonstrated the potential success of RNAi in cancer therapy. *In vitro* study targeting mutated oncogene K-Ras, its expression level was strongly inhibited, vs no inhibition of the wild-type. Upon injection of siRNA treated cells into nude mice, tumor formation was dramatically inhibited<sup>[77]</sup>. Another study targeted the epidermal growth factor receptor; epidermal growth factor receptor (EGFR), which confers an oncogenic activity when mutated leading to promotion of proliferation and survival of the cancerous cell. *In vitro* targeting of EGFR displayed as with K-Ras inhibited expression of mutated EGFR while the wild rapid and massive apoptosis<sup>[79]</sup>. Another potential target for cancer therapy by RNAi is P-glycoprotein; the product of the multidrug resistance gene.

**miRNAs biomarker:** miRNAs are regulatory factors that function to repress the transcription of mRNA. Because each miR contains a seed sequence that is complementary to the UTR region of up to around 50 mRNA, the biological impact from the modulation of just a single miR can be significant. Expression profiles are deregulated in cells undergoing pathophysiologic stress suggesting potential as markers of disease states. Based on numerous favorable characteristics of miRNAs as biomarkers as miRNAs are short, protein bound, highly stable in the circulation, and often travel encapsulated in micro vesicles have revealed their potential as diagnostic, prognostic, and treatment response biomarkers.

Several miRNA databases as miRBASE<sup>[80]</sup>, biological databases as those of National Center for Biotechnology Information, and others, ontologies as Gene Ontology, and pathway networks allow investigators to augment and validate relations between miRNA and other information on cellular locations and molecular processes, as well as pathways they contribute to<sup>[81,82]</sup>.

In particular, genetic and epigenetic changes in cells and high frequency of methylated genes in tumors

lead to adenocarcinoma and may serve as a promising marker in the detection of cancer DNA<sup>[83,84]</sup>. Identification of a panel of biomarker alterations can give us a recognizable pattern of molecular alterations in the HCC which can serve as a "signature" specific for each tumor.

### **Advanced methods used in identifying biomarkers related to HCC**

Numerous recent technologies such as next-generation sequencing (NGS)<sup>[85]</sup> and microarray technologies<sup>[86,87]</sup> have adopted in searching for different biomarkers emerged in era of "omics"<sup>[88,89]</sup>. The progress in high-throughput technologies used in ease way to examine a whole tumor genome (genomics, transcriptomics, proteomics) feature important advances in understanding of the underlying sophisticated pathomechanism for carcinogenesis and metastasis of HCC leading to discover of promising biomarkers with clinical potential. Involving loss of heterogeneity, copy number variations, single nucleotide polymorphism aneuploidy<sup>[90,91]</sup>, transcriptome<sup>[92,93]</sup>, proteome<sup>[94,95]</sup>, epigenome<sup>[83,84]</sup>, metabolome<sup>[96,97]</sup>, and miRNA profile<sup>[98]</sup>.

The use of genomics and bioinformatics techniques are inevitable for the generation and analysis of comprehensive datasets from patient samples, targeting the detection of hundreds thousands of genetic entities. They have facilitated the investigation of biological entities associated with the progression of tumors - array comparative genomic hybridization (aCGH) array platform have been applied to HCC samples to better deep understand the role of DNA genomic aberrations. Different microarrays companies began by Affymetrix Inc. then applied by Illumina Inc have developed similar approaches containing SNP probes. Numerous studies have used either CGH or aCGH techniques to investigate chromosomal alterations associated with HCC<sup>[40]</sup>. These array assays based on identification of critical regions commonly exhibit either increased or deletion dosage of gene, leading to alterations in DNA CNVs, aberrations or abnormal LOH blocks in different malignancies, involving liver cancer<sup>[99,100]</sup>.

## **HUMAN LIVER CANCER PCR ARRAY**

Liver cancer PCR Array profiles the expression of many important genes included in the development of HCC. Since numerous microarray studies have identified many deregulated genes, which are important for cellular signaling and other normal biological processes. RT Profiler PCR Array System directed at these genes may yield insights into the molecular mechanisms underlying liver carcinogenesis. This array includes genes commonly up- and down-regulated in HCC, genes involved of signal transduction pathways, and also genes involved in other deregulated biological pathways such as cell cycle, epithelial to mesenchymal transition, inflammation and apoptosis.

### **Next-generation sequencing**

Next-generation sequencing as Roche 454 and Illumina

has been recently introduced to enable massive parallel measurement of mRNA and miRNA expression<sup>[101]</sup>. NGS technology, once reserved for the largest and busiest of research centers, is now attainable to enterprises of all sizes discovering new knowledge on cancer, microbiology, agriculture, genetic disease, reproductive health, and forensics, and other emerging areas. Cancer Sequencing output data are of great important helping in shed light promising newer cancer diagnostics and making data potential of clinical use<sup>[102]</sup>.

### **Emerging therapeutic strategies for HCC**

HCV related HCC is a big health problem in our country Egypt due to the high prevalence of HCV infection among Egyptians. The national committee for viral hepatitis control has started emerging antiviral therapies known as direct acting antivirals. These drugs showed promising increase in the sustained virological response. But what about the role of these drugs on guarding against HCC development?

It is thought that it is early to have a conclusive answer about this question because these drugs act only on the HCV not on liver cells. Moreover, most of the candidate patients for use of these drugs have different grades of liver fibrosis. Can antivirals induce regression of liver fibrosis or even cause stasis of this liver inflammation? The answer for this question needs to follow-up these patients for 20 years at least to detect natural course of liver fibrosis among these patients after control the HCV. Gene therapy for liver cancer means transfer of genetic material to malignant cells, initiating therapeutic effect. This type of emerging therapy may complement or substitute the conventional treatment for HCC. It is important to minimize the transfer of genetic materials to nonmalignant tissues of the liver especially that HCC usually develops on top of cirrhotic liver, thus transfer of genetic material to adjacent non-malignant tissues will accelerate the deterioration of liver functions<sup>[103,104]</sup>.

Inside the target cells, gene expression could be regulated by tumor specific promoters (transcriptional) as surviving or AFP promoters or by targeting messenger RNA of the therapeutic gene (post-transcriptional) for destruction by micro-RNAs. Therapeutic genes used cause cell death through cytotoxicity; inhibition of oncogenic pathway function or stimulation of antitumor immune response<sup>[104]</sup>.

Viral gene therapy procedures aiming to deliver RNAi have shown promising response in animal models of liver cancer through aiming oncogenic pathways involving p28GANK<sup>[105]</sup>, survivin<sup>[106,107]</sup>, VEGF<sup>[108]</sup> and URG11<sup>[109,110]</sup>.

Other emerging tool of gene therapy for HCC are Replicating virus vectors. Using of specific retroviral replicating vector (RRV) could inhibit the growth of HCC tumor and generate suicide gene therapy effectively with no detectable RRV signal in extratumoral tissues. The resulted tumor-specific suicide-gene-encoding RRV may achieve the engagement application of retroviral gene therapy for HCC cancer<sup>[111]</sup>.



As discussed above, the possible use of miRNA expression in liver cancer as early detectors, miRNAs may themselves be used as therapeutic agents. Different studies clarify the role of miRNAs in HCC tumorigenesis. It was found that miR-26a which is involved in inducing arrest of cell cycle and known to be down-regulated in both human and mouse malignancies, including HCC. In a mouse model of liver cancer, miRNA expression from an AAV vector led to suppression of malignant cell growth and stimulates tumor-specific apoptosis<sup>[112]</sup>.

Recuperation of expression of miRNA 223<sup>[113]</sup>, miRNA 122, lead to decreased metastasis and angiogenesis<sup>[114]</sup>. Restoration of miRNA101 expression sensitized cells to killing by conventional chemotherapy<sup>[115,116]</sup>. Silencing of up regulated miRNAs in HCC may lead to disruption of pathways important to tumor survival and development. In this core, it was found that silencing of HCC through specific miR-21 and miR-221 each resulted in reduction of viable and tumor of HCC cells<sup>[117,118]</sup>.

## CONCLUSION

There is increasing evidence for HCC tumorigenesis in patients with HCV involves accumulation of genetic alterations. Underlying pathogenic mechanisms of HCC is complex and heterogeneous disease with multiple and variable of risk factors. Thus, signatures of a combination of non-invasive and cost-effective biomarkers may be more valuable for the diagnosis, staging, and prognosis of HCC. Multiple factors contribute in liver carcinogenesis during HCV infection, these factors may act in parallel to each other or they may act in intersecting lines, each in his role. Altogether, these players represent a big challenge in front of conventional therapeutic modalities for HCC. So we think that it's time to try to discover the underlying mechanism for hepatocarcinogenesis to pave the way for development of tailored therapy for those patients changing the basic researches into applied researches. More efforts must be paid by hepatologists especially in countries with high prevalence of HCV infection to identify the underlying genetic mechanisms for liver carcinogenesis among these patients. Cooperation between scientists all over the world with use of recent technology and bioinformatics will build up a strong network for diagnostic markers for HCC and help in early detection of this malignant disease. This network will act as a platform for development of tailored therapy for HCV related HCC.

## REFERENCES

- 1 Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 9-29 [PMID: 24399786 DOI: 10.3322/caac.21208]
- 2 Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; **131**: 174-181 [PMID: 10428733 DOI: 10.7326/0003-4819-131-3-199908030-00003]
- 3 Yang JD, Roberts LR. Hepatocellular carcinoma: A global view. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 448-458 [PMID: 20628345 DOI: 10.1038/nrgastro.2010.100]
- 4 Chung RT, Baumert TF. Curing chronic hepatitis C--the arc of a medical triumph. *N Engl J Med* 2014; **370**: 1576-1578 [PMID: 24720678 DOI: 10.1056/NEJMp1400986]
- 5 van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Heathcote EJ, Manns MP, Kuske L, Zeuzem S, Hofmann WP, de Knecht RJ, Hansen BE, Janssen HL. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012; **308**: 2584-2593 [PMID: 23268517 DOI: 10.1001/jama.2012.144878]
- 6 Agrawal A, Kumar D, Verma T, Gupta R. A case of transfusion related acute lung injury in a thalassemic child. *Med J Armed Forces India* 2015; **71**: S224-S226 [PMID: 26265840 DOI: 10.1016/j.mjafi.2014.04.001]
- 7 Koike K. Molecular basis of hepatitis C virus-associated hepatocarcinogenesis: lessons from animal model studies. *Clin Gastroenterol Hepatol* 2005; **3**: S132-S135 [PMID: 16234061 DOI: 10.1016/S1542-3565(05)00700-7]
- 8 Shlomai A, de Jong YP, Rice CM. Virus associated malignancies: the role of viral hepatitis in hepatocellular carcinoma. *Semin Cancer Biol* 2014; **26**: 78-88 [PMID: 24457013 DOI: 10.1016/j.semcancer.2014.01.004]
- 9 Hoshida Y, Fuchs BC, Bardeesy N, Baumert TF, Chung RT. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. *J Hepatol* 2014; **61**: S79-S90 [PMID: 25443348 DOI: 10.1016/j.jhep.2014.07.010]
- 10 Tsai WL, Chung RT. Viral hepatocarcinogenesis. *Oncogene* 2010; **29**: 2309-2324 [PMID: 20228847 DOI: 10.1038/ncr.2010.36]
- 11 Anzola M. Hepatocellular carcinoma: role of hepatitis B and hepatitis C viruses proteins in hepatocarcinogenesis. *J Viral Hepat* 2004; **11**: 383-393 [PMID: 15357643 DOI: 10.1111/j.1365-2893.2004.00521.x]
- 12 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. *Hepatology* 2007; **46**: 1357-1364 [PMID: 17657816 DOI: 10.1002/hep.21836]
- 13 Fishman SL, Factor SH, Balestrieri C, Fan X, Dibisceglie AM, Desai SM, Benson G, Branch AD. Mutations in the hepatitis C virus core gene are associated with advanced liver disease and hepatocellular carcinoma. *Clin Cancer Res* 2009; **15**: 3205-3213 [PMID: 19383824 DOI: 10.1158/1078-0432.CCR-08-2418]
- 14 Ray RB, Steele R, Meyer K, Ray R. Transcriptional repression of p53 promoter by hepatitis C virus core protein. *J Biol Chem* 1997; **272**: 10983-10986 [PMID: 9110985 DOI: 10.1074/jbc.272.17.10983]
- 15 Cho J, Baek W, Yang S, Chang J, Sung YC, Suh M. HCV core protein modulates Rb pathway through pRb down-regulation and E2F-1 up-regulation. *Biochim Biophys Acta* 2001; **1538**: 59-66 [PMID: 11341983 DOI: 10.1016/S0167-4889(00)00137-3]
- 16 Alisi A, Giambartolomei S, Cupelli F, Merlo P, Fontemaggi G, Spaziani A, Balsano C. Physical and functional interaction between HCV core protein and the different p73 isoforms. *Oncogene* 2003; **22**: 2573-2580 [PMID: 12730672 DOI: 10.1038/sj.onc.1206333]
- 17 Yamanaka T, Kodama T, Doi T. Subcellular localization of HCV core protein regulates its ability for p53 activation and p21 suppression. *Biochem Biophys Res Commun* 2002; **294**: 528-534 [PMID: 12056798 DOI: 10.1016/S0006-291X(02)00508-9]
- 18 Kwun HJ, Jang KL. Dual effects of hepatitis C virus Core protein on the transcription of cyclin-dependent kinase inhibitor p21 gene. *J Viral Hepat* 2003; **10**: 249-255 [PMID: 12823590 DOI: 10.1046/j.1365-2893.2003.00434.x]
- 19 Tsutsumi T, Suzuki T, Moriya K, Shintani Y, Fujie H, Miyoshi H, Matsuura Y, Koike K, Miyamura T. Hepatitis C virus core protein

- activates ERK and p38 MAPK in cooperation with ethanol in transgenic mice. *Hepatology* 2003; **38**: 820-828 [PMID: 14512869 DOI: 10.1002/hep.1840380408]
- 20 **Levrero M.** Viral hepatitis and liver cancer: the case of hepatitis C. *Oncogene* 2006; **25**: 3834-3847 [PMID: 16799625 DOI: 10.1038/sj.onc.1209562]
  - 21 **Matsuzaki K,** Murata M, Yoshida K, Sekimoto G, Uemura Y, Sakaida N, Kaibori M, Kamiyama Y, Nishizawa M, Fujisawa J, Okazaki K, Seki T. Chronic inflammation associated with hepatitis C virus infection perturbs hepatic transforming growth factor beta signaling, promoting cirrhosis and hepatocellular carcinoma. *Hepatology* 2007; **46**: 48-57 [PMID: 17596875 DOI: 10.1002/hep.21672]
  - 22 **Wang XW,** Hussain SP, Huo TI, Wu CG, Forgues M, Hofseth LJ, Brechot C, Harris CC. Molecular pathogenesis of human hepatocellular carcinoma. *Toxicology* 2002; **181-182**: 43-47 [PMID: 12505283 DOI: 10.1016/S0300-483X(02)00253-6]
  - 23 **Sakamuro D,** Furukawa T, Takegami T. Hepatitis C virus nonstructural protein NS3 transforms NIH 3T3 cells. *J Virol* 1995; **69**: 3893-3896 [PMID: 7745741]
  - 24 **Zemel R,** Gerechet S, Greif H, Bachmatove L, Birk Y, Golan-Goldhirsh A, Kunin M, Berdichevsky Y, Benhar I, Tur-Kaspa R. Cell transformation induced by hepatitis C virus NS3 serine protease. *J Viral Hepat* 2001; **8**: 96-102 [PMID: 11264729 DOI: 10.1046/j.1365-2893.2001.00283.x]
  - 25 **Kwon HJ,** Jung EY, Ahn JY, Lee MN, Jang KL. p53-dependent transcriptional repression of p21(waf1) by hepatitis C virus NS3. *J Gen Virol* 2001; **82**: 2235-2241 [PMID: 11514734 DOI: 10.1099/0022-1317-82-9-2235]
  - 26 **Deng L,** Nagano-Fujii M, Tanaka M, Nomura-Takigawa Y, Ikeda M, Kato N, Sada K, Hotta H. NS3 protein of Hepatitis C virus associates with the tumour suppressor p53 and inhibits its function in an NS3 sequence-dependent manner. *J Gen Virol* 2006; **87**: 1703-1713 [PMID: 16690937 DOI: 10.1099/vir.0.81735-0]
  - 27 **Hassan M,** Ghozlan H, Abdel-Kader O. Activation of c-Jun NH2-terminal kinase (JNK) signaling pathway is essential for the stimulation of hepatitis C virus (HCV) non-structural protein 3 (NS3)-mediated cell growth. *Virology* 2005; **333**: 324-336 [PMID: 15721365 DOI: 10.1016/j.virol.2005.01.008]
  - 28 **Hassan M,** Selimovic D, Ghozlan H, Abdel-Kader O. Induction of high-molecular-weight (HMW) tumor necrosis factor(TNF) alpha by hepatitis C virus (HCV) non-structural protein 3 (NS3) in liver cells is AP-1 and NF-kappaB-dependent activation. *Cell Signal* 2007; **19**: 301-311 [PMID: 16916598 DOI: 10.1016/j.cellsig.2006.07.002]
  - 29 **Dapito DH,** Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, Caviglia JM, Khiabanian H, Adeyemi A, Batailler R, Lefkowitz JH, Bower M, Friedman R, Sartor RB, Rabadan R, Schwabe RF. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 2012; **21**: 504-516 [PMID: 22516259 DOI: 10.1016/j.ccr.2012.02.007]
  - 30 **Haybaeck J,** Zeller N, Wolf MJ, Weber A, Wagner U, Kurrer MO, Bremer J, Iezzi G, Graf R, Clavien PA, Thimme R, Blum H, Nedospasov SA, Zatloukal K, Ramzan M, Ciesek S, Pietschmann T, Marche PN, Karin M, Kopf M, Browning JL, Aguzzi A, Heikenwalder M. A lymphotoxin-driven pathway to hepatocellular carcinoma. *Cancer Cell* 2009; **16**: 295-308 [PMID: 19800575 DOI: 10.1016/j.ccr.2009.08.021]
  - 31 **Mocini A,** Cornella H, Villanueva A. Emerging signaling pathways in hepatocellular carcinoma. *Liver Cancer* 2012; **1**: 83-93 [PMID: 24159576 DOI: 10.1159/000342405]
  - 32 **Hanahan D,** Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]
  - 33 **Hammond SM.** MicroRNAs as tumor suppressors. *Nature Genetics* 2007; **39**: 582-583 [PMID: 17460676 DOI: 10.1038/ng0507-582]
  - 34 **Shibata T,** Aburatani H. Exploration of liver cancer genomes. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 340-349 [PMID: 24473361 DOI: 10.1038/nrgastro.2014.6]
  - 35 **Amr KS,** Ezzat WM, Elhosary YA, Hegazy AE, Fahim HH, Kamel RR. The potential role of miRNAs 21 and 199-a in early diagnosis of hepatocellular carcinoma. *Gene* 2016; **575**: 66-70 [PMID: 26302751 DOI: 10.1016/j.gene.2015.08.038]
  - 36 **Macdonald GA,** Greenson JK, Saito K, Cherian SP, Appelman HD, Boland CR. Microsatellite instability and loss of heterozygosity at DNA mismatch repair gene loci occurs during hepatic carcinogenesis. *Hepatology* 1998; **28**: 90-97 [PMID: 9657101 DOI: 10.1002/hep.510280114]
  - 37 **Blum HE,** Moradpour D. Viral pathogenesis of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2002; **17** Suppl 3: S413-S420 [PMID: 12472973 DOI: 10.1046/j.1440-1746.17.s3.37.x]
  - 38 **Albertson DG,** Collins C, McCormick F, Gray JW. Chromosome aberrations in solid tumors. *Nat Genet* 2003; **34**: 369-376 [PMID: 12923544 DOI: 10.1038/ng1215]
  - 39 **Pollack JR,** Sørle T, Perou CM, Rees CA, Jeffrey SS, Lonning PE, Tibshirani R, Botstein D, Børresen-Dale AL, Brown PO. Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. *Proc Natl Acad Sci USA* 2002; **99**: 12963-12968 [PMID: 12297621 DOI: 10.1073/pnas.162471999]
  - 40 **Moizadeh P,** Breuhahn K, Stützer H, Schirmacher P. Chromosome alterations in human hepatocellular carcinomas correlate with aetiology and histological grade--results of an explorative CGH meta-analysis. *Br J Cancer* 2005; **92**: 935-941 [PMID: 15756261 DOI: 10.1038/sj.bjc.6602448]
  - 41 **Zhao X,** Weir BA, LaFramboise T, Lin M, Beroukhi R, Garraway L, Beheshti J, Lee JC, Naoki K, Richards WG, Sugarbaker D, Chen F, Rubin MA, Jänne PA, Girard L, Minna J, Christiani D, Li C, Sellers WR, Meyerson M. Homozygous deletions and chromosome amplifications in human lung carcinomas revealed by single nucleotide polymorphism array analysis. *Cancer Res* 2005; **65**: 5561-5570 [PMID: 15994928 DOI: 10.1158/0008-5472.CAN-04-4603]
  - 42 **Herceg Z.** Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. *Mutagenesis* 2007; **22**: 91-103 [PMID: 17284773 DOI: 10.1093/mutage/gel068]
  - 43 **Vaissière T,** Sawan C, Herceg Z. Epigenetic interplay between histone modifications and DNA methylation in gene silencing. *Mutat Res* 2008; **659**: 40-48 [PMID: 18407786 DOI: 10.1016/j.mrrev.2008.02.004]
  - 44 **Nishida N,** Goel A. Genetic and epigenetic signatures in human hepatocellular carcinoma: a systematic review. *Curr Genomics* 2011; **12**: 130-137 [PMID: 21966251 DOI: 10.2174/138920211795564359]
  - 45 **Herceg Z,** Paliwal A. Epigenetic mechanisms in hepatocellular carcinoma: how environmental factors influence the epigenome. *Mutat Res* 2011; **727**: 55-61 [PMID: 21514401 DOI: 10.1016/j.mrrev.2011.04.001]
  - 46 **Müller C.** Chronic Hepatitis B and C--current treatment and future therapeutic prospects. *Wien Med Wochenschr* 2006; **156**: 391-396 [PMID: 16937041 DOI: 10.1007/s10354-006-0314-5]
  - 47 **Benegiamo G,** Vinciguerra M, Mazzocchi G, Piepoli A, Andriulli A, Pazienza V. DNA methyltransferases 1 and 3b expression in Huh-7 cells expressing HCV core protein of different genotypes. *Dig Dis Sci* 2012; **57**: 1598-1603 [PMID: 22526584 DOI: 10.1007/s10620-012-2160-1]
  - 48 **Duong FH,** Christen V, Lin S, Heim MH. Hepatitis C virus-induced up-regulation of protein phosphatase 2A inhibits histone modification and DNA damage repair. *Hepatology* 2010; **51**: 741-751 [PMID: 20043320 DOI: 10.1002/hep.23388]
  - 49 **Miura K,** Taura K, Kodama Y, Schnabl B, Brenner DA. Hepatitis C virus-induced oxidative stress suppresses hepcidin expression through increased histone deacetylase activity. *Hepatology* 2008; **48**: 1420-1429 [PMID: 18671304 DOI: 10.1002/hep.22486]
  - 50 **Chen CZ.** MicroRNAs as oncogenes and tumor suppressors. *N Engl J Med* 2005; **353**: 1768-1771 [PMID: 16251533 DOI: 10.1056/Nejmp058190]
  - 51 **Borel F,** Konstantinova P, Jansen PL. Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular

- carcinoma. *J Hepatol* 2012; **56**: 1371-1383 [PMID: 22314424 DOI: 10.1016/j.jhep.2011.11.026]
- 52 **Ladeiro Y**, Couchy G, Balabaud C, Bioulac-Sage P, Pelletier L, Rebouissou S, Zucman-Rossi J. MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations. *Hepatology* 2008; **47**: 1955-1963 [PMID: 18433021 DOI: 10.1002/hep.22256]
  - 53 **Anwar SL**, Lehmann U. MicroRNAs: Emerging Novel Clinical Biomarkers for Hepatocellular Carcinomas. *J Clin Med* 2015; **4**: 1631-1650 [PMID: 26295264 DOI: 10.3390/jcm4081631]
  - 54 **Huang S**, He X. The role of microRNAs in liver cancer progression. *Br J Cancer* 2011; **104**: 235-240 [PMID: 21102580 DOI: 10.1038/sj.bjc.6606010]
  - 55 **Hou J**, Lin L, Zhou W, Wang Z, Ding G, Dong Q, Qin L, Wu X, Zheng Y, Yang Y, Tian W, Zhang Q, Wang C, Zhang Q, Zhuang SM, Zheng L, Liang A, Tao W, Cao X. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell* 2011; **19**: 232-243 [PMID: 21316602 DOI: 10.1016/j.ccr.2011.01.001]
  - 56 **Li D**, Liu X, Lin L, Hou J, Li N, Wang C, Wang P, Zhang Q, Zhang P, Zhou W, Wang Z, Ding G, Zhuang SM, Zheng L, Tao W, Cao X. MicroRNA-99a inhibits hepatocellular carcinoma growth and correlates with prognosis of patients with hepatocellular carcinoma. *J Biol Chem* 2011; **286**: 36677-36685 [PMID: 21878637 DOI: 10.1074/jbc.M111.270561]
  - 57 **Wang Y**, Lee AT, Ma JZ, Wang J, Ren J, Yang Y, Tantoso E, Li KB, Ooi LL, Tan P, Lee CG. Profiling microRNA expression in hepatocellular carcinoma reveals microRNA-224 up-regulation and apoptosis inhibitor-5 as a microRNA-224-specific target. *J Biol Chem* 2008; **283**: 13205-13215 [PMID: 18319255 DOI: 10.1074/jbc.M707629200]
  - 58 **Zhuang LP**, Meng ZQ. Serum miR-224 reflects stage of hepatocellular carcinoma and predicts survival. *Biomed Res Int* 2015; **2015**: 731781 [PMID: 25688365 DOI: 10.1155/2015/731781]
  - 59 **Ali S**, Almhanna K, Chen W, Philip PA, Sarkar FH. Differentially expressed miRNAs in the plasma may provide a molecular signature for aggressive pancreatic cancer. *Am J Transl Res* 2010; **3**: 28-47 [PMID: 21139804]
  - 60 **Sethi S**, Kong D, Land S, Dyson G, Sakr WA, Sarkar FH. Comprehensive molecular oncogenomic profiling and miRNA analysis of prostate cancer. *Am J Transl Res* 2013; **5**: 200-211 [PMID: 23573364]
  - 61 **Shen Q**, Fan J, Yang XR, Tan Y, Zhao W, Xu Y, Wang N, Niu Y, Wu Z, Zhou J, Qiu SJ, Shi YH, Yu B, Tang N, Chu W, Wang M, Wu J, Zhang Z, Yang S, Gu J, Wang H, Qin W. Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. *Lancet Oncol* 2012; **13**: 817-826 [PMID: 22738799 DOI: 10.1016/S1470-2045(12)70233-4]
  - 62 **Zhao YJ**, Ju Q, Li GC. Tumor markers for hepatocellular carcinoma. *Mol Clin Oncol* 2013; **1**: 593-598 [PMID: 24649215 DOI: 10.3892/mco.2013.119]
  - 63 **Hong Y**, Long J, Li H, Chen S, Liu Q, Zhang B, He X, Wang Y, Li H, Li Y, Zhang T, Lu C, Yan H, Zhang M, Li Q, Cao B, Bai Z, Wang J, Zhang Z, Zhu S, Zheng J, Ou X, Ma H, Jia J, You H, Wang S, Huang J. An Analysis of Immunoreactive Signatures in Early Stage Hepatocellular Carcinoma. *EBioMedicine* 2015; **2**: 438-446 [PMID: 26137588 DOI: 10.1016/j.ebiom.2015.03.010]
  - 64 **Wu JT**. Serum alpha-fetoprotein and its lectin reactivity in liver diseases: a review. *Ann Clin Lab Sci* 1990; **20**: 98-105 [PMID: 1691611]
  - 65 **Khien VV**, Mao HV, Chinh TT, Ha PT, Bang MH, Lac BV, Hop TV, Tuan NA, Don LV, Taketa K, Satomura S. Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology-proven hepatocellular carcinoma. *Int J Biol Markers* 2001; **16**: 105-111 [PMID: 11471892]
  - 66 **Poon RT**, Ng IO, Lau C, Yu WC, Fan ST, Wong J. Correlation of serum basic fibroblast growth factor levels with clinicopathologic features and postoperative recurrence in hepatocellular carcinoma. *Am J Surg* 2001; **182**: 298-304 [PMID: 11587697 DOI: 10.1016/S0002-9610(01)00708-5]
  - 67 **Safran H**, Charpentier K, Dubel G. Gastrointestinal Cancers Symposium, 2010: Abstract
  - 68 **Lo YM**, Chiu RW. The biology and diagnostic applications of plasma RNA. *Ann N Y Acad Sci* 2004; **1022**: 135-139 [PMID: 15251952 DOI: 10.1196/annals.1318.022]
  - 69 **Chan AK**, Chiu RW, Lo YM. Cell-free nucleic acids in plasma, serum and urine: a new tool in molecular diagnosis. *Ann Clin Biochem* 2003; **40**: 122-130 [PMID: 12662399 DOI: 10.1258/000456303763046030]
  - 70 **Zhu K**, Dai Z, Pan Q, Wang Z, Yang GH, Yu L, Ding ZB, Shi GM, Ke AW, Yang XR, Tao ZH, Zhao YM, Qin Y, Zeng HY, Tang ZY, Fan J, Zhou J. Metadherin promotes hepatocellular carcinoma metastasis through induction of epithelial-mesenchymal transition. *Clin Cancer Res* 2011; **17**: 7294-7302 [PMID: 21976539 DOI: 10.1158/1078-0432.CCR-11-1327]
  - 71 **Zhou SL**, Dai Z, Zhou ZJ, Wang XY, Yang GH, Wang Z, Huang XW, Fan J, Zhou J. Overexpression of CXCL5 mediates neutrophil infiltration and indicates poor prognosis for hepatocellular carcinoma. *Hepatology* 2012; **56**: 2242-2254 [PMID: 22711685 DOI: 10.1002/hep.25907]
  - 72 **Wang Y**, Chen Y, Ge N, Zhang L, Xie X, Zhang J, Chen R, Wang Y, Zhang B, Xia J, Gan Y, Ren Z, Ye S. Prognostic significance of alpha-fetoprotein status in the outcome of hepatocellular carcinoma after treatment of transarterial chemoembolization. *Ann Surg Oncol* 2012; **19**: 3540-3546 [PMID: 22532305 DOI: 10.1245/s10434-012-2368-5]
  - 73 **Scartozzi M**, Faloppi L, Bianconi M, Giampieri R, Maccaroni E, Bittoni A, Del Prete M, Loretelli C, Belvederesi L, Svegliati Baroni G, Cascinu S. The role of LDH serum levels in predicting global outcome in HCC patients undergoing TACE: implications for clinical management. *PLoS One* 2012; **7**: e32653 [PMID: 22461886 DOI: 10.1371/journal.pone.0032653]
  - 74 **Pompili M**, Rapaccini GL, de Luca F, Caturelli E, Astone A, Siena DA, Villani MR, Grattagliano A, Cedrone A, Gasbarrini G. Risk factors for intrahepatic recurrence of hepatocellular carcinoma in cirrhotic patients treated by percutaneous ethanol injection. *Cancer* 1997; **79**: 1501-1508 [PMID: 9118030 DOI: 10.1002/(SICI)1097-0142(19970415)]
  - 75 **Chung GE**, Kim W, Lee JH, Kim YJ, Yoon JH, Lee JM, Lee JY, Kim SH, Kim D, Lee HS. Negative hepatitis B envelope antigen predicts intrahepatic recurrence in hepatitis B virus-related hepatocellular carcinoma after ablation therapy. *J Gastroenterol Hepatol* 2011; **26**: 1638-1645 [PMID: 22011297 DOI: 10.1111/j.1440-1746.2011.06777.x]
  - 76 **Ishii H**, Okada S, Nose H, Okusaka T, Nagahama H, Nakayama H, Nakasuka H, Yoshimori M. Predictive factors for recurrence after percutaneous ethanol injection for solitary hepatocellular carcinoma. *Hepatogastroenterology* 1996; **43**: 938-943 [PMID: 8884317]
  - 77 **Jana S**, Chakraborty C, Nandi S, Deb JK. RNA interference: potential therapeutic targets. *Appl Microbiol Biotechnol* 2004; **65**: 649-657 [PMID: 15372214 DOI: 10.1007/s00253-004-1732-1]
  - 78 **Takeshita F**, Ochiya T. Therapeutic potential of RNA interference against cancer. *Cancer Sci* 2006; **97**: 689-696 [PMID: 16863503 DOI: 10.1111/j.1349-7006.2006.00234.x]
  - 79 **Pai SI**, Lin YY, Macaes B, Meneshian A, Hung CF, Wu TC. Prospects of RNA interference therapy for cancer. *Gene Ther* 2006; **13**: 464-477 [PMID: 16341059 DOI: 10.1038/sj.gt.3302694]
  - 80 **Kozomara A**, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res* 2011; **39**: D152-D157 [PMID: 21037258 DOI: 10.1093/nar/gkq1027]
  - 81 **Huang da W**, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 2009; **4**: 44-57 [PMID: 19131956 DOI: 10.1038/nprot.2008.211]
  - 82 **Huang da W**, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 2009; **37**: 1-13 [PMID: 19033363 DOI: 10.1093/nar/gkn923]
  - 83 **Scott KL**, Nogueira C, Heffernan TP, van Doorn R, Dhakal S,



- Hanna JA, Min C, Jaskelioff M, Xiao Y, Wu CJ, Cameron LA, Perry SR, Zeid R, Feinberg T, Kim M, Vande Woude G, Granter SR, Bosenberg M, Chu GC, DePinho RA, Rimm DL, Chin L. Proinvasion metastasis drivers in early-stage melanoma are oncogenes. *Cancer Cell* 2011; **20**: 92-103 [PMID: 21741599 DOI: 10.1016/j.ccr.2011.05.025]
- 84 **Bai DS**, Dai Z, Zhou J, Liu YK, Qiu SJ, Tan CJ, Shi YH, Huang C, Wang Z, He YF, Fan J. Capn4 overexpression underlies tumor invasion and metastasis after liver transplantation for hepatocellular carcinoma. *Hepatology* 2009; **49**: 460-470 [PMID: 19053044 DOI: 10.1002/hep.22638]
- 85 **Cho W**, Ziogas DE, Katsios C, Roukos DH. Emerging personalized oncology: sequencing and systems strategies. *Future Oncol* 2012; **8**: 637-641 [PMID: 22764759 DOI: 10.2217/fon.12.44]
- 86 **Bostjancic E**, Zidar N, Glavac D. MicroRNA microarray expression profiling in human myocardial infarction. *Dis Markers* 2009; **27**: 255-268 [PMID: 20075508 DOI: 10.3233/DMA-2009-0671]
- 87 **Leivonen SK**, Mäkelä R, Ostling P, Kohonen P, Haapa-Paananen S, Kleivi K, Enerly E, Aakula A, Hellström K, Sahlberg N, Kristensen VN, Børresen-Dale AL, Saviranta P, Perälä M, Kallioniemi O. Protein lysate microarray analysis to identify microRNAs regulating estrogen receptor signaling in breast cancer cell lines. *Oncogene* 2009; **28**: 3926-3936 [PMID: 19684618 DOI: 10.1038/onc.2009.241]
- 88 **Dexlin L**, Ingvarsson J, Frendéus B, Borrebaeck CA, Wingren C. Design of recombinant antibody microarrays for cell surface membrane proteomics. *J Proteome Res* 2008; **7**: 319-327 [PMID: 18047267 DOI: 10.1021/pr070257x]
- 89 **Aravalli RN**, Steer CJ, Cressman EN. Molecular mechanisms of hepatocellular carcinoma. *Hepatology* 2008; **48**: 2047-2063 [PMID: 19003900 DOI: 10.1002/hep.22580]
- 90 **Marquardt JU**, Galle PR, Teufel A. Molecular diagnosis and therapy of hepatocellular carcinoma (HCC): an emerging field for advanced technologies. *J Hepatol* 2012; **56**: 267-275 [PMID: 21782758 DOI: 10.1016/j.jhep.2011.07.007]
- 91 **Villanueva A**, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. *Semin Liver Dis* 2007; **27**: 55-76 [PMID: 17295177 DOI: 10.1055/s-2006-960171]
- 92 **Kumar V**, Kato N, Urabe Y, Takahashi A, Muroyama R, Hosono N, Otsuka M, Tateishi R, Omata M, Nakagawa H, Koike K, Kamatani N, Kubo M, Nakamura Y, Matsuda K. Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. *Nat Genet* 2011; **43**: 455-458 [PMID: 21499248 DOI: 10.1038/ng.809]
- 93 **Krawczyk M**, Müllenbach R, Weber SN, Zimmer V, Lammert F. Genome-wide association studies and genetic risk assessment of liver diseases. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 669-681 [PMID: 21045792 DOI: 10.1038/ngastro.2010.170]
- 94 **You JS**, Jones PA. Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell* 2012; **22**: 9-20 [PMID: 22789535 DOI: 10.1016/j.ccr.2012.06.008]
- 95 **Hoshida Y**, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009; **69**: 7385-7392 [PMID: 19723656 DOI: 10.1158/0008-5472.CAN-09-1089]
- 96 **Ke AW**, Shi GM, Zhou J, Huang XY, Shi YH, Ding ZB, Wang XY, Devbhandari RP, Fan J. CD151 amplifies signaling by integrin  $\alpha 6 \beta 1$  to PI3K and induces the epithelial-mesenchymal transition in HCC cells. *Gastroenterology* 2011; **140**: 1629-1641.e15 [PMID: 21320503 DOI: 10.1053/j.gastro.2011.02.008]
- 97 **Iijichi M**, Takayama T, Matsumura M, Shiratori Y, Omata M, Makuuchi M. alpha-Fetoprotein mRNA in the circulation as a predictor of postsurgical recurrence of hepatocellular carcinoma: a prospective study. *Hepatology* 2002; **35**: 853-860 [PMID: 11915031 DOI: 10.1053/jhep.2002.32100]
- 98 **Yin S**, Li J, Hu C, Chen X, Yao M, Yan M, Jiang G, Ge C, Xie H, Wan D, Yang S, Zheng S, Gu J. CD133 positive hepatocellular carcinoma cells possess high capacity for tumorigenicity. *Int J Cancer* 2007; **120**: 1444-1450 [PMID: 17205516 DOI: 10.1002/ijc.22476]
- 99 **Cifola I**, Spinelli R, Beltrame L, Peano C, Fasoli E, Ferrero S, Bosari S, Signorini S, Rocco F, Perego R, Proserpio V, Raimondo F, Mocarelli P, Battaglia C. Genome-wide screening of copy number alterations and LOH events in renal cell carcinomas and integration with gene expression profile. *Mol Cancer* 2008; **7**: 6 [PMID: 18194544 DOI: 10.1186/1476-4598-7-6]
- 100 **Tsafirir D**, Bacolod M, Selvanayagam Z, Tsafirir I, Shia J, Zeng Z, Liu H, Krier C, Stengel RF, Barany F, Gerald WL, Paty PB, Domany E, Notterman DA. Relationship of gene expression and chromosomal abnormalities in colorectal cancer. *Cancer Res* 2006; **66**: 2129-2137 [PMID: 16489013 DOI: 10.1158/0008-5472.CAN-05-2569]
- 101 **Morin RD**, O'Connor MD, Griffith M, Kuchenbauer F, Delaney A, Prabhu AL, Zhao Y, McDonald H, Zeng T, Hirst M, Eaves CJ, Marra MA. Application of massively parallel sequencing to microRNA profiling and discovery in human embryonic stem cells. *Genome Res* 2008; **18**: 610-621 [PMID: 18285502 DOI: 10.1101/gr.7179508]
- 102 **Basho RK**, Eterovic AK, Meric-Bernstam F. Clinical Applications and Limitations of Next-Generation Sequencing. *Amer Emat Onco* 2015; **11**: 17-22
- 103 **Okuda K**. Hepatocellular carcinoma. *J Hepatol* 2000; **32**: 225-237 [PMID: 10728807]
- 104 **Severi T**, van Malenstein H, Verslype C, van Pelt JF. Tumor initiation and progression in hepatocellular carcinoma: risk factors, classification, and therapeutic targets. *Acta Pharmacol Sin* 2010; **31**: 1409-1420 [PMID: 20953207 DOI: 10.1038/aps.2010.142]
- 105 **Li H**, Fu X, Chen Y, Hong Y, Tan Y, Cao H, Wu M, Wang H. Use of adenovirus-delivered siRNA to target oncoprotein p28GANK in hepatocellular carcinoma. *Gastroenterology* 2005; **128**: 2029-2041 [PMID: 15940635 DOI: 10.1053/j.gastro.2005.03.001]
- 106 **Zhang R**, Ma L, Zheng M, Ren J, Wang T, Meng Y, Zhao J, Jia L, Yao L, Han H, Li K, Yang A. Survivin knockdown by short hairpin RNA abrogates the growth of human hepatocellular carcinoma xenografts in nude mice. *Cancer Gene Ther* 2010; **17**: 275-288 [PMID: 19876077 DOI: 10.1038/cgt.2009.68]
- 107 **Lu X**, Zheng Q, Xiong J. Effect of siRNA targeting survivin gene on the biological behavior of hepatocellular carcinoma. *J Huazhong Univ Sci Technolog Med Sci* 2005; **25**: 48-50, 58 [PMID: 15934307]
- 108 **Raskopf E**, Vogt A, Sauerbruch T, Schmitz V. siRNA targeting VEGF inhibits hepatocellular carcinoma growth and tumor angiogenesis in vivo. *J Hepatol* 2008; **49**: 977-984 [PMID: 18845354 DOI: 10.1016/j.jhep.2008.07.022]
- 109 **Fan R**, Li X, Du W, Zou X, Du R, Zhao L, Luo G, Mo P, Xia L, Pan Y, Shi Y, Lian Z, Feitelson MA, Nie Y, Liu J, Fan D. Adenoviral-mediated RNA interference targeting URG11 inhibits growth of human hepatocellular carcinoma. *Int J Cancer* 2011; **128**: 2980-2993 [PMID: 20725996 DOI: 10.1002/ijc.25624]
- 110 **Binny C**, Peruta MD, Nathwani AC. Targeted Gene Therapy for Hepatocellular Carcinoma: A Reality? In *Clinical Dilemmas in Primary Liver Cancer*, Wiley-Blackwell: Oxford, 2011 [DOI: 10.1002/9781119962205.ch26]
- 111 **Lai YH**, Lin CC, Chen SH, Tai CK. Tumor-specific suicide gene therapy for hepatocellular carcinoma by transcriptionally targeted retroviral replicating vectors. *Gene Ther* 2015; **22**: 155-162 [PMID: 25354682 DOI: 10.1038/gt.2014.98]
- 112 **Kota J**, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, Chang TC, Vivekanandan P, Torbenson M, Clark KR, Mendell JR, Mendell JT. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* 2009; **137**: 1005-1017 [PMID: 19524505 DOI: 10.1016/j.cell.2009.04.021]
- 113 **Tanaka S**, Arii S. Medical treatments: in association or alone, their role and their future perspectives: novel molecular-targeted therapy for hepatocellular carcinoma. *J Hepatobiliary Pancreat Sci* 2010;



- 17: 413-419 [PMID: 19941009 DOI: 10.1007/s00534-009-0238-8]
- 114 **Hoshida Y**, Toffanin S, Lachenmayer A, Villanueva A, Minguez B, Llovet JM. Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. *Semin Liver Dis* 2010; **30**: 35-51 [PMID: 20175032 DOI: 10.1055/s-0030-1247131]
- 115 **Tsai WC**, Hsu PW, Lai TC, Chau GY, Lin CW, Chen CM, Lin CD, Liao YL, Wang JL, Chau YP, Hsu MT, Hsiao M, Huang HD, Tsou AP. MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. *Hepatology* 2009; **49**: 1571-1582 [PMID: 19296470 DOI: 10.1002/hep.22806]
- 116 **Li S**, Fu H, Wang Y, Tie Y, Xing R, Zhu J, Sun Z, Wei L, Zheng X. MicroRNA-101 regulates expression of the v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS) oncogene in human hepatocellular carcinoma. *Hepatology* 2009; **49**: 1194-1202 [PMID: 19133651 DOI: 10.1002/hep.22757]
- 117 **Connolly E**, Melegari M, Landgraf P, Tchaikovskaya T, Tennant BC, Slagle BL, Rogler LE, Zavolan M, Tuschl T, Rogler CE. Elevated expression of the miR-17-92 polycistron and miR-21 in hepadnavirus-associated hepatocellular carcinoma contributes to the malignant phenotype. *Am J Pathol* 2008; **173**: 856-864 [PMID: 18688024 DOI: 10.2353/ajpath.2008.080096]
- 118 **Gramantieri L**, Fornari F, Ferracin M, Veronese A, Sabbioni S, Calin GA, Grazi GL, Croce CM, Bolondi L, Negrini M. MicroRNA-221 targets Bmf in hepatocellular carcinoma and correlates with tumor multifocality. *Clin Cancer Res* 2009; **15**: 5073-5081 [PMID: 19671867 DOI: 10.1158/1078-0432.CCR-09-0092]

**P- Reviewer:** Cheikhrouhou LK, Ozden S **S- Editor:** Qiu S

**L- Editor:** A **E- Editor:** Li D



## Case Control Study

# Frontal assessment battery: A tool for screening minimal hepatic encephalopathy?

Karina Zamprogno de Souza, Maria Penha Zago-Gomes

Karina Zamprogno de Souza, Maria Penha Zago-Gomes, the Center of Health Science, Federal University of Espírito Santo, Vitória 29043-900, Brazil

**Author contributions:** de Souza KZ performed the research and wrote the paper; de Souza KZ and Zago-Gomes MP designed the research and analyzed the data.

**Institutional review board statement:** The study was reviewed and approved by the ethics committee of the Center of Health Science, Federal University of Espírito Santo.

**Informed consent statement:** All patients or their legal guardians provided informed written consent.

**Conflict-of-interest statement:** The authors have no conflicts of interest to declare.

**Data sharing statement:** The technical appendix, statistical code, and dataset are available from the corresponding author at [karinaz\\_med\\_ufes@yahoo.com.br](mailto:karinaz_med_ufes@yahoo.com.br). Participants gave informed consent for data sharing; however, the presented data are anonymized and the risk of identification is low.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Karina Zamprogno de Souza, MD, The Center of Health Science, Federal University of Espírito Santo, Marechal Campos Avenue, Number 1468, Vitória 29043-900, Brazil. [karinaz\\_med\\_ufes@yahoo.com.br](mailto:karinaz_med_ufes@yahoo.com.br)  
Telephone: +55-27-996236404  
Fax: +55-27-33357215

Received: April 23, 2016

Peer-review started: April 23, 2016

First decision: June 12, 2016

Revised: August 13, 2016

Accepted: August 27, 2016

Article in press: August 29, 2016

Published online: October 28, 2016

## Abstract

### AIM

To apply the Frontal Assessment Battery to cirrhotic patients with or without overt hepatic encephalopathy (OHE) and controls.

### METHODS

The frontal assessment battery (FAB) was applied to 87 patients with liver cirrhosis (16 with and 71 without OHE) and 40 control subjects without cirrhosis treated at the alcohol and liver outpatient clinics and the gastroenterology ward of the Cassiano Antônio de Moraes University Hospital (Hospital Universitário Cassiano Antônio de Moraes - HUCAM), Espírito Santo, Brazil.

### RESULTS

The average FAB score was lower for the cirrhotic than for the non-cirrhotic patients ( $10.6 \pm 3.67$  vs  $12.25 \pm 2.72$ ,  $P = 0.015$ ). The FAB score was lower for the cirrhotic patients with OHE than for the patients without OHE ( $8.25 \pm 4.55$  vs  $11.14 \pm 3.25$ ,  $P = 0.027$ ). The total FAB score was lower for the cirrhotic patients without OHE than for the non-cirrhotic patients, although this difference was not significant ( $11.14 \pm 3.25$  vs  $12.25 \pm 2.72$ ,  $P = 0.067$ ). Nevertheless, the difference in the scores on the subtest that assessed the ability to inhibit a response previously conditioned to a stimulus was significant ( $1.72 \pm 0.93$  vs  $2.2 \pm 0.85$ ,  $P = 0.011$ ).

### CONCLUSION

The present study indicates that the FAB is a promising

tool for outpatient minimal HE screening and the assessment of HE severity.

**Key words:** Executive functions; Frontal lobe; Hepatic encephalopathy; Minimal hepatic encephalopathy; Liver cirrhosis; Frontal assessment battery

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The diagnosis of hepatic encephalopathy is based on the West Haven classification. Minimal hepatic encephalopathy is defined by cognitive changes in patients with liver cirrhosis or portosystemic shunting without changes in their physical examination. The diagnosis is performed by neurophysiological and/or neuropsychological tests that are difficult to apply and are expensive. The frontal assessment battery (FAB), which is quick and easy to apply, can be used by the clinician. In the present study, the FAB score was lower in cirrhotic patients, especially those with hepatic encephalopathy. The FAB is a promising test for minimal hepatic encephalopathy screening at the bedside and in outpatient clinics.

de Souza KZ, Zago-Gomes MP. Frontal assessment battery: A tool for screening minimal hepatic encephalopathy? *World J Hepatol* 2016; 8(30): 1262-1268 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i30/1262.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i30.1262>

## INTRODUCTION

Hepatic encephalopathy (HE) comprises a heterogeneous group of neuropsychiatric disorders that occur in patients with liver cirrhosis (LC) or portosystemic shunting in the absence of other known brain diseases<sup>[1]</sup>. The traditional HE classification comprises four grades based on the West Haven criteria for the semi-quantitative grading of mental status<sup>[2,3]</sup>. Overt HE (OHE) is characterized by the presence of clinical manifestations that are easily recognizable in clinical interviews and physical examinations and corresponds to grades 2 to 4. Sub-clinical HE includes forms of the disease that are not easily recognizable in the clinical interview and physical examination, such as West Haven grade 1 and minimal HE (MHE), in which the cognitive deficits can only be detected using specialized tests<sup>[4,5]</sup>. The cognitive deficits caused by MHE have negative impacts on the social and occupational lives of the patients and thus impair their quality of life<sup>[2]</sup> and are associated with a higher risk of accidents<sup>[6]</sup>. Because MHE cannot be recognized during a physical examination, its diagnosis is established through the application of tests to apparently normal individuals who exhibit risk factors for the development of this condition<sup>[7]</sup>.

Traditionally, the MHE diagnosis is established based on neurophysiological and/or neuropsychological tests<sup>[4]</sup>.

The majority of these tests are extensive and require a significant amount of time to perform; additionally, they are difficult to interpret and require the expertise of neuropsychiatry professionals<sup>[8]</sup>. The frontal assessment battery (FAB) developed by Dubois *et al*<sup>[9]</sup> is a quick tool; its application requires approximately 10 min. It is well accepted by patients and is useful for identifying the presence and assessing the severity of dysexecutive syndromes that affect cognition and motor behavior<sup>[10]</sup>. The FAB comprises six subtests (conceptualization, lexical fluency, motor series, conflicting instructions, inhibitory control, and automatic behavior)<sup>[9,11]</sup>. Originally, the FAB was used to assess patients with Parkinson's disease who exhibited abnormalities on the Wisconsin Card Sorting Test, Trail Making Test and verbal fluency tests<sup>[9,12]</sup> and patients with abnormal perfusion of the frontal lobe on imaging tests<sup>[13-17]</sup>. These studies suggest that the FAB evaluates executive functions of the frontal lobe. Additionally, it has proven useful for distinguishing between Alzheimer's disease (AD) and frontotemporal dementia (FTD)<sup>[18]</sup>, detecting subclinical dysexecutive alterations in alcoholic subjects, formulating differentiated therapeutic strategies for the management of alcoholic patients on an individual basis<sup>[19]</sup>, and correlating the use of crack cocaine with a decline in the frontal executive functions as a function of the duration of drug use<sup>[20]</sup>. The present study represents the first application of the FAB in patients with chronic liver disease. The results are compared between individuals with OHE and subclinical HE and the controls.

The aim of the present study was to determine whether the FAB could detect differences between patients with LC and the controls and between cirrhotic patients with and without OHE and to investigate whether this tool might be indicated for outpatient screening for MHE.

## MATERIALS AND METHODS

### Patients

The present study assessed 127 individuals treated at the gastroenterology ward and liver and alcohol outpatient clinics of Cassiano Antônio de Moraes University Hospital, Federal University of Espírito Santo (Hospital Universitário Cassiano Antônio de Moraes, da Universidade Federal do Espírito Santo), Espírito Santo, Brazil. A total of 87 patients had LC, including 16 with OHE and 71 without HE. The remaining 40 patients were defined as controls and were matched according to gender and the cirrhosis etiology (alcoholism, hepatitis B, or hepatitis C). All LC patients were assessed and classified based on the Child-Pugh classification for the severity of liver disease. Individuals with clinical manifestations of psychiatric diseases, those who had consumed alcohol in the past 15 d, and those under 18 years of age were excluded from the study.

### LC diagnosis

The LC diagnosis was established based on the com-

**Table 1 Frontal assessment battery**

Frontal assessment battery	
(1) Similarities	
In what way are they alike?	
(a) A banana and an orange	
(b) A table and a chair	
(c) A tulip, a rose, and a daisy	
3 correct: 3	
2 correct: 2	
1 correct: 1	
None correct: 0	
(2) Lexical fluency	
Say as many words as you can begin with the letter "S", except for proper nouns	
(3) Motor series	
Fist, palm, edge (first together, then alone)	
6 correct consecutive series alone: 3	
At least 3 correct consecutive series alone: 2	
3 correct consecutive series with the examiner: 1	
Cannot perform 3 correct consecutive series even with the examiner: 0	
(4) Conflicting instructions	
Tap twice when I tap once	
Tap once when I tap twice	
No errors: 3	
1-2 errors: 2	
More than 2 errors: 1	
Patient imitates the taps of the examiner at least 4 consecutive times: 0	
(5) Go/No Go	
Tap once when I tap once	
Do not tap when I tap twice	
No errors: 3	
1-2 errors: 2	
More than 2 errors: 1	
Patient imitates the taps of the examiner at least 4 consecutive times: 0	
(6) Behavior	
Do not touch my hands	
The patient's hands should be on his/her knees with the palm up.	
Without saying anything, the examiner brings his/her own hands close to the patient's. If the patient touches the examiner's hands, the examiner says: Now, do not touch my hands. Then, a new attempt begins	
Patient does not touch the examiner's hands: 3	
Patient hesitates and asks what he/she has to do: 2	
Patient touches the examiner's hands without hesitation: 1	
Patient touches the examiner's hands even after he/she was told not to do so: 0	

bination of clinical criteria and the results of imaging and/or histopathological tests.

### HE diagnosis

Patients with LC and neuropsychiatric disorders detected during the clinical interviews and physical examinations in the absence of other known brain diseases were diagnosed with OHE<sup>[1]</sup>. The following clinical manifestations were considered: Behavioral alterations, sleep disorders, irritability, and depression. The psychomotor abnormalities included asterixis, bradykinesia, tremors, and rigidity. Additionally, mental confusion and acute temporal-spatial disorientation were considered<sup>[21]</sup>.

### FAB

The FAB was applied to patients individually by the same examiner using only a paper and pencil; the tests

were applied to inpatients at the bedside and at the outpatient clinics. The FAB is described in Table 1.

To compare the FAB scores to dichotomous categorical variables, the scores were categorized as high ( $> 11$ ) or low ( $\leq 11$ ) based on the median scores of the LC patients.

### Ethics

The present study was approved by the ethics committee of the Center of Health Sciences, Federal University of Espírito Santo (Universidade Federal do Espírito Santo), which is associated with the National Commission of Research Ethics (Comissão Nacional de Ética em Pesquisa - CONEP). After receiving a detailed explanation of the study, the participants signed an informed consent form approved by the institutional medical ethics committee.

### Statistical analysis

The statistical analysis was performed with the Epi Info 6.04 e BioEstat 5.3 software. The data were subjected to a descriptive analysis, including the frequency distribution (in the case of qualitative variables), are expressed as absolute numbers ( $n$ ) and percentages (%), and the means and standard deviations (SD) were calculated. The means of data with a normal distribution were compared using Student's  $t$ -test. Categorical variables were subjected to a cross-tabulation analysis with a  $\chi^2$  test. Fisher's exact test was used to compare two variables when the expected frequency according to the null hypothesis was less than five, and the maximum likelihood ratio was used when the exposure variable comprised more than two categories.

The non-parametric Mann-Whitney test was used for continuous variables with a non-normal distribution. Associations with a value of  $P < 0.05$  were considered statistically significant.

## RESULTS

### Patient characteristics

The demographic characteristics of the sample are described in Table 2. The predominant cause of cirrhosis was alcoholism (65.5%). The cirrhosis etiology and the underlying diseases of the control subjects are described in Table 3. No significant differences were detected in the FAB scores related to the liver cirrhosis etiology.

Among the 87 cirrhotic patients, 33 were classified as Child-Pugh class A, 36 were classified as class B, and 17 were classified as class C. Most of the cirrhotic patients with OHE were classified as Child-Pugh class C (56% vs 11% of the cirrhotic patients without OHE,  $P < 0.001$ ).

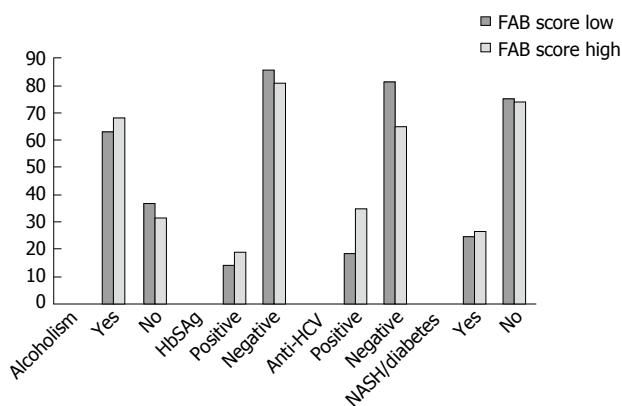
Figure 1 shows that no difference was observed in the FAB scores as a function of the cause of disease when the cirrhotic patients and controls were compared.

The FAB score was lower (mean 10.6, SD  $\pm 3$ , maximum score 18) for the cirrhotic patients than for the controls (mean 12.5, SD  $\pm 2.72$ ,  $P = 0.015$ ). For the cirrhotic patients with OHE, the average score was



**Table 2** Characterization of the cirrhotic and non-cirrhotic patients *n* (%)

Variables	Cirrhotic ( <i>n</i> = 87)	Non-cirrhotic ( <i>n</i> = 40)	<i>P</i> -value
Gender			0.814
Male	68 (78)	32 (80)	
Female	19 (22)	8 (20)	
Age range			0.001
50 yr or older	69 (79)	20 (50)	
Under 50 yr	18 (21)	20 (50)	
Formal schooling			0.577
None	5 (6)	3 (7.5)	
1 to 3 yr	15 (17)	3 (7.5)	
4 to 7 yr	33 (38)	18 (45)	
8 to 11 yr	28 (32)	12 (30)	
12 or more years	6 (7)	4 (10)	

**Figure 1** Percent distribution of the frontal assessment battery score, categorized as high or low, of the investigated patients (cases and controls) compared according to the cause of liver cirrhosis (Fisher's exact test). FAB: Frontal assessment battery; HbSAg: Hepatitis B virus surface antigen; HCV: Hepatitis C virus; NASH: Non-alcoholic steatohepatitis.

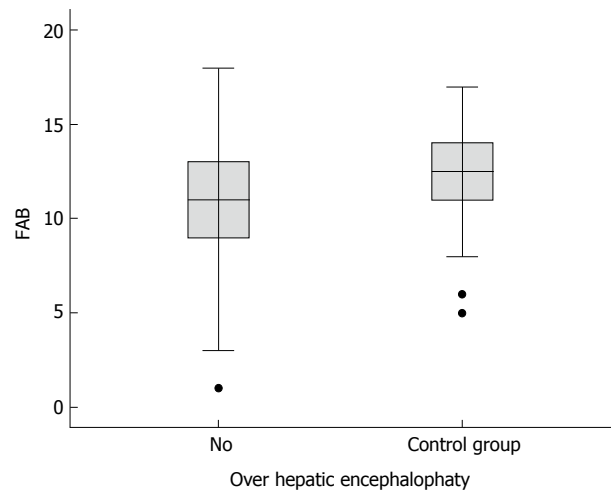
8.25 (SD  $\pm$  4.55) and the median score was 7.5. In comparison, the scores of the cirrhotic patients without HE were higher (mean 11.4, SD  $\pm$  3.25, median 11,  $P$  = 0.027). The FAB score was lower for the cirrhotic patients without OHE than for the controls, although the difference was not significant ( $P$  = 0.067) (Figure 2).

The poorest scores in the cirrhotic group corresponded to the inhibitory control subtest (GO/NO-GO) (mean 1.61, SD  $\pm$  0.98) compared with the controls (mean 2.2, SD  $\pm$  0.85,  $P$  = 0.02). The GO/NO-GO scores were lower for the cirrhotic group without OHE (mean 1.72; SD  $\pm$  0.93) than for the controls (mean 2.2, SD  $\pm$  0.85,  $P$  = 0.011). The GO/NO-GO scores were also lower for the cirrhotic group with OHE (mean 1.13, SD  $\pm$  1.09) than for the cirrhotic group without OHE (mean 1.72, SD  $\pm$  0.93,  $P$  = 0.02), as shown in Figure 3. In this subtest, the individuals were required to inhibit a learned behavior (clap once when the examiner claps twice) and then perform a different task (do not clap when the examiner claps twice). No significant differences were detected in any of the other subtests in the comparison between the cirrhotic patients without OHE and the control group (Figure 4).

Of the 16 cirrhotic patients with OHE, 12 exhibited

**Table 3** Comparison of causes of cirrhosis and the underlying diseases of non-cirrhotic patients *n* (%)

Variables	Cirrhotic ( <i>n</i> = 87)	Non-cirrhotic ( <i>n</i> = 40)	<i>P</i> -value
Alcoholism			0.434
Yes	57 (65.5)	29 (72.5)	
No	30 (34.5)	11 (27.5)	
Hepatitis B surface antigen			0.864
Positive	14 (16)	7 (17.5)	
Negative	72 (84)	33 (82.5)	
Anti-hepatitis C virus antibodies			0.316
Positive	22 (25.6)	7 (17.5)	
Negative	64 (74)	33 (82.5)	
Non-alcoholic steatohepatitis/ diabetes			0.007
Yes	22 (25)	2 (5)	
No	65 (75)	38 (95)	

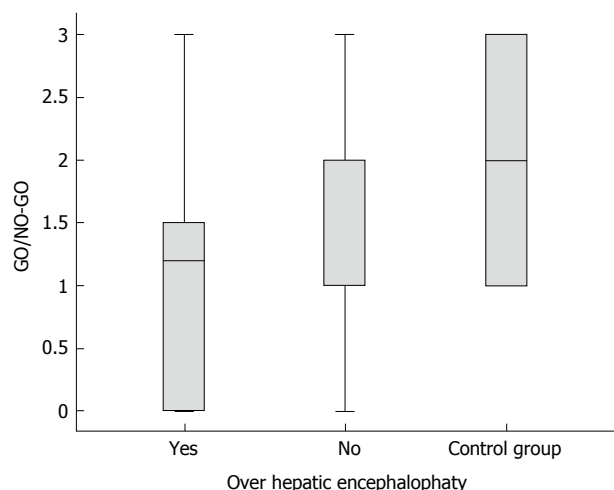
**Figure 2** Median (interquartile range) total scores on the frontal assessment battery for cirrhotic patients with overt hepatic encephalopathy (*n* = 16) vs cirrhotic patients without overt hepatic encephalopathy (*n* = 71) (Student's *t*-test) and the control group (non-cirrhotic patients, *n* = 40) vs the case group (cirrhotic patients with and without overt hepatic encephalopathy, *n* = 87) (Mann-Whitney test). FAB: Frontal assessment battery.

low FAB scores (12/16, 75%). Low FAB scores were exhibited by 37/71 (52%) cirrhotic patients without HE and 17/40 (43%) controls. The linear association test detected a significant difference among the groups ( $P$  = 0.038).

## DISCUSSION

Several studies have shown that MHE causes abnormalities in the attention, social interactions, behavior, and quality of sleep of patients, with consequent impairment of the performance of the activities of daily living. Additionally, interference with more complex activities, such as driving ability or planning a trip, impairs the quality of life of patients and may increase the risk of accidents involving themselves and others<sup>[2]</sup>.

The investigation of patients with cirrhosis by the West Haven test is not sufficient to identify subclinical forms of encephalopathy<sup>[22]</sup>. Traditionally, the MHE



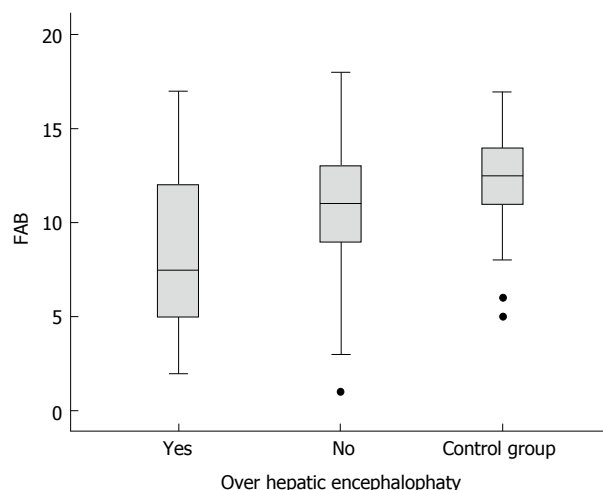
**Figure 3** Median (interquartile range) total scores on the inhibitory control subtest (GO/NO-GO) for cirrhotic patients with overt hepatic encephalopathy ( $n = 16$ ) vs cirrhotic patients without overt hepatic encephalopathy ( $n = 71$ ) (Student's  $t$ -test) and the control group (non-cirrhotic patients,  $n = 40$ ) vs the case group (cirrhotic patients with and without overt hepatic encephalopathy,  $n = 87$ ) (Mann-Whitney test).

diagnosis is established based on the detection of neurological dysfunctions in neurophysiological and/or neuropsychological tests<sup>[4]</sup>. However, these tests are rather long, time consuming, and difficult to interpret<sup>[8]</sup>. Dhiman *et al.*<sup>[3]</sup> suggested that the Psychometric Hepatic Encephalopathy Score, which is a battery of neuropsychological tests that can detect abnormalities such as alterations of motor function, visuospatial orientation, visual perception, visual construction, attention, concentration, and (with somewhat lower efficacy) memory disorders, should be considered the gold standard for the assessment of MHE, whereas computer-based tests such as Critical Flicker Frequency and Inhibitory Control Test should be used for screening. However, neurophysiological tests are difficult to apply in an outpatient setting because they require modern facilities and equipment<sup>[2]</sup>.

A study was conducted in which the Mini-Mental State Examination was applied to patients with LC and OHE, MHE, or without HE to establish whether this test might be used as a screening method for MHE and HE West Haven grades 1 and 2. However, a significant difference was not found between the scores of patients with MHE and those without HE. Moreover, alteration of the mental status was only detected in patients with OHE West Haven grade 3, which could be clinically detected without additional assessment methods<sup>[21]</sup>.

Citro *et al.*<sup>[22]</sup> conducted a study that applied the Trail Making Test (a simple inexpensive test) in a recent series evidenced a poor psychometric performance in more than half of the patients who were free of manifest encephalopathy. The authors also observed that subclinical hepatic encephalopathy was mostly present in patients with HCV-related cirrhosis. In the present study, there was no difference in FAB scores related to the cirrhosis etiology.

The present study used the FAB described by



**Figure 4** Median (interquartile range) total scores on the frontal assessment battery for cirrhotic patients without overt hepatic encephalopathy vs the control group (non-cirrhotic patients) (Mann-Whitney test). FAB: Frontal assessment battery.

Dubois *et al.*<sup>[9]</sup> as a useful and practical tool to establish the presence and severity of dysexecutive syndromes affecting cognition and motor behavior<sup>[9]</sup> and to assess patients with and without LC.

Some studies have indicated that the FAB evaluates the executive functions of the frontal lobe. In clinical practice, the FAB has also been used to distinguish between AD and FTD at the bedside, even in the earliest stages of disease<sup>[23]</sup>. One case-control study compared a group of 170 alcoholic subjects to a group of 40 non-alcoholic controls to assess frontal functions in different categories of alcoholism according to the Lesch typology. The use of the FAB as an assessment instrument allowed the detection of subclinical dysexecutive abnormalities among the alcoholic subjects. These data might serve to formulate differentiated therapeutic strategies for the management of alcoholic patients on an individual basis<sup>[20]</sup>.

The FAB was also used as a tool in a descriptive cross-sectional case series study of 72 crack cocaine users in which their patterns of drug use, global cognition, and frontal executive functions were assessed. The results indicated a decline in executive functions associated with the duration of drug use, especially for the functions investigated by the Automatic behavior subtest<sup>[20]</sup>.

No reports exist in the literature of studies investigating the frontal executive functions in cirrhotic patients. Therefore, no reference values for normality existed to compare with the results of the cirrhotic and non-cirrhotic patients. In the present case-control study, the FAB was applied to cirrhotic and non-cirrhotic patients. The performance of the former group was poorer, suggesting a considerable difference in the performance on the FAB between these two patient groups. The lower scores exhibited by the cirrhotic patients might be attributed to the presence of MHE, which is a subclinical condition that affects less than 15% of cirrhotic Child-Pugh class A patients and approximately

50% of patients classified as Child-Pugh class B or C<sup>[10]</sup>. Among patients with advanced liver disease according to the Child-Pugh classification, the scores of patients classified as Child-Pugh class C were not significantly different from the overall group of cirrhotic patients who exhibited the lowest FAB scores. However, the cirrhotic patients classified as Child-Pugh class C exhibited poorer performance in the GO/NO-GO and Automatic behavior subtests.

Abnormalities in the FAB GO/NO-GO subtest indicate difficulty in inhibiting inappropriate responses due to injury to the ventral part of the frontal lobe<sup>[9]</sup>. In the present study, the scores exhibited by cirrhotic patients were significantly lower than the scores of the non-cirrhotic patients. This finding might be explained by the executive dysfunction caused by MHE<sup>[8]</sup>.

No other FAB subtest scores (conceptualization, verbal fluency, motor series, conflicting instructions, and automatic behavior) were significantly different between the cirrhotic and non-cirrhotic patients.

Detecting minimal hepatic encephalopathy in patients with cirrhosis may help improve their quality of life<sup>[22]</sup>. The FAB score was lower for patients with OHE than for cirrhotic patients without OHE. This finding indicates that the FAB can detect the psychomotor abnormalities characteristic of OHE<sup>[1]</sup>.

Regarding the FAB subtests, patients with OHE exhibited lower scores on the Motor series subtest, which indicated injury to the frontal lobe that impaired temporal organization and the maintenance and execution of successive actions. Additionally, this group of patients exhibited lower scores on the GO/NO-GO subtest, in which abnormalities indicated difficulty inhibiting inappropriate responses. These findings show that the FAB detects HE-related cognitive dysfunctions in the early subclinical stage of disease<sup>[2]</sup> via the GO/NO-GO subtest as well as the psychomotor abnormalities characteristic of OHE, which are recognizable in the physical examination<sup>[3]</sup> via the Motor series subtest.

Although the FAB score exhibited by the cirrhotic patients without MHE was lower than the average score of the controls, the difference was not significant. Further studies with larger sample sizes are needed to thoroughly investigate this difference. The score on the GO/NO-GO subtest was significantly lower for the cirrhotic patients without OHE than for the non-cirrhotic patients. We may infer that this difference is due to the presence of MHE-related executive dysfunctions in some cirrhotic patients who do not have clinical OHE manifestations.

Currently, no studies have applied the FAB to cirrhotic patients with or without complications such as HE to establish the cutoff point for defining normality. However, the differences in the FAB scores between the cirrhotic and non-cirrhotic patients and between patients with and without HE in the present study demonstrate its value. Further studies are needed to determine whether the FAB could be used as a screening tool for all grades of OHE severity and particularly as a helpful tool for the assessment of West Haven grade 1 and MHE in clinical

practice<sup>[1]</sup>.

In conclusion, the FAB is a promising tool with an easy and quick application that may be used by trained general practitioners in both the outpatient setting and at the bedside as a screening method for West Haven grade 1 HE and MHE. Further studies are needed to validate this tool and compare it to other neuropsychometric batteries currently used to detect MHE.

## ACKNOWLEDGMENTS

I thank my professor, Dr. Maria da Penha Zago-Gomes; my patients; and the institution Universidade Federal do Espírito Santo, where I graduated and developed my study.

## COMMENTS

### Background

The usual diagnostic minimal hepatic encephalopathy (MHE) tests are time consuming and require the participation of specialized professionals. The delay in diagnosis has a negative impact on the quality of life of patients and leads to a higher risk of progression to overt HE. The availability of an easily applicable screening test for MHE, such as the frontal assessment battery (FAB), allows early detection of MHE and its treatment.

### Research frontiers

The FAB is a battery of tests with easy and quick application that was originally used for the evaluation of patients with dysexecutive syndromes of the frontal lobe, such as Parkinson's disease, chronic alcohol abusers and crack users. The FAB can also be useful in differentiating the frontotemporal dementia of Alzheimer's disease. Therefore, the FAB would be a useful tool for evaluating the presence and severity of dysexecutive syndromes that affect cognition and motor behavior in liver cirrhosis patients.

### Innovations and breakthroughs

Previous articles in the literature unsuccessfully proposed simple and inexpensive screening tests for MHE, such as the Mini Mental and Trail Making Test. However, the application of the FAB for the evaluation of MHE and HE in patients with liver cirrhosis is unique. Their study suggests that the FAB should be considered a promising tool for screening MHE and HE by the clinician.

### Applications

The FAB is a promising tool for screening HE and MHE in patients both at the bedside and in outpatient clinics.

### Peer-review

It is a good paper.

## REFERENCES

- 1 Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy--definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002; **35**: 716-721 [PMID: 11870389 DOI: 10.1053/jhep.2002.31250]
- 2 Dhiman RK, Saraswat VA, Sharma BK, Sarin SK, Chawla YK, Butterworth R, Duseja A, Aggarwal R, Amarapurkar D, Sharma P, Madan K, Shah S, Seth AK, Gupta RK, Koshy A, Rai RR, Dilawari JB, Mishra SP, Acharya SK. Minimal hepatic encephalopathy: consensus statement of a working party of the Indian National Association for Study of the Liver. *J Gastroenterol Hepatol* 2010; **25**: 1029-1041 [PMID: 20594216 DOI: 10.1111/j.1440-1746.2010.06318.x]

- 3 **Dhiman RK**, Chawla YK. Minimal hepatic encephalopathy. *Indian J Gastroenterol* 2009; **28**: 5-16 [PMID: 19529896 DOI: 10.1007/s12664-009-0003-6]
- 4 **Córdoba J**. New assessment of hepatic encephalopathy. *J Hepatol* 2011; **54**: 1030-1040 [PMID: 21145874 DOI: 10.1016/j.jhep.2010.11.015]
- 5 **Bajaj JS**, Pinkerton SD, Sanyal AJ, Heuman DM. Diagnosis and treatment of minimal hepatic encephalopathy to prevent motor vehicle accidents: a cost-effectiveness analysis. *Hepatology* 2012; **55**: 1164-1171 [PMID: 22135042 DOI: 10.1002/hep.25507]
- 6 **Bajaj JS**, Saeian K, Schubert CM, Hafeezullah M, Franco J, Varma RR, Gibson DP, Hoffmann RG, Stravitz RT, Heuman DM, Sterling RK, Shiffman M, Topaz A, Boyett S, Bell D, Sanyal AJ. Minimal hepatic encephalopathy is associated with motor vehicle crashes: the reality beyond the driving test. *Hepatology* 2009; **50**: 1175-1183 [PMID: 19670416 DOI: 10.1002/hep.23128]
- 7 **Sharma P**. Minimal hepatic encephalopathy. *J Assoc Physicians India* 2009; **57**: 760-763 [PMID: 20329443]
- 8 **Ortiz M**, Jacas C, Córdoba J. Minimal hepatic encephalopathy: diagnosis, clinical significance and recommendations. *J Hepatol* 2005; **42** Suppl: S45-S53 [PMID: 15777572 DOI: 10.1016/j.jhep.2004.11.028]
- 9 **Dubois B**, Slachevsky A, Litvan I, Pillon B. The FAB: a Frontal Assessment Battery at bedside. *Neurology* 2000; **55**: 1621-1626 [PMID: 11113214 DOI: 10.1212/wnl.55.11.1621]
- 10 **Moorhouse P**, Gorman M, Rockwood K. Comparison of EXIT-25 and the Frontal Assessment Battery for evaluation of executive dysfunction in patients attending a memory clinic. *Dement Geriatr Cogn Disord* 2009; **27**: 424-428 [PMID: 19372680 DOI: 10.1159/000212755]
- 11 **Beato RG**, Nitrini R, Formigoni AP, Caramelli P. Brazilian version of the frontal assessment battery (FAB). *Dement Neuropsychol* 2007; **1**: 59-65
- 12 **Cohen OS**, Vakil E, Tanne D, Molshatzki N, Nitsan Z, Hassin-Baer S. The frontal assessment battery as a tool for evaluation of frontal lobe dysfunction in patients with Parkinson disease. *J Geriatr Psychiatry Neurol* 2012; **25**: 71-77 [PMID: 22689698 DOI: 10.1177/0891988712445087]
- 13 **Guedj E**, Allali G, Goetz C, Le Ber I, Volteau M, Lacomblez L, Vera P, Hitzel A, Hannequin D, Decousus M, Thomas-Antérion C, Magne C, Vercelletto M, Bernard AM, Didic M, Lotterie JA, Puel M, Brice A, Habert MO, Dubois B. Frontal Assessment Battery is a marker of dorsolateral and medial frontal functions: A SPECT study in frontotemporal dementia. *J Neurol Sci* 2008; **273**: 84-87 [PMID: 18938766 DOI: 10.1016/j.jns.2008.06.035]
- 14 **Kim TH**, Huh Y, Choe JY, Jeong JW, Park JH, Lee SB, Lee JJ, Jhoo JH, Lee DY, Woo JI, Kim KW. Korean version of frontal assessment battery: psychometric properties and normative data. *Dement Geriatr Cogn Disord* 2010; **29**: 363-370 [PMID: 20424455 DOI: 10.1159/000297523]
- 15 **Oshima E**, Terada S, Sato S, Ikeda C, Nagao S, Takeda N, Honda H, Yokota O, Uchitomi Y. Frontal assessment battery and brain perfusion imaging in Alzheimer's disease. *Int Psychogeriatr* 2012; **24**: 994-1001 [PMID: 22217392 DOI: 10.1017/s1041610211002481]
- 16 **Yoshida H**, Terada S, Sato S, Kishimoto Y, Ata T, Oshima E, Honda H, Ishihara T, Kuroda S. Frontal assessment battery and brain perfusion imaging in early dementia. *Dement Geriatr Cogn Disord* 2009; **27**: 133-138 [PMID: 19182480 DOI: 10.1159/000198687]
- 17 **Benke T**, Karner E, Delazer M. FAB-D: German version of the Frontal Assessment Battery. *J Neurol* 2013; **260**: 2066-2072 [PMID: 23649609 DOI: 10.1007/s00415-013-6929-8]
- 18 **Slachevsky A**, Villalpando JM, Sarazin M, Hahn-Barma V, Pillon B, Dubois B. Frontal assessment battery and differential diagnosis of frontotemporal dementia and Alzheimer disease. *Arch Neurol* 2004; **61**: 1104-1107 [PMID: 15262742 DOI: 10.1001/archneur.61.7.1104]
- 19 **Zago-Gomes MP**, Nakamura-Palacios EM. Cognitive components of frontal lobe function in alcoholics classified according to Lesch's typology. *Alcohol Alcohol* 2009; **44**: 449-457 [PMID: 19666906 DOI: 10.1093/alcalc/agg043]
- 20 **Moscon J**. Avaliação dos padrões de consumo, cognição global e de funções executivas em usuários de crack em ambulatório especializado de alta demanda. Tese de mestrado apresentada ao programa de pós-graduação em ciências fisiológicas do centro de ciências da saúde da Universidade Federal do Espírito Santo, ES, 2013
- 21 **Koziarska D**, Wunsch E, Milkiewicz M, Wójcicki M, Nowacki P, Milkiewicz P. Mini-Mental State Examination in patients with hepatic encephalopathy and liver cirrhosis: a prospective, quantified electroencephalography study. *BMC Gastroenterol* 2013; **13**: 107 [PMID: 23815160 DOI: 10.1186/1471-230x-13-107]
- 22 **Citro V**, Milan G, Tripodi FS, Gennari A, Sorrentino P, Gallotta G, Postiglione A, Tarantino G. Mental status impairment in patients with West Haven grade zero hepatic encephalopathy: the role of HCV infection. *J Gastroenterol* 2007; **42**: 79-82 [PMID: 17322997 DOI: 10.1007/s00535-006-1978-8]
- 23 **Lipton AM**, Ohman KA, Womack KB, Hynan LS, Ninman ET, Lacritz LH. Subscores of the FAB differentiate frontotemporal lobar degeneration from AD. *Neurology* 2005; **65**: 726-731 [PMID: 16157906 DOI: 10.1212/01.wnl.0000174437.73416.7b]

P- Reviewer: Tarantino G S- Editor: Qiu S L- Editor: A  
E- Editor: Li D





Retrospective Study

## Retrospective study of the associations between hepatitis C virus infection and metabolic factors

Shira Yair-Sabag, Elchanan Nussinson, Ofir Ben-Assuli, Fahmi Shibli, Azmi Shahbari, Shira Zelber-Sagi

Shira Yair-Sabag, Elchanan Nussinson, Fahmi Shibli, Azmi Shahbari, Gastroenterology Institute, Emek Medical Center, Afula 18742, Israel

Shira Yair-Sabag, Shira Zelber-Sagi, School of Public Health, University of Haifa, Haifa 3498838, Israel

Ofir Ben-Assuli, Ono Academic College, Kiryat Ono 5545173, Israel

**Author contributions:** Yair-Sabag S performed the research and drafted and wrote the paper; Yair-Sabag S, Nussinson E and Zelber-Sagi S contributed equally to this manuscript; Nussinson E wrote the paper and supervised the study; Ben-Assuli O provided analytical oversight; Shibli F and Shahbari A provided administrative support; Zelber-Sagi S designed and supervised the study.

**Institutional review board statement:** This study was reviewed and approved by the Haifa University and Emek Medical Center Institutional Review Boards.

**Informed consent statement:** All study participants provided verbal informed consent prior to study enrollment.

**Conflict-of-interest statement:** There are no conflicts of interest to declare.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Elchanan Nussinson, MD, Gastro-

enterology Institute, Emek Medical Center, Dufifat 7 St, Afula 18742, Israel. [elchanann@gmail.com](mailto:elchanann@gmail.com)  
 Telephone: +972-54-4943922  
 Fax: +972-4-6523745

Received: May 8, 2016

Peer-review started: May 9, 2016

First decision: June 13, 2016

Revised: July 28, 2016

Accepted: September 13, 2016

Article in press: September 18, 2016

Published online: October 28, 2016

### Abstract

#### AIM

To evaluate the bidirectional association between metabolic syndrome (MS) components and antiviral treatment response for chronic hepatitis C virus (HCV) infection.

#### METHODS

This retrospective cohort study included 119 HCV + patients treated with pegylated-interferon- $\alpha$  and ribavirin. Metabolic characteristics and laboratory data were collected from medical records. Differences in baseline clinical and demographic risk factors between responders and non-responders were assessed using independent samples *t*-tests or  $\chi^2$  tests. The effects of sustained viral response (SVR) to antiviral treatment on *de novo* impairments in MS components, including impaired fasting glucose (IFG) and type 2 diabetes mellitus (T2DM), were assessed using univariable and multivariable logistic regression analysis, while the effect of MS components on SVR was assessed using univariable logistic regression analysis.

#### RESULTS

Of the 119 patients, 80 (67%) developed SVR over the

average  $54 \pm 13$  mo follow-up. The cumulative risks for *de novo* T2DM and IFG were 5.07- (95%CI: 1.261-20.4,  $P = 0.022$ ) and 3.87-fold higher (95%CI: 1.484-10.15,  $P = 0.006$ ), respectively for non-responders than responders, when adjusted for the baseline risk factors age, sex, HCV genotype, high viral load, and steatosis. Post-treatment triglyceride levels were significantly lower in non-responders than in responders (OR = 0.27; 95%CI: 0.069-0.962,  $P = 0.044$ ). Age and HCV genotype 3 were significantly different between responders and non-responders, and MS components were not significantly associated with SVR. Steatosis tended to attenuate SVR (OR = 0.596; 95%CI: 0.331-1.073,  $P = 0.08$ ).

## CONCLUSION

SVR was associated with lower *de novo* T2DM and IFG incidence and higher triglyceride levels. Patients infected with HCV should undergo T2DM screening and antidiabetic treatment.

**Key words:** Hepatitis C virus; Type 2 diabetes mellitus; Antiviral therapy; Sustained viral response; Metabolic syndrome; Hepatic steatosis; Peg interferon alpha; Ribavirin; Direct acting antiviral agents

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatitis C virus (HCV) is associated with a unique metabolic syndrome (MS) type: Insulin resistance with type 2 diabetes mellitus (T2DM), hypocholesterolemia, and liver steatosis. We retrospectively investigated the association between MS components and HCV infection, including antiviral therapy response, for 119 patients infected with HCV treated with interferon alpha and ribavirin. After long-term follow-up, *de novo* T2DM incidence significantly decreased, and triglyceride levels significantly increased in treatment responders. Only steatosis tended to affect treatment response. The association between HCV and lipid metabolic pathways may be important even with new direct antiviral agents. Patients infected with HCV should be screened for T2DM.

Yair-Sabag S, Nussinson E, Ben-Assuli O, Shibli F, Shahbari A, Zelber-Sagi S. Retrospective study of the associations between hepatitis C virus infection and metabolic factors. *World J Hepatol* 2016; 8(30): 1269-1278 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i30/1269.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i30.1269>

## INTRODUCTION

A mutual association between hepatitis C virus (HCV) infection and host metabolism has been demonstrated in several studies. HCV depends on host lipids for entry into the hepatocytes and for its replication; in return,

HCV also affects the metabolism of host lipids<sup>[1-3]</sup>.

HCV causes insulin resistance, hepatic steatosis, type 2 diabetes mellitus (T2DM), and low serum cholesterol and triglyceride (TG) levels. Insulin resistance contributes to HCV-related disruption of glucose and lipid metabolism<sup>[4]</sup>, and it is a key factor in metabolic syndrome (MS). In addition, HCV infection might lead to hepatic steatosis *via* several pathways.

Hepatic steatosis might aggravate MS directly by causing further insulin resistance<sup>[5]</sup> or indirectly because of resultant hepatic fibrosis<sup>[6]</sup> or cirrhosis<sup>[4,7,8]</sup>. After HCV infection, cholesterol and TG levels decrease, creating a different lipid profile from that for MS<sup>[9]</sup>. However, T2DM might be twice as prevalent in patients infected with HCV compared to the general population<sup>[5]</sup>. HCV has been associated with a unique type of MS called hepatitis C-associated dysmetabolic syndrome (HCADS), which includes liver steatosis, insulin resistance, and hypocholesterolemia<sup>[5,10]</sup>. Reversal of hypocholesterolemia and steatosis after achieving sustained viral response (SVR) with antiviral therapy has been observed in several studies<sup>[11-13]</sup>.

Therefore, although MS is not clearly associated with HCV, there is an association between HCV and some MS components. HCV-induced fatty liver and insulin resistance leads to T2DM; with the additional presence of MS, HCV replication is accelerated by activation of hepatocyte transcription factors, leading to increased lipogenesis and the provision of lipids for HCV replication<sup>[5,9,10]</sup>. Furthermore, in patients with MS, immune responses to HCV can be attenuated by leptin resistance or other changes in adipokine secretion<sup>[5]</sup>. Thus, MS might interfere with SVR after treatment<sup>[11,14-17]</sup>.

Previous studies showed that HCV eradication decreases the risk of *de novo* glucose abnormalities and insulin resistance. On the other hand, some studies reported neither an association between metabolic syndrome and HCV infection<sup>[18]</sup> nor reduced incidence of *de novo* glucose abnormalities in responders to treatment with interferon alpha and ribavirin<sup>[19]</sup>.

Our study aimed to assess the association between MS components and HCV infection based on the response to the therapy as well as to evaluate the influence of MS components on the response to antiviral therapy in a younger cohort of HCV-infected patients with a long term follow-up.

## MATERIALS AND METHODS

During 2004-2008, 119 patients diagnosed with chronic HCV infection, based on positive HCV RNA findings on polymerase chain reaction (PCR), were treated with combination pegylated-interferon  $\alpha$  (Peg-IFN $\alpha$ ) and ribavirin in the department of gastroenterology at Emek Medical Center in Afula, Israel. All patients were eligible for the Peg-IFN $\alpha$  and ribavirin treatment, which consisted of 180  $\mu$ g Peg-IFN $\alpha$  administered subcutaneously once a week and 800-1200 mg ribavirin administered orally

daily. Treatment lasted 24 wk for patients with genotypes 2/3 and 48 wk for those with genotypes 1/4.

Patients with hepatocellular carcinoma (HCC), human immunodeficiency virus infection, other serious conditions, or evidence of drug abuse or excessive alcohol consumption during the year preceding the enrollment were excluded.

### Metabolic and demographic characteristics

Patients were evaluated before treatment, 6 mo after treatment, and every year after the end of treatment until 2011. Age, body mass index (BMI), serum fasting blood glucose, TG, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, aspartate transaminase, alanine transaminase, viral load, blood pressure, liver biopsy results, and liver ultrasonography were obtained from medical records and were not available in some cases. To reduce data bias, all data were extracted from several independent sources, including patient hospital files, electronic files from the family physician, and laboratory results every year after the end of treatment. The study was performed after obtaining local ethics committee approval.

Impaired fasting glucose (IFG) was defined as a serum fasting glucose level  $> 100$  mg/dL, and T2DM was identified based on diagnoses documented in medical records, serum fasting glucose level  $> 126$  mg/dL, or use of antidiabetic drugs. MS was defined using the World Health Organization clinical criteria for MS<sup>[20]</sup>.

Serum anti-HCV antibodies were measured using a 2<sup>nd</sup> generation immunoassay, and HCV RNA was measured using real time-PCR (RT-PCR; Amplicor HCV test, Roche Diagnostic; detection rate = 50 IU/mL). HCV genotyping was determined using RT-PCR (HCV genotyping, DNA immunoassay).

Most, but not all, patients agreed to and underwent a baseline liver biopsy to determine hepatic inflammation and steatosis. In addition, ultrasonography was performed before treatment and during the follow-up period to determine hepatic steatosis.

### Statistical analysis

The statistical methods of this study were reviewed by Shira Zelber-Sagi from the School of Public Health at the University of Haifa and Ofir Ben-Assuli from Ono Academic College.

Continuous variables (MS components) are presented as means  $\pm$  SD. Statistical analyses were performed using SPSS version 21 (IBM Corp., Armonk, NY, United States), and  $P < 0.05$  was considered statistically significant for all analyses.

The response to treatment was the independent variable, and metabolic components were initially evaluated separately as dependent variables using independent samples *t*-tests or Mann-Whitney U tests, when appropriate, for continuous variables (e.g., age and BMI) and Pearson's  $\chi^2$  tests and odds ratios (ORs)

for categorical variables (e.g., sex and genotype).

To test differences from baseline to the average follow-up duration in continuous variables between the treatment responders and non-responders, independent samples *t*-tests were performed.

The difference in *de novo* occurrence of MS components (0 = normal values; 1 = abnormal values indicating presence of the component) between responders and non-responders was calculated after excluding patients with MS components prior to antiviral treatment. Then, logistic regression analysis of *de novo* occurrence of T2DM and other MS components was conducted at different time intervals using an unadjusted (univariable) logistic regression (model 1) and an adjusted (multivariable) logistic regression (model 2, adjusted for age, sex, BMI, and genotype). Additionally, the cumulative rates of the patients without *de novo* occurrence of IFG, T2DM, and MS were estimated using the Kaplan-Meier method and compared between responders and non-responders using the log-rank test.

The effects of metabolic, demographic, and clinical variables on treatment response were determined using univariable logistic regression analysis.

## RESULTS

The mean age of the 119 HCV-positive patients treated with Peg-IFN $\alpha$  and ribavirin (57% men, 43% women) was  $41 \pm 11.3$  years (Table 1). The sample population primarily included immigrants from the Union of Soviet Socialist Republics (77%). The proportions of patients with HCV genotypes 1, 2, 3 and 4 were 66%, 9.2%, 22% and 1.7%, respectively (Table 1). The mean follow-up duration for MS components after treatment was  $47.5 \pm 13.3$  mo.

Regarding MS components, hypertension, T2DM, and IFG were present in 17%, 9.2%, and 27.7% of patients, respectively (Table 1). Serum HDL values were within the lower limit of the normal range, and serum TG levels were within the normal range. Mean BMI was in the overweight range ( $27 \pm 5.4$  kg/m<sup>2</sup>). Steatosis was present in 36% or 16.5% of patients, as determined with liver biopsy or abdominal ultrasound, respectively (Table 1).

SVR was obtained in 67% ( $n = 80$ ) of patients. Only baseline age and HCV genotype 3 were significantly different between responders and non-responders (Table 2). Non-responders were significantly older ( $P = 0.017$ ), and significantly fewer non-responders had HCV genotype 3 ( $P = 0.005$ ).

In the unadjusted regression analysis of the effect of the baseline metabolic factors on treatment response, metabolic syndrome and metabolic components (except T2DM) negatively affected treatment response (OR = 0.448; OR = 0.597; respectively), though none of them were significantly associated with treatment response ( $P = 0.847$  and  $P = 0.483$ ; respectively) (Table 3).

**Table 1** Baseline characteristics of the hepatitis C virus-infected patients treated with pegylated-interferon  $\alpha$  and ribavirin

Variable (normal values)	<i>n</i> <sup>1</sup>	
Age (yr)	119	41 ± 11.3
Sex (men %)		57.1
Birth place (%)		
Israel		12.6
Union of Soviet Socialist Republics		77.3
Other (Europe, North America, South Africa, Georgia)		10
Viral load (IU/mL)	114	461.234 ± 251.445
HCV genotype (%)		
1		66.4
2		9.2
3		22.7
4		1.7
BMI (19-25 kg/m <sup>2</sup> )	81	27.0 ± 5.4
Systolic BP (15.99 kPa)	108	15.59 ± 2.26
Diastolic BP (10.66 kPa)	108	9.06 ± 1.56
Serum glucose (70-100 mg/dL)	98	96.96 ± 20.5
Cholesterol (100-200 mg/dL)	117	176 ± 49
Triglycerides (30-150 mg/dL)	90	116 ± 65
HDL (40-60 mg/dL)	74	48.8 ± 12
AST (3-32 IU)	119	51 ± 32
ALT (3-33 IU)	119	77 ± 59
T2DM (diagnosis, fasting blood glucose > 126 mg/dL, or use of anti-diabetic drugs) (%)		9.2
IFG or T2DM (fasting blood glucose > 100 mg/dL) (%)		27.7
Steatosis determined using liver biopsy ( <i>n</i> = 85) <sup>2</sup>		
Without steatosis (%)		36
Mild		37.3
Moderate		25.3
Severe		1.3
Steatosis determined using abdominal ultrasound ( <i>n</i> = 110; with steatosis) (%)		16.5

Values are reported as mean ± SD or %. <sup>1</sup>Data were not available for some patients due to the retrospective nature of the study;

<sup>2</sup>Some of the patients refused to undergo liver biopsy. HCV: Hepatitis C virus; BMI: Body mass index; BP: Blood pressure; HDL: High-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; T2DM: Type 2 diabetes mellitus; IFG: Impaired fasting glucose.

**Table 2** Comparison of baseline characteristics between responders and non-responders to treatment with pegylated-interferon  $\alpha$  and ribavirin

Variable	Non-responders	Responders	<i>P</i> value <sup>1</sup>
Age (yr)	44.62 ± 11.22	39.42 ± 11.14	0.017
BMI (kg/m <sup>2</sup> )	26.88 ± 5.11	27.34 ± 5.70	0.717
Genotype 3 (%)	2.7	17.4	0.005
Male sex (%)	20.8	35.6	0.736

The values are reported as mean ± SD or percentages. <sup>1</sup>*P* values determined using independent-samples *t*-tests. BMI: Body mass index.

In the unadjusted regression analysis of the effect of the baseline demographic and clinical variables on treatment response, the ORs for HCV genotype 3 and age were significant (OR = 5.35; 95%CI: 1.48-19.3; *P* = 0.01 and OR = 0.959; 95%CI: 0.926-9.93; *P* = 0.019; respectively), suggesting positive effects of genotype 3 and relatively young age on response to treatment (Table 4). While the rate of hepatic steatosis as determined using abdominal ultrasound (16.5%) was not significant, the rate of hepatic steatosis as determined by liver biopsy (64%) tended to result in a better response (*P* = 0.085) (Table 4).

**Table 3** Unadjusted logistic regression analysis of the association between baseline metabolic components and antiviral treatment response (*n* = 115)

Variables	Crude OR	95%CI ( <i>P</i> value)
BMI > 30 kg/m <sup>2</sup>	0.825	0.303-2.243 (0.706)
IFG (> 100 mg/dL)	0.609	0.266-1.393 (0.140)
T2DM (diagnosis, fasting blood glucose > 126 mg/dL, or use of anti-diabetic drugs)	1.094	0.301-3.975 (0.892)
High blood pressure (> 16/10.66 kPa)	0.713	0.269-1.889 (0.495)
High triglycerides	1.075	0.338-2.978 (0.889)
High cholesterol and low HDL levels	0.782	0.367-1.666 (0.523)
Presence of any metabolic syndrome components (high cholesterol levels, hyperlipidemia, high BP, or BMI > 30), without T2DM	0.448	0.551-1.301 (0.847)
Metabolic syndrome	0.597	0.141-2.520 (0.483)

The non-responder group is the reference group. OR: Odds ratio; BMI: Body mass index; IFG: Impaired fasting glucose; T2DM: Type 2 diabetes mellitus; HDL: High-density lipoprotein cholesterol; BP: Blood pressure.

Univariable and multivariable logistic regression analyses of the effect of antiviral therapy response on the *de novo* impaired MS components resulted in significant



**Table 4** Univariate analysis of the association between baseline demographic, clinical, and laboratory variables and successful treatment response

Variables	Crude OR	95%CI (P value)
Sex (n = 115)	0.878	0.412-1.873 (0.737)
Mean age (yr) (n = 115)	0.959	0.926-9.93 (0.019)
Birth place (Israel/Union of Soviet Socialist Republics/other) (n = 115)	0.839	0.530-1.329 (0.455)
Current smoker (yes/no) (n = 105)	1.487	0.762-2.901 (0.245)
Alcohol consumption (none/past) (n = 103)	1.133	0.266-4.824 (0.866)
Drug use (none/past user) (n = 106)	1.476	0.550-3.961 (0.439)
Genotype 3 (n = 115) (genotypes 1, 2, and 4 are grouped as the reference)	5.35	1.48-19.3 (0.010)
Liver steatosis determined by biopsy (yes/no) (n = 74)	0.596	0.331-1.079 (0.085)
Liver steatosis determined by ultrasound (yes/no) (n = 107)	0.515	0.181-1.462 (0.213)

Data were not available for some patients due to the retrospective nature of the study. OR: Odds ratio.

**Table 5** Unadjusted (model 1) and adjusted (model 2) logistic regression analyses of the association between the response to hepatitis C antiviral treatment and the de novo occurrence of metabolic syndrome components

Variable	Model 1			Model 2		
	n	OR	95%CI (P value)	n	OR	95%CI (P value)
T2DM (diagnosis, fasting blood glucose > 126 mg/dL, or use of anti-diabetic drugs)	83	5.07	1.261-20.494 (0.022)	-		
IFG (fasting blood glucose > 100 mg/dL)	83	3.87	1.484-10.154 (0.006)	53	4.7 <sup>1</sup>	1.280-17.316 (0.020)
Hypertriglyceridemia (triglycerides > 150 mg/dL)	96	0.27	0.069-0.967 (0.044)	-		
Low HDL levels	54	0.70	0.188-2.607 (0.595)	39	1.524 <sup>1</sup>	0.185-12.588 (0.695)
Men: HDL ≤ 35 mg/dL						-
Women: HDL ≤ 39 mg/dL						
Obesity (BMI > 30 kg/m <sup>2</sup> )	96	1.12	0.178-7.030 (0.91)	96	0.78 <sup>2</sup>	0.115-5.339 (0.80)
Hypertension (defined by WHO)	95	1.176	0.379-3.626 (0.782)	62	1.92 <sup>1</sup>	0.246-5.636 (0.458)
Hepatic steatosis determined by ultrasound	90	2.66	0.929-7.636 (0.068)	64	2.151 <sup>1</sup>	0.555-8.33 (0.268)

<sup>1</sup>Adjusted for sex, age, and BMI; <sup>2</sup>Adjusted for sex, age, and genotype. The responders group is the reference group for all the dependent variables. Multivariable analysis could not be conducted owing to the small number of responders with *de novo* DM (n = 3) or small number of non-responders with hypertriglyceridemia (n = 3). OR: Odds ratio; BMI: Body mass index; IFG: Impaired fasting glucose; T2DM: Type 2 diabetes mellitus; HDL: High-density lipoprotein cholesterol; WHO: World Health Organization; DM: Diabetes mellitus.

crude ORs of 3.87 for IFG and 5.07 for T2DM ( $P = 0.006$  and  $0.022$ , respectively) in the unadjusted model, and a significant OR of 4.7 for IFG in the adjusted model ( $P = 0.02$ ; model 2). Because of the low incidence of T2DM in responders ( $n = 3$ ), T2DM could not be evaluated in the adjusted model (model 2; multivariable analysis) (Table 5). The crude OR for hypertriglyceridemia was 0.27 ( $P = 0.044$ ). Because of the low occurrence of hypertriglyceridemia in the non-responders ( $n = 3$ ), hypertriglyceridemia could not be evaluated in the adjusted model.

According to Kaplan-Meier analyses, there were lower rates of IFG and T2DM in responders than in the non-responders ( $P = 0.006$  and  $0.023$ , respectively) (Figure 1). Overall, the occurrence of MS in responders was not different from that in non-responders (Figure 2).

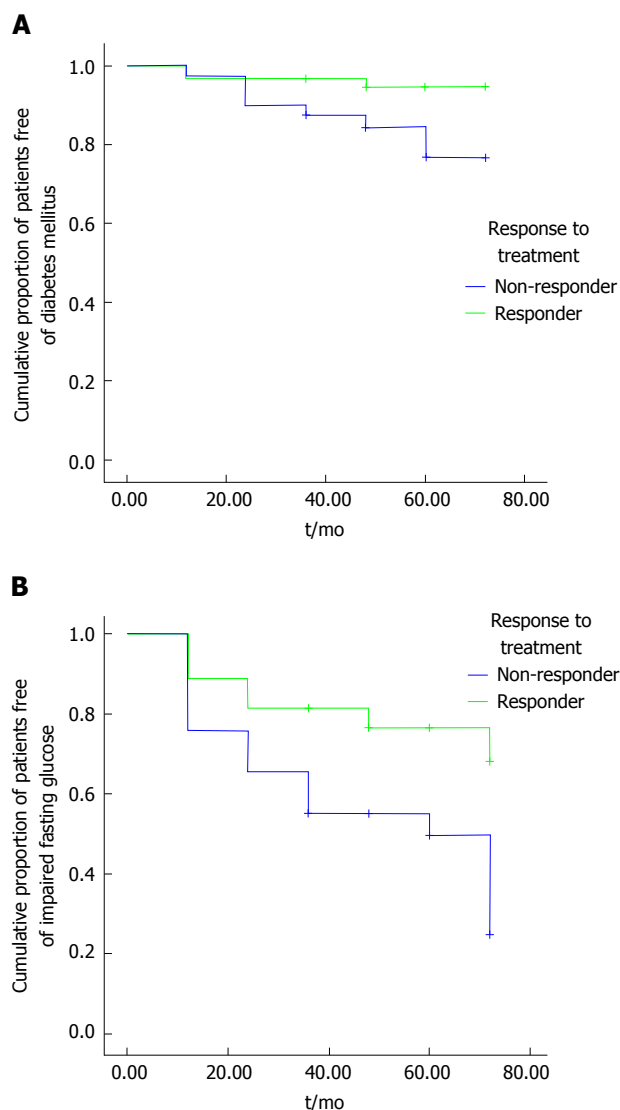
## DISCUSSION

Our study, which aimed to examine the association between MS components and HCV infection based on treatment response and the influence of MS components on the success of antiviral therapy, did not detect any differences in most pre- or post-treatment MS com-

ponent values between responders and non-responders to antiviral therapy. However, *de novo* IFG and T2DM occurred significantly more often in non-responders than in responders.

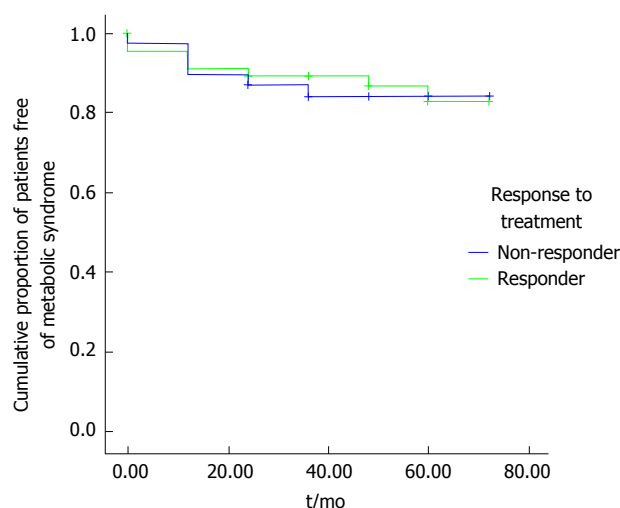
Our results regarding T2DM are consistent with those of other studies conducted with larger community cohorts. In a study conducted in Taiwan with a 7-year follow-up period, the cumulative incidence of T2DM was 14.3% in anti-HCV-positive patients and 8.6% in seronegative individuals ( $P < 0.0001$ )<sup>[21]</sup>. In another study conducted in Japan, 143 of 2842 HCV-positive patients treated with IFN $\alpha$  monotherapy or IFN $\alpha$  and ribavirin combination therapy developed T2DM during a mean observation period of 6.4 years, and only 26 of these patients with T2DM had SVR<sup>[22]</sup>.

HCV infection causes insulin resistance in the very early stage hepatic lesions (fibrosis stage 0 or 1). The progression of fibrosis, primarily owing to insulin resistance, worsens insulin resistance<sup>[23]</sup>, which may lead to T2DM in predisposed individuals. In addition to SVR, fibrosis stage is independently associated with T2DM in HCV-infected patients<sup>[24]</sup>. Furthermore, a recent meta-analysis<sup>[25]</sup> and systematic review included 11 studies, of which only five examined the influence of



**Figure 1** Kaplan-Meier analysis of the cumulative ratio of hepatitis C virus-positive patients. A: Without type 2 diabetes mellitus, based on the response to antiviral treatment; B: Without impaired fasting glucose, based on the response to antiviral treatment.

HCV eradication on the risk of *de novo* glucose abnormalities<sup>[19,24,26,27]</sup> and insulin resistance<sup>[27]</sup>. Of the 2 studies with long follow-up periods<sup>[19,26]</sup>, one (8-year follow-up) failed to demonstrate reduced *de novo* T2DM in patients with SVR<sup>[19]</sup>. The other (follow-up of  $5.7 \pm 2$  years) showed that SVR reduced *de novo* glucose abnormalities in patients with chronic HCV. However, patient ages were not reported<sup>[26]</sup>. In another study with a relatively short follow-up (24 mo), SVR in patients with chronic HCV who did not have T2DM ( $51.8 \pm 12.2$  years old) prevented the development of *de novo* insulin resistance<sup>[28]</sup>. A significant two-third reduction in T2DM development was reported in a large cohort of patients with HCV and SVR ( $51.8 \pm 9$  years old) after IFN $\alpha$  monotherapy or combination therapy with IFN $\alpha$  and ribavirin<sup>[22]</sup>. Thus, curing HCV infections decreases the incidence of T2DM or improves homeostatic model assessment insulin resistance (HOMA-IR) in most studies<sup>[22-33]</sup>. These effects



**Figure 2** Kaplan-Meier analysis of the cumulative ratio of hepatitis C virus-positive patients without metabolic syndrome, based on the response to antiviral treatment.

might be specific to patients with particular genotypes, with a reduction in HOMA-IR in patients with HCV genotype 1 but not those with genotypes 2/3<sup>[23]</sup>. Antiviral therapy might improve insulin resistance independent of virological outcomes<sup>[32]</sup> although a greater reduction in HOMA was observed in the patients who achieved persistent HCV clearance<sup>[33]</sup>. Antiviral therapy might also improve hepatic steatosis and fibrosis<sup>[29,34,35]</sup>. Thus, there might be an association between HCV and MS, and patients with HCV infection and MS have higher HOMA-IR values.

Several other components of MS, including waist circumference, BMI, and arterial hypertension, have been reported more frequently in non-responders to antiviral therapy<sup>[15]</sup>. The present study failed to demonstrate a significant relationship between baseline metabolic factors and treatment responses, potentially owing to the relatively young sample population. However, some metabolic factors (apart from T2DM) showed trends for differences based on the treatment response (e.g., *de novo* hypertriglyceridemia was 3.7 times more frequent in the responders than in the non-responders).

A bidirectional relationship between serum lipid levels and success of antiviral therapy for HCV has been reported<sup>[36]</sup>. Successful antiviral therapy might reverse the low LDL cholesterol, HDL cholesterol, and TG levels associated with HCV infection<sup>[7,12,13]</sup>. Low serum LDL levels in HCV infection result from the utilization of geranyl-geranyl phosphate, a product of the mevalonate pathway that is an early branch point of the cholesterol synthetic pathway, for HCV replication<sup>[1,7]</sup>. Higher baseline serum LDL cholesterol and lower serum TG levels were associated with higher rates of SVR<sup>[34]</sup>, and lower serum LDL cholesterol levels correlated with low rates of SVR<sup>[33,35]</sup> in non-diabetic, non-cirrhotic patients infected with genotype 1 HCV. High serum LDL cholesterol levels

might improve the rates of SVR by competing with binding to hepatocyte LDL receptors and subsequently reducing the infection of hepatocytes with HCV<sup>[4,11,34]</sup>. In contrast, HDL cholesterol enhances HCV infection by facilitating its entry into hepatocytes<sup>[2]</sup>. However, high baseline serum HDL cholesterol levels reportedly interfere with the early viral response, but not with SVR<sup>[3,4]</sup>, while serum HDL cholesterol inversely correlates with the rate of SVR in men but not in women, resulting in a lack of an association between overall baseline HDL levels and SVR<sup>[11]</sup>.

Hepatic steatosis might also attenuate the antiviral treatment response, a trend that was demonstrated in the present study based on steatosis identified *via* liver biopsy. The insulin resistance and hepatic steatosis present during HCV infection are genotype-specific. Lower HOMA values are reported in patients infected with genotype 3 than in those infected with genotype 1. Insulin resistance-associated steatosis, which is present in patients with HCV genotype 3, is caused mainly by viral inhibition of the enzyme microsomal triglyceride transfer protein (viral steatosis), which might resolve with successful antiviral therapy. With the other HCV genotypes, steatosis is due to insulin resistance, stimulation of fatty acid synthesis, and inhibition of mitochondrial  $\beta$ -oxidation (metabolic steatosis)<sup>[37-39]</sup>. Metabolic steatosis might be associated with a high BMI and central obesity, which are not usually improved by viral eradication. HCV 1 and 4 core proteins might cause insulin resistance by functionally inhibiting insulin signaling pathways *via* increased levels of pro-inflammatory cytokines including tumor necrosis factor (TNF)- $\alpha$  and suppressors of cytokine signaling (SOCS) proteins, which impair insulin signaling and activate sterol regulatory element binding proteins, resulting in increased hepatic lipid synthesis<sup>[37-39]</sup>. The increased levels of hepatic proinflammatory cytokines have additional effect of negative regulate IFN $\alpha$  transduction.

This might explain the molecular link between insulin resistance and the nonresponse to antiviral therapy<sup>[4]</sup>. Furthermore, steatosis has been demonstrated to decrease SVR in HCV genotype 1 but not in HCV genotype 3, although steatosis is a predictor of HCV infection relapse with genotype 3 HCV<sup>[16]</sup>. This effect of steatosis on SVR might be the result of its association with insulin resistance<sup>[40]</sup>, which is caused by excretion of TNF $\alpha$  and SOCS protein from the increased trunk fat in HCV infection. The lower level of PPAR- $\alpha$  mRNA also mediates genotype 3 hepatic steatogenesis<sup>[7,8,39]</sup>. However, Peg-IFN $\alpha$  and ribavirin treatment response with HCV genotype 3 infection is better than with HCV genotype 1, despite more severe steatosis and lower cholesterol levels<sup>[10,37,40]</sup>. This might be related to the association between steatosis and higher BMI with genotypes 1 and 4<sup>[14,15]</sup>.

It is noteworthy that, even in the era of direct acting antiviral (DAA; *e.g.*, telaprevir and boceprevir)-based

triple therapy, some baseline metabolic variables might affect SVR, albeit to a lesser degree than with IFN and ribavirin combination therapy<sup>[41]</sup>. However, insulin resistance does not predict SVR to telaprevir-based triple therapy or to the protease inhibitor danoprevir monotherapy<sup>[16,42]</sup>, while low serum LDL levels might affect SVR in telaprevir-treated patients, and obesity impairs SVR in patients treated with boceprevir-based regimen<sup>[16,41]</sup>. An additional link between DAA and MS has been demonstrated with danoprevir monotherapy, an inhibitor of NS3/3A HCV serine protease, which might increase insulin sensitivity considerably, independent of its antiviral effect<sup>[15,38,42]</sup>. Furthermore, DAAs are less effective against genotype 3 HCV infection, partly due to steatosis<sup>[43]</sup> and relapse after IFN-free therapy with the polymerase inhibitor sofosbuvir and ribavirin is associated with a low baseline LDL level.

This study has certain limitations. First, the study was retrospective, and some data were missing for some cases. Serum glucose levels were examined for diagnosis and monitoring glycemic control of T2DM and IFG. However, due to the retrospective nature of this manuscript, HbA1c and glycated albumin (GA) values were not available in most of the patients' charts. Nevertheless, liver cirrhosis and INF  $\alpha$  treatment may falsely decrease HbA1c owing to hemolysis. On the other hand, GA as a glycemic control marker in patients with chronic liver disease may be overestimated, due to prolonged albumin half-life.

Additionally, the small sample size resulted in a small number of responders with *de novo* T2DM and non-responders with *de novo* hypertriglyceridemia, which limited the ability to assess these data in the multivariable logistic regression analysis.

However, the strengths of the study include the relatively young age of the patients and the relatively long follow-up period for all MS components after antiviral treatment, which enabled us to observe the effect of SVR on the cumulative incidences of IFG and T2DM. MS, including insulin resistance, hyperglycemia, high BMI, and liver steatosis, might complicate the disease course of patients infected with HCV, by enhancing cirrhosis, HCC, and cardiovascular disease<sup>[29,43-47]</sup>. Thus, it is worthwhile to screen these patients for T2DM<sup>[46,47]</sup>. Furthermore, with T2DM in the presence of HCV, there are specific considerations for antidiabetic treatment. Insulin and sulfonylurea administration might increase the risk of HCC<sup>[45,48]</sup>, while the insulin sensitizers metformin and pioglitazone might decrease the risk of HCC and steatosis. However, these agents are harmful and might cause lactic acidosis and hepatic toxicity, respectively, in patients with liver cirrhosis<sup>[45]</sup>. The new dipeptidyl peptidase-4 inhibitors appear promising<sup>[45,47,49]</sup>.

In conclusion, the results presented here suggest that MS components did not have any significant effect on the response to antiviral therapy, although hepatic steatosis tended to impair the response to antiviral treatment.

There were no differences in the post-treatment changes in most MS components between responders and non-responders to antiviral therapy. However, the incidences of *de novo* T2DM and IFG were significantly higher in non-responders. Given the younger age of the patient population in the present study compared to previous similar studies, the findings might suggest a direct effect of HCV on the development of T2DM independent of fibrosis or cirrhosis. The higher serum TG levels after SVR exemplify the interaction between HCV infection and lipid metabolic pathways. Due to the increased risk of HCV infection with T2DM, it might be appropriate to screen HCV patients for T2DM and insulin resistance and to consider treatment of T2DM in the presence of HCV with new antidiabetic agents.

## COMMENTS

### Background

A bidirectional association exists between chronic hepatitis C virus (HCV) infection and some components of metabolic syndrome (MS).

### Research frontiers

Most, but not all, previous studies showed an association between chronic HCV infection and MS components, including type 2 diabetes mellitus (T2DM), insulin resistance, elevated body mass index, and hepatic steatosis. Several host MS components might affect the disease course and sustained virological response (SVR) of HCV-infected patients treated with pegylated-interferon  $\alpha$  (Peg-IFN $\alpha$ ) and ribavirin. On the other hand, SVR can affect some MS components, mainly decreased insulin resistance (IR) and decreased *de novo* occurrence of T2DM.

### Innovations and breakthroughs

This study, which included a younger patient cohort, was designed to assess associations between MS components and HCV infection, but it did not show increased prevalence of T2DM with HCV infection. However, after long term follow-up T2DM was more frequent in non-responders to treatment with Peg-IFN $\alpha$  and ribavirin.

### Applications

Patients with HCV infection should be frequently monitored for T2DM and treated appropriately, considering the increased risk of cirrhosis and HCC in young patients with HCV-associated diabetes mellitus. The authors think that further studies are needed to evaluate the mutual association of MS components with direct acting antiviral (DAA) drug therapy in chronic HCV infection.

### Terminology

MS components refer to the metabolic syndrome factors; IR refers to insulin resistance; SVR refers to sustained virological response, which means viral eradication and cure; DAA refers to direct acting antiviral agents.

### Peer-review

This is a very interesting study which shows the association between metabolic syndrome mechanism and response to antiviral treatment for chronic HCV infection. Patients were followed up for about 4 years; univariable and multivariable logistic regression analysis were applied. Data are well presented and discussed.

## REFERENCES

- Felmlee DJ, Hafirassou ML, Lefevre M, Baumert TF, Schuster C. Hepatitis C virus, cholesterol and lipoproteins—impact for the viral life cycle and pathogenesis of liver disease. *Viruses* 2013; **5**: 1292-1324 [PMID: 23698400 DOI: 10.3390/v5051292]
- Pécheur EI. Lipoprotein receptors and lipid enzymes in hepatitis C virus entry and early steps of infection. *Scientifica* (Cairo) 2012; **2012**: 709853 [PMID: 24278733 DOI: 10.6064/2012/709853]
- Ye J. Reliance of host cholesterol metabolic pathways for the life cycle of hepatitis C virus. *PLoS Pathog* 2007; **3**: e108 [PMID: 17784784 DOI: 10.1371/journal.ppat.0030108]
- Kawaguchi Y, Mizuta T. Interaction between hepatitis C virus and metabolic factors. *World J Gastroenterol* 2014; **20**: 2888-2901 [PMID: 24659880 DOI: 10.3748/wjg.v20.i11.2888]
- Adinolfi LE, Restivo L, Zampino R, Lonardo A, Loria P. Metabolic alterations and chronic hepatitis C: treatment strategies. *Expert Opin Pharmacother* 2011; **12**: 2215-2234 [PMID: 21883025 DOI: 10.1517/14656566.2011.597742]
- Lecube A, Hernández C, Genescà J, Simó R. Glucose abnormalities in patients with hepatitis C virus infection: Epidemiology and pathogenesis. *Diabetes Care* 2006; **29**: 1140-1149 [PMID: 16644655 DOI: 10.2337/dc05-1995]
- Negro F. Mechanisms and significance of liver steatosis in hepatitis C virus infection. *World J Gastroenterol* 2006; **12**: 6756-6765 [PMID: 17106922 DOI: 10.3748/wjg.v12.i42.6756]
- Lonardo A, Adinolfi LE, Restivo L, Ballestri S, Romagnoli D, Baldelli E, Nascimbeni F, Loria P. Pathogenesis and significance of hepatitis C virus steatosis: an update on survival strategy of a successful pathogen. *World J Gastroenterol* 2014; **20**: 7089-7103 [PMID: 24966582 DOI: 10.3748/wjg.v20.i23.7089]
- Negro F. HCV infection and metabolic syndrome: which is the chicken and which is the egg? *Gastroenterology* 2012; **142**: 1288-1292 [PMID: 22537435 DOI: 10.1053/j.gastro.2011.12.063]
- Adinolfi LE, Restivo L, Marrone A. The predictive value of steatosis in hepatitis C virus infection. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 205-213 [PMID: 23445230 DOI: 10.1586/egh.13.7]
- Ramcharran D, Wahed AS, Conjeevaram HS, Evans RW, Wang T, Belle SH, Yee LJ. Associations between serum lipids and hepatitis C antiviral treatment efficacy. *Hepatology* 2010; **52**: 854-863 [PMID: 20690192 DOI: 10.1002/hep.23796]
- Fernández-Rodríguez CM, López-Serrano P, Alonso S, Gutiérrez ML, Lledó JL, Pérez-Calle JL, Temiño R, Cacho G, Nevado M, Casas ML, Gasalla JM, Bonet B. Long-term reversal of hypocholesterolaemia in patients with chronic hepatitis C is related to sustained viral response and viral genotype. *Aliment Pharmacol Ther* 2006; **24**: 507-512 [PMID: 16886916 DOI: 10.1111/j.1365-2036.2006.03000.x]
- Kuo YH, Chuang TW, Hung CH, Chen CH, Wang JH, Hu TH, Lu SN, Lee CM. Reversal of hypolipidemia in chronic hepatitis C patients after successful antiviral therapy. *J Formos Med Assoc* 2011; **110**: 363-371 [PMID: 21741004 DOI: 10.1016/S0929-6646(11)60054-5]
- Soresi M, Tripi S, Franco V, Giannitrapani L, Alessandri A, Rappa F, Vuturo O, Montalto G. Impact of liver steatosis on the antiviral response in the hepatitis C virus-associated chronic hepatitis. *Liver Int* 2006; **26**: 1119-1125 [PMID: 17032413 DOI: 10.1111/j.1478-3231.2006.01347.x]
- Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; **38**: 639-644 [PMID: 12939590 DOI: 10.1053/jhep.2003.50350]
- Cheng FK, Torres DM, Harrison SA. Hepatitis C and lipid metabolism, hepatic steatosis, and NAFLD: still important in the era of direct acting antiviral therapy? *J Viral Hepat* 2014; **21**: 1-8 [PMID: 24329852 DOI: 10.1111/jvh.12172]
- Tarantino G, Conca P, Sorrentino P, Ariello M. Metabolic factors involved in the therapeutic response of patients with hepatitis C virus-related chronic hepatitis. *J Gastroenterol Hepatol* 2006; **21**: 1266-1268 [PMID: 16872307 DOI: 10.1111/j.1440-1746.2006.04394.x]
- Cheng YL, Wang YC, Lan KH, Huo TI, Huang YH, Su CW, Lin HC, Lee FY, Wu JC, Lee SD. Anti-hepatitis C virus seropositivity is not associated with metabolic syndrome irrespective of age, gender and fibrosis. *Ann Hepatol* 2015; **14**: 181-189 [PMID: 25671827]
- Giordanino C, Bugianesi E, Smedile A, Ciancio A, Abate ML,



- Olivero A, Pellicano R, Cassader M, Gambino R, Bo S, Ciccone G, Rizzetto M, Saracco G. Incidence of type 2 diabetes mellitus and glucose abnormalities in patients with chronic hepatitis C infection by response to treatment: results of a cohort study. *Am J Gastroenterol* 2008; **103**: 2481-2487 [PMID: 18702647 DOI: 10.1111/j.1572-0241.2008.02002.x]
- 20 Grundy SM, Brewer HB, Cleeman JI, Smith SC, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Arterioscler Thromb Vasc Biol* 2004; **24**: e13-e18 [PMID: 14766739 DOI: 10.1161/01.ATV.0000111245.75752.C6]
- 21 Wang CS, Wang ST, Yao WJ, Chang TT, Chou P. Hepatitis C virus infection and the development of type 2 diabetes in a community-based longitudinal study. *Am J Epidemiol* 2007; **166**: 196-203 [PMID: 17496314 DOI: 10.1093/aje/kwm061]
- 22 Arase Y, Suzuki F, Suzuki Y, Akuta N, Kobayashi M, Kawamura Y, Yatsuji H, Sezaki H, Hosaka T, Hirakawa M, Ikeda K, Kumada H. Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. *Hepatology* 2009; **49**: 739-744 [PMID: 19127513 DOI: 10.1002/hep.22703]
- 23 Thompson AJ, Patel K, Chuang WL, Lawitz EJ, Rodriguez-Torres M, Rustgi VK, Flisiak R, Pianko S, Diago M, Arora S, Foster GR, Torbenson M, Benhamou Y, Nelson DR, Sulkowski MS, Zeuzem S, Pulkstenis E, Subramanian GM, McHutchison JG. Viral clearance is associated with improved insulin resistance in genotype 1 chronic hepatitis C but not genotype 2/3. *Gut* 2012; **61**: 128-134 [PMID: 21873466 DOI: 10.1136/gut.2010.236158]
- 24 Romero-Gómez M, Fernández-Rodríguez CM, Andrade RJ, Diago M, Alonso S, Planas R, Solá R, Pons JA, Salmerón J, Barcena R, Perez R, Carmona I, Durán S. Effect of sustained virological response to treatment on the incidence of abnormal glucose values in chronic hepatitis C. *J Hepatol* 2008; **48**: 721-727 [PMID: 18308416 DOI: 10.1016/j.jhep.2007.11.022]
- 25 Zhang W, Rao HY, Feng B, Liu F, Wei L. Effects of interferon-alpha treatment on the incidence of hyperglycemia in chronic hepatitis C patients: a systematic review and meta-analysis. *PLoS One* 2012; **7**: e39272 [PMID: 22768067 DOI: 10.1371/journal.pone.0039272]
- 26 Simó R, Lecube A, Genescà J, Esteban JI, Hernández C. Sustained virological response correlates with reduction in the incidence of glucose abnormalities in patients with chronic hepatitis C virus infection. *Diabetes Care* 2006; **29**: 2462-2466 [PMID: 17065685 DOI: 10.2337/dc06-0456]
- 27 Kawaguchi Y, Mizuta T, Oza N, Takahashi H, Ario K, Yoshimura T, Eguchi Y, Ozaki I, Hisatomi A, Fujimoto K. Eradication of hepatitis C virus by interferon improves whole-body insulin resistance and hyperinsulinaemia in patients with chronic hepatitis C. *Liver Int* 2009; **29**: 871-877 [PMID: 19302179 DOI: 10.1111/j.1478-3231.2009.01993.x]
- 28 Aghemo A, Prati GM, Rumi MG, Soffredini R, D'Ambrosio R, Orsi E, De Nicola S, Degasperis E, Grancini V, Colombo M. Sustained virological response prevents the development of insulin resistance in patients with chronic hepatitis C. *Hepatology* 2012; **56**: 1681-1687 [PMID: 22619107 DOI: 10.1002/hep.25867]
- 29 Pearlman BL, Traub N. Sustained virologic response to antiviral therapy for chronic hepatitis C virus infection: a cure and so much more. *Clin Infect Dis* 2011; **52**: 889-900 [PMID: 21427396 DOI: 10.1093/cid/cir076]
- 30 Kawaguchi T, Ide T, Taniguchi E, Hirano E, Itou M, Sumie S, Nagao Y, Yanagimoto C, Hanada S, Koga H, Sata M. Clearance of HCV improves insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate 1 and 2. *Am J Gastroenterol* 2007; **102**: 570-576 [PMID: 17222321 DOI: 10.1111/j.1572-0241.2006.01038.x]
- 31 Kim HJ, Park JH, Park DI, Cho YK, Sohn CI, Jeon WK, Kim BI. Clearance of HCV by Combination Therapy of Pegylated Interferon alpha-2a and Ribavirin Improves Insulin Resistance. *Gut Liver* 2009; **3**: 108-115 [PMID: 20431732 DOI: 10.5009/gnl.2009.3.2.108]
- 32 Brandman D, Bacchetti P, Ayala CE, Maher JJ, Khalili M. Impact of insulin resistance on HCV treatment response and impact of HCV treatment on insulin sensitivity using direct measurements of insulin action. *Diabetes Care* 2012; **35**: 1090-1094 [PMID: 22399695 DOI: 10.2337/dc11-1837]
- 33 Petta S, Cammà C, Di Marco V, Cabibi D, Ciminnisi S, Caldarella R, Licata A, Massenti MF, Marchesini G, Craxi A. Time course of insulin resistance during antiviral therapy in non-diabetic, non-cirrhotic patients with genotype 1 HCV infection. *Antivir Ther* 2009; **14**: 631-639 [PMID: 19704165]
- 34 Gopal K, Johnson TC, Gopal S, Walfish A, Bang CT, Suwandhi P, Pena-Sahdala HN, Clain DJ, Bodenheimer HC, Min AD. Correlation between beta-lipoprotein levels and outcome of hepatitis C treatment. *Hepatology* 2006; **44**: 335-340 [PMID: 16871569 DOI: 10.1002/hep.21261]
- 35 Poynard T, Ratziu V, McHutchison J, Manns M, Goodman Z, Zeuzem S, Younossi Z, Albrecht J. Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. *Hepatology* 2003; **38**: 75-85 [PMID: 12829989 DOI: 10.1053/jhep.2003.50267]
- 36 Dai CY, Yeh ML, Huang CF, Hou CH, Hsieh MY, Huang JF, Lin IL, Lin ZY, Chen SC, Wang LY, Chuang WL, Yu ML, Tung HD. Chronic hepatitis C infection is associated with insulin resistance and lipid profiles. *J Gastroenterol Hepatol* 2015; **30**: 879-884 [PMID: 23808794 DOI: 10.1111/jgh.12313]
- 37 Negro F, Alaei M. Hepatitis C virus and type 2 diabetes. *World J Gastroenterol* 2009; **15**: 1537-1547 [PMID: 19340895 DOI: 10.3748/wjg.15.1537]
- 38 Patel A, Harrison SA. Hepatitis C virus infection and nonalcoholic steatohepatitis. *Gastroenterol Hepatol (N Y)* 2012; **8**: 305-312 [PMID: 22933860]
- 39 Bugianesi E, Salamone F, Negro F. The interaction of metabolic factors with HCV infection: does it matter? *J Hepatol* 2012; **56** Suppl 1: S56-S65 [PMID: 22300466 DOI: 10.1016/S0168-8278(12)60007-5]
- 40 Basaranoglu M, Basaranoglu G. Pathophysiology of insulin resistance and steatosis in patients with chronic viral hepatitis. *World J Gastroenterol* 2011; **17**: 4055-4062 [PMID: 22039318 DOI: 10.3748/wjg.v17.i36.4055]
- 41 Grasso A, Malfatti F, Testa R. Are metabolic factors still important in the era of direct antiviral agents in patients with chronic hepatitis C? *World J Gastroenterol* 2013; **19**: 6947-6956 [PMID: 24222938 DOI: 10.3748/wjg.v19.i41.6947]
- 42 Moucari R, Forestier N, Larrey D, Guyader D, Couzigou P, Benhamou Y, Voitot H, Vidaud M, Seiwert S, Bradford B, Zeuzem S, Marcellin P. Danoprevir, an HCV NS3/4A protease inhibitor, improves insulin sensitivity in patients with genotype 1 chronic hepatitis C. *Gut* 2010; **59**: 1694-1698 [PMID: 20861007 DOI: 10.1136/gut.2010.219089]
- 43 Lim T. Metabolic syndrome in chronic hepatitis C infection: does it still matter in the era of directly acting antiviral therapy? *Hepat Med* 2014; **6**: 113-118 [PMID: 25506251 DOI: 10.2147/HMER.S60083]
- 44 Negro F. Facts and fictions of HCV and comorbidities: steatosis, diabetes mellitus, and cardiovascular diseases. *J Hepatol* 2014; **61**: S69-S78 [PMID: 25443347 DOI: 10.1016/j.jhep.2014.08.003]
- 45 Kawaguchi T, Sata M. Importance of hepatitis C virus-associated insulin resistance: therapeutic strategies for insulin sensitization. *World J Gastroenterol* 2010; **16**: 1943-1952 [PMID: 20419831 DOI: 10.3748/wjg.v16.i16.1943]
- 46 Hammerstad SS, Grock SF, Lee HJ, Hasham A, Sundaram N, Tomer Y. Diabetes and Hepatitis C: A Two-Way Association. *Front Endocrinol (Lausanne)* 2015; **6**: 134 [PMID: 26441826 DOI: 10.3389/fendo.2015.00134]
- 47 García-Compeán D, González-González JA, Lavalle-González FJ, González-Moreno EI, Villarreal-Pérez JZ, Maldonado-Garza HJ. Current Concepts in Diabetes Mellitus and Chronic Liver Disease: Clinical Outcomes, Hepatitis C Virus Association, and Therapy. *Dig Dis Sci* 2016; **61**: 371-380 [PMID: 26462490 DOI: 10.1007/s10620-015-3907-2]

- 48 **Kawaguchi T**, Taniguchi E, Morita Y, Shirachi M, Tateishi I, Nagata E, Sata M. Association of exogenous insulin or sulphonylurea treatment with an increased incidence of hepatoma in patients with hepatitis C virus infection. *Liver Int* 2010; **30**: 479-486 [PMID: 20040053 DOI: 10.1111/j.1478-3231.2009.02191.x]
- 49 **Itou M**, Kawaguchi T, Taniguchi E, Sata M. Dipeptidyl peptidase-4: a key player in chronic liver disease. *World J Gastroenterol* 2013; **19**: 2298-2306 [PMID: 23613622 DOI: 10.3748/wjg.v19.i15.2298]

**P- Reviewer:** Osna NA, Tanaka Y    **S- Editor:** Qiu S    **L- Editor:** A  
**E- Editor:** Li D



Prospective Study

## Reversibility of minimal hepatic encephalopathy following liver transplantation in Egyptian cirrhotic patients

Mahmoud A Osman, Moataz M Sayed, Khaled A Mansour, Shereen A Saleh, Wesam A Ibrahim, Sara M Abdelhakam, Mohamed Bahaa, Wael A Yousry, Hosam S Elbaz, Reginia N Mikhail, Azza M Hassan, Ehab H Elsayed, Dalia A Mahmoud

Mahmoud A Osman, Moataz M Sayed, Khaled A Mansour, Shereen A Saleh, Wesam A Ibrahim, Wael A Yousry, Hosam S Elbaz, Reginia N Mikhail, Department of Internal Medicine, Hepatology and Gastroenterology, Faculty of Medicine, Ain Shams University, Cairo 11341, Egypt

Sara M Abdelhakam, Department of Tropical Medicine, Faculty of Medicine, Ain Shams University, Cairo 11341, Egypt

Mohamed Bahaa, Department of Hepatobiliary Surgery, Faculty of Medicine, Ain Shams University, Cairo 11341, Egypt

Azza M Hassan, Department of Community, Environmental and Occupational Medicine, Faculty of Medicine, Ain Shams University, Cairo 11341, Egypt

Ehab H Elsayed, Department of Internal Medicine, Hepatology and Gastroenterology, National Research Center, Cairo 11341, Egypt

Dalia A Mahmoud, Department of Neuropsychiatry, Faculty of Medicine, Ain Shams University, Cairo 11341, Egypt

**Author contributions:** Osman MA and Mahmoud DA contributed equally to this work; Osman MA, Sayed MM, Mansour KA, Saleh SA, Abdelhakam SM, Bahaa M and Mahmoud DA designed the research; Osman MA, Ibrahim WA, Bahaa M, Yousry WA, Elbaz HS, Mikhail RN and Mahmoud DA performed the research; Osman MA, Elbaz HS, Mikhail RN, Elsayed EH, Hassan AM and Mahmoud DA analyzed the data; Sayed MM, Mansour KA, Saleh SA, Ibrahim WA, Abdelhakam SM, Yousry WA and Hassan AM contributed analytic tools; Sayed MM, Saleh SA, Ibrahim WA, Abdelhakam SM and Yousry WA wrote the paper.

**Institutional review board statement:** This study was reviewed and approved by the Research Ethics Committee of Faculty of Medicine, Ain Shams University Institutional Review Board.

**Clinical trial registration statement:** This study is registered at (<https://clinicaltrials.gov/show/NCT02767622>). The registration

identification number is (NCT02767622 Unique Protocol ID: 875).

**Informed consent statement:** All study participants provided written informed consent prior to study enrollment.

**Conflict-of-interest statement:** None of the authors have any conflicts of interests or any financial disclosures.

**Data sharing statement:** The technical appendix, statistical code and dataset are available from the corresponding author at [saratropical@yahoo.com](mailto:saratropical@yahoo.com). The participants gave informed consent for the data sharing.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Sara M Abdelhakam, MD, Assistant Professor of Tropical Medicine, Department of Tropical Medicine, Faculty of Medicine, Ain Shams University, Khalifa El-Maamon St., Abbassia, Cairo 11341, Egypt. [saratropical@yahoo.com](mailto:saratropical@yahoo.com)  
 Telephone: +20-100-1601548  
 Fax: +20-22-2598751

**Received:** May 28, 2016

**Peer-review started:** May 30, 2016

**First decision:** July 20, 2016

**Revised:** August 6, 2016

**Accepted:** September 13, 2016

**Article in press:** September 18, 2016

**Published online:** October 28, 2016

## Abstract

### AIM

To evaluate the reversibility of minimal hepatic encephalopathy (MHE) following liver transplantation (LT) in Egyptian cirrhotic patients.

### METHODS

This prospective study included twenty patients with biopsy-proven liver cirrhosis listed for LT and twenty age- and sex-matched healthy control subjects. All underwent neuro-psychiatric examination, laboratory investigations, radiological studies and psychometric tests including trail making test A (TMT A), TMT B, digit symbol test and serial dotting test. The psychometric hepatic encephalopathy score (PHES) was calculated for patients to diagnose MHE. Psychometric tests were repeated six months following LT in the cirrhotic patient group.

### RESULTS

Before LT, psychometric tests showed highly significant deficits in cirrhotic patients in comparison to controls ( $P < 0.001$ ). There was a statistically significant improvement in test values in the patient group after LT; however, their values were still significantly worse than those of the controls ( $P < 0.001$ ). The PHES detected MHE in 16 patients (80%) before LT with a median value of  $-7 \pm 3.5$ . The median PHES value was significantly improved following LT, reaching  $-4.5 \pm 5$  ( $P < 0.001$ ), and the number of patients with MHE decreased to 11 (55%). The pre-transplant model for end-stage liver disease (MELD) score  $\geq 15$  was significantly related to the presence of post-transplant MHE ( $P = 0.005$ ). More patients in whom reversal of MHE was observed had a pre-transplant MELD score  $< 15$ .

### CONCLUSION

Reversal of MHE in cirrhotic patients could be achieved by LT, especially in those with a MELD score  $< 15$ .

**Key words:** Liver transplantation; Model for end-stage liver disease score; Psychometric tests; Minimal hepatic encephalopathy; Cirrhosis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We evaluated the reversibility of minimal hepatic encephalopathy (MHE) following liver transplantation (LT) in Egyptian cirrhotic patients. Twenty patients with biopsy-proven liver cirrhosis listed for LT and twenty age- and sex-matched healthy controls were included. All underwent psychometric tests including trail making test A, trail making test B, digit symbol test and serial dotting test. Psychometric hepatic encephalopathy score was calculated for patients to diagnose MHE. Psychometric tests were repeated six months following LT in the cirrhotic patient group. We found that the reversal of MHE could be achieved by LT especially in those with a model for end-stage liver disease score  $< 15$ .

Osman MA, Sayed MM, Mansour KA, Saleh SA, Ibrahim WA, Abdelhakam SM, Bahaa M, Yousry WA, Elbaz HS, Mikhail RN, Hassan AM, Elsayed EH, Mahmoud DA. Reversibility of minimal hepatic encephalopathy following liver transplantation in Egyptian cirrhotic patients. *World J Hepatol* 2016; 8(30): 1279-1286 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i30/1279.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i30.1279>

## INTRODUCTION

The prevalence of overt hepatic encephalopathy (HE) in patients with decompensated liver cirrhosis ranges from 16% to 21%, while that of minimal HE (MHE) or covert HE is 20%-80%<sup>[1]</sup>.

MHE impairs daily functioning, driving performance, work capability and learning ability in cirrhotic patients. It also predisposes to overt HE and increased mortality<sup>[2]</sup>. There are several methods of diagnosing MHE, such as comprehensive neuropsychological examinations, standard psychometric batteries, and computerized testing<sup>[3]</sup>.

The psychometric hepatic encephalopathy score (PHES) battery can detect neuropsychiatric abnormalities and MHE. It assesses visual perception, construction, visual/spatial orientation, motor speed and accuracy, concentration, and attention in cirrhotic patients with end-stage liver disease. When PHES was compared to the standard methods of determining HE, its sensitivity and specificity were 96% and 100%, respectively<sup>[4]</sup>.

The PHES was initially composed of seven tests. The portosystemic encephalopathy battery was introduced later to exclude tests with poor sensitivity. It includes the line tracing test (LTT) and/or the digit symbol test, in addition to the number connection tests A and B (NCT A and B)<sup>[5]</sup>. The sum of the scores of these tests ranges between +5 and -15. A score of below or equal to -4 is diagnostic for MHE<sup>[4]</sup>.

The LTT requires the longest time to calculate its score, and there is an existing controversy in interpretation of its two outcomes: Time and errors. Thus, only three of the four tests, NCT-A, NCT-B and DST, have been commonly used for MHE detection<sup>[6]</sup>. The result of any test was regarded to be abnormal if it was beyond the 2 standard deviation range of the control subjects. In some previous studies, MHE was diagnosed when two of these tests were abnormal<sup>[7]</sup>. In others, it was diagnosed when only one test was abnormal<sup>[8]</sup>.

Liver transplantation (LT) is now considered an established effective and innovative treatment option for patients with end-stage liver diseases for a wide range of indications over the last fifty years<sup>[9]</sup>. The surgical outcomes and survival rates following LT have been previously estimated; however, the effect of LT on MHE has not been properly studied. A few studies have compared the cognitive performance of cirrhotic patients before and after LT. Some demonstrated cognitive improvement, and others have suggested reversibility of MHE after LT<sup>[10,11]</sup>.



This study aimed to evaluate the reversibility of MHE following liver transplantation in Egyptian cirrhotic patients.

## MATERIALS AND METHODS

This prospective study was conducted at Ain Shams Center for Organ Transplant, Ain Shams Specialized Hospital, Cairo, Egypt from June 2014 to April 2015. It included twenty right-handed patients with biopsy-proven liver cirrhosis listed for LT.

In addition, twenty age- and sex-matched healthy persons were enrolled, constituting the control group. The groups were similar regarding number of education years and handedness. The healthy controls were collected from the outpatient clinics among those coming for pre-employment screenings. Liver and systemic diseases were excluded by history, physical examination, laboratory and radiologic assessment.

Written informed consent was obtained from patients and controls prior to inclusion in the study. The study protocol was approved by the Research Ethical Committee of Faculty of Medicine, Ain Shams University according to the ethical guidelines of the 1975 Declaration of Helsinki.

### Patients' selection

**Exclusion criteria:** (1) patients with clinical or laboratory evidence of any concomitant infection, severe gastrointestinal bleeding, anemia, electrolyte abnormalities, or renal insufficiency; (2) overt hepatic encephalopathy (persistent or episodic) as revealed by a standard clinical neurological examination; (3) significant cortical atrophy or other structural brain changes as revealed by conventional neurological imaging studies; (4) regular use of psychotropic drugs, such as benzodiazepines; (5) known major psychiatric disorder; (6) patients unable to perform the tests (illiterate or with upper limb motor handicaps); (7) less than 6 mo of complete alcohol abstinence; and (8) post-transplant toxic levels of immunosuppressive drugs.

All of the following were performed to recruited patients and controls: (1) full history taking together with a full clinical, neurological and psychiatric examination done by both an experienced hepatologist and neuropsychiatrist; (2) laboratory investigations including complete blood count; liver function tests: Alanine transaminase, aspartate transaminase, total and direct bilirubin, international normalized ratio (INR), prothrombin time, serum albumin; kidney function tests and full electrolytes including blood urea nitrogen, creatinine, sodium, potassium, magnesium, calcium, phosphorus; C-reactive protein to exclude patients with any infections; and post-transplant immunosuppressive drug levels for patients only to exclude those with toxic levels. The modified Child-Pugh score was calculated for patients, and each patient was categorized as A, B or C. Additionally, the model for end-stage liver disease (MELD) score was calculated for patients using laboratory results collected immediately before LT with no adjustments

for malignancy. We calculated the MELD score using the following formula:  $MELD = [0.957 \times \ln(\text{creatinine mg/dL}) + 0.378 \times \ln(\text{bilirubin mg/dL}) + 1.12 \times \ln(\text{INR}) + 0.643 \times 10^8]$ ; (3) radiological studies included pelvi-abdominal ultrasound with examination of liver size, echogenicity, splenomegaly, amount of ascites, portal vein diameter and patency, presence of any hepatic focal lesions or any abdominal malignancy and a detailed kidney examination pre- and post-transplantation (Hitachi, EUB-5500). A computerized tomography (CT) for the brain was performed to all patients to exclude any brain pathology (Toshiba, High Speed 16 Slice); and (4) psychometric tests included the following neuropsychological tests.

**Trail making test A:** Patient should draw a line from number (1) to number (2) and from (2) to number (3) till reaching number (24), without elevating the pencil from the paper. The time was recorded in seconds. If the patient made an error, the examiner told him to correct it, but the timing was not stopped. The average score was 29 s, while the deficient score was > 78 s and the rule of thumb was that most completed it in 90 s. The rule of thumb is a broadly accurate guide or principle, based on practice rather than theory<sup>[12,13]</sup>.

**Trail making test B:** Patient should draw a line from number (1) to letter (A), then from letter (A) to number (2), then from number (2) to letter (B), and so on, alternating the number and letter respecting the alphabetical order till letter (L). After explaining the test to the patient, timing should be started and recorded in seconds, including time needed to correct any error done. The average score was 75 s, while > 273 s was considered deficient and the rule of thumb was that most completed it in 3 min.

**Digit symbol (substitution) test:** A coding key was presented consisting of nine abstract symbols, each paired with a number. The patient was required to scan the key and write down the symbol corresponding to each number as rapidly as possible. Ninety seconds were given to the patient and when the time was finished, the number of symbols performed by the patient was counted. The score was recorded in points. If the patient made any errors, timing continued towards their 90 s, and the patient might lose time. A healthy individual should be able to complete the test in 90 s or less. A fall of 1 to 1.5 SD below the mean is considered suggestive of cerebral dysfunction.

**Serial dotting test:** Also called the circle dotting test, the serial dotting test (SDT) was used to test pure motor speed. The patient was asked to put a dot in each of the 100 circles given on the sheet after being prepared first by dotting the 20 circles at the top of the sheet.

The results of the trail making test A (TMT A), TMT B, and SDT were measured in seconds, including the time needed to correct any errors, and the results of digit symbol (substitution) test (DST) were measured as

points.

Accordingly, a better performance was reflected by a higher result of DST and lower results of other tests.

**Interpretation of the score:** To obtain the measure of overall visual-motor and visual-constructive performance, we calculated the average percentile score of the 4 selected tests: TMT A, TMT B, DST and SDT. The average score of these tests was arbitrarily named the visual-motor and visual-constructive performance (VMCP) score or PHES. The patient was diagnosed to have MHE when his total score was equal or below -4.

### Post-transplantation follow-up

The immunosuppressive regimen included cyclosporine or tacrolimus, mycophenolate mofetil (MMF), and corticosteroids in all patients except those transplanted for hepatocellular carcinoma (HCC). In patients transplanted for HCC, the regimen included calcineurin inhibitors and steroids only. Trough levels of cyclosporine were maintained between 200 and 300 ng/mL while those of tacrolimus were maintained between 8 and 12 ng/mL. Rapid withdrawal of corticosteroids within three months was routine in all patients.

In cases of acute rejection, the first-line therapy consisted of optimization of the maintenance level of immunosuppression. If there was no response, then MMF or rapamycin were added to the patient's regimen, if not already being taken. In some cases, a shift from cyclosporine to tacrolimus was beneficial. A small dose of steroids was used if all other measures failed.

The complete psychometric battery was repeated six months following LT in the cirrhotic patient group. The post-transplant testing was done while the patient was in a stable condition with no clinical or laboratory evidence of any concomitant infection, anemia, electrolyte abnormalities, acute transplant rejection episode or other severe clinical problems.

### Statistical analysis

Data were analyzed using the SPSS software computer program version 18 (SPSS, Chicago, IL, United States). They were described as the mean  $\pm$  standard deviation (SD) for quantitative (parametric) variables and as median  $\pm$  inter-quartile range (IQR) for quantitative (non-parametric) variables. Qualitative (categorical) variables were presented as frequency and percentage. The independent samples *t*-test was used for the comparison of quantitative parametric variables among two independent groups and the Mann Whitney *U* test was used for non-parametric data. The Wilcoxon Signed Ranks test was used for the comparison of quantitative non-parametric variables among two dependent groups (before and after transplantation). The  $\chi^2$  test (or Fisher's exact test when appropriate) was used for comparison of distribution of qualitative variables among different groups.

Significance level (*P*) value: (1)  $P \leq 0.05$  was significant; (2)  $P < 0.01$  was highly significant; and (3)  $P >$

0.05 was non-significant.

The statistical methods of this study were reviewed by Azza M Hassan, Department of Community, Environmental and Occupational Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

## RESULTS

This prospective study included twenty patients with biopsy-proven hepatitis C virus (HCV)-related liver cirrhosis listed for LT. Their mean age was  $53.2 \pm 5.39$  years and they consisted of 17 males (85%) and 3 females (15%). Five patients were diagnosed with hepatocellular carcinoma on top of liver cirrhosis.

In addition, twenty age- and sex-matched healthy subjects were enrolled, constituting the control group. Their mean age was  $53.4 \pm 6.49$  years and they consisted of 15 males (75%) and 5 females (25%).

Before LT, the median  $\pm$  IQR of Child-Pugh score of the enrolled patients was  $9 \pm 4.5$ ; five patients (25%) were Child A, six (30%) were B and nine (45%) patients were Child C. Their median  $\pm$  IQR of MELD score was  $14.5 \pm 6.5$ , where 50% (10 patients) had a MELD score below 15 and 50% (10 patients) had a MELD score above 15.

Six months following LT, the median  $\pm$  IQR of Child-Pugh and MELD scores were  $6 \pm 1.8$  and  $11.5 \pm 4.5$ , respectively, with a statistically significant improvement ( $P < 0.001$  and  $0.002$ , respectively) (Table 1).

Table 2 shows the analysis of the median score values of different psychometric tests (TMT A, TMT B, DST and SDT) and the VMCP score in patients before and after LT, as well as in healthy control subjects. Before LT, the psychometric tests and the VMCP score showed highly significant deficits in cirrhotic patients in comparison to controls ( $P < 0.001$ ).

After LT, there were statistically significant improvements in test values in the patient group when compared to their values before LT. However, the values of patients after LT were still significantly worse than those of the control subjects ( $P < 0.001$ ).

Among the studied 20 cirrhotic patients, the PHES, represented by the VMCP score, detected MHE in 16 patients (80%) before LT, with a median value of  $-7 \pm 3.5$ . The median PHES value was significantly improved following LT, reaching  $-4.5 \pm 5$  ( $P < 0.001$ ), and the number of patients with MHE decreased to 11 (55%) post-LT.

Table 3 shows that the pre-transplant MELD score  $\geq 15$  was significantly related to the presence of post-transplant MHE ( $P = 0.005$ ). In cirrhotic patients with a pre-transplant MELD score  $\geq 15$ , 100% had pre-transplant MHE and 90% had post-transplant MHE. On the other hand, among those with a MELD score  $< 15$ , 60% had pre-transplant MHE and 20% had post-transplant MHE. A higher number of patients in whom reversal of MHE was observed had a pre-transplant MELD score  $< 15$ .

**Table 1** Laboratory data, Child-Pugh and model for end-stage liver disease scores before and after liver transplantation in the patients' group

Variable	Before LT (median $\pm$ IQR)	After LT (median $\pm$ IQR)	Z	P value
INR	1.5 $\pm$ 0.6	1.4 $\pm$ 0.4	2.198	0.029 <sup>1</sup>
ALT (N: 7-40 IU/L)	24.5 $\pm$ 35.25	19.5 $\pm$ 29.8	2.201	0.026 <sup>1</sup>
AST (N: 7-37 IU/L)	44.5 $\pm$ 28	22.5 $\pm$ 13.75	2.918	0.002 <sup>2</sup>
Total bilirubin (N: 0.2-1.2 mg/dL)	2 $\pm$ 2.45	1.4 $\pm$ 0.8	2.156	0.029 <sup>1</sup>
Albumin (N: 3.5-5.3 g/dL)	2.3 $\pm$ 1.15	3.3 $\pm$ 0.9	3.021	0.021 <sup>1</sup>
Creatinine (N: 0.5-1.2 mg/dL)	0.9 $\pm$ 0.4	1 $\pm$ 0.2	0.176	0.893
BUN (N: 20-40 mg/dL)	12 $\pm$ 6.8	11.5 $\pm$ 4.8	0.218	0.839
Sodium (N: 135-147 mEq/L)	132.5 $\pm$ 11.75	134.5 $\pm$ 10.8	0.197	0.856
Potassium (N: 3.5-5.3 mEq/L)	3.8 $\pm$ 0.7	4.4 $\pm$ 0.8	2.469	0.011 <sup>1</sup>
Calcium (N: 9-11 mg/dL)	8.6 $\pm$ 1.1	8.9 $\pm$ 1.3	2.584	0.007 <sup>2</sup>
Phosphorus (N: 3-4.5 mg/dL)	3.2 $\pm$ 0.6	3.6 $\pm$ 1.3	3.219	< 0.001 <sup>2</sup>
Magnesium (N: 1.8-3.6 mg/dL)	2.1 $\pm$ 0.6	2.2 $\pm$ 0.7	1.777	0.076
Child-Pugh score	9 $\pm$ 4.5	6 $\pm$ 1.8	3.549	< 0.001 <sup>2</sup>
MELD score	14.5 $\pm$ 6.5	11.5 $\pm$ 4.5	2.928	0.002 <sup>2</sup>

<sup>1</sup>Significant; <sup>2</sup>Highly significant. Z: Wilcoxon signed ranks test; IQR: Inter-quartile range; INR: International normalized ratio; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood urea nitrogen; MELD: Model for end-stage liver disease; LT: Liver transplantation; N: Normal range.

**Table 2** Median score values of different psychometric tests in controls and patients before and after liver transplantation

	Controls	Patients before LT	Patients after LT	Patients before LT vs after LT (P value) <sup>1</sup>	Controls vs patients before LT (P value) <sup>2</sup>	Controls vs patients after LT (P value) <sup>2</sup>
TMT A (median $\pm$ IQR)	27 $\pm$ 8	110 $\pm$ 32.5	80 $\pm$ 30.8	0.010	< 0.001	< 0.001
TMT B (median $\pm$ IQR)	62 $\pm$ 17.3	282.5 $\pm$ 137.5	167.5 $\pm$ 72.0	0.002	< 0.001	< 0.001
DST (median $\pm$ IQR)	60 $\pm$ 4.75	22 $\pm$ 6	28.5 $\pm$ 14.5	0.001	< 0.001	< 0.001
SDT (median $\pm$ IQR)	34 $\pm$ 3.75	62 $\pm$ 20.75	51 $\pm$ 27.5	0.002	< 0.001	< 0.001
VMCP (median $\pm$ IQR)	1 $\pm$ 1	-7 $\pm$ 3.5	-4.5 $\pm$ 5.0	< 0.001	< 0.001	< 0.001

<sup>1</sup>Wilcoxon signed ranks test; <sup>2</sup>Mann Whitney U test. TMT: Trail making test; DST: Digit symbol test; SDT: Serial dotting test; VMCP: Visual-motor and visual-constructive performance score; LT: Liver transplantation; IQR: Inter-quartile range.

**Table 3** Relation between pre-transplant model for end-stage liver disease score and the presence of pre- and post-transplant minimal hepatic encephalopathy n (%)

		Pre-transplant MELD score		$\chi^2$	P value
		< 15 (n = 10)	$\geq$ 15 (n = 10)		
Pre-transplant	-ve	4 (40)	0 (0)	5.000 <sup>1</sup>	0.087
MHE	+ve	6 (60)	10 (100)		
Post-transplant	-ve	8 (80)	1 (10)	9.899 <sup>1</sup>	0.005 <sup>2</sup>
MHE	+ve	2 (20)	9 (90)		

<sup>1</sup>Fisher's exact test; <sup>2</sup>Highly significant. MELD: Model for end-stage liver disease; MHE: Minimal hepatic encephalopathy; -ve: Negative; +ve: Positive.

Table 4 shows comparison between patients who recovered from MHE ( $n = 5$ ) and those who didn't recover ( $n = 11$ ) regarding age, sex, pre-transplant lab investigations and pre-transplant Child and MELD scores. We found that non-recovered patients had significantly higher INR, total bilirubin, Child and MELD scores than recovered ones ( $P = 0.027$ ,  $0.013$ ,  $0.038$  and  $0.009$ , respectively).

## DISCUSSION

In the current study, twenty cirrhotic patients listed for

LT and twenty healthy controls were included. Patients with pre or post-transplant clinical or laboratory evidence of infection, electrolyte imbalance, renal impairment or immunosuppressive drugs toxicity were excluded from the study. The etiology of liver cirrhosis in the included patients was chronic hepatitis C. Egypt has the highest prevalence of HCV worldwide, with an exceptionally high burden of liver disease<sup>[14]</sup>.

A neuropsychological test battery, consisting of TMT A, TMT B, SDT and DST was applied to both the cirrhotic patient and control groups before and six months after LT. These are the same tests used by Wang *et al.*<sup>[3]</sup> and Tsai *et al.*<sup>[15]</sup> to diagnose MHE. They have high sensitivity and specificity and are easily applied with no difficulty in their score calculation<sup>[15,16]</sup>. These tests monitor changes in attention, motor speed and executive functions, which are the first to improve in the post transplantation period<sup>[3,16]</sup>. The TMT is a measure of attention, speed, and mental flexibility. It also tests spatial organization, visual pursuits, recall, and recognition<sup>[12]</sup>. Part A tests visual scanning, numeric sequencing, and visuo-motor speed, while part B tests cognitive demands including visual motor, visual spatial abilities and mental flexibility<sup>[16,17]</sup>. The DST measures the perceptual ability<sup>[18]</sup>, while the SDT tests the pure motor speed<sup>[19]</sup>.

The total score of the four tests (TMT A, TMT B, SDT

**Table 4** Comparison between recovered and non-recovered patients regarding age, sex, pre-transplant laboratory investigations and pre-transplant Child and model for end-stage liver disease scores

Variable	Recovered (n = 5) (median ± IQR)	Non-recovered (n = 11) (median ± IQR)	Z <sup>1</sup>	P value
INR	1.5 ± 0.5	1.7 ± 0.5	2.231	0.027
ALT	25 ± 51	38 ± 36	0.397	0.743
AST	49 ± 45.5	53 ± 39	0.283	0.827
Total bilirubin	1.4 ± 1	3.1 ± 2.9	2.437	0.013
Albumin	2.3 ± 1	2.3 ± 0.5	1.208	0.267
Creatinine	0.7 ± 0.5	0.9 ± 0.4	0.517	0.661
BUN	11 ± 11.5	12 ± 5	0.514	0.636
Sodium	139 ± 12.5	132 ± 7	0.910	0.377
Potassium	4 ± 1.2	3.6 ± 0.8	1.310	0.221
Calcium	8.9 ± 0.7	8.3 ± 1.3	0.746	0.482
Phosphorus	2.7 ± 0.8	3.2 ± 1.3	1.204	0.259
Magnesium	2.3 ± 0.6	2 ± 0.6	0.969	0.355
Child-Pugh score	9 ± 1.5	11 ± 1	2.140	0.038
MELD score	13 ± 3.5	17 ± 5	2.557	0.009
Age (mean ± SD)	56.6 ± 1.7	51.2 ± 6.1	1.911 <sup>2</sup>	0.077
Sex				
Male (n, %)	4 (80)	9 (81.8)	0.007 <sup>3</sup>	1.000
Female (n, %)	1 (20)	2 (18.2)		

<sup>1</sup>Mann Whitney U test; <sup>2</sup>Independent samples t-test; <sup>3</sup> $\chi^2$ , Fisher's exact test. INR: International normalized ratio; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood urea nitrogen; MELD: Model for end-stage liver disease; IQR: Inter-quartile range; SD: Standard deviation.

and DST), which represents the VMCP score or PHES, has a cutoff level of -4. Any patient with a score below or equal to -4 is defined as having MHE<sup>[3,15,16]</sup>.

With regard to the scores of TMT A, TMT B, DST and SDT, and the total PHES score in the current study; significant differences were found between patients and control subjects, together with the significant improvement in the patient scores after LT. This is in agreement with Mattarozzi *et al*<sup>[20]</sup>.

The significant improvement of the patients' MELD score after LT agrees with Lin *et al*<sup>[10]</sup> and Mattarozzi *et al*<sup>[20]</sup>.

The present study supports the relation between MHE and higher values of a MELD score. This is also in agreement with Mattarozzi *et al*<sup>[20]</sup> and Montagnese *et al*<sup>[21]</sup>. More patients with a pre-transplant MELD score > 15 experienced pre- and post-transplant MHE. More patients in whom reversal of MHE was observed had a pre-transplant MELD score < 15, indicating that early LT for patients with a MELD score < 15 may be associated with a higher incidence of reversal of MHE and could save the brain from the irreversible damages associated with end-stage liver disease. These findings may change the LT priority for patients with MHE with a MELD score < 15 receiving priority over those with a MELD > 15.

In a trial to find the factors affecting the reversibility of MHE, comparison between patients who recovered and those who didn't was done in the present study. Pre-transplant Child and MELD scores were significantly lower in patients who recovered from MHE. Age and sex differences were insignificant between those who recovered and those who didn't.

This is different from the study of Mechtcheriakov *et al*<sup>[22]</sup>, in which the duration of liver cirrhosis and its severity (as determined by the Child classification) did not influence the improvement after LT. However, O'Carroll *et al*<sup>[23]</sup> reported that severe liver disease at pre-transplant assessment was associated with more slowing of reaction times and increased bioelectric dysfunction of the brain. In the study of Mechtcheriakov *et al*<sup>[22]</sup>, patients' age was not related to recovery from MHE after LT which is similar to our study.

Although there was improvement in the cognitive function after LT in the current study, it did not reach the normal optimal levels of the healthy controls. This observation agrees with O'Carroll *et al*<sup>[23]</sup>, Tarter *et al*<sup>[24]</sup> and Garcia-Martinez *et al*<sup>[25]</sup> indicating that MHE and the deterioration in cognitive function in liver disease patients are not completely reversible after LT.

It was hypothesized by Rose *et al*<sup>[26]</sup> that hepatic encephalopathy may be manifested by either "delirium-like" or "dementia-like" clinical features. The former is likely to be metabolic in origin, whereas the latter is likely to be due to a structural brain lesion, which may be specific to liver disease.

Ammonia has been suggested to have a role in the metabolic pathogenesis of MHE. Hyperammonemia in patients with liver cirrhosis may result in an increase in the brain glutamine with subsequent reduction in the brain magnetization-transfer ratio<sup>[25]</sup>.

Teperman<sup>[11]</sup> demonstrated that patients who survived 10 years post-LT had significant cognitive dysfunction and poor health-related quality of life. This supports the evidence for a "dementia-like" parameter



of MHE that is irreversible after LT. Lin *et al.*<sup>[10]</sup> showed improvement of both the extracellular cerebral edema and the demyelination of white matter in patients with MHE following LT, but they still did not reach the control level.

In the current study, gross structural brain lesions were excluded by CT brain before and after LT. Future studies should expand and should include larger sample size in order to investigate different metabolic, neurological and physical tests that could identify the exact causes of incomplete recovery of the brain cognitive functions.

In conclusion, the reversal of minimal hepatic encephalopathy in cirrhotic patients can be achieved by liver transplantation, especially in those with a pre-transplant MELD score < 15.

## COMMENTS

### Background

Minimal hepatic encephalopathy (MHE) impairs daily functioning, driving performance, work capability and learning ability in cirrhotic patients. It also predisposes to overt hepatic encephalopathy and increases mortality. The psychometric hepatic encephalopathy score (PHES) battery can detect neuropsychiatric abnormalities. It assesses visual perception, construction, visual/spatial orientation, motor speed and accuracy, concentration, and attention in cirrhotic patients with end-stage liver disease. Liver transplantation (LT) is now considered an established effective and innovative treatment option for patients with end-stage liver diseases. The effects of LT on MHE are poorly studied.

### Research frontiers

The authors evaluated the reversibility of MHE following LT in Egyptian cirrhotic patients. Twenty right-handed patients with biopsy-proven liver cirrhosis listed for LT and twenty age- and sex-matched healthy control subjects were included. All underwent psychometric tests including trail making test A (TMT A), TMT B, the digit symbol test and the serial dotting test. The PHES was calculated to diagnose MHE. Psychometric tests were repeated six months following LT in cirrhotic patient group. They found that reversal of MHE in cirrhotic patients could be achieved by LT, especially in those with a MELD score < 15.

### Innovations and breakthroughs

This is the first Egyptian study that addresses the reversibility of minimal hepatic encephalopathy following LT.

### Applications

The findings of this study may represent a future strategy, indicating that early LT for patients with a MELD score < 15 may be associated with a higher incidence of reversal of MHE and could save the brain from the irreversible damage associated with end-stage liver disease.

### Terminology

MHE is a neuropsychiatric syndrome that may occur in cirrhotic patients with no recognizable clinical symptoms of hepatic encephalopathy but with mild cognitive and psychomotor deficits, impairing daily functioning.

### Peer-review

This study provides very interesting results which indicate that liver transplantation cannot fully recover all Egyptian patients from MHE caused by hepatitis C virus-induced cirrhosis, and has the most beneficial effect in patients with pre-transplant MELD score less than 15. This is the first study that investigated the reversibility of MHE in Egyptian population after liver transplantation, and provides important information regarding the effect of transplantation on the course of MHE.

## REFERENCES

- 1 Jepsen P, Ott P, Andersen PK, Sørensen HT, Vilstrup H. Clinical course of alcoholic liver cirrhosis: a Danish population-based cohort study. *Hepatology* 2010; **51**: 1675-1682 [PMID: 20186844 DOI: 10.1002/hep.23500]
- 2 Agrawal S, Umapathy S, Dhiman RK. Minimal hepatic encephalopathy impairs quality of life. *J Clin Exp Hepatol* 2015; **5**: S42-S48 [PMID: 26041957 DOI: 10.1016/j.jceh.2014.11.006]
- 3 Wang JY, Zhang NP, Chi BR, Mi YQ, Meng LN, Liu YD, Wang JB, Jiang HX, Yang JH, Xu Y, Li X, Xu JM, Zhang G, Zhou XM, Zhuge YZ, Tian DA, Ye J, Liu YL. Prevalence of minimal hepatic encephalopathy and quality of life evaluations in hospitalized cirrhotic patients in China. *World J Gastroenterol* 2013; **19**: 4984-4991 [PMID: 23946605 DOI: 10.3748/wjg.v19.i30.4984]
- 4 Lv XF, Liu K, Qiu YW, Cai PQ, Li J, Jiang GH, Deng YJ, Zhang XL, Wu PH, Xie CM, Wen G. Anomalous gray matter structural networks in patients with hepatitis B virus-related cirrhosis without overt hepatic encephalopathy. *PLoS One* 2015; **10**: e0119339 [PMID: 25786256 DOI: 10.1371/journal.pone.0119339]
- 5 Nabi E, Bajaj JS. Useful tests for hepatic encephalopathy in clinical practice. *Curr Gastroenterol Rep* 2014; **16**: 362 [PMID: 24357348 DOI: 10.1007/s11894-013-0362-0]
- 6 Riggio O, Ridola L, Pasquale C, Pentassuglio I, Nardelli S, Moscucci F, Merli M, Montagnese S, Amodio P, Merkel C. A simplified psychometric evaluation for the diagnosis of minimal hepatic encephalopathy. *Clin Gastroenterol Hepatol* 2011; **9**: 613-616.e1 [PMID: 21440091 DOI: 10.1016/j.cgh.2011.03.017]
- 7 Sharma P, Sharma BC, Sarin SK. Critical flicker frequency for diagnosis and assessment of recovery from minimal hepatic encephalopathy in patients with cirrhosis. *Hepatobiliary Pancreat Dis Int* 2010; **9**: 27-32 [PMID: 20133225]
- 8 Marić D, Klasnja B, Filipović D, Brkić S, Ruzić M, Bugarski V. Minimal hepatic encephalopathy in patients with decompensated liver cirrhosis. *Acta Clin Croat* 2011; **50**: 375-380 [PMID: 22384773]
- 9 Shukla A, Vadeyar H, Rela M, Shah S. Liver Transplantation: East versus West. *J Clin Exp Hepatol* 2013; **3**: 243-253 [PMID: 25755506 DOI: 10.1016/j.jceh.2013.08.004]
- 10 Lin WC, Chou KH, Chen CL, Chen HL, Lu CH, Li SH, Huang CC, Lin CP, Cheng YF. Longitudinal brain white matter alterations in minimal hepatic encephalopathy before and after liver transplantation. *PLoS One* 2014; **9**: e105887 [PMID: 25166619 DOI: 10.1371/journal.pone.0105887]
- 11 Teperman LW. Impact of pretransplant hepatic encephalopathy on liver posttransplantation outcomes. *Int J Hepatol* 2013; **2013**: 952828 [PMID: 24324895 DOI: 10.1155/2013/952828]
- 12 Gaudino EA, Geisler MW, Squires NK. Construct validity in the Trail Making Test: what makes Part B harder? *J Clin Exp Neuropsychol* 1995; **17**: 529-535 [PMID: 7593473 DOI: 10.1080/01688639508405143]
- 13 Lezak MD, Howieson DB, Loring DW. Neuropsychological Assessment. 4th ed. New York, NY: Oxford University Press, 2004: 1016
- 14 Lavanchy D. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011; **17**: 107-115 [PMID: 21091831 DOI: 10.1111/j.1469-0691.2010.03432.x]
- 15 Tsai CF, Chu CJ, Huang YH, Wang YP, Liu PY, Lin HC, Lee FY, Lu CL. Detecting minimal hepatic encephalopathy in an endemic country for hepatitis B: the role of psychometrics and serum IL-6. *PLoS One* 2015; **10**: e0128437 [PMID: 26039496 DOI: 10.1371/journal.pone.0128437]
- 16 Zhang Y, Feng Y, Cao B, Tian Q. Effects of SIBO and rifaximin therapy on MHE caused by hepatic cirrhosis. *Int J Clin Exp Med* 2015; **8**: 2954-2957 [PMID: 25932262]
- 17 Reitan RM. Validity of the Trail Making test as an indicator of organic brain damage. *Percept Mot Skills* 1958; **8**: 271-276 [DOI: 10.2466/PMS.8.7.271-276]
- 18 Bettcher BM, Libon DJ, Kaplan E, Swenson R, Penney DL. Digit Symbol Substitution Test. In: Kreutzer JS, DeLuca J, Caplan B. Encyclopedia of Clinical Neuropsychology. New York: Springer,

- 2011: 849-853 [DOI: 10.1007/978-0-387-79948-3\_1289]
- 19 **Kharbanda PS**, Saraswat VA, Dhiman RK. Minimal hepatic encephalopathy: diagnosis by neuropsychological and neurophysiologic methods. *Indian J Gastroenterol* 2003; **22** Suppl 2: S37-S41 [PMID: 15025253]
- 20 **Mattarozzi K**, Stracciari A, Vignatelli L, D'Alessandro R, Morelli MC, Guarino M. Minimal hepatic encephalopathy: longitudinal effects of liver transplantation. *Arch Neurol* 2004; **61**: 242-247 [PMID: 14967773 DOI: 10.1001/archneur.61.2.242]
- 21 **Montagnese S**, Balistreri E, Schiff S, De Rui M, Angeli P, Zanus G, Cillo U, Bombonato G, Bolognesi M, Sacerdoti D, Gatta A, Merkel C, Amodio P. Covert hepatic encephalopathy: agreement and predictive validity of different indices. *World J Gastroenterol* 2014; **20**: 15756-15762 [PMID: 25400460 DOI: 10.3748/wjg.v20.i42.15756]
- 22 **Mechtcheriakov S**, Graziadei IW, Mattedi M, Bodner T, Kugener A, Hinterhuber HH, Marksteiner J, Vogel W. Incomplete improvement of visuo-motor deficits in patients with minimal hepatic encephalopathy after liver transplantation. *Liver Transpl* 2004; **10**: 77-83 [PMID: 14755782 DOI: 10.1002/lt.20009]
- 23 **O'Carroll RE**, Couston M, Cossar J, Masterton G, Hayes PC. Psychological outcome and quality of life following liver transplantation: a prospective, national, single-center study. *Liver Transpl* 2003; **9**: 712-720 [PMID: 12827558 DOI: 10.1053/jlts.2003.50138]
- 24 **Tarter RE**, Hegedus AM, Van Thiel DH, Edwards N, Schade RR. Neurobehavioral correlates of cholestatic and hepatocellular disease: differentiation according to disease specific characteristics and severity of the identified cerebral dysfunction. *Int J Neurosci* 1987; **32**: 901-910 [PMID: 3596934 DOI: 10.3109/00207458709043346]
- 25 **Garcia-Martinez R**, Rovira A, Alonso J, Jacas C, Simón-Talero M, Chavarria L, Vargas V, Córdoba J. Hepatic encephalopathy is associated with posttransplant cognitive function and brain volume. *Liver Transpl* 2011; **17**: 38-46 [PMID: 21254343 DOI: 10.1002/lt.22197]
- 26 **Rose C**, Jalan R. Is minimal hepatic encephalopathy completely reversible following liver transplantation? *Liver Transpl* 2004; **10**: 84-87 [PMID: 14755783 DOI: 10.1002/lt.20030]

**P- Reviewer:** McMillin MA, Stanojlovic O **S- Editor:** Gong ZM  
**L- Editor:** A **E- Editor:** Li D



Prospective Study

## Regulatory and activated effector T cells in chronic hepatitis C virus: Relation to autoimmunity

Hanan Fouad, Maissa El Raziky, Eman Medhat Hassan, Ghada Mahmoud Abdel Aziz, Samar K Darweesh, Ahmed Reda Sayed

Hanan Fouad, Department of Medical Biochemistry, Faculty of Medicine, Cairo University, Cairo 11562, Egypt

Hanan Fouad, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Hail University, Hail 81442, Saudi Arabia

Maissa El Raziky, Eman Medhat Hassan, Samar K Darweesh, Department of Hepato-gastroenterology and Tropical Medicine, Faculty of Medicine, Cairo University, Cairo 11562, Egypt

Ghada Mahmoud Abdel Aziz, Ahmed Reda Sayed, Department of Medical Biochemistry, Faculty of Medicine, Beni Suef University, Beni Suef 19206, Egypt

**Author contributions:** All authors contributed to this work.

**Institutional review board statement:** The research Ethics Committee of Faculty of Medicine, Cairo University approved the study.

**Informed consent statement:** Written consent was signed from all subjects of the study.

**Conflict-of-interest statement:** There are no conflicts of interest.

**Data sharing statement:** We have no data sharing statement.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Hanan Fouad, Professor, Department of Medical Biochemistry, Faculty of Medicine, Cairo University,

110 Manial Street, Manial El-Roda District, Cairo 11562, Egypt. [hanan.fouad@kasralainy.edu.eg](mailto:hanan.fouad@kasralainy.edu.eg)  
Telephone: +20-22-5256002  
Fax: +20-22-3632297

Received: April 23, 2016

Peer-review started: April 25, 2016

First decision: June 6, 2016

Revised: July 30, 2016

Accepted: August 27, 2016

Article in press: August 29, 2016

Published online: October 28, 2016

### Abstract

#### AIM

To investigate how Tregs are regulated in chronic hepatitis C virus (HCV) patients *via* assessment of Tregs markers (granzyme 2, CD69 and FoxP3), Teffs markers [TNFRSF4 (OX40), INFG] and *CD4*, *CD25* genes.

#### METHODS

A prospective study was conducted on 120 subjects divided into 4 groups: Group I ( $n = 30$ ) treatment naïve chronic HCV patients; Group II ( $n = 30$ ) chronic HCV treated with Peg/Riba; Group III ( $n = 30$ ) chronic HCV associated with non-organ specific autoantibody and Group IV ( $n = 30$ ) healthy persons as a control group. Tregs and Teffs markers were assessed in peripheral blood mononuclear cells by quantitative real time reverse transcriptase-polymerase chain reaction.

#### RESULTS

Chronic HCV patients exhibited significant higher levels of both Teffs and Tregs in comparison to healthy control group. Tregs markers were significantly decreased in Peg/Riba treated HCV patients in comparison to treatment naïve HCV group. In HCV patients with antinuclear

antibody (ANA) +ve, Tregs markers were significantly decreased in comparison to all other studied groups. Teffs markers were significantly elevated in all HCV groups in comparison to control and in HCV group with ANA +ve in comparison to treatment naïve HCV group.

## CONCLUSION

Elevated Tregs cells in chronic HCV patients dampen both CD4<sup>+</sup> and CD8<sup>+</sup> autologous T cell immune response. Interferon- $\alpha$  and ribavirin therapy suppress proliferation of Tregs. More significant suppression of Tregs was observed in HCV patients with autoantibodies favoring pathological autoimmune response.

**Key words:** Autoimmunity; T regulatory cells; Hepatitis C virus; T activator cells; Interferon

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** A prospective study conducted on 120 subjects divided into: Treatment naïve hepatitis C virus (HCV) patients, HCV patients treated with old standard of care, HCV associated with antinuclear antibody (ANA) and healthy control group. Teffs/Tregs imbalance was evaluated. Results showed that HCV patients exhibited significant higher levels of both Teffs and Tregs markers. Interferon- $\alpha$  and ribavirin therapy suppresses proliferation of Tregs. More significant suppression of Tregs was observed in HCV patients with autoantibodies favoring pathological autoimmune response. Teffs markers were significantly elevated in HCV treated group and in HCV group with ANA +ve in comparison to treatment naïve HCV group.

Fouad H, El Raziky M, Hassan EM, Aziz GMA, Darweesh SK, Sayed AR. Regulatory and activated effector T cells in chronic hepatitis C virus: Relation to autoimmunity. *World J Hepatol* 2016; 8(30): 1287-1294 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i30/1287.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i30.1287>

## INTRODUCTION

Over 200 million people worldwide are suffering from chronic hepatitis C virus (HCV) infection and liver cirrhosis will be developed in about a quarter of these patients<sup>[1]</sup>. The prevalence rate of HCV genotype 4 in high risk populations in Egypt ranges from 73% to 90% and it was also found to be highly prevalent in sub-Saharan Africa and in the Middle East<sup>[2,3]</sup>.

T lymphocytes play a major role in cell mediated immunity<sup>[4,5]</sup>. The several subsets of T cells have distinct functions and the majority is part of the adaptive immune system. Other subtypes can effectively present antigens to other T cells and are considered to be part of the innate immune system<sup>[6]</sup>.

HCV is accompanied with different autoimmune manife-

stations<sup>[7]</sup>, and could be a stimulator for the autoimmune reactions causing production of autoantibodies<sup>[8]</sup>. More recently, Acay *et al*<sup>[9]</sup> stated that the auto-antibodies in chronic HCV infection are highly incident. The authors stated that high percentages of patients with chronic hepatitis C had anti-mitochondrial antibodies, anti-smooth muscle antibodies, antinuclear antibody (ANA), thyroid antibody and anti-liver kidney microsomal antibodies.

The old HCV therapeutic protocol recommended by National Institutes of Health<sup>[10]</sup> was pegylated interferon (PEG-IFN) and ribavirin. Either endogenous or exogenous IFN- $\alpha$  leads to down regulation of CD4<sup>+</sup> FoxP3<sup>hi</sup> IFN- $\gamma$ <sup>neg</sup> activated T regulatory cells (aTregs) while at the same time induces induction of CD4<sup>+</sup> FoxP3<sup>low/neg</sup> IFN- $\gamma$ <sup>pos</sup> T-activated cells (aTeffs). IFN- $\alpha$  play an essential role in suppression of Tregs *via* inhibition of interleukin-2 secretion<sup>[11]</sup>.

Together, these observations support the fact that in early antiviral response there is a production of IFN- $\alpha$  which enhances CD4 effector functions by inhibiting Tregs activation, whereas sustained elevation of IFN- $\alpha$  reverses Tregs/Teffs balance towards Teffs activation, generation of auto antibody and development of autoimmunity.

The objective of the present study is to evaluate the extent of Teffs/Tregs imbalance in chronic HCV and its association with old standard of care as well as the presence of ANA.

## MATERIALS AND METHODS

### Study outcomes

Our research hypothesis was that HCV with or without IFN- $\alpha$  and ribavirin is usually associated with Tregs/Teffs imbalance with subsequent generation of autoantibodies.

The primary outcome for this study was to evaluate Teffs/Tregs balance and regulation in chronic HCV through assessment of Tregs markers (granzyme 2, CD69 and FoxP3), Teffs markers (TNFRSF4, INF $\gamma$ ) and CD4, CD25 genes. Assessment of the effect of IFN- $\alpha$  and ribavirin on Teffs/Tregs balance as well as the association of Teffs/Tregs balance with the presence of antinuclear antibody were also conducted.

### Study population

This was a prospective study conducted in Biochemistry and Molecular Biology Unit, Cairo University, Faculty of Medicine. The study included one hundred and twenty subjects categorized into 4 groups: Group I (30 patients) treatment naïve chronic HCV patients; Group II (30 patients) chronic HCV patients treated with the old standard of care therapy; Peg-IFN- $\alpha$  and ribavirin (Peg/Riba), group III (30 patients) chronic HCV patients associated with non-organ specific autoantibody and group IV, 30 healthy persons served as a control group. The patients attended the Internal Medicine Department at Beni-Sueif General Hospital. Healthy controls matched the age and sex of other patients. Cairo University Institutional



**Table 1** Primers for *TNFRSF4(OX40)*, *granzyme 2*, *CD69*, *CD4*, *CD25*, *FoxP3* and *interferon  $\gamma$*  genes

<i>TNFRSF4OX 40</i>	Forward: '5 GCA ATA GCT CGG ACG CAA TCT 3'
DQ032625.1	Reverse: '5 GAG GGT CCC TGT GAG GTT CT 3'
<i>Granzyme 2</i>	Forward: '5 TAC CAT TGA GTT GTG CGT GGG 3'
NM_004131.4	Reverse: '5 GCC ATT GTT TCG TCC ATA GGA GA 3'
<i>CD69</i>	Forward: '5 GGT CAC CCA TGG AAG TGG TC 3'
NM_001781.2	Reverse: '5 GAC TTC GGA CCA CAG AGC AG 3'
<i>CD4</i> NM_001195017.2	Forward: '5 CTG CAA GTT CTC ACA CCG TC 3'
	Reverse: '5 CTA GAG TTG CCT GCT CTG CC 3'
<i>CD25</i> IL2R NM_000417.2	Forward: '5 GCT CTA CAC AGA GGT CCT GC 3'
	Reverse: '5 AGC ACA ACG GAT GTC TCC TG 3'
<i>FoxP3</i>	Forward: '5 CCC ATC CCC AGG AGT CTT G 3'
NG_007392.1	Reverse: '5 ACC ATG ACT AGG GGC ACT GTA 3'
<i>Interferon <math>\gamma</math></i>	Forward: '5 ATG GTT GTC CTG CCT GCA AT 3'
NG_015840.1	Reverse: '5 CTT GCT TAG GTT GGC TGC CT 3'

review board in Faculty of Medicine approved the study. Informed written consent was signed by all subjects of the study.

The eligibility of selected patients included: (1) age between 18 and 65 years old; (2) anti-HCV positive serum; (3) positive HCV RNA detected by reverse-transcription/polymerase chain reaction (RT/PCR); (4) non-organ specific autoantibody by positive ANA test (titer > 1/32) in group III only and < 1/16 in all other groups; and (5) white blood cell > 3.500/mm<sup>3</sup>.

A signed informed consent was got in accordance with Declaration of Helsinki ethics guidelines.

Exclusion criteria include patients with: Hepatocellular carcinoma, HBV co-infection, severe psychiatric disease, HIV-positive patients, co-morbid serious conditions, schistosomiasis mansoni, past history of alcohol abuse or long use of hepatotoxic drugs.

All HCV-infected patients in the treated group had a 48 wk course of old standard of care (Peg/Riba therapy) and achieved sustained virologic response. The T cells markers were analyzed after more than 6 mo of the end of the Peg/Riba course.

### Study analytic procedure

Whole blood was obtained from all subjects of the study. The mononuclear cell layer was isolated using Ficoll (Sigma, St. Louis, MO, United States) and centrifugation was conducted for 30 min at 400 g in cooling centrifuge.

**RNA extraction:** Total RNA was isolated from mononuclear cell layer using Qiagen purification reagent (Qiagen, CA, United States). The extracted RNA was quantified and checked for purity using a spectrophotometer (260/280 w.l.).

**Primer sequence:** PCR primers were got from GenBank RNA sequences cited at the following website: <http://www.ncbi.nlm.nih.gov/tools/primer-blast> (Table 1).

**Real-time quantitative PCR using SYBR Green I:** Step One plus real-time PCR system was used in the analysis using software version 3.1 (Applied Biosystems,

United States). Optimization of the annealing temperature was conducted for the PCR protocol and for the primer sets.

All cDNA were prepared for all gene markers, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and for non-template negative control.

Five microliter of total RNA was used to generate cDNA using 20 pmol antisense primer and 0.8  $\mu$ L AMV reverse transcriptase at 37 °C for 60 min. The relative abundance of mRNA species was evaluated using the SYBR® Green method (Applied Biosystems, CA, United States).

Annealing temperature of 60 °C was optimized for all primer sets. Real time polymerase reaction was performed in 25  $\mu$ L reaction volume consisting of Mater Mix of SYBR Green, 3  $\mu$ L of cDNA, 900 nmol/L of every primer. Amplification conditions were conducted according to the manufacturer specifications: 2 min at 50 °C, 10 min at 95 °C, 40 repeated cycles with 15 s denaturation and 10 min of annealing/extension at 60 °C.

### Calculation of relative quantification (relative expression)

The resulting data were expressed in Cycle threshold (Ct). The PCR data results show Ct values of all studied genes (*CD69*, *CD4*, *granzyme 2*, *TNFRSF4*, *FoxP3*, *CD25* and *IFN $\gamma$* ) and the house keeping gene (GAPDH). A negative control sample was no template cDNA was used. Target gene expression was related to GAPDH.

Data were calculated using the Applied Biosystems Step One plus software. Relative gene expressions of all assessed genes were calculated using the comparative Ct method. All values were normalized to GAPDH house-keeping gene and expressed as fold changes relative to the background levels found in the control samples.

### Statistical analysis

Statistical Package of Social Studies (SPSS) version 16.0.1 (SPSS Inc., Chicago, IL, United States) was utilized. Numerical data were presented as mean  $\pm$  standard deviation. The null hypothesis was calculated for multiple groups by a single-factor ANOVA and for two groups by

**Table 2** Relative gene expression of Tregs and Teffs specific genes in the four groups

	Group I (naïve)	Group II (Peg/Riba)	Group III (ANA+)	Group IV (control)
Granzyme 2 Tregs	0.704 ± 0.039	0.603 ± 0.046	0.400 ± 0.042	0.489 ± 0.053
CD69 Tregs	0.647 ± 0.037	0.54 ± 0.049	0.306 ± 0.036	0.383 ± 0.043
FoxP3 Tregs	0.531 ± 0.033	0.444 ± 0.046	0.330 ± 0.039	0.39 ± 0.030
TNFRSF4OX 40	0.596 ± 0.047	0.688 ± 0.057	0.707 ± 0.05	0.482 ± 0.056
CD4	0.584 ± 0.034	0.677 ± 0.048	0.712 ± 0.042	0.528 ± 0.05
CD25	0.595 ± 0.039	0.684 ± 0.053	0.73 ± 0.053	0.495 ± 0.041
Interferon $\gamma$	0.466 ± 0.035	0.483 ± 0.035	0.522 ± 0.044	0.357 ± 0.038

ANA: Antinuclear antibody.

**Table 3** Multiple comparisons of gene expression of Tregs markers in the four studied groups

	Group I naïve	Group II Peg/Riba	Group III ANA+	Group IV control
FOX3				
Group I naïve		<sup>a</sup> $P \leq 0.05$	<sup>d</sup> $P \leq 0.001$	<sup>b</sup> $P \leq 0.01$
Group II Peg/Riba	<sup>a</sup> $P \leq 0.05$		<sup>a</sup> $P \leq 0.05$	<sup>b</sup> $P \leq 0.01$
Group III ANA+	<sup>d</sup> $P \leq 0.001$	<sup>a</sup> $P \leq 0.05$		<sup>d</sup> $P \leq 0.001$
Group IV control	<sup>b</sup> $P \leq 0.01$	<sup>b</sup> $P \leq 0.01$	<sup>d</sup> $P \leq 0.001$	
CD69				
Group I naïve		<sup>b</sup> $P \leq 0.01$	<sup>b</sup> $P \leq 0.01$	<sup>d</sup> $P \leq 0.001$
Group II Peg/Riba	<sup>b</sup> $P \leq 0.01$		<sup>b</sup> $P \leq 0.01$	<sup>d</sup> $P \leq 0.001$
Group III ANA+	<sup>b</sup> $P \leq 0.01$	<sup>b</sup> $P \leq 0.01$		<sup>d</sup> $P \leq 0.001$
Group IV control	<sup>d</sup> $P \leq 0.001$	<sup>d</sup> $P \leq 0.001$	<sup>d</sup> $P \leq 0.001$	
Granzyme				
Group I naïve		<sup>d</sup> $P \leq 0.001$	<sup>d</sup> $P \leq 0.001$	<sup>d</sup> $P \leq 0.001$
Group II Peg/Riba	<sup>d</sup> $P \leq 0.001$		<sup>b</sup> $P \leq 0.01$	<sup>d</sup> $P \leq 0.001$
Group III ANA+	<sup>d</sup> $P \leq 0.001$	<sup>b</sup> $P \leq 0.01$		<sup>d</sup> $P \leq 0.001$
Group IV control	<sup>d</sup> $P \leq 0.001$	<sup>d</sup> $P \leq 0.001$	<sup>d</sup> $P \leq 0.001$	

ANA: Antinuclear antibody.

unpaired *t*-test. Statistically significant was considered if *P* value was < 0.05.

## RESULTS

This study included 120 subjects divided into four groups. There weren't any difference between the four groups with statistical significance regarding age, sex distribution, albumin and T.bilirubin values (*P*-value > 0.05).

Findings of the present study exhibited that chronic HCV patients exhibited significant higher levels of both Teffs and Tregs markers as compared to healthy control group. Tregs markers (granzyme 2, CD69, FoxP3) were significantly decreased in Peg/Riba treated HCV patients in comparison to treatment naïve HCV group (Tables 2 and 3).

In HCV patients with autoantibodies, Tregs markers were significantly decreased in comparison to all the other studied groups (Tables 2 and 3, Figure 1).

Teffs specific genes (*TNFRSF4* and *IFN- $\gamma$* ) and *CD4*, *CD25* showed significant elevation in treatment naïve HCV group in comparison to control group (Tables 2 and 4, Figure 2).

More significant elevation in *Teffs* genes was observed in both Peg/Riba treated HCV and HCV with autoantibodies groups as compared to treatment naïve HCV and control groups (Tables 2 and 4, Figure 2).

## DISCUSSION

HCV is reported to suppress immune system to sustain chronic infection. Accumulation of Tregs and activation of inhibitory signaling pathways play essential roles in suppressing antiviral effector T cells (Teffs). The mechanisms by which HCV impairs Teffs include: Induction of Tregs, Th1 deficiency or Th2 dominance, blunted T cell activation, T cell apoptosis and T cell anergy<sup>[12]</sup>.

The objectives of the present study were to assess the extent of upregulation of Tregs in HCV patients whether or not associated with auto-antibodies. In the present study, we have evaluated certain markers of Tregs and Teffs in peripheral blood mononuclear cells.

FOXP3 (forkhead box P3) is a member of forkhead/winged-helix family of transcriptional regulators which are master regulators in the development and function of Tregs<sup>[13,14]</sup>.

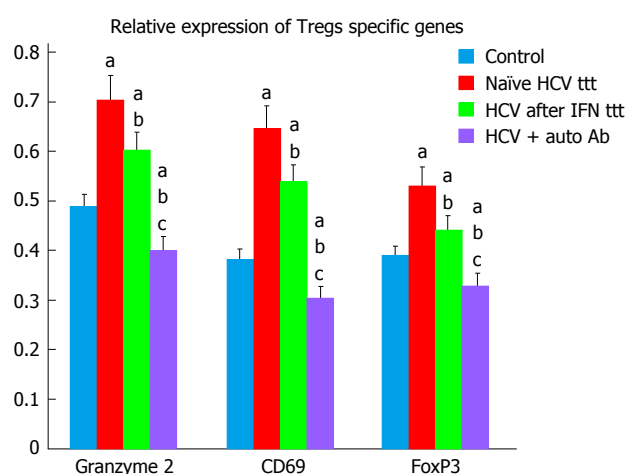
Our results exhibited significant upregulation of FOXP3 in all HCV patients groups as compared to healthy controls. Other studies also confirmed accumulation of FOXP3<sup>+</sup> Tregs in most chronic viral infections with subsequent suppression of antiviral CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses<sup>[15-18]</sup>.

Moreover, findings of our study demonstrated more significant decrease in Tregs specific genes (*CD69*, *FoxP3*, and *granzyme 2*) in HCV patients group after Peg/

**Table 4 Multiple comparisons of gene expression of Teffs markers in the four studied groups**

	Group I naïve	Group II Peg/Riba	Group III ANA+	Group IV control
<b>TNFRSF4OX 40</b>				
Group I naïve		<sup>b</sup> $P \leq 0.01$	<sup>a</sup> $P \leq 0.05$	<sup>a</sup> $P \leq 0.05$
Group II Peg/Riba	<sup>b</sup> $P \leq 0.01$		NS ( $P > 0.05$ )	<sup>b</sup> $P \leq 0.01$
Group III ANA+	<sup>a</sup> $P \leq 0.05$	NS ( $P > 0.05$ )		<sup>d</sup> $P \leq 0.001$
Group IV control	<sup>a</sup> $P \leq 0.05$	<sup>b</sup> $P \leq 0.01$	<sup>d</sup> $P \leq 0.001$	
<b>CD4</b>				
Group I naïve		<sup>b</sup> $P \leq 0.01$	<sup>a</sup> $P \leq 0.05$	<sup>a</sup> $P \leq 0.05$
Group II Peg/Riba	<sup>b</sup> $P \leq 0.01$		NS ( $P > 0.05$ )	<sup>a</sup> $P \leq 0.05$
Group III ANA+	<sup>a</sup> $P \leq 0.05$	NS ( $P > 0.05$ )		<sup>b</sup> $P \leq 0.01$
Group IV control	<sup>a</sup> $P \leq 0.05$	<sup>a</sup> $P \leq 0.05$	<sup>b</sup> $P \leq 0.01$	
<b>CD25</b>				
Group I naïve		<sup>b</sup> $P \leq 0.01$	<sup>a</sup> $P \leq 0.05$	<sup>a</sup> $P \leq 0.05$
Group II Peg/Riba	<sup>b</sup> $P \leq 0.01$		NS ( $P > 0.05$ )	<sup>b</sup> $P \leq 0.01$
Group III ANA+	<sup>a</sup> $P \leq 0.05$	NS ( $P > 0.05$ )		<sup>d</sup> $P \leq 0.001$
Group IV control	<sup>a</sup> $P \leq 0.05$	<sup>b</sup> $P \leq 0.01$	<sup>d</sup> $P \leq 0.001$	
<b>Interferon-<math>\gamma</math></b>				
Group I naïve		<sup>b</sup> $P \leq 0.001$	<sup>b</sup> $P \leq 0.001$	<sup>a</sup> $P \leq 0.05$
Group II Peg/Riba	<sup>b</sup> $P \leq 0.001$		NS ( $P > 0.05$ )	<sup>a</sup> $P \leq 0.05$
Group III ANA+	<sup>b</sup> $P \leq 0.001$	NS ( $P > 0.05$ )		<sup>b</sup> $P \leq 0.01$
Group IV control	<sup>a</sup> $P \leq 0.05$	<sup>a</sup> $P \leq 0.05$	<sup>b</sup> $P \leq 0.01$	

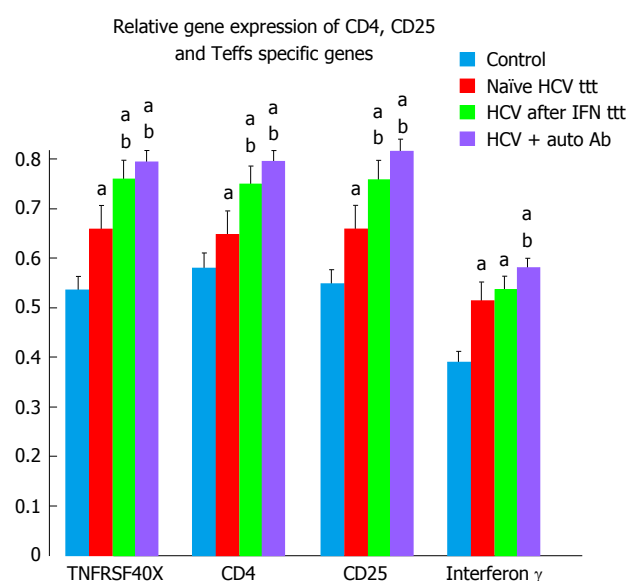
ANA: Antinuclear antibody; NS: Non significant.

**Figure 1 Relative gene expression of Tregs specific genes in the studied groups.** <sup>a</sup>Significant difference vs control group; <sup>b</sup>Significant difference vs treatment naïve HCV group; <sup>c</sup>Significant difference vs HCV group after treatment. HCV: Hepatitis C virus; IFN: Interferon.

Riba therapy. Similar findings were reported by Langhans *et al.*<sup>[19]</sup> who stated that ribavirin can inhibit functions of HCV-specific Tregs beside its immuno-stimulatory effects on TH1 cells. Ribavirin can subsequently inhibit Treg-mediated suppression of Teffs in chronic HCV infections pushing the disease towards autoimmune responses.

Golding *et al.*<sup>[11]</sup> stated that IFN- $\alpha$ , promotes proliferation of FoxP3<sup>Low/Neg</sup>IFN- $\gamma$ <sup>Pos</sup> activated Teffs while simultaneously suppresses the development of FoxP3<sup>HI</sup>IFN- $\gamma$ <sup>Neg</sup> activated Tregs. These data coincided with our findings in group II in relation to the other groups.

CD4 gene product is a membrane glycoprotein of T lymphocytes that mediates initiation and augments early phase of T-cell activation<sup>[20-22]</sup>. Findings of the present study demonstrated significant elevation of CD4 gene in

**Figure 2 Relative gene expression of Teffs specific genes and CD4, CD25 in the studied groups.** <sup>a</sup>Significant difference vs control group; <sup>b</sup>Significant difference vs treatment naïve HCV group. HCV: Hepatitis C virus.

all HCV groups in comparison to control group suggesting increase in activated T cells function. More significant elevation of CD4 was observed in HCV after treatment and in HCV with auto-antibodies groups.

CD25 is a type I transmembrane protein present on activated T cells, activated B cells and in memory CD8 T cells<sup>[23-27]</sup>. Our results demonstrated significant elevation of CD25 in all HCV studied groups with more significant elevation after Peg/Riba therapy. Similar findings were reported by Caetano *et al.*<sup>[28]</sup> in chronic HCV patients during Peg/Riba treatment who presented an amplified CD8 T-cell responses specific to HCV and more increase Teffs.

Moorman *et al.*<sup>[12]</sup> showed that many inhibitory signaling pathways were up-regulated during chronic HCV infection, resulting in expansion of Tregs and contraction of Teffs. Thus, this inhibitory pathway may not only regulate proliferation and differentiation of naïve T cells, but also control responses of Teffs, memory cells, and expansion of Tregs<sup>[29]</sup>.

These facts coincided with our results that showed significant elevation of Tregs specific genes (*CD69*, *FoxP3*, and *granzyme 2*) and significant elevation of Teffs specific genes (*TNFRSF4*, *INF- $\gamma$* ) and *CD4*, *CD25* genes in both groups of HCV whether naïve or after treatment in comparison to healthy controls.

Granzyme B encodes a protein that is essential in induction of cell-mediated immune response for the faster initiation of target cell apoptosis by cytolytic T lymphocytes<sup>[30]</sup>. Tregs possess granzyme B, enabling them to induce apoptosis in effector T-cells<sup>[31,32]</sup>. Our results demonstrated that granzyme B was found to be significantly elevated in naïve HCV patients with significant decrease after Peg/Riba therapy. More significant decrease in its levels was observed in HCV patients with autoantibodies favoring autoimmune response in those patients.

CD69 expression was studied by Colbeck *et al.*<sup>[33]</sup> and they stated that Tregs expressing CD69(+) are more proliferative and more suppressive than their CD69(-) counterparts. This finding explains our results that showed significant lower CD69 expression in HCV patients with autoantibodies suggesting inhibition of Tregs activity favoring autoimmune environment.

IFN- $\gamma$  is a member of the type II class of interferons<sup>[34]</sup>. Longhi *et al.*<sup>[31]</sup> stated that CD8<sup>+</sup> T cells when cultured on their own secrete much higher levels of IFN- $\gamma$  in patients with autoimmune hepatitis when compared to normal subjects and have a high proliferation rate. This finding coincided with our results that demonstrated significant higher levels of IFN- $\gamma$  in patients with autoantibodies than healthy controls.

The protein encoded by the *TNFRSF4* gene belongs to tumor necrosis factor - receptor superfamily that has essential roles in CD4<sup>+</sup> T cell response<sup>[35,36]</sup>.

Our findings showed that, in HCV patients with ANA +ve, *TNFRSF4* and *INF- $\gamma$*  (T effector gene) were significantly higher as compared to HCV naïve and healthy control groups. This means that T effector cells dominate over T regulatory cells favoring autoimmunity and initiating pathological immune response with production of autoantibodies. Similar findings were reported by González-Amaro *et al.*<sup>[37]</sup>.

Also, our results agreed with similar findings reported by Longhi *et al.*<sup>[31]</sup> who stated that regulatory T cells are decreased numerically and impaired functionally in autoimmune hepatitis. Similar to our results, several studies reported that HCV infection induces a dramatic increase in Tregs, which contributes to the immune response failure during HCV infection<sup>[38]</sup>.

In conclusion, chronic HCV patients exhibited signi-

ficant higher levels of both Teffs and Tregs in comparison to healthy controls. Moreover, elevated levels of Treg cells in patients with chronic HCV dampen both the CD4<sup>+</sup> and CD8<sup>+</sup> autologous T cell immune response. IFN- $\alpha$  and ribavirin therapy suppress proliferation of Tregs and do not restore the Teffs/Tregs imbalance. More significant suppression of Tregs was observed in HCV patients with autoantibodies favoring pathological autoimmune response.

## COMMENTS

### Background

Hepatitis C virus (HCV) is accompanied with different autoimmune manifestations, and could be a stimulator for the autoimmune reactions causing production of autoantibodies. More recently, Acay *et al* stated that the auto-antibodies in chronic HCV infection are highly incident. The authors stated that high percentages of patients with chronic hepatitis C had anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies (ASMA), anti-nuclear antibodies (ANA), thyroid antibody and anti-liver kidney microsomal antibodies (anti-LKM-1). The old HCV therapeutic protocol was pegylated interferon (IFN) and ribavirin. IFN- $\alpha$  leads to down regulation of CD4<sup>+</sup> FoxP3<sup>hi</sup> IFN- $\gamma$ <sup>neg</sup> activated T regulatory cells (aTregs) while at the same time induces induction of CD4<sup>+</sup> FoxP3<sup>low/neg</sup> IFN- $\gamma$ <sup>pos</sup> T-activated cells (aTeffs). Together, these observations support the fact that sustained elevation of IFN- $\alpha$  reverses Tregs/Teffs balance towards Teffs activation, generation of auto antibody and development of autoimmunity. The objective of the present study is to evaluate the extent of Teffs/Tregs imbalance in chronic HCV and its association with old standard of care as well as the presence of ANA.

### Research frontiers

IFN- $\alpha$ /ribavirin old therapeutic protocol enhances CD4 effector (Teffs) functions by inhibiting Tregs activation. The old protocol reverses Tregs/Teffs balance towards Teffs activation, generation of auto antibody and development of autoimmunity.

### Innovations and breakthroughs

New direct acting antiviral drugs do not induce Tregs/Teffs imbalance, whereas the old standard of care IFN- $\alpha$  and ribavirin induce Tregs/Teffs imbalance. Replacing the old therapeutic protocol by the new direct acting antiviral drugs is mandatory because beside its efficacy, the new direct acting antiviral drugs do not induce autoimmunity.

### Applications

Chronic HCV patients exhibited significant higher levels of both Teffs and Tregs in comparison to healthy controls. Moreover, elevated levels of Treg cells in patients with chronic HCV dampen both the CD4<sup>+</sup> and CD8<sup>+</sup> autologous T cell immune response. IFN- $\alpha$  and ribavirin therapy suppress proliferation of Tregs and do not restore the Teffs/Tregs imbalance. More significant suppression of Tregs was observed in HCV patients with autoantibodies favoring pathological autoimmune response. Replacing the old therapeutic protocol; IFN- $\alpha$  and ribavirin by the new direct acting antiviral drugs is mandatory and is also essential avoid Teffs/Tregs imbalance.

### Terminology

Tregs also known as suppressor T cells, are a subpopulation of T cells that down-regulates or suppress induction, proliferation and activation of effector T cells. Tregs also maintain tolerance to self-antigens and prevent autoimmunity. Teffs includes various T cell types that actively respond to antigenic stimuli, such as co-stimulation. This includes helper T cells, cytotoxic or killer T cells, and potentially other T cell types as memory cells. HCV-induced autoantibodies involves: AMA, ASMA, ANA, thyroid antibody and anti-LKM-1. Forkhead box P3 is a member of forkhead/winged-helix transcriptional regulators which master the development and function of Tregs. *CD4* gene product is a membrane glycoprotein of T lymphocytes that mediates initiation and augments early phase of T-cell activation. Granzyme B is a protein that enables Tregs to induce apoptosis in effector T-cells. *TNFRSF4* gene belongs to tumor necrosis factor



-receptor superfamily that plays essential roles in CD4<sup>+</sup> T cell response.

## Peer-review

The manuscript presents the gene expression of immune molecules in peripheral blood mononuclear cell by reverse transcription polymerase chain reaction. And to investigate the immune changes induced by HCV, as well as after PR therapy. This paper may be interest to the readers of the journal and provide some knowledge for clinicians and researchers.

## REFERENCES

- Jiménez-Sousa MA**, Fernández-Rodríguez A, Guzmán-Fulgencio M, García-Álvarez M, Resino S. Meta-analysis: implications of interleukin-28B polymorphisms in spontaneous and treatment-related clearance for patients with hepatitis C. *BMC Med* 2013; **11**: 6 [PMID: 23298311 DOI: 10.1186/1741-7015-11-6]
- AASLD/IDSA HCV Guidance Panel**. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology* 2015; **62**: 932-954 [PMID: 26111063 DOI: 10.1002/HEP.27950]
- Mohamoud YA**, Mumtaz GR, Riome S, Miller D, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. *BMC Infect Dis* 2013; **13**: 288 [PMID: 23799878 DOI: 10.1186/1471-2334-13-288]
- McClory S**, Hughes T, Freud AG, Briercheck EL, Martin C, Trimboli AJ, Yu J, Zhang X, Leone G, Nuovo G, Caligiuri MA. Evidence for a stepwise program of extrathymic T cell development within the human tonsil. *J Clin Invest* 2012; **122**: 1403-1415 [PMID: 22378041 DOI: 10.1172/JCI46125]
- Alberts B**, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell. 4th ed. New York: Garland Science, 2002: 1367. Lymphocytes and the Cellular Basis of Adaptive Immunity. Available from: URL: <http://www.ncbi.nlm.nih.gov/books/NBK26921/>
- Vantourout P**, Hayday A. Six-of-the-best: unique contributions of  $\gamma\delta$  T cells to immunology. *Nat Rev Immunol* 2013; **13**: 88-100 [PMID: 23348415 DOI: 10.1038/nri3384]
- Jadali Z**, Alavian SM. Autoimmune diseases co-existing with hepatitis C virus infection. *Iran J Allergy Asthma Immunol* 2010; **9**: 191-206 [PMID: 21131699]
- Himoto T**, Masaki T. Extrahepatic manifestations and autoantibodies in patients with hepatitis C virus infection. *Clin Dev Immunol* 2012; **2012**: 871401 [PMID: 22988469 DOI: 10.1155/2012/871401]
- Acay A**, Demir K, Asik G, Tunay H, Acarturk G. Assessment of the Frequency of Autoantibodies in Chronic Viral Hepatitis. *Pak J Med Sci* 2015; **31**: 150-154 [PMID: 25878633 DOI: 10.12669/pjms.311.6053]
- National Institutes of Health**. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C: 2002--June 10-12, 2002. *Hepatology* 2002; **36**: S3-S20 [PMID: 12407572 DOI: 10.1053/jhep.2002.37117]
- Golding A**, Rosen A, Petri M, Akhter E, Andrade F. Interferon-alpha regulates the dynamic balance between human activated regulatory and effector T cells: implications for antiviral and autoimmune responses. *Immunology* 2010; **131**: 107-117 [PMID: 20465564 DOI: 10.1111/j.1365-2567.2010.03280.x]
- Moorman JP**, Wang JM, Zhang Y, Ji XJ, Ma CJ, Wu XY, Jia ZS, Wang KS, Yao ZQ. Tim-3 pathway controls regulatory and effector T cell balance during hepatitis C virus infection. *J Immunol* 2012; **189**: 755-766 [PMID: 22706088 DOI: 10.4049/jimmunol.1200162]
- Zhang L**, Zhao Y. The regulation of Foxp3 expression in regulatory CD4(+)CD25(+)T cells: multiple pathways on the road. *J Cell Physiol* 2007; **211**: 590-597 [PMID: 17311282 DOI: 10.1002/jcp.21001]
- Marson A**, Kretschmer K, Frampton GM, Jacobsen ES, Polansky JK, MacIsaac KD, Levine SS, Fraenkel E, von Boehmer H, Young RA. Foxp3 occupancy and regulation of key target genes during T-cell stimulation. *Nature* 2007; **445**: 931-935 [PMID: 17237765 DOI: 10.1038/nature05478]
- Hori S**, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; **299**: 1057-1061 [PMID: 12522256 DOI: 10.1126/science.1079490]
- Walker CM**. Adaptive immunity to the hepatitis C virus. *Adv Virus Res* 2010; **78**: 43-86 [PMID: 21040831 DOI: 10.1016/B978-0-12-385032-4.00002-1]
- Belkaid Y**, Chen W. Regulatory ripples. *Nat Immunol* 2010; **11**: 1077-1078 [PMID: 21079629 DOI: 10.1038/ni1210-1077]
- Zhai N**, Chi X, Li T, Song H, Li H, Jin X, Crispe IN, Su L, Niu J, Tu Z. Hepatitis C virus core protein triggers expansion and activation of CD4(+)CD25(+) regulatory T cells in chronic hepatitis C patients. *Cell Mol Immunol* 2015; **12**: 743-749 [PMID: 25531392 DOI: 10.1038/cmi.2014.119]
- Langhans B**, Nischalke HD, Arndt S, Braunschweiger I, Nattermann J, Sauerbruch T, Spengler U. Ribavirin exerts differential effects on functions of Cd4+ Th1, Th2, and regulatory T cell clones in hepatitis C. *PLoS One* 2012; **7**: e42094 [PMID: 22848715 DOI: 10.1371/journal.pone.0042094]
- Geer LY**, Marchler-Bauer A, Geer RC, Han L, He J, He S, Liu C, Shi W, Bryant SH. The NCBI BioSystems database. *Nucleic Acids Res* 2010; **38**: D492-D496 [PMID: 19854944 DOI: 10.1093/nar/gkp858]
- Sayers EW**, Barrett T, Benson DA, Bolton E, Bryant SH, Canese K, Chetvernin V, Church DM, Dicuccio M, Federhen S, Feolo M, Geer LY, Helmberg W, Kapustin Y, Landsman D, Lipman DJ, Lu Z, Madden TL, Madej T, Maglott DR, Marchler-Bauer A, Miller V, Mizrahi I, Ostell J, Panchenko A, Pruitt KD, Schuler GD, Sequeira E, Sherry ST, Shumway M, Sirotkin K, Slotta D, Souvorov A, Starchenko G, Tatusova TA, Wagner L, Wang Y, John Wilbur W, Yaschenko E, Ye J. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* 2010; **38**: D5-D16 [PMID: 19910364 DOI: 10.1093/NAR/GKP967]
- Soldevila B**, Alonso N, Martínez-Arconada MJ, Morillas RM, Planas R, Sanmartí AM, Martínez-Cáceres EM. A prospective study of T- and B-lymphocyte subpopulations, CD81 expression levels on B cells and regulatory CD4(+) CD25(+) CD127(low/-) FoxP3(+) T cells in patients with chronic HCV infection during pegylated interferon-alpha2a plus ribavirin treatment. *J Viral Hepat* 2011; **18**: 384-392 [PMID: 20487258 DOI: 10.1111/J.1365-2893.2010.01317.X]
- Starbeck-Miller GR**, Xue HH, Harty JT. IL-12 and type I interferon prolong the division of activated CD8 T cells by maintaining high-affinity IL-2 signaling in vivo. *J Exp Med* 2014; **211**: 105-120 [PMID: 24367005 DOI: 10.1084/JEM.20130901]
- Janeway CA**, Travers P, Walport M, Shlomchik MJ. Immunobiology: The Immune System in Health and Disease. 5th ed. New York: Garland Science, 2001. Available from: URL: <http://www.ncbi.nlm.nih.gov/books/NBK10757/>
- Triplett TA**, Curti BD, Bonafede PR, Miller WL, Walker EB, Weinberg AD. Defining a functionally distinct subset of human memory CD4+ T cells that are CD25POS and FOXP3NEG. *Eur J Immunol* 2012; **42**: 1893-1905 [PMID: 22585674 DOI: 10.1002/eji.201242444]
- Wherry EJ**, Ahmed R. Memory CD8 T-cell differentiation during viral infection. *J Virol* 2004; **78**: 5535-5545 [PMID: 15140950 DOI: 10.1128/JVI.78.11.5535-5545.2004]
- Burchill MA**, Golden-Mason L, Wind-Rotolo M, Rosen HR. Memory re-differentiation and reduced lymphocyte activation in chronic HCV-infected patients receiving direct-acting antivirals. *J Viral Hepat* 2015; **22**: 983-991 [PMID: 26482547 DOI: 10.1111/jvh.12465]
- Caetano J**, Martinho A, Paiva A, Pais B, Valente C, Luxo C. Differences in hepatitis C virus (HCV)-specific CD8 T-cell phenotype during pegylated alpha interferon and ribavirin treatment are related to response to antiviral therapy in patients chronically infected with HCV. *J Virol* 2008; **82**: 7567-7577 [PMID: 18480446 DOI: 10.1128/JVI.02175-07]
- Mengshol JA**, Golden-Mason L, Arikawa T, Smith M, Niki T, McWilliams R, Randall JA, McMahan R, Zimmerman MA, Rangachari M, Dobrinskikh E, Busson P, Polyak SJ, Hirashima

- M, Rosen HR. A crucial role for Kupffer cell-derived galectin-9 in regulation of T cell immunity in hepatitis C infection. *PLoS One* 2010; **5**: e9504 [PMID: 20209097 DOI: 10.1371/journal.pone.0009504]
- 30 **Dahl CA**, Bach FH, Chan W, Huebner K, Russo G, Croce CM, Herfurth T, Cairns JS. Isolation of a cDNA clone encoding a novel form of granzyme B from human NK cells and mapping to chromosome 14. *Hum Genet* 1990; **84**: 465-470 [PMID: 2323780]
- 31 **Longhi MS**, Hussain MJ, Mitry RR, Arora SK, Mieli-Vergani G, Vergani D, Ma Y. Functional study of CD4+CD25+ regulatory T cells in health and autoimmune hepatitis. *J Immunol* 2006; **176**: 4484-4491 [PMID: 16547287 DOI: 10.4049/jimmunol.176.7.4484]
- 32 **Azzi J**, Skartsis N, Mounayar M, Magee CN, Batal I, Ting C, Moore R, Riella LV, Ohori S, Abdoli R, Smith B, Fiorina P, Heathcote D, Bakhos T, Ashton-Rickardt PG, Abdi R. Serine protease inhibitor 6 plays a critical role in protecting murine granzyme B-producing regulatory T cells. *J Immunol* 2013; **191**: 2319-2327 [PMID: 23913965 DOI: 10.4049/jimmunol.1300851]
- 33 **Colbeck EJ**, Hindley JP, Smart K, Jones E, Bloom A, Bridgeman H, McPherson RC, Turner DG, Ladell K, Price DA, O'Connor RA, Anderton SM, Godkin AJ, Gallimore AM. Eliminating roles for T-bet and IL-2 but revealing superior activation and proliferation as mechanisms underpinning dominance of regulatory T cells in tumors. *Oncotarget* 2015; **6**: 24649-24659 [PMID: 26433463 DOI: 10.18632/oncotarget.5584]
- 34 **Gray PW**, Goeddel DV. Structure of the human immune interferon gene. *Nature* 1982; **298**: 859-863 [PMID: 6180322 DOI: 10.1038/298859a0]
- 35 **Latza U**, Dürkop H, Schnittger S, Ringeling J, Eitelbach F, Hummel M, Fonatsch C, Stein H. The human OX40 homolog: cDNA structure, expression and chromosomal assignment of the ACT35 antigen. *Eur J Immunol* 1994; **24**: 677-683 [PMID: 7510240 DOI: 10.1002/eji.1830240329]
- 36 **Keoshkerian E**, Helbig K, Beard M, Zaunders J, Seddiki N, Kelleher A, Hampartoumian T, Zekry A, Lloyd AR. A novel assay for detection of hepatitis C virus-specific effector CD4(+) T cells via co-expression of CD25 and CD134. *J Immunol Methods* 2012; **375**: 148-158 [PMID: 22019644 DOI: 10.1016/j.jim.2011.10.004]
- 37 **González-Amaro R**, Cortés JR, Sánchez-Madrid F, Martín P. Is CD69 an effective brake to control inflammatory diseases? *Trends Mol Med* 2013; **19**: 625-632 [PMID: 23954168 DOI: 10.1016/J.MOLMED.2013.07.006]
- 38 **Hashemipoor T**, Bamdad T, Merat S, Janzamin E, Nemati L, Jabbari H, Sharifi AH, Zamini H. Expansion of CD4+ CD25+ FoxP3+ regulatory T cells in chronic hepatitis C virus infection. *Iran J Immunol* 2010; **7**: 177-185 [PMID: 20876988]

**P- Reviewer:** Baddour N, Barili F, Ji FP **S- Editor:** Gong XM

**L- Editor:** A **E- Editor:** Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 November 8; 8(31): 1295-1342







## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verducci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



**REVIEW**

- 1295 Alcohol use disorder and its impact on chronic hepatitis C virus and human immunodeficiency virus infections

*Fuster D, Sanvisens A, Bolao F, Rivas I, Tor J, Muga R*

**MINIREVIEWS**

- 1309 Prophylactic liver transplantation for high-risk recurrent hepatocellular carcinoma

*Yang PC, Ho CM, Hu RH, Ho MC, Wu YM, Lee PH*

**ORIGINAL ARTICLE****Retrospective Cohort Study**

- 1318 Safe and effective sofosbuvir-based therapy in patients with mental health disease on hepatitis C virus treatment

*Tang LSY, Masur J, Sims Z, Nelson A, Osinusi A, Kohli A, Kattakuzhy S, Polis M, Kottitil S*

**Retrospective Study**

- 1327 Liver resection for early hepatocellular cancer: Comparison of centers in 3 different countries

*Wong LL, Hernandez BY, Shvetsov YB, Kawano Y, Tang ZY, Ji JF*

**Prospective Study**

- 1336 Mortality and rebleeding following variceal haemorrhage in liver cirrhosis and periportal fibrosis

*Mohammed SEA, Abdo AE, Mudawi HMY*

## Contents

*World Journal of Hepatology*  
Volume 8 Number 31 November 8, 2016

### ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Dr. Yu-Bao Zheng, MD, PhD, Associate Professor, Chief Doctor, Teacher, Department of Infectious Diseases, the Third Affiliated Hospital, Sun Yat-sen University, Guangzhou 510630, Guangdong Province, China

### AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

### FLYLEAF

#### I-IV Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Department of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL BOARD MEMBERS  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

EDITORIAL OFFICE  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
November 8, 2016

COPYRIGHT  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS  
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION  
<http://www.wjgnet.com/esps/>

## Alcohol use disorder and its impact on chronic hepatitis C virus and human immunodeficiency virus infections

Daniel Fuster, Arantza Sanvisens, Ferran Bolao, Inmaculada Rivas, Jordi Tor, Robert Muga

Daniel Fuster, Arantza Sanvisens, Jordi Tor, Robert Muga, Department of Internal Medicine, Addiction Unit, Hospital Universitari Germans Trias i Pujol, 08916 Badalona, Spain

Ferran Bolao, Department of Internal Medicine, Hospital Universitari de Bellvitge, IDIBELL, 08907 L'Hospitalet de Llobregat, Spain

Inmaculada Rivas, Municipal Center for Substance Abuse Treatment (Centro Delta), IMSP, 08916 Badalona, Spain

**Author contributions:** Fuster D performed the literature search and drafted the first version of the manuscript; Sanvisens A and Muga R provided feedback for the first version and suggested additional references; all authors edited and provided feedback around the updated version of the review and approved the final version of the manuscript.

**Supported by** Ministry of Economy and Competitiveness, Institute of Health Carlos, ISCIII: European fund for regional development (FEDER), Nos. RETICS RD 12/0028/0006 and RD16/0017/0003; Ministry of Health, Social Services, and Equality, Nos. PNSD 2014/042 and PNSD 2015/027.

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Daniel Fuster, MD, PhD, Department of Internal Medicine, Addiction Unit, Hospital Universitari Germans Trias i Pujol, Carretera de Canyet, S/N, 08916 Badalona, Spain. [dfuster.germanstrias@gencat.cat](mailto:dfuster.germanstrias@gencat.cat)  
Telephone: +34-934-978908  
Fax: +34-934-978768

Received: May 4, 2016

Peer-review started: May 6, 2016

First decision: July 4, 2016

Revised: August 4, 2016

Accepted: August 27, 2016

Article in press: August 29, 2016

Published online: November 8, 2016

### Abstract

Alcohol use disorder (AUD) and hepatitis C virus (HCV) infection frequently co-occur. AUD is associated with greater exposure to HCV infection, increased HCV infection persistence, and more extensive liver damage due to interactions between AUD and HCV on immune responses, cytotoxicity, and oxidative stress. Although AUD and HCV infection are associated with increased morbidity and mortality, HCV antiviral therapy is less commonly prescribed in individuals with both conditions. AUD is also common in human immunodeficiency virus (HIV) infection, which negatively impacts proper HIV care and adherence to antiretroviral therapy, and liver disease. In addition, AUD and HCV infection are also frequent within a proportion of patients with HIV infection, which negatively impacts liver disease. This review summarizes the current knowledge regarding pathological interactions of AUD with hepatitis C infection, HIV infection, and HCV/HIV co-infection, as well as relating to AUD treatment interventions in these individuals.

**Key words:** Hepatitis C virus; Human immunodeficiency virus; Hepatitis C virus/human immunodeficiency virus co-infection; Liver; Alcohol

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The present review is focused on alcohol use disorder and hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infection, as well as HCV/

## HIV co-infection.

Fuster D, Sanvisens A, Bolao F, Rivas I, Tor J, Muga R. Alcohol use disorder and its impact on chronic hepatitis C virus and human immunodeficiency virus infections. *World J Hepatol* 2016; 8(31): 1295-1308 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i31/1295.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i31.1295>

## INTRODUCTION

Alcohol abuse is a major cause of preventable liver disease worldwide, and alcohol use disorder (AUD) is associated with substantial disease burden in western countries<sup>[1]</sup>. According to 5<sup>th</sup> edition of the Diagnostic and Statistical Manual of Mental Disorders<sup>[2]</sup>, AUD encompasses both alcohol abuse and alcohol dependence. Table 1 presents the diagnostic criteria for AUD and other definitions of unhealthy alcohol use, such as the recommendations of the United States National Institute on Alcohol Abuse and Alcoholism.

In the United States, almost 9% of the adult population meets the AUD criteria and alcohol contributes to 79000 deaths annually<sup>[3]</sup>. Within the European Union, alcohol misuse causes 14% of deaths in men and nearly 8% of deaths in women, with alcohol-related mortality disproportionately impacting young people<sup>[4]</sup>. In Spain, unhealthy alcohol use is exhibited by 5% of the population between 15 and 64 years old, and 15% report at least one binge drinking episode within the prior year<sup>[5]</sup>. Moreover, the pattern of binge drinking is becoming increasingly prevalent, mainly among young individuals.

Per capita alcohol consumption is strongly correlated with liver cirrhosis mortality rates globally<sup>[6]</sup>. However, the short- and long-term impacts of binge drinking with regards to the development and severity of alcoholic liver disease (ALD) are not yet known. Per capita alcohol consumption is strongly correlated with liver cirrhosis mortality rates across countries<sup>[5]</sup>. Notably, the medical literature reveals wide heterogeneity in the methods used to assess alcohol exposure, and it can be challenging to analyze time-varying exposures like alcohol consumption over time<sup>[7]</sup>.

### Epidemiology of AUD in hepatitis C virus and human immunodeficiency virus infection

Addressing alcohol use is critical in the management of hepatitis C virus (HCV)-infected patients, as AUD is associated with poor clinical outcomes and liver-related deaths in this patient group<sup>[8]</sup>. Compared to the general population, HCV-infected adults tend to consume greater amounts of ethanol<sup>[9]</sup>, being over twice as likely to consume more than one alcoholic drink per day (34% vs 14%) and almost 8 times more likely to consume over three drinks per day (19% vs 2%)<sup>[10]</sup>.

Moreover, alcohol abuse is associated with concomitant use of illegal substances, and 30% to 50% of patients with a history of substance abuse consume alcohol<sup>[11]</sup>. This is highly important since 2/3 of new HCV infections in the western world are associated with drug injection<sup>[12]</sup>. Accordingly, the prevalence of HCV infection is higher among patients with AUD who are current or past injecting drug users<sup>[13]</sup>. Within a cohort of patients with AUD admitted for hospital detoxification in the Barcelona area, HCV prevalence was as high as 20%<sup>[14]</sup>. However, other researchers in Spain reported a much lower prevalence of 3.5%<sup>[13]</sup>, possibly due to differences in patient selection.

The prevalence of HCV infection is confounded by the degree of liver disease. Cross-sectional studies performed in hepatology clinics showed that HCV prevalence was higher among patients with advanced liver fibrosis, and almost universal among HCV-infected patients with hepatocellular carcinoma<sup>[15,16]</sup>. On the other hand, HCV prevalence ranged from 1% to 10% in community-oriented studies of individuals with AUD but without clinically apparent liver disease<sup>[17,18]</sup>. A recent meta-analysis including 24 studies reported that the average weighted prevalence of HCV infection among patients with AUD was 16.3%<sup>[13]</sup>.

AUD may also be common among human immunodeficiency virus (HIV)/AIDS patients, with a prevalence ranging from 30% to 50%<sup>[19]</sup>. High prevalences of alcohol consumption have been reported in HIV/AIDS cohort studies from the United States<sup>[20,21]</sup>, Europe<sup>[22-24]</sup>, South Africa<sup>[25]</sup>, and other parts of the world<sup>[26]</sup>. In the Women's Interagency HIV Study, 14%-24% of female HIV/AIDS participants reported hazardous alcohol use within the past year<sup>[27]</sup>. On the other hand, patients with AUD show a lower prevalence of HIV infection than HCV infection<sup>[14]</sup>, which is confounded by prevalence of injection drug use.

## AUD AND CHRONIC HCV INFECTION

### Effect of alcohol on HCV replication

Alcohol metabolites apparently enhance viral protein expression as well as the heterogeneity of HCV quasi-species<sup>[28]</sup>. Some authors describe RNA-HCV increases among patients who use alcohol<sup>[29]</sup>. However, a meta-analysis performed by Anand *et al*<sup>[30]</sup> in 2005 showed no association between RNA-HCV and alcohol consumption.

### Impact of alcohol on HCV infection persistence

Spontaneous resolution of HCV infection requires an early and wide immune response against HCV viral proteins<sup>[31]</sup>. Once acute HCV infection is controlled, the presence of memory T-cell populations is associated with reduced persistence of infection in re-exposed individuals<sup>[32]</sup>. HCV infection persistence is also associated with loss of specific T-cell proliferation, and reduced migration of effector T cells to the liver<sup>[33]</sup>. HCV-infected patients with AUD show functional impairment of dendritic cells<sup>[34]</sup>, which partly explains the association between alcohol use



**Table 1** Diagnostic criteria for alcohol use disorder and other definitions of unhealthy alcohol use

## AUD (DSM-5)

In the past year<sup>[2]</sup>, have you<sup>1</sup>

Had times when you ended up drinking more, or longer than you intended?

More than once wanted to cut down or stop drinking, or tried to, but couldn't?

Spent a lot of time drinking? Or being sick or getting over the aftereffects?

Experienced craving - a strong need, or urge, to drink?

Found that drinking or being sick from drinking often interfered with taking care of your home or family? Or caused job troubles? Or school problems?

Continued to drink even though it was causing trouble with your family or friends?

Given up or cut back on activities that were important or interesting to you, or gave you pleasure, in order to drink?

More than once gotten into situations while or after drinking that increased your chances of getting hurt (such as driving, swimming, using machinery, walking in a dangerous area, or having unsafe sex)?

Continued to drink even though it was making you feel depressed or anxious or adding to another health problem? Or after having had a memory blackout?

Had to drink much more than you once did to get the effect you want? Or found that your usual number of drinks had much less effect than before?

Found that when the effects of alcohol were wearing off, you had withdrawal symptoms, such as trouble sleeping, shakiness, irritability, anxiety, depression, restlessness, nausea, or sweating? Or sensed things that were not there?

Risky alcohol use<sup>[178]</sup>

Drinking more than the recommended amount by the National Institute on Alcohol Abuse and Alcoholism

&gt; 14 drinks per week or &gt; 4 drinks on any day for men

&gt; 7 drinks per week or &gt; 3 drinks on any day for women or men &gt; 65 yr

## Problem drinking

Use of alcohol accompanied by alcohol-related consequences but not meeting criteria for AUD

<sup>1</sup>Meeting any two of the 11 criteria during the same 12-mo period is consistent with AUD. The severity of an AUD-mild, moderate, or severe-is based on the number of criteria met. AUD: Alcohol use disorder; DSM-5: Diagnostic and statistical manual of mental disorders.

and lower odds of spontaneous HCV resolution<sup>[35,36]</sup>.

**Effect of alcohol on HCV-related immunity**

Mice that are chronically exposed to ethanol exhibit diminished immune responses to HCV-core protein, mainly due to impaired maturation of dendritic cells<sup>[34]</sup>. In HCV-infected patients, dendritic cells present impaired allostimulation capacity, which is more apparent in the presence of alcohol<sup>[34]</sup>. Alcohol and HCV infection exert synergistic effects, suppressing major histocompatibility complex class II<sup>[37]</sup> *via* functional impairment of the proteasome (intracellular protein complexes that degrade unnecessary or damaged proteins) and alterations in interferon signaling<sup>[38]</sup>. This could partly explain the lower efficacy of interferon-based HCV treatment regimens among patients with AUD<sup>[39]</sup>.

**Effect of alcohol on cytotoxicity**

Enhanced hepatocyte apoptosis is observed in HCV infection, which is apparently associated with impaired immune responses rather than directly attributable to the viral infection<sup>[40]</sup>. Hepatocyte apoptosis is mediated by cytotoxic T cells and natural killer cells *via* caspase activity<sup>[40]</sup>. BCL-2 protein is associated with mitochondrial permeability, and its expression is reduced in HCV-infected hepatocytes<sup>[41]</sup>. Alcohol seems to enhance hepatocyte apoptosis through down-regulation of BCL-2 expression<sup>[40]</sup>.

**Alcohol and oxidative stress**

The HCV core viral protein is associated with higher oxidative stress. It binds the mitochondrial wall, facilitating calcium entrance, electron transport, and increased

reactive oxygen species, which results in increased oxidative stress that damages the cell<sup>[42]</sup>. This protein also targets microsomal triglyceride transfer protein activity, thus modifying hepatic very-low-density lipoprotein particle assembly and secretion, which leads to liver steatosis<sup>[43]</sup>. Moreover, the HCV core viral protein alters the oxidant/antioxidant state of the liver in the absence of inflammation, consequently producing mitochondrial DNA damage<sup>[44]</sup>.

In HCV-core transgenic mice, chronic ethanol administration is associated with higher lipid peroxidation and synergic induction of TGF- $\beta$ 1 and hepatic stellate cells<sup>[45]</sup>. The HCV-core protein cooperates with ethanol to activate some p38 mitogen-activated protein kinase pathways, resulting in polygene modulation, and contributing to liver disease pathogenesis<sup>[46]</sup>. In alcohol-fed NS5A transgenic mice, the synergistic effect between HCV infection and alcohol is dependent on mechanisms involving Toll-like receptor 4, which belongs to the innate immune system<sup>[47]</sup>. Alcohol consumption and HCV infection impact FOXO3 expression, thus impairing antioxidant capacity in the liver<sup>[48]</sup>.

In humans, indirect evidence suggests that oxidative stress is associated with more extensive liver injury in patients with AUD and HCV infection, as they tend to show higher serum levels of malondialdehyde (a lipid peroxidation product), poor glutathione peroxidase activity, and stimulation of Th1 response cytokines<sup>[49]</sup>. Moreover, patients with AUD present major lipid peroxidation, and the loss of antioxidant capacity is associated with liver fibrosis<sup>[50]</sup>. Among HCV-infected patients who drink alcohol, liver fibrosis is independently associated with liver steatosis, oxidative stress, age, and iron

deposits in the liver<sup>[51]</sup>.

### **Alcohol and progression of HCV-related liver disease**

Alcohol consumption is associated with more extensive progression of HCV-related liver damage<sup>[52,53]</sup>. No safe level of alcohol consumption has been described, as even HCV-infected patients who drink moderate amounts of alcohol (30 g/d) experience progressive liver fibrosis<sup>[54-56]</sup>. A meta-analysis assessed 20 studies that were published between 1995 and 2004, and found that the relative risk of progression to liver cirrhosis or decompensated liver disease among HCV-infected patients was 2.3 times higher, with a 95%CI of 1.7-3.3, among those who drank alcohol compared to abstainers<sup>[52]</sup>. However, the majority of included studies were performed in liver units, and thus might be biased towards patients with more severe forms of liver disease<sup>[52]</sup>. Alcohol consumption is also associated with higher risks of cirrhosis decompensation and liver-related death<sup>[57]</sup>. Moreover, alcohol consumption has a synergistic effect with chronic hepatitis C, increasing the risk of liver cancer<sup>[58]</sup>.

### **Assessment of liver disease in patients with AUD and HCV infection**

In both HCV infection and ALD, liver fibrosis is the main prognostic factor of liver disease progression<sup>[59,60]</sup>. Although liver biopsy is the gold standard for liver fibrosis assessment<sup>[61]</sup>, it is associated with several rare complications and is not usually performed in patients with substance use disorders<sup>[62]</sup>. Recent reports describe the estimation of liver fibrosis using several non-invasive biological markers derived from laboratory parameters routinely used in clinical practice, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and platelet count.

Of these potential markers, FIB-4<sup>[63]</sup> and the aspartate aminotransferase/platelet ratio index (APRI)<sup>[64]</sup> have been validated against the gold standard of liver biopsy in HCV-monoinfected patients as well as HCV/HIV-coinfected patients<sup>[65-68]</sup>. These markers perform better for detecting either the absence of liver fibrosis or the presence of advanced liver fibrosis<sup>[63,64]</sup>. However, clinical experience using these markers in patients with AUD is limited<sup>[69]</sup>, and concerns have been raised about the possibility of overestimating liver fibrosis in patients with alcoholic steatohepatitis. Moreover, ALD is a formal contraindication for the use of Pohl's score<sup>[70]</sup>-an index that uses aminotransferase levels and platelet count. Transient elastography has also been used to assess liver fibrosis in ALD<sup>[71]</sup>, but the presence of severe liver steatosis may distort results, leading to overestimation of advanced liver fibrosis<sup>[72]</sup>.

In prior studies, we have defined alcohol-related liver disease (ARLD) as the presence of any two of the following criteria: Elevated AST to between 74 and 300 U/L, AST/ALT  $\geq 2$ , and total bilirubin  $> 1.2$  mg/dL<sup>[73,74]</sup>. Within a cohort of AUD patients admitted for hospital detoxification in metropolitan Barcelona, Spain, 14.6%

met those criteria, and ARLD was associated with mid-term mortality<sup>[75]</sup>.

### **Impact of HCV infection on hospitalizations and mortality of patients with AUD**

As previously mentioned, alcohol use is associated with worse prognosis in HCV-related liver disease. It is estimated that 36% of liver cirrhosis among HCV-infected individuals is attributable to alcohol use<sup>[76]</sup>. HCV infection also has a deleterious impact on clinical outcomes among patients with AUD<sup>[77-80]</sup>. Tsui *et al.*<sup>[77]</sup> identified 6354 AUD-related hospital admissions, and reported that the HCV-positive patients were twice as likely to die (4.4% vs 2.4%,  $P < 0.01$ ), and showed significantly longer hospital stays (19% longer, 95%CI: 12%-27%). Another study included patients from the United States Nationwide Inpatient Sample Dataset who had a primary or a secondary discharge diagnosis of alcoholic hepatitis, and reported that HCV-positive patients had higher mortality with an odds ratio (OR) of 1.29 (95%CI: 1.12-1.49,  $P < 0.01$ )<sup>[78]</sup>.

Patients with AUD who are exposed to HCV infection probably differ from those who are not exposed with regards to co-morbidities or behaviors associated with poorer survival, such as the use of illicit drugs<sup>[81]</sup>. However, even in studies that have accounted for various lifestyle factors, HCV infection remains associated with both overall mortality, showing a hazard ratio (HR) of 2.55 (95%CI: 1.50-4.33,  $P < 0.01$ ), and liver-related mortality (HR = 3.24, 95%CI: 1.18-8.94,  $P = 0.02$ )<sup>[79]</sup>.

In our study of 675 AUD patients admitted for hospital detoxification, we examined the impact of HCV infection on mortality. Our results showed that HCV infection was associated with higher mortality, and that this effect was more apparent in patients with younger ages at admission (HR = 3.1, 95%CI: 1.3-7.3,  $P < 0.01$ ) and those who were co-infected with HCV/HIV (HR = 3.9, 95%CI: 2.1-7.1,  $P < 0.01$ )<sup>[80]</sup>. In the same Barcelona cohort, we recently reported that AUD patients with HCV mono-infection showed an increased risk of liver-related death in comparison to AUD patients without HCV-infection (HR = 3.92, 95%CI: 2.03-7.59)<sup>[82]</sup>.

### **Interferon-based treatment of HCV infection in patients with AUD**

In the era of HCV antiviral therapy including interferon, infection treatment was challenging in individuals who consumed alcohol<sup>[8]</sup>. In fact, alcohol use was a major reason for a lack of HCV treatment<sup>[83,84]</sup>. Several researchers analyzed strategies to extend HCV treatment to patients with unhealthy alcohol use. Le Lan *et al.*<sup>[85]</sup> performed an observational study of HCV treatment in alcohol-drinking patients, in which drinking in moderation was encouraged but not required. Of the study population, 30% continuously abstained, 34% consumed low-risk amounts of alcohol, and 36% continued to drink risky amounts. The overall sustained viral response (SVR) rate was 48% with no difference observed between

**Table 2 Treatment interventions for unhealthy alcohol use and alcohol use disorder**

Condition	Intervention
Unhealthy alcohol use	Brief intervention
AUD	Motivational interviewing
	Hospital detoxification
	Individual and group therapy
	Approved pharmacological treatments:
	Disulfiram
	Acamprosate
	Naltrexone
	Nalmefene
	Investigational treatments:
	Baclofene
	Topiramate
	Gabapentin

AUD: Alcohol use disorder.

abstainers and low-risk drinkers<sup>[85]</sup>, confirming prior results in a Swiss HCV cohort<sup>[86]</sup>.

Evon *et al*<sup>[87]</sup> performed a randomized clinical trial in the United States, which included 9-mo intervention comprising counseling, case management, and motivational interviewing for patients ineligible for HCV treatment (31% due to alcohol abuse). The intervention was associated with a 2.38 relative risk of being deemed eligible (95%CI: 1.21-4.68). The groups did not differ with regards to the proportion of patients that eventually received HCV antiviral therapy<sup>[87]</sup>.

### **Interferon-free treatment of HCV infection in patients with AUD**

The advent of direct-acting antivirals and interferon-free regimens has dramatically changed the landscape of HCV treatment, with most registration trials and pilot real-life experiences reporting SVR rates of over 90%<sup>[88]</sup>. Although treatment is now more feasible for patients with substance use disorders<sup>[89,90]</sup>, to date, very few patients with AUD have been included in clinical trials<sup>[91-93]</sup>.

The current American Association for the Study of Liver Diseases - Infectious Diseases of America guidelines for HCV treatment advocate abstinence from alcohol<sup>[94]</sup>. When appropriate, these guidelines suggest interventions to facilitate the cessation of alcohol consumption, ranging from brief interventions for patients with low alcohol intake<sup>[94]</sup>, to referral to mutual help groups and specialty treatment for patients with established AUD<sup>[94]</sup>. While alcohol consumption is not a formal contraindication for HCV treatment, a year of abstinence from alcohol is thought to be necessary to achieve adequate treatment adherence<sup>[95]</sup>.

There remains a need for a change in the provision of HCV treatment such that patients with AUD and HCV infection can benefit from viral eradication. Expansion of the capacity of primary care clinics or addiction clinics to provide HCV treatment has been successfully tested in several areas of the United States<sup>[96]</sup> and Australia<sup>[90]</sup>. These experiences should be replicated worldwide to

more effectively treat difficult-to-reach populations<sup>[97]</sup>.

### **AUD treatment in patients with HCV infection**

Brief interventions involving feedback and discussion of the negative consequences of alcohol abuse are efficacious at motivating reduced alcohol consumption among among patients with unhealthy alcohol use<sup>[98]</sup>, but not patients with alcohol dependence. Such brief interventions can be targeted towards patients with HCV infection, with delivery at the primary care level or in hepatology clinics<sup>[94,99]</sup>. More intensive treatments, such as motivational enhancement therapy, can also reduce the number of drinking days among patients with chronic HCV infection<sup>[100]</sup>. Other type of interventions, such as group therapy, can reportedly motivate abstinence from alcohol in 44% of patients in an HCV clinic<sup>[101]</sup>.

Table 2 summarizes the various treatment strategies for patients with AUD. Specialty treatment should be favored in such cases, and patients should be offered detoxification; specific pharmacotherapy including disulfiram, acamprosate, naltrexone, or nalmefene; and psychosocial support<sup>[3]</sup>. Some researchers have reported satisfactory results with baclofene in patients with overt end-stage liver disease<sup>[102]</sup>.

## **AUD AND HIV INFECTION**

### **Effect of alcohol on the immune system**

The combined effects of alcohol and HIV on the immune system have been investigated in simian models<sup>[103]</sup>. Alcohol and HIV infection show a synergistic impact on gastrointestinal tract integrity, causing initial depletion of intestinal CD4 cells<sup>[104,105]</sup>. Loss of intestinal wall integrity is associated with increased permeability, microbial translocation, and immune activation<sup>[106]</sup>. Immune activation is crucial for HIV disease progression<sup>[107]</sup>, and is reportedly a better predictor of disease progression than HIV viral load<sup>[106,108]</sup>. While alcohol seems to impact the adaptive immune responses to HIV infection in animal models, the results in humans are mixed<sup>[103]</sup>. In a study of HIV-infected patients, blood alcohol levels relative to alcohol intake were higher before antiretroviral treatment compared to after treatment<sup>[109]</sup>.

### **Alcohol and HIV disease progression**

Prior to widespread use of antiretroviral therapy (ART), epidemiological data suggested that alcohol use was not associated with HIV disease progression<sup>[110,111]</sup>. However, following the advent of ART, several authors have reported reduced ART effectiveness among patients with AUD<sup>[19,112]</sup>. In 2003, Samet *et al*<sup>[113]</sup> investigated a cohort of HIV-infected patients, and reported cross-sectional data suggesting that alcohol consumption negatively impacted HIV disease progression. Alcohol consumption was associated with lower CD4 cell counts and higher HIV viral loads in patients receiving ART. A later longitudinal study of the same cohort demonstrated that heavy alcohol use in patients not receiving ART was

associated with lower CD4 cell counts but not with HIV viral load<sup>[114]</sup>.

Chander *et al.*<sup>[115]</sup> at John Hopkins University reported that heavy alcohol consumption was associated with reduced viral suppression of HIV infection and lower treatment adherence. Wu *et al.*<sup>[116]</sup> investigated 325 subjects receiving ART and found that, after adjusting for adherence, daily drinkers showed a nearly four-fold increase in the odds of detectable HIV viral load. This association was non-significant for regular drinkers. Their results further showed that alcohol use was not associated with CD4 cell count, and that alcohol consumption was not associated with HIV viral load among patients not receiving ART<sup>[116]</sup>. On the other hand, Baum *et al.*<sup>[117]</sup> investigated HIV-infected patients receiving ART, and reported that alcohol use was associated with lower CD4 cell counts, greater risk of showing a CD4 cell count of < 200, and an increased HIV viral load over time.

More recent studies indicate that the benefits of ART seem to outweigh the detrimental effects of alcohol use, reinforcing the importance of initiating ART and ensuring adequate treatment adherence<sup>[118]</sup>. A study in a Swiss HIV cohort revealed no effect of alcohol consumption on either virological failure or CD4 cell count, both among ART-receiving and ART-naïve patients<sup>[119]</sup>. That study also demonstrated that heavy drinkers were more likely to interrupt ART; however, only 2.8% of participants were heavy drinkers<sup>[119]</sup>. A recent French study of HIV/AIDS patients reported that low levels of alcohol consumption (< 10 g/d) were associated with higher CD4 counts compared to in abstainers<sup>[120]</sup>. However, the beneficial effects of such low levels of alcohol consumption may be confounded by other healthier behaviors exhibited by moderate drinkers<sup>[121]</sup>.

Overall, evidence acquired during the first decade of ART use suggested that AUD may impact HIV disease progression; however, more recent studies do not support those findings. These contradictory results may be partly explained by poor adherence to treatment and barriers to proper medical care associated with AUD.

### **Alcohol and comorbidities**

Alcohol use is associated with unprotected sex and syringe sharing, thus elevating the risks of HIV acquisition and transmission<sup>[122-124]</sup>. Moreover, alcohol use is associated with higher prevalence of depressive symptoms<sup>[125]</sup>, which can influence ART initiation<sup>[126]</sup>, treatment adherence<sup>[127]</sup>, treatment discontinuation<sup>[128]</sup>, and disease progression<sup>[129,130]</sup>. Other substance use disorders frequently co-exist in patients who exhibit alcohol abuse<sup>[11]</sup>, which is also associated with poorer treatment adherence, reduced HIV viral suppression, and lower retention in care<sup>[112,131]</sup>.

Heavy alcohol use is related to liver disease among patients with HIV infection<sup>[132,133]</sup>, and is also associated with cardiovascular disease<sup>[134]</sup> and exacerbations of chronic obstructive pulmonary disease<sup>[135]</sup>. A systematic review of 13 studies reported that heavy alcohol use was associated with elevated risk of cardiovascular

disease, with a risk ratio of 1.78 (95%CI: 1.09-2.93)<sup>[134]</sup>.

### **Alcohol and mortality in HIV infection**

Alcohol is commonly regarded as an underappreciated modifiable risk factor in individuals with HIV infection, with or without HCV co-infection<sup>[116]</sup>. A retrospective study from northern California evaluated data from between 1996 and 2005, and found that higher mortality rates were associated with diagnosis of a substance use disorder (alcohol only, drug only, or alcohol and drug)<sup>[136]</sup>. In the HIV-LIVE cohort of HIV-positive patients with alcohol problems, short-term mortality was associated with homelessness and drug use<sup>[137]</sup>, and long-term mortality was associated with HCV infection and high levels of inflammation markers<sup>[79,138]</sup>. A study from the VACS cohort revealed that even non-hazardous levels of alcohol consumption were associated with decreased survival<sup>[139]</sup>. Recent data from the same VACS cohort shows that among HIV-positive participants, alcohol use was associated with greater physiological injury. Moreover, within this cohort, a greater risk of mortality was associated with an Alcohol Use Disorders Identification Test value of  $\geq 4$  drinks/mo (HR = 1.25, 95%CI: 1.09-1.44), and of  $\geq 30$  drinks/mo (HR = 1.30, 95%CI: 1.14-1.50)<sup>[140]</sup>.

### **HIV treatment in patients with AUD**

Alcohol use co-existing with other substance use is associated with lower quality of HIV care<sup>[141]</sup> and poor retention in care<sup>[131]</sup>. A systematic review of 53 studies published between 2010 and 2015 showed that 77% of studies revealed that alcohol use was negatively associated with the HIV treatment cascade, *i.e.*, access to care, ART prescription, and treatment adherence<sup>[142]</sup>. This suggests that unhealthy alcohol use should be targeted to increase the proportion of HIV/AIDS patients who achieve viral suppression.

Even modest alcohol consumption has been associated with poor ART adherence<sup>[139]</sup>. Hendershot *et al.*<sup>[143]</sup> performed a meta-analysis of 40 studies, and showed that patients who drank relatively more were 50%-60% less likely to adhere to ART compared with those who abstained or drank relatively less. Alcohol consumption appears to be dose-dependently related to ART adherence<sup>[115]</sup>, and shows a temporal relationship to missed ART treatments<sup>[144]</sup>.

### **AUD treatment in HIV-infected patients**

Among HIV/AIDS patients who drink alcohol, brief interventions are reportedly efficacious for reducing the frequency of alcohol use and the frequency of unprotected sex<sup>[145,146]</sup>. However, patients abusing alcohol might need more intensive treatment. Some authors report that the addition of motivational interviewing<sup>[147]</sup> and problem solving therapy may be necessary to improve ART adherence<sup>[148]</sup>. An intervention called retention through enhanced personal contact has also been tested to improve retention among HIV-positive patients with alcohol use or mental illness<sup>[149]</sup>.



**Table 3** Non-invasive methods for analyzing liver fibrosis in patients with alcohol use disorder, hepatitis C virus infection and hepatitis C virus - human immunodeficiency virus co-infection

Ref.	Setting	Non-invasive method	Method for detecting alcohol consumption	Finding
Lieber <i>et al</i> <sup>[69]</sup>	VA studies (2) of alcoholic liver disease	APRI <sup>1</sup>	Average alcohol intake	Low sensitivity and specificity of APRI in comparison to liver biopsy, especially in subjects with HCV
Chaudhry <i>et al</i> <sup>[169]</sup>	HIV Hopkins clinical cohort	APRI	Past 6-mo hazardous drinking	No effect of alcohol on APRI values in HCV/HIV co-infection
Blackard <i>et al</i> <sup>[170]</sup>	WIHS cohort	FIB-4 <sup>2</sup>	Recent drinking	No association between alcohol intake and FIB-4 values in HCV/HIV co-infection
Muga <i>et al</i> <sup>[171]</sup>	AUD patients admitted for detoxification	FIB-4	Past 6-mo unhealthy drinking	No association between FIB-4 and alcohol use in HCV/HIV co-infection
Fuster <i>et al</i> <sup>[173]</sup>	HIV-live cohort	FIB-4 and APRI	LDH	No association between LDH and liver fibrosis measured with FIB-4 or APRI
Lim <i>et al</i> <sup>[174]</sup>	VACS cohort	FIB-4	AUDIT-C <sup>3</sup>	Advanced liver fibrosis correlated with alcohol use

<sup>1</sup>APRI: AST to platelet ratio index= {[AST/AST upper limit of normal (IU/L)]/platelet count (10<sup>9</sup>/L)} × 100<sup>[64]</sup>; <sup>2</sup>FIB-4 = age × AST (IU/L)/platelet count (10<sup>9</sup>/L) × ALT (IU/L)<sup>1/2</sup><sup>[63]</sup>; <sup>3</sup>AUDIT-C: Alcohol Use Disorders Identification Test<sup>[79]</sup>. HIV: Human immunodeficiency virus; AUD: Alcohol use disorder; APRI: Aminotransferase/platelet ratio index; HCV: Hepatitis C virus; LDH: Lifetime drinking history; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; VA: United States Department of Veteran Affairs; WIHS: Women's Interagency HIV study; VACS: Veterans Aging Cohort study.

Chander *et al*<sup>[150]</sup> recently performed a cross-sectional survey among HIV care providers, and found that although the majority reported that they usually screen for alcohol use, only 10% used a formal screening tool. Moreover, knowledge of pharmacotherapy for AUD was low, and most care providers referred patients to outside resources for treatment<sup>[150]</sup>.

## AUD AND HCV/HIV CO-INFECTION

A proportion of patients with both AUD and HCV infection also have HIV infection. In fact, HCV/HIV co-infection is clinically relevant among individuals with history of injection drug use<sup>[151]</sup>. HIV infection is associated with faster progression of HCV-related liver fibrosis<sup>[152,153]</sup> as well as earlier occurrence of decompensated liver disease<sup>[154,155]</sup>, liver cancer<sup>[156]</sup>, and liver-related death<sup>[157]</sup>. During the interferon era, co-infection with HIV compromised HCV treatment response<sup>[158,159]</sup>. However, interferon-free regimens have greatly increased the efficacy of HCV antiviral treatment among co-infected patients, both in clinical trials<sup>[160]</sup> and in real-life scenarios<sup>[161,162]</sup>. On the other hand, HCV infection is associated with increased risk of ART-related liver toxicity<sup>[163]</sup>, which is even higher with concurrent alcohol use<sup>[164]</sup>. In cases of HCV/HIV co-infection, alcohol use is also associated with poorer treatment adherence<sup>[165]</sup>, and seems to increase HCV RNA levels<sup>[166,167]</sup>.

Until recently, the impact of alcohol use on HCV-related liver disease in HIV-infected patients had not received much attention in the literature. Older studies suggest that alcohol use is associated with biopsy-proven liver fibrosis in cases of co-infection<sup>[152,168]</sup>. However, studies using non-invasive methods have produced mixed results, highlighting the shortcomings of non-invasive methods-including methods relying on ALT, AST, and platelets-in patients with ALD<sup>[70,69]</sup>. Table 3

summarizes the different studies that have used non-invasive methods to evaluate liver fibrosis in patients with AUD and HCV infection or HCV/HIV co-infection.

A cross-sectional study in an urban HIV/AIDS cohort revealed that heavy alcohol use was associated with advanced liver fibrosis measured using the APRI score<sup>[169]</sup>. However, when the patients were stratified by HCV infection, high APRI score was associated with hazardous alcohol use only among patients without HCV infection<sup>[169]</sup>. Blackard *et al*<sup>[170]</sup> investigated a cohort of women, and demonstrated that alcohol use was not associated with FIB-4 values among HCV/HIV co-infected patients. Within our cohort of AUD patients, FIB-4 was significantly higher among HCV/HIV co-infected patients compared to in HCV monoinfected patients<sup>[171]</sup>. In the HIV-LIVE cohort, lifetime alcohol consumption<sup>[172]</sup> was not associated with the absence of liver fibrosis (FIB-4 < 1.45), and similar results were found for the presence of advanced liver fibrosis (FIB-4 ≥ 3.25) and among patients with HCV infection<sup>[173]</sup>. A study in the VACS cohort-which included a larger number of patients and a different measure of alcohol consumption-reported greater risks of advanced liver fibrosis (measured based on FIB-4) among co-infected patients who exhibited nonhazardous drinking (OR = 14.2, 95%CI: 5.91-34.0) or hazardous/binge drinking (OR = 18.9, 95%CI: 7.98-44.8), or who had alcohol-related diagnoses (OR = 25.2, 95%CI: 10.6-59.7) relative to uninfected individuals who were nonhazardous drinkers<sup>[174]</sup>. The somewhat discordant results among studies may be partly due to differences in the methods used to describe alcohol use and other characteristics of the study population<sup>[169-174]</sup>.

French researchers investigating HCV/HIV co-infected patients recently found that advanced liver fibrosis (measured with transient elastography) was more common among those with an alcohol-related diagnosis (OR = 3.06, 95%CI: 1.42-6.60) compared

to non-hazardous drinkers<sup>[175]</sup>. Elastography may be more reliable than laboratory markers for assessing liver fibrosis in HCV/HIV co-infected patients with AUD. Additionally, the combination of HCV infection and alcohol use is associated with greater mortality within HIV/AIDS cohorts<sup>[79,176]</sup>, highlighting the need to further address alcohol use in co-infection. Although it can be challenging, it is feasible to reduce alcohol use in the setting of HCV/HIV co-infection<sup>[177]</sup>.

## CONCLUSION

To reduce the impact of HCV, HIV and ethanol on liver disease, patients with AUD should be screened for HCV and HIV infection, and interventions should focus on both reducing alcohol consumption and treating viral infections. Moreover, patients with HCV infection or HCV/HIV co-infection should be screened for unhealthy alcohol use to prevent end-stage liver disease. Several treatment interventions are efficacious for reducing alcohol consumption among individuals with HCV infection or HCV/HIV co-infection.

In settings where AUD often coexists with other substance use and viral co-infections, higher levels of co-morbidities are expected. Health care facilities for treatment interventions and multidisciplinary approaches must be widely accessible for managing AUD and associated diseases.

## REFERENCES

- 1 **Saitz R.** Clinical practice. Unhealthy alcohol use. *N Engl J Med* 2005; **352**: 596-607 [PMID: 15703424 DOI: 10.1056/NEJMcP042262]
- 2 **American Psychiatric Association.** Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, USA: American Psychiatric Association Publishing [DOI: 10.1176/appi.books.9780890425596]
- 3 **Friedmann PD.** Clinical practice. Alcohol use in adults. *N Engl J Med* 2013; **368**: 365-373 [PMID: 23343065 DOI: 10.1056/NEJMcP1204714]
- 4 **World Health Organization.** Alcohol in the European Union. Consumption, harm and policy approaches. Copenhagen, Denmark: World Health Organization; 2012. Available from: URL: [http://www.euro.who.int/\\_data/assets/pdf\\_file/0003/160680/e96457.pdf](http://www.euro.who.int/_data/assets/pdf_file/0003/160680/e96457.pdf)
- 5 **Ministerio de Sanidad, Servicios Sociales e Igualdad.** Estudio Edades. Encuesta sobre alcohol y drogas en España. Plan Nacional sobre Drogas; 2013. Available from: URL: <http://www.pnsd.msssi.gob.es/profesionales/sistemasInformacion/sistemaInformacion/pdf/EDADES2013.pdf>
- 6 **Rehm J, Taylor B, Mohapatra S, Irving H, Baliunas D, Patra J, Roerecke M.** Alcohol as a risk factor for liver cirrhosis: a systematic review and meta-analysis. *Drug Alcohol Rev* 2010; **29**: 437-445 [PMID: 20636661 DOI: 10.1111/j.1465-3362.2009.00153.x]
- 7 **Cook RL, Kelso NE, Brumback BA, Chen X.** Analytic strategies to evaluate the association of time-varying exposures to HIV-related outcomes: Alcohol consumption as an example. *Curr HIV Res* 2016; **14**: 85-92 [PMID: 26511345 DOI: 10.2174/1570162X13666151029101919]
- 8 **Fuster D, Tor J, Rey-Joly C, Muga R.** [Pathogenic interactions between alcohol and hepatitis C]. *Med Clin (Barc)* 2012; **138**: 627-632 [PMID: 21696783 DOI: 10.1016/j.medcli.2011.04.019]
- 9 **Stoller EP, Hund AJ, Webster NJ, Bixen CE, Perzynski AT, McCormick RA, Kanuch SW, Dawson NV.** Alcohol consumption within the context of hepatitis C: a qualitative study of non-problematic drinkers. *Alcohol Alcohol* 2006; **41**: 546-552 [PMID: 16855001 DOI: 10.1093/alcalc/agl055]
- 10 **Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ.** The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705-714 [PMID: 16702586 DOI: 10.7326/0003-4819-144-10-200605160-00004]
- 11 **Campbell JV, Hagan H, Latka MH, Garfein RS, Golub ET, Coady MH, Thomas DL, Strathdee SA.** High prevalence of alcohol use among hepatitis C virus antibody positive injection drug users in three US cities. *Drug Alcohol Depend* 2006; **81**: 259-265 [PMID: 16129567 DOI: 10.1016/j.drugalcdep.2005.07.005]
- 12 **Alter MJ.** Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007; **13**: 2436-2441 [PMID: 17552026 DOI: 10.3748/wjg.v13.i17.2436]
- 13 **Novo-Veleiro I, Calle Cde L, Domínguez-Quibén S, Pastor I, Marcos M, Laso FJ.** Prevalence of hepatitis C virus infection in alcoholic patients: cohort study and systematic review. *Alcohol Alcohol* 2013; **48**: 564-569 [PMID: 23690232 DOI: 10.1093/alcalc/agt044]
- 14 **Rivas I, Sanvisens A, Bolao F, Fuster D, Tor J, Pujol R, Torrens M, Rey-Joly C, Muga R.** Impact of medical comorbidity and risk of death in 680 patients with alcohol use disorders. *Alcohol Clin Exp Res* 2013; **37** Suppl 1: E221-E227 [PMID: 23320801 DOI: 10.1111/j.1530-0277.2012.01861.x]
- 15 **Bruix J, Barrera JM, Calvet X, Ercilla G, Costa J, Sanchez-Tapias JM, Ventura M, Vall M, Bruguera M, Bru C.** Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet* 1989; **2**: 1004-1006 [PMID: 2572739 DOI: 10.1016/S0140-6736(89)91015-5]
- 16 **Rosman AS, Paronetto F, Galvin K, Williams RJ, Lieber CS.** Hepatitis C virus antibody in alcoholic patients. Association with the presence of portal and/or lobular hepatitis. *Arch Intern Med* 1993; **153**: 965-969 [PMID: 7683191 DOI: 10.1001/archinte.1993.00410080031005]
- 17 **Rosman AS, Waraich A, Galvin K, Casiano J, Paronetto F, Lieber CS.** Alcoholism is associated with hepatitis C but not hepatitis B in an urban population. *Am J Gastroenterol* 1996; **91**: 498-505 [PMID: 8633498]
- 18 **Bellentani S, Saccoccio G, Costa G, Tiribelli C, Manenti F, Sodde M, Saveria Crocè L, Sasso F, Pozzato G, Cristianini G, Brandi G.** Drinking habits as cofactors of risk for alcohol induced liver damage. The Dionysos Study Group. *Gut* 1997; **41**: 845-850 [PMID: 9462221 DOI: 10.1136/gut.41.6.845]
- 19 **Hahn JA, Samet JH.** Alcohol and HIV disease progression: weighing the evidence. *Curr HIV/AIDS Rep* 2010; **7**: 226-233 [PMID: 20814765 DOI: 10.1007/s11904-010-0060-6]
- 20 **Samet JH, Walley AY, Bridden C.** Illicit drugs, alcohol, and addiction in human immunodeficiency virus. *Panminerva Med* 2007; **49**: 67-77 [PMID: 17625483]
- 21 **Chander G, Josephs J, Fleishman JA, Korthuis PT, Gaist P, Hellinger J, Gebo K.** Alcohol use among HIV-infected persons in care: results of a multi-site survey. *HIV Med* 2008; **9**: 196-202 [PMID: 18366443 DOI: 10.1111/j.1468-1293.2008.00545.x]
- 22 **Rosenthal E, Salmon-Ceron D, Lewden C, Bouteloup V, Pialoux G, Bonnet F, Karmochkine M, May T, François M, Burty C, Jouglé E, Costagliola D, Morlat P, Chêne G, Cacoub P; Mortavic/Mortalité 2005 Study Group.** Liver-related deaths in HIV-infected patients between 1995 and 2005 in the French GERMIVIC Joint Study Group Network (Mortavic 2005 study in collaboration with the Mortalite 2005 survey, ANRS EN19). *HIV Med* 2009; **10**: 282-289 [PMID: 199226410 DOI: 10.1111/j.1468-1293.2008.00686.x]
- 23 **Krupitsky EM, Horton NJ, Williams EC, Lioznov D, Kuznetsova M, Zvartau E, Samet JH.** Alcohol use and HIV risk behaviors among HIV-infected hospitalized patients in St. Petersburg, Russia. *Drug Alcohol Depend* 2005; **79**: 251-256 [PMID: 16002034 DOI: 10.1016/j.drugalcdep.2005.01.015]
- 24 **Conen A, Fehr J, Glass TR, Furrer H, Weber R, Vernazza P, Hirschel B, Cavassini M, Bernasconi E, Bucher HC, Battegay M.** Self-reported alcohol consumption and its association with

- adherence and outcome of antiretroviral therapy in the Swiss HIV Cohort Study. *Antivir Ther* 2009; **14**: 349-357 [PMID: 19474469]
- 25 **Scott-Sheldon LA**, Carey KB, Carey MP, Cain D, Simbayi LC, Kalichman SC. Alcohol use disorder, contexts of alcohol use, and the risk of HIV transmission among South African male patrons of shebeens. *Drug Alcohol Depend* 2014; **140**: 198-204 [PMID: 24854966 DOI: 10.1016/j.drugalcdep.2014.04.022]
  - 26 **Soboka M**, Tesfaye M, Feyissa GT, Hanlon C. Alcohol use disorders and associated factors among people living with HIV who are attending services in south west Ethiopia. *BMC Res Notes* 2014; **7**: 828 [PMID: 25417542 DOI: 10.1186/1756-0500-7-828]
  - 27 **Cook RL**, Zhu F, Belnap BH, Weber K, Cook JA, Vlahov D, Wilson TE, Hessel NA, Plankey M, Howard AA, Cole SR, Sharp GB, Richardson JL, Cohen MH. Longitudinal trends in hazardous alcohol consumption among women with human immunodeficiency virus infection, 1995-2006. *Am J Epidemiol* 2009; **169**: 1025-1032 [PMID: 19270052 DOI: 10.1093/aje/kwp004]
  - 28 **Seronello S**, Montanez J, Presleigh K, Barlow M, Park SB, Choi J. Ethanol and reactive species increase basal sequence heterogeneity of hepatitis C virus and produce variants with reduced susceptibility to antivirals. *PLoS One* 2011; **6**: e27436 [PMID: 22087316 DOI: 10.1371/journal.pone.0027436]
  - 29 **Siu L**, Foont J, Wands JR. Hepatitis C virus and alcohol. *Semin Liver Dis* 2009; **29**: 188-199 [PMID: 19387918 DOI: 10.1055/s-0029-1214374]
  - 30 **Anand BS**, Thornby J. Alcohol has no effect on hepatitis C virus replication: a meta-analysis. *Gut* 2005; **54**: 1468-1472 [PMID: 16162952 DOI: 10.1136/gut.2004.056697]
  - 31 **Thimme R**, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 2001; **194**: 1395-1406 [PMID: 11714747]
  - 32 **Mehta SH**, Cox A, Hoover DR, Wang XH, Mao Q, Ray S, Strathdee SA, Vlahov D, Thomas DL. Protection against persistence of hepatitis C. *Lancet* 2002; **359**: 1478-1483 [PMID: 11988247 DOI: 10.1016/S0140-6736(02)08435-0]
  - 33 **Thimme R**, Bukh J, Spangenberg HC, Wieland S, Pemberton J, Steiger C, Govindarajan S, Purcell RH, Chisari FV. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci USA* 2002; **99**: 15661-15668 [PMID: 12441397 DOI: 10.1073/pnas.202608299]
  - 34 **Dolganic A**, Kodys K, Kopasz A, Marshall C, Mandrekar P, Szabo G. Additive inhibition of dendritic cell allostimulatory capacity by alcohol and hepatitis C is not restored by DC maturation and involves abnormal IL-10 and IL-2 induction. *Alcohol Clin Exp Res* 2003; **27**: 1023-1031 [PMID: 12824825 DOI: 10.1097/01.ALC.0000071745.63433.32]
  - 35 **Piasecki BA**, Lewis JD, Reddy KR, Bellamy SL, Porter SB, Weinrieb RM, Stieritz DD, Chang KM. Influence of alcohol use, race, and viral coinfections on spontaneous HCV clearance in a US veteran population. *Hepatology* 2004; **40**: 892-899 [PMID: 15382122 DOI: 10.1002/hep.20384]
  - 36 **Grebely J**, Grady B, Hajarizadeh B, Page K, Dore GJ. Disease progression during advanced fibrosis: IL28B genotype or HCV RNA levels? *Hepatology* 2014; **59**: 1650-1651 [PMID: 23929769 DOI: 10.1002/hep.26639]
  - 37 **Osna NA**. Hepatitis C virus and ethanol alter antigen presentation in liver cells. *World J Gastroenterol* 2009; **15**: 1201-1208 [PMID: 19291820 DOI: 10.3748/wjg.15.1201]
  - 38 **McCartney EM**, Beard MR. Impact of alcohol on hepatitis C virus replication and interferon signaling. *World J Gastroenterol* 2010; **16**: 1337-1343 [PMID: 20238400 DOI: 10.3748/wjg.16.1337]
  - 39 **Anand BS**, Currie S, Dieperink E, Bini EJ, Shen H, Ho SB, Wright T. Alcohol use and treatment of hepatitis C virus: results of a national multicenter study. *Gastroenterology* 2006; **130**: 1607-1616 [PMID: 16697724 DOI: 10.1053/j.gastro.2006.02.023]
  - 40 **Kountouras J**, Zavos C, Chatzopoulos D. Apoptosis in hepatitis C. *J Viral Hepat* 2003; **10**: 335-342 [PMID: 12969183]
  - 41 **Nakamoto Y**, Kaneko S, Kobayashi K. Increased susceptibility to apoptosis and attenuated Bcl-2 expression in T lymphocytes and monocytes from patients with advanced chronic hepatitis C. *J Leukoc Biol* 2002; **72**: 49-55 [PMID: 12101262]
  - 42 **Szabo G**, Wands JR, Eken A, Osna NA, Weinman SA, Machida K, Joe Wang H. Alcohol and hepatitis C virus--interactions in immune dysfunctions and liver damage. *Alcohol Clin Exp Res* 2010; **34**: 1675-1686 [PMID: 20608905 DOI: 10.1111/j.1530-0277.2010.01255.x]
  - 43 **Perlemuter G**, Sabile A, Letteron P, Vona G, Topilco A, Chrétien Y, Koike K, Pessayre D, Chapman J, Barba G, Bréchot C. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *FASEB J* 2002; **16**: 185-194 [PMID: 11818366 DOI: 10.1096/fj.01-0396com]
  - 44 **Moriya K**, Nakagawa K, Santa T, Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Miyazawa T, Ishibashi K, Horie T, Imai K, Todoroki T, Kimura S, Koike K. Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. *Cancer Res* 2001; **61**: 4365-4370 [PMID: 11389061]
  - 45 **Perlemuter G**, Lettéron P, Carnot F, Zavala F, Pessayre D, Nalpas B, Bréchot C. Alcohol and hepatitis C virus core protein additively increase lipid peroxidation and synergistically trigger hepatic cytokine expression in a transgenic mouse model. *J Hepatol* 2003; **39**: 1020-1027 [PMID: 14642621]
  - 46 **Tsutsumi T**, Suzuki T, Moriya K, Shintani Y, Fujie H, Miyoshi H, Matsuura Y, Koike K, Miyamura T. Hepatitis C virus core protein activates ERK and p38 MAPK in cooperation with ethanol in transgenic mice. *Hepatology* 2003; **38**: 820-828 [PMID: 14512869 DOI: 10.1053/jhep.2003.50399]
  - 47 **Machida K**, Tsukamoto H, Mkrtchyan H, Duan L, Dynnyk A, Liu HM, Asahina K, Govindarajan S, Ray R, Ou JH, Seki E, Deshaies R, Miyake K, Lai MM. Toll-like receptor 4 mediates synergism between alcohol and HCV in hepatic oncogenesis involving stem cell marker Nanog. *Proc Natl Acad Sci USA* 2009; **106**: 1548-1553 [PMID: 19171902 DOI: 10.1073/pnas.0807390106]
  - 48 **Tikhanovich I**, Kuravi S, Campbell RV, Kharbanda KK, Artigues A, Villar MT, Weinman SA. Regulation of FOXO3 by phosphorylation and methylation in hepatitis C virus infection and alcohol exposure. *Hepatology* 2014; **59**: 58-70 [PMID: 23857333]
  - 49 **Castellano-Higuera A**, González-Reimers E, Alemán-Valls MR, Abreu-González P, Santolaria-Fernández F, De La Vega-Prieto MJ, Gómez-Sirvent JL, Peláez-González R. Cytokines and lipid peroxidation in alcoholics with chronic hepatitis C virus infection. *Alcohol Alcohol* 2008; **43**: 137-142 [PMID: 18184121 DOI: 10.1093/alcal/agm171]
  - 50 **Rigamonti C**, Mottaran E, Reale E, Rolla R, Cipriani V, Capelli F, Boldorini R, Vidali M, Sartori M, Albano E. Moderate alcohol consumption increases oxidative stress in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 42-49 [PMID: 18216180 DOI: 10.1053/jhep.2003.50275]
  - 51 **Vidali M**, Occhino G, Ivaldi A, Rigamonti C, Sartori M, Albano E. Combination of oxidative stress and steatosis is a risk factor for fibrosis in alcohol-drinking patients with chronic hepatitis C. *Am J Gastroenterol* 2008; **103**: 147-153 [PMID: 18184121 DOI: 10.1111/j.1572-0241.2007.01596.x]
  - 52 **Hutchinson SJ**, Bird SM, Goldberg DJ. Influence of alcohol on the progression of hepatitis C virus infection: a meta-analysis. *Clin Gastroenterol Hepatol* 2005; **3**: 1150-1159 [PMID: 16271348]
  - 53 **Pace CA**, Samet JH. In the Clinic. Substance Use Disorders. *Ann Intern Med* 2016; **164**: ITC49-ITC64 [PMID: 27043992 DOI: 10.7326/AITC201604050]
  - 54 **Hézode C**, Lonjon I, Roudot-Thoraval F, Pawlotsky JM, Zafrani ES, Dhumeaux D. Impact of moderate alcohol consumption on histological activity and fibrosis in patients with chronic hepatitis C, and specific influence of steatosis: a prospective study. *Aliment Pharmacol Ther* 2003; **17**: 1031-1037 [PMID: 12694085]
  - 55 **Brognez V**, Nyssen-Behets C, Grégoire V, Reyckers H, Lengelé B. Implant osseointegration in the irradiated mandible. A comparative study in dogs with a microradiographic and histologic assessment. *Clin Oral Implants Res* 2002; **13**: 234-242 [PMID: 12010153]



- 56 **Monto A**, Patel K, Bostrom A, Pianko S, Pockros P, McHutchison JG, Wright TL. Risks of a range of alcohol intake on hepatitis C-related fibrosis. *Hepatology* 2004; **39**: 826-834 [PMID: 14999703 DOI: 10.1002/hep.20127]
- 57 **Harris HE**, Ramsay ME, Andrews N, Eldridge KP. Clinical course of hepatitis C virus during the first decade of infection: cohort study. *BMJ* 2002; **324**: 450-453 [PMID: 11859045]
- 58 **Hassan MM**, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, Beasley P, Patt YZ. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. *Hepatology* 2002; **36**: 1206-1213 [PMID: 12395331 DOI: 10.1053/jhep.2002.36780]
- 59 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI24282]
- 60 **Ghany MG**, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374 [PMID: 19330875 DOI: 10.1002/hep.22759]
- 61 **Gebo KA**, Herlong HF, Torbenson MS, Jenckes MW, Chander G, Ghanem KG, El-Kamary SS, Sulkowski M, Bass EB. Role of liver biopsy in management of chronic hepatitis C: a systematic review. *Hepatology* 2002; **36**: S161-S172 [PMID: 12407590 DOI: 10.1053/jhep.2002.36989]
- 62 **Sanvisens A**, Fuster D, Serra I, Tor J, Tural C, Rey-Joly C, Muga R. Estimated liver fibrosis and its impact on all-cause mortality of HCV-monoinfected and HCV/HIV-coinfected drug users. *Curr HIV Res* 2011; **9**: 256-262 [PMID: 21675942]
- 63 **Sterling RK**, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325 [PMID: 16729309 DOI: 10.1002/hep.21178]
- 64 **Wai CT**, Greenston JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]
- 65 **Loko MA**, Castera L, Dabis F, Le Bail B, Winnock M, Coureau G, Bioulac-Sage P, de Ledinghen V, Neau D. Validation and comparison of simple noninvasive indexes for predicting liver fibrosis in HIV-HCV-coinfected patients: ANRS CO3 Aquitaine cohort. *Am J Gastroenterol* 2008; **103**: 1973-1980 [PMID: 18796094 DOI: 10.1111/j.1572-0241.2008.01954.x]
- 66 **Nunes D**, Fleming C, Offner G, O'Brien M, Tumilty S, Fix O, Heeren T, Koziel M, Graham C, Craven DE, Stuver S, Horsburgh CR. HIV infection does not affect the performance of noninvasive markers of fibrosis for the diagnosis of hepatitis C virus-related liver disease. *J Acquir Immune Defic Syndr* 2005; **40**: 538-544 [PMID: 16284529]
- 67 **Sebastiani G**, Halfon P, Castera L, Pol S, Thomas DL, Mangia A, Di Marco V, Pirisi M, Voiculescu M, Guido M, Bourliere M, Noventa F, Alberti A. SAFE biopsy: a validated method for large-scale staging of liver fibrosis in chronic hepatitis C. *Hepatology* 2009; **49**: 1821-1827 [PMID: 19291784 DOI: 10.1002/hep.22859]
- 68 **Vallet-Pichard A**, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**: 32-36 [PMID: 17567829 DOI: 10.1002/hep.21669]
- 69 **Lieber CS**, Weiss DG, Morgan TR, Paronetto F. Aspartate aminotransferase to platelet ratio index in patients with alcoholic liver fibrosis. *Am J Gastroenterol* 2006; **101**: 1500-1508 [PMID: 16863553 DOI: 10.1111/j.1572-0241.2006.00610.x]
- 70 **Pohl A**, Behling C, Oliver D, Kilani M, Monson P, Hassanein T. Serum aminotransferase levels and platelet counts as predictors of degree of fibrosis in chronic hepatitis C virus infection. *Am J Gastroenterol* 2001; **96**: 3142-3146 [PMID: 11721762 DOI: 10.1111/j.1572-0241.2001.05268.x]
- 71 **Mueller S**, Millonig G, Sarovska L, Friedrich S, Reimann FM, Pritsch M, Eisele S, Stickel F, Longerich T, Schirmacher P, Seitz HK. Increased liver stiffness in alcoholic liver disease: differentiating fibrosis from steatohepatitis. *World J Gastroenterol* 2010; **16**: 966-972 [PMID: 20180235 DOI: 10.3748/wjg.16.966]
- 72 **Mueller S**, Englert S, Seitz HK, Badea RI, Erhardt A, Bozaari B, Beaugrand M, Lupşor-Platon M. Inflammation-adapted liver stiffness values for improved fibrosis staging in patients with hepatitis C virus and alcoholic liver disease. *Liver Int* 2015; **35**: 2514-2521 [PMID: 26121926 DOI: 10.1111/liv.12904]
- 73 **O'Shea RS**, Dasarathy S, McCullough AJ. Alcoholic liver disease. *Hepatology* 2010; **51**: 307-328 [PMID: 20034030 DOI: 10.1002/hep.23258]
- 74 **Lucey MR**, Mathurin P, Morgan TR. Alcoholic hepatitis. *N Engl J Med* 2009; **360**: 2758-2769 [PMID: 19553649 DOI: 10.1056/NEJMra0805786]
- 75 **Fuster D**, Sanvisens A, Bolao F, Zuluaga P, Rivas I, Tor J, Muga R. Markers of inflammation and mortality in a cohort of patients with alcohol dependence. *Medicine (Baltimore)* 2015; **94**: e607 [PMID: 25761182 DOI: 10.1097/MD.0000000000000607]
- 76 **Innes HA**, Hutchinson SJ, Barclay S, Cadzow E, Dillon JF, Fraser A, Goldberg DJ, Mills PR, McDonald SA, Morris J, Stanley A, Hayes P. Quantifying the fraction of cirrhosis attributable to alcohol among chronic hepatitis C virus patients: implications for treatment cost-effectiveness. *Hepatology* 2013; **57**: 451-460 [PMID: 22961861 DOI: 10.1002/hep.26051]
- 77 **Tsui JI**, Pletcher MJ, Vittinghoff E, Seal K, Gonzales R. Hepatitis C and hospital outcomes in patients admitted with alcohol-related problems. *J Hepatol* 2006; **44**: 262-266 [PMID: 16226823 DOI: 10.1016/j.jhep.2005.07.027]
- 78 **Singal AK**, Kuo YF, Anand BS. Hepatitis C virus infection in alcoholic hepatitis: prevalence patterns and impact on in-hospital mortality. *Eur J Gastroenterol Hepatol* 2012; **24**: 1178-1184 [PMID: 22735607 DOI: 10.1097/MEG.0b013e328355cce0]
- 79 **Fuster D**, Cheng DM, Quinn EK, Nunes D, Saitz R, Samet JH, Tsui JI. Chronic hepatitis C virus infection is associated with all-cause and liver-related mortality in a cohort of HIV-infected patients with alcohol problems. *Addiction* 2014; **109**: 62-70 [PMID: 24112091 DOI: 10.1111/add.12367]
- 80 **Fuster D**, Sanvisens A, Bolao F, Serra I, Rivas I, Tor J, Muga R. Impact of hepatitis C virus infection on the risk of death of alcohol-dependent patients. *J Viral Hepat* 2015; **22**: 18-24 [PMID: 25131721 DOI: 10.1111/jvh.12290]
- 81 **Grebely J**, Dore GJ. What is killing people with hepatitis C virus infection? *Semin Liver Dis* 2011; **31**: 331-339 [PMID: 22189973 DOI: 10.1055/s-0031-1297922]
- 82 **Sanvisens A**, Bolao F, Jarrin I, Fuster D, Zuluaga P, Tor J, Muga R. Impact of Hepatitis C Virus infection in the liver-related mortality of patients with alcohol use disorder. In: International Liver Congress. 2016 April 13-17; Barcelona, Spain
- 83 **Grebely J**, Haire B, Taylor LE, Macneill P, Litwin AH, Swan T, Byrne J, Levin J, Bruggmann P, Dore GJ. Excluding people who use drugs or alcohol from access to hepatitis C treatments - Is this fair, given the available data? *J Hepatol* 2015; **63**: 779-782 [PMID: 26254264 DOI: 10.1016/j.jhep.2015.06.014]
- 84 **Okazaki T**, Yoshihara H, Suzuki K, Yamada Y, Tsujimura T, Kawano K, Yamada Y, Abe H. Efficacy of interferon therapy in patients with chronic hepatitis C. Comparison between non-drinkers and drinkers. *Scand J Gastroenterol* 1994; **29**: 1039-1043 [PMID: 7871371]
- 85 **Le Lan C**, Guillygomarc'h A, Danielou H, Le Dréau G, Lainé F, Védelhié C, Deugnier Y, Brissot P, Guyader D, Moirand R. A multi-disciplinary approach to treating hepatitis C with interferon and ribavirin in alcohol-dependent patients with ongoing abuse. *J Hepatol* 2012; **56**: 334-340 [PMID: 21756854 DOI: 10.1016/j.jhep.2011.05.021]
- 86 **Bruggmann P**, Dampz M, Gerlach T, Kravec L, Falcato L. Treatment outcome in relation to alcohol consumption during hepatitis C therapy: an analysis of the Swiss Hepatitis C Cohort Study. *Drug Alcohol Depend* 2010; **110**: 167-171 [PMID: 20334985 DOI: 10.1016/j.drugalcdep.2010.02.016]
- 87 **Evon DM**, Simpson K, Kixmiller S, Galanko J, Dougherty K, Golin



- C, Fried MW. A randomized controlled trial of an integrated care intervention to increase eligibility for chronic hepatitis C treatment. *Am J Gastroenterol* 2011; **106**: 1777-1786 [PMID: 21769136 DOI: 10.1038/ajg.2011.219]
- 88 **Afdhal NH**, Zeuzem S, Schooley RT, Thomas DL, Ward JW, Litwin AH, Razavi H, Castera L, Poynard T, Muir A, Mehta SH, Dee L, Graham C, Church DR, Talal AH, Sulkowski MS, Jacobson IM. The new paradigm of hepatitis C therapy: integration of oral therapies into best practices. *J Viral Hepat* 2013; **20**: 745-760 [PMID: 24168254 DOI: 10.1111/jvh.12173]
  - 89 **Lalezari J**, Sullivan JG, Varunok P, Galen E, Kowdley KV, Rustgi V, Aguilar H, Felizarta F, McGovern B, King M, Polepally AR, Cohen DE. Ombitasvir/paritaprevir/r and dasabuvir plus ribavirin in HCV genotype 1-infected patients on methadone or buprenorphine. *J Hepatol* 2015; **63**: 364-369 [PMID: 25839406 DOI: 10.1016/j.jhep.2015.03.029]
  - 90 **Grebely J**, Alavi M, Micallef M, Dunlop AJ, Balcomb AC, Phung N, Weltman MD, Day CA, Treloar C, Bath N, Haber PS, Dore GJ. Treatment for hepatitis C virus infection among people who inject drugs attending opioid substitution treatment and community health clinics: the ETHOS Study. *Addiction* 2016; **111**: 311-319 [PMID: 26451534 DOI: 10.1111/add.13197]
  - 91 **Feld JJ**, Jacobson IM, Hézode C, Asselah T, Ruane PJ, Gruener N, Abergel A, Mangia A, Lai CL, Chan HL, Mazzotta F, Moreno C, Yoshida E, Shafraun SD, Townner WJ, Tran TT, McNally J, Osinusi A, Svarovskaia E, Zhu Y, Brainard DM, McHutchison JG, Agarwal K, Zeuzem S. Sofosbuvir and Velpatasvir for HCV Genotype 1, 2, 4, 5, and 6 Infection. *N Engl J Med* 2015; **373**: 2599-2607 [PMID: 26571066 DOI: 10.1056/NEJMoa1512610]
  - 92 **Charlton M**, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS, Fried MW, Terrault NA, O'Leary JG, Vargas HE, Kuo A, Schiff E, Sulkowski MS, Gilroy R, Watt KD, Brown K, Kwo P, Pungpapong S, Korenblat KM, Muir AJ, Teperman L, Fontana RJ, Denning J, Arterburn S, Dvory-Sobol H, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal N. Ledipasvir and Sofosbuvir Plus Ribavirin for Treatment of HCV Infection in Patients With Advanced Liver Disease. *Gastroenterology* 2015; **149**: 649-659 [PMID: 25985734 DOI: 10.1053/j.gastro.2015.05.010]
  - 93 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]
  - 94 **American Association for the Study of Liver Diseases-Infectious Diseases Society of America (AASLD-IDS)**. HCV Guidance: Recommendations for testing, managing, and treating hepatitis C. 2016. Available from: URL: <http://www.hcvguidelines.org>
  - 95 **North CS**, Sims O, Hong BA, Jain MK, Brown G, Lisker-Melman M, Pollio DE. An empirical study of alcohol consumption by patients considering HCV treatment. *Am J Drug Alcohol Abuse* 2014; **40**: 484-489 [PMID: 25140981 DOI: 10.3109/00952990.2014.945592]
  - 96 **Mitruka K**, Thornton K, Cusick S, Orme C, Moore A, Manch RA, Box T, Carroll C, Holtzman D, Ward JW. Expanding primary care capacity to treat hepatitis C virus infection through an evidence-based care model--Arizona and Utah, 2012-2014. *MMWR Morb Mortal Wkly Rep* 2014; **63**: 393-398 [PMID: 24807237]
  - 97 **Muga R**, Zuluaga P, Sanvisens A, Rivas I, Fuster D, Bolao F, Tor J. Hepatitis C associated to substance abuse: ever closer to a treatment without Interferon. *Adicciones* 2015; **27**: 141-149 [PMID: 26132303 DOI: 10.20882/adicciones.698]
  - 98 **Saitz R**. Treatment of alcohol and other drug dependence. *Liver Transpl* 2007; **13**: S59-S64 [PMID: 17969089 DOI: 10.1002/lt.21339]
  - 99 **Dieperink E**, Ho SB, Heit S, Durfee JM, Thuras P, Willenbring ML. Significant reductions in drinking following brief alcohol treatment provided in a hepatitis C clinic. *Psychosomatics* 2010; **51**: 149-156 [PMID: 20332290 DOI: 10.1176/appi.psy.51.2.149]
  - 100 **Dieperink E**, Fuller B, Isenhardt C, McMaken K, Lenox R, Pocha C, Thuras P, Hauser P. Efficacy of motivational enhancement therapy on alcohol use disorders in patients with chronic hepatitis C: a randomized controlled trial. *Addiction* 2014; **109**: 1869-1877 [PMID: 25040898 DOI: 10.1111/add.12679]
  - 101 **Proeschold-Bell RJ**, Patkar AA, Naggie S, Coward L, Mannelli P, Yao J, Bixby P, Muir AJ. An integrated alcohol abuse and medical treatment model for patients with hepatitis C. *Dig Dis Sci* 2012; **57**: 1083-1091 [PMID: 22134784 DOI: 10.1007/s10620-011-1976-4]
  - 102 **Addolorato G**, Leggio L, Agabio R, Colombo G, Gasbarrini G. Baclofen: a new drug for the treatment of alcohol dependence. *Int J Clin Pract* 2006; **60**: 1003-1008 [PMID: 16893442 DOI: 10.1111/j.1742-1241.2006.01065.x]
  - 103 **Bagby GJ**, Amedee AM, Siggins RW, Molina PE, Nelson S, Veazey RS. Alcohol and HIV Effects on the Immune System. *Alcohol Res* 2015; **37**: 287-297 [PMID: 26695751]
  - 104 **Sandler NG**, Douek DC. Microbial translocation in HIV infection: causes, consequences and treatment opportunities. *Nat Rev Microbiol* 2012; **10**: 655-666 [PMID: 22886237 DOI: 10.1038/nrmicro2848]
  - 105 **Douek D**. HIV disease progression: immune activation, microbes, and a leaky gut. *Top HIV Med* 2007; **15**: 114-117 [PMID: 17720995]
  - 106 **Deeks SG**, Kitchen CM, Liu L, Guo H, Gascon R, Narváez AB, Hunt P, Martin JN, Kahn JO, Levy J, McGrath MS, Hecht FM. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. *Blood* 2004; **104**: 942-947 [PMID: 15117761 DOI: 10.1182/blood-2003-09-3333]
  - 107 **Klatt NR**, Harris LD, Vinton CL, Sung H, Briant JA, Tabb B, Morcock D, McGinty JW, Lifson JD, Lafont BA, Martin MA, Levine AD, Estes JD, Brechley JM. Compromised gastrointestinal integrity in pigtail macaques is associated with increased microbial translocation, immune activation, and IL-17 production in the absence of SIV infection. *Mucosal Immunol* 2010; **3**: 387-398 [PMID: 20357762 DOI: 10.1038/mi.2010.14]
  - 108 **Hunt PW**, Sinclair E, Rodriguez B, Shive C, Clagett B, Funderburg N, Robinson J, Huang Y, Epling L, Martin JN, Deeks SG, Meinert CL, Van Natta ML, Jabs DA, Lederman MM. Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. *J Infect Dis* 2014; **210**: 1228-1238 [PMID: 24755434 DOI: 10.1093/infdis/jiu238]
  - 109 **McCance-Katz EF**, Lum PJ, Beatty G, Gruber VA, Peters M, Rainey PM. Untreated HIV infection is associated with higher blood alcohol levels. *J Acquir Immune Defic Syndr* 2012; **60**: 282-288 [PMID: 22495786 DOI: 10.1097/QAI.0b013e318256625f]
  - 110 **Kaslow RA**, Blackwelder WC, Ostrow DG, Yerg D, Palenicek J, Coulson AH, Valdiserri RO. No evidence for a role of alcohol or other psychoactive drugs in accelerating immunodeficiency in HIV-1-positive individuals. A report from the Multicenter AIDS Cohort Study. *JAMA* 1989; **261**: 3424-3429 [PMID: 2524608]
  - 111 **Coates RA**, Farewell VT, Raboud J, Read SE, MacFadden DK, Calzavara LM, Johnson JK, Shepherd FA, Fanning MM. Cofactors of progression to acquired immunodeficiency syndrome in a cohort of male sexual contacts of men with human immunodeficiency virus disease. *Am J Epidemiol* 1990; **132**: 717-722 [PMID: 2403112]
  - 112 **Lucas GM**, Gebo KA, Chaisson RE, Moore RD. Longitudinal assessment of the effects of drug and alcohol abuse on HIV-1 treatment outcomes in an urban clinic. *AIDS* 2002; **16**: 767-774 [PMID: 11964533]
  - 113 **Samet JH**, Horton NJ, Traphagen ET, Lyon SM, Freedberg KA. Alcohol consumption and HIV disease progression: are they related? *Alcohol Clin Exp Res* 2003; **27**: 862-867 [PMID: 12766632 DOI: 10.1097/01.ALC.0000065438.80967.56]
  - 114 **Samet JH**, Cheng DM, Libman H, Nunes DP, Alperen JK, Saitz R. Alcohol consumption and HIV disease progression. *J Acquir Immune Defic Syndr* 2007; **46**: 194-199 [PMID: 17667330 DOI: 10.1097/QAI.0b013e318142aabb]
  - 115 **Chander G**, Lau B, Moore RD. Hazardous alcohol use: a risk factor for non-adherence and lack of suppression in HIV infection. *J Acquir Immune Defic Syndr* 2006; **43**: 411-417 [PMID: 17099312]

- DOI: 10.1097/01.qai.0000243121.44659.a4]
- 116 **Wu ES**, Metzger DS, Lynch KG, Douglas SD. Association between alcohol use and HIV viral load. *J Acquir Immune Defic Syndr* 2011; **56**: e129-e130 [PMID: 21532918 DOI: 10.1097/QAI.0b013e31820dc1c8]
  - 117 **Baum MK**, Rafie C, Lai S, Sales S, Page JB, Campa A. Alcohol use accelerates HIV disease progression. *AIDS Res Hum Retroviruses* 2010; **26**: 511-518 [PMID: 20455765 DOI: 10.1089/aid.2009.0211]
  - 118 **Kowalski S**, Colantuoni E, Lau B, Keruly J, McCaul ME, Hutton HE, Moore RD, Chander G. Alcohol consumption and CD4 T-cell count response among persons initiating antiretroviral therapy. *J Acquir Immune Defic Syndr* 2012; **61**: 455-461 [PMID: 22955054 DOI: 10.1097/QAI.0b013e3182712d39]
  - 119 **Conen A**, Wang Q, Glass TR, Fux CA, Thurnheer MC, Orasch C, Calmy A, Bernasconi E, Vernazza P, Weber R, Bucher HC, Battegay M, Fehr J. Association of alcohol consumption and HIV surrogate markers in participants of the swiss HIV cohort study. *J Acquir Immune Defic Syndr* 2013; **64**: 472-478 [PMID: 23892243 DOI: 10.1097/QAI.0b013e3182a61ea9]
  - 120 **Carrieri MP**, Protopopescu C, Raffi F, March L, Reboud P, Spire B, Leport C. Low alcohol consumption as a predictor of higher CD4+ cell count in HIV-treated patients: a french paradox or a proxy of healthy behaviors? The ANRS APROCO-COPILOTE CO-08 cohort. *J Acquir Immune Defic Syndr* 2014; **65**: e148-e150 [PMID: 24346641 DOI: 10.1097/QAI.0000000000000087]
  - 121 **Naimi TS**, Babor T, Chikritzhs T, Stockwell TR, McCambridge J, Miller P, Xuan Z, Bradley K, Blanchette JG, Kypri K, Saitz R. Let's Not "Relax" Evidence Standards when Recommending Risky Preventive Therapeutic Agents. *Alcohol Clin Exp Res* 2015; **39**: 1275-1276 [PMID: 25912415 DOI: 10.1111/acer.12724]
  - 122 **Samet JH**, Pace CA, Cheng DM, Coleman S, Bridden C, Paredesi M, Saggurti N, Raj A. Alcohol use and sex risk behaviors among HIV-infected female sex workers (FSWs) and HIV-infected male clients of FSWs in India. *AIDS Behav* 2010; **14**: S74-S83 [PMID: 20544381 DOI: 10.1007/s10461-010-9723-y]
  - 123 **Chaudhry AA**, Botsko M, Weiss L, Egan JE, Mitty J, Estrada B, Lucas GM, Woodson T, Flanigan TP, Fiellin DA. Participant characteristics and HIV risk behaviors among individuals entering integrated buprenorphine/naloxone and HIV care. *J Acquir Immune Defic Syndr* 2011; **56** Suppl 1: S14-S21 [PMID: 21317589 DOI: 10.1097/QAI.0b013e318209d3b9]
  - 124 **Hasse B**, Ledergerber B, Hirschel B, Vernazza P, Glass TR, Jeannin A, Evison JM, Elzi L, Cavassini M, Bernasconi E, Nicca D, Weber R. Frequency and determinants of unprotected sex among HIV-infected persons: the Swiss HIV cohort study. *Clin Infect Dis* 2010; **51**: 1314-1322 [PMID: 21034200 DOI: 10.1086/656809]
  - 125 **Sullivan LE**, Saitz R, Cheng DM, Libman H, Nunes D, Samet JH. The impact of alcohol use on depressive symptoms in human immunodeficiency virus-infected patients. *Addiction* 2008; **103**: 1461-1467 [PMID: 18637000 DOI: 10.1111/j.1360-0443.2008.02245.x]
  - 126 **Goodness TM**, Palfai TP, Cheng DM, Coleman SM, Bridden C, Blokhina E, Krupitsky E, Samet JH. Depressive symptoms and antiretroviral therapy (ART) initiation among HIV-infected Russian drinkers. *AIDS Behav* 2014; **18**: 1085-1093 [PMID: 24337725 DOI: 10.1007/s10461-013-0674-y]
  - 127 **Gonzalez JS**, Batchelder AW, Psaros C, Safren SA. Depression and HIV/AIDS treatment nonadherence: a review and meta-analysis. *J Acquir Immune Defic Syndr* 2011; **58**: 181-187 [PMID: 21857529 DOI: 10.1097/QAI.0b013e31822d490a]
  - 128 **Kim TW**, Palepu A, Cheng DM, Libman H, Saitz R, Samet JH. Factors associated with discontinuation of antiretroviral therapy in HIV-infected patients with alcohol problems. *AIDS Care* 2007; **19**: 1039-1047 [PMID: 17852002 DOI: 10.1080/09540120701294245]
  - 129 **Conaty TP**. More about March editorial. *J Am Dent Assoc* 2008; **139**: 659-660 [PMID: 18519980 DOI: 10.1097/PSY.0b013e3181777a5f]
  - 130 **Ghebremichael M**, Paintsil E, Ickovics JR, Vlahov D, Schuman P, Boland R, Schoenbaum E, Moore J, Zhang H. Longitudinal association of alcohol use with HIV disease progression and psychological health of women with HIV. *AIDS Care* 2009; **21**: 834-841 [PMID: 20024739 DOI: 10.1080/09540120802537864]
  - 131 **Westergaard RP**, Hess T, Astemborski J, Mehta SH, Kirk GD. Longitudinal changes in engagement in care and viral suppression for HIV-infected injection drug users. *AIDS* 2013; **27**: 2559-2566 [PMID: 23770493 DOI: 10.1097/QAD.0b013e328363b6f2]
  - 132 **Salmon-Ceron D**, Lewden C, Morlat P, Bévilacqua S, Jouglu E, Bonnet F, Héripert L, Costagliola D, May T, Chêne G. Liver disease as a major cause of death among HIV infected patients: role of hepatitis C and B viruses and alcohol. *J Hepatol* 2005; **42**: 799-805 [PMID: 15973779]
  - 133 **Joshi D**, O'Grady J, Dieterich D, Gazzard B, Agarwal K. Increasing burden of liver disease in patients with HIV infection. *Lancet* 2011; **377**: 1198-1209 [PMID: 21459211 DOI: 10.1016/S0140-6736(10)62001-6]
  - 134 **Kelso NE**, Sheps DS, Cook RL. The association between alcohol use and cardiovascular disease among people living with HIV: a systematic review. *Am J Drug Alcohol Abuse* 2015; **41**: 479-488 [PMID: 26286352 DOI: 10.3109/00952990.2015.1058812]
  - 135 **Depp TB**, McGinnis KA, Kraemer K, Akgün KM, Edelman EJ, Fiellin DA, Butt AA, Crystal S, Gordon AJ, Freiberg M, Gibert CL, Rimland D, Bryant KJ, Crothers K. Risk factors associated with acute exacerbation of chronic obstructive pulmonary disease in HIV-infected and uninfected patients. *AIDS* 2016; **30**: 455-463 [PMID: 26765938 DOI: 10.1097/QAD.0000000000000940]
  - 136 **DeLorenze GN**, Weisner C, Tsai AL, Satre DD, Quesenberry CP. Excess mortality among HIV-infected patients diagnosed with substance use dependence or abuse receiving care in a fully integrated medical care program. *Alcohol Clin Exp Res* 2011; **35**: 203-210 [PMID: 21058961 DOI: 10.1111/j.1530-0277.2010.01335.x]
  - 137 **Walley AY**, Cheng DM, Libman H, Nunes D, Horsburgh CR, Saitz R, Samet JH. Recent drug use, homelessness and increased short-term mortality in HIV-infected persons with alcohol problems. *AIDS* 2008; **22**: 415-420 [PMID: 18195568 DOI: 10.1097/QAD.0b013e3282f423f8]
  - 138 **Fuster D**, Cheng DM, Quinn EK, Armah KA, Saitz R, Freiberg MS, Samet JH, Tsui JI. Inflammatory cytokines and mortality in a cohort of HIV-infected adults with alcohol problems. *AIDS* 2014; **28**: 1059-1064 [PMID: 24401638 DOI: 10.1097/QAD.0000000000000184]
  - 139 **Braithwaite RS**, Conigliaro J, Roberts MS, Shechter S, Schaefer A, McGinnis K, Rodriguez MC, Rabeneck L, Bryant K, Justice AC. Estimating the impact of alcohol consumption on survival for HIV+ individuals. *AIDS Care* 2007; **19**: 459-466 [PMID: 17453583 DOI: 10.1080/09540120601095734]
  - 140 **Justice AC**, McGinnis KA, Tate JP, Braithwaite RS, Bryant KJ, Cook RL, Edelman EJ, Fiellin LE, Freiberg MS, Gordon AJ, Kraemer KL, Marshall BD, Williams EC, Fiellin DA. Risk of mortality and physiologic injury evident with lower alcohol exposure among HIV infected compared with uninfected men. *Drug Alcohol Depend* 2016; **161**: 95-103 [PMID: 26861883 DOI: 10.1016/j.drugalcdep.2016.01.017]
  - 141 **Korthuis PT**, Fiellin DA, McGinnis KA, Skanderson M, Justice AC, Gordon AJ, Doebler BA, Asch SM, Fiellin LE, Bryant K, Gibert CL, Crystal S, Goetz MB, Rimland D, Rodriguez-Barradas MC, Kraemer KL. Unhealthy alcohol and illicit drug use are associated with decreased quality of HIV care. *J Acquir Immune Defic Syndr* 2012; **61**: 171-178 [PMID: 22820808 DOI: 10.1097/QAI.0b013e31826741aa]
  - 142 **Nagasawa M**, Kanbayashi S, Mogi K, Serpell JA, Kikusui T. Comparison of behavioral characteristics of dogs in the United States and Japan. *J Vet Med Sci* 2016; **78**: 231-238 [PMID: 26412048 DOI: 10.1007/s11904-015-0285-5]
  - 143 **Hendershot CS**, Stoner SA, Pantalone DW, Simoni JM. Alcohol use and antiretroviral adherence: review and meta-analysis. *J Acquir Immune Defic Syndr* 2009; **52**: 180-202 [PMID: 19668086 DOI: 10.1097/QAI.0b013e3181b18b6e]
  - 144 **Braithwaite RS**, McGinnis KA, Conigliaro J, Maisto SA, Crystal S, Day N, Cook RL, Gordon A, Bridges MW, Seiler JF, Justice AC. A temporal and dose-response association between alcohol

- consumption and medication adherence among veterans in care. *Alcohol Clin Exp Res* 2005; **29**: 1190-1197 [PMID: 16046874]
- 145 **Brown JL**, DeMartini KS, Sales JM, Swartzendruber AL, DiClemente RJ. Interventions to reduce alcohol use among HIV-infected individuals: a review and critique of the literature. *Curr HIV/AIDS Rep* 2013; **10**: 356-370 [PMID: 23990322 DOI: 10.1007/s11904-013-0174-8]
  - 146 **Chander G**, Hutton HE, Lau B, Xu X, McCaul ME. Brief Intervention Decreases Drinking Frequency in HIV-Infected, Heavy Drinking Women: Results of a Randomized Controlled Trial. *J Acquir Immune Defic Syndr* 2015; **70**: 137-145 [PMID: 25967270 DOI: 10.1097/QAI.0000000000000679]
  - 147 **Hasin DS**, Aharonovich E, O'Leary A, Greenstein E, Pavlicova M, Arunajadai S, Waxman R, Wainberg M, Helzer J, Johnston B. Reducing heavy drinking in HIV primary care: a randomized trial of brief intervention, with and without technological enhancement. *Addiction* 2013; **108**: 1230-1240 [PMID: 23432593 DOI: 10.1111/add.12127]
  - 148 **Parry CD**, Morojele NK, Myers BJ, Kekwaletswe CT, Manda SO, Sorsdahl K, Ramjee G, Hahn JA, Rehm J, Shuper PA. Efficacy of an alcohol-focused intervention for improving adherence to antiretroviral therapy (ART) and HIV treatment outcomes - a randomised controlled trial protocol. *BMC Infect Dis* 2014; **14**: 500 [PMID: 25212696 DOI: 10.1186/1471-2334-14-500]
  - 149 **Gardner LI**, Marks G, Shahani L, Giordano TP, Wilson TE, Drainoni ML, Keruly JC, Batey DS, Metsch LR. Assessing efficacy of a retention-in-care intervention among HIV patients with depression, anxiety, heavy alcohol consumption and illicit drug use. *AIDS* 2016; **30**: 1111-1119 [PMID: 26760454 DOI: 10.1097/QAD.0000000000001019]
  - 150 **Chander G**, Monroe AK, Crane HM, Hutton HE, Saag MS, Cropsey K, Eron JJ, Quinlivan EB, Geng E, Mathews WC, Boswell S, Rodriguez B, Ellison M, Kitahata MM, Moore RD, McCaul ME. HIV primary care providers--Screening, knowledge, attitudes and behaviors related to alcohol interventions. *Drug Alcohol Depend* 2016; **161**: 59-66 [PMID: 26857898 DOI: 10.1016/j.drugalcdep.2016.01.015]
  - 151 **Sulkowski MS**, Thomas DL. Hepatitis C in the HIV-infected patient. *Clin Liver Dis* 2003; **7**: 179-194 [PMID: 12691466]
  - 152 **Benhamou Y**, Bochet M, Di Martino V, Charlotte F, Azria F, Coutellier A, Vidaud M, Bricaire F, Opolon P, Katlama C, Poynard T. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfecting patients. The Multivirc Group. *Hepatology* 1999; **30**: 1054-1058 [PMID: 10498659 DOI: 10.1002/hep.510300409]
  - 153 **Sulkowski MS**, Mehta SH, Torbenson MS, Higgins Y, Brinkley SC, de Oca RM, Moore RD, Afdhal NH, Thomas DL. Rapid fibrosis progression among HIV/hepatitis C virus-co-infected adults. *AIDS* 2007; **21**: 2209-2216 [PMID: 18090048 DOI: 10.1097/QAD.0b013e3282f10de9]
  - 154 **Pineda JA**, García-García JA, Aguilar-Guisado M, Ríos-Villegas MJ, Ruiz-Morales J, Rivero A, del Valle J, Luque R, Rodríguez-Baño J, González-Serrano M, Camacho A, Macías J, Grilo I, Gómez-Mateos JM. Clinical progression of hepatitis C virus-related chronic liver disease in human immunodeficiency virus-infected patients undergoing highly active antiretroviral therapy. *Hepatology* 2007; **46**: 622-630 [PMID: 17659577 DOI: 10.1002/hep.21757]
  - 155 **Lo Re V**, Kallan MJ, Tate JP, Localio AR, Lim JK, Goetz MB, Klein MB, Rimland D, Rodriguez-Barradas MC, Butt AA, Gibert CL, Brown ST, Park L, Dubrow R, Reddy KR, Kostman JR, Strom BL, Justice AC. Hepatic decompensation in antiretroviral-treated patients co-infected with HIV and hepatitis C virus compared with hepatitis C virus-monoinfected patients: a cohort study. *Ann Intern Med* 2014; **160**: 369-379 [PMID: 24723077 DOI: 10.7326/M13-1829]
  - 156 **Limketkai BN**, Mehta SH, Sutcliffe CG, Higgins YM, Torbenson MS, Brinkley SC, Moore RD, Thomas DL, Sulkowski MS. Relationship of liver disease stage and antiviral therapy with liver-related events and death in adults coinfecting with HIV/HCV. *JAMA* 2012; **308**: 370-378 [PMID: 22820790 DOI: 10.1001/jama.2012.7844]
  - 157 **Gray JW**, Carrano AV, Moore DH, Steinmetz LL, Minkler J, Mayall BH, Mendelsohn ML, Van Dilla MA. High-speed quantitative karyotyping by flow microfluorometry. *Clin Chem* 1975; **21**: 1258-1262 [PMID: 1170959 DOI: 10.1086/318501]
  - 158 **Soriano V**, Puoti M, Sulkowski M, Cargnel A, Benhamou Y, Peters M, Mauss S, Bräu N, Hatzakis A, Pol S, Rockstroh J. Care of patients coinfecting with HIV and hepatitis C virus: 2007 updated recommendations from the HCV-HIV International Panel. *AIDS* 2007; **21**: 1073-1089 [PMID: 17502718 DOI: 10.1097/QAD.0b013e3281084e4d]
  - 159 **Nunes D**, Saitz R, Libman H, Cheng DM, Vidaver J, Samet JH. Barriers to treatment of hepatitis C in HIV/HCV-coinfecting adults with alcohol problems. *Alcohol Clin Exp Res* 2006; **30**: 1520-1526 [PMID: 16930214 DOI: 10.1111/j.1530-0277.2006.00183.x]
  - 160 **Sulkowski MS**, Eron JJ, Wyles D, Trinh R, Lalezari J, Wang C, Slim J, Bhatti L, Gathe J, Ruane PJ, Elion R, Bredeek F, Brennan R, Blick G, Khatri A, Gibbons K, Hu YB, Fredrick L, Schnell G, Pilot-Matias T, Tripathi R, Da Silva-Tillmann B, McGovern B, Campbell AL, Podsadecki T. Ombitasvir, paritaprevir co-dosed with ritonavir, dasabuvir, and ribavirin for hepatitis C in patients co-infected with HIV-1: a randomized trial. *JAMA* 2015; **313**: 1223-1231 [PMID: 25706092 DOI: 10.1001/jama.2015.1328]
  - 161 **Christensen S**, Mauss S, Hueppe D, Lutz T, Schewe K, Rockstroh JK, Baumgarten A, Simon KG, Busch H, Ingiliz P. Directly acting agents against HCV- Results from the German Hepatitis C cohort (GECCO). In: Conference on Retroviruses and Opportunistic Infections (CROI). 2016 Feb 22-25; Boston, USA
  - 162 **Piroth L**, Wittkop L, Lacombe K, Rosenthal E, Gilbert C, Carrieri P, Dabis F, Sogni P, Dominique Salmon-Ceron for the the ANRS CO13 HEPACVIH Study Group. Response to DAA-based regimens in HIV-HCV co-infected patients in real-life, France. In: Conference on Retroviruses and Opportunistic Infections (CROI). 2016 Feb 22-25; Boston, USA
  - 163 **Sulkowski MS**, Mehta SH, Chaisson RE, Thomas DL, Moore RD. Hepatotoxicity associated with protease inhibitor-based antiretroviral regimens with or without concurrent ritonavir. *AIDS* 2004; **18**: 2277-2284 [PMID: 15577540]
  - 164 **Kottliil S**, Polis MA, Kovacs JA. HIV Infection, hepatitis C infection, and HAART: hard clinical choices. *JAMA* 2004; **292**: 243-250 [PMID: 15249574 DOI: 10.1001/jama.292.2.243]
  - 165 **Marcellin F**, Lions C, Winnock M, Salmon D, Durant J, Spire B, Mora M, Loko MA, Dabis F, Dominguez S, Roux P, Carrieri MP. Self-reported alcohol abuse in HIV-HCV co-infected patients: a better predictor of HIV virological rebound than physician's perceptions (HEPAVIH ARNS CO13 cohort). *Addiction* 2013; **108**: 1250-1258 [PMID: 23421419 DOI: 10.1111/add.12149]
  - 166 **Cooper CL**, Cameron DW. Effect of alcohol use and highly active antiretroviral therapy on plasma levels of hepatitis C virus (HCV) in patients coinfecting with HIV and HCV. *Clin Infect Dis* 2005; **41** Suppl 1: S105-S109 [PMID: 16265607 DOI: 10.1086/429506]
  - 167 **Fishbein DA**, Lo Y, Netski D, Thomas DL, Klein RS. Predictors of hepatitis C virus RNA levels in a prospective cohort study of drug users. *J Acquir Immune Defic Syndr* 2006; **41**: 471-476 [PMID: 16652056 DOI: 10.1097/01.qai.0000218360.28712.f3]
  - 168 **Tural C**, Fuster D, Tor J, Ojanguren I, Sirera G, Ballesteros A, Lasanta JA, Planas R, Rey-Joly C, Clotet B. Time on antiretroviral therapy is a protective factor for liver fibrosis in HIV and hepatitis C virus (HCV) co-infected patients. *J Viral Hepat* 2003; **10**: 118-125 [PMID: 12614468]
  - 169 **Chaudhry AA**, Sulkowski MS, Chander G, Moore RD. Hazardous drinking is associated with an elevated aspartate aminotransferase to platelet ratio index in an urban HIV-infected clinical cohort. *HIV Med* 2009; **10**: 133-142 [PMID: 19207596 DOI: 10.1111/j.1468-1293.2008.00662.x]
  - 170 **Blackard JT**, Welge JA, Taylor LE, Mayer KH, Klein RS, Celentano DD, Jamieson DJ, Gardner L, Sherman KE. HIV mono-infection is associated with FIB-4 - A noninvasive index of liver fibrosis - in women. *Clin Infect Dis* 2011; **52**: 674-680 [PMID: 21468129 DOI: 10.1093/cid/cir214]

- 21248367 DOI: 10.1093/cid/ciq199]
- 171 **Muga R**, Sanvisens A, Fuster D, Tor J, Martínez E, Pérez-Hoyos S, Muñoz A. Unhealthy alcohol use, HIV infection and risk of liver fibrosis in drug users with hepatitis C. *PLoS One* 2012; **7**: e46810 [PMID: 23056462 DOI: 10.1371/journal.pone.0046810]
  - 172 **Skinner HA**, Sheu WJ. Reliability of alcohol use indices. The Lifetime Drinking History and the MAST. *J Stud Alcohol* 1982; **43**: 1157-1170 [PMID: 7182675]
  - 173 **Fuster D**, Tsui JI, Cheng DM, Quinn EK, Bridden C, Nunes D, Libman H, Saitz R, Samet JH. Impact of lifetime alcohol use on liver fibrosis in a population of HIV-infected patients with and without hepatitis C coinfection. *Alcohol Clin Exp Res* 2013; **37**: 1527-1535 [PMID: 23647488]
  - 174 **Lim JK**, Tate JP, Fultz SL, Goulet JL, Conigliaro J, Bryant KJ, Gordon AJ, Gibert C, Rimland D, Goetz MB, Klein MB, Fiellin DA, Justice AC, Lo Re V. Relationship between alcohol use categories and noninvasive markers of advanced hepatic fibrosis in HIV-infected, chronic hepatitis C virus-infected, and uninfected patients. *Clin Infect Dis* 2014; **58**: 1449-1458 [PMID: 24569533 DOI: 10.1093/cid/ciu097]
  - 175 **Marcellin F**, Roux P, Loko MA, Lions C, Caumont-Prim A, Dabis F, Salmon-Ceron D, Spire B, Carrieri MP. High levels of alcohol consumption increase the risk of advanced hepatic fibrosis in HIV/hepatitis C virus-coinfected patients: a sex-based analysis using transient elastography at enrollment in the HEPAVIH ANRS CO13 cohort. *Clin Infect Dis* 2014; **59**: 1190-1192 [PMID: 25015913 DOI: 10.1093/cid/ciu525]
  - 176 **Obel N**, Omland LH, Kronborg G, Larsen CS, Pedersen C, Pedersen G, Sørensen HT, Gerstoft J. Impact of non-HIV and HIV risk factors on survival in HIV-infected patients on HAART: a population-based nationwide cohort study. *PLoS One* 2011; **6**: e22698 [PMID: 21799935 DOI: 10.1371/journal.pone.0022698]
  - 177 **Tsui JI**, Saitz R, Cheng DM, Nunes D, Libman H, Alperen JK, Samet JH. Awareness of hepatitis C diagnosis is associated with less alcohol use among persons co-infected with HIV. *J Gen Intern Med* 2007; **22**: 822-825 [PMID: 17503108 DOI: 10.1007/s11606-007-0147-y]
  - 178 **National Institute on Alcohol Abuse and Alcoholism**. Helping patients who drink too much: a clinician's guide. 2007 ed. Bethesda, MD; 2007
  - 179 **Bush K**, Kivlahan DR, McDonell MB, Fihn SD, Bradley KA. The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking. Ambulatory Care Quality Improvement Project (ACQUIP). Alcohol Use Disorders Identification Test. *Arch Intern Med* 1998; **158**: 1789-1795 [PMID: 9738608]

**P- Reviewer:** Chen YD, Kawasaki H, Liu HF **S- Editor:** Qi Y

**L- Editor:** A **E- Editor:** Li D







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



## Prophylactic liver transplantation for high-risk recurrent hepatocellular carcinoma

Po-Chih Yang, Cheng-Maw Ho, Rey-Heng Hu, Ming-Chih Ho, Yao-Ming Wu, Po-Huang Lee

Po-Chih Yang, Department of Surgery, National Taiwan University Hospital Hsinchu Branch, Hsinchu City 300, Taiwan

Cheng-Maw Ho, Rey-Heng Hu, Ming-Chih Ho, Yao-Ming Wu, Po-Huang Lee, Department of Surgery, National Taiwan University Hospital, Taipei 10002, Taiwan

**Author contributions:** Yang PC performed the majority of the writing, prepared the figures and tables; Ho CM designed the outline and coordinated the writing of the paper; Hu RH and Ho MC performed data accusation and writing; Wu YM provided the input in writing the paper; Lee PH assisted with the design and interpretation of this study.

**Conflict-of-interest statement:** There is no conflict of interest associated with any of the senior authors or other coauthors who contributed to this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Cheng-Maw Ho, MD, PhD, Clinical Assistant Professor, Department of Surgery, National Taiwan University Hospital, 7 Chung-Shan S. Rd., Taipei 10002, Taiwan. [mingho@ntu.edu.tw](mailto:mingho@ntu.edu.tw)  
 Telephone: +886-2-23123456  
 Fax: +886-2-23568810

Received: June 27, 2016

Peer-review started: June 27, 2016

First decision: August 18, 2016

Revised: August 24, 2016

Accepted: September 13, 2016

Article in press: September 18, 2016

Published online: November 8, 2016

### Abstract

Hepatocellular carcinoma (HCC) is the second most common cause of cancer-related death in the world. Radical treatment of HCC in early stages results in a long disease-free period and improved overall survival. The choice of optimal management strategy for HCC mainly depends on the severity of the underlying liver disease. For patients with decompensated liver cirrhosis and HCC within Milan criteria (MC), liver transplant (LT) is the choice of treatment. However, for patients with good residual liver reserve and HCC within MC, selection of other curative treatments such as liver resection (LR) or radiofrequency ablation may be a reasonable alternative. For patients without cirrhosis, LR can result in an overall survival similar to that provided by LT. Therefore, it is an accepted alternative to LT especially in areas with organ shortage. However, the cumulative 5-year recurrence rate of HCC post LR might be as high as 70%. For initial transplant-eligible (within MC) patients with recurrent HCC post LR, salvage liver transplant (SLT) was first proposed in 2000. However, most patients with recurrent HCC considered for SLT are untransplantable cases due to HCC recurrence beyond MC or comorbidity. Thus, the strategy of opting for SLT results in the loss of the opportunity of LT for these patients. Some authors proposed the concept of "de principe liver transplant" (*i.e.*, prophylactic LT before HCC recurrence) to prevent losing the chance of LT for these potential candidates. Factors associated with the failure of SLT will be dissected and discussed in three parts: Patient, tumor, and underlying liver disease. Regarding patient-related factors, the rate of transplantability depends on patient compliance. Patients without regular follow-up tend to develop HCC recurrence beyond MC at the time of tumor detection. Advancing age is another factor related to severe comorbidities when LT is considered for HCC recurrence, and these elderly candidates become ineligible as time goes by. Regarding tumor-related factors, histopathological features of the resected specimen are used mostly for determining the prognosis of early HCC recurrences. Such

prognostic factors include the presence of microvascular invasion, poor tumor differentiation, the presence of microsatellites, the presence of multiple tumors, and the presence of the gene-expressing signature associated with aggressive HCC. These prognostic factors might be used as a selection tool for SLT or prophylactic LT, while remaining mindful of the fact that most of them are also prognostic factors for post-transplant HCC recurrence. Regarding underlying liver disease-related factors, progression of chronic viral hepatitis and high viral load may contribute to the development of late (*de novo*) HCC recurrence as a consequence of sustained inflammatory reaction. However, correlation between the severity of liver fibrosis and tumor recurrence is still controversial. Some prognostic scoring systems that integrate these three factors have been proposed to predict recurrence patterns after LR for HCC. Theoretically, after excluding patients with high risk of post-transplant HCC recurrence, either by observation of a cancer-free period or by measurement of biological factors (such as alpha fetoprotein), prophylactic LT following curative resection of HCC could be considered for selected patients with high risk of recurrence to provide longer survival.

**Key words:** Liver transplant; Hepatocellular carcinoma; Salvage; Risk factor; Resection; Microvascular invasion; Recurrence; Prophylactic

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In this minireview, we discuss about the strategy of prophylactic liver transplant after liver resection for patients with a high risk of recurrence. Prognostic risk factors and scoring systems for recurrence are also analyzed.

Yang PC, Ho CM, Hu RH, Ho MC, Wu YM, Lee PH. Prophylactic liver transplantation for high-risk recurrent hepatocellular carcinoma. *World J Hepatol* 2016; 8(31): 1309-1317 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i31/1309.htm> DOI: <http://dx.doi.org/10.4254/wj.h.v8.i31.1309>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer. It has a high prevalence in Asia and sub-Saharan Africa due to the high incidence of chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in these regions. It is much more common in men than in women. In men, HCC is the second leading cause of cancer-related death in developing countries and worldwide<sup>[1]</sup>.

It is well established that liver transplant (LT) is the treatment of choice for patients with early HCC and decompensated liver disease<sup>[2]</sup>. The most notable criteria for transplant in HCC cases is the Milan criteria (MC)

described by Mazzaferro *et al.*<sup>[3]</sup> in 1996. In selected patients with a single tumor less than 5 cm in diameter, or no more than 3 tumors each 3 cm or less in diameter, LT can offer a > 70% 5-year survival and a < 10% 5-year recurrence rate<sup>[4]</sup>. However, for patients with early HCC and cirrhotic liver with preserved function, the choice between liver resection (LR) and LT has been an issue of debate<sup>[5]</sup>. Donor organ shortage is the major problem with using LT for this group of patients<sup>[6]</sup>. Primary LR can achieve comparable 5-year overall survival rates (> 70%) with proper patient selection and application of advanced surgical techniques over the last decades<sup>[7-10]</sup>. However, the intrahepatic recurrence rate within 5 years of LR in cirrhotic patients is > 70%<sup>[11]</sup>. In the era of organ shortage, Majno *et al.*<sup>[12]</sup> first proposed a treatment strategy that involves performing LR as the first-line treatment for patients with single small HCC and preserved liver function and reserving LT for patients with recurrent HCC within MC. This is the so-called "salvage liver transplant (SLT)" strategy. Most patients with HCC recurrence cannot benefit by this strategy in the real-world clinical setting due to recurrent HCC beyond MC at detection or poor general condition unsuitable for LT. We speculate whether early LT before the development of untransplantable recurrence can save their lives and eradicate the cancer. This concept of prophylactic LT for high-risk recurrent HCC before the development of recurrence is also called "de principe LT"<sup>[13]</sup>. Recently, some authors suggested the use of the histopathological features of the specimen of the resected tumor as the selection tool for LT to improve the outcome of cases with high recurrence rate after LR<sup>[13-16]</sup>. However, most of these histopathological features are also prognostic factors of post-transplant HCC recurrence. This review will discuss the treatment strategy of LT before HCC recurrence (*de principe*) and at recurrence (*salvage*) for initial transplant-eligible patients developing recurrent tumors after LR. Poor prognostic clinicopathological factors associated with early and late HCC recurrence are also reviewed in three parts, "patient", "tumor", and "underlying liver disease". At last, we introduce some scoring systems for predicting HCC recurrence after LR.

## LT AT HCC RECURRENCE: SALVAGE LT

LR as the first-line treatment for primary small HCC in compensated cirrhotic liver is widely adopted with an acceptable survival rate but a high recurrence rate. No treatment guidelines exist for recurrent HCC after LR. Salvage curative treatment for recurrent HCC following primary LR includes SLT, repeat LR, and radiofrequency ablation (RFA). In our group, Lee *et al.*<sup>[17]</sup> first reported in 1995 that the cumulative 5-year survival rates in patients undergoing repeated hepatic resection after the first operation was 65.1%, and according to Ho *et al.*<sup>[18]</sup>, the latest 5-year survival rates after recurrence in patients receiving repeat hepatectomy was 72%,

which is similar to that of patients who have undergone primary resection and have no recurrence. Chan *et al.*<sup>[19]</sup> report comparable survival rates and tumor-free survival rates in SLT and repeat LR, but RFA yields poorer outcome than SLT and repeat LR (5-year survival rates in SLT, repeat LR, and RFA: 50.0%, 48.0%, 11.4%, respectively; 5-year tumor-free survival rates in SLT, repeat LR, and RFA: 57.9%, 49.3%, 10.6%, respectively). RFA is associated with poor survival rates but can be considered for patients not suitable for LR. In another series by Yamashita *et al.*<sup>[20]</sup> which compared the outcomes between repeat LR and SLT, the perioperative outcomes including the operation time, intraoperative blood loss, the length of hospital stay, and post-operative morbidity, were all significant worse in the SLT group. No significant difference was observed in the overall survival between these two groups, but patients who underwent SLT had better disease-free survival<sup>[20,21]</sup>. The difference between the results of these two salvage treatments is similar to the difference between primary LT and initial LR for early HCC in compensated liver. However, in areas without sufficient donors, repeat LR is the only treatment for patients with recurrent HCC and enough remnant liver that can provide an overall survival comparable to SLT. Mise *et al.*<sup>[22]</sup> report the result of third or more repeat hepatectomies for recurrent HCC. The 5- and 10-year overall survival rates from the initial hepatectomy are 91.4% and 75.5% respectively, and the 5-year disease-free survival rate after the second hepatectomy is 17.9%.

Comparison of primary LT and SLT for HCC within MC in recent studies revealed similar perioperative course, morbidity, overall survival, and disease-free survival<sup>[16,23-28]</sup>, while a previous study showed the association of LT after resection and higher operative mortality, an increase of recurrence, and poorer outcomes<sup>[29]</sup>. In the systemic review by Chan *et al.*<sup>[30]</sup> the median 5-year overall and disease-free survival rates in SLT are 62% and 67%, respectively. In the era of organ shortage, LR should be considered as the primary curative treatment for resectable tumors in compensated livers, and SLT is a safe and effective strategy for initial transplant-eligible patients when recurrent HCC or hepatic function deterioration occur<sup>[12]</sup>.

The SLT strategy is widely acceptable for patients with previous transplant-eligible HCC. However, some authors also advocate the strategy of performing LR as one of the locoregional therapies for tumor downstaging in patients with initial HCC beyond LT criteria and performing LT after HCC recurrence<sup>[31]</sup>. The results of this downstaging strategy showed better survival outcomes as compared with patients with HCC recurrence who undergo LR without SLT. However, for post-LR recurrent HCC beyond MC, the results of SLT are not beneficial and not recommended in a recent report<sup>[22]</sup>. Prospective studies are needed to examine the long-term outcomes of extending the criteria of LT for intermediate-advanced HCC either before or after tumor recurrence.

## LT BEFORE RECURRENCE: CONCEPT OF PROPHYLACTIC LT

As previous study stated, SLT has been proven effective for patients with recurrent HCC within the criteria of the following: Tumor recurrence within MC, patient adherence to a regular follow-up with imaging to detect early recurrence, and good general patient condition for LT. However, the intention-to-treat analysis by Fuks *et al.*<sup>[32]</sup> showed that nearly half of the patients with recurrent HCC following LR did not undergo LT, including one-third due to recurrence beyond MC. Other studies also report that 20% to 80% of the patients considered for SLT are not transplantable due to recurrence beyond transplant criteria or advanced age with significant comorbidity<sup>[8,15,29,33,34]</sup>. This means that with the strategy of SLT, we lose the chance of LT in originally transplantable patient. Sala *et al.*<sup>[13]</sup> first reported four cases of prophylactic LT, performed based on the expectation of early recurrence according to the gross and microscopic features of the resected specimen, including microvascular invasion and additional nodules. Patients with high risk of recurrence as identified by histopathological findings were enlisted for LT. Scatton *et al.*<sup>[14]</sup> predicted the risk of HCC recurrence after LR on the basis of the histological features of the resected specimen (including Edmondson score, vascular invasion, nuclear grade, and architectural growth pattern), which are used as the selection tool for LT. In this series, six patients were enlisted and underwent prophylactic LT without evidence of residual disease. However, the population of this study was heterogeneous, with three of the six patients in this study having HCC beyond MC at resection, and the other three patients having resected HCC within MC. These six patients are all alive without recurrence with mean follow-up of 55 mo.

Tribillon *et al.*<sup>[34]</sup> report the largest series of prophylactic LT in intention-to-treat analysis of 63 patients with intermediate or bad pathological factors (microvascular invasion and/or moderate/poor differentiation) in the resected specimen being enlisted for LT prior to recurrence (de principe group). The overall survival of this group was compared to 48 patients with favorable pathological features being enlisted for LT at the time of HCC recurrence (salvage group). The 5-year survival rate since primary LR was significantly better in the de principe group as compared with the salvage group (84.6% vs 74.8%), and the 5-year disease survival rate was also better in the de principe group (79.3% vs 72.3%).

This active attitude of enlisting patients for LT prior to recurrence can treat both potential recurrent HCC and underlying liver disease. However, literature about this strategy is scarce. The most important viewpoint discussed in the literature about this prophylactic strategy is preventing original transplant-eligible patients from developing beyond MC at recurrence and provide longer survival. However, if more stringent follow-ups and increased accuracy of imaging studies lead to



**Table 1 Comparison between prophylactic liver transplant and wait-and-see before hepatocellular carcinoma recurrence**

The strategy	Prophylactic LT	Wait-and-see
Immunosuppressant exposure	Life-long	Nil
Surgical morbidity and mortality	Present	Nil
Long-term HCC recurrence	Lower <sup>[32]</sup>	Higher <sup>[11]</sup>
Survival benefit (5-year survival rate)	84.6% <sup>[32]</sup>	Around 70% <sup>[7-10]</sup>
Further management after recurrence	Hepatectomy, RFA, TACE, Sorafenib, Yttrium-90	SLT, repeat hepatectomy, RFA, TACE, Sorafenib, Yttrium-90

LT: Liver transplant; RFA: Radiofrequency ablation; TACE: Transcatheter arterial chemoembolization; HCC: Hepatocellular carcinoma.

**Table 2 Prognostic factors of early hepatocellular carcinoma recurrence after liver resection and after liver transplantation**

Risk factor of HCC recurrence	After liver resection	After liver transplantation
Serological		
AFP	> 400 ng/mL <sup>[49]</sup>	> 1000 ng/mL <sup>[34,35]</sup>
Tumor gross		
Tumor size	> 3 cm <sup>[30]</sup> or > 5 cm <sup>[37,41,65]</sup>	> 6 cm <sup>[35]</sup>
Tumor number	> 3 <sup>[65]</sup>	≥ 4 <sup>[35]</sup>
Satellite nodules	Yes <sup>[30,63,66]</sup>	Yes <sup>[33]</sup>
Tumor microscopic		
Tumor differentiation	Intermediate, or poor differentiation, or undifferentiation <sup>[30,49,65]</sup>	Poor differentiation, or undifferentiation <sup>[33]</sup>
Microvascular invasion	Yes <sup>[30,37,41,49,64-66]</sup>	Yes <sup>[33,34]</sup>
Liver parenchyma		
Severity of cirrhosis	Controversial <sup>[67-69]</sup>	No
Milan criteria	Yes <sup>[68]</sup> (predict recurrence within/beyond MC)	Yes <sup>[3]</sup>

HCC: Hepatocellular carcinoma; AFP: Alpha-fetoprotein; MC: Milan criteria.

early detection of recurrent tumor for these patients, does the result still justify this novel strategy? Salvage treatment after detection of recurrent HCC includes LT, repeat hepatectomy, RFA, transcatheter arterial chemoembolization, sorafenib, and trans-arterial radio-rembolization (Yttrium-90). The choice of these salvage treatment depends on the extent of underlying liver disease, the aggressiveness of tumor at recurrence, and the general condition of the patient. LT has been proven to be correlated with better overall survival and disease-free survival rates with careful patient selection as a curative method, as compared with other salvage treatments previously stated<sup>[17-19]</sup>. However, for cases without evidence of recurrence, it is unclear if we should choose prophylactic LT for patients with a high risk of recurrence or just close follow-ups and salvage treatment at recurrence. The accompanying morbidity and mortality with prophylactic LT and the limited number of organs also hinder this aggressive strategy. The comparison of the benefits and risks between prophylactic LT and the wait-and-see strategy followed by salvage treatment is listed in Table 1.

On the other hand, is a higher probability of recurrence after initial hepatectomy equivalent to a shorter disease-free survival after salvage or prophylactic LT? If HCC recurs easily after salvage or prophylactic LT, this strategy became meaningless. Most prognostic factors associated with recurrence after LR are also relevant to post-transplant recurrence, including microvascular invasion of HCC, larger tumor size, higher tumor number, poorer differentiation of the tumor, and higher level of alpha-fetoprotein (AFP)<sup>[35-37]</sup> (Table 2). It is difficult to

distinguish patients with higher recurrence after hepatectomy from those with possible post-transplant HCC recurrence. A period of observation should be considered after primary LR to identify the aggressiveness of occult HCC in the absence of specific predicting factors. Further investigation is needed to stratify patients for better application of treatment after hepatectomy.

## CLINICOPATHOLOGICAL FACTORS ASSOCIATED WITH HIGH-RISK RECURRENCE

The most important issue in adopting prophylactic LT is the identification of prognostic factors associated with high-risk recurrence. Tumor dissemination from primary tumor before resection and new lesion development in underlying oncogenic cirrhotic parenchyma are two major pathways leading to recurrence<sup>[38-42]</sup>. The former is associated with early recurrence within 2 years after primary resection, while the latter is more likely associated with late recurrence<sup>[42-45]</sup>. We summarize the recent data in the literature on the clinicopathological factors linked with HCC recurrence.

## PATIENT-RELATED FACTORS: DEMOGRAPHIC AND BIOCHEMICAL FACTORS

The age factor associated with recurrence after resection remains controversial. Older age at resection may be

suggestive of long-standing chronic liver disease and higher susceptibility to HCC recurrence over time. Older age (65 years or more) is an independent risk factor for tumor recurrence, as shown in the recent major series by Fan *et al.*<sup>[46]</sup> and Pompili *et al.*<sup>[47]</sup>. However, in the series of HBV-related HCC by Mathews *et al.*<sup>[48]</sup> younger age (40 years or less) was closely associated with more aggressive disease and shorter disease-free survival after resection. The other major series by Hung *et al.*<sup>[49]</sup> does not show old age (60 years or more) to be a poor independent factor for tumor recurrence.

Serum AFP level has been conventionally used as a simple and effective tool for routine surveillance of HCC and for monitoring recurrence following treatment<sup>[50]</sup>. Elevated serum AFP level at the time of resection has been frequently reported to predict the risk of post-resection recurrence of HCC<sup>[51-56]</sup>. Many studies have proposed the relationship between the pretreatment AFP level and tumor-free survival using different cut-off values of AFP level (for example, 20, 100, 400 or 1000 ng/mL)<sup>[44,49,57,58]</sup>. Higher pretreatment serum AFP level is associated with shorter disease-free period. Ho *et al.*<sup>[51]</sup> proposed the value of 400 ng/mL as the cut-off AFP level to predict untransplantable recurrence after primary curative resection of HCC. However, in another study by Shim *et al.*<sup>[59]</sup> the result of a test based on propensity score, included 525 patients who underwent HCC resection and showed no correlation between preoperative serum AFP level and the risk of recurrence. Serum AFP level can also be abnormally high in chronic hepatitis C and advanced cirrhotic liver without HCC<sup>[60]</sup>. It is controversial to use serum AFP level as the predictor of HCC recurrence. Instead of predicting the risk of recurrence, the higher level of serum AFP should be considered as the consequence of aggressive tumor features such as microvascular invasion and poorer tumor differentiation, which indicate worse prognosis<sup>[61]</sup>. Serum AFP level > 1000 ng/mL is also reported to be associated with higher post-transplant recurrence due to the correlation with more aggressive tumor biology<sup>[35-37]</sup>.

## TUMOR-RELATED FACTORS: HISTOPATHOLOGICAL FACTORS

It is well known that early recurrence after HCC resection is related to tumor dissemination prior to operation<sup>[42]</sup>. The histopathological profile obtained from the resected specimen has been used to predict the risk of tumor dissemination and as an objective selection tool for LT in the last decade<sup>[13,14,32,34]</sup>. Among these factors, microvascular invasion of the tumor is the most critical factor in disease dissemination. As seen in most cancers, angiogenesis, or new vessel formation, is essential for HCC growth<sup>[62]</sup>. In advanced stages of tumor progression, HCC cells develop the ability to invade adjacent blood vessels and potentially begin to metastasize. The presence of microvascular invasion is the hallmark of aggressive tumor behavior

and associated with high recurrence rate after curative resection<sup>[63]</sup>. Sumie *et al.*<sup>[64]</sup> report 3-year recurrence-free survival rates in HCC with and without microvascular invasion to be 27.7% and 67.5%, respectively. Other poor histopathological features, like the presence of satellite nodules and poor tumor differentiation, are also recognized, along with microvascular invasion, to predict early recurrence<sup>[32,39,43,65-68]</sup>. Most of these poor histopathological factors associated with early recurrence after LR are also predictors of recurrence after LT, including larger tumor size, larger tumor number, satellite nodules, poorer tumor differentiation, and microvascular invasion (Table 2). These features are linked to the aggressiveness of the tumor biology and predict the recurrence both after LR and LT. Patients with a tendency of post-LR recurrence may also develop a risk of post-LT recurrence. While considering prophylactic LT for patients with these poor histopathological features, cut-off criteria should be made to exclude those with more aggressive HCC and also potentially easy recurrence after LT.

## UNDERLYING LIVER DISEASE-RELATED FACTORS: VIROLOGICAL FACTORS

The preneoplastic status of underlying liver disease is considered to relate with elevated carcinogenesis and de novo tumor development in late phase recurrence (2 years after resection)<sup>[42]</sup>. The correlation between stage of liver fibrosis and disease-free survival is controversial. Grazi *et al.*<sup>[69]</sup> and Taura *et al.*<sup>[70]</sup> showed that HCC without cirrhosis has better disease-free survival compared with HCC with cirrhosis after curative resection in Asia, while Beard *et al.*<sup>[71]</sup> showed the reverse results for western countries. Instead of the severity of liver cirrhosis, the sustained necroinflammatory reaction resulting from higher hepatitis activity may play a more important role in the development of secondary primary HCC two years after resection. Initial high HBV viral loads > 2000 IU/mL<sup>[72]</sup> or 10<sup>6</sup> copies/mL<sup>[45]</sup> at the time of HBV-related HCC resection or one month post resection HBV DNA > 20000 IU/mL<sup>[49]</sup> are all proven to be independent risk factors for tumor recurrence. Ongoing HBV replication can induce active hepatitis and subsequent inflammation in oncogenic liver parenchyma leading to *de novo* recurrent HCC. Regarding Hepatitis C, patients with HCV infection tend to have higher hepatitis activity, which is related to elevated carcinogenesis, than patients with HBV infection<sup>[42]</sup>. However, the difference in recurrence-free survival is not significant between patients with HBV infection or those with HCV infection<sup>[73,74]</sup>. A recent national study of 11950 patients in Japan by Utsunomiya *et al.*<sup>[75]</sup> showed that patients without viral hepatitis have a significant lower risk of HCC recurrence than those with HBV or HCV infection.

## SCORING SYSTEM

Some authors propose the scoring system that integrated

**Table 3** Scoring systems for predicting hepatocellular carcinoma recurrence

Ref.	Basis of scoring system	Prognostic factors	Discriminated scores
Pan <i>et al</i> <sup>[76]</sup>	Glasgow prognostic score	Preoperative CRP > 10 mg/L (1 point) Albumin < 3.5 g/L (1 point)	0, 1, 2
Fuks <i>et al</i> <sup>[32]</sup>	Histological features	Microscopic vascular invasion Presence of satellite nodules Tumor size > 3 cm Poor differentiated tumor Cirrhosis	< 3 factors ≥ 3 factors
Roayaie <i>et al</i> <sup>[66]</sup>	Degree of vascular invasion	Invasion of a vessel with a muscular wall (1 point) Invasion of a vessel ≥ 1 cm from the tumor capsule (1 point)	0, 1, 2
Lee <i>et al</i> <sup>[68]</sup>	Clinical risk score	Initial disease beyond Milan criteria Microsatellites or multiple tumors Lymphovascular invasion (1 point for each factor)	0, 1, 2, 3

Higher scores indicate higher recurrence rate. CRP: C-reactive protein.

clinical, biochemical, and histopathological factors to classify the risk of HCC recurrence after resection<sup>[32,66,68,76]</sup> (Table 3). Most scoring systems consist of the extent of tumor invasiveness, while the Glasgow prognostic score originally used in the prediction of outcomes among non-small-cell lung cancer patients<sup>[77]</sup> is composed of the serum levels of C-reactive protein (CRP) and albumin. The higher serum level of CRP and lower serum level of albumin present in the systemic inflammatory response is associated with a more active viral hepatitis in the remnant liver parenchyma<sup>[76]</sup>. The higher scores in each system indicate shorter disease-free period and poorer outcome. The clinical risk score system by Lee *et al*<sup>[68]</sup> uses pathological factors to predict the likelihood of recurrence after LR, and it can be used to identify patients who may lose the chance of SLT at recurrence. Whether this strategy system applies to prophylactic liver transplantation needs further validation.

## CONCLUSION

Prophylactic LT is a novel concept for patients with high-risk recurrent HCC after primary resection before recurrence. Microvascular invasion, larger tumor size, larger tumor number, and poor tumor differentiation are all predictors for recurrence after LR and LT, while serum AFP level > 1000 ng/mL is the unique feature for predicting recurrence after LT. The length of observation after prophylactic LT should be established to examine the occult aggressiveness of the HCC resulting in recurrence after LT. It is safe and effective when patients who fulfilled MC at the time of resection are carefully selected. Large prospective studies are required to clarify the long-term results of this strategy.

## REFERENCES

- 1 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]
- 2 Mor E, Tur-Kaspa R, Sheiner P, Schwartz M. Treatment of hepatocellular carcinoma associated with cirrhosis in the era of liver transplantation. *Ann Intern Med* 1998; **129**: 643-653 [PMID: 9786813 DOI: 10.7326/0003-4819-129-8-199810150-00013]
- 3 Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]
- 4 Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]
- 5 Poon RT. Optimal initial treatment for early hepatocellular carcinoma in patients with preserved liver function: transplantation or resection? *Ann Surg Oncol* 2007; **14**: 541-547 [PMID: 17103069 DOI: 10.1245/s10434-006-9156-z]
- 6 Yamamoto J, Iwatsuki S, Kosuge T, Dvorchik I, Shimada K, Marsh JW, Yamasaki S, Starzl TE. Should hepatomas be treated with hepatic resection or transplantation? *Cancer* 1999; **86**: 1151-1158 [PMID: 10506698 DOI: 10.1002/(SICI)1097-0142(19991001)86]
- 7 Fan ST, Mau Lo C, Poon RT, Yeung C, Leung Liu C, Yuen WK, Ming Lam C, Ng KK, Ching Chan S. Continuous improvement of survival outcomes of resection of hepatocellular carcinoma: a 20-year experience. *Ann Surg* 2011; **253**: 745-758 [PMID: 21475015 DOI: 10.1097/SLA.0b013e3182111195]
- 8 Poon RT, Fan ST, Lo CM, Liu CL, Wong J. Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: implications for a strategy of salvage transplantation. *Ann Surg* 2002; **235**: 373-382 [PMID: 11882759 DOI: 10.1097/00000658-200203000-00009]
- 9 Ho CM, Lee PH, Chen CL, Ho MC, Wu YM, Hu RH. Long-term outcomes after resection versus transplantation for hepatocellular carcinoma within UCSF criteria. *Ann Surg Oncol* 2012; **19**: 826-833 [PMID: 21879276 DOI: 10.1245/s10434-011-1975-x]
- 10 Yamashita Y, Tsujita E, Takeishi K, Ishida T, Ikegami T, Ezaki T, Maeda T, Utsunomiya T, Nagasue N, Shirabe K, Maehara Y. Trends in surgical results of hepatic resection for hepatocellular carcinoma: 1,000 consecutive cases over 20 years in a single institution. *Am J Surg* 2014; **207**: 890-896 [PMID: 24144344 DOI: 10.1016/j.amjsurg.2013.07.028]
- 11 Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-

- 6736(11)61347-0]
- 12 **Majno PE**, Sarasin FP, Mentha G, Hadengue A. Primary liver resection and salvage transplantation or primary liver transplantation in patients with single, small hepatocellular carcinoma and preserved liver function: an outcome-oriented decision analysis. *Hepatology* 2000; **31**: 899-906 [PMID: 10733546 DOI: 10.1053/he.2000.5763]
  - 13 **Sala M**, Fuster J, Llovet JM, Navasa M, Solé M, Varela M, Pons F, Rimola A, García-Valdecasas JC, Brú C, Bruix J. High pathological risk of recurrence after surgical resection for hepatocellular carcinoma: an indication for salvage liver transplantation. *Liver Transpl* 2004; **10**: 1294-1300 [PMID: 15376311 DOI: 10.1002/lt.20202]
  - 14 **Scatton O**, Zalinski S, Terris B, Lefevre JH, Casali A, Massault PP, Conti F, Calmus Y, Soubrane O. Hepatocellular carcinoma developed on compensated cirrhosis: resection as a selection tool for liver transplantation. *Liver Transpl* 2008; **14**: 779-788 [PMID: 18508370 DOI: 10.1002/lt.21431]
  - 15 **Cherqui D**, Laurent A, Mocellin N, Tayar C, Luciani A, Van Nhieu JT, Decaens T, Hurtova M, Memeo R, Mallat A, Duvoux C. Liver resection for transplantable hepatocellular carcinoma: long-term survival and role of secondary liver transplantation. *Ann Surg* 2009; **250**: 738-746 [PMID: 19801927 DOI: 10.1097/SLA.0b013e3181bd582b]
  - 16 **Maggs JR**, Suddle AR, Aluvihare V, Heneghan MA. Systematic review: the role of liver transplantation in the management of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2012; **35**: 1113-1134 [PMID: 22432733 DOI: 10.1111/j.1365-2036.2012.05072.x]
  - 17 **Lee PH**, Lin WJ, Tsang YM, Hu RH, Sheu JC, Lai MY, Hsu HC, May W, Lee CS. Clinical management of recurrent hepatocellular carcinoma. *Ann Surg* 1995; **222**: 670-676 [PMID: 7487215]
  - 18 **Ho CM**, Lee PH, Shau WY, Ho MC, Wu YM, Hu RH. Survival in patients with recurrent hepatocellular carcinoma after primary hepatectomy: comparative effectiveness of treatment modalities. *Surgery* 2012; **151**: 700-709 [PMID: 22284764 DOI: 10.1016/j.surg.2011.12.015]
  - 19 **Chan AC**, Chan SC, Chok KS, Cheung TT, Chiu DW, Poon RT, Fan ST, Lo CM. Treatment strategy for recurrent hepatocellular carcinoma: salvage transplantation, repeated resection, or radiofrequency ablation? *Liver Transpl* 2013; **19**: 411-419 [PMID: 23447460 DOI: 10.1002/lt.23605]
  - 20 **Yamashita Y**, Tsujita E, Takeishi K, Ishida T, Ikegami T, Ezaki T, Maeda T, Utsunomiya T, Nagasue N, Shirabe K, Maehara Y. Trends in surgical results of hepatic resection for hepatocellular carcinoma: 1,000 consecutive cases over 20 years in a single institution. *Am J Surg* 2014; **207**: 890-896 [PMID: 24144344 DOI: 10.1016/j.amjsurg.2013.07.028]
  - 21 **Chan DL**, Morris DL, Chua TC. Clinical efficacy and predictors of outcomes of repeat hepatectomy for recurrent hepatocellular carcinoma - a systematic review. *Surg Oncol* 2013; **22**: e23-e30 [PMID: 23535302 DOI: 10.1016/j.suronc.2013.02.009]
  - 22 **Mise Y**, Hasegawa K, Shindoh J, Ishizawa T, Aoki T, Sakamoto Y, Sugawara Y, Makuuchi M, Kokudo N. The Feasibility of Third or More Repeat Hepatectomy for Recurrent Hepatocellular Carcinoma. *Ann Surg* 2015; **262**: 347-357 [PMID: 25185473 DOI: 10.1097/SLA.0000000000000882]
  - 23 **Belghiti J**, Cortes A, Abdalla EK, Régimbeau JM, Prakash K, Durand F, Sommacale D, Dondero F, Lesurtel M, Sauvanet A, Farges O, Kianmanesh R. Resection prior to liver transplantation for hepatocellular carcinoma. *Ann Surg* 2003; **238**: 885-892; discussion 892-893 [PMID: 14631225 DOI: 10.1097/01.sla.0000098621.74851.65]
  - 24 **Del Gaudio M**, Ercolani G, Ravaioli M, Cescon M, Lauro A, Vivarelli M, Zanella M, Cucchetti A, Vetrone G, Tuci F, Ramacciato G, Grazi GL, Pinna AD. Liver transplantation for recurrent hepatocellular carcinoma on cirrhosis after liver resection: University of Bologna experience. *Am J Transplant* 2008; **8**: 1177-1185 [PMID: 18444925 DOI: 10.1111/j.1600-6143.2008.02229.x]
  - 25 **Moon JI**, Kwon CH, Joh JW, Choi GS, Jung GO, Kim JM, Shin M, Choi SJ, Kim SJ, Lee SK. Primary versus salvage living donor liver transplantation for patients with hepatocellular carcinoma: impact of microvascular invasion on survival. *Transplant Proc* 2012; **44**: 487-493 [PMID: 22410053 DOI: 10.1016/j.transproceed.2011.11.009]
  - 26 **Hwang S**, Lee SG, Moon DB, Ahn CS, Kim KH, Lee YJ, Ha TY, Song GW. Salvage living donor liver transplantation after prior liver resection for hepatocellular carcinoma. *Liver Transpl* 2007; **13**: 741-746 [PMID: 17457860 DOI: 10.1002/lt.21157]
  - 27 **Liu F**, Wei Y, Wang W, Chen K, Yan L, Wen T, Zhao J, Xu M, Li B. Salvage liver transplantation for recurrent hepatocellular carcinoma within UCSF criteria after liver resection. *PLoS One* 2012; **7**: e48932 [PMID: 23145027 DOI: 10.1371/journal.pone.0048932]
  - 28 **Kaido T**, Mori A, Ogura Y, Hata K, Yoshizawa A, Iida T, Yagi S, Uemoto S. Living donor liver transplantation for recurrent hepatocellular carcinoma after liver resection. *Surgery* 2012; **151**: 55-60 [PMID: 21943635 DOI: 10.1016/j.surg.2011.06.032]
  - 29 **Adam R**, Azoulay D, Castaing D, Eshkenazy R, Pascal G, Hashizume K, Samuel D, Bismuth H. Liver resection as a bridge to transplantation for hepatocellular carcinoma on cirrhosis: a reasonable strategy? *Ann Surg* 2003; **238**: 508-518; discussion 518-519 [PMID: 14530722 DOI: 10.1097/01.sla.0000090449.87109.44]
  - 30 **Chan DL**, Alzahrani NA, Morris DL, Chua TC. Systematic review of efficacy and outcomes of salvage liver transplantation after primary hepatic resection for hepatocellular carcinoma. *J Gastroenterol Hepatol* 2014; **29**: 31-41 [PMID: 24117517 DOI: 10.1111/jgh.12399]
  - 31 **Tuci F**, Vitale A, D'Amico F, Gringeri E, Neri D, Zanusi G, Bassi D, Polacco M, Boetto R, Lodo E, Germani G, Burra P, Angeli P, Cillo U. Survival benefit of transplantation for recurrence of hepatocellular carcinoma after liver resection. *Transplant Proc* 2014; **46**: 2287-2289 [PMID: 25242770 DOI: 10.1016/j.transproceed.2014.07.031]
  - 32 **Fuks D**, Dokmak S, Paradis V, Diouf M, Durand F, Belghiti J. Benefit of initial resection of hepatocellular carcinoma followed by transplantation in case of recurrence: an intention-to-treat analysis. *Hepatology* 2012; **55**: 132-140 [PMID: 21932387 DOI: 10.1002/hep.24680]
  - 33 **Margarit C**, Escartin A, Castells L, Vargas V, Allende E, Bilbao I. Resection for hepatocellular carcinoma is a good option in Child-Turcotte-Pugh class A patients with cirrhosis who are eligible for liver transplantation. *Liver Transpl* 2005; **11**: 1242-1251 [PMID: 16184539 DOI: 10.1002/lt.20398]
  - 34 **Tribillon E**, Barbier L, Goumard C, Irtan S, Perdigo-Cotta F, Durand F, Paradis V, Belghiti J, Scatton O, Soubrane O. When Should We Propose Liver Transplant After Resection of Hepatocellular Carcinoma? A Comparison of Salvage and De Principe Strategies. *J Gastrointest Surg* 2016; **20**: 66-76; discussion 76 [PMID: 26582597 DOI: 10.1007/s11605-015-3018-6]
  - 35 **Agopian VG**, Harlander-Locke M, Zarrinpar A, Kaldas FM, Farmer DG, Yersiz H, Finn RS, Tong M, Hiatt JR, Busuttil RW. A novel prognostic nomogram accurately predicts hepatocellular carcinoma recurrence after liver transplantation: analysis of 865 consecutive liver transplant recipients. *J Am Coll Surg* 2015; **220**: 416-427 [PMID: 25690672 DOI: 10.1016/j.jamcollsurg.2014.12.025]
  - 36 **Hameed B**, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level > 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. *Liver Transpl* 2014; **20**: 945-951 [PMID: 24797281 DOI: 10.1002/lt.23904]
  - 37 **Duvoux C**, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, Francoz C, Compagnon P, Vanlemmens C, Dumortier J, Dharancy S, Gugenheim J, Bernard PH, Adam R, Radenne S, Muscari F, Conti F, Hardwigsen J, Pageaux GP, Chazouillères O, Salame E, Hilleret MN, Lebray P, Abergel A, Debbete-Gratien M, Kluger MD, Mallat A, Azoulay D, Cherqui D. Liver transplantation for hepatocellular carcinoma: a model including  $\alpha$ -fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012; **143**: 986-994.e3; quiz e14-e15 [PMID: 22750200 DOI: 10.1053/j.gastro.2012.05.052]



- 38 **Arii S**, Monden K, Niwano M, Furutani M, Mori A, Mizumoto M, Imamura M. Results of surgical treatment for recurrent hepatocellular carcinoma; comparison of outcome among patients with multicentric carcinogenesis, intrahepatic metastasis, and extrahepatic recurrence. *J Hepatobiliary Pancreat Surg* 1998; **5**: 86-92 [PMID: 9683759]
- 39 **Cha C**, Fong Y, Jarnagin WR, Blumgart LH, DeMatteo RP. Predictors and patterns of recurrence after resection of hepatocellular carcinoma. *J Am Coll Surg* 2003; **197**: 753-758 [PMID: 14585409 DOI: 10.1016/j.jamcollsurg.2003.07.003]
- 40 **Arii S**, Teramoto K, Kawamura T, Okamoto H, Kaido T, Mori A, Imamura M. Characteristics of recurrent hepatocellular carcinoma in Japan and our surgical experience. *J Hepatobiliary Pancreat Surg* 2001; **8**: 397-403 [PMID: 11702247 DOI: 10.1007/s005340100000]
- 41 **Poon RT**, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. *Cancer* 2000; **89**: 500-507 [PMID: 10931448]
- 42 **Imamura H**, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003; **38**: 200-207 [PMID: 12547409 DOI: 10.1016/S0168-8278(02)00360-4]
- 43 **Cheng Z**, Yang P, Qu S, Zhou J, Yang J, Yang X, Xia Y, Li J, Wang K, Yan Z, Wu D, Zhang B, Hüser N, Shen F. Risk factors and management for early and late intrahepatic recurrence of solitary hepatocellular carcinoma after curative resection. *HPB (Oxford)* 2015; **17**: 422-427 [PMID: 25421805 DOI: 10.1111/hpb.12367]
- 44 **Jeng KS**, Sheen IS, Tsai YC. Does the presence of circulating hepatocellular carcinoma cells indicate a risk of recurrence after resection? *Am J Gastroenterol* 2004; **99**: 1503-1509 [PMID: 15307868 DOI: 10.1111/j.1572-0241.2004.30227.x]
- 45 **Wu JC**, Huang YH, Chau GY, Su CW, Lai CR, Lee PC, Huo TI, Sheen IJ, Lee SD, Lui WY. Risk factors for early and late recurrence in hepatitis B-related hepatocellular carcinoma. *J Hepatol* 2009; **51**: 890-897 [PMID: 19747749 DOI: 10.1016/j.jhep.2009.07.009]
- 46 **Fan ST**, Poon RT, Yeung C, Lam CM, Lo CM, Yuen WK, Ng KK, Liu CL, Chan SC. Outcome after partial hepatectomy for hepatocellular cancer within the Milan criteria. *Br J Surg* 2011; **98**: 1292-1300 [PMID: 21656513 DOI: 10.1002/bjs.7583]
- 47 **Pompili M**, Saviano A, de Matthaeis N, Cucchetti A, Ardito F, Federico B, Brunello F, Pinna AD, Giorgio A, Giulini SM, De Sio I, Torzilli G, Fornari F, Capussotti L, Guglielmi A, Piscaglia F, Aldrighetti L, Caturelli E, Calise F, Nuzzo G, Rapaccini GL, Giulante F. Long-term effectiveness of resection and radiofrequency ablation for single hepatocellular carcinoma  $\leq 3$  cm. Results of a multicenter Italian survey. *J Hepatol* 2013; **59**: 89-97 [PMID: 23523578 DOI: 10.1016/j.jhep.2013.03.009]
- 48 **Mathews P**, Lee D, Chung YH, Kim JA, Lee JH, Jin YJ, Park W, Lyu H, Jaffee E, Zheng L, Yu E, Lee YJ. Effects of genomic changes in hepatitis B virus on postoperative recurrence and survival in patients with hepatocellular carcinoma. *Ann Surg Oncol* 2013; **20**: 1216-1222 [PMID: 23104706 DOI: 10.1245/s10434-012-2706-7]
- 49 **Hung IF**, Wong DK, Poon RT, Fong DY, Chui AH, Seto WK, Fung JY, Chan AC, Yuen JC, Tiu R, Choi O, Lai CL, Yuen MF. Risk Factors and Post-Resection Independent Predictive Score for the Recurrence of Hepatitis B-Related Hepatocellular Carcinoma. *PLoS One* 2016; **11**: e0148493 [PMID: 26901762 DOI: 10.1371/journal.pone.0148493]
- 50 **Johnson PJ**. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. *Clin Liver Dis* 2001; **5**: 145-159 [PMID: 11218912 DOI: 10.1016/S1089-3261(05)70158-6]
- 51 **Ho CM**, Wu CY, Lee PH, Lai HS, Ho MC, Wu YM, Hu RH. Analysis of the risk factors of untransplantable recurrence after primary curative resection for patients with hepatocellular carcinoma. *Ann Surg Oncol* 2013; **20**: 2526-2533 [PMID: 23504121 DOI: 10.1245/s10434-013-2940-7]
- 52 **Nanashima A**, Taura N, Abo T, Ichikawa T, Sakamoto I, Nagayasu T, Nakao K. Tumor marker levels before and after curative treatment of hepatocellular carcinoma as predictors of patient survival. *Dig Dis Sci* 2011; **56**: 3086-3100 [PMID: 21706206 DOI: 10.1007/s10620-011-1796-6]
- 53 **Zhang XF**, Qi X, Meng B, Liu C, Yu L, Wang B, Lv Y. Prognosis evaluation in alpha-fetoprotein negative hepatocellular carcinoma after hepatectomy: comparison of five staging systems. *Eur J Surg Oncol* 2010; **36**: 718-724 [PMID: 20538423 DOI: 10.1016/j.ejso.2010.05.022]
- 54 **Tangkijvanich P**, Anukulkarnkusol N, Suwangool P, Lertmaharit S, Hanvivatvong O, Kullavanijaya P, Poovorawan Y. Clinical characteristics and prognosis of hepatocellular carcinoma: analysis based on serum alpha-fetoprotein levels. *J Clin Gastroenterol* 2000; **31**: 302-308 [PMID: 11129271 DOI: 10.1097/00004836-200012000-00007]
- 55 **Santambrogio R**, Opocher E, Costa M, Barabino M, Zuin M, Bertolini E, De Filippi F, Bruno S. Hepatic resection for "BCLC stage A" hepatocellular carcinoma. The prognostic role of alpha-fetoprotein. *Ann Surg Oncol* 2012; **19**: 426-434 [PMID: 21732145 DOI: 10.1245/s10434-011-1845-6]
- 56 **Yamamoto K**, Imamura H, Matsuyama Y, Hasegawa K, Beck Y, Sugawara Y, Makuuchi M, Kokudo N. Significance of alpha-fetoprotein and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma undergoing hepatectomy. *Ann Surg Oncol* 2009; **16**: 2795-2804 [PMID: 19669841 DOI: 10.1245/s10434-009-0618-y]
- 57 **Yang SL**, Liu LP, Yang S, Liu L, Ren JW, Fang X, Chen GG, Lai PB. Preoperative serum  $\alpha$ -fetoprotein and prognosis after hepatectomy for hepatocellular carcinoma. *Br J Surg* 2016; Epub ahead of print [PMID: 26996727 DOI: 10.1002/bjs.10093]
- 58 **Ma WJ**, Wang HY, Teng LS. Correlation analysis of preoperative serum alpha-fetoprotein (AFP) level and prognosis of hepatocellular carcinoma (HCC) after hepatectomy. *World J Surg Oncol* 2013; **11**: 212 [PMID: 23981851 DOI: 10.1186/1477-7819-11-212]
- 59 **Shim JH**, Yoon DL, Han S, Lee YJ, Lee SG, Kim KM, Lim YS, Lee HC, Chung YH, Lee YS. Is serum alpha-fetoprotein useful for predicting recurrence and mortality specific to hepatocellular carcinoma after hepatectomy? A test based on propensity scores and competing risks analysis. *Ann Surg Oncol* 2012; **19**: 3687-3696 [PMID: 22644512 DOI: 10.1245/s10434-012-2416-1]
- 60 **Sterling RK**, Wright EC, Morgan TR, Seeff LB, Hoefs JC, Di Bisceglie AM, Dienstag JL, Lok AS. Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. *Am J Gastroenterol* 2012; **107**: 64-74 [PMID: 21931376 DOI: 10.1038/ajg.2011.312]
- 61 **Pang RW**, Joh JW, Johnson PJ, Monden M, Pawlik TM, Poon RT. Biology of hepatocellular carcinoma. *Ann Surg Oncol* 2008; **15**: 962-971 [PMID: 18236113 DOI: 10.1245/s10434-007-9730-z]
- 62 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]
- 63 **Rodríguez-Perálvarez M**, Luong TV, Andreana L, Meyer T, Dhillon AP, Burroughs AK. A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. *Ann Surg Oncol* 2013; **20**: 325-339 [PMID: 23149850 DOI: 10.1245/s10434-012-2513-1]
- 64 **Sumie S**, Kuromatsu R, Okuda K, Ando E, Takata A, Fukushima N, Watanabe Y, Kojiro M, Sata M. Microvascular invasion in patients with hepatocellular carcinoma and its predictable clinicopathological factors. *Ann Surg Oncol* 2008; **15**: 1375-1382 [PMID: 18324443 DOI: 10.1245/s10434-008-9846-9]
- 65 **Villanueva A**, Hoshida Y, Battiston C, Tovar V, Sia D, Alsinet C, Cornella H, Liberzon A, Kobayashi M, Kumada H, Thung SN, Bruix J, Newell P, April C, Fan JB, Roayaie S, Mazzaferro V, Schwartz ME, Llovet JM. Combining clinical, pathology, and gene expression data to predict recurrence of hepatocellular carcinoma. *Gastroenterology* 2011; **140**: 1501-1512.e2 [PMID: 21320499 DOI: 10.1053/j.gastro.2011.02.006]

- 66 **Roayaie S**, Blume IN, Thung SN, Guido M, Fiel MI, Hiotis S, Labow DM, Llovet JM, Schwartz ME. A system of classifying microvascular invasion to predict outcome after resection in patients with hepatocellular carcinoma. *Gastroenterology* 2009; **137**: 850-855 [PMID: 19524573 DOI: 10.1053/j.gastro.2009.06.003]
- 67 **Shah SA**, Cleary SP, Wei AC, Yang I, Taylor BR, Hemming AW, Langer B, Grant DR, Greig PD, Gallinger S. Recurrence after liver resection for hepatocellular carcinoma: risk factors, treatment, and outcomes. *Surgery* 2007; **141**: 330-339 [PMID: 17349844 DOI: 10.1016/j.surg.2006.06.028]
- 68 **Lee SY**, Konstantinidis IT, Eaton AA, Gönen M, Kingham TP, D'Angelica MI, Allen PJ, Fong Y, DeMatteo RP, Jarnagin WR. Predicting recurrence patterns after resection of hepatocellular cancer. *HPB (Oxford)* 2014; **16**: 943-953 [PMID: 25041404 DOI: 10.1111/hpb.12311]
- 69 **Grazi GL**, Cescon M, Ravaioli M, Ercolani G, Gardini A, Del Gaudio M, Vetrone G, Cavallari A. Liver resection for hepatocellular carcinoma in cirrhotics and noncirrhotics. Evaluation of clinicopathologic features and comparison of risk factors for long-term survival and tumour recurrence in a single centre. *Aliment Pharmacol Ther* 2003; **17** Suppl 2: 119-129 [PMID: 12786623]
- 70 **Taura K**, Ikai I, Hatano E, Yasuchika K, Nakajima A, Tada M, Seo S, Machimoto T, Uemoto S. Influence of coexisting cirrhosis on outcomes after partial hepatic resection for hepatocellular carcinoma fulfilling the Milan criteria: an analysis of 293 patients. *Surgery* 2007; **142**: 685-694 [PMID: 17981188 DOI: 10.1016/j.surg.2007.05.009]
- 71 **Beard RE**, Hanto DW, Gautam S, Miksad RA. A comparison of surgical outcomes for noncirrhotic and cirrhotic hepatocellular carcinoma patients in a Western institution. *Surgery* 2013; **154**: 545-555 [PMID: 23777589 DOI: 10.1016/j.surg.2013.02.019]
- 72 **Hung IF**, Poon RT, Lai CL, Fung J, Fan ST, Yuen MF. Recurrence of hepatitis B-related hepatocellular carcinoma is associated with high viral load at the time of resection. *Am J Gastroenterol* 2008; **103**: 1663-1673 [PMID: 18616655 DOI: 10.1111/j.1572-0241.2008.01872.x]
- 73 **Takenaka K**, Yamamoto K, Taketomi A, Itasaka H, Adachi E, Shirabe K, Nishizaki T, Yanaga K, Sugimachi K. A comparison of the surgical results in patients with hepatitis B versus hepatitis C-related hepatocellular carcinoma. *Hepatology* 1995; **22**: 20-24 [PMID: 7601413]
- 74 **Kao WY**, Su CW, Chau GY, Lui WY, Wu CW, Wu JC. A comparison of prognosis between patients with hepatitis B and C virus-related hepatocellular carcinoma undergoing resection surgery. *World J Surg* 2011; **35**: 858-867 [PMID: 21207029 DOI: 10.1007/s00268-010-0928-z]
- 75 **Utsunomiya T**, Shimada M, Kudo M, Ichida T, Matsui O, Izumi N, Matsuyama Y, Sakamoto M, Nakashima O, Ku Y, Takayama T, Kokudo N. A comparison of the surgical outcomes among patients with HBV-positive, HCV-positive, and non-B non-C hepatocellular carcinoma: a nationwide study of 11,950 patients. *Ann Surg* 2015; **261**: 513-520 [PMID: 25072437 DOI: 10.1097/SLA.0000000000000821]
- 76 **Pan QX**, Zhang JH, Su ZJ, Wang CR, Ke SY. The Glasgow Prognostic Score is an independent prognostic predictor of hepatocellular carcinoma following radical resection. *Oncol Res Treat* 2014; **37**: 192-197 [PMID: 24732643 DOI: 10.1159/000361082]
- 77 **Forrest LM**, McMillan DC, McArdle CS, Angerson WJ, Dunlop DJ. Evaluation of cumulative prognostic scores based on the systemic inflammatory response in patients with inoperable non-small-cell lung cancer. *Br J Cancer* 2003; **89**: 1028-1030 [PMID: 12966420 DOI: 10.1038/sj.bjc.6601242]

P- Reviewer: Sipos F S- Editor: Qi Y L- Editor: A  
E- Editor: Li D



Retrospective Cohort Study

## Safe and effective sofosbuvir-based therapy in patients with mental health disease on hepatitis C virus treatment

Lydia Shuk Yee Tang, Jack Masur, Zayani Sims, Amy Nelson, Anu Osinusi, Anita Kohli, Sarah Kattakuzhy, Michael Polis, Shyam Kottlil

Lydia Shuk Yee Tang, Jack Masur, Amy Nelson, Sarah Kattakuzhy, Shyam Kottlil, Institute of Human Virology, Division of Infectious Diseases, University of Maryland School of Medicine, Baltimore, MD 21201, United States

Zayani Sims, Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, MD 20892, United States

Anu Osinusi, Gilead Sciences Inc, Foster City, CA 94404, United States

Anita Kohli, Creighton University School of Medicine, St Joseph's Hospital, Phoenix, AZ 85013, United States

Michael Polis, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, United States

**Author contributions:** Tang LSY, Masur J, Sims Z, Polis M and Kottlil S designed the research; Tang LSY, Masur J, Sims Z, Nelson A, Osinusi A, Kohli A and Kattakuzhy S performed the research; Tang LSY, Masur J, Sims Z and Kattakuzhy S analyzed the data; Tang LSY, Masur J, Sims Z and Kottlil S wrote the paper; all authors contributed critical revisions related to important intellectual content of the manuscript and had final approval of the version of the article to be published.

**Institutional review board statement:** Each study was approved by the institutional review board of NIAID and was conducted in compliance with the Good Clinical Practice guidelines, the Declaration of Helsinki and regulatory requirements. An independent safety monitor participated in the interim safety and efficacy analysis for SPARE. The Regulatory Compliance and Human Participants Protection Branch of NIAID served as the study sponsor and medical monitor.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** Dr. Lydia Shuk Yee Tang receives funding from Gilead Sciences' Frontlines of Communities in the

United States (FOCUS) program. Dr. Anu Osinusi is an employee of Gilead Sciences Inc. None of the other authors have any conflict of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Shyam Kottlil, MD, PhD, Professor of Medicine, Institute of Human Virology, Division of Infectious Diseases, University of Maryland School of Medicine, 725 West Lombard Street, Baltimore, MD 21201, United States. [skottlil@ihv.umaryland.edu](mailto:skottlil@ihv.umaryland.edu)  
**Telephone:** +1-410-7068614  
**Fax:** +1-410-7061952

**Received:** May 29, 2016

**Peer-review started:** May 29, 2016

**First decision:** July 29, 2016

**Revised:** August 19, 2016

**Accepted:** September 8, 2016

**Article in press:** September 9, 2016

**Published online:** November 8, 2016

## Abstract

### AIM

To study impact of baseline mental health disease on hepatitis C virus (HCV) treatment; and Beck's Depression Inventory (BDI) changes with sofosbuvir- and

interferon-based therapy.

## METHODS

This is a retrospective cohort study of participants from 5 studies enrolled from single center trials conducted at the Clinical Research Center of the National Institutes of Health, Bethesda, MD, United States. All participants were adults with chronic HCV genotype 1 infection and naïve to HCV therapy. Two of the studies included HCV mono-infected participants only (SPARE, SYNERGY-A), and 3 included human immunodeficiency virus (HIV)/HCV co-infected participants only (ERADICATE, PFINPK, and ALBIN). Patients were treated for HCV with 3 different regimens: Sofosbuvir and ribavirin in the SPARE trial, ledipasvir and sofosbuvir in SYNERGY-A and ERADICATE trials, and pegylated interferon (IFN) and ribavirin for 48 wk in the PIFNPK and ALBIN trials. Participants with baseline mental health disease (MHD) were identified (defined as either a DSM IV diagnosis of major depression, bipolar disorder, schizophrenia, generalized anxiety, and post-traumatic stress disorder or requiring anti-depressants, antipsychotics, mood stabilizers or psychotropics prescribed by a psychiatrist). For our first aim, we compared sustained virologic response (SVR) and adherence (pill counts, study visits, and in 25 patients, blood levels of the sofosbuvir metabolite, GS-331007) within each study. For our second aim, only patients with HIV coinfection were evaluated. BDI scores were obtained pre-treatment, during treatment, and post-treatment among participants treated with sofosbuvir-based therapy, and compared to scores from participants treated with interferon-based therapy. Statistical differences for both aims were analyzed by Fisher's Exact, and *t*-test with significance defined as a *P* value less than 0.05.

## RESULTS

Baseline characteristics did not differ significantly between all participants with and without MHD groups treated with sofosbuvir-based therapy. Among patients treated with sofosbuvir-based therapy, the percentage of patients with MHD who achieved SVR was the same as those without (SPARE: 60.9% of those MHD compared to 67.6% in those without, *P* = 0.78; SYNERGY-A: 100% of both groups; ERADICATE: 100% compared to 97.1%). There was no statistically significant difference in pill counts, adherence to study visits between groups, nor mean serum concentrations of GS-331007 for each group at week 2 of treatment (*P* = 0.72). Among patients with HIV co-infection, pre-treatment BDI scores were similar among patients treated with sofosbuvir, and those treated with interferon (sofosbuvir-based 5.24, IFN-based 6.96; *P* = 0.14); however, a dichotomous effect on was observed during treatment. Among participants treated with directly acting antiviral (DAA)-based therapy, mean BDI scores decreased from 5.24 (pre-treatment) to 3.28 during treatment (1.96 decrease, *P* = 0.0034) and 2.82 post-treatment. The decrease in mean score from pre- to post-treatment was statistically significant (-2.42, *P* = 0.0012). Among participants treated with IFN-based therapy, mean BDI

score increased from 6.96 at pre-treatment to 9.19 during treatment (an increase of 2.46 points, *P* = 0.1), and then decreased back to baseline post-treatment (mean BDI score 6.3, *P* = 0.54). Overall change in mean BDI scores from pre-treatment to during treatment among participants treated with DAA-based and IFN-therapy was statistically significant (-1.96 and +2.23, respectively; *P* = 0.0032). This change remained statistically significant when analysis was restricted to participants who achieved SVR (-2.0 and +4.36, respectively; *P* = 0.0004).

## CONCLUSION

Sofosbuvir-based therapy is safe and well tolerated in patients with MHD. A decline in BDI associated with sofosbuvir-based HCV treatment suggests additional MHD benefits, although the duration of these effects is unknown.

**Key words:** Sofosbuvir; Direct acting antivirals; Directly acting antiviral; Hepatitis C; Mental health disease; Depression; Interferon; Beck's Depression Inventory

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The prevalence of mental health disease (MHD) among patients with chronic hepatitis C virus (HCV) can be high. However, patients with MHD may be marginalized with respect to HCV therapy and MHD is one of the most frequently cited reason for exclusion from HCV therapy. HCV therapy has evolved from interferon-based to directly acting antiviral (DAA)-based therapy with excellent tolerability and efficacy. Our study found that baseline MHD did not impact efficacy nor treatment adherence to sofosbuvir-based therapy. Furthermore, we found that Beck's Depression Inventory scores improved with sofosbuvir-based therapy, suggesting that HCV treatment with the newer DAA therapies may have additional mental health benefits.

Tang LSY, Masur J, Sims Z, Nelson A, Osinusi A, Kohli A, Kattakuzhy S, Polis M, Kottitil S. Safe and effective sofosbuvir-based therapy in patients with mental health disease on hepatitis C virus treatment. *World J Hepatol* 2016; 8(31): 1318-1326 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i31/1318.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i31.1318>

## INTRODUCTION

An estimated 185 million people worldwide are currently infected with chronic hepatitis C virus (HCV), and approximately 3 to 4 million new infections occur each year<sup>[1]</sup>. Chronic infection with HCV is a leading cause of progressive liver disease, end-stage liver disease, hepatocellular cancer, and remains the leading indication for liver transplantation in the United States<sup>[2-4]</sup>.

A complex interplay exists between mental health



disorders (MHD) and HCV infection<sup>[5-9]</sup>. In some cohorts, 30%-44% of all patients with HCV have an active psychiatric-most frequently depression-disorder<sup>[7,10-14]</sup>. MHD is one of the most frequently cited reason for exclusion from HCV therapy, contributing to 44% of exclusions in one study<sup>[13]</sup>. Exacerbation of psychiatric complications, adherence, concurrent substance abuse, and concern for reinfection are just some of the barriers to treatment<sup>[5]</sup>. These concerns are primarily related to interferon-based therapies, which require long treatment durations of 24 to 48 wk, high pill burdens, and multiple associated dose-limiting neuropsychiatric side effects<sup>[8,14-16]</sup>. Recent reports, however, suggest that patients with MHD can successfully achieve sustained virologic response (SVR, considered cure) rates with interferon-based regimens comparable to those without MHD<sup>[8,14,17]</sup>.

Treatment has shifted to combination all-oral, interferon-free directly acting antiviral (DAA) therapy characterized by short treatment durations of 8-24 wk<sup>[18-22]</sup>, low pill burdens, improved tolerability, and achieving SVR rates of over 90% for both treatment naïve and interferon treatment experienced<sup>[20-24]</sup>. In this study, we present data from five National Institute of Allergy and Infectious Diseases (NIAID) trials - SPARE<sup>[19]</sup> (using sofosbuvir and ribavirin), SYNERGY-A<sup>[18]</sup>, and ERADICATE<sup>[25]</sup> (both using ledipasvir and sofosbuvir), and PIFNPK<sup>[26]</sup> and ALBIN<sup>[27]</sup> [studies of interferon (IFN)-ribavirin-based therapy among patients with human immunodeficiency virus (HIV)/HCV co-infection]. This study has two aims. Firstly, we address the impact of baseline MHD on SVR and adherence to sofosbuvir-based, interferon-free therapy. Secondly, we characterize the change in Beck's Depression Inventory (BDI) scores among patients with HIV/HCV co-infection treated with sofosbuvir-based therapy and with interferon-based therapy. For our first aim we present data from three NIAID trials - SPARE<sup>[19]</sup> (using sofosbuvir and ribavirin), SYNERGY-A<sup>[18]</sup>, and ERADICATE<sup>[25]</sup> (both using ledipasvir and sofosbuvir). For our second aim, BDI scores were obtained from patients enrolled in ERADICATE, PIFNPK and ALBIN.

## MATERIALS AND METHODS

### Patients

Patients for all studies were enrolled from single center trials conducted at the Clinical Research Center of the National Institutes of Health, Bethesda, MD, United States. Adult patients with chronic HCV genotype 1 (GT-1) infection naïve to HCV therapy were included. Written or oral informed consent approved by the NIAID Institutional Review Board was obtained from all patients. Full eligibility criteria for all 5 studies are as previously published<sup>[18,19,25-28]</sup>.

### Baseline MHD and impact on SVR and adherence

SPARE was a 2-part, randomized controlled trial from October 2011 through April 2012<sup>[19]</sup>. Patients with early

to moderate liver fibrosis were treated for 24 wk with 400 mg/d of sofosbuvir and weight-based ribavirin, 400 mg in the morning, 600 mg in the evening if < 75 kg or 600 mg twice a day if > 75 kg). A second cohort of patients with all stages of fibrosis (including compensated cirrhosis) was randomized to receive 400 mg/d of sofosbuvir in combination with either weight-based ribavirin or low-dose (600 mg/d) ribavirin for 24 wk<sup>[19]</sup>.

SYNERGY-A was a phase 2a cohort study<sup>[18]</sup>. From January 2013 to December 2013, twenty GT-1 HCV-infected patients were treated for 12 wk with ledipasvir 90 mg and sofosbuvir 400 mg administered as a single combination pill (ledipasvir-sofosbuvir) taken once daily. Neither patient nor investigators were blinded<sup>[18]</sup>.

ERADICATE was an open-label phase 2b trial of ledipasvir-sofosbuvir once daily to non-cirrhotic GT-1 HCV-infected patients with stable HIV-disease<sup>[25]</sup>. From June 2013 to February 2014, fifty HCV GT-1 patients were treated for 12 wk with ledipasvir and sofosbuvir.

### Identification of baseline mental health disorders

In all three clinical trials of sofosbuvir-based therapy, patients with MHD were included. We retrospectively identified patients with baseline MHD defined as either: (1) a DSM IV diagnosis of major depression, bipolar disorder, schizophrenia, generalized anxiety, and post-traumatic stress disorder; or (2) requiring anti-depressants, anti-psychotics, mood stabilizers or psychotropics prescribed by a psychiatrist.

### Efficacy assessment

SVR, defined as undetectable HCV RNA 12 wk post completion of treatment, was the primary outcome. Plasma HCV RNA levels were measured using the real time HCV assay (Abbott), with a lower limit of quantification (LLOQ) of 12 IU/mL, and COBAS TaqMan HCV RNA assay version 2.0 (Roche), with an LLOQ of 43 IU/mL.

### Adherence assessment

For all 3 studies, we performed pill counts at set time points: Sofosbuvir and ribavirin counts in SPARE; ledipasvir-sofosbuvir counts in SYNERGY-A and ERADICATE. Sofosbuvir adherence was documented during 11 time points based on participant recall and pill counts. Missed doses were recorded only through the time of treatment discontinuation in patients who stopped treatment early. We compared the percentage of patients who completed treatment with 3 or less missed pills to those who missed more than 3. For SYNERGY-A and ERADICATE, the average number of sofosbuvir-ledipasvir pills taken by patients in each group was also calculated.

In SPARE, attendance at study visits for all participants was monitored. In addition, pharmacokinetics and pharmacodynamics of sofosbuvir and its metabolite GS-331007 were obtained and calculated on 25 participants. Levels in serum were measured at 0, 1, 2, 4, 8, 12, 24, 36 h and at 14 d after administration of sofosbuvir and ribavirin using a high-performance liquid

**Table 1** Baseline demographics of participants with mental health disease treated with ledipasvir-sofosbuvir *n* (%)

	SPARE ( <i>n</i> = 23)	SYNERGY A ( <i>n</i> = 7)	ERADICATE ( <i>n</i> = 15)	<i>P</i> value
Demographic				
Age, mean ± SD	54 ± 6	52 ± 10	56 ± 8	0.44
Male gender	14 (61)	5 (71)	9 (60)	0.86
Race or ethnicity				0.68
White	4 (17)	2 (29)	2 (13)	-
Black	19 (83)	5 (71)	13 (87)	-
Hispanic	0	0	0	-
HCV genotype 1 subtype				0.46
1A	18 (78)	4 (57)	12 (80)	-
1B	5 (22)	3 (43)	3 (20)	-
HIV +	0 (0)	0 (0)	15 (100)	< 0.0001
Mental health disorder				
Depression	14 (61)	4 (57)	8 (53)	0.89
Anxiety	4 (17)	3 (43)	1 (7)	0.12
Bipolar disorder	4 (17)	3 (43)	5 (33)	0.32
Post-traumatic stress disorder	2 (7)	1 (14)	3 (20)	0.60
Schizophrenia	0	1 (14)	0	-

HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

chromatography mass spectrometry bioanalytical technique (QPS LLC) as previously described<sup>[19]</sup>.

### Change in BDI among patients with HIV/HCV coinfection

PIFNPK and ALBIN were open label, non-randomized studies designed to look at the safety, toxicity, pharmacokinetics, and efficacy of interferon in combination with ribavirin. In the PIFNPK study, peginterferon alfa-2a 180 µg twice weekly (Pegasys; Roche Laboratories, Palo Alto, CA), was evaluated. In the ALBIN study, Alb-interferon (albumin/interferon alfa 2b fusion protein 900 µg subcutaneous injection every two weeks, Peg-Intron; Schering-Plough) was evaluated. Eligibility criteria as previously published<sup>[26-28]</sup>.

BDI scores were collected prior to treatment (baseline), during treatment, and one to eight weeks post-end of treatment on participants with HIV/HCV co-infection from ERADICATE, PIFNPK, and ALBIN. BDI scores for all participants, and those who achieved SVR were evaluated.

### Statistical analysis

Statistical differences in mean and observed frequencies for both aims were analyzed by Fisher's Exact, and *t*-test with significance defined as a *P* value less than 0.05. Changes in observed frequencies in SVR and adherence between participants with MHD and those without for aim 1 were analyzed by Fisher's Exact test within each study. Analyses were performed using PRISM 6.0 (GraphPad).

## RESULTS

### SVR and adherence among patients with MHD treated with sofosbuvir-based therapy

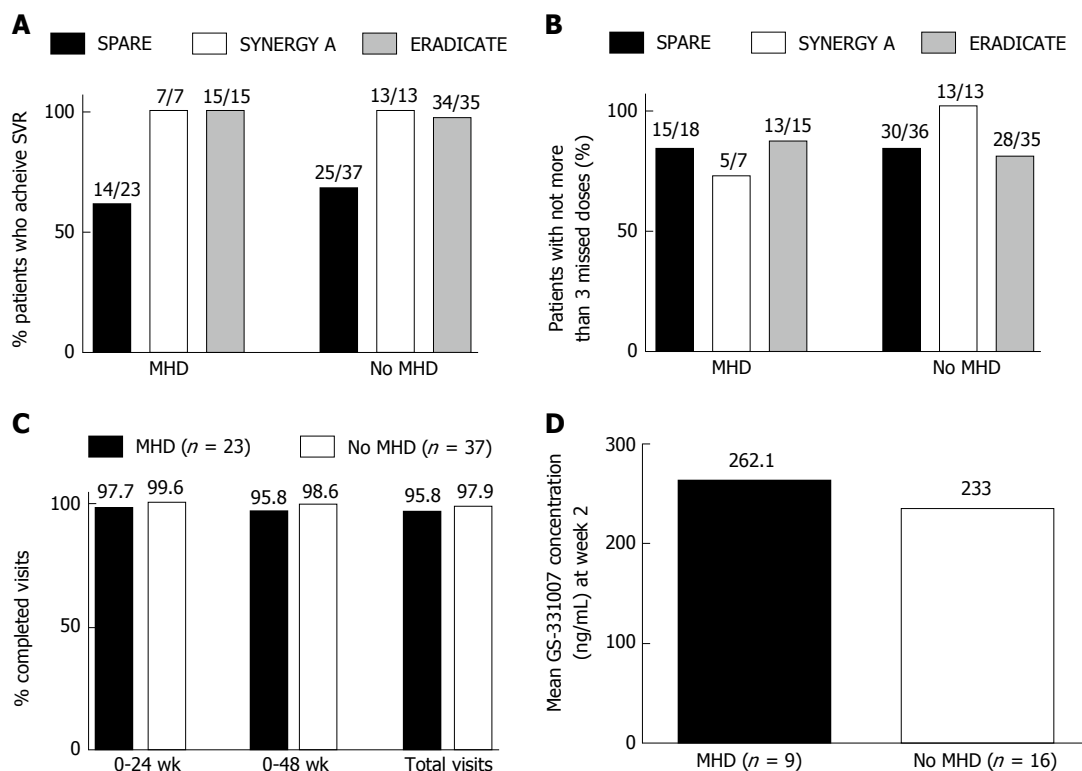
**Baseline demographics:** In all 3 studies, differences in baseline characteristics were not statistically significant between treatment groups (Table 1). The average age

was 50-60 years, and participants were predominantly African American males with GT-1a genotype.

**Mental health disease:** Thirty-eight percent of participants in SPARE, 35% in SYNERGY-A, and 30% in ERADICATE were classified as having baseline MHD. The prevalence of disorders for each study is shown in Table 1. Depression was the most common disorder and many participants had more than one diagnosis.

**Treatment outcome:** In all 3 studies the percentage of patients with MHD who achieved SVR was not statistically different from those without MHD (Figure 1A). In SPARE, 60.9% of those with MHD achieved SVR compared to 67.6% in those without (*P* = 0.78). In SYNERGY-A, 100% of both groups achieved SVR and in ERADICATE 100% of those with and 97.1% of those without MHD achieved SVR.

**Patient adherence:** (1) pill counts; in SYNERGY-A those with MHD and those without took, on average, 96.9% and 98.9% of their pills, respectively (*P* = 0.5). Both groups in ERADICATE took, on average, 97.8% of their pills. There was no statistically significant difference between groups with respect to the numbers that completed treatment with no more than 3 missed pills (Figure 1B): In SPARE, 83% in both groups completed treatment with no more than 3 missed pills. In SYNERGY-A, 5 of the 7 patients (71%) with MHD missed no more than 3 pills compared to 13 out of 13 (100%) among those without MHD (*P* = 0.11). In ERADICATE 13 out of 15 (87%) of those with MHD compared to 28 out of 35 (80%) of those without completed treatment with no more than 3 missed pills (*P* = 0.7); (2) adherence to study visit-SPARE; there was no difference in the proportion of patients with MHD and those without who completed the total required visits (95.8% vs 97.9%,



**Figure 1** Sustained virologic response and measures of adherence among participants with and without mental health disease. A: Sustained virologic response (SVR) achieved (ERADICATE, SYNERGY-A, SPARE). The comparisons of achieved SVR between groups within each study showed no significant differences for those with mental health disease (MHD) from those without MHD; B: Patients with not more than 3 missed doses (ERADICATE, SYNERGY-A, SPARE). The comparisons of adherence tracked by number of patients who had 3 or fewer total missed doses by pill count showed no significant differences for those with MHD from those without MHD in each study; C: Visit adherence (SPARE). Comparisons of total numbers of required visits completed showed no difference between those with MHD and those without during treatment (0-24 wk), through SVR24 (0-48 wk) and overall ( $P = 0.12$ ); D: GS-331007 concentration at week 2 (SPARE). Comparisons of mean GS-331007 concentration showed no difference between those with MHD and those without at week 2 of treatment ( $P = 0.72$ ).

$P = 0.12$ ) (Figure 1C); and (3) Serum levels of GS-331007-SPARE; We obtained pharmacodynamic and pharmacokinetic data for GS-331007 from 25 patients. Nine had MHD compared to 16 without. There was no statistically significant difference in the mean serum concentrations of GS-331007 for each group at week 2 of treatment ( $P = 0.72$ ) (Figure 1D).

#### BDI scores among patients with HIV/HCV co-infection treated with sofosbuvir- and IFN-based therapy

Table 2 shows the baseline characteristics of the patients treated with ledipasvir-sofosbuvir and IFN-based therapy. Age and sex were similar in both groups; however, the ledipasvir-sofosbuvir-treatment group included more African-American and fewer patients with late stage 2-4 disease. While more participants had baseline MHD in the IFN-based treatment group, baseline BDI scores were similar among the patients of both treatment groups (ledipasvir-sofosbuvir  $5.24 \pm \text{SD } 5.48$ , IFN-based  $6.96 \pm \text{SD } 8.67$ ;  $P = 0.14$ ).

#### BDI scores among all patients

**Patients treated with ledipasvir-sofosbuvir:** Mean BDI scores decreased from 5.24 at baseline to 3.28 during treatment (1.96 decrease,  $\pm \text{SD } 4.50$ ,  $P = 0.0034$ ) and 2.82 post-treatment. The decrease in

mean score from baseline to post-treatment was also statistically significant ( $-2.42 \pm \text{SD } 4.99$ ,  $P = 0.0012$ , Figure 2A).

**Patients treated with IFN:** Mean BDI score increased from 6.96 at day zero to 9.19 during treatment. This change was not statistically significant (an increase of  $2.46 \pm \text{SD } 8.96$ ;  $P = 0.1$ ), and then decreased back to baseline post-treatment (mean BDI score  $6.3 \pm \text{SD } 7.91$ ;  $P = 0.54$ , Figure 2A).

**Comparison of change in BDI scores from baseline to during and post-treatment:** The overall change in BDI scores from baseline to during treatment among patients treated with ledipasvir-sofosbuvir ( $-1.96 \pm \text{SD } 4.5$ ) compared to the change among those treated with IFN ( $+2.23 \pm \text{SD } 7.5$ ) was statistically significant ( $P = 0.0032$ , Figure 2A). However, change from baseline to post-treatment was not statistically significant ( $-2.42 \pm \text{SD } 4.99$  vs  $-0.65 \pm \text{SD } 6.24$ ;  $P = 0.18$ ).

#### BDI scores among patients who achieved SVR

**Patients treated with ledipasvir-sofosbuvir who achieved SVR:** Forty-nine out of the 50 (98%) patients treated achieved SVR. Baseline BDI was  $5.35 \pm \text{SD } 5.48$ . Mean BDI decreased to  $3.35 \pm \text{SD } 4.75$  during treatment

**Table 2** Baseline demographics of participants with hepatitis C/human immunodeficiency virus coinfection who had baseline, during, and post-treatment Beck's Depression Inventory scoring analyzed *n* (%)

Demographic	Sofosbuvir-based therapy ( <i>n</i> = 50)	Interferon-based therapy ( <i>n</i> = 26)	<i>P</i> value
Age, median	58	47	
Male	37 (74)	22 (84)	0.79
African American	42 (84)	11 (42)	0.0004
Fibrosis			0.00132
F0-1	35 (70)	10 (38)	
F2-4	15 (30)	16 (62)	
SVR	49 (98)	13 (50)	0.0001
Baseline mental health disease	15 (30)	15 (58)	0.0264

SVR: Sustained virologic response.

(an overall change of  $-2 \pm \text{SD } 4.45$ ,  $P = 0.0034$ ), and further decreased to  $2.88 \pm \text{SD } 5$  post-treatment. The change in mean BDI from baseline to post-treatment was statistically significant ( $-2.47 \pm \text{SD } 5.03$ ,  $P = 0.0012$ ).

**Patients treated with IFN and achieved SVR:** Eleven out of 26 (42%) patients achieved SVR. Among these participants, mean baseline BDI was  $4.55 \pm \text{SD } 4.48$ . This increased to  $8.91 \pm \text{SD } 7.67$  during treatment ( $P = 0.07$ ) and then returned to baseline of  $5.81 \pm \text{SD } 6.4$ .

#### Comparison of change in BDI scores from baseline to during and post-treatment among patients who achieved SVR

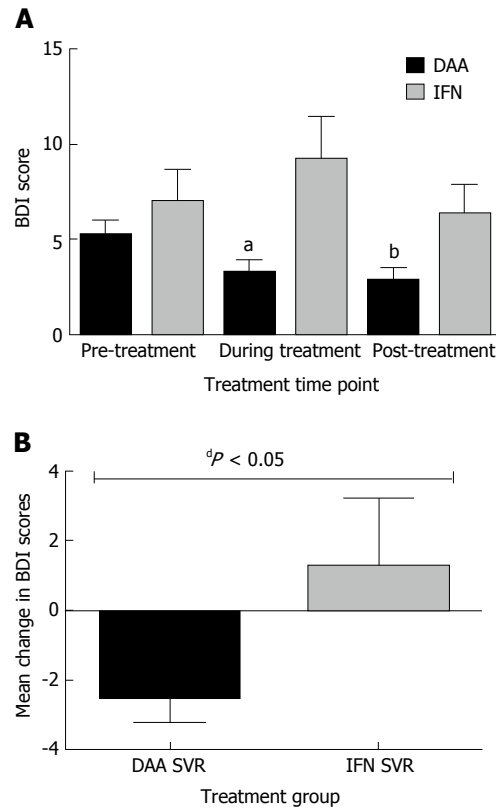
Mean BDI among participants treated with ledipasvir-sofosbuvir decreased  $2 \pm \text{SD } 4.54$  from baseline to during-treatment, whereas among participants treated with IFN-based therapy, mean BDI increased by  $4.36 \pm \text{SD } 7.2$ . This difference in changes in mean BDI scores was statistically significant ( $P = 0.0004$ ).

Similarly, the difference in change in mean BDI from baseline to post-treatment was also statistically significant ( $-2.47 \pm \text{SD } 5.03$ , compared to  $+1.27 \pm \text{SD } 6.36$ ,  $P = 0.038$ ).

## DISCUSSION

Sofosbuvir-based therapy was equally effective among participants with MHD, and among those without MHD. Patients with baseline MHD demonstrated similar levels of adherence with study visits, study drugs, and achieved similar rates of SVR to those who did not have a baseline MHD diagnosis. Furthermore, among participants with HIV/HCV coinfection treated with DAA therapy, we observed a statistically significant improvement in BDI scores during and after the end of treatment time point (post-treatment) compared to baseline (pre-treatment), while participants treated with IFN-based therapy saw no significant change in BDI scores.

In a study of 4084 United States veterans, psychiatric disease was identified as a predictor of non-treatment (odds ratio = 9.45)<sup>[29]</sup>. Furthermore, due to shared



**Figure 2** Beck's Depression Inventory scores among participants with hepatitis C/human immunodeficiency virus coinfection and treated with ledipasvir-sofosbuvir and interferon-based therapy. A: Mean BDI scores pre-, during, and post-treatment among all participants; Decrease in mean score from baseline to during treatment, <sup>a</sup> $P = 0.0034$ , DAA vs IFN; Decrease in mean score from baseline to post-treatment, <sup>b</sup> $P = 0.0012$ , DAA vs IFN; B: Change in mean BDI scores from baseline to post-treatment among participants who achieved SVR, <sup>d</sup> $P = 0.0004$ , DAA vs IFN. SVR: Sustained virologic response; BDI: Beck's Depression Inventory; DAA: Directly acting antiviral; IFN: Interferon.

transmission routes, HIV/HCV coinfection can be high among certain cohorts. A cross sectional study of a large cohort of patients with HIV found that 21% were coinfecting with HCV. Among these patients with HIV/HCV coinfection, depression severity scores were higher, and antidepressant medications were more often prescribed, compared to patients with HIV mono-infection<sup>[30]</sup>. Concern for emergent or worsening of neuropsychiatric side effects associated with interferon-based HCV therapy resulted in treatment deferment even for patients with stable MHD<sup>[14,31,32]</sup>. The treatment of chronic HCV, however, has evolved to interferon-free, all-oral, DAA regimens. Concerns regarding adherence to and subsequent success with DAA regimens among patients with MHD remains to be addressed.

This study has 2 aims: Firstly, to address the impact of baseline MHD on adherence to and subsequent success with DAA regimens among patients with MHD; and secondly, to describe the change in BDI scores among patients treated with a sofosbuvir-based regimen (ledipasvir-sofosbuvir) and compare this to the change among patients treated with IFN-based therapy.

In the current study, we combined results from three studies using interferon-free regimens. We compared the



effect of MHD on outcome (SVR) and three modalities of adherence (pill count, study visits and serum levels of GS-331007). The prevalence of MHD among the 3 studies (approximately 35%) was comparable to the baseline prevalence reported in other studies<sup>[7,10-14]</sup>. This study demonstrates that patients with MHD can achieve SVR at rates comparable to those without MHD. Six patients from SPARE did not complete treatment, 5 of which were identified as suffering from MHD but only one discontinuation from study could be attributed to MHD as determined by evaluation by the principal investigator. This suggests that these interferon-free regimens did not affect adherence and subsequent efficacy of therapy.

For our second aim, we analyzed baseline pre-treatment, during treatment, and post-treatment BDI scores among HIV/HCV coinfecting participants treated with a sofosbuvir-based regimen (ledipasvir-sofosbuvir) in the ERADICATE study and coinfecting participants treated with IFN-based therapy (PFINPK and ALBIN). Mean changes from baseline to during and to post-treatment were compared within, and between, each treatment group. We demonstrate that despite similar baseline BDI scores in both treatment groups, participants treated with ledipasvir-sofosbuvir saw a decrease in BDI scores during treatment and post-treatment compared to baseline. However, participants treated with IFN-based therapy did not see any change in BDI scores. In fact, when treatment groups were compared to each other and adjusted for participants who achieved SVR, the overall decrease in BDI score from baseline to post-treatment among participants treated with ledipasvir-sofosbuvir was significantly different compared to the overall increase in BDI score among participants treated with IFN-based therapy. It is conceivable that successful treatment of HCV alone should be associated with improvement in mental health. However, our findings suggest that sofosbuvir-based (and possibly any IFN-free) anti-HCV therapy may have additional mental health benefits beyond end of treatment.

This study is strengthened by multiple measures of adherence: Pill counts for all 3 studies; and in SPARE study visits with the additional objective measure of serum levels of the sofosbuvir metabolite GS-331007 at week 2 of therapy. Adherence to oral DAA therapy was high in all 3 studies, regardless of baseline MHD status, with no significant differences in pill counts, study visits, and serum GS-331007 levels between those with and without MHD. Whether the high adherence was a consequence of participant selection and the more intensive adherence interventions and counseling that are inherent to clinical trials, or related to the improved tolerability and ease of administration of the medications cannot be determined.

Limitations of this study include its small sample sizes for the three patient groups analyzed and therefore may not be sufficiently powered to detect differences. This did not allow for addressing the hypothesis that a regimen of fewer pills for shorter duration (1 pill once a day in ERADICATE and SYNERGY-A for 12 wk

compared to several pills a day for 24 wk in SPARE) was associated with significantly higher adherence. Furthermore, combining more than one study did result in a non-homogenous study population, most evident in the difference in baseline fibrosis stage among the participants undergoing BDI evaluation. Finally, this study did not include patients with severe, uncontrolled MHD therefore may have been biased towards those who already had a background of good adherence or lower BDI scores.

We hope that these preliminary findings will open the dialogue to further expand eligibility to those with MHD and lead to further, larger studies involving patients with more challenging characteristics: Not just those with baseline MHD, but also those with substance abuse - two diagnoses which are frequently paired<sup>[5-8]</sup>. Inclusion of these marginalized groups will be necessary if we are to gain an advantage in the battle to eradicate hepatitis C.

In conclusion, our study supports that patients with baseline MHD can be successfully engaged and treated with DAA therapies, and that sofosbuvir-based therapy is associated with improvement in BDI scores.

## COMMENTS

### Background

The treatment of chronic hepatitis C has evolved from interferon-based to direct acting antiviral-based therapy, with high tolerability and efficacy. Much focus has now shifted to increasing access to these new agents. However, the prevalence of mental health disease (MHD) is high among patients with hepatitis C and MHD is one of the most frequently cited reasons for withholding hepatitis C therapy.

### Research frontiers

The author's group focuses on hepatitis C eradication strategies among urban populations with unique patient populations that are predominantly African American, with advanced liver fibrosis, and high prevalence of human immunodeficiency virus co-infection and MHD. The authors believe that patients with MHD can be safely and effectively treated for hepatitis C with direct acting antiviral therapy. The findings of this study supports this hypothesis.

### Innovations and breakthroughs

Patients with mental health diseases may be excluded from hepatitis C therapy due to concerns for exacerbation of psychiatric complications, adherence, concurrent substance abuse, and reinfection due to continued high-risk behaviors. Hepatitis C therapy has evolved from interferon-based to direct acting antiviral agents, which are associated with minimal side effects. However, the high cost of these agents has led to restricted access to these medications. This study addresses the concerns regarding adherence to and subsequent success with directly acting antiviral regimens among patients with MHD. Furthermore, the findings of this study suggest that sofosbuvir-based therapy may be associated with improvements in Beck's Depression Inventory (BDI) scores.

### Applications

The findings of this study supports policy change to increase eligibility and access to hepatitis C therapy with new direct acting antiviral therapies among patients with mental health disease.

### Terminology

Sofosbuvir is a direct acting antiviral nucleotide inhibitor that acts upon the hepatitis C NS5B polymerase, preventing viral replication, and is the backbone to several hepatitis C treatment regimens. BDI is a multiple-choice tool for

measuring the severity of depression.

## Peer-review

Study of mental health in hepatitis C virus treated patients is considered to be a good practical point.

## REFERENCES

- 1 **Mohd Hanafiah K**, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]
- 2 **Armstrong GL**, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705-714 [PMID: 16702586 DOI: 10.7326/0003-4819-144-10-2006-05160-00004]
- 3 **Kim WR**. The burden of hepatitis C in the United States. *Hepatology* 2002; **36**: S30-S34 [PMID: 12407574 DOI: 10.1002/hep.1840360705]
- 4 **Davis GL**, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology* 2010; **138**: 513-521, 521.e1-e6 [PMID: 19861128 DOI: 10.1053/j.gastro.2009.09.067]
- 5 **Loftis JM**, Matthews AM, Hauser P. Psychiatric and substance use disorders in individuals with hepatitis C: epidemiology and management. *Drugs* 2006; **66**: 155-174 [PMID: 16451091 DOI: 10.2165/00003495-200666020-00003]
- 6 **Fireman M**, Indest DW, Blackwell A, Whitehead AJ, Hauser P. Addressing tri-morbidity (hepatitis C, psychiatric disorders, and substance use): the importance of routine mental health screening as a component of a comanagement model of care. *Clin Infect Dis* 2005; **40** Suppl 5: S286-S291 [PMID: 15768336 DOI: 10.1086/427442]
- 7 **el-Serag HB**, Kunik M, Richardson P, Rabeneck L. Psychiatric disorders among veterans with hepatitis C infection. *Gastroenterology* 2002; **123**: 476-482 [PMID: 12145801 DOI: 10.1053/gast.2002.34750]
- 8 **Sockalingam S**, Blank D, Banga CA, Mason K, Dodd Z, Powis J. A novel program for treating patients with trimorbidity: hepatitis C, serious mental illness, and active substance use. *Eur J Gastroenterol Hepatol* 2013; **25**: 1377-1384 [PMID: 23680911 DOI: 10.1097/MEG.0b013e3283624a28]
- 9 **Rosenberg SD**, Goodman LA, Osher FC, Swartz MS, Essock SM, Butterfield MI, Constantine NT, Wolford GL, Salyers MP. Prevalence of HIV, hepatitis B, and hepatitis C in people with severe mental illness. *Am J Public Health* 2001; **91**: 31-37 [PMID: 11189820 DOI: 10.2105/AJPH.91.1.31]
- 10 **Tavakkoli M**, Ferrando SJ, Rabkin J, Marks K, Talal AH. Depression and fatigue in chronic hepatitis C patients with and without HIV co-infection. *Psychosomatics* 2013; **54**: 466-471 [PMID: 23756122 DOI: 10.1016/j.psych.2013.02.009]
- 11 **Falck-Ytter Y**, Kale H, Mullen KD, Sarbah SA, Sorescu L, McCullough AJ. Surprisingly small effect of antiviral treatment in patients with hepatitis C. *Ann Intern Med* 2002; **136**: 288-292 [PMID: 11848726 DOI: 10.7326/0003-4819-136-4-200202190-00008]
- 12 **Yovtcheva SP**, Rifai MA, Moles JK, Van der Linden BJ. Psychiatric comorbidity among hepatitis C-positive patients. *Psychosomatics* 2001; **42**: 411-415 [PMID: 11739908 DOI: 10.1176/appi.psy.42.5.411]
- 13 **Rowan PJ**, Tabasi S, Abdul-Latif M, Kunik ME, El-Serag HB. Psychosocial factors are the most common contraindications for antiviral therapy at initial evaluation in veterans with chronic hepatitis C. *J Clin Gastroenterol* 2004; **38**: 530-534 [PMID: 15220690 DOI: 10.1097/01.mcg.0000123203.36471.70]
- 14 **Wu JY**, Shadbolt B, Teoh N, Blunn A, To C, Rodriguez-Morales I, Chitturi S, Kaye G, Rodrigo K, Farrell G. Influence of psychiatric diagnosis on treatment uptake and interferon side effects in patients with hepatitis C. *J Gastroenterol Hepatol* 2014; **29**: 1258-1264 [PMID: 24955454 DOI: 10.1111/jgh.12515]
- 15 **Sherman KE**, Flamm SL, Afdhal NH, Nelson DR, Sulkowski MS, Everson GT, Fried MW, Adler M, Reesink HW, Martin M, Sankoh AJ, Adda N, Kauffman RS, George S, Wright CI, Poordad F. Response-guided telaprevir combination treatment for hepatitis C virus infection. *N Engl J Med* 2011; **365**: 1014-1024 [PMID: 21916639 DOI: 10.1056/NEJMoa1014463]
- 16 **Kwo PY**, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, Galati JS, Gordon SC, Ravendhran N, Rossaro L, Anderson FH, Jacobson IM, Rubin R, Koury K, Pedicone LD, Brass CA, Chaudhri E, Albrecht JK. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naïve patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010; **376**: 705-716 [PMID: 20692693 DOI: 10.1016/S0140-6736(10)60934-8]
- 17 **Kelly EM**, Corace K, Emery J, Cooper CL. Bipolar patients can safely and successfully receive interferon-based hepatitis C antiviral treatment. *Eur J Gastroenterol Hepatol* 2012; **24**: 811-816 [PMID: 22495398 DOI: 10.1097/MEG.0b013e3283535c56]
- 18 **Kohli A**, Osinusi A, Sims Z, Nelson A, Meissner EG, Barrett LL, Bon D, Marti MM, Silk R, Kotb C, Gross C, Jolley TA, Sidharthan S, Petersen T, Townsend K, Egerson D, Kapoor R, Spurlin E, Sneller M, Proschan M, Herrmann E, Kwan R, Teferi G, Talwani R, Diaz G, Kleiner DE, Wood BJ, Chavez J, Abbott S, Symonds WT, Subramanian GM, Pang PS, McHutchison J, Polis MA, Fauci AS, Masur H, Kottitil S. Virological response after 6 week triple-drug regimens for hepatitis C: a proof-of-concept phase 2A cohort study. *Lancet* 2015; **385**: 1107-1113 [PMID: 25591505 DOI: 10.1016/S0140-6736(14)61228-9]
- 19 **Osinusi A**, Meissner EG, Lee YJ, Bon D, Heytens L, Nelson A, Sneller M, Kohli A, Barrett L, Proschan M, Herrmann E, Shivakumar B, Gu W, Kwan R, Teferi G, Talwani R, Silk R, Kotb C, Wroblewski S, Fishbein D, Dewar R, Highbarger H, Zhang X, Kleiner D, Wood BJ, Chavez J, Symonds WT, Subramanian M, McHutchison J, Polis MA, Fauci AS, Masur H, Kottitil S. Sofosbuvir and ribavirin for hepatitis C genotype 1 in patients with unfavorable treatment characteristics: a randomized clinical trial. *JAMA* 2013; **310**: 804-811 [PMID: 23982366 DOI: 10.1001/jama.2013.109309]
- 20 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]
- 21 **Lawitz E**, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naïve and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; **383**: 515-523 [PMID: 24209977 DOI: 10.1016/S0140-6736(13)62121-2]
- 22 **Lawitz E**, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, DeJesus E, Pearlman B, Rabinovitz M, Gitlin N, Lim JK, Pockros PJ, Scott JD, Fevery B, Lambrecht T, Ouwerkerk-Mahadevan S, Callewaert K, Symonds WT, Picchio G, Lindsay KL, Beumont M, Jacobson IM. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naïve patients: the COSMOS randomised study. *Lancet* 2014; **384**: 1756-1765 [PMID: 25078309 DOI: 10.1016/S0140-6736(14)61036-9]
- 23 **Sulkowski MS**, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, McPhee F, Sherman D, Hindes R, Symonds W, Pasquinelli C, Grasela DM. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV

- infection. *N Engl J Med* 2014; **370**: 211-221 [PMID: 24428467 DOI: 10.1056/NEJMoa1306218]
- 24 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]
- 25 **Osinusi A**, Townsend K, Kohli A, Nelson A, Seamon C, Meissner EG, Bon D, Silk R, Gross C, Price A, Sajadi M, Sidharthan S, Sims Z, Herrmann E, Hogan J, Teferi G, Talwani R, Proschan M, Jenkins V, Kleiner DE, Wood BJ, Subramanian GM, Pang PS, McHutchison JG, Polis MA, Fauci AS, Masur H, Kottlilil S. Virologic response following combined ledipasvir and sofosbuvir administration in patients with HCV genotype 1 and HIV co-infection. *JAMA* 2015; **313**: 1232-1239 [PMID: 25706232 DOI: 10.1001/jama.2015.1373]
- 26 **Murphy AA**, Herrmann E, Osinusi AO, Wu L, Sachau W, Lempicki RA, Yang J, Chung TL, Wood BJ, Haagmans BL, Kottlilil S, Polis MA. Twice-weekly pegylated interferon- $\alpha$ -2a and ribavirin results in superior viral kinetics in HIV/hepatitis C virus co-infected patients compared to standard therapy. *AIDS* 2011; **25**: 1179-1187 [PMID: 21593619 DOI: 10.1097/QAD.0b013e3283471d53]
- 27 **Osinusi A**, Bon D, Nelson A, Lee YJ, Poonia S, Shivakumar B, Cai SY, Wood B, Haagmans B, Lempicki R, Herrmann E, Sneller M, Polis M, Masur H, Kottlilil S. Comparative efficacy, pharmacokinetic, pharmacodynamic activity, and interferon stimulated gene expression of different interferon formulations in HIV/HCV genotype-1 infected patients. *J Med Virol* 2014; **86**: 177-185 [PMID: 24166150 DOI: 10.1002/jmv.23773]
- 28 **Osinusi A**, Rasimas JJ, Bishop R, Proschan M, McLaughlin M, Murphy A, Cortez KJ, Polis MA, Masur H, Rosenstein D, Kottlilil S. HIV/Hepatitis C virus-coinfected virologic responders to pegylated interferon and ribavirin therapy more frequently incur interferon-related adverse events than nonresponders do. *J Acquir Immune Defic Syndr* 2010; **53**: 357-363 [PMID: 20101190 DOI: 10.1097/QAI.0b013e3281c7a29d]
- 29 **Bini EJ**, Bräu N, Currie S, Shen H, Anand BS, Hu KQ, Jeffers L, Ho SB, Johnson D, Schmidt WN, King P, Cheung R, Morgan TR, Awad J, Pedrosa M, Chang KM, Aytaman A, Simon F, Hagedorn C, Moseley R, Ahmad J, Mendenhall C, Waters B, Strader D, Sasaki AW, Rossi S, Wright TL. Prospective multicenter study of eligibility for antiviral therapy among 4,084 U.S. veterans with chronic hepatitis C virus infection. *Am J Gastroenterol* 2005; **100**: 1772-1779 [PMID: 16086714 DOI: 10.1111/j.1572-0241.2005.41860.x]
- 30 **Yoon JC**, Crane PK, Ciechanowski PS, Harrington RD, Kitahata MM, Crane HM. Somatic symptoms and the association between hepatitis C infection and depression in HIV-infected patients. *AIDS Care* 2011; **23**: 1208-1218 [PMID: 21562994 DOI: 10.1080/09540121.2011.555739]
- 31 **Lim C**, Olson J, Zaman A, Phelps J, Ingram KD. Prevalence and impact of manic traits in depressed patients initiating interferon therapy for chronic hepatitis C infection. *J Clin Gastroenterol* 2010; **44**: e141-e146 [PMID: 20495465 DOI: 10.1097/MCG.0b013e3281dc24f8]
- 32 **Alavi M**, Grebely J, Matthews GV, Petoumenos K, Yeung B, Day C, Lloyd AR, Van Beek I, Kaldor JM, Hellard M, Dore GJ, Haber PS. Effect of pegylated interferon- $\alpha$ -2a treatment on mental health during recent hepatitis C virus infection. *J Gastroenterol Hepatol* 2012; **27**: 957-965 [PMID: 22142332 DOI: 10.1111/j.1440-1746.2011.07035.x]

**P- Reviewer:** Garcia-Olmo D, Marchan-Lopez A, Shiha G

**S- Editor:** Yu J **L- Editor:** A **E- Editor:** Li D



## Retrospective Study

# Liver resection for early hepatocellular cancer: Comparison of centers in 3 different countries

Linda L Wong, Brenda Y Hernandez, Yurii B Shvetsov, Yoichi Kawano, Zhao-You Tang, Jun-Fang Ji

Linda L Wong, Brenda Y Hernandez, Yurii B Shvetsov, Cancer Center, Department of Surgery, John A Burns School of Medicine, University of Hawaii, Honolulu, HI 96813, United States

Yoichi Kawano, Department of Surgery, Nippon Medical School, Tokyo 113-0022, Japan

Zhao-You Tang, Liver Cancer Institute and Zhongshan Hospital, Fudan University, Shanghai 200043, China

Jun-Fang Ji, Life Sciences Institute, Zhejiang University, Hangzhou 310058, Zhejiang Province, China

**Author contributions:** All the authors contributed to the manuscript.

**Institutional review board statement:** This study was reviewed and approved by the University of Hawaii Institutional Review Board. Data from Shanghai is from a cohort in which the anonymous data is publically available. Data from the Nippon Medical Center did not require Institutional review as it is retrospective anonymous data.

**Informed consent statement:** This is not applicable since this is a retrospective study. Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

**Conflict-of-interest statement:** Dr Wong is a speaker for Bayer Healthcare. The other authors have no conflicts of interest to declare.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

[licenses/by-nc/4.0/](#)

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Linda L Wong, MD, Professor, Cancer Center, Department of Surgery, John A Burns School of Medicine, University of Hawaii, 550 South Beretania Street, Suite 403, Honolulu, HI 96813, United States. [hepatoma@aol.com](mailto:hepatoma@aol.com)  
**Telephone:** +1-808-5235033  
**Fax:** +1-808-5284940

**Received:** May 13, 2016

**Peer-review started:** May 14, 2016

**First decision:** June 14, 2016

**Revised:** June 28, 2016

**Accepted:** August 15, 2016

**Article in press:** August 16, 2016

**Published online:** November 8, 2016

## Abstract

### AIM

To compare patients who underwent resection of early stage hepatocellular cancer (HCC) in three different countries.

### METHODS

This retrospective study characterizes 573 stage I / II HCC patients treated with liver resection in 3 tertiary-referral centers: Tokyo ( $n = 250$ ), Honolulu ( $n = 146$ ) and Shanghai ( $n = 177$ ).

### RESULTS

Shanghai patients were younger, predominantly male, hepatitis-B seropositive (94%) and cirrhotic (93%). Tokyo patients were older and more likely to have hepatitis-C (67%), smaller tumors, low albumin, and normal alpha-fetoprotein. The Honolulu cohort had the largest tumors and 30% had no viral hepatitis. Age-adjusted mortality at 1 and 5-years were lower in the



Tokyo cohort compared to Honolulu and there was no difference in mortality between Shanghai and Honolulu cohorts. Elevated alpha-fetoprotein, low albumin and tumor > 5 cm were associated with increased 1-year mortality. These factors and cirrhosis were independently associated with increased 5-year mortality. Independent risk factors of survival varied when examined separately by center.

### CONCLUSION

The profile of early-stage HCC patients is strikingly different across countries and likely contributes to survival differences. Underlying differences in patient populations including risk factors/comorbidities influencing disease progression may also account for variation in outcomes.

**Key words:** Hepatocellular cancer; Liver resection; Viral hepatitis

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Treatment for hepatocellular cancer (HCC) depends on stage and liver function. Single-institution studies have characterized resection for HCC but this unique study combines the experience of three large hepatobiliary centers in different countries with 573 resections for stage I / II HCC in Tokyo ( $n = 250$ ), Honolulu ( $n = 146$ ) and Shanghai ( $n = 177$ ). Groups differed in viral hepatitis, tumor size, alpha fetal protein (AFP) and cirrhosis. One and 5-year mortality was lowest in the Tokyo cohort. Elevated AFP, low albumin, tumor > 5 cm and cirrhosis were independently-associated with increased 5-year mortality. The profile of early-stage HCC patients is strikingly different across countries and likely contributes to survival differences.

Wong LL, Hernandez BY, Shvetsov YB, Kawano Y, Tang ZY, Ji JF. Liver resection for early hepatocellular cancer: Comparison of centers in 3 different countries. *World J Hepatol* 2016; 8(31): 1327-1335 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i31/1327.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i31.1327>

### INTRODUCTION

Hepatocellular cancer (HCC) is the fifth most common cancer in males and the ninth in females worldwide and is the second most deadly cancer. In 2012, there were 782000 HCC cases and 745000 deaths. HCC is more prominent in less-developed countries and more than 50% of cases were diagnosed in Asia<sup>[1]</sup>. In the United States, there were 35000 new cases of liver and intrahepatic bile duct cancer in 2015 and HCC is one of the few cancers that is increasing in both incidence and mortality<sup>[2]</sup>. The best treatments for early stage HCC include liver resection for those with adequate liver function and liver transplant for those with decompensated

cirrhosis or tumor that is not amenable to resection. Multiple single center studies have demonstrated success with liver resection and transplant, but patient populations largely differ in underlying risk factors (viral hepatitis, diabetes, obesity, alcohol and smoking) and may differ by technique of resection, indications for resection, patient management, use of adjuvant therapy, and follow-up<sup>[3-8]</sup>. The use of liver resection may also vary depending on the availability of liver transplantation. Countries with relatively limited donor liver availability or new transplant programs may depend more on resection for curative therapy. Because of limited donor livers, some countries, such as Japan, have made great efforts at developing successful surveillance and diagnosis programs that detect more than 60% of HCC at a very early stage<sup>[9]</sup>. Early detection allows more patients to undergo resection or liver-directed therapy such as local ablation with curative intent.

Because of potential differences in surveillance, tumor size, and available therapies, it is difficult to directly compare a particular therapy for HCC in different countries. The aim of the present study is to compare patient and clinical characteristics and survival of early (stage I , II ) HCC patients treated by resection in three different countries. These centers include large tertiary referral centers for HCC in Shanghai (China), Nippon (Japan) and Hawaii, the United States with the highest incidence of HCC.

### MATERIALS AND METHODS

This is a retrospective analysis of 573 liver resections performed in 3 tertiary referral centers for liver disease performed in 3 different countries.

#### Honolulu cohort (United States)

The Honolulu cohort consisted of 936 HCC cases referred between 1993 and 2014 to the only liver transplant program in Hawaii and the only referral center for liver disease/surgery for the American territories of the Pacific Basin (including Samoa, Guam, Saipan, and the Marshall Islands). Patients were primarily United States citizen of diverse racial/ethnic backgrounds including Whites, Asians, and Pacific Islanders but also included foreign nationals from Asian countries who sought medical care in the United States. Race/ethnicity and birthplace were assessed as risk factors for HCC were previously shown to vary by these demographic characteristics in this study population<sup>[10]</sup>. This clinic and the transplant center were initially affiliated with Hawaii Medical Center-East (formerly St. Francis Medical Center) and after 2012, the Queens Medical Center. This center sees about 60%-70% of the HCC cases in Hawaii. Liver resections were performed by a single group of hepatobiliary/transplant surgeons, with about 80% of these cases done by a single surgeon (LW).

HCC was confirmed histologically by percutaneous biopsy or at surgery. In the first decade, HCC consistent

with the previous United Network for Organ Sharing policy regarded transplant for HCC patients without biopsy. More recently, the diagnosis of HCC was made with only imaging if a dynamic contrast-enhanced study showed typical arterial enhancement with venous “washout” as described by the American Association for the Study of Liver Disease guidelines<sup>[11,12]</sup>.

Data collected included demographic data (age, sex, birthplace, self-reported ethnicity) and the presence of diabetes mellitus, hyperlipidemia, smoking, viral hepatitis, alcohol abuse, obesity and other chronic liver diseases. Laboratory data collected included bilirubin, albumin, prothrombin time, creatinine, alanine aminotransferase, aspartate aminotransferase, platelet count, Model for End-stage Liver Disease score and alpha fetal protein (AFP). The size, number, and location of the tumor(s) were used to determine the Tumor Node Metastases stage according to the American Joint Commission on Cancer (AJCC) staging manual<sup>[13]</sup>.

After excluding patients who presented with ruptured HCC and underwent embolization prior to resection, 146 HCC cases were included in the study. During this time period, 84 patients underwent liver transplant for HCC. This study was approved by the University of Hawaii Institutional Review Board.

#### **Shanghai cohort (China)**

The Shanghai cohort was comprised of 241 HCC cases diagnosed between 2002–2003 and followed for up to 70 mo. Patients were diagnosed and treated at Zhongshan Hospital (Fudan University) in Shanghai, China. Zhongshan Hospital is a major teaching hospital affiliated with the Ministry of Health of China. This is a 1700 bed medical facility that serves approximately 80000 inpatients and 3 million outpatients/emergency visits annually.

All patients were of Chinese ethnicity and were initially seen by medical organizations in the surrounding areas but the final diagnosis was made in this facility. Patients were diagnosed based on imaging criteria, as well as with a history of chronic viral hepatitis and elevated AFP. Three surgeons including Dr. Zhao-You Tang (author) performed all of the liver resections in this cohort. The diagnosis of HCC was confirmed by two independent pathologists.

The patient enrollment criteria included those with detailed information on clinical presentation and pathological characteristics; and detailed follow-up data for at least 3 years, which included recurrence-free survival, overall survival, as well as the cause of death. The detailed clinical presentation characteristics included but were not limited to sex, age, OKUDA staging, CLIP staging, BCLC staging, Child-Pugh score, TNM staging, multiple nodules, satellite nodule, tumor size, tumor capsule, cirrhosis, tumor thrombosis, lymph node, alanine transaminase, Albumin, international normalized ratio, hepatitis B surface (HBV) Ag, hepatitis C antibody, HBV viral status, pre-treatment AFP, preoperative therapy,

and postoperative other therapies. A majority of patients were long-term carriers of HBV (94%). The updated TNM classification was used in this cohort, and 177 early stage HCC patients (TNM stage I and II) with survival information were therefore chosen to perform comparison analysis in our study. Data for this cohort are publically available and have been used in many HCC translational research studies<sup>[14,15]</sup>.

#### **Tokyo cohort (Japan)**

The Tokyo cohort consisted of 504 HCC cases diagnosed between 1986 and 2014 in the Department of Surgery at Nippon Medical School, which has a primary medical center (1000 beds) and 3 smaller branch hospitals. Decisions on therapy were made by hepatologists and surgeons and all liver resections were performed at the primary medical center by members of a dedicated liver surgery team (10 hepatobiliary surgeons, surgical residents and medical students). Living-donor liver transplantation is done in this medical center, but only 15 cases have been done and no deceased-donor liver transplants were performed during this time period.

Although the treatment strategy has been changing in Japan, decisions on therapy were based on an algorithm for treatment of HCC reported by Makuuchi *et al.*<sup>[16,17]</sup>. This algorithm was based on three factors: Degree of liver damage (Childs A, B or C), number of tumors (single, 2–3 or 4 or more), and tumor diameter ( $\leq 3$  cm or  $> 3$  cm). Indications for surgery were according to modified-Makuuchi criteria incorporating the indocyanine green test<sup>[18]</sup>. The final diagnosis of HCC was histologically confirmed at surgery by a group of expert pathologists. Use of transplantation for HCC was extremely limited because of scarcity of organs from deceased donors. Hepatectomy is generally the first choice for Child-Pugh class A and selected class B cirrhotic patients.

In this cohort of 504 patients with HCC, the vast majority of patients were Japanese. The pre-operative diagnosis of HCC was made primarily with imaging and confirmed at resection. Liver biopsy prior to surgery was rarely performed. Data collected in this cohort included: Age, gender, HBV, hepatitis C virus (HCV), presence of coma, ascites, bilirubin, albumin, protime, AFP, Childs-Pugh class, presence of cirrhosis, stage, tumor size, recurrence and survival. Additional data that were collected but not used in this analysis included ICG (indocyanine green), AFP-LC, PIVKA, tumor differentiation, vascular invasion and details on the segments of liver that were removed. Of the 504 HCC patients in this cohort, 250 diagnosed at TNM stage I and II were included in the present analysis.

#### **Statistical analysis**

All analyses were conducted with SAS version 9.3 (SAS Institute, Inc., Cary NC). All *P*-values were two-sided, and *P* < 0.05 was defined as significant. Characteristics of Honolulu, Tokyo and Shanghai HCC patients were compared using generalized linear models (continuous variables) and  $\chi^2$  tests (categorical variables). Differences

**Table 1** Characteristics of resected stage 1 and 2 hepatocellular cancer patients: Honolulu, Tokyo and Shanghai

Characteristic	Honolulu ( <i>n</i> = 146)	Tokyo ( <i>n</i> = 250)	Shanghai ( <i>n</i> = 177)	<i>P</i> value
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	
Mean age in years	62.7 (SD 11.4)	67.0 (SD 8.7)	50.6 (SD 11.1)	< 0.0001
Age < 50 yr	18 (12.3)	8 (3.2)	83 (46.9)	< 0.0001
Males	100 (68.5)	180 (72.0)	145 (81.9)	0.01
Hepatitis B positive	62 (42.5)	35 (14.3)	167 (94.4)	< 0.0001
Hepatitis C positive	39 (26.7)	163 (66.8)	5 (3.3)	< 0.0001
Hepatitis B and C positive	5 (3.4)	4 (1.6)	3 (2.0)	0.50
Stage I	129 (88.4)	68 (27.2)	91 (51.4)	< 0.0001
Childs A	143 (99.3)	224 (89.6)	172 (97.2)	< 0.0001
Cirrhosis	60 (41.1)	133 (56.8)	163 (92.6)	< 0.0001
Mean tumor size (cm)	6.4 (SD 4.6)	3.0 (SD 2.1)	3.9 (SD 2.6)	< 0.0001
Tumor size < 5.0 cm	75 (51.4)	213 (85.2)	137 (77.4)	< 0.0001
AFP < 20 ng/mL	72 (49.3)	144 (57.6)	70 (39.6)	0.0011
Albumin < 3.5 g/dL	23 (15.8)	85 (34)	21 (11.9)	< 0.0001

Hepatitis B surface (*n* = 6); hepatitis C virus (*n* = 32); Child-Pugh (*n* = 2); cirrhosis (*n* = 17); albumin *n* = 17). AFP: Alpha fetal protein.

in HCC mortality among patients treated in Honolulu, Tokyo and Shanghai were examined using Kaplan-Meier estimates and Cox proportional hazards regression. Survival period was computed from the date of HCC diagnosis to the date of death from any cause. Patients alive at the end of the follow-up period were considered censored. The proportional hazard assumption for Cox models was checked by plotting scaled Schoenfeld residuals against time to event<sup>[19]</sup>. There was evidence of non-proportionality of hazards with respect to time. For this reason and due to the uneven follow-up period between the three centers, survival was partitioned into two time periods: Survival at 1 year after diagnosis and at 5 years following diagnosis were modeled separately. Analyses were adjusted for patients' age at time of diagnosis. Predictors of overall survival were also evaluated in 1-year and 5-year models. Univariate analyses were used to model age (< 50 year; ≥ 50 year), sex (male; female); stage (I; II); Child-Pugh Score (A; B); tumor size (< 5 cm; ≥ 5 cm) presence/absence of cirrhosis; AFP (< 20 ng/mL; ≥ 20 ng/mL); albumin levels (< 3.5 g/dL; ≥ 3.5 g/dL), HBV (positive; negative); and HCV (positive; negative). The three center locations were modeled as indicator variables with Honolulu as the reference. Along with age, factors found to be significant at the  $P \leq 0.10$  level in univariate analyses were included in the full multivariate models. A statistical review of the study was performed by a biomedical statistician.

## RESULTS

A total of 573 HCC patients diagnosed at AJCC stage I or II who underwent resection were included in the present analyses. Patients included 146 from Honolulu, 250 from Tokyo, and 177 from Shanghai. Patient and clinical characteristics varied widely across countries (Table 1). Patients were youngest in Shanghai and oldest in Tokyo ( $P < 0.0001$ ). Males comprised 82% of Shanghai patients, 72% of Tokyo cases, and 69% of Honolulu cases ( $P =$

0.01). HBV seropositivity was highest among Shanghai HCC cases (94%), followed by Honolulu (43%) and Tokyo (14%) cases ( $P < 0.0001$ ). Conversely, HCV seropositivity was highest among Tokyo cases (67%), followed by Honolulu (27%) and Shanghai (3%) patients ( $P < 0.0001$ ). Stage I cases were predominant in Honolulu (89%), compared to Shanghai (51%) and Tokyo (27%) cases ( $P < 0.0001$ ). Cirrhosis was present in most Shanghai cases (93%), compared to 57% of Tokyo and 41% of Honolulu cases ( $P < 0.0001$ ). Mean tumor size was largest in Honolulu cases (6.4 cm), compared to Shanghai and Tokyo cases (3.9 cm and 3.0 cm, respectively) ( $P < 0.0001$ ). Elevated AFP levels were present in 60% of Shanghai patients, 51% of Honolulu cases, and 42% of Tokyo patients ( $P = 0.001$ ). Abnormal albumin levels (< 3.5 g/dL) were present in 34% of Tokyo patients compared to 16% and 12% in the Honolulu and Shanghai cohorts, respectively ( $P < 0.0001$ ).

Overall, 1-year and 5-year mortality varied across the three centers (Figure 1). Thirty-day mortality was 2.8%, 1.6% and 0% for Honolulu, Tokyo and Shanghai groups, respectively. Mortality was compared across the three centers with Honolulu as the reference (Table 2). (Estimates adjusted for age at diagnosis only and additionally adjusted for the year of surgery were comparable. Therefore, estimates adjusted for age at diagnosis only are reported). During the 1-year survival and 5-year periods, Tokyo patients had lower mortality than those in Honolulu (age-adjusted HR = 0.28; 95%CI: 0.15-0.51 and age-adjusted HR = 0.70; 95%CI: 0.50-0.98, respectively). One-year and 5-year survival did not differ between the Shanghai and Honolulu cohorts.

Predictors of overall 1-year and 5-year survival were examined (Table 3). For 1-year survival, the multivariate model included age, AFP, tumor size, albumin and center as covariates. In the final multivariate model, the following were positively associated with increased risk of mortality at 1-year: AFP levels ≥ 20 (adjusted HR = 2.27; 95%CI: 1.32-3.90), tumor size ≥ 5 cm

**Table 2 Overall age-adjusted survival in resected stage 1 and 2 hepatocellular cancer patients: Honolulu, United States, Tokyo, Japan and Shanghai, China**

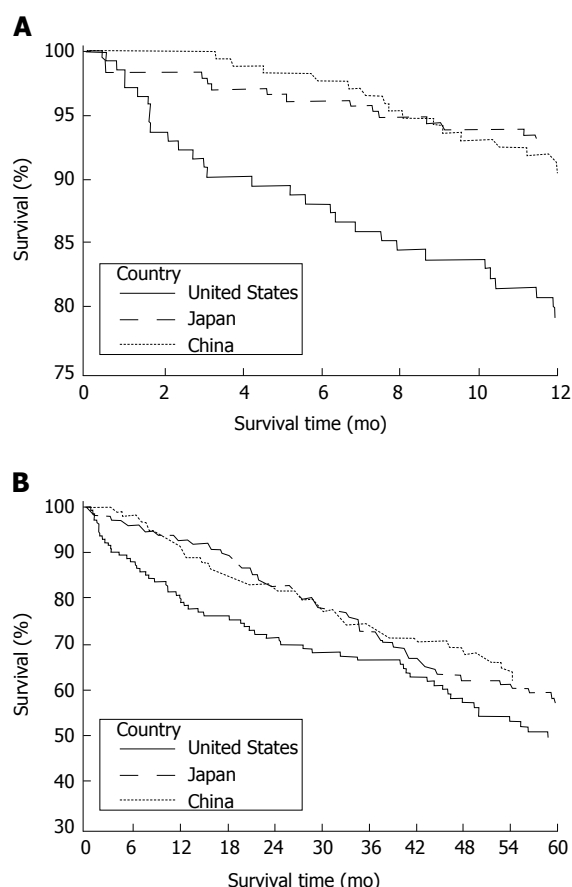
Center		Honolulu	Tokyo	Shanghai
No. patients		146	250	177
No. deaths		29	17	6
Mean follow-up (yr)		4.04	3.75	3.55
Median follow-up (yr)		3.33	2.83	4.36
1-yr survival	Hazard ratio <sup>1</sup>	1	0.28	0.63
	95%CI	Reference	0.15-0.51	0.32-1.21
	P value		< 0.0001	0.17
5-yr survival	Hazard ratio	1	0.7	0.74
	95%CI	Reference	0.50-0.98	0.51-1.09
	P value		0.04	0.13

<sup>1</sup>Age-adjusted.

(adjusted HR = 2.00; 95%CI: 1.17-3.43) and albumin < 3.5 g/dL (adjusted HR = 2.10; 95%CI: 1.20-3.68). The Tokyo cohort had lower 1-year mortality than Honolulu (adjusted HR = 0.33; 95%CI: 0.17-0.65). For 5-year survival, the multivariate model included age, stage, Child-Pugh Score, AFP, albumin, cirrhosis, tumor size, and center as covariates. In the final multivariate model, predictors of 5-year survival were AFP  $\geq$  20 (adjusted HR = 1.57; 95%CI: 1.17-2.10), cirrhosis (adjusted HR = 1.59; 95%CI: 1.12-2.26), tumor size  $\geq$  5 cm (adjusted HR = 1.84; 95%CI: 1.34-2.54), and albumin < 3.5 g/dL (adjusted HR = 1.72; 95%CI: 1.21-2.46). Both Tokyo and Shanghai centers had better 5-year survival than the Honolulu cohort (adjusted HR = 0.51; 95%CI: 0.33-0.78 and adjusted HR = 0.47; 95%CI: 0.31-0.72). Predictors of overall 1-year and 5-year survival were examined separately by center. In Honolulu, predictors of both 1-year and 5-year survival were AFP  $\geq$  20 (1-year: adjusted HR = 3.21; 95%CI: 1.36-7.56; 5-year: adjusted HR = 3.18; 95%CI: 1.35-7.51) and albumin < 3.5 g/dL (1-year: adjusted HR = 4.17; 95%CI: 1.92-9.04; 5-year: adjusted HR = 4.13; 95%CI: 1.90-8.98). In the Tokyo cohort, there were no significant predictors of 1-year survival. Five-year survival was associated with cirrhosis (adjusted HR = 1.90; 95%CI: 1.17-3.09) and tumor size  $\geq$  5 cm (adjusted HR = 2.29; 95%CI: 1.33-3.94). For the Shanghai cohort, 1-year mortality risk was associated with tumor size  $\geq$  5 cm (adjusted HR = 2.99; 95%CI: 1.14-7.80) and 5-year predictors included AJCC stage 2 (vs 1) (adjusted HR = 2.30; 95%CI: 1.35-3.92) and Child-Pugh Score (B vs A) (adjusted HR = 4.51; 95%CI: 1.59-12.81).

## DISCUSSION

Therapy for hepatocellular cancer has evolved and there are current practice guidelines based on Barcelona Clinic Liver Cancer (BCLC) staging<sup>[11,12]</sup>. These guidelines provide a general framework, but what occurs in the real world is likely center and country specific. In developed countries with resources to perform liver transplant, multiple studies have compared outcome between resection



**Figure 1 Overall survival analysis of hepatocellular cancer patients from Honolulu, Tokyo, and Shanghai.** A: Kaplan-Meier survival curves for one-year survival analysis; B: Kaplan-Meier survival curves for five-year survival analysis; A and B: Log-rank test were used for statistical analysis.

and transplant and those treated with transplant have better survival and less recurrence<sup>[7,20-30]</sup>. Nonetheless, widespread use of transplant is constrained by the availability of donor livers. Other reports compared liver resection and local ablation. Although ablation was effective especially for tumors less than 2.0 cm and can be performed with fewer complications, liver resections had better long-term, recurrence free-survival in some series<sup>[3,31-34]</sup>. Good short-term outcomes occur in small tumors, whether resected, ablated or transplanted, but recurrence rates, cost and donor livers are major factors in the decision-making. Worldwide, strategies to treat liver cancer have evolved based on the burden of liver cancer and the available resources in that particular area.

This is the first study that attempts to assess one surgical modality in 3 different countries, each of which has a high burden of disease, but different resources and treatment strategies. We chose liver resection because this was uniformly available and not dependent on technology or donor livers. Rather than comparing data in the form of a meta-analysis or systematic review, we developed a working relationship between the surgeons and scientists in these three centers. In this study, we selected only stage I / II HCC who underwent liver resection, in an attempt to make a comparison in as



**Table 3** Predictors of 1-year and 5-year overall mortality in resected stage 1 and 2 hepatocellular cancer patients in Honolulu, Tokyo and Shanghai

Covariates	Univariate			Multivariate		
	Hazard ratio	Confidence interval	P value	Hazard ratio <sup>1</sup>	Confidence interval	P value
<b>1-yr</b>						
Age (yr, $\geq 50$ vs $< 50$ )	1.29	0.66-2.55	0.46	1.41	0.68-2.92	0.36
Sex (male vs female)	1.39	0.82-2.38	0.22			
AFP (ng/mL, $\geq 20$ vs $< 20$ )	2.28	1.33-3.91	0.003	2.27	1.32-3.90	0.003
Cirrhosis (yes vs no)	1.13	0.65-1.95	0.66			
Tumor size (cm, $\geq 5$ vs $< 5$ )	2.64	1.60-4.34	0.0001	2.00	1.17-3.43	0.01
Albumin (g/dL, $< 3.5$ vs $\geq 3.5$ )	1.72	1.01-2.94	0.045	2.10	1.20-3.68	0.01
AJCC stage (2 vs 1)	0.90	0.55-1.49	0.16			
Childs Pugh (B vs A)	1.27	0.46-3.49	0.64			
Hepatitis B (+ vs -)	0.77	0.47-1.28	0.33			
Hepatitis C (+ vs -)	0.73	0.42-1.25	0.25			
Tokyo vs Honolulu	0.32	0.17-0.58	0.0002	0.33	0.17-0.65	0.001
Shanghai vs Honolulu	0.43	0.23-0.78	0.005	0.54	0.28-1.03	0.06
<b>5-yr</b>						
Age (yr, $\geq 50$ vs $< 50$ )	1.12	0.79-1.60	0.52	1.14	0.77-1.69	0.53
Sex (male vs female)	1.14	0.84-1.56	0.40			
AFP (ng/mL, $\geq 20$ vs $< 20$ )	1.56	1.17-2.06	0.002	1.57	1.17-2.10	0.002
Cirrhosis (yes vs no)	1.32	0.97-1.80	0.08	1.59	1.12-2.26	0.009
Tumor size (cm, $\geq 5$ vs $< 5$ )	1.66	1.24-2.23	0.0007	1.84	1.34-2.54	0.0002
Albumin (g/dL, $< 3.5$ vs $\geq 3.5$ )	1.91	1.41-2.58	$< 0.0001$	1.72	1.21-2.46	0.003
AJCC stage (2 vs 1)	1.30	0.98-1.72	0.07			
Childs Pugh (B vs A)	1.78	1.05-3.02	0.03	1.33	0.73-2.43	0.35
Hepatitis B (+ vs -)	0.87	0.65-1.15	0.31			
Hepatitis C (+ vs -)	1.11	0.83-1.49	0.48			
Tokyo vs Honolulu	0.74	0.53-1.03	0.08	0.51	0.33-0.78	0.002
Shanghai vs Honolulu	0.66	0.46-0.94	0.02	0.47	0.31-0.72	0.0006

<sup>1</sup>Multivariate model adjusted for covariates listed. AFP: Alpha fetal protein; AJCC: American Joint Commission on Cancer.

homogeneous a group as possible. We showed that although the survival outcomes are different in various centers, overall survival is mostly dependent on tumor factors and underlying liver function. Although the patients in each center differ in many respects (mean age, gender, viral risk factor, tumor size, mean tumor size and AFP), all centers had excellent 30-d mortality. Our study showed that tumor size, AFP, and albumin were factors associated with early mortality. By 5 years post-resection, these same factors in addition to the presence of cirrhosis were predictors of mortality. Both the Tokyo and Shanghai cohorts had better 1- and 5-year survival compared to the Honolulu cohort even after adjustment for clinical factors. Differences in patient populations across the centers may account for these differences. Compared to the generally homogeneous Tokyo and Shanghai patients, the Honolulu cohort was comprised of racially and ethnic diverse individuals born within and outside the United States. Many Honolulu patients had comorbidities including those that may contribute to disease progression (obesity, type-2 diabetes, excess alcohol consumption and past intravenous drug use)<sup>[10]</sup>. We were unable to account for these differences in comorbidities and risk factors as this information was not available for the Tokyo and Shanghai cohorts. Differences in the patient populations are further supported by our observation that independent risk factors of survival differed across centers. In Honolulu, elevated AFP and albumin were associated with both 1-year and 5-year

survival. In the Tokyo cohort, cirrhosis large and tumor size were associated with 1-year survival. For the Shanghai cohort, tumor size was a predictor of 1-year survival while AJCC stage and Child-Pugh Score were associated with 5-year mortality risk.

Single-center studies have similarly demonstrated that tumor characteristics (size, vascular invasion) and underlying liver function are predictors of survival<sup>[3,5,21,35,36]</sup>. Kao *et al.*<sup>[3]</sup> examining 1265 liver resections for early stage HCC, showed that low albumin, AFP  $> 20$  ng/mL, and tumor size  $> 3$  cm affected mortality. Kang *et al.*<sup>[34]</sup> studying 353 South Korean patients, found that vascular invasion and thrombocytopenia were risk factors for poor disease-free survival. Many of these studies were large series of liver resections in centers outside the United States. Large United States studies of liver resection for HCC have been primarily based on cancer databases with limited information on underlying liver function<sup>[30,33,37]</sup>, or were conducted in single centers that focused on the comparison between liver transplant and resection<sup>[29-38]</sup>.

A few studies have also compared the outcome of liver resections in patients with HBV vs HCV. Chen *et al.*<sup>[38]</sup> studying 2920 patients in Taiwan, showed that patients with HBV were younger, had higher AFP and larger tumor size and lower mean survival (11.1 mo vs 23.9 mo with HCV). Dohmen *et al.*<sup>[39]</sup> demonstrated that among 692 patients in Japan, HBV patients were younger, presented with more advanced stage and had poorer overall survival. Wu *et al.*<sup>[40]</sup> reported that

among 110 Taiwanese patients who underwent hepatic resection for HCC, neither underlying cirrhosis nor viral status affected operative morbidity or mortality, but the poorer liver reserve in HCV cirrhotic patients resulted in worse survival compared to the HBV patients. Franssen *et al.*<sup>[37]</sup> reported that among 567 United States patients who underwent liver resection, HBV rather than HCV-related HCC had better survival and less recurrence. Our study allowed comparison of a primarily HBV-related HCC group of patients (China), a primarily HCV-related HCC group (Japan) and a mixed group (Hawaii), and when considered together viral hepatitis status had little bearing on overall 1- and 5-year survival as the cirrhosis, tumor size, AFP and underlying liver function had the greatest effect on outcome.

This study is limited in that the time frame was different in the three groups. In the Tokyo and Honolulu cohorts, this study represented a 20+ year experience, whereas the Shanghai cohort underwent liver resection over a 2-year period. Because this study was done retrospectively, each group collected different parameters, so there was limited data collected by all groups that could be directly compared. There are also likely differences in the quality of long-term follow up between the centers. This study also has variable data on recurrence of HCC and treatment of these recurrences, which may affect long-term survival. Survival was also expressed as all-cause survival so it is difficult to determine the contribution of HCC to overall patient outcome. Finally, differences in survival after liver resection may be due to availability of liver transplant and other locoregional therapies in a particular country. The increased availability of liver transplant in the Honolulu group may have prompted fewer resections in those with smaller tumors, leaving liver resections for larger tumors with reasonable liver function. Unfortunately, we would not be able to determine this without information on all HCC referred to each of these centers.

In spite of these differences and limitations, this study represents a large experience of liver resections by expert hepatobiliary surgeons in their respective countries. In the final analysis, the very early outcome after liver resection for HCC is similar in specialized centers in different countries but later survival is better in the Tokyo and Shanghai groups. Tumor factors, underlying liver function, comorbidities and availability of other therapies may be playing a role patient selection for resection and the ultimate outcome. Nevertheless, this study demonstrates that collaborations at an international level will be important for understanding how to better manage and treat HCC.

## COMMENTS

### Background

Treatment for hepatocellular cancer depends on stage and liver function. How liver cancer is treated in different country may also depend on available therapy. Liver transplant has the best long-term disease free survival for early liver cancer, however the availability of liver transplant differs in various countries

and may limit this therapy.

### Research frontiers

Single-institution studies have characterized resection for hepatocellular but this unique study combines the experience of three large hepatobiliary centers in different countries with 573 resections for stage I/II hepatocellular cancer in Tokyo ( $n = 250$ ), Honolulu ( $n = 146$ ) and Shanghai ( $n = 177$ ).

### Innovations and breakthroughs

Groups differed in viral hepatitis, tumor size, alpha fetal protein (AFP) and cirrhosis. One and 5-year mortality was lowest in the Tokyo cohort. Elevated AFP, low albumin, tumor > 5 cm and cirrhosis were independently-associated with increased 5-year mortality. The profile of early-stage hepatocellular patients is strikingly different across countries and likely contributes to survival differences.

### Applications

This study is important as it demonstrates the importance of collaboration between centers in different countries so that we can better diagnose and manage hepatocellular cancer.

### Terminology

Liver resection is a surgical procedure involving removal of a portion of liver that has a malignant cancer. Liver transplantation is performed for those patients with hepatocellular cancer and poor underlying liver function.

### Peer-review

The authors of this paper observed excellent early outcomes after liver resection for early stage hepatocellular cancer. Differences in longer term survival were likely related to tumor size, albumin, AFP and the presence of cirrhosis.

## REFERENCES

- 1 Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]
- 2 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; **65**: 5-29 [PMID: 25559415 DOI: 10.3322/caac.21254]
- 3 Kao WY, Chao Y, Chang CC, Li CP, Su CW, Huo TI, Huang YH, Chang YJ, Lin HC, Wu JC. Prognosis of Early-Stage Hepatocellular Carcinoma: The Clinical Implications of Substages of Barcelona Clinic Liver Cancer System Based on a Cohort of 1265 Patients. *Medicine* (Baltimore) 2015; **94**: e1929 [PMID: 26512620 DOI: 10.1097/MD.0000000000001929]
- 4 Yim SY, Seo YS, Jung CH, Kim TH, Lee JM, Kim ES, Keum B, Jong YK, An H, Kim JH, Yim HJ, Kim DS, Jeon YT, Yeon JE, Lee HS, Chun HJ, Byun KS, Um SH, Kim CD, Ryu HS. The management and prognosis of patients with hepatocellular carcinoma: what has changed in 20 years? *Liver Int* 2016; **36**: 445-453 [PMID: 26352789 DOI: 10.1111/liv.12960]
- 5 Lee WC, Lee CF, Cheng CH, Wu TJ, Chou HS, Wu TH, Soong RS, Chan KM, Yu MC, Chen MF. Outcomes of liver resection for hepatocellular carcinoma in liver transplantation era. *Eur J Surg Oncol* 2015; **41**: 1144-1152 [PMID: 26163047 DOI: 10.1016/j.ejso.2015.05.024]
- 6 Zhu Q, Li N, Zeng X, Han Q, Li F, Yang C, Lv Y, Zhou Z, Liu Z. Hepatocellular carcinoma in a large medical center of China over a 10-year period: evolving therapeutic option and improving survival. *Oncotarget* 2015; **6**: 4440-4450 [PMID: 25686836 DOI: 10.18632/oncotarget.2913]
- 7 Yamashita Y, Tsuijita E, Takeishi K, Ishida T, Ikegami T, Ezaki T, Maeda T, Utsunomiya T, Nagasue N, Shirabe K, Maehara Y. Trends in surgical results of hepatic resection for hepatocellular carcinoma: 1,000 consecutive cases over 20 years in a single institution. *Am J Surg* 2014; **207**: 890-896 [PMID: 24144344 DOI: 10.1016/j.amjsurg.2013.07.028]
- 8 Hsueh KC, Lee TY, Kor CT, Chen TM, Chang TM, Yang SF,

- Hsieh CB. The role of liver transplantation or resection for patients with early hepatocellular carcinoma. *Tumour Biol* 2016; **37**: 4193-4201 [PMID: 26490991 DOI: 10.1007/s13277-015-4243-z]
- 9 Kudo M. Surveillance, diagnosis, treatment, and outcome of liver cancer in Japan. *Liver Cancer* 2015; **4**: 39-50 [PMID: 26020028 DOI: 10.1159/000367727]
  - 10 Wong LL, Hernandez B, Kwee S, Albright CL, Okimoto G, Tsai N. Healthcare disparities in Asians and Pacific Islanders with hepatocellular cancer. *Am J Surg* 2012; **203**: 726-732 [PMID: 22227170 DOI: 10.1016/j.amjsurg.2011.06.055]
  - 11 Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
  - 12 Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
  - 13 Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. AJCC cancer staging manual. 7<sup>th</sup> Edition. New York: Springer, 2009: 191-199
  - 14 Ji J, Shi J, Budhu A, Yu Z, Forgues M, Roessler S, Ambs S, Chen Y, Meltzer PS, Croce CM, Qin LX, Man K, Lo CM, Lee J, Ng IO, Fan J, Tang ZY, Sun HC, Wang XW. MicroRNA expression, survival, and response to interferon in liver cancer. *N Engl J Med* 2009; **361**: 1437-1447 [PMID: 19812400 DOI: 10.1056/NEJMoa0901282]
  - 15 Budhu A, Jia HL, Forgues M, Liu CG, Goldstein D, Lam A, Zanetti KA, Ye QH, Qin LX, Croce CM, Tang ZY, Wang XW. Identification of metastasis-related microRNAs in hepatocellular carcinoma. *Hepatology* 2008; **47**: 897-907 [PMID: 18176954 DOI: 10.1002/hep.22160]
  - 16 Makuuchi M, Kokudo N. Clinical practice guidelines for hepatocellular carcinoma: the first evidence based guidelines from Japan. *World J Gastroenterol* 2006; **12**: 828-829 [PMID: 16521207]
  - 17 Liver Cancer Study Group of Japan. General rules for the clinical and pathological study of primary liver cancer. Second English edition. Kanehara & Co., Ltd., Tokyo: 2003
  - 18 Imamura H, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Assessment of hepatic reserve for indication of hepatic resection: decision tree incorporating indocyanine green test. *J Hepatobiliary Pancreat Surg* 2005; **12**: 16-22 [PMID: 15754094]
  - 19 Grambsch PM, Therneau TM, Fleming TR. Diagnostic plots to reveal functional form for covariates in multiplicative intensity models. *Biometrics* 1995; **51**: 1469-1482 [PMID: 8589234]
  - 20 Scatton O, Goumard C, Cauchy F, Fartoux L, Perdigo F, Conti F, Calmus Y, Boelle PY, Belghiti J, Rosmorduc O, Soubrane O. Early and resectable HCC: Definition and validation of a subgroup of patients who could avoid liver transplantation. *J Surg Oncol* 2015; **118**: 1007-1015 [PMID: 25918872 DOI: 10.1002/jso.23916]
  - 21 Wong RJ, Wantuck J, Valenzuela A, Ahmed A, Bonham C, Gallo A, Melcher ML, Lutchman G, Concepcion W, Esquivel C, Garcia G, Daugherty T, Nguyen MH. Primary surgical resection versus liver transplantation for transplant-eligible hepatocellular carcinoma patients. *Dig Dis Sci* 2014; **59**: 183-191 [PMID: 24282054 DOI: 10.1007/s10620-013-2947-8]
  - 22 Chirica M, Tranchart H, Tan V, Faron M, Balladur P, Paye F. Infection with hepatitis C virus is an adverse prognostic factor after liver resection for early-stage hepatocellular carcinoma: implications for the management of hepatocellular carcinoma eligible for liver transplantation. *Ann Surg Oncol* 2013; **20**: 2405-2412 [PMID: 23338483 DOI: 10.1245/s10434-012-2861-x]
  - 23 Ho CM, Lee PH, Chen CL, Ho MC, Wu YM, Hu RH. Long-term outcomes after resection versus transplantation for hepatocellular carcinoma within UCSF criteria. *Ann Surg Oncol* 2012; **19**: 826-833 [PMID: 21879276 DOI: 10.1245/s10434-011-1975-x]
  - 24 Koniaris LG, Levi DM, Pedrosa FE, Franceschi D, Tzakis AG, Santamaria-Barria JA, Tang J, Anderson M, Misra S, Solomon NL, Jin X, DiPasco PJ, Byrne MM, Zimmers TA. Is surgical resection superior to transplantation in the treatment of hepatocellular carcinoma? *Ann Surg* 2011; **254**: 527-537; discussion 537-538 [PMID: 21865950 DOI: 10.1097/SLA.0b013e31822ca66f]
  - 25 Huang J, Hernandez-Alejandro R, Croome KP, Yan L, Wu H, Chen Z, Prasoon P, Zeng Y. Radiofrequency ablation versus surgical resection for hepatocellular carcinoma in Childs A cirrhotics-a retrospective study of 1,061 cases. *J Gastrointest Surg* 2011; **15**: 311-320 [PMID: 21052859 DOI: 10.1007/s11605-010-1372-y]
  - 26 Cherqui D, Laurent A, Mocellin N, Tayar C, Luciani A, Van Nhieu JT, Decaens T, Hurtova M, Memeo R, Mallat A, Duvoux C. Liver resection for transplantable hepatocellular carcinoma: long-term survival and role of secondary liver transplantation. *Ann Surg* 2009; **250**: 738-746 [PMID: 19801927 DOI: 10.1097/SLA.0b013e3181bd582b]
  - 27 Bellavance EC, Lumpkins KM, Mentha G, Marques HP, Capussotti L, Pulitano C, Majno P, Mira P, Rubbia-Brandt L, Ferrero A, Aldrighetti L, Cunningham S, Russolillo N, Philosophe B, Barroso E, Pawlik TM. Surgical management of early-stage hepatocellular carcinoma: resection or transplantation? *J Gastrointest Surg* 2008; **12**: 1699-1708 [PMID: 18709418 DOI: 10.1007/s11605-008-0652-2]
  - 28 Chapman WC, Klintmalm G, Hemming A, Vachharajani N, Majella Doyle MB, DeMatteo R, Zaydfudim V, Chung H, Cavaness K, Goldstein R, Zendajas I, Melstrom LG, Nagorney D, Jarnagin W. Surgical treatment of hepatocellular carcinoma in North America: can hepatic resection still be justified? *J Am Coll Surg* 2015; **220**: 628-637 [PMID: 25728142 DOI: 10.1016/j.jamcollsurg.2014.12.030]
  - 29 Seshadri RM, Besur S, Niemeyer DJ, Templin M, McKillop IH, Swan RZ, Martinie JB, Russo MW, Iannitti DA. Survival analysis of patients with stage I and II hepatocellular carcinoma after a liver transplantation or liver resection. *HPB (Oxford)* 2014; **16**: 1102-1109 [PMID: 24964271 DOI: 10.1111/hpb.12300]
  - 30 Liu PH, Hsu CY, Lee YH, Hsia CY, Huang YH, Su CW, Chiou YY, Lin HC, Huo TI. When to Perform Surgical Resection or Radiofrequency Ablation for Early Hepatocellular Carcinoma?: A Nomogram-guided Treatment Strategy. *Medicine (Baltimore)* 2015; **94**: e1808 [PMID: 26512576 DOI: 10.1097/MD.0000000000001808]
  - 31 Liu PH, Hsu CY, Hsia CY, Lee YH, Huang YH, Chiou YY, Lin HC, Huo TI. Surgical Resection Versus Radiofrequency Ablation for Single Hepatocellular Carcinoma  $\leq$  2 cm in a Propensity Score Model. *Ann Surg* 2016; **263**: 538-545 [PMID: 25775062 DOI: 10.1097/SLA.0000000000001178]
  - 32 Li GZ, Speicher PJ, Lidsky ME, Darrabie MD, Scarborough JE, White RR, Turley RS, Clary BM. Hepatic resection for hepatocellular carcinoma: do contemporary morbidity and mortality rates demand a transition to ablation as first-line treatment? *J Am Coll Surg* 2014; **218**: 827-834 [PMID: 24655879 DOI: 10.1016/j.jamcollsurg.2013.12.036]
  - 33 Zhou Z, Lei J, Li B, Yan L, Wang W, Wei Y, Cheng K. Liver resection and radiofrequency ablation of very early hepatocellular carcinoma cases (single nodule & lt; 2 cm): a single-center study. *Eur J Gastroenterol Hepatol* 2014; **26**: 339-344 [PMID: 24150522 DOI: 10.1097/MEG.000000000000012]
  - 34 Kang CM, Choi GH, Kim DH, Choi SB, Kim KS, Choi JS, Lee WJ. Revisiting the role of nonanatomic resection of small (& lt; or = 4 cm) and single hepatocellular carcinoma in patients with well-preserved liver function. *J Surg Res* 2010; **160**: 81-89 [PMID: 19577249 DOI: 10.1016/j.jss.2009.01.021]
  - 35 Dahiya D, Wu TJ, Lee CF, Chan KM, Lee WC, Chen MF. Minor versus major hepatic resection for small hepatocellular carcinoma (HCC) in cirrhotic patients: a 20-year experience. *Surgery* 2010; **147**: 676-685 [PMID: 20004441 DOI: 10.1007/s00534-010-0286-0]
  - 36 Ulahannan SV, Duffy AG, McNeel TS, Kish JK, Dickie LA, Rahma OE, McGlynn KA, Greten TF, Altekruse SF. Earlier presentation and application of curative treatments in hepatocellular carcinoma. *Hepatology* 2014; **60**: 1637-1644 [PMID: 24996116 DOI: 10.1002/hep.27288]
  - 37 Franssen B, Alshebeeb K, Tabrizian P, Marti J, Pierobon ES, Lubezky N, Roayaie S, Florman S, Schwartz ME. Differences in surgical outcomes between hepatitis B- and hepatitis C-related hepatocellular carcinoma: a retrospective analysis of a single North American center. *Ann Surg* 2014; **260**: 650-656; discussion 656-658

[PMID: 25203882 DOI: 10.1097/SLA.0000000000000917]

- 38 **Chen CH**, Huang GT, Yang PM, Chen PJ, Lai MY, Chen DS, Wang JD, Sheu JC. Hepatitis B- and C-related hepatocellular carcinomas yield different clinical features and prognosis. *Eur J Cancer* 2006; **42**: 2524-2529 [PMID: 16920352]
- 39 **Dohmen K**, Shigematsu H, Irie K, Ishibashi H. Comparison of the clinical characteristics among hepatocellular carcinoma of hepatitis

B, hepatitis C and non-B non-C patients. *Hepatogastroenterology* 2003; **50**: 2022-2027 [PMID: 14696457 DOI: 10.1016/j.jeca.2006.06.007]

- 40 **Wu CC**, Tang JS, Lin MC, Yeh DC, Liu TJ, P'eng FK. Comparison of liver resection for hepatocellular carcinoma in hepatitis B and hepatitis C-related cirrhotic patients. *Hepatogastroenterology* 1999; **46**: 651-655 [PMID: 10370591]

**P-Reviewer:** Bramhall S, He ST, Sipos F **S-Editor:** Qiu S  
**L-Editor:** A **E-Editor:** Li D





## Prospective Study

# Mortality and rebleeding following variceal haemorrhage in liver cirrhosis and periportal fibrosis

Sara Elfadil Abbas Mohammed, Abdelmunem Eltayeb Abdo, Hatim Mohamed Yousif Mudawi

Sara Elfadil Abbas Mohammed, Hatim Mohamed Yousif Mudawi, Department of Internal Medicine, Faculty of Medicine, University of Khartoum, Khartoum 11111, Sudan

Abdelmunem Eltayeb Abdo, National Centre for Gastro-intestinal and Liver Disease, Ibn Sina specialized Hospital, Khartoum 11111, Sudan

**Author contributions:** Mohammed SEA contributed to concept, design, data collection, data analysis and drafting of the manuscript; Abdo AE contributed to concept, design, critical revision and final approval of the manuscript; Mudawi HMY contributed to concept, design, data interpretation, drafting and final approval of the manuscript; all authors read and approved the final manuscript.

**Institutional review board statement:** The study was approved by the Research and Ethics Review Committee at Mohamed Salih Idris Bleeding Centre, Khartoum, Sudan.

**Clinical trial registration statement:** Not applicable, there were no interventions in this study.

**Informed consent statement:** All participants in the study or their legal guardian provided their informed consent before being enrolled in the study.

**Conflict-of-interest statement:** The authors of this manuscript have no conflict of interest to declare.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Hatim Mohamed Yousif Mudawi, FRCP,

Professor, Department of Internal Medicine, Faculty of Medicine, University of Khartoum, PO Box 2245, Khartoum 11111, Sudan. [hmudawi@hotmail.com](mailto:hmudawi@hotmail.com)  
 Telephone: +249-91-2202600  
 Fax: +249-15-5117877

Received: May 20, 2016

Peer-review started: May 20, 2016

First decision: July 4, 2016

Revised: August 5, 2016

Accepted: August 27, 2016

Article in press: August 29, 2016

Published online: November 8, 2016

## Abstract

### AIM

To investigate mortality and rebleeding rate and identify associated risk factors at 6 wk and 5 d following acute variceal haemorrhage in patients with liver cirrhosis and schistosomal periportal fibrosis.

### METHODS

This is a prospective study conducted during the period from March to December 2014. Patients with portal hypertension presenting with acute variceal haemorrhage secondary to either liver cirrhosis (group A) or schistosomal periportal fibroses (group B) presenting within 24 h of the onset of the bleeding were enrolled in the study and followed for a period of 6 wk. Analysis of data was done by Microsoft Excel and comparison between groups was done by Statistical Package of Social Sciences version 20 to calculate means and find the levels of statistical differences and define the mortality rates, the *P* value of < 0.05 was considered to be significant.

### RESULTS

A total of 94 patients were enrolled in the study. Thirty-two patients (34%) had liver cirrhosis (group A) and

62 (66%) patients had periportal fibrosis (group B). Mortality: The 6-wk and 5-d mortality were 53% and 16% respectively in group A compared to 10% and 0% in group B ( $P$  value  $< 0.000$  and  $< 0.004$ ). In group A; a Child-Turcotte-Pugh class C and rebleeding within 5 d were significantly associated with 5-d mortality ( $P$  value  $< 0.029$  and  $< 0.049$  respectively) and Child-Turcotte-Pugh class C was also a significant risk factor for 6-wk mortality ( $P$  value  $< 0.018$ ). In group B; mortality was significantly associated with rebleeding within the 6-wk follow-up period and requirement for blood transfusion on admission ( $P$  value  $< 0.005$  and  $< 0.049$ ). Rebleeding: The 6-wk and 5-d rebleeding rate in group A were 56% and 25% respectively compared to 32% and 3% in group B ( $P$  value  $< 0.015$  and  $< 0.002$ ). Clinical presentation with encephalopathy was a significant risk factor for 5 d rebleeding in group A ( $P$  value  $< 0.005$ ) while grade III periportal fibrosis and requirement for blood transfusion on admission were significant risk factors for 6-wk rebleeding in group B ( $P$  value  $< 0.004$  and  $< 0.02$ ).

### CONCLUSION

The 6-wk and 5-d mortality and rebleeding rate were significantly higher in patients with liver cirrhosis compared to patients with schistosomal periportal fibrosis.

**Key words:** Variceal haemorrhage; Periportal fibrosis; Liver cirrhosis; Mortality; Rebleeding

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This study was conducted to investigate the rate and risk factors associated with rebleeding and mortality at 6 wk and 5 d following acute variceal haemorrhage in patients with liver cirrhosis and schistosomal periportal fibrosis (PPF). The 6-wk and 5-d mortality in cirrhosis were 56% and 16% compared to 10% and 0% in patients with schistosomal PPF ( $P$  value  $< 0.000$  and  $< 0.004$ ). The 6-wk and 5-d rebleeding rate in cirrhosis were also high at 53% and 25% compared to 32% and 3% respectively in patients with schistosomal PPF ( $P$  value  $< 0.015$  and  $< 0.002$ ). In conclusion the 6-wk and 5-d mortality and rebleeding were significantly higher in patients with liver cirrhosis compared to patients with schistosomal periportal fibrosis.

Mohammed SEA, Abdo AE, Mudawi HMY. Mortality and rebleeding following variceal haemorrhage in liver cirrhosis and periportal fibrosis. *World J Hepatol* 2016; 8(31): 1336-1342 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i31/1336.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i31.1336>

### INTRODUCTION

Variceal bleeding is a devastating complication of portal hypertension. The 6-wk mortality in patients with liver cirrhosis is between 17%-28%<sup>[1-3]</sup> and the risk of

rebleeding after acute variceal haemorrhage (AVH) is highest within the first 6 wk with a peak in the first 5 d<sup>[2]</sup>. In Sudan, a country endemic with schistosomiasis, AVH in the majority of cases is caused by portal hypertension due to schistosomal periportal fibrosis (PPF), while cirrhosis is less common<sup>[4]</sup>. Published data from Sudan showed that the crude mortality rate of schistosomiasis in a village in the Gezira state; an area endemic for schistosomiasis was 51/100000 per year with a case fatality per year of 1/1000 in those secreting eggs and 11/100 in those with bleeding varices<sup>[5]</sup>. Significant risk factors for variceal bleeding in patients with schistosomal portal hypertension, after at least one episode of bleeding compared to patients with schistosomal PPF without bleeding were splenic longitudinal dimension of more than 11 cm and PPF of  $\geq$  grade III<sup>[6]</sup>. The 6-wk and 5-d mortality and rebleeding rate following AVH in schistosomal portal hypertension have not been well studied.

The objectives of this study were to investigate mortality and rebleeding rate and identify associated risk factors for 6 wk and 5 d following acute variceal haemorrhage in patients with portal hypertension secondary to liver cirrhosis and schistosomal PPF.

### MATERIALS AND METHODS

This is a prospective study conducted at Mohamed Salih Idris bleeding centre in Khartoum state, Sudan from March to December 2014. Patients presenting with acute upper gastrointestinal (GI) bleeding, whether it was their first bleeding episode or otherwise, were included if they were  $> 16$  years old, had portal hypertensive variceal bleeding (diagnosed on upper gastrointestinal endoscopy) secondary to liver cirrhosis (group A) or PPF (group B) presenting within 24 h of the onset of the bleeding. Patients were excluded if they were  $\leq 16$  years, had hepatocellular carcinoma or had non variceal upper gastrointestinal bleeding. Data were collected in a specially designed sheet which included patients' demographics and clinical presentation, baseline laboratory tests done upon initial presentation (CBC, RFT, LFT's and INR), patients' hemodynamic status upon presentation, the number of blood units transfused, the amount of the vasopressor agent Terlipressin acetate (Glypressin<sup>®</sup>, Ferring, Germany) used, endoscopic findings and endoscopic management done. All patients received Terlipressin 2 mg intravenously bolus dose followed by 1 mg 6 hourly whenever the drug was available. All patients underwent upper gastrointestinal endoscopy within 12 h of admission to identify the source of bleeding. Oesophageal varices were graded according to Paquet<sup>[7]</sup> and gastric varices were graded according to Sarin *et al*<sup>[8]</sup>. Oesophageal varices and junctional varices (GOV1) were treated with injection sclerotherapy or band ligation, whichever was available, 5% ethanolamine oleate was the agent used for injection sclerotherapy and the volume used for each patient was documented, junctional varices (GOV2) and isolated gastric varices

**Table 1** Demographic criteria and clinical presentation in 94 patients with liver cirrhosis and periportal fibrosis presenting with acute variceal haemorrhage *n* (%)

Variable	PPF ( <i>n</i> = 62)	Cirrhosis ( <i>n</i> = 32)	<i>P</i> value
Males	56 (90.3)	27 (84.4)	0.395
Females	6 (9.7)	5 (15.6)	
Mean age (yr)	49.3 ± 13.4	48.7 ± 15.1	0.840
HBV	4 (6.5)	8 (25)	0.004 <sup>1</sup>
HCV	2 (3.2)	1 (3)	
HBV and HCV	0 (0)	3 (9.4)	
SBP > 100, PR < 100	50 (80.6)	18 (56.2)	0.012 <sup>1</sup>
SBP < 100, PR > 100	12 (19.4)	14 (43.8)	
Hb < 5 g/dL	10 (16.1)	4 (12.5)	0.236
Hb 5-10 g/dL	36 (58.1)	24 (75)	
Hb > 10 g/dL	16 (25.8)	4 (12.5)	

<sup>1</sup>Significant risk factor. HBV: Hepatitis B virus; HCV: Hepatitis C virus; SBP: Systolic blood pressure; PR: Pulse rate; Hb: Haemoglobin.

were treated with the injection of cyanoacrylate (Histoacryl®, B Braun, Spain) and Sengstaken Blakemore tube was used to control bleeding if initial endoscopy was not successful. Cirrhotic patients were covered with prophylactic antibiotics. An appointment for secondary prophylactic endoscopic treatment was given within 2 wk and B-blockers were prescribed if not contraindicated. The diagnosis of cirrhosis was established by clinical, radiological and laboratory findings and the cause of cirrhosis was looked for and documented. Child-Turcotte-Pugh (CTP) classification and the model for end stage liver disease (MELD) score were calculated for each patient. In the case of schistosomal PPF, the severity of liver fibrosis was graded as described by Homeida *et al.*<sup>[9]</sup> from I - III as follows: Grade I : Mild echogenic thickening of one or two portal vein radicles with little change in the walls of the portal vein; Grade II : Moderate to severe periportal irregular thickening of most of the portal vein radicles, with marked narrowing of the central lucency, marked thickening at the bifurcation of the portal vein, and mild thickening of the main portal vein; and Grade III: Marked thickening of the walls of the portal vein radicles with obliteration of the central lucency in the peripheral branches forming thick irregular echogenic 10-20 mm bands reaching the periphery of the liver with thickening down to main portal vein walls.

Patients were followed every 24 h for the first 5 d and then at 6 wk for mortality and rebleeding. Rebleeding was defined according to the Baveno V consensus as a single episode of clinically significant rebleeding from portal hypertensive sources (recurrent melena, hematemesis resulting in hospital admission, blood transfusion, 3 g drop in haemoglobin or death). Rebleeding during the first 120 h (5 d) was regarded as treatment failure, whereas rebleeding up to 6 wk was regarded as failure of secondary prophylaxis<sup>[10]</sup>.

The primary endpoint of this study was the rate of variceal rebleeding and mortality at 5 d and at 6 wk following AVH in portal hypertension secondary to cirrhosis or PPF. The secondary endpoints were to determine risk factors associated with variceal rebleeding and

mortality at 5 d and 6 wk among the study population.

The study was reviewed and approved by the Research and Ethics Review Committee at Mohamed Salih Idris Centre. All study participants or their legal guardian provided their informed consent before being enrolled in the study.

### Statistical analysis

Analysis of data was done by Microsoft Excel and comparison between groups was done by Statistical Package of Social Sciences version 20 to calculate means and find the levels of statistical differences and define the mortality rates, the *P* value of < 0.05 was considered to be significant.

## RESULTS

A total of 94 patients were enrolled in the study with a mean age of 49 ± 1.0 years, and males constituted 88% with M:F ratio of 7:1.

### Group A (liver cirrhosis)

There were 32 patients (34%) in group A. Demographic criteria and clinical presentation are shown in Table 1, clinical findings and medical management in Table 2 and endoscopic findings/management are shown in Table 3. After discharge from the bleeding centre, patients were advised to attend for further endoscopic management in order to eradicate the varices with the earliest session to be done at 2 wk. A total of 19% underwent upper endoscopy at less than 2 wk because of rebleeding, 31% performed the session at the advised 2 wk, 13% at 3 wk, 3% at 4 wk and 3% beyond 4 wk, whereas 31% did not attend for the second endoscopy session.

### Group B (schistosomal PPF)

There were 62 patients (66%) in group B. The clinical and demographic data are shown in Table 1, ultrasound findings and medical management in Table 2 and endoscopic findings/management are presented in Table 3.

After discharge from the bleeding centre patients were advised to continue endoscopic management in order to eradicate the varices with the earliest session to be done at 2 wk. A total of 5% of the patients underwent upper endoscopy at less than 2 wk because of rebleeding, 48% performed the session at the advised 2 wk, 23% at 3 wk, 8% at 4 wk and 11% beyond 4 wk while 5% did not attend for the second endoscopy session.

### Overall mortality

The 6-wk and 5-d mortality were 53% and 16% respectively in group A compared to 10% and 0% in group B (*P* value < 0.000 and < 0.004 respectively) (Table 4).

### Factors related to mortality

In group A, the CTP class C and rebleeding within 5 d were significant risk factors for 5 d mortality (*P* value < 0.029 and < 0.049 respectively). CTP class C was also

**Table 2 Clinical findings and medical management provided in 94 patients with liver cirrhosis and periportal fibrosis presenting with acute variceal haemorrhage *n* (%)**

Variable	PPF ( <i>n</i> = 62)	Cirrhosis ( <i>n</i> = 32)	<i>P</i> value
Jaundice	0 (0)	16 (50)	0.000 <sup>1</sup>
Ascites	5 (8.1)	16 (50)	0.000 <sup>1</sup>
Encephalopathy	3 (4.8)	9 (28.1)	0.001 <sup>1</sup>
Child class A	-	9 (28)	-
Child class B	-	13 (41)	-
Child class C	-	10 (31)	-
MELD score < 18		19 (59.4)	-
MELD score > 18		13 (40.6)	-
PPF grade II	29	-	-
PPF grade III	71	-	-
Mean portal vein diameter	17.4 ± 3.3 mm	16.4 ± 3.1 mm	0.155
Terlipressin stat dose 2 mg IV	48 (77.4)	27 (84.4)	0.426
Terlipressin 6 hourly over 24 h	15 (24.2)	14 (43.8)	0.052
Requirement for blood transfusion (mean number of units)	2 ± 1 units	2 ± 1 units	-

<sup>1</sup>Significant risk factor. PPF: Periportal fibrosis; MELD: Model for end stage liver disease.**Table 3 Endoscopy findings and endoscopic management in 94 patients with liver cirrhosis and periportal fibrosis presenting with acute variceal haemorrhage *n* (%)**

Variable	PPF ( <i>n</i> = 62)	Cirrhosis ( <i>n</i> = 32)	<i>P</i> value
Grade II OV	5 (8.1)	2 (6.3)	0.961
Grade III OV	17 (27.4)	10 (31.3)	
Grade IV OV	23 (37.1)	14 (43.8)	
Gastric varices	17 (27.4)	6 (18.8)	
Band ligation	8 (12.9)	1 (3.1)	0.318
Sclerotherapy	45 (72.6)	28 (87.5)	
Histoacryl injection	4 (6.5)	2 (6.3)	
Both histoacryl/sclerotherapy/band	5 (8.1)	1 (3.1)	

OV: Oesophageal varices; PPF: Periportal fibrosis.

a significant risk factor for 6-wk mortality (*P* value < 0.018) (Table 5).

In group B, rebleeding within the 6-wk follow-up period and blood transfusion on admission were significant risk factors for mortality (*P* value < 0.005 and < 0.049 respectively) (Table 6).

### Rebleeding rate

The 6-wk and 5-d rebleeding rate in group A were 56% and 25% respectively compared to 32% and 3% in group B (*P* value < 0.015 and < 0.002) (Table 4).

### Factors related to variceal rebleeding within 6 wk

In group A, no significant factors were related to rebleeding within 6 wk.

In group B, grade III PPF and blood transfusion on admission were significant risk factors associated with rebleeding in this group with a *P* value < 0.004 and < 0.02 respectively (Tables 5 and 6).

### Factors related to variceal rebleeding within 5 d

In group A, clinical presentation with encephalopathy was a significant risk factor for rebleeding within 5 d (*P* value < 0.005). In group B there were no significant factors

**Table 4 Study outcomes in 94 patients with liver cirrhosis and periportal fibrosis presenting with acute variceal haemorrhage *n* (%)**

	PPF ( <i>n</i> = 62)	Cirrhosis ( <i>n</i> = 32)	<i>P</i> value
Mortality at 6 wk	10	53	0.000 <sup>1</sup>
Mortality at 5 d	0	16	0.004 <sup>1</sup>
Rebleeding at 6 wk	32	56	0.015 <sup>1</sup>
Rebleeding at 5 d	3	25	0.002 <sup>1</sup>

<sup>1</sup>Significant risk factor. PPF: Periportal fibrosis.

contributing to rebleeding within 5 d (Tables 5 and 6).

## DISCUSSION

In this study, we evaluated early mortality and rebleeding following AVH. In this part of Africa minimal data is available with regards to mortality following AVH due to scarcity of endoscopy services. This study is unique because we evaluated the patients in two groups according to the etiology of the underlying liver disease, either liver cirrhosis or schistosomal periportal fibrosis. Previous studies on early mortality following AVH were done exclusively on patients with liver cirrhosis<sup>[3,11-13]</sup>.

In this study, in the cirrhosis group, the 6-wk and 5-d mortality were both high at 53% and 16% respectively, whereas the 6-wk mortality following AVH in patients with schistosomal PPF was 10% with no deaths reported during the first 5 day (*P* value < 0.000 and < 0.004). This high rate of mortality in cirrhosis following variceal bleeding is well described by D'Amico; where four clinical stages of cirrhosis were agreed upon in the Baveno IV consensus conference. Each stage with different features and a different prognosis as follows: Stage 1 no varices or ascites, mortality rate is 1%, stage 2 varices without ascites and without bleeding, mortality rate is 3.4% per year, stage 3 is characterised by ascites with or without varices but never bled, mortality rate is 20% per year, stage 4 is characterised by GI bleeding with or without ascites, in this stage the one year mortality is 57% and



**Table 5** Factors associated with mortality and rebleeding in 32 patients with liver cirrhosis

Study outcome	Factors	P value
Mortality at 6 wk	CTP score C	0.018 <sup>1</sup>
Mortality at 5 d	CTP score C	0.029 <sup>1</sup>
	Rebleeding within 5 d	0.049 <sup>1</sup>
Rebleeding at 6 wk	Non	Non
Rebleeding at 5 d	Encephalopathy	0.005 <sup>1</sup>

<sup>1</sup>Significant risk factor. CTP: Child-Turcotte-Pugh.

nearly half of these deaths occur within 6 wk from the initial episode of bleeding<sup>[14]</sup>.

This difference in mortality rate between the two groups is most likely due to preserved liver cell function in most patients with PPF compared to patients with liver cirrhosis<sup>[15]</sup>. In this study we observed that only a minority of patients with schistosomal PPF presented with clinical evidence of liver cell failure; mainly ascites in 2% and encephalopathy in 3%, it has been reported that a few patients with schistosomiasis do evolve to an end stage of the disease with hepatocellular failure, this is known as decompensated schistosomiasis<sup>[15]</sup>.

Le Moine *et al*<sup>[3]</sup> reported predictive factors for mortality at 6 wk in cirrhotic patients being prolonged prothrombin time, encephalopathy and number of blood units transfused. Krige *et al*<sup>[12]</sup> found in a study done exclusively in alcoholic cirrhosis, that CTP class C, encephalopathy, ascites, bilirubin > 51 mmol/L, INR > 2.3, albumin < 25 g/L and patients who require balloon tamponade were factors related to 6-wk mortality. In this study we also found that the CTP class C was significantly related to 6-wk mortality (*P* value < 0.02) this was similar to findings in other studies<sup>[3,12,13]</sup>. Furthermore factors related to mortality within the first 5 d following AVH in cirrhosis were again the CTP class C and the rebleeding within these 5 d. Bambha *et al*<sup>[11]</sup> suggested that the MELD score rather than CTP class was more powerful in predicting 6-wk mortality. In this study, the MELD score was not a significant risk factor for mortality. We found that mortality following AVH in schistosomal PPF (10%) is much less when compared to cirrhotic patients (56%). There is scanty data on the early outcomes of patients with schistosomal PPF presenting with AVH, however, a study from Tanzania found that mortality following AVH in patients with schistosomal PPF after 8 wk of follow-up was quite similar to this study at 10%<sup>[16]</sup>. In this study, no deaths occurred within the first 5 d in the PPF group.

Factors significantly contributing to the 6-wk mortality in PPF included blood transfusion within the first 24 h and rebleeding within the 6-wk follow-up period (*P* values < 0.049, < 0.005 respectively). The 6-wk rebleeding rate in the PPF group was (32%), less than cirrhosis group at 56% (*P* value < 0.004). Significant factors contributing to the 6-wk rebleeding rate in PPF group were grade III PPF on abdominal ultrasound and blood transfusion on admission (*P* value < 0.004 and < 0.02 respectively). A previous study from Sudan demonstrated that rebleeding

**Table 6** Factors associated with mortality and rebleeding in 62 patients with periportal fibrosis

Study outcome	Factors	P value
Mortality at 6 wk	Blood transfusion	0.049 <sup>1</sup>
	Rebleeding within 6 wk	0.005 <sup>1</sup>
Mortality at 5 d	Non	Non
Rebleeding at 6 wk	Blood transfusion	0.021 <sup>1</sup>
	Grade III PPF	0.004 <sup>1</sup>
Rebleeding at 5 d	Non	Non

<sup>1</sup>Significant risk factor. PPF: Periportal fibrosis.

was more in grade III PPF<sup>[17]</sup>. A study from Brazil also found that the sonographic grade of periportal fibrosis was an important tool in predicting variceal complications in patients with schistosomal PPF<sup>[18]</sup>, whereas the longitudinal spleen dimension of more than 11 cm, was an important tool in predicting variceal bleeding in another study<sup>[6]</sup>. It is well known that a conservative blood transfusion strategy is associated with better survival outcomes in patients with upper gastrointestinal bleeding<sup>[19]</sup>. In this study blood transfusion was provided to 58% of patients with PPF with a mean of 2 ± 1 units of blood. Requirement of blood transfusion was a significant factor for both mortality and rebleeding in PPF group (*P* value < 0.049 and < 0.02 respectively).

Rebleeding within 5 d in PPF occurred in two patients (3%), which is much less than in cirrhosis group at 25% (*P* value < 0.002). Both patients had grade III PPF, were hemodynamically stable and did not require blood transfusion, however, none of the factors evaluated contributed to rebleeding within 5 d. Further studies are needed to reveal other causes contributing to rebleeding such as the portal vein pressure and intra variceal pressure.

In this study, the rebleeding rate among cirrhosis group was high at 56% and 25% of these patients developed rebleeding within the first 5 d. The 6-wk rebleeding rate reported in literature was 16.2%, 30% and 24.2%<sup>[2,12,13]</sup>.

It has been reported that the severity of liver disease in terms of presence of ascites and encephalopathy contributes to the rebleeding rate<sup>[3,12]</sup>. In this study, the presence of hepatic encephalopathy in patients with cirrhosis was a significant factor for rebleeding within 5 d of AVH (*P* value < 0.005). Non of the other factors evaluated were found significant for 6-wk rebleeding in cirrhosis, perhaps larger studies with bigger sample sizes are needed to reveal the factors contributing to rebleeding in patients with cirrhosis in our region.

It is known that the use of vasopressor agents improve outcomes following AVH<sup>[20]</sup>. In this study, Terlipressin was provided to patients whenever available, however there was no significant difference on survival or rebleeding rate between patients with liver cirrhosis and patients PPF with use of Terlipressin.

In patients with PPF and cirrhosis the mean portal vein diameter was 17.4 ± 3.3 mm and 16.4 ± 3.1 mm

respectively, reflecting high portal pressure and hence high variceal pressure. Therefore, effective lowering of portal pressure should be paramount in order to prevent the dreadful complication of variceal bleeding.

HBsAg seroprevalence among PPF patients was 6%, similar to seroprevalence of HBsAg among the general population in central Sudan<sup>[21]</sup>, hence HBV screening and vaccination in patients with PPF should be encouraged.

In conclusion, this study has demonstrated that the 6-wk and 5-d mortality and rebleeding are significantly higher in patients with liver cirrhosis compared to patients with schistosomal periportal fibrosis. Effective secondary prophylaxis after AVH needs to be adhered to and when resuscitating patients with AVH, blood transfusion should be given carefully and HBV vaccination should be actively encouraged.

## COMMENTS

### Background

Variceal bleeding is a devastating complication of portal hypertension. In patients with liver cirrhosis the risk of rebleeding after acute variceal haemorrhage is highest within the first 6 wk with a peak in the first 5 day.

### Research frontiers

In Sudan, a country endemic with schistosomiasis, acute variceal haemorrhage (AVH) in the majority of cases is caused by portal hypertension due to schistosomal periportal fibrosis, while cirrhosis is less common. The 6-wk and 5-d mortality and rebleeding following AVH in schistosomal portal hypertension have not been well studied.

### Innovations and breakthroughs

In this study, the authors evaluated early mortality and rebleeding following AVH. In this part of Africa minimal data is available with regards to mortality following AVH due to scarcity of endoscopy services. This study is unique because the authors evaluated the patients in two groups according to the etiology of the underlying liver disease, either liver cirrhosis or schistosomal periportal fibrosis. Previous studies on early mortality following AVH were done exclusively on patients with liver cirrhosis.

### Applications

This study has demonstrated that the 6-wk and 5-d mortality and rebleeding are significantly higher in patients with liver cirrhosis compared to patients with schistosomal periportal fibrosis.

### Terminology

Schistosomiasis is endemic in Sudan; the mortality of schistosoma mansoni infection is mostly due to development of periportal fibrosis with subsequent development of portal hypertension and oesophageal varices causing significant morbidity and mortality.

### Peer-review

This is a well conducted prospective study about variceal bleeding complications in African Setting.

## REFERENCES

- 1 **Thomopoulos K**, Theocharis G, Mimidis K, Lampropoulou-Karatzach, Alexandridis E, Nikolopoulou V. Improved survival of patients presenting with acute variceal bleeding. Prognostic indicators of short- and long-term mortality. *Dig Liver Dis* 2006; **38**: 899-904 [PMID: 17005458 DOI: 10.1016/j.dld.2006.08.002]
- 2 **D'Amico G**, De Franchis R; Cooperative Study Group. Upper dig-

- estive bleeding in cirrhosis. Post-therapeutic outcome and prognostic indicators. *Hepatology* 2003; **38**: 599-612 [PMID: 12939586 DOI: 10.1053/jhep.2003.50385]
- 3 **Le Moine O**, Adler M, Bourgeois N, Delhay M, Devière J, Gelin M, Vandermeeren A, Van Gossum A, Vereerstraeten A, Vereerstraeten P. Factors related to early mortality in cirrhotic patients bleeding from varices and treated by urgent sclerotherapy. *Gut* 1992; **33**: 1381-1385 [PMID: 1446864 DOI: 10.1136/gut.33.10.1381]
- 4 **Khamis H**, Abdul Wahab SM. An overview of Mohammed Salih Idris (MSI) centre for acute gastrointestinal bleeding: a look into one year experience in acute GI bleeding. *Sudan Medical Monitor* 2008; **3**: 135-138
- 5 **Kheir MM**, Eltoum IA, Saad AM, Ali MM, Baraka OZ, Homeida MM. Mortality due to schistosomiasis mansoni: a field study in Sudan. *Am J Trop Med Hyg* 1999; **60**: 307-310 [PMID: 10072156]
- 6 **Eltoum IA**, Taha TE, Saad AM, Suliman SM, Bennett JL, Nash TE, Homeida MM. Predictors of upper gastrointestinal bleeding in patients with schistosomal periportal fibrosis. *Br J Surg* 1994; **81**: 996-999 [PMID: 7922096 DOI: 10.1002/bjs.1800810722]
- 7 **Paquet KJ**. Prophylactic endoscopic sclerosing treatment of the esophageal wall in varices - a prospective controlled randomized trial. *Endoscopy* 1982; **14**: 4-5 [PMID: 7035153 DOI: 10.1055/s-2007-1021560]
- 8 **Sarin SK**, Lahoti D, Saxena SP, Murthy NS, Makwana UK. Prevalence, classification and natural history of gastric varices: a long-term follow-up study in 568 portal hypertension patients. *Hepatology* 1992; **16**: 1343-1349 [PMID: 1446890 DOI: 10.1002/hep.1840160607]
- 9 **Homeida M**, Abdel-Gadir AF, Cheever AW, Bennett JL, Arbab BM, Ibrahim SZ, Abdel-Salam IM, Dafalla AA, Nash TE. Diagnosis of pathologically confirmed Symmers' periportal fibrosis by ultrasonography: a prospective blinded study. *Am J Trop Med Hyg* 1988; **38**: 86-91 [PMID: 3124648]
- 10 **de Franchis R**. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2010; **53**: 762-768 [PMID: 20638742 DOI: 10.1016/j.jhep.2010.06.004]
- 11 **Bambha K**, Kim WR, Pedersen R, Bida JP, Kremers WK, Kamath PS. Predictors of early re-bleeding and mortality after acute variceal haemorrhage in patients with cirrhosis. *Gut* 2008; **57**: 814-820 [PMID: 18250126 DOI: 10.1136/gut.2007.137489]
- 12 **Krige JE**, Kotze UK, Distiller G, Shaw JM, Bornman PC. Predictive factors for rebleeding and death in alcoholic cirrhotic patients with acute variceal bleeding: a multivariate analysis. *World J Surg* 2009; **33**: 2127-2135 [PMID: 19672651 DOI: 10.1007/s00268-009-0172-6]
- 13 **Altamirano J**, Zapata L, Agustin S, Muntaner L, González-Angulo A, Ortiz AL, Degiau L, Garibay J, Camargo L, Genesca J. Predicting 6-week mortality after acute variceal bleeding: role of Classification and Regression Tree analysis. *Ann Hepatol* 2009; **8**: 308-315 [PMID: 20009129]
- 14 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]
- 15 **Andrade ZA**. Schistosomal hepatopathy. *Mem Inst Oswaldo Cruz* 2004; **99**: 51-57 [PMID: 15486635 DOI: 10.1590/S0074-02762004000900009]
- 16 **Chofle AA**, Jaka H, Koy M, Smart LR, Kabangila R, Ewings FM, Mazigo HD, Johnson WD, Fitzgerald DW, Peck RN, Downs JA. Oesophageal varices, schistosomiasis, and mortality among patients admitted with haematemesis in Mwanza, Tanzania: a prospective cohort study. *BMC Infect Dis* 2014; **14**: 303 [PMID: 24894393 DOI: 10.1186/1471-2334-14-303]
- 17 **Mudawi HM**, Ibrahim KB. Endoscopic variceal sclerotherapy in patients with Symmers periportal fibroses. *Trop Doct* 2007; **37**: 179-181 [PMID: 17716514 DOI: 10.1258/004947507781524719]
- 18 **Richter J**, CorreiaDacal AR, VergettiSiqueira JG, Poggensee G, Mannsmann U, Deelder A, Feldmeier H. Sonographic prediction of variceal bleeding in patients with liver fibrosis due to

- Schistosomamansoni. *Trop Med Int Health* 1998; **3**: 728-735 [PMID: 9754668 DOI: 10.1046/j.1365-3156.1998.00285.x]
- 19 **Villanueva C**, Colomo A, Bosch A, Concepción M, Hernandez-Gea V, Aracil C, Graupera I, Poca M, Alvarez-Urturi C, Gordillo J, Guarner-Argente C, Santaló M, Muñoz E, Guarner C. Transfusion strategies for acute upper gastrointestinal bleeding. *N Engl J Med* 2013; **368**: 11-21 [PMID: 23281973 DOI: 10.1056/NEJMoa1211801]
  - 20 **D'Amico G**, Pietrosi G, Tarantino I, Pagliaro L. Emergency sclerotherapy versus vasoactive drugs for variceal bleeding in cirrhosis: a Cochrane meta-analysis. *Gastroenterology* 2003; **124**: 1277-1291 [PMID: 12730868 DOI: 10.1016/S0016-5085(03)00269-5]
  - 21 **Mudawi HM**. Epidemiology of viral hepatitis in Sudan. *Clin-ExpGastroenterol* 2008; **1**: 9-13 [PMID: 21677820 DOI: 10.2147/CEG.S3887]
- P- Reviewer:** Boetto R, Facciorusso A, Fargion S, Gencdal G, Haddad LBD, Obed A **S- Editor:** Gong XM **L- Editor:** A **E- Editor:** Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>





# World Journal of *Hepatology*

*World J Hepatol* 2016 November 18; 8(32): 1343-1418





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vysloulzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*



Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**REVIEW**

- 1343 Anti-hepatitis C virus drugs and kidney

*Carrier P, Essig M, Debette-Gratien M, Sautereau D, Rousseau A, Marquet P, Jacques J, Loustaud-Ratti V*

- 1354 Toll-like receptors in pathophysiology of liver diseases

*Kiziltas S*

**ORIGINAL ARTICLE****Basic Study**

- 1370 Changes in cellular proliferation and plasma products are associated with liver failure

*Melgaço JG, Soriani FM, Sucupira PHF, Pinheiro LA, Vieira YR, de Oliveira JM, Lewis-Ximenez LL, Araújo CCV, Pacheco-Moreira LF, Menezes GB, Cruz OG, Vitral CL, Pinto MA*

**Retrospective Study**

- 1384 Systemic-to-pulmonary artery pressure ratio as a predictor of patient outcome following liver transplantation

*Rebel A, Nguyen D, Bauer B, Sloan PA, DiLorenzo A, Hassan ZU*

**Observational Study**

- 1392 Novel non-invasive biological predictive index for liver fibrosis in hepatitis C virus genotype 4 patients

*Khattab M, Sakr MA, Fattah MA, Mousa Y, Soliman E, Breedy A, Fathi M, Gaber S, Altaweil A, Osman A, Hassouna A, Motawea I*

**Randomized Clinical Trial**

- 1402 Telbivudine vs tenofovir in hepatitis B e antigen-negative chronic hepatitis B patients: OPTIMA roadmap study

*Krastev Z, Petrova D, Kotzev I, Celen MK, Mendelson M, Chandra R, Pandey P, Hamed K*

**CASE REPORT**

- 1414 Spontaneous liver rupture as first sign of polyarteritis nodosa

*Gómez-Luque I, Alconchel F, Ciria R, Ayllón MD, Luque A, Sánchez M, López-Cillero P, Briceño J*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Hong-Chuan Zhao, MD, PhD, Associate Professor, Attending Doctor, Director, Editor, Surgeon, Department of General Surgery, the First Affiliated Hospital of Anhui Medical University, Hefei 230022, Anhui Province, China

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL BOARD MEMBERS**  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

**EDITORIAL OFFICE**  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
November 18, 2016

**COPYRIGHT**  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
<http://www.wjgnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esps/>

## Anti-hepatitis C virus drugs and kidney

Paul Carrier, Marie Essig, Marilyne Debette-Gratien, Denis Sautereau, Annick Rousseau, Pierre Marquet, Jérémie Jacques, Véronique Loustaud-Ratti

Paul Carrier, Marie Essig, Marilyne Debette-Gratien, Annick Rousseau, Pierre Marquet, Véronique Loustaud-Ratti, U850 INSERM, Université de Limoges, 87000 Limoges, France

Paul Carrier, Marilyne Debette-Gratien, Denis Sautereau, Jérémie Jacques, Véronique Loustaud-Ratti, Service d'Hépatogastroentérologie, CHU Limoges, 87042 Limoges, France

**Author contributions:** Carrier P and Loustaud-Ratti V wrote the manuscript; Essig M, Debette-Gratien M, Sautereau D, Rousseau A, Marquet P and Jacques J read the manuscript and conducted a critical analysis.

**Conflict-of-interest statement:** All authors have no conflict of interest concerning this work.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Paul Carrier, MD, Service d'Hépatogastroentérologie, CHU Limoges, 2, Avenue Martin Luther King, 87042 Limoges, France. [pcarrier@hotmail.fr](mailto:pcarrier@hotmail.fr)  
 Telephone: +33-5-55058726  
 Fax: +33-5-55056767

Received: March 26, 2016

Peer-review started: March 26, 2016

First decision: May 23, 2016

Revised: July 27, 2016

Accepted: September 13, 2016

Article in press: September 18, 2016

Published online: November 18, 2016

### Abstract

Hepatitis C virus (HCV) mainly targets the liver but can

also induce extrahepatic manifestations. The kidney may be impacted *via* an immune mediated mechanism or a cytopathic effect. HCV patients are clearly at a greater risk of chronic kidney disease (CKD) than uninfected patients are, and the presence of CKD increases mortality. Interferon-based therapies and ribavirin are difficult to manage and are poorly effective in end-stage renal disease and hemodialysis. These patients should be given priority treatment with new direct anti-viral agents (DAAs) while avoiding peginterferon and ribavirin. The first results were convincing. To aid in the correct use of these drugs in patients with renal insufficiency, their pharmacokinetic properties and potential renal toxicity must be known. The renal toxicity of these new drugs was not a safety signal in clinical trials, and the drugs are generally efficient in these frail populations. These drugs are usually well tolerated, but recent cohort studies have demonstrated that these new regimens may be associated with renal side effects, especially when using sofosbuvir combinations. HCV, renal diseases and comorbidities are intimately linked. The close monitoring of renal function is required, particularly for at-risk patients (transplanted, HIV-coinfected, CKD, hypertensive or diabetic patients). New DAA regimens, which will soon be approved, will probably change the landscape.

**Key words:** Nephrotoxicity; Hepatitis C; Direct anti-viral agents; Kidney; End-stage renal disease

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatitis C patients are clearly at risk of chronic kidney disease (CKD). New direct anti-viral agents (DAAs) with different pharmacokinetic properties are generally efficient in such populations. However, renal toxicity has been described in frail patients such as patients with CKD, transplants and human immunodeficiency virus co-infections under real-life conditions, especially with sofosbuvir combinations. New DAAs, which will be soon approved, will probably change the



landscape favorably. Close monitoring of renal function is required for at-risk patients, but patients without comorbidities are probably at a very low risk of renal toxicity.

Carrier P, Essig M, Debette-Gratien M, Sautereau D, Rousseau A, Marquet P, Jacques J, Loustaud-Ratti V. Anti-hepatitis C virus drugs and kidney. *World J Hepatol* 2016; 8(32): 1343-1353 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i32/1343.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i32.1343>

## INTRODUCTION

Hepatitis C virus (HCV) mainly targets the liver but also targets the kidney *via* either an immune mediated mechanism (cryoglobulinemic vasculitis) or a cytopathic effect<sup>[1-3]</sup>.

Epidemiological studies show that the risk of chronic kidney disease (CKD) is 20% higher in HCV patients than in uninfected individuals<sup>[4]</sup>. HCV increases the risk of both end-stage renal disease (ESRD)<sup>[5]</sup> and renal mortality<sup>[6]</sup>. Moreover, patients who are infected with HCV exhibit an increased risk of developing diabetes, high blood pressure and secondary vascular renal diseases<sup>[7]</sup>. Finally, chronic hepatitis C is the most commonly seen viral infection in patients with renal insufficiency<sup>[8]</sup>; its treatment is warranted and remains a great challenge.

Historically, interferon-based therapy was considered nephrotoxic in a dose-dependent or idiosyncratic manner<sup>[9]</sup>. First-generation protease inhibitors (*i.e.*, telaprevir and boceprevir in association with peginterferon and ribavirin) have also been implicated<sup>[10]</sup>, although their role remains controversial<sup>[11]</sup>. Although ribavirin is not nephrotoxic, it accumulates in patients with CKD, and its secondary effects (particularly anemia) are much more severe.

Although new direct anti-viral agents (DAAs) were very well tolerated in phase III trials, recent real-life studies have demonstrated some nephrotoxicity in frail populations that were treated with sofosbuvir-based regimens<sup>[12,13]</sup>.

After a brief review of the pharmacokinetics of anti-HCV drugs, we review their potential renal toxicity and clinical experiences related to the use of these drugs in populations at risk of renal disease.

## PHARMACOKINETICS OF HCV

### TREATMENTS

#### *Treatments that are available in 2016*

The combination of pegylated interferon and ribavirin with or without first-generation protease inhibitors (boceprevir and telaprevir) is no longer used in many developed countries<sup>[14-16]</sup>. However, it may still be relevant in developing countries.

Standard care in countries where DAAs are available is based on the combinations of two or three DAAs from

different families: Second-generation protease inhibitors, NS5B polymerase inhibitors, and NS5A inhibitors. Ribavirin may be added in cirrhotic patients to shorten treatment duration.

All but two DAA phase III studies did not include patients with severe renal insufficiency (4-5 CKD stages)<sup>[17,18]</sup>. Sparse data are thus available, and guidelines recommend that these patients be referred to expert centers<sup>[14]</sup>.

To justify the proper use of HCV treatments in renal insufficiency, the pharmacokinetic properties of these drugs should be remembered.

#### *Pharmacokinetics of interferon, pegylated interferons and ribavirin*

Interferons are natural cytokines. Alpha interferon and its pegylated form are active against viral replication. Pegylation prolongs the half-life of interferon, thus necessitating fewer injections<sup>[19-21]</sup>. The kidney plays a central role in interferon clearance. Interferon is filtered through glomeruli and undergoes lysosomal proteolytic degradation during proximal tubular reabsorption<sup>[22,23]</sup>.

Ribavirin is a guanosine analog that exhibits broad-spectrum activity against DNA and RNA viruses. Its mechanism of action is based on the erroneous incorporation of ribavirin triphosphate into replicating RNA strands, thereby inhibiting chain elongation<sup>[24]</sup>. When used with interferon, ribavirin acts synergistically, preventing relapses and breakthroughs, and remains relevant in the DAA era in special circumstances. The major side effects of ribavirin are hemolytic anemia and teratogenicity. The renal excretion of ribavirin and its metabolites accounts for 40% of its clearance; the remainder is eliminated through the spleen *via* its principal metabolite, ribavirin triphosphate, which is captured in erythrocytes. Based on the product characteristics, the ribavirin area under the concentration curve (AUC) is doubled when calculating estimated glomerular filtration rates (eGFRs) between 30 and 45 mL/min per 1.73 m<sup>2</sup> and is tripled when calculating eGFRs between 13 and 30 mL/min per 1.73 m<sup>2</sup><sup>[25]</sup>.

#### *Pharmacokinetics of DAAs*

**First-generation protease inhibitors:** Telaprevir and boceprevir are significantly high CYP3A4, P-glycoprotein (P-gp) inhibitors and are also OATP1B1/2 and OCT 1 and 2 inhibitors, respectively.

Thus, they interact significantly with calcineurin inhibitors in transplant patients and with some human immunodeficiency virus (HIV)-specific medications, thereby increasing the renal toxicity of these drugs by increasing their exposure<sup>[26,27]</sup>. These drugs are poorly eliminated by the kidney (1% for telaprevir<sup>[28]</sup>, 9% for boceprevir<sup>[29]</sup>). Telaprevir is excreted by the tubular cells through organic cation transporter 2 (OCT2) and presents a risk of interaction with medications such as dolutegravir<sup>[30]</sup>.

**New DAAs:** Most new DAAs are eliminated in the bile, with the exception of sofosbuvir, which is the keystone

of the main approved DAA regimens.

Sofosbuvir weakly inhibits CYP3A4, intestinal P-gp, and BCRP. Seventy-two percent of sofosbuvir is eliminated by the kidney, primarily as its main metabolite GS-331007<sup>[31]</sup>. The mechanism of clearance warrants study, even if it is reasonable to evoke tubular excretion by analogy with HIV or hepatitis B virus (HBV) analogs. GS-331007 AUC is greater than 55%, 88% and 451% in cases of mild, moderate and severe renal insufficiency, respectively. GS-331007 exposure is increased by at least 10 to 20 times in patients with ESRD<sup>[32]</sup>.

**Several DAAs can be used in combination with sofosbuvir:** (1) NS3/4 protease inhibitor: Simeprevir moderately inhibits CYP3A and intestinal P-gp and potentially inhibits OATP1B1 and MRP2. Its urinary excretion is less than 1%<sup>[33]</sup>. On average, the simeprevir AUC is increased by 62% in subjects with severe renal impairment. The drug is not eliminated by dialysis; and (2) NS5A inhibitors: Daclatasvir is a substrate of CYP3A4 and P-gp and moderately inhibits OATP1B1/3 and P-gp. Its excretion in urine is < 1%. In case of severe renal insufficiency, AUC is increased by 27%, but no dose adjustment is needed<sup>[34]</sup>. Ledipasvir is a weak inhibitor of P-gp and BCRP. Its renal excretion is < 1%<sup>[35]</sup>, and its pharmacokinetics are not altered by severe renal impairment<sup>[36]</sup>. Velpatasvir moderately interacts with CYP3A4, CYP2C8, OATP and P-gp<sup>[37]</sup> and is primarily eliminated in the feces (> 99%). The sofosbuvir/velpatasvir combination will be available soon. According to very preliminary data, this combination appears well tolerated in subjects with severe renal impairment. Velpatasvir AUC is approximately 50% higher in these subjects than in subjects with normal function<sup>[38]</sup>.

**Other combinations exist:** (1) paritaprevir/ritonavir (anti-protease inhibitor), ombitasvir (anti-NS5A inhibitor) and dasabuvir (anti-polymerase inhibitor). Paritaprevir/ritonavir is a powerful CYP3A4 inhibitor. Ritonavir is a well-known inhibitor of many renal transporters including OAT1, OAT2, MRP2, MRP4 and MATE1<sup>[39]</sup>. The four-drug combination is a substrate of P-gp and CYP3A4 and is mainly eliminated in the bile<sup>[40,41]</sup>. In case of CKD 1, paritaprevir and dasabuvir AUCs are increased by 20%, and ritonavir AUC is increased by 42%. In patients with CKD 2 and 3, paritaprevir and dasabuvir AUCs are increased by 37% and ritonavir AUC is increased by 80%. In patients with CKD 4, paritaprevir and dasabuvir AUCs are increased by 50%, and ritonavir AUC is increased by 114%. Ombitasvir AUC remains unchanged<sup>[42]</sup>, and (2) grazoprevir and elbasvir: This regimen will be available soon. Both molecules are substrates of CYP3A4, OATP and P-gp<sup>[43]</sup>. Less than 1% of grazoprevir and elbasvir are excreted by the kidney; the AUC<sub>0-24h</sub> values of grazoprevir and elbasvir are higher in subjects with severe renal insufficiency relative to controls [1.65- (1.09, 2.49) and 1.86-fold (1.38, 2.51) (90%CI), respectively]. Drug removal by hemodialysis is negligible<sup>[44]</sup>. Clinical

experience shows that dose adjustment is not needed in the setting of non-dialysis-dependent stage 4-5 CKD and dialysis-dependent stage 5 CKD<sup>[17]</sup>.

## SPECIFIC NEPHROTOXICITY OF HCV DRUGS

### *Interferon or pegylated interferon and ribavirin*

A dose-dependent or idiosyncratic renal toxicity of alpha interferon and pegylated interferon is well established although rare<sup>[45]</sup>. This nephrotoxicity is mostly reported in cases of malignancy<sup>[46,47]</sup>. However, no correlations were found among the occurrence of renal involvement, the type of interferon used, administration route, treatment dosage and duration, and the patient's profile. The histological features are nonspecific and various, mainly involving minimal forms of glomerular damage, including cellular hyperplasia and focal segmental glomerulosclerosis, which are often associated with nephrotic syndrome<sup>[45,48-51]</sup>. Interferon may worsen any pre-existing glomerular lesions<sup>[52]</sup>. Microangiopathic thrombosis has also been described<sup>[53,54]</sup>. More rarely, interstitial fibrosis (usually mild) as well as nonspecific interstitial inflammation and tubular atrophy, and interstitial nephritis associated with nephrotic syndrome<sup>[55]</sup> or acute tubulopathy<sup>[47,56,57]</sup> have been reported.

Proteinuria (usually a self-limited proteinuria that does not exceed 1 g/d) is observed in 15% to 20% of patients taking interferon<sup>[58,59]</sup>. Nevertheless, hepatitis C-associated glomerulonephritis may be cured with alpha interferon-based treatment, independent of SVR<sup>[60]</sup>.

Renal failure generally occurs during the first weeks of treatment and rarely occurs after several months<sup>[61]</sup>.

The involved physiopathological mechanisms are not clear. In a cellular model, Lechner *et al.*<sup>[62]</sup> demonstrated that interferon directly affects tubular barrier function in renal epithelial cells in a reversible time- and dose-dependent manner. More recently, the same team showed that alpha interferon can activate caspase-3, -8 and -9, which favors the apoptosis cascade in renal proximal tubular epithelia. Gresser *et al.*<sup>[63]</sup> showed that the daily administration of interferon to newborn mice can lead to severe glomerulopathy associated with glomerular sclerosis and IgG and C3 deposits<sup>[64]</sup>.

Ribavirin renal toxicity has not been documented and is not probable in monotherapy<sup>[65,66]</sup>. Nevertheless, by analogy with the ribavirin apoptotic activity observed in K562 leukemia cells, potential tubular toxicity has been hypothesized<sup>[65,67]</sup>.

### *New treatments and nephrotoxicity*

**Boceprevir and telaprevir:** The first-generation protease inhibitors boceprevir and telaprevir have been combined with pegylated interferon and ribavirin. No renal side effect was found in phase III studies<sup>[68-74]</sup>, which is consistent with the weak renal clearance of these drugs. Nevertheless, in a large cohort (1486 patients),

Mauss *et al.*<sup>[10]</sup> showed a reversible decrease of eGFR in patients taking telaprevir or boceprevir. Similar reports involving telaprevir therapy confirmed this observation and suggested a link with anemia occurrence<sup>[75-78]</sup>. Recently, Kunze *et al.*<sup>[30]</sup> described competition between telaprevir and OCT2, which interacts with creatinine tubular transport and is involved in proximal tubular secretion. Our team validated this hypothesis with a predictive model suggesting that the clinically observed creatinine increase is not due to renal toxicity of the drug<sup>[11]</sup>. Independent of this pharmacological effect, one of our patients experienced acute renal failure at week 20 of telaprevir treatment. In addition to extra-membranous glomerulonephritis, the renal biopsy showed particularly intense interstitial fibrosis that would exceptionally be described by pegylated interferon and probably implies telaprevir or a combination of telaprevir-pegylated interferon<sup>[3]</sup>.

**New DAAs:** The renal toxicity of new DAA was not a safety signal in phase III clinical trials<sup>[79-83]</sup>; however, most of the included patients presented with eGFR values of greater than 60 mL/min per 1.73 m<sup>2</sup> and few comorbidities. The prescription of sofosbuvir is not desirable for patients with an eGFR of less than 30 mL/min per 1.73 m<sup>2</sup>. In practice, however, half of the daily dose<sup>[84]</sup> or a full dose taken every other day<sup>[85]</sup> was found safe. Various recommendations<sup>[14-16]</sup> specify that renal function should be monitored during treatment with sofosbuvir (grade B). Indeed, on the one hand, the drug is cleared by the kidney; on the other hand, a structural analogy with HBV nucleotide analogs is observed. Therefore, competitive risks with other drugs (antiviral or anti-calcineurins) that are eliminated by the tubule are awaited. In a prospective unselected HCV population, we were unable to find evidence for the induction of subclinical tubulopathy by the antiviral treatment when using tools for the early detection of proximal tubular injury (unpublished data). However, potential proximal tubular toxicity can be hypothesized.

DAAs are usually combined with sofosbuvir, *i.e.*, simeprevir, daclatasvir and ledipasvir do not appear to increase renal risk, although it is difficult to distinguish between the contributions of sofosbuvir and other drugs with which it is combined to the occurrence of renal failure: (1) simeprevir: Renal failure resulting from simeprevir therapy was not found in phase III studies<sup>[86,87]</sup>, except in association with sofosbuvir<sup>[13,88]</sup>; (2) daclatasvir: No renal warning was observed in phase III studies<sup>[89]</sup>, except when daclatasvir was associated with sofosbuvir in liver transplant patients<sup>[90]</sup>; and (3) ledipasvir: One case report suggested possible acute renal toxicity, but this occurred in association with sofosbuvir<sup>[91]</sup>.

Concerning the combination ombitasvir, paritaprevir/ritonavir, dasabuvir, plasma creatinine increase was described in 2 of the 293 patients who had experienced previous interferon-based treatment<sup>[92]</sup>. Other phase III studies did not describe any renal adverse event<sup>[93-96]</sup>.

## EXPERIENCES ON ANTI-HCV THERAPIES IN POPULATIONS AT RENAL RISK

### ESRD and hemodialysis

HCV prevalence is high among patients on long-term dialysis (5% to 10% in Europe and in the United States and 10% to 70% in developing countries)<sup>[97]</sup>. HCV decreases global survival in this population<sup>[98]</sup>.

HCV screening is recommended once yearly in hemodialysis patients. Patients generally present with normal transaminase levels<sup>[99]</sup>, low viral load<sup>[100]</sup>, and moderate fibrosis stage<sup>[8,101,102]</sup>, although fibrosis appears to progress more rapidly in this population. For these reasons, anti-HCV treatment is warranted.

Three meta-analyses of historical treatment with pegylated alpha interferon and ribavirin showed a 40% SVR in ESRD<sup>[103-105]</sup>. The results obtained did not differ between alpha interferon and pegylated alpha interferon<sup>[106]</sup>. Ribavirin is generally contra-indicated in patients with eGFR values of less than 50 mL/min due to the high risk of ribavirin metabolite accumulation in erythrocytes, which increases the amplitude of hemolytic anemia<sup>[24,107]</sup>. However, at minimal doses, ribavirin was used after each dialysis session<sup>[108]</sup> or 5 d per week<sup>[109]</sup>. The erythropoietin doses were usually increased<sup>[109]</sup>.

First-generation protease inhibitors in combination with pegylated interferon and ribavirin gave potentially interesting results<sup>[110-113]</sup>, but the observed high antiviral efficacy was accompanied by numerous serious adverse effects<sup>[112]</sup>.

ESRD and dialysis patients should be given priority treatment with new DAAs while avoiding peginterferon and ribavirin.

The currently available data on the approved DAAs are sparse. The adequate dose of sofosbuvir is unknown, and ribavirin should be avoided (see above).

Small preliminary studies, mainly based on the sofosbuvir/simeprevir combination<sup>[84,114,115]</sup>, have shown a SVR rate of between 87% and 100% in ESRD genotype 1 patients. In a real-life TARGET cohort evaluating a sofosbuvir and simeprevir regimen, similar results were observed, with an increased benefit when adding ribavirin; however, anemia risk was increased<sup>[13]</sup>. In summary, the safety of sofosbuvir in ESRD is unclear, and larger trials are awaited.

Recently, preliminary results of the RUBY-1 trial including 20 patients with CKD 4 renal insufficiency receiving the approved regimen of ombitasvir, paritaprevir/ritonavir, and dasabuvir with (genotype 1a) or without (genotype 1b) ribavirin showed a SVR of 90%; however, ribavirin had to be stopped in 9 of the 13 G1a patients<sup>[18]</sup>.

More recently, elbasvir and grazoprevir were administered together once daily in the largest trial to date (the Phase III C-SURFER study); the trial included 224 ESRD patients, 179 of whom were hemodialysis dependent, and achieved a 99% SVR12 in genotype 1 patients<sup>[17]</sup>. Elbasvir and grazoprevir are expected to be approved shortly.

Thus, two regimens are or will be recommended in genotype 1 patients with severe renal insufficiency: Paritaprevir-ritonavir-ombitasvir-dasabuvir for patients with G1b and grazoprevir-elbasvir for patients with all G1 subtypes.

### **Patients with renal impairment**

In the TARGET cohort, the sofosbuvir/simeprevir combination (with or without ribavirin or pegylated interferon) was found to be efficacious and safe in HCV-infected patients of differing CKD stage. Compared with patients without renal insufficiency, these patients experienced a deterioration of their eGFR (25% with an initial eGFR < 30 mL/min per 1.73 m<sup>2</sup>, 13% with an eGFR of between 31 and 45 mL/min per 1.73 m<sup>2</sup>, and 1% to 2% with an eGFR > 45 mL/min per 1.73 m<sup>2</sup>). These results suggest that sofosbuvir-based treatments used in kidney patients warrant close monitoring<sup>[13]</sup>. In the TARGET cohort, patients with a basal eGFR of less than 30 mL/min per 1.73 m<sup>2</sup> showed a high risk of acute renal insufficiency (25%)<sup>[13]</sup>.

### **Kidney transplantation**

HCV prevalence among kidney transplant patients is approximately 10%, and most of the patients are viremic<sup>[116]</sup>. HCV decreases global survival in this population<sup>[117]</sup>.

HCV also increases sepsis, diabetes, glomerulonephritis and rejection<sup>[102,117-120]</sup>.

Anti-viral treatment is recommended for preventing fibrosis progression, risk of fibrosing cholestatic hepatitis and sepsis. Interferon is no longer recommended in this setting due to the strong risk of rejection<sup>[121]</sup>, although this risk has been shown to be lower than expected<sup>[122,123]</sup>. Moreover, meta-analyses have demonstrated a weak SVR rate (18% to 26.9%) and a high rate of withdrawal: 21.1% to 35% with alpha interferon<sup>[124,125]</sup> and 40.6% with pegylated interferon<sup>[125]</sup>. No data with pegylated interferon and boceprevir or telaprevir-based triple therapy are available. However, the data obtained from liver transplant experience show that it is very difficult to manage drug interactions with calcineurin inhibitors, thus leading to serious adverse events<sup>[26]</sup>.

A few published preliminary studies using sofosbuvir-based combinations showed a SVR > 95%; however, the immunosuppressing drug concentrations varied, a finding that should be studied and monitored<sup>[126-130]</sup>. Liver transplantation experience is more important, and treatment of such patients has shown good results in terms of efficacy, tolerance and medication interactions<sup>[90,131]</sup>.

Recently, the concept of pre-transplant treatment has become preeminent, especially for patients of genotypes 1 and 4, due to the availability of regimens avoiding sofosbuvir<sup>[17]</sup>. However, patients with genotypes 2 and 3 for whom sofosbuvir-based regimens are recommended should be treated after kidney transplantation while awaiting new pangenotypic combinations<sup>[132]</sup>.

### **Liver transplantation**

In the French CUPILT cohort of liver transplant patients who were treated with sofosbuvir and daclatasvir,

37.1% experienced a 25% decrease of GFR during or after treatment; however, in 10.9% of the cases, this GFR decrease was not reversible. The existence of prior kidney disease and fibrosing cholestatic hepatitis were both independent predictors of decreased GFR. The authors emphasized the importance of close renal function monitoring in this population<sup>[12]</sup>. These data were confirmed in an American multicenter study<sup>[133]</sup>. Moreover, patients with fibrosing cholestatic hepatitis who were treated with sofosbuvir, ribavirin and pegylated interferon ( $n = 8$ ) or daclatasvir, sofosbuvir and ribavirin ( $n = 14$ ) experienced high rates of renal failure (4/8 and 7/14, respectively), including 1 with creatinine clearance of less than 30 mL/min per 1.73 m<sup>2</sup><sup>[90]</sup>.

### **Coinfected patients**

In coinfecting patients of the ION-4 study, who were treated with sofosbuvir/ledipasvir, 4 of the 335 patients exhibited worsened renal function (a creatinine increase of 35  $\mu$ mol/L or more); tenofovir AUC<sub>tau</sub> increased by 20% and 30% in two patients, one patient discontinued tenofovir, and the drug dose was reduced for one patient<sup>[134]</sup>.

Renal function improved in all patients after treatment discontinuation.

### **Particular cases**

**Acute renal insufficiency:** Acute renal insufficiency has mainly been reported in cohorts with high renal risk. Recently, the first case of acute kidney injury, as documented by renal biopsy, was described in a patient receiving sofosbuvir and ledipasvir and suffering from hypertension and diabetes mellitus type 2: The biopsy showed an acute allergic interstitial nephritis with diabetic nephropathy. Corticosteroid therapy was introduced, and this stabilized the renal function<sup>[91]</sup>.

**Adolescents and children:** The pharmacokinetics of new antiviral drugs are not known in this population. To our knowledge, only one study using ledipasvir/sofosbuvir (90/400 mg) in 100 adolescent patients (12 to 17 years old) with HCV genotype 1 for 12 wk resulted in an SVR12 rate of 97%, a similar result to that obtained in adults. Ledipasvir/sofosbuvir was well tolerated with no grade 3-4 adverse events, serious adverse events, or treatment discontinuations due to adverse events<sup>[135]</sup> in particular renal events. In the context of the universal use of new DAAs, a study in children aged 3 to < 12 years is ongoing (ClinicalTrials.gov Identifier: NCT02249182).

In summary, sofosbuvir-based combinations have exhibited renal toxicity in frail patients such as CKD, transplant and HIV co-infected patients under real-life conditions. Real-life studies suggest a risk of eGFR deterioration in patients with previous renal impairment, suggesting that these combinations be used cautiously in this setting including, in particular, diabetes mellitus and hypertension.

Physiopathologically, tubular toxicity can be suggested by structural analogy between this drug and antiretroviral analogs; however, this was not demonstrated in patients



with normal renal function. Nevertheless, these new anti-HCV DAAs appear to act synergistically with drugs that are known to exert a toxic action on the tubule, such as anticalcineurins and tenofovir. Finally, a classic drug-induced renal tubulointerstitial disease of immunological origin has recently been described in at least one documented case with renal biopsy.

New combinations, such as paritaprevir-ritonavir-ombitasvir-dasbuvir for genotype 1b and grazoprevir-elbasvir for all genotype 1 subtypes, show promise in patients with severe renal impairment.

## CONCLUSION

HCV treatment should be offered to all patients with ESRD or kidney transplant candidates, regardless of liver fibrosis stage, due to the intimate link between HCV, renal diseases and comorbidities such as cardiovascular complications and diabetes and because of the impact of HCV on mortality.

There is no clear recommendation for the use of currently approved DAAs in cases of severe renal insufficiency; these drugs may be prescribed under certain conditions, preferably without ribavirin. However, expert opinions are needed.

New DAAs, which will soon be approved, will probably favorably change the landscape.

DAA regimens can present renal side effects, especially sofosbuvir combinations. Close monitoring of renal function is required in at-risk patients comprising patients with CKD, ESRD and hemodialysis, hypertension and diabetes, HIV coinfection, and transplant patients. Current recommendations require the universal monitoring of renal function in patients treated with DAAs. However, patients with none of the above described comorbidities are probably at very low risk of renal toxicity and will no longer require such close monitoring in future.

## ACKNOWLEDGMENTS

We thank Céline Rigaud for her help and Sarah Demai for her English proofreading.

## REFERENCES

- 1 Wörnle M, Schmid H, Banas B, Merkle M, Henger A, Roeder M, Blattner S, Bock E, Kretzler M, Gröne HJ, Schlöndorff D. Novel role of toll-like receptor 3 in hepatitis C-associated glomerulonephritis. *Am J Pathol* 2006; **168**: 370-385 [PMID: 16436653 DOI: 10.2353/ajpath.2006.050491]
- 2 Fabrizi F, Plaisier E, Saadoun D, Martin P, Messa P, Cacoub P. Hepatitis C virus infection, mixed cryoglobulinemia, and kidney disease. *Am J Kidney Dis* 2013; **61**: 623-637 [PMID: 23102733 DOI: 10.1053/j.ajkd.2012.08.040]
- 3 Carrier P, Chambaraud T, Vong C, Guillaudeau A, Debette-Gratien M, Jacques J, Legros R, Sautereau D, Essig M, Loustaud-Ratti V. Severe renal impairment during triple therapy with telaprevir. *Clin Res Hepatol Gastroenterol* 2014; **38**: e69-e71 [PMID: 24461554 DOI: 10.1016/j.clinre.2013.12.005]
- 4 Park H, Adeyemi A, Henry L, Stepanova M, Younossi Z. A meta-analytic assessment of the risk of chronic kidney disease in patients with chronic hepatitis C virus infection. *J Viral Hepat* 2015; **22**: 897-905 [PMID: 25904153 DOI: 10.1111/jvh.12413]
- 5 Hsu YC, Ho HJ, Huang YT, Wang HH, Wu MS, Lin JT, Wu CY. Association between antiviral treatment and extrahepatic outcomes in patients with hepatitis C virus infection. *Gut* 2015; **64**: 495-503 [PMID: 25398770 DOI: 10.1136/gutjnl-2014-308163]
- 6 Lee MH, Yang HI, Lu SN, Jen CL, You SL, Wang LY, Wang CH, Chen WJ, Chen CJ. Chronic hepatitis C virus infection increases mortality from hepatic and extrahepatic diseases: a community-based long-term prospective study. *J Infect Dis* 2012; **206**: 469-477 [PMID: 22811301 DOI: 10.1093/infdis/jis385]
- 7 Petta S, Macaluso FS, Craxi A. Cardiovascular diseases and HCV infection: a simple association or more? *Gut* 2014; **63**: 369-375 [PMID: 24295849 DOI: 10.1136/gutjnl-2013-306102]
- 8 Kamar N, Alric L, Izopet J, Rostaing L. Hepatitis C virus and kidney disease. *Clin Res Hepatol Gastroenterol* 2013; **37**: 328-333 [PMID: 23522570 DOI: 10.1016/j.clinre.2013.02.010]
- 9 Izzedine H, Launay-Vacher V, Bourry E, Brocheriou I, Karie S, Deray G. Drug-induced glomerulopathies. *Expert Opin Drug Saf* 2006; **5**: 95-106 [PMID: 16370959 DOI: 10.1517/14740338.5.1.95]
- 10 Mauss S, Hueppe D, Alshuth U. Renal impairment is frequent in chronic hepatitis C patients under triple therapy with telaprevir or boceprevir. *Hepatology* 2014; **59**: 46-48 [PMID: 23813604 DOI: 10.1002/hep.26602]
- 11 Loustaud-Ratti V, Rousseau A, Carrier P, Vong C, Chambaraud T, Jacques J, Debette-Gratien M, Sautereau D, Essig M. eGFR decrease during antiviral C therapy with first generation protease inhibitors: a clinical significance? *Liver Int* 2015; **35**: 71-78 [PMID: 25039814 DOI: 10.1111/liv.12631]
- 12 Coilly A, Fougerou C, De Ledinghen V, Houssel-Debry P, Duvoux C, Di Martino V, Radenne S, Kamar N, D'Alteroche L, Leroy V, Canva V, Lebray P, Moreno C, Dumortier J, Silvain C, Besch C, Perre P, Botta-Fridlund D, Anty R, Rohel A, Renault A, Danjou H, Duclos-Vallee J-C, Pageaux G-P. G15: The association of sofosbuvir and daclatasvir for treating severe recurrence of HCV infection after liver transplantation: Results from a large french prospective multicentric ANRS CO23 CUPILT cohort. *J Hepatol* 2015; **62** (Suppl 2): S236-7 [DOI: 10.1016/S0168-8278(15)30103-3]
- 13 Saxena V, Korashy FM, Sise ME, Lim JK, Schmidt M, Chung RT, Liapakis A, Nelson DR, Fried MW, Terrault NA. Safety and efficacy of sofosbuvir-containing regimens in hepatitis C-infected patients with impaired renal function. *Liver Int* 2016; **36**: 807-816 [PMID: 26923436 DOI: 10.1111/liv.13102]
- 14 AFEF, Société Française d'Hépatologie, Recommandations sur la prise en charge des hépatites virales C, février 2016. Available from: URL: [http://www.afef.asso.fr/ckfinder/userfiles/files/recommandations-textes-officiels/Recommandations\\_AFEF\\_Hepati teC\\_Final-02-2016.pdf](http://www.afef.asso.fr/ckfinder/userfiles/files/recommandations-textes-officiels/Recommandations_AFEF_Hepati teC_Final-02-2016.pdf)
- 15 European Association for Study of Liver. EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol* 2015; **63**: 199-236 [PMID: 25911336 DOI: 10.1016/j.jhep.2015.03.025]
- 16 AASLD/IDSA HCV Guidance Panel. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology* 2015; **62**: 932-954 [PMID: 26111063 DOI: 10.1002/hep.27950]
- 17 Roth D, Nelson DR, Bruchfeld A, Liapakis A, Silva M, Monsour H, Martin P, Pol S, Londoño MC, Hassanein T, Zamor PJ, Zuckerman E, Wan S, Jackson B, Nguyen BY, Robertson M, Barr E, Wahl J, Greaves W. Grazoprevir plus elbasvir in treatment-naïve and treatment-experienced patients with hepatitis C virus genotype 1 infection and stage 4-5 chronic kidney disease (the C-SURFER study): a combination phase 3 study. *Lancet* 2015; **386**: 1537-1545 [PMID: 26456905 DOI: 10.1016/S0140-6736(15)00349-9]
- 18 Pockros PJ, Reddy KR, Mantry PS, Cohen E, Bennett M, Sulkowski MS, Bernstein DE, Cohen DE, Shulman NS, Wang D, Khatri A, Abunimeh M, Podsadecki T, Lawitz E. Efficacy of Direct-Acting Antiviral Combination for Patients With Hepatitis C Virus Genotype 1 Infection and Severe Renal Impairment or End-Stage Renal Disease. *Gastroenterology* 2016; **150**: 1590-1598 [PMID: 26976799 DOI: 10.1053/j.gastro.2016.02.078]

- 19 **Glue P**, Fang JW, Rouzier-Panis R, Raffanel C, Sabo R, Gupta SK, Salfi M, Jacobs S. Pegylated interferon-alpha2b: pharmacokinetics, pharmacodynamics, safety, and preliminary efficacy data. Hepatitis C Intervention Therapy Group. *Clin Pharmacol Ther* 2000; **68**: 556-567 [PMID: 11103758 DOI: 10.1067/mcp.2000.110973]
- 20 **European Medicines Agency**. Résumé des caractéristiques du produit Pegasys. Available from: URL: [http://www.ema.europa.eu/docs/fr\\_FR/document\\_library/EPAR\\_-\\_Product\\_Information/human/000395/WC500039195.pdf](http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/000395/WC500039195.pdf)
- 21 **European Medicines Agency**. Résumé des caractéristiques du produit Pegatron. Available from: URL: [http://www.ema.europa.eu/docs/fr\\_FR/document\\_library/EPAR\\_-\\_Product\\_Information/human/000280/WC500039388.pdf](http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/000280/WC500039388.pdf)
- 22 **Gad SC**. Handbook of Pharmaceutical Biotechnology. John Wiley & Sons; 2007
- 23 **Wills RJ**. Clinical pharmacokinetics of interferons. *Clin Pharmacokinet* 1990; **19**: 390-399 [PMID: 1702693 DOI: 10.2165/00003088-199019050-00003]
- 24 **Loustaud-Ratti V**, Rousseau A, Marquet P, Denis F, Alain S. Ribavirin in chronic hepatitis C: past and future. *Expert Rev Anti Infect Ther* 2009; **7**: 249-253 [PMID: 19344238 DOI: 10.1586/eri.09.5]
- 25 **European Medicines Agency**. Résumé des caractéristiques du produit Rebetol. Available from: URL: [http://www.ema.europa.eu/docs/fr\\_FR/document\\_library/EPAR\\_-\\_Product\\_Information/human/000246/WC500048210.pdf](http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/000246/WC500048210.pdf)
- 26 **Coilly A**, Dumortier J, Botta-Fridlund D, Latournerie M, Leroy V, Pageaux GP, Agostini H, Giostra E, Moreno C, Roche B, Antonini TM, Guillaud O, Lebray P, Radenne S, Saouli AC, Calmus Y, Alric L, Debette-Gratien M, De Ledinghen V, Durand F, Duvoux C, Samuel D, Duclos-Vallée JC. Multicenter Experience with Boceprevir or Telaprevir to Treat Hepatitis C Recurrence after Liver Transplantation: When Present Becomes Past, What Lessons for Future? *PLoS One* 2015; **10**: e0138091 [PMID: 26394142 DOI: 10.1371/journal.pone.0138091]
- 27 **Antonini TM**, Furlan V, Teicher E, Haim-Boukobza S, Sebah M, Coilly A, Bonhomme-Faivre L, Roque-Afonso AM, Vittecoq D, Samuel D, Taburet AM, Duclos-Vallée JC. Therapy with boceprevir or telaprevir in HIV/hepatitis C virus co-infected patients to treat recurrence of hepatitis C virus infection after liver transplantation. *AIDS* 2015; **29**: 53-58 [PMID: 25387314 DOI: 10.1097/QAD.0000000000000516]
- 28 **European Medicines Agency**. Résumé des caractéristiques du produit Incivo. Available from: URL: [http://www.ema.europa.eu/docs/fr\\_FR/document\\_library/EPAR\\_-\\_Product\\_Information/human/002313/WC500115529.pdf](http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/002313/WC500115529.pdf)
- 29 **European Medicines Agency**. Résumé des caractéristiques du produit Victrelis. Available from: URL: [http://www.ema.europa.eu/docs/fr\\_FR/document\\_library/EPAR\\_-\\_Product\\_Information/human/002332/WC500109786.pdf](http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/002332/WC500109786.pdf)
- 30 **Kunze A**, Huwyler J, Camenisch G, Gutmann H. Interaction of the antiviral drug telaprevir with renal and hepatic drug transporters. *Biochem Pharmacol* 2012; **84**: 1096-1102 [PMID: 22902721 DOI: 10.1016/j.bcp.2012.07.032]
- 31 **European Medicines Agency**. Résumé des caractéristiques du produit Sovaldi. Available from: URL: [http://www.ema.europa.eu/docs/fr\\_FR/document\\_library/EPAR\\_-\\_Product\\_Information/human/002798/WC500160597.pdf](http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/002798/WC500160597.pdf)
- 32 **Kirby BJ**, Symonds WT, Kearney BP, Mathias AA. Pharmacokinetic, Pharmacodynamic, and Drug-Interaction Profile of the Hepatitis C Virus NS5B Polymerase Inhibitor Sofosbuvir. *Clin Pharmacokinet* 2015; **54**: 677-690 [PMID: 25822283 DOI: 10.1007/s40262-015-0261-7]
- 33 **European Medicines Agency**. Résumé des caractéristiques du produit Olysio. Available from: URL: [http://www.ema.europa.eu/docs/fr\\_FR/document\\_library/EPAR\\_-\\_Product\\_Information/human/002777/WC500167867.pdf](http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/002777/WC500167867.pdf)
- 34 **European Medicines Agency**. Résumé des caractéristiques du produit Daklinza. Available from: URL: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_-\\_Product\\_Information/human/003768/WC500172848.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/003768/WC500172848.pdf)
- 35 **European Medicines Agency**. Résumé des caractéristiques du produit Harvoni. Available from: URL: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_-\\_Product\\_Information/human/003850/WC500177995.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/003850/WC500177995.pdf)
- 36 **Mogalian E**. The Pharmacokinetics of Ledipasvir, an HCV Specific NS5A Inhibitor, in HCV-Uninfected Subjects with Severe Renal Impairment. Available from: URL: <http://liverlearning.aasld.org/aasld/2014/thelivermeeting/61996/erik.mogalian.the.pharmacokinetics.of.ledipasvir.an.hcv.specific.ns5a.html?f=m311343>
- 37 **Mogalian E**, German P, Kearney BP, Yang CY, Brainard D, McNally J, Moorehead L, Mathias A. Use of Multiple Probes to Assess Transporter- and Cytochrome P450-Mediated Drug-Drug Interaction Potential of the Pangenotypic HCV NS5A Inhibitor Velpatasvir. *Clin Pharmacokinet* 2016; **55**: 605-613 [PMID: 26519191 DOI: 10.1007/s40262-015-0334-7]
- 38 **Mogalian E**, Mathias A, Brainard D, Shen G, McNally J, Sajwani K, Robson R, Ries D, Lasseter K, Marbury T. P0712: The pharmacokinetics of GS-5816, a pangenotypic HCV-specific NS5A inhibitor, in HCV-uninfected subjects with severe renal impairment. *J Hepatol* 2015; **62** Suppl 2: S590-S591 [DOI: 10.1016/S0168-8278(15)30915-6]
- 39 **Gutiérrez F**, Fulladosa X, Barril G, Domingo P. Renal tubular transporter-mediated interactions of HIV drugs: implications for patient management. *AIDS Rev* 2014; **16**: 199-212 [PMID: 25350530]
- 40 **European Medicines Agency**. Résumé des caractéristiques du produit Exviera. Available from: URL: [http://www.ema.europa.eu/docs/fr\\_FR/document\\_library/EPAR\\_-\\_Product\\_Information/human/003837/WC500182233.pdf](http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/003837/WC500182233.pdf)
- 41 **European Medicines Agency**. Résumé des caractéristiques du produit Viekirax. Available from: URL: [http://www.ema.europa.eu/docs/fr\\_FR/document\\_library/EPAR\\_-\\_Product\\_Information/human/003839/WC500183997.pdf](http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/003839/WC500183997.pdf)
- 42 **Khatrri A**, Dutta S, Marbury TC, Preston RA, Rodrigues L Jr, Wang H, Awni W, Menon RM. Pharmacokinetics and Tolerability of Anti-Hepatitis C Virus Treatment with Ombitasvir, Paritaprevir, Ritonavir, with or Without Dasabuvir, in Subjects with Renal Impairment. *Clin Pharmacokinet* 2016; Epub ahead of print [PMID: 27389403 DOI: 10.1007/s40262-016-0429-9]
- 43 **Sulejmani N**, Jafri SM, Gordon SC. Pharmacodynamics and pharmacokinetics of elbasvir and grazoprevir in the treatment of hepatitis C. *Expert Opin Drug Metab Toxicol* 2016; **12**: 353-361 [PMID: 26849059 DOI: 10.1517/17425255.2016.1148685]
- 44 **Yeh WW**, Caro L, Guo Z, Feng HP, Davis HU, Kozisek M, Stypinski D, Feng C, Mitchell C, Gillespie A, Ichhpurani N, Marshall WL, Lasseter KC, Marbury TC, Butterson JR. Pharmacokinetics of co-administered HCV protease inhibitor MK-5172 and NS5A inhibitor MK-8742 in volunteers with end-stage renal disease on hemodialysis or severe renal impairment not on hemodialysis. *Hepatology* 2014; **60** S1: 1137A [DOI: 10.1002/hep.27533]
- 45 **Russo MW**, Fried MW. Side effects of therapy for chronic hepatitis C. *Gastroenterology* 2003; **124**: 1711-1719 [PMID: 12761728]
- 46 **Dimitrov Y**, Heibel F, Marcellin L, Chantrel F, Moulin B, Hannedouche T. Acute renal failure and nephrotic syndrome with alpha interferon therapy. *Nephrol Dial Transplant* 1997; **12**: 200-203 [PMID: 9027801]
- 47 **Ault BH**, Stapleton FB, Gaber L, Martin A, Roy S, Murphy SB. Acute renal failure during therapy with recombinant human gamma interferon. *N Engl J Med* 1988; **319**: 1397-1400 [PMID: 3141812 DOI: 10.1056/NEJM198811243192107]
- 48 **Shah M**, Jenis EH, Mookerjee BK, Schriber JR, Baer MR, Herzig GP, Wetzler M. Interferon-alpha-associated focal segmental glomerulosclerosis with massive proteinuria in patients with chronic myeloid leukemia following high dose chemotherapy. *Cancer* 1998; **83**: 1938-1946 [PMID: 9806652]
- 49 **Ohta S**, Yokoyama H, Wada T, Sakai N, Shimizu M, Kato T, Furuichi K, Segawa C, Hisada Y, Kobayashi K. Exacerbation of glomerulonephritis in subjects with chronic hepatitis C virus

- infection after interferon therapy. *Am J Kidney Dis* 1999; **33**: 1040-1048 [PMID: 10352191]
- 50 **Al Harbi A**, Al Ghamdi S, Subaity Y, Khalil A. Interferon-induced acute renal failure in nephrotic syndrome. *Nephrol Dial Transplant* 1998; **13**: 1316-1318 [PMID: 9623580]
  - 51 **Kanungo S**, Tamirisa S, Gopalakrishnan R, Salinas-Madrigal L, Bastani B. Collapsing glomerulopathy as a complication of interferon therapy for hepatitis C infection. *Int Urol Nephrol* 2010; **42**: 219-222 [PMID: 19496019 DOI: 10.1007/s11255-009-9594-1]
  - 52 **Fisher ME**, Rossini M, Simmons E, Harris RC, Moeckel G, Zent R. A woman with chronic hepatitis C infection and nephrotic syndrome who developed multiple renal lesions after interferon alfa therapy. *Am J Kidney Dis* 2004; **44**: 567-573 [PMID: 15332232]
  - 53 **Jadoul M**, Piessevaux H, Ferrant A, Cosyns JP, van Ypersele de Strihou C. Renal thrombotic microangiopathy in patients with chronic myelogenous leukaemia treated with interferon-alpha 2b. *Nephrol Dial Transplant* 1995; **10**: 111-113 [PMID: 7724004]
  - 54 **Stratta P**, Canavese C, Dogliani M, Thea A, Degani G, Mairone L, Vercellone A. Hemolytic-uremic syndrome during recombinant alpha-interferon treatment for hairy cell leukemia. *Ren Fail* 1993; **15**: 559-561 [PMID: 8210571]
  - 55 **Averbuch SD**, Austin HA, Sherwin SA, Antonovych T, Bunn PA, Longo DL. Acute interstitial nephritis with the nephrotic syndrome following recombinant leukocyte a interferon therapy for mycosis fungoides. *N Engl J Med* 1984; **310**: 32-35 [PMID: 6689738 DOI: 10.1056/NEJM198401053100107]
  - 56 **Gordon A**, Menahem S, Mitchell J, Jenkins P, Dowling J, Roberts SK. Combination pegylated interferon and ribavirin therapy precipitating acute renal failure and exacerbating IgA nephropathy. *Nephrol Dial Transplant* 2004; **19**: 2155 [PMID: 15252182 DOI: 10.1093/ndt/gfh336]
  - 57 **Fahal IH**, Murry N, Chu P, Bell GM. Acute renal failure during interferon treatment. *BMJ* 1993; **306**: 973 [PMID: 8490476]
  - 58 **Quesada JR**, Talpaz M, Rios A, Kurzrock R, Gutterman JU. Clinical toxicity of interferons in cancer patients: a review. *J Clin Oncol* 1986; **4**: 234-243 [PMID: 2418169]
  - 59 **Kurschel E**, Metz-Kurschel U, Niederle N, Aulbert E. Investigations on the subclinical and clinical nephrotoxicity of interferon alpha-2B in patients with myeloproliferative syndromes. *Ren Fail* 1991; **13**: 87-93 [PMID: 1957045]
  - 60 **Al-Wakeel J**, Mitwalli A, Tarif N, Al-Mohaya S, Malik G, Khalil M. Role of interferon-alpha in the treatment of primary glomerulonephritis. *Am J Kidney Dis* 1999; **33**: 1142-1146 [PMID: 10352204]
  - 61 **Lederer E**, Truong L. Unusual glomerular lesion in a patient receiving long-term interferon alpha. *Am J Kidney Dis* 1992; **20**: 516-518 [PMID: 1442766]
  - 62 **Lechner J**, Malloth N, Seppi T, Beer B, Jennings P, Pfaller W. IFN-alpha induces barrier destabilization and apoptosis in renal proximal tubular epithelium. *Am J Physiol Cell Physiol* 2008; **294**: C153-C160 [PMID: 18032529 DOI: 10.1152/ajpcell.00120.2007]
  - 63 **Gresser I**, Aguet M, Morel-Maroger L, Woodrow D, Puvion-Dutilleul F, Guillon JC, Maury C. Electrophoretically pure mouse interferon inhibits growth, induces liver and kidney lesions, and kills suckling mice. *Am J Pathol* 1981; **102**: 396-402 [PMID: 6163363]
  - 64 **Morel-Maroger L**, Sloper JC, Vinter J, Woodrow D, Gresser I. An ultrastructural study of the development of nephritis in mice treated with interferon in the neonatal period. *Lab Invest* 1978; **39**: 513-522 [PMID: 153433]
  - 65 **Fabrizi F**, Aghemo A, Fogazzi GB, Moroni G, Passerini P, D'Ambrosio R, Messa P. Acute tubular necrosis following interferon-based therapy for hepatitis C: case study with literature review. *Kidney Blood Press Res* 2013; **38**: 52-60 [PMID: 24556714 DOI: 10.1159/000355753]
  - 66 **Schalm SW**, Hansen BE, Chemello L, Bellobuono A, Brouwer JT, Weiland O, Cavalletto L, Schvarcz R, Ideo G, Alberti A. Ribavirin enhances the efficacy but not the adverse effects of interferon in chronic hepatitis C. Meta-analysis of individual patient data from European centers. *J Hepatol* 1997; **26**: 961-966 [PMID: 9186825]
  - 67 **Kökény S**, Papp J, Weber G, Vaszkó T, Carmona-Saez P, Oláh E. Ribavirin acts via multiple pathways in inhibition of leukemic cell proliferation. *Anticancer Res* 2009; **29**: 1971-1980 [PMID: 19528454]
  - 68 **McHutchison JG**, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ; PROVE1 Study Team. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; **360**: 1827-1838 [PMID: 19403902 DOI: 10.1056/NEJMoa0806104]
  - 69 **Hézode C**, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, Bourlière M, Gharakhanian S, Bengtsson L, McNair L, George S, Kieffer T, Kwong A, Kauffman RS, Alam J, Pawlotsky JM, Zeuzem S; PROVE2 Study Team. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; **360**: 1839-1850 [PMID: 19403903 DOI: 10.1056/NEJMoa0807650]
  - 70 **McHutchison JG**, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, Zeuzem S, Reesink HW, Garg J, Bsharat M, George S, Kauffman RS, Adda N, Di Bisceglie AM; PROVE3 Study Team. Telaprevir for previously treated chronic HCV infection. *N Engl J Med* 2010; **362**: 1292-1303 [PMID: 20375406 DOI: 10.1056/NEJMoa0908014]
  - 71 **Jacobson IM**, Mchutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]
  - 72 **Kwo PY**, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, Galati JS, Gordon SC, Ravendhran N, Rossaro L, Anderson FH, Jacobson IM, Rubin R, Koury K, Pedicone LD, Brass CA, Chaudhri E, Albrecht JK; SPRINT-1 investigators. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naïve patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010; **376**: 705-716 [PMID: 20692693 DOI: 10.1016/S0140-6736(10)60934-8]
  - 73 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP; SPRINT-2 investigators. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]
  - 74 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R; HCV RESPOND-2 Investigators. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]
  - 75 **Virlogeux V**, Pradat P, Bailly F, Funingana G, Gonçalves F, Maynard M, Hartig-Lavie K, Amiri M, Zoulim F. Boceprevir and telaprevir-based triple therapy for chronic hepatitis C: virological efficacy and impact on kidney function and model for end-stage liver disease score. *J Viral Hepat* 2014; **21**: e98-e107 [PMID: 24612466 DOI: 10.1111/jvh.12237]
  - 76 **Karino T**, Ozeki I, Hige S, Kimura M, Arakawa T, Nakajima T, Kuwata Y, Sato T, Ohmura T, Toyota J. Telaprevir impairs renal function and increases blood ribavirin concentration during telaprevir/pegylated interferon/ribavirin therapy for chronic hepatitis C. *J Viral Hepat* 2014; **21**: 341-347 [PMID: 24001168 DOI: 10.1111/jvh.12162]
  - 77 **Rémy AJ**, Lesgourgues B, Nalet B, Causse X, Henrion J, Denis J, Arotçarena R, Hagège H, Pariente A; APROVIE group of Association Nationale des Gastroentérologues des Hôpitaux Généraux (ANGH). Renal dysfunction associated with telaprevir-containing triple therapy for chronic hepatitis C: is early prediction possible? *Eur J Gastroenterol Hepatol* 2014; **26**: 996-1002 [PMID: 25072384 DOI: 10.1097/MEG.0000000000000081]
  - 78 **Kozielewicz D**, Dybowska D, Karwowska K, Wietlicka-Piszc M.



- Renal impairment in patients with chronic hepatitis C treated with first generation protease inhibitors. *Expert Opin Drug Saf* 2015; **14**: 1815-1825 [PMID: 26513231 DOI: 10.1517/14740338.2015.102882]
- 79 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]
  - 80 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]
  - 81 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Di Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]
  - 82 **Alqahtani SA**, Afdhal N, Zeuzem S, Gordon SC, Mangia A, Kwo P, Fried M, Yang JC, Ding X, Pang PS, McHutchison JG, Pound D, Reddy KR, Marcellin P, Kowdley KV, Sulkowski M. Safety and tolerability of ledipasvir/sofosbuvir with and without ribavirin in patients with chronic hepatitis C virus genotype 1 infection: Analysis of phase III ION trials. *Hepatology* 2015; **62**: 25-30 [PMID: 25963890 DOI: 10.1002/hep.27890]
  - 83 **Nelson DR**, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R, Oguchi G, Thuluvath PJ, Ortiz-Lasanta G, Rabinovitz M, Bernstein D, Bennett M, Hawkins T, Ravendhran N, Sheikh AM, Varunok P, Kowdley KV, Hennicken D, McPhee F, Rana K, Hughes EA. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology* 2015; **61**: 1127-1135 [PMID: 25614962 DOI: 10.1002/hep.27726]
  - 84 **Bhamidimarri KR**, Czul F, Peyton A, Levy C, Hernandez M, Jeffers L, Roth D, Schiff E, O'Brien C, Martin P. Safety, efficacy and tolerability of half-dose sofosbuvir plus simeprevir in treatment of Hepatitis C in patients with end stage renal disease. *J Hepatol* 2015; **63**: 763-765 [PMID: 26095179 DOI: 10.1016/j.jhep.2015.06.004]
  - 85 **Desnoyer A**, Pospai D, Lê MP, Gervais A, Heurgué-Berlot A, Laradi A, Harent S, Pinto A, Salmon D, Hillaire S, Fontaine H, Zucman D, Simonpoli AM, Muret P, Larrouy L, Bernard Chabert B, Descamps D, Yazdanpanah Y, Peytavin G. Pharmacokinetics, safety and efficacy of a full dose sofosbuvir-based regimen given daily in hemodialysis patients with chronic hepatitis C. *J Hepatol* 2016; **65**: 40-47 [PMID: 26952005 DOI: 10.1016/j.jhep.2016.02.044]
  - 86 **Jacobson IM**, Dore GJ, Foster GR, Fried MW, Radu M, Rafalsky VV, Moroz L, Craxi A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Scott J, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2014; **384**: 403-413 [PMID: 24907225 DOI: 10.1016/S0140-6736(14)60494-3]
  - 87 **Manns M**, Marcellin P, Poordad F, de Araujo ES, Buti M, Horsmans Y, Janczewska E, Villamil F, Scott J, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2014; **384**: 414-426 [PMID: 24907224 DOI: 10.1016/S0140-6736(14)60538-9]
  - 88 **Sulkowski MS**, Vargas HE, Di Bisceglie AM, Kuo A, Reddy KR, Lim JK, Morelli G, Darling JM, Feld JJ, Brown RS, Frazier LM, Stewart TG, Fried MW, Nelson DR, Jacobson IM; HCV-TARGET Study Group. Effectiveness of Simeprevir Plus Sofosbuvir, With or Without Ribavirin, in Real-World Patients With HCV Genotype 1 Infection. *Gastroenterology* 2016; **150**: 419-429 [PMID: 26497081 DOI: 10.1053/j.gastro.2015.10.013]
  - 89 **Sulkowski MS**, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinesstrosa F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, McPhee F, Sherman D, Hines R, Symonds W, Pasquinelli C, Grasela DM. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med* 2014; **370**: 211-221 [PMID: 24428467 DOI: 10.1056/NEJMoa1306218]
  - 90 **Leroy V**, Dumortier J, Coilly A, Sebahg M, Fougerou-Leurent C, Radenne S, Botta D, Durand F, Silvain C, Lebray P, Houssel-Deby P, Kamar N, D'Alteroche L, Petrov-Sanchez V, Diallo A, Pageaux GP, Duclos-Vallee JC; Agence Nationale de Recherches sur le SIDA et les Hépatites Virales CO23 Compassionate Use of Protease Inhibitors in Viral C in Liver Transplantation Study Group. Efficacy of Sofosbuvir and Daclatasvir in Patients With Fibrosing Cholestatic Hepatitis C After Liver Transplantation. *Clin Gastroenterol Hepatol* 2015; **13**: 1993-2001.e1-2 [PMID: 26044317 DOI: 10.1016/j.cgh.2015.05.030]
  - 91 **Wanchoo R**, Thakkar J, Schwartz D, Jhaveri KD. Harvoni (Ledipasvir With Sofosbuvir)-Induced Renal Injury. *Am J Gastroenterol* 2016; **111**: 148-149 [PMID: 26785666 DOI: 10.1038/ajg.2015.391]
  - 92 **Zeuzem S**, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourlière M, Sulkowski MS, Wedemeyer H, Tam E, Desmond P, Jensen DM, Di Bisceglie AM, Varunok P, Hassanein T, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1604-1614 [PMID: 24720679 DOI: 10.1056/NEJMoa1401561]
  - 93 **Feld JJ**, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, Weiland O, Aguilar H, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1594-1603 [PMID: 24720703 DOI: 10.1056/NEJMoa1315722]
  - 94 **Poordad F**, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, Shiffman ML, Wedemeyer H, Berg T, Yoshida EM, Forns X, Lovell SS, Da Silva-Tillmann B, Collins CA, Campbell AL, Podsadecki T, Bernstein B. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med* 2014; **370**: 1973-1982 [PMID: 24725237 DOI: 10.1056/NEJMoa1402869]
  - 95 **Ferenci P**, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A, Box TD, Younes Z, Enayati P, Green S, Baruch Y, Bhandari BR, Caruntu FA, Sepe T, Chulanov V, Janczewska E, Rizzardini G, Gervain J, Planas R, Moreno C, Hassanein T, Xie W, King M, Podsadecki T, Reddy KR; PEARL-III Study; PEARL-IV Study. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *N Engl J Med* 2014; **370**: 1983-1992 [PMID: 24795200 DOI: 10.1056/NEJMoa1402338]
  - 96 **Andreone P**, Colombo MG, Enejosa JV, Koksai I, Ferenci P, Maieron A, Müllhaupt B, Horsmans Y, Weiland O, Reesink HW, Rodrigues L, Hu YB, Podsadecki T, Bernstein B. ABT-450, ritonavir, ombitasvir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. *Gastroenterology* 2014; **147**: 359-365.e1 [PMID: 24818763 DOI: 10.1053/j.gastro.2014.04.045]
  - 97 **Fabrizi F**. Hepatitis C virus infection and dialysis: 2012 update. *ISRN Nephrol* 2013; **2013**: 159760 [PMID: 24959533 DOI: 10.5402/2013/159760]
  - 98 **Fabrizi F**, Takkouche B, Lunghi G, Dixit V, Messa P, Martin P. The



- impact of hepatitis C virus infection on survival in dialysis patients: meta-analysis of observational studies. *J Viral Hepat* 2007; **14**: 697-703 [PMID: 17875004 DOI: 10.1111/j.1365-2893.2007.00868.x]
- 99 **Fabrizi F**, Lunghi G, Andrucci S, Pagliari B, Mangano S, Faranna P, Pagano A, Locatelli F. Influence of hepatitis C virus (HCV) viraemia upon serum aminotransferase activity in chronic dialysis patients. *Nephrol Dial Transplant* 1997; **12**: 1394-1398 [PMID: 9249775]
  - 100 **Fabrizi F**, Martin P, Dixit V, Brezina M, Russell J, Conrad A, Schmid P, Gerosa S, Gitnick G. Detection of de novo hepatitis C virus infection by polymerase chain reaction in hemodialysis patients. *Am J Nephrol* 1999; **19**: 383-388 [PMID: 10393375 DOI: 10.1159/000013482]
  - 101 **Alric L**, Di-Martino V, Selves J, Cacoub P, Charlotte F, Reynaud D, Piette JC, Péron JM, Vinel JP, Durand D, Izopet J, Poynard T, Duffaut M, Rostaing L. Long-term impact of renal transplantation on liver fibrosis during hepatitis C virus infection. *Gastroenterology* 2002; **123**: 1494-1499 [PMID: 12404224]
  - 102 **Roth D**, Gaynor JJ, Reddy KR, Ciano G, Sageshima J, Kupin W, Guerra G, Chen L, Burke GW. Effect of kidney transplantation on outcomes among patients with hepatitis C. *J Am Soc Nephrol* 2011; **22**: 1152-1160 [PMID: 21546575 DOI: 10.1681/ASN.2010060668]
  - 103 **Alavian SM**, Tabatabaei SV. Meta-analysis of factors associated with sustained viral response in patients on hemodialysis treated with standard or pegylated interferon for hepatitis C infection. *Iran J Kidney Dis* 2010; **4**: 181-194 [PMID: 20622305]
  - 104 **Fabrizi F**, Ganeshan SV, Lunghi G, Messa P, Martin P. Antiviral therapy of hepatitis C in chronic kidney diseases: meta-analysis of controlled clinical trials. *J Viral Hepat* 2008; **15**: 600-606 [PMID: 18444984 DOI: 10.1111/j.1365-2893.2008.00990.x]
  - 105 **Gordon CE**, Uhlig K, Lau J, Schmid CH, Levey AS, Wong JB. Interferon for hepatitis C virus in hemodialysis--an individual patient meta-analysis of factors associated with sustained virological response. *Clin J Am Soc Nephrol* 2009; **4**: 1449-1458 [PMID: 19643927 DOI: 10.2215/CJN.01850309]
  - 106 **Gordon CE**, Uhlig K, Lau J, Schmid CH, Levey AS, Wong JB. Interferon treatment in hemodialysis patients with chronic hepatitis C virus infection: a systematic review of the literature and meta-analysis of treatment efficacy and harms. *Am J Kidney Dis* 2008; **51**: 263-277 [PMID: 18215704 DOI: 10.1053/j.ajkd.2007.11.003]
  - 107 **Kamar N**, Chatelut E, Manolis E, Lafont T, Izopet J, Rostaing L. Ribavirin pharmacokinetics in renal and liver transplant patients: evidence that it depends on renal function. *Am J Kidney Dis* 2004; **43**: 140-146 [PMID: 14712437]
  - 108 **Carriero D**, Fabrizi F, Uriel AJ, Park J, Martin P, Dieterich DT. Treatment of dialysis patients with chronic hepatitis C using pegylated interferon and low-dose ribavirin. *Int J Artif Organs* 2008; **31**: 295-302 [PMID: 18432584]
  - 109 **Deltenre P**, Moreno C, Tran A, Ollivier I, Provôt F, Stanke F, Lazrek M, Castel H, Canva V, Louvet A, Colin M, Glowacki F, Dharancy S, Henrion J, Hazzan M, Noel C, Mathurin P. Anti-viral therapy in haemodialysed HCV patients: efficacy, tolerance and treatment strategy. *Aliment Pharmacol Ther* 2011; **34**: 454-461 [PMID: 21682756 DOI: 10.1111/j.1365-2036.2011.04741.x]
  - 110 **Mehawej M**, Rostaing L, Alric L, Del Bello A, Izopet J, Kamar N. Boceprevir-Based Triple Antiviral Therapy for Chronic Hepatitis C Virus Infection in Kidney-Transplant Candidates. *J Transplant* 2015; **2015**: 159795 [PMID: 26257919 DOI: 10.1155/2015/159795]
  - 111 **Dumortier J**, Guillaud O, Gagnieu MC, Janbon B, Juillard L, Morelon E, Leroy V. Anti-viral triple therapy with telaprevir in haemodialysed HCV patients: is it feasible? *J Clin Virol* 2013; **56**: 146-149 [PMID: 23149155 DOI: 10.1016/j.jcv.2012.10.009]
  - 112 **Wiegand J**, Maasoumy B, Buggisch P, Buslau A, Schiefke I, Berg T, Wedemeyer H, Sarrazin C, Hinrichsen H. Letter: Telaprevir triple therapy in chronic hepatitis C genotype 1 patients receiving haemodialysis. *Aliment Pharmacol Ther* 2014; **39**: 1342-1344 [PMID: 24803258 DOI: 10.1111/apt.12748]
  - 113 **Knapstein J**, Galle PR, Zimmermann T. Antiviral triple therapy with boceprevir in a chronic hepatitis C haemodialysis patient awaiting kidney re-transplantation. *Dig Liver Dis* 2014; **46**: 88-89 [PMID: 24054768 DOI: 10.1016/j.dld.2013.08.133]
  - 114 **Bhamidimarri KR**, Gutierrez JA, Grigorian A, Peyton L, Levy C, O'Brien C, Martin P. Urgent Treatment With Sofosbuvir Based Regimen For Hepatitis C Genotype 1 Patients With Severe Renal Insufficiency (GFR <30ml/min). Available from: URL: <http://liverlearning.aasld.org/aasld/2014/thelivermeeting/61050/kalyan.bhamidimarri.urgent.treatment.with.sofosbuvir.based.regimen.for.html?f=p16m2t1370l1343>
  - 115 **Nazario HE**, Ndungu M, Modi AA. Sofosbuvir and simeprevir in hepatitis C genotype 1-patients with end-stage renal disease on haemodialysis or GFR < 30 ml/min. *Liver Int* 2016; **36**: 798-801 [PMID: 26583882 DOI: 10.1111/liv.13025]
  - 116 **Fabrizi F**, Martin P, Dixit V, Messa P. Meta-analysis of observational studies: hepatitis C and survival after renal transplant. *J Viral Hepat* 2014; **21**: 314-324 [PMID: 24716634 DOI: 10.1111/jvh.12148]
  - 117 **Bruchfeld A**, Wilczek H, Elinder CG. Hepatitis C infection, time in renal-replacement therapy, and outcome after kidney transplantation. *Transplantation* 2004; **78**: 745-750 [PMID: 15371680]
  - 118 **Kamar N**, Mariat C, Delahousse M, Dantal J, Al Najjar A, Cassuto E, Lefrançois N, Cointault O, Touchard G, Villemain F, Di Giambattista F, Benhamou PY, Diapason Study Group. Diabetes mellitus after kidney transplantation: a French multicentre observational study. *Nephrol Dial Transplant* 2007; **22**: 1986-1993 [PMID: 17400559 DOI: 10.1093/ndt/gfm011]
  - 119 **Scott DR**, Wong JK, Spicer TS, Dent H, Mensah FK, McDonald S, Levy MT. Adverse impact of hepatitis C virus infection on renal replacement therapy and renal transplant patients in Australia and New Zealand. *Transplantation* 2010; **90**: 1165-1171 [PMID: 20861806 DOI: 10.1097/TP.0b013e3181f92548]
  - 120 **Corogue M**, Vallet-Pichard A, Pol S. HCV and the kidney. *Liver Int* 2016; **36** Suppl 1: 28-33 [PMID: 26725894 DOI: 10.1111/liv.13022]
  - 121 Viral hepatitis guidelines in hemodialysis and transplantation. *Am J Transplant* 2004; **4** Suppl 10: 72-82 [PMID: 15504218 DOI: 10.1111/j.1600-6135.2004.00676.x]
  - 122 **López-Medrano F**, Fernández-Ruiz M, Morales JM, San-Juan R, Cervera C, Carratalá J, Torre-Cisneros J, Gavalda J, Muñoz P, Len O, Martín-Dávila P, Ramos A, Montejo M, Lumbreras C, Moreno A, Aguado JM; Spanish Network for the Research of Infection in Transplantation/Network of Research in Infectious Diseases (RESITRA/REIPI) Study Group. Impact of hepatitis C virus infection on the risk of infectious complications after kidney transplantation: data from the RESITRA/REIPI cohort. *Transplantation* 2011; **92**: 543-549 [PMID: 21869745 DOI: 10.1097/TP.0b013e318225dbae]
  - 123 **Forman JP**, Tolkoff-Rubin N, Pascual M, Lin J. Hepatitis C, acute humoral rejection, and renal allograft survival. *J Am Soc Nephrol* 2004; **15**: 3249-3255 [PMID: 15579529 DOI: 10.1097/01.ASN.0000145896.16153.43]
  - 124 **Fabrizi F**, Lunghi G, Dixit V, Martin P. Meta-analysis: anti-viral therapy of hepatitis C virus-related liver disease in renal transplant patients. *Aliment Pharmacol Ther* 2006; **24**: 1413-1422 [PMID: 17081162 DOI: 10.1111/j.1365-2036.2006.03151.x]
  - 125 **Wei F**, Liu J, Liu F, Hu H, Ren H, Hu P. Interferon-based anti-viral therapy for hepatitis C virus infection after renal transplantation: an updated meta-analysis. *PLoS One* 2014; **9**: e90611 [PMID: 24699257 DOI: 10.1371/journal.pone.0090611]
  - 126 **Sawinski D**, Kaur N, Ajeti A, Trofe-Clark J, Lim M, Bleicher M, Goral S, Forde KA, Bloom RD. Successful Treatment of Hepatitis C in Renal Transplant Recipients With Direct-Acting Antiviral Agents. *Am J Transplant* 2016; **16**: 1588-1595 [PMID: 26604182 DOI: 10.1111/ajt.13620]
  - 127 **Kamar N**, Marion O, Rostaing L, Cointault O, Ribes D, Lavayssière L, Esposito L, Del Bello A, Métivier S, Barange K, Izopet J, Alric L. Efficacy and Safety of Sofosbuvir-Based Antiviral Therapy to Treat Hepatitis C Virus Infection After Kidney Transplantation. *Am J Transplant* 2016; **16**: 1474-1479 [PMID: 26587971 DOI: 10.1111/ajt.13518]
  - 128 **Gutierrez JA**, Carrion AF, Avalos D, O'Brien C, Martin P,

- Bhamidimarri KR, Peyton A. Sofosbuvir and simeprevir for treatment of hepatitis C virus infection in liver transplant recipients. *Liver Transpl* 2015; **21**: 823-830 [PMID: 25825070 DOI: 10.1002/lt.24126]
- 129 **Lin MV**, Sise ME, Pavlakis M, Hanifi JM, Rutherford AE, Elias N, Heher EC, Curry MP, Riella LV. LP42 : Safety and efficacy of novel antivirals in kidney transplant recipients with chronic hepatitis c virus (HCV) infection. *J Hepatol* 2015; **62**: S284-S285 [DOI: 10.1016/S0168-8278(15)30196-3]
- 130 **Huard G**, Kim B, Patel A. Early Safety and Efficacy Profiles of Renal Transplant Recipients with Chronic Hepatitis C Treated with Sofosbuvir and Ribavirin. Available from: URL: <http://liverlearning.aasld.org/aasld/2014/thelivermeeting/60747/genevieve.huard.early.safety.and.efficacy.profiles.of.renal.transplant.html?f=p14m2s169355>
- 131 **Forns X**, Charlton M, Denning J, McHutchison JG, Symonds WT, Brainard D, Brandt-Sarif T, Chang P, Kivett V, Castells L, Prieto M, Fontana RJ, Baumert TF, Coilly A, Londoño MC, Habersetzer F. Sofosbuvir compassionate use program for patients with severe recurrent hepatitis C after liver transplantation. *Hepatology* 2015; **61**: 1485-1494 [PMID: 25557906 DOI: 10.1002/hep.27681]
- 132 **Belga S**, Doucette KE. Hepatitis C in non-hepatic solid organ transplant candidates and recipients: A new horizon. *World J Gastroenterol* 2016; **22**: 1650-1663 [PMID: 26819530 DOI: 10.3748/wjg.v22.i4.1650]
- 133 **Te H**, Ahn J, Schiano T, Diesing A, Aronsohn A, Robertazzi S, Myers L, Satoskar R. O110 : Simeprevir sofosbuvir combination therapy for recurrent genotype-1 hepatitis C in liver transplant recipients : A real-life multicenter experience. *J Hepatol* 2015; **62**: S248 [DOI: 10.1016/S0168-8278(15)30129-X]
- 134 **Naggie S**, Cooper C, Saag M, Workowski K, Ruane P, Towner WJ, Marks K, Luetkemeyer A, Baden RP, Sax PE, Gane E, Santana-Bagur J, Stamm LM, Yang JC, German P, Dvory-Sobol H, Ni L, Pang PS, McHutchison JG, Stedman CA, Morales-Ramirez JO, Bräu N, Jayaweera D, Colson AE, Tebas P, Wong DK, Dieterich D, Sulkowski M; ION-4 Investigators. Ledipasvir and Sofosbuvir for HCV in Patients Coinfected with HIV-1. *N Engl J Med* 2015; **373**: 705-713 [PMID: 26196665 DOI: 10.1056/NEJMoa1501315]
- 135 **Schwarz K**, Murray KF, Rosenthal P, Bansal S, Lin CH, Ni L, Kanwar B, Fraser J, German P, Brainard DM, Wen J, Gonzalez-Peralta R, Jonas MM, Balistreri W. GS17: High Rates Of Svr12 In Adolescents Treated With The Combination Of Ledipasvir/ Sofosbuvir. Proceedings of The International Liver Congress™ 2016 - 51st annual meeting of the European Association for the Study of the Liver; April 13-17, 2016, Barcelona, Spain. *J Hepatol* 2016; **64**: S184-S185

**P- Reviewer:** Maan R, Rosencrantz RA, Yao DF **S- Editor:** Gong ZM

**L- Editor:** A **E- Editor:** Li D



## Toll-like receptors in pathophysiology of liver diseases

Safak Kiziltas

Safak Kiziltas, Department of Gastroenterology, Baskent University Istanbul Hospital, 34662 Istanbul, Turkey

**Author contributions:** Kiziltas S performed conception and design of the paper and drafting and revising the article and had primary responsibility for final content.

**Conflict-of-interest statement:** Author declares no conflict of interests for this article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Safak Kiziltas, MD, Assistant Professor, Department of Gastroenterology, Baskent University Istanbul Hospital, Oymaci Street, No:7, 34662 Altunizade, Istanbul, Turkey. [safakkiziltas@hotmail.com](mailto:safakkiziltas@hotmail.com)  
Telephone: +90-216-5541500  
Fax: +90-216-6519858

Received: June 12, 2016  
Peer-review started: June 17, 2016  
First decision: July 11, 2016  
Revised: August 17, 2016  
Accepted: September 21, 2016  
Article in press: September 22, 2016  
Published online: November 18, 2016

### Abstract

Toll-like receptors (TLRs) are pattern recognition receptors that participate in host defense by recognizing pathogen-associated molecular patterns alongside inflammatory processes by recognizing damage associated

molecular patterns. Given constant exposure to pathogens from gut, strict control of TLR-associated signaling pathways is essential in the liver, which otherwise may lead to inappropriate production of pro-inflammatory cytokines and interferons and may generate a predisposition to several autoimmune and chronic inflammatory diseases. The liver is considered to be a site of tolerance induction rather than immunity induction, with specificity in hepatic cell functions and distribution of TLR. Recent data emphasize significant contribution of TLR signaling in chronic liver diseases *via* complex immune responses mediating hepatocyte (*i.e.*, hepatocellular injury and regeneration) or hepatic stellate cell (*i.e.*, fibrosis and cirrhosis) inflammatory or immune pathologies. Herein, we review the available data on TLR signaling, hepatic expression of TLRs and associated ligands, as well as the contribution of TLRs to the pathophysiology of hepatic diseases.

**Key words:** Toll-like receptors; Innate immunity; Liver disease; Pathophysiology; Signaling

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Toll-like receptors (TLRs) are known to be pattern recognition receptors that recognize pathogen- and damage-associated molecular pattern molecules and thus participate in the activation of innate immune system. TLR signaling plays a significant role in liver diseases, whereas inflammatory or immune pathologies targeting distinct liver cells are based on complex immune responses. Herein, we review the current data on TLR signaling, hepatic expression of TLRs and associated ligands, as well as the contribution of TLRs to the pathophysiology of hepatic diseases.

Kiziltas S. Toll-like receptors in pathophysiology of liver diseases. *World J Hepatol* 2016; 8(32): 1354-1369 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i32/1354.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i32.1354>

## INTRODUCTION

Liver, main filter organ acting as a first line of defense, is continuously exposed to massive gut-derived antigenic load *via* the portal vein, whereas inflammatory signs occur under normal conditions owing to highly specific immune properties leading to immune tolerance<sup>[1-7]</sup>.

Pathogen-associated molecular patterns (PAMP) are specific signature molecules essential to entire categories of microorganisms<sup>[8-11]</sup>. Innate immune system recognizes PAMPs *via* pattern recognition receptors (PRRs)<sup>[7-9,12,13]</sup> and consequent downstream signaling cascades for proper host recognition and prevention of immune system hyperactivation<sup>[7-9,14]</sup>.

Toll-like receptors (TLRs) are a family of PRRs that induce innate immune system by recognizing PAMPs and damage-associated molecular pattern molecules (DAMPs)<sup>[15-18]</sup>. Although the recognition of PAMPs enables a prompt and effective protection against invading pathogens<sup>[5,11,12]</sup>, TLRs also contribute to the activation of adaptive immune responses, epithelial regeneration and carcinogenesis and regulation of sterile inflammation<sup>[5,19,20]</sup>.

Consistent with their extensive hepatocellular expression<sup>[7,18,21,22]</sup>, TLRs have recently been recognized as principal elements of the hepatic immune system that also play a crucial role in liver physiology and pathophysiology<sup>[11,15,23]</sup>. Despite being constantly exposed to gut-derived PAMPs, healthy liver is free of inflammation risk due to presence of "liver tolerance" in which modulation of TLR signals also plays a role<sup>[5,15,23-25]</sup>. A tight regulation of TLR activation occurs at many levels involving the receptor itself, the signaling cascade and a distinct compartmentalization of TLRs<sup>[24,26,27]</sup>. Acute and chronic liver diseases are highly associated with triggering TLR signaling by gut-derived microbiota in the breakdown of the tolerance and sterile insult-associated products of damaged cells<sup>[28]</sup>.

Ligand mediated stimulation of TLRs activates downstream adaptor molecules, including myeloid differentiation primary response protein 88 (MyD88), myeloid toll/interleukin (IL)-1 receptor (TIR)-domain-containing adaptor-inducing interferon- $\beta$  (TRIF) and TRIF-related adaptor molecule (TRAM). This triggers signaling cascades that converge on nuclear factor- $\kappa$ B (NF- $\kappa$ B), interferon (IFN) response factors (IRFs) and mitogen-activated protein (MAP) kinases<sup>[23,29-32]</sup>. As a result, transcription of certain proinflammatory agents including IL-6, IL-12, IL-23, and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) is induced<sup>[23,29-32]</sup>.

TLR-mediated inflammatory-signaling pathways are shown to be associated with entire spectrum of liver diseases, from hepatitis, liver fibrosis and cirrhosis to alcoholic and nonalcoholic liver disease, ischemia/reperfusion injury, liver regeneration and hepatocellular carcinoma<sup>[4,5,7,8,15,18,23,33]</sup>.

Herein, we review the available literature on TLR signaling, hepatic expression of TLRs and associated ligands, as well as the contribution of TLRs to the patho-

physiology of hepatic diseases.

## TLR FAMILY, DISTRIBUTION, LIGANDS

TLRs are a group of evolutionarily conserved type I transmembrane proteins responsible for innate immune and inflammatory responses<sup>[34-38]</sup>. They comprise an extracellular domain with receptor specific leucine-rich repeat motifs and a highly conserved cytosolic domain alike to the IL-1 receptor called TIR<sup>[13,29,36,37]</sup>.

Of 13 TLRs exist in mammals, only TLRs 1-10 exist in humans<sup>[9,26,39-41]</sup>. The presence of multiple widely expressed TLRs enables recognition of different pathogens and thus initiation of appropriate immunologic response by the innate immunity system<sup>[30,42,43]</sup>. PAMPs include microbial molecular structures such as Gram-negative related lipopolysaccharide (LPS); Gram-positive bacteria related lipoteichoic acid and peptidoglycan (PGN); lipoglycans, lipoarabinomannan, lipopeptides and lipomannans from mycobacteria; zymosan from yeast; and DNA from viruses and bacteria<sup>[34,44]</sup>.

DAMP include extracellular matrix and plasma membrane components, nuclear and cytosolic proteins and elements of damaged organelles<sup>[9,34,45,46]</sup>.

Each TLR is able to recognize a particular molecular pattern<sup>[29]</sup>. TLR1, TLR2, TLR4, TLR5 and TLR6 bind to molecules associated with bacterial membrane such as LPS, lipoprotein and PGN, whereas TLR3, TLR7, TLR8 and TLR9 detect viral and bacterial or endogenous nucleic acids, including ssRNA, dsRNA, and unmethylated cytosine phosphate guanine (CpG)-containing DNA<sup>[29]</sup>. TLR4 along with TLR2 can recognize antigens from bacteria, fungi, parasites, viruses and DAMPs<sup>[47,48]</sup>. TLR10 is the only family member among humans with no definite ligand, function or localization<sup>[9,13]</sup>.

Given their ability to detect wide range of non-microbial host-derived stimuli and their extensive expression in various cell types, TLRs are considered to participate in development, progression and resolution of several noninfectious inflammatory and immune diseases<sup>[37,49]</sup>.

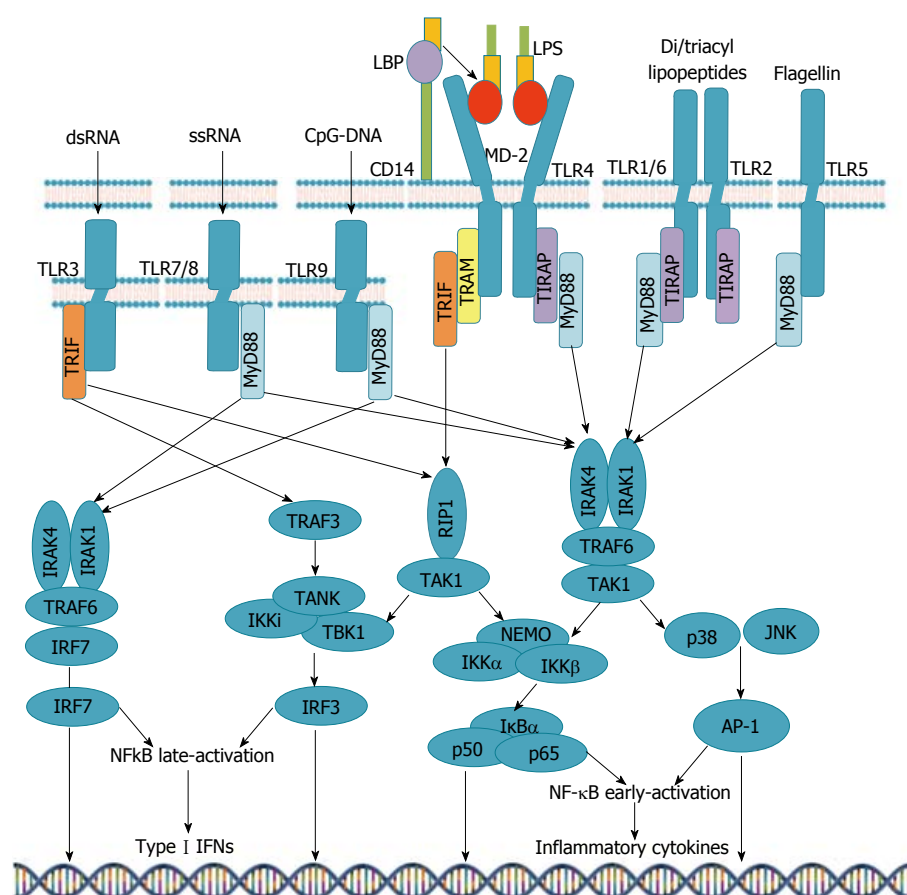
## TLR SIGNALING PATHWAYS

Healthy liver contains low mRNA levels of TLRs and shows no activation of TLR-signaling pathways<sup>[5,50,51]</sup>. However, in the case of a breakdown in TLR tolerance against endogenous ligands under pathologic conditions, the TLR-related immune response induces TLR-ligand complex activated expression of proinflammatory/anti-inflammatory cytokines and interferons<sup>[7,9,27,52]</sup>.

The differential host cell response after TLR ligand stimulation is associated with the fact that TLRs selectively use four main adaptor molecules, including MyD88, TIR domain-containing adaptor protein (TIRAP, or MyD88 adaptor-like), TIR domain-containing adaptor protein inducing interferon- $\beta$  (TRIF) and TRAM<sup>[7,9,27,30,52]</sup>.

Signal transduction pathways following ligand-induced receptor dimerization involve one or more TIR-containing adaptor molecules, such as IL-1 receptor-associated





**Figure 1** Toll-like receptors signaling pathways. TLR: Toll-like receptors; LPS: Lipopolysaccharide; NF-κB: Nuclear factor; IFNs: Interferons; LBP: LPS-binding protein; TRIF: Toll/interleukin-1 receptor-domain-containing adaptor-inducing interferon-β; MyD88: Myeloid differentiation primary response protein 88; TRAM: TRIF-related adaptor molecule; TIRAP: TIR domain-containing adaptor protein; IRAK: IL-1 receptor-associated kinase; TRAF: Tumor necrosis factor receptor-associated factor; TBK1: TANK binding kinase-1; IKK: IκB kinase; AP: Activator protein; JNK: c-Jun N-terminal kinase.

kinase (IRAK)-1, IRAK-4, TNF receptor-associated factor (TRAF)-6 and TANK binding kinase (TBK)-1, MAP kinases and IκB kinase (IKK). This leads to activation of the nuclear transcriptional factor kappa-B (NF-κB), interferon (IFN) regulatory factor 3 (IRF-3) and activator protein (AP)-1<sup>[37,53]</sup>.

Upon binding with their ligand, all superfamily receptors except TLR3 use MyD88 to initiate signaling which may also act along with other adaptors, such as TIRAP, in the response induced by TLR4, TLR1/2, and TLR2/6. Activation of TLRs 5, 7, 8 and 9 also leads to NF-κB and AP-1 production, with no need for TIRAP to stimulate MyD88. TLRs 7 and 9 act through IRAK-1, 4 and TRAF-6, phosphorylate IRF-7 and lead to type 1 interferon mRNA expression. TLR3-mediated signaling uses only the TRIF adaptor molecule, which is also recruited by TLR4 in concert with another adaptor called TRAM<sup>[9,12,23,32,39,54]</sup> (Figure 1).

Hence, while intracellular signaling is similar, the final outcome of TLR activation differs depending on the nature of PAMPs, concomitantly activated TLRs and PRRs, the level of cytokines, and the cell stimulated<sup>[13,27,55-57]</sup>. Moreover, chronically activated signaling pathways is likely to induce transcription of oncogenic factors, which adds a further level of complexity to the intracellular

signaling for these receptors<sup>[13,27,58]</sup>.

## TLR EXPRESSION AND SIGNALING IN HEPATIC CELL POPULATIONS

Under constant exposure to gut-derived microbiota, strict regulation of TLR signaling pathways is crucial in the liver, which otherwise may lead to inappropriate production of proinflammatory cytokines and interferons creating a predisposition to several autoimmune and chronic inflammatory diseases<sup>[9]</sup>.

Liver cells are classified as parenchymal or non-parenchymal cells. Hepatocytes comprise 60%-80% of the parenchymal cells, whereas the remaining population of non-parenchymal cells include Kupffer cells (KCs), sinusoidal endothelial cells (SECs), hepatic stellate cells (HSCs), dendritic cells (DCs), biliary epithelial cells (BECs) and intrahepatic lymphocytes<sup>[1,9,33]</sup>.

Besides distinct function of liver cells with a highly specific distribution of TLR<sup>[1,33]</sup>, liver comprises many populations of cells with immune competence that may respond to TLR signals, indicating the complexity of immune responses underlying inflammatory or immune pathologies associated with the liver cells<sup>[10]</sup>.

mRNA levels of TLR1, TLR2, TLR4, TLR6, TLR7, TLR8,

**Table 1** Toll-like receptor expression and their signaling in the liver<sup>[5,9,11,15,23,33,49]</sup>

TLR subfamily	Members	Expression of cell population in the liver (protein level)	Location	Ligand (origin)	Signaling	Final product-effect
TLR2 subfamily	TLR1/2	NK cells, DCs (h)	Plasma membrane	Bacterial lipoproteins Triacylated lipopeptides	TIRAP-MyD88-NF-κB/AP-1/IRF5 pathway	Pro- and anti-inflammatory cytokines excluding type 1 IFNs; the apoptotic cascade <i>via</i> recruiting FADD leading to caspase-8 activation
	TLR2/6	Hepatocytes, Kupffer cells, NK cells, B cells, activated T cells, DCs (m), biliary epithelial cells		Diacylated lipopeptides LPS of Gram-positive bacteria Fungal zymosan Mycoplasma lipopeptides	TIRAP-MyD88-NF-κB/AP-1 pathway	
	TLR10	Unknown		ND		
TLR3 subfamily	TLR3	Hepatocytes, LSECs, Kupffer cells, NK cells, NKT cells, activated T cells, cDCs (m), biliary epithelial cells	Endosome	Double-stranded RNA (viruses)	PI3K/TRIF-IRF3 pathway TRAM-TRIF-NF-κB pathway PI3K/TRIF-RIP1-NF-κB pathway	Production of type 1 IFNs; the apoptotic cascade <i>via</i> recruiting FADD leading to caspase 8 activation; DC maturation
TLR4 subfamily	TLR4 <sup>1</sup>	Hepatocytes, LSECs, Kupffer cells, NK cells, B cells, activated T cells, DCs (m), biliary epithelial cells, HSCs	Plasma membrane	LPS of Gram-negative bacteria; fusion protein (respiratory syncytial virus), envelope protein (mouse mammary-tumor virus); HMGB1, hyaluronan, HSP60, free fatty acids (endogenous ligands); HSP72 (cells during stress and injury) surfactant protein A; fibrinogen; fibronectin extra domain A	TIRAP-MyD88-NF-κB/AP-1 pathway TRAM-TRIF-NF-κB/IRF3 pathway	Pro- and anti-inflammatory cytokines excluding type 1 IFNs; the apoptotic cascade <i>via</i> recruiting FADD leading to caspase 8 activation; DC maturation; activating caspase-1 through adaptor molecule apoptosis associated speck-like protein <sup>2</sup>
TLR5 subfamily	TLR5	Biliary epithelial cells	Plasma membrane	Flagellin protein (bacteria)	MyD88-NF-κB/IRF5 pathway	Pro- and anti-inflammatory cytokines excluding type 1 IFNs
TLR9 subfamily	TLR7/8	NK cells, B cells, DCs (h), DCs (m)	Endosome	Single-stranded RNA (viruses), double-stranded, shortinterfering RNA (siRNA)	MyD88 and endosomal acidification (maturation)-IRF7 pathway; MyD88-NF-κB pathway	High levels of type 1 IFN production in pDCs; proinflammatory cytokine production
	TLR9	LSECs, Kupffer cells, NK cells, B in mDCs and macrophages		Imidazoquinoline CpG-containing viral or bacterial DNA Endogenous host-DNA		

<sup>1</sup>TLR4 requires LPS-binding protein (LBP), CD14 and MD2 to recognize LPS; <sup>2</sup>Containing a caspase recruitment domain (ASC)<sup>[33]</sup>. RIP1: Receptor-interacting protein 1; FADD: Fas-associated death domain; TLR: Toll-like receptors; LPS: Lipopolysaccharide; DCs: Dendritic cells; HSCs: Hepatic stellate cells; LSECs: Liver sinusoidal endothelial cells; IFNs: Interferons; DC: Dendritic cell; MyD88: Myeloid differentiation primary response protein 88.

TLR9, TLR10 and signaling molecules such as MD-2 and MyD88 are lower in liver as compared with the levels observed in other organs<sup>[50,51,59]</sup>. This discrepancy indicates the high tolerance to TLR ligands from the intestinal microbiota in liver<sup>[11]</sup>, whereas no specific liver cell population is considered central in TLR-mediated pathologies, with the different effects of TLR ligation varying from cell to cell<sup>[10]</sup> (Table 1).

### Hepatocytes

Constituting 60% of liver cells, hepatocytes are the principal site for PRR production<sup>[5,33]</sup>. They express mRNA for all TLRs and are responsive to multiple PAMPs, while respond fairly weakly to TLR2 and TLR4 ligands<sup>[5,9,33]</sup>. While TLR4 expression in hepatocytes is not upregulated by proinflammatory mediators, hepatocytes show increased responsiveness to TLR2 ligands under inflammatory conditions leading to up-regulation of TLR2 expression by LPS, TNF-α, bacterial lipoprotein, and IL-1β in an NF-κB-dependent manner<sup>[5,11,33,60,61]</sup>.

### Kupffer cells

Accounting for approximately 20% of non-parenchymal cells, KCs play a significant role in host defense by orchestrating the inflammatory response *via* functional properties, including phagocytosis, antigen processing and presentation, and secretion of proinflammatory mediators such as cytokines, prostanoids, nitric oxide, and reactive oxygen intermediates<sup>[5,9,11,33,62]</sup>.

KCs express TLRs 2, 3, 4 and 9 and have a higher threshold for activation when compared with other immune cells given their milieu<sup>[5,9,33,63]</sup>.

KCs are less responsive to "LPS tolerance" in the physiological environment, whereas upon activation, they produce several pro-inflammatory (IL-6, IL-12, IL-18 and TNFα) and anti-inflammatory (IL-10) mediators<sup>[33,64-66]</sup>. Additionally, KCs produce IFN-β, upregulate the expression of MHC-II/costimulatory molecules and promote T cell proliferation and IFN-γ production; when stimulated with TLR3/TLR4 ligands; TLR1/TLR8 ligands and TLR1/2/4/6 ligands, respectively<sup>[22,33]</sup>.

### Hepatic stellate cells

Constituting < 1% of non-parenchymal cells, HSCs undergo an activation process after liver injury and become the main liver cell type that produce extracellular matrix, contributing onset of liver fibrosis<sup>[67-70]</sup>.

HSCs express TLRs 4 and 9, whereas expression of TLR2 is induced by TLR4 stimulation in HSCs<sup>[68-70]</sup>. Activated HSCs express TLR4 and CD14 and respond to LPS upon the activation of IKK/NF- $\kappa$ B and c-Jun N-terminal kinase (JNK) as well as the secretion of proinflammatory cytokines such as transforming growth factor (TGF)- $\beta$ , IL-6, IL-8 and several chemokines such as MCP-1, MIP-2, intercellular cell adhesion molecule 1, vascular cell adhesion molecule 1, and E-selectin<sup>[9,33,70]</sup>. TLR4 enhances TGF- $\beta$  signaling, and stellate cell activation was shown to promote hepatic fibrosis<sup>[71]</sup>. In chimeric C3H/HeJ mice with TLR4 mutation in HSC or KCs, amelioration of hepatic fibrosis by LPS indicated a cardinal role for KCs and HSC in hepatic inflammation and fibrosis<sup>[9,72]</sup>. LPS was shown to downregulate the TGF- $\beta$  pseudoreceptor BAMBI in quiescent HSCs to induce TGF- $\beta$  signaling and stellate cell activation<sup>[71]</sup>. Additionally, TLR9 signaling activated *via* DNA from apoptotic hepatocytes was shown to modulate liver fibrosis *via* its effects on HSC differentiation through increased collagen production and inhibited HSC migration<sup>[73]</sup>. Hence, LPS and other TLR ligands are suggested to facilitate fibrogenic responses in the liver *via* their direct effects on HSCs<sup>[9,11,33]</sup>.

### Biliary epithelial cells

Accounting for approximately 5% of non-parenchymal cell population in the liver, BECs are commonly exposed to several gut-derived microbes<sup>[74,75]</sup>. BECs mainly express TLRs 2, 3, 4 and 5, which are upregulated by IFN- $\gamma$  stimulation<sup>[74,75]</sup>. TLR2 and TLR4 activation results in increased IRAK-M expression and provide negative feedback in human intrahepatic BECs<sup>[76]</sup>.

Under normal conditions, increased IRAK-M expression is critical in preventing undesired induction of the TLR signaling cascade, while in case of inflammatory conditions, upregulation of BEC-associated TLRs leads to IFN- $\gamma$  and TNF- $\alpha$  exposure, participating in biliary pathogenic responses<sup>[9,75]</sup>.

### Sinusoidal endothelial cells

Making up 50% of the non-parenchymal cells, SECs function in hepatic perfusion and nutrient supply<sup>[66,77-79]</sup>. They express TLR3, 4 and 9 and show increased NF- $\kappa$ B activation and CD54 expression alongside a limited ability to trigger leukocyte adhesion after LPS stimulation<sup>[66,77-79]</sup>. Although these effects indicate a scavenging role and thus the likelihood of SECs acting as antigen presenting cells, the exact role of the TLR signaling in inflammatory process in SEC remains inconclusive<sup>[9,11,33,66,77-79]</sup>.

Isolated SECs from WT mice were shown to respond to TLR1, 2, 6 and 9 ligands *via* producing TNF- $\alpha$ ; to TLR3 ligands by producing TNF- $\alpha$ , IL-6 and IFN- $\beta$ ; and to TLR4 ligands *via* production of TNF- $\alpha$  and IL-6<sup>[22,33]</sup>. Upon TLR8

ligand binding, SECs leads to TNF- $\alpha$  production alongside upregulation of major histocompatibility complex (MHC)-II and co-stimulatory molecules. Stimulation of SECs by TLR1, 2 or 6 ligands is suggested to be associated with activation of allogeneic T cells, as evaluated by the mixed lymphocyte reaction<sup>[22,33]</sup>. The SEC immune response is also modulated by LPS tolerance, which appears to be based on prostanoid expression rather than regulation at the level of TLR4 surface expression<sup>[78]</sup>. Although SECs have been suggested to be involved in the hepatic uptake of LPS in some studies, several studies have not confirmed such a role<sup>[33,80,81]</sup>.

### Hepatic dendritic cells

Comprising < 1% of non-parenchymal cells, hepatic DCs are recruited into the liver sinusoids during inflammation and then they may migrate to periportal and pericentral areas<sup>[5,33,82,83]</sup>. Plasmacytoid DCs (pDCs), myeloid DCs, lymphoid DCs, mixed lymphoid + myeloid DCs and natural killer DCs are amongst the DC subsets, whereas lymphoid and myeloid DCs are considered conventional DCs<sup>[33,82,83]</sup>.

Each DC subset show distinct TLR expression pattern in humans with TLR1, 7 and 9 expression *via* pDCs, while expression of all TLRs excluding TLR9 by other DC subsets<sup>[20,33,84]</sup>. Cytokines TNF- $\alpha$ , IL-6 and IL-12 TLR7 are produced by hepatic pDCs upon TLR7 and TLR9 activation, whereas TNF- $\alpha$  and IL-6 in response to TLR2, TLR3 and TLR4 activation<sup>[50,85]</sup>.

## TLRs IN THE PATHOPHYSIOLOGY OF LIVER DISEASES

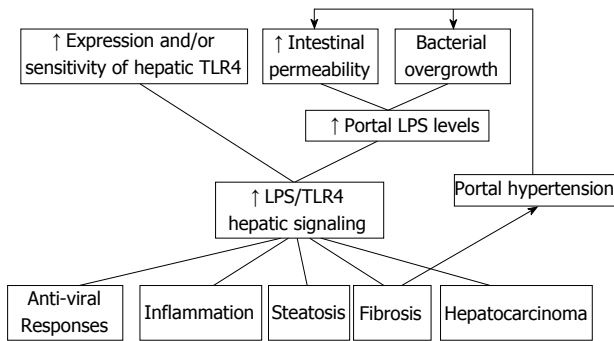
Increasing evidence suggests that TLRs have significant contribution to the pathogenesis and progression of several liver diseases, *i.e.*, non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), viral hepatitis, autoimmune liver disease and hepatic inflammation-fibrosis-carcinoma (IFC) sequence including hepatic fibrosis and/or cirrhosis and hepatocarcinoma<sup>[9,11,13,15,23,33]</sup>.

LPS/TLR4 and TLR2 signaling have been suggested to be principal actors in the human hepatic IFC sequence associated with viral chronic hepatitis<sup>[86]</sup>, while the participation of TLR3 in the pathophysiology of several liver diseases has also been suggested in the recent studies<sup>[11,15,23,87]</sup> (Figure 2).

### NAFLD and steatohepatitis

NAFLD and steatohepatitis is characterized by a pathologic spectrum that ranges from fatty liver (hepatic steatosis) to cirrhosis with intervening non-alcoholic steatohepatitis (NASH) and usually occurs in association with obesity and insulin resistance<sup>[13,72,88-90]</sup>.

Increased serum PAMP levels were observed in both experimental models and in NAFLD patients<sup>[9,18,91-96]</sup>. A shift in microbial populations to adopt an "obese" phenotype in NAFLD is referred to as "metabolic endotoxaemia", in which a high-fat diet is associated with



**Figure 2 Enhanced lipopolysaccharide/toll-like receptors 4 signaling in chronic liver diseases.** Induction of anti-viral responses, inflammation, steatosis, fibrosis, and hepatocarcinoma via LPS/TLR4 signaling alongside hepatic fibrosis mediated portal hypertension which further increases bacterial overgrowth and intestinal permeability, creating a positive feedback process. TLR4: Toll-like receptors 4; LPS: Lipopolysaccharide.

elevated levels of LPS translocation<sup>[27,90,97]</sup>.

While TLR2, TLR4 and TLR9 participate in the development of NASH and NAFLD, LPS-TLR4 is considered to be the main pathway for the progression of NAFLD<sup>[98-100]</sup>. The role of bacterial overgrowth has also been associated with development of NASH, emphasizing the interaction between bacterial overgrowth, gut permeability and liver injury<sup>[90,101,102]</sup>.

While the role of adipose tissue macrophages in the development of NAFLD is not yet clear, KCs are known to play a pivotal role in the development of NAFLD alongside accompanying hepatic inflammation and related complications<sup>[18,98]</sup>.

When inflammation occurs in NAFLD, NF- $\kappa$ B and transcriptional factor AP1 are activated, stimulating the production of TNF- $\alpha$  and IL-10, in particular, by KCs<sup>[23,103]</sup>. Studies in animal models indicated the likelihood of TLRs 2, 4 and 9 to participate in NAFLD onset or progression<sup>[9,18,91,104]</sup>. LPS/TLR4 and TLR9 signaling in KCs have been associated with both onset and progression of NAFLD by inducing reactive oxygen species (ROS)-dependent activation of X-box binding protein-1 and IL-1b, respectively, whereas induction of hepatic steatosis occurs independent of TLR2 signaling in KCs<sup>[18,104-106]</sup> (Figure 2).

While free fatty acids and denatured host DNA are considered to be potential candidates to activate TLR2, TLR4 and TLR9 signals, no clear-cut evidence exists to confirm their capacity to activate TLRs in NAFLD<sup>[18]</sup>. TLR4 signaling has been considered to play a major role in the pathogenesis of NAFLD that operates *via* KCs stimulation and increased ROS and TNF- $\alpha$  production<sup>[13]</sup>.

## ALD

ALD is described along a disease spectrum ranging from steatosis and steatohepatitis to fibrosis and cirrhosis and potential development of hepatocellular carcinoma (HCC)<sup>[90,107]</sup>.

Despite a strong association between alcohol and hepatotoxicity, the exact pathogenesis has not yet been

elucidated<sup>[90]</sup>. Involvement of the gut microbiota *via* a "leaky" gut has been indicated in the development of ALD<sup>[18]</sup>, whereas the role of alcohol has also been suggested in increasing gut permeability by disrupting tight junctions<sup>[108,109]</sup>. Increased plasma LPS levels and hepatic endotoxin levels, which leads to increased TLR4 signaling on KCs, HSC, LSECs and hepatocytes and thus the release of pro-inflammatory cytokines have been associated with inflammation and liver damage<sup>[9,107,108,110]</sup>.

Recent studies indicate significant contribution of TLR4 signaling and thus the crucial role of both KCs and HSCs in development of gut-derived endotoxin related effects in ALD<sup>[18]</sup>. Chronic alcohol consumption is also associated with the increased expression of TLR1, TLR2, TLR4 and TLR6-TLR9, which further potentiates the secretion of the pro-inflammatory TNF- $\alpha$  in response to LPS<sup>[111]</sup>.

KCs produce pro-inflammatory cytokines (TNF- $\alpha$ , IL-1, IL-6 and IL-8, chemokines) and profibrogenic factors (TGF- $\beta$ ) under post-LPS mediated TLR4-dependent stimulation, and consequent liver inflammation and stellate cell activation induce liver fibrosis<sup>[9,15,112,113]</sup>. The TLR4-dependent downstream signaling cascade in ALD was shown to proceed *via* the MyD88-independent pathway, possibly *via* adapter molecule TRIF<sup>[114]</sup>. Nonetheless, increased expression of not only TLR4 but also other TLRs such as TLR1, 2, 6, 7, 8 and 9 was shown in an experimental chronic alcohol model<sup>[115]</sup>.

Although activation of KCs *via* TLR4 signaling is a key event in the pathogenesis of alcohol-induced liver injury<sup>[18]</sup>, recent data emphasize the activation of TLR4 signaling in HSCs as well, indicating the their contribution to alcohol-induced hepatocyte injury, steatosis, inflammation, and fibrogenesis<sup>[18,116]</sup>. In HSCs, activated TLR4 signaling downregulates TGF- $\beta$  pseudoreceptor BMP and activin membrane-bound inhibitor (BAMBI), resulting in enhancement of TGF- $\beta$  signaling, whereas BAMBI downregulation is dependent on MyD88 but not TRIF<sup>[18,110]</sup>. The TLR4-TRIF-IRF3-dependent pathway associated with bone marrow-derived cells including KCs is considered to be more important than the TLR4-MyD88-dependent pathway in the development of alcoholic steatohepatitis<sup>[18,110,114]</sup>.

Acting through upregulation of TLR4 and MD-2 and induction of a Th1-type immune response, bacterial DNA recognition by TLR9 was also shown to be associated with LPS induced liver injury<sup>[117]</sup>, indicating the likelihood of TLR9 signaling to contribute to pathogenesis of ALD<sup>[18]</sup>.

## Hepatic fibrosis and cirrhosis

The development of hepatic fibrosis and consequent cirrhosis upon continued liver insults may occur in any type of chronic hepatic injury, including viral hepatitis, alcohol, autoimmune and metabolic disease<sup>[9,67]</sup>.

Prolonged or repeated liver injury leads to a maladaptive interplay of hepatocytes, HSCs and KCs in association with TLR expression, eventually resulting in abnormal extracellular matrix protein deposition in the



liver<sup>[35,67,118]</sup>.

LPS-TLR4 activation is considered essential for hepatic fibrogenesis, whereas TLR4 is expressed on KCs and HSCs, the key mediators of hepatic fibrogenesis<sup>[27,75,80,81]</sup>.

KCs express the highest levels of TLR4 and act as the principal target of LPS leading to release of several pro-inflammatory and pro-fibrogenic mediators<sup>[5,27,71,114,119]</sup>. However, HSCs are crucial in the pathogenesis of fibrosis and cirrhosis given their myofibroblastic phenotype and ability to produce collagen, the principal component of fibrotic tissue<sup>[9,120]</sup>.

Activation of HSC occurs either *via* pro-inflammatory cytokines and growth factors secreted by LPS-TLR4-stimulated KCs, or directly *via* LPS-TLR4-dependent HSC stimulation<sup>[9,71]</sup>. LPS/TLR4 signaling in HSCs is essential for development of liver fibrosis and acts *via* stimulating production of chemokines that recruit KCs alongside enabling unrestricted activation of HSCs by KCs-derived profibrogenic cytokine TGF- $\beta$ <sup>[11,13,103,121]</sup> (Figure 2).

TLR4 activation in HSCs is considered to be the main step for collagen production and the main mediator of fibrosis and cirrhosis<sup>[9,11,67,70,71]</sup>.

KCs induce fibrogenesis by means of proinflammatory and profibrogenic cytokine secretion, whereas HSCs are the leading source of extracellular matrix production in the fibrotic liver<sup>[11,67]</sup>.

TLR9 signaling-associated metabolic pathways are also considered important in the genesis of hepatic fibrosis *in vivo*, leading to activation of pathways such as IL-1 production and thus HSCs by upregulating profibrogenic genes, such as procollagen type I and tissue inhibitor metalloproteinase-1<sup>[16,69,103,104]</sup>.

Moreover, a deficiency of TLR3-mediated NK cell-dependent apoptosis of HSCs has been linked to the progression of alcohol-induced liver fibrosis<sup>[122,123]</sup>. Upregulation of TLR2 was shown to promote liver inflammation and fibrogenesis in NASH<sup>[106]</sup> and HSCs activation and inflammation response during carbon tetrachloride-induced liver fibrosis mediated *via* MAPK and NF- $\kappa$ B signaling pathways<sup>[124]</sup>, whereas TLR5 was also shown to be directly involved in the progression of fibrosis *via* activation of the NF- $\kappa$ B and MAPK signaling pathways<sup>[52]</sup>.

### Hepatitis B

Hepatitis B virus (HBV) is a DNA virus responsible for acute hepatitis, which is self-limiting in 80%-90% of adults and chronic in 10%-20% of cases<sup>[5,125]</sup>. Hepatitis B is associated with an increased risk of developing cirrhosis, hepatic decompensation and HCC, but prognosis shows interpersonal variation depending on the viral susceptibility and induction of antiviral immune response<sup>[126,127]</sup>.

Indicating the role of TLRs in HBV infection, the activation of TLR3, TLR7 and TLR9 as well as TLR4 and TLR5, has been associated with blockage of viral replication *via* IFN-dependent inhibition of HBV<sup>[76,128,129]</sup>. Moreover, HBV leads to TLR downregulation alongside restriction of receptor activity, increasing the likelihood of persistent infection<sup>[27]</sup>.

*In vitro* HBV studies on TLR expression in HepG2 cells revealed elevated expression of TLRs 2, 3, 4, 5, 6, 7 and 9 mRNA upon ligand binding along with an induced IFN response and abolished HBV DNA replication and RNA transcription, whereas no or very limited expression of TLRs 1, 8 and 10<sup>[9,130]</sup>. Furthermore, transfection of HBV-positive cell lines with TLR adaptor molecules was shown to be associated with elevated TLR activity and a consequent reduction in HBV DNA and mRNA levels<sup>[131]</sup>, whereas HBV replication was completely abolished after injection of TLR3, TLR4, TLR5, TLR7 and TLR9 ligands into HBV transgenic mice<sup>[129]</sup>.

TLR1, TLR2, TLR4 and TLR6 were shown to be down-regulated in HBV-infected peripheral blood monocytes along with a decreased cytokine response to TLR2 and TLR4 ligands<sup>[132]</sup>. Downregulation of TLR2 on hepatocytes and hepatic KCs was demonstrated in HBeAg-positive CHB-infected patients, whereas upregulation of TLR2 and cytokine expression was observed in HBeAg-negative CHB patients<sup>[133]</sup>. Hence, HBeAg-induced downregulation of TLR2 *via* precore protein has been accused for the accelerated progression of disease in HBeAg-positive patients<sup>[9,133]</sup>.

Although HBV is able to downregulate TLRs and thus avoid anti-viral pathways, prolonged infection and loss of HBeAg is considered likely to upregulate TLR signaling pathways such as TLR2 that are not primarily involved in anti-HBV responses while trigger hepatic inflammation and disease progression<sup>[11]</sup>.

*In vitro* analysis of HBV-Met cells revealed that TLR-treated KCs and SECs to have a modulatory effect on HBV replication<sup>[134]</sup>. TLR3- and TLR4-stimulated KCs and TLR3-activated SECs were shown to affect HBV replication *via* MyD88-independent pathway<sup>[66]</sup>. HBV-suppressing effect was mediated by IFN- $\beta$  in case of TLR3 ligand activation, whereas by cytokines of an undefined nature in case of TLR4-activated KCs<sup>[66]</sup>.

HBV is a stealth virus and thus does not induce an IFN response during the early phase of infection, whereas its recognition by liver resident cells is considered likely to activate innate immune responses without IFN induction<sup>[107,135]</sup>. Notably, HBV was shown to be recognized by hepatic NPCs, mainly by KCs, leading to NF- $\kappa$ B-dependent induction of the release of the inflammatory cytokines IL-6, IL-8, TNF- $\alpha$  and IL-1 $\beta$  as well as reduced expression of transcription factors essential for HBV gene expression and replication including hepatocyte nuclear factor (HNF) 1 $\alpha$  and HNF4 $\alpha$ <sup>[136]</sup>.

### Hepatitis C

Hepatitis C virus (HCV) is a hepatotropic virus responsible for development of chronic hepatitis and related complications such as liver cirrhosis, liver failure or HCC<sup>[137,138]</sup>.

Similarly to HBV, current evidence indicates that HCV selectively impairs activation of TLR signaling controlling HCV replication, while it concomitantly stimulates TLR pathways that generate a chronic inflammatory state

leading to persistent liver injury<sup>[11,27,139,140]</sup>.

HCV-induced inhibition of TLR signaling contributes to its chronicity related to virus dissemination, inflammation and eventual progression to fibrosis and cirrhosis<sup>[9,11]</sup>.

Regulation of HCV replication by non-parenchymal liver cells occurs through the production of IFN- $\beta$  upon their stimulation by TLR3 and TLR4<sup>[141]</sup>. The inhibitory effect of HCV proteins on TLR7 and TLR9, is also likely to prevent virus clearance<sup>[27]</sup>. Furthermore, activation of TLR2 along with TLR1 and TLR6 and possibly TLR4 by HCV core protein and NS3 promotes hepatic inflammation and injury<sup>[142-145]</sup>.

In the presence of HCV, significantly decreased TLR7 expression along with TLR7-independent activation of IRF-7 pathway was demonstrated both *in vitro* and *in vivo*<sup>[146]</sup>.

The NS3/4A serine protease of HCV, HCV NS3 protein and HCV NS5A act *via* three signaling pathways including the TLR3-TRIF-TBK1-IRF-3, TLRMyD88, and RIG-I/MDA5-IPS-1 pathways to enable HCV to evade innate immune signaling<sup>[33]</sup>. Moreover, LPS, the HCV core protein and IFN- $\gamma$  have been suggested to amplify inflammatory monocyte/macrophage activation *via* formation of MyD88-IRAK complexes, increased NF- $\kappa$ B activation and increased production of TNF- $\alpha$ , leading to the loss of TLR tolerance<sup>[147]</sup>.

Based on these findings, both host- and virus derived factors have been considered likely to act on macrophages to induce persistent inflammation during chronic HCV infection<sup>[53,107]</sup>.

### Hepatocarcinoma

Diseases associated with uncontrolled innate immunity related to TLR ligand exposure in the liver (fibrosis, hepatitis B and C infection, ALD and NASH) are also among the etiologies for HCC. Therefore, it appears likely that TLRs play a role in the development of inflammation-associated liver cancer and are involved in the progression of HCC<sup>[18,107]</sup>. Hence, chronic hepatic inflammation and fibrosis, as regulated by TLR activation, promotes HCC formation in approximately 10% of cases of cirrhosis<sup>[9,54]</sup>.

TLRs, TLR4 in particular, are considered to play a significant role in associating hepatic chronic inflammation and hepatocarcinoma<sup>[13]</sup>. A significant regression in liver tumors in TLR4 and MyD88 deficient mice indicates a prominent contribution of TLR signaling to hepatocarcinogenesis<sup>[23,148]</sup>.

HCC has been indicated to be promoted *via* gut microbiota and TLR4 in association with increased production of proinflammatory cytokines (TNF- $\alpha$ , IL-6), hepatomitogen epiregulin expression and prevention of apoptosis, whereas a reduction in the development of HCC was shown *via* gut sterilization, germ-free status or TLR4 inactivation<sup>[18,149,150]</sup>.

Activation of KCs *via* TLRs is considered to be involved in the process of tumorigenesis<sup>[18]</sup> by inducing proinflammatory cytokines and hepatomitogens responsible for enhanced development of HCC<sup>[150,151]</sup>, whereas TLR4

expression on non-marrow-derived resident liver cells is considered to be required for the promotion of HCC<sup>[149]</sup>.

TLR4 contributes significantly to hepatic inflammation and fibrosis, whereas upregulation of inflammatory factors such as COX-2 and NF- $\kappa$ B by TLR4 as well as the TLR adaptor protein Myd-88 is also important in hepatocarcinogenesis<sup>[148,152-155]</sup>. TLR3 expression is suggested to contribute to hepatocarcinoma *via* proapoptotic activity, while activation of TLR9 *via* CpG DNA of HBV has been associated with malignant transformation in liver cells<sup>[27,156,157]</sup>.

Although, TLR2 binding with ligands such as HMGB1 and HSPA1A is associated with tumor enhancement, the effect of TLR2 activation is considered likely to differ according to the phase of HCC carcinogenesis, with anti-oncogenic potential slowing down the onset and development of HCC in earlier phases, whereas pro-oncogenic potential during later stages that promotes the progression of inflammation and fibrosis<sup>[158]</sup>.

Activation of the NF- $\kappa$ B and JNK pathways and higher expression levels of IKK $\alpha$  and IKK $\beta$  are considered critical in the production of the cytokines related to TLR-induced liver damage and HCC progression<sup>[107]</sup>.

Recently, spontaneous HCC development was demonstrated in hepatocyte-specific TAK1 deleted (TAK1DHEP) mice along with a resistance for HCC development that occurs *via* deletion of MyD88, TLR4 or TLR9 signaling<sup>[159]</sup>.

Alcohol and HCV are suggested to interact in causing progression of liver disease and malignancy, whereas TLR4, TLR4 downstream gene Nanog and activated LPS-TLR4 are also considered to contribute to this synergy *via* triggering proliferative and anti-apoptotic signals to non-marrow-derived resident liver cells and thus HCC progression<sup>[9,149,150,160]</sup>.

### Ischemic/reperfusion injury and liver allograft rejection

Ischemia-reperfusion (I/R) injury in partial hepatectomy and liver transplantation is associated with the release of various endogenous ligands for hepatic tissue TLRs and thus the activation of complex signaling pathways that induce neutrophilic and T-lymphocytic tissue inflammation and injury<sup>[53,161,162]</sup>.

Among the most studied TLRs in hepatic I/R, TLR4 was shown to participate in certain acute sterile injury models, including liver I/R, by mobilizing the immune system upon detection of endogenous ligands, whereas limited data are available on TLR2 and TLR9<sup>[163,164]</sup>.

MyD88-independent activation of TLR4 by DAMPs is considered central to the inflammatory process observed in I/R lesions<sup>[165-167]</sup>, whereas HSP, heparan sulfate, fibronectin, fibrinogen, hyaluronan and HMGB1 are known to act as endogenous ligands for TLR4 activation in hepatic I/R injury<sup>[5,163]</sup>.

Release of HMGB1 activates the cell surface TLR4 on KCs and leads to a subsequent release of cytotoxic mediators (TNF- $\alpha$ , IL-6 and chemokine IP-10), alongside an inappropriate activation of the pro-apoptotic protein kinase JNK and stress-responsive NF- $\kappa$ B, all of which are mediators of cell injury<sup>[5,163,168,169]</sup>. Cellular expression

of TLR4 is further upregulated *via* newly synthesized mediators such as TNF- $\alpha$ , leading to formation of a vicious cycle of proinflammatory cytokine production<sup>[61,163,170]</sup>.

Downstream TLR4 signaling pathways in I/R injury seems to be independent of MyD88 signaling, whereas TRIF-dependent activation of the interferon response and IRF1 expression is considered critical for mediating I/R injury in hepatocytes in terms of releasing the danger signal HMGB1<sup>[164,171,172]</sup>. Hence, TLR4, IRF1 and HMGB1 are considered three important and interacting mediators of I/R injury<sup>[164]</sup>.

Albeit not consistent, available data suggest that besides lack of TLR4, downregulation of TLR2 expression in the donor organ also suppress I/R injury<sup>[27,165,173]</sup>. Accordingly, given the amelioration of liver injury in I/R *via* non-selective inhibition of TLR2 and TLR4 activation by certain molecules such as bicyclol or N-acetylcysteine, role of TLRs in I/R lesion has been emphasized<sup>[27,174,175]</sup>.

TLR9, which shows affinity toward both pathogen-derived and endogenous host DNA, is considered to play a crucial role in non-pathogen-induced hepatic I/R injury by causing neutrophil activation, liver necrosis, and inflammatory cytokine release<sup>[163,176,177]</sup>.

Although TLR signaling dependent early activation of the innate immune system is consistently reported in the setting of I/R injury, additional studies are required to fully explore the roles of other TLRs and TLR signaling pathways in I/R injury<sup>[163,164]</sup>.

### **Liver regeneration after partial hepatectomy**

Recognizing the mechanism of liver regeneration is important not only for managing acute liver failure and post-transplant hepatic dysfunction but also for disturbed liver regeneration in NASH or NAFLD and advanced liver fibrosis<sup>[178]</sup>. The deposition of excessive amounts of extracellular matrix, the presence of persistent inflammation, the transformation of SECs and HSCs, portal blood flow reduction and increased JNK activity are considered among the factors associated with the regenerative ability of fibrotic livers<sup>[178,179]</sup>.

TLR/MyD88-mediated pathways are associated with onset of liver regeneration after partial hepatectomy (PH) *via* activation of NF- $\kappa$ B, release of TNF- $\alpha$  and IL-6 and the expression of the immediate early genes for cell replication in hepatocytes, whereas distinct TLR ligands responsible for the priming process have not yet been clarified<sup>[33,178]</sup>. No contribution of TLR2, TLR4 or TLR9 to MyD88-mediated pathways and no influence of TLR2 or TLR4 on proinflammatory cytokine production or gene replication have been reported for liver regeneration after PH<sup>[33,180,181]</sup>.

In fact, given the inhibition of regenerative process *via* excessive TLR signaling produced by LPS injection after PH, the magnitude of TLR signaling is considered critical for intact liver regeneration<sup>[178,182]</sup>.

TLR3 signaling, which utilizes a distinct adaptor protein, TRIF, is considered to attenuate the initiation of liver regeneration *via* TLR3-dependent NF- $\kappa$ B activation in hepatocytes and TLR3-induced IFN- $\gamma$  through

STAT1 and consequent induction of the IRF-1 and p21 pathways<sup>[178,183,184]</sup>.

In addition, although a non-TLR MyD88-dependent pathway with IL-1 and IL-18 has been suggested to play a role in allograft rejection initially, findings on the existence of normal liver regeneration after PH in caspase 1-deficient mice indicate unremarkable participation of IL-1 $\beta$  and IL-18 in liver regeneration<sup>[178]</sup>.

### **Hepatic autoimmune disorders**

Although antibody formation against self-antigens is key to the development of autoimmune hepatic diseases, including autoimmune hepatitis, primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC)<sup>[185]</sup>, recently the influence of gut microbiota on the propagation of these diseases has been indicated<sup>[90]</sup>.

Given that the liver is considered a classical immunoprivileged site, TLR signals may act as an important promoter for overcoming this immunoprivilege and inducing hepatic autoimmune disease<sup>[11,13,186]</sup>.

Previous studies have suggested regulator role of gut-derived products on T cell function within the liver<sup>[90]</sup>, based on the connection between TLR4 signaling and the trapping of CD8+ T cells in the murine liver<sup>[187]</sup>, as well as contribution of TLR9 to the homing and stimulation of hepatic NKT cells *via* a KC and IL-12 dependent process<sup>[188]</sup>. The role of LPS/TLR4 signaling has been indicated in the pathogenesis of PBC and PSC<sup>[13]</sup>. Monocytes from PBC patients have been suggested to show increased sensitivity to activation of selective TLRs (TLR2, TLR4, TLR3, TLR5 and TLR9), while the subsequent release of proinflammatory cytokines has been associated with development of self-tolerance and autoimmune progression<sup>[189]</sup> (Figure 2).

LPS was shown to accumulate in significant amounts in the biliary epithelia of PBC patients, whereas positivity for IgM antibodies against lipid A, an immunogenic and toxic component of LPS, is confirmed in 64% of PBC sera<sup>[190,191]</sup>. TLR4 expression is significantly elevated in BECs, periportal hepatocytes and blood monocytes of PBC patients<sup>[192,193]</sup>, whereas LPS/TLR4 signaling has been associated with an increased release of proinflammatory cytokines such as IL-1b, IL-6, IL-8 and TNF- $\alpha$ <sup>[189]</sup>. TLR4 ligand-stimulated NK cells have been suggested to be associated with BEC damage in the presence of TLR3 ligand-activated monocytes among PBC patients<sup>[194]</sup>. Despite similar levels of TLRs in BECs isolated from livers from patients and controls, stimulation *via* TLR3 agonist poly I:C and co-culture with liver-infiltrating mononuclear cells resulted in elevated chemokine levels in livers from patients<sup>[195]</sup>. Moreover, when compared to patients with autoimmune hepatitis and Hepatitis C, patients with PBC showed higher levels of TLR3 and IFN- $\alpha/\beta$  in portal tracts and liver parenchyma<sup>[196]</sup>. Furthermore, TLR9 ligand (CpG) stimulation of peripheral blood monocytes from PBC patients was demonstrated to activate IgM-producing B cells and to increase TLR9 expression on these cells<sup>[197,198]</sup>. These findings emphasize the role of innate immunity not only in the pathogenesis and pro-

gression of PBC but also in the regulation of adaptive immune responses<sup>[9]</sup>.

The role of TLRs in PSC has not been extensively studied<sup>[11]</sup>. Abnormal LPS accumulation was demonstrated in BECs in PSC<sup>[190]</sup>. Stimulating isolated BECs with anti-BEC antibodies from patients with PSC leads to increased expression of TLR4 along with higher levels of inflammatory cytokines in the presence of LPS<sup>[199]</sup>.

Accordingly, increased LPS accumulation and TLR4 expression in BECs has been suggested to induce breakdown of self-tolerance and onset of bile duct damage in PBC and PSC thorough their stimulatory effects on selective pro-inflammatory cytokines with a critical role<sup>[13]</sup>. Given the signs of inflammatory bowel disease to exist in most patients with PSC and the likelihood of gut factors to induce response onset per se with no preceding immune cell dysfunction, future investigations are needed addressing the role of gut microbiota in conjunction with PSC and PBC to provide a better understanding of the mechanisms and treatment of these complex diseases<sup>[90]</sup>.

## CONCLUSION

TLRs have been recognized as key regulators of innate and adaptive immune responses in the liver, although growing evidence suggests the critical role of TLR dysregulation in the pathogenesis and progression of many liver diseases<sup>[9,107]</sup>. TLRs, mainly TLR4 and TLR2, play a fundamental role in the inflammation and fibrosis of the liver and promote the progression of chronic liver diseases<sup>[27,35,86]</sup>. Indeed, LPS/TLR4 signaling is enhanced and essential in liver diseases such as ALD, NAFLD, PSC, CBP and fibrosis, and inhibition of TLR4 has been associated with amelioration of liver injury, emphasizing the contribution of LPS/TLR4 signaling to the pathogenesis of liver diseases<sup>[13]</sup>.

The local innate immune system represented by liver cells participates in tolerance induction or inflammation alongside its interaction with the adaptive immune system, whereas suppression of the TLR system in the liver by pathogens enhance chronicity of infection<sup>[107]</sup>. Therefore, targeting TLR signaling at different levels of cascade appears to offer therapeutic potential in the management of chronic liver disease<sup>[11]</sup>.

LPS/TLR4 signaling pathway has been recognized as an important pharmacological target in chronic liver diseases. Suppression of TLR4 signaling *via* modulation of LPS production, TLR and co-receptor expression and downstream signaling molecules has been shown to ameliorate liver injury, indicating the contribution of LPS/TLR4 signaling to the pathogenesis of chronic liver diseases. Given the likelihood of systemic suppression of TLR4 to disable responding pattern of TLR4 to invading pathogens, modulation of intestinal microbiota *via* probiotics and symbiotics become a preferred therapeutic strategy for liver diseases, associated with favorable tolerability and safety<sup>[13,23]</sup>. Besides, certain synthetic ligands of TLRs have been considered to act as target molecules for drug

development given their effects on regulation of innate and adaptive immune responses, including TLR activators (for infections and certain cancers), TLR inhibitors (for inflammatory diseases and sepsis) as well as TLR neutralizing antibodies<sup>[34,37]</sup>. Further investigation of the role of TLR pathways in liver diseases addressing the downstream mediators and regulation of TLR signaling, the specific cell populations involved, the role of TLR polymorphisms and the mechanisms underlying liver tumorigenesis is needed to transfer knowledge on TLR pathophysiology into clinical practice in treating human liver diseases<sup>[5,23]</sup>.

## REFERENCES

- 1 **Racanelli V**, Rehermann B. The liver as an immunological organ. *Hepatology* 2006; **43**: S54-S62 [PMID: 16447271 DOI: 10.1002/hep.21060]
- 2 **Tiegs G**, Lohse AW. Immune tolerance: what is unique about the liver. *J Autoimmun* 2010; **34**: 1-6 [PMID: 19717280 DOI: 10.1016/j.jaut.2009.08.008]
- 3 **Crispe IN**. Immune tolerance in liver disease. *Hepatology* 2014; **60**: 2109-2117 [PMID: 24913836 DOI: 10.1002/hep.27254]
- 4 **Henao-Mejia J**, Elinav E, Thaiss CA, Licona-Limon P, Flavell RA. Role of the intestinal microbiome in liver disease. *J Autoimmun* 2013; **46**: 66-73 [PMID: 24075647 DOI: 10.1016/j.jaut.2013.07.001]
- 5 **Schwabe RF**, Seki E, Brenner DA. Toll-like receptor signaling in the liver. *Gastroenterology* 2006; **130**: 1886-1900 [PMID: 16697751 DOI: 10.1053/j.gastro.2006.01.038]
- 6 **Doherty DG**. Immunity, tolerance and autoimmunity in the liver: A comprehensive review. *J Autoimmun* 2016; **66**: 60-75 [PMID: 26358406 DOI: 10.1016/j.jaut.2015.08.020]
- 7 **Norberto C**, Chávez-Tapia, Leticia González-Rodríguez, MinSeung Jeong, Yanine López-Ramírez, Varenka Barbero-Becerra, Eva Juárez-Hernández, Juan L. Romero-Flores, Marco Arrese, Nahúm Méndez-Sánchez, Misael Uribe. Current evidence on the use of probiotics in liver diseases. *J Functional Foods* 2015; **17**: 137-151 [DOI: 10.1016/j.jff.2015.05.009]
- 8 **Beutler BA**. TLRs and innate immunity. *Blood* 2009; **113**: 1399-1407 [PMID: 18757776 DOI: 10.1182/blood-2008-07-019307]
- 9 **Kesar V**, Odin JA. Toll-like receptors and liver disease. *Liver Int* 2014; **34**: 184-196 [PMID: 24118797 DOI: 10.1111/liv.12315]
- 10 **Bigorgne AE**, Crispe IN. TLRs in Hepatic Cellular Crosstalk. *Gastroenterol Res Pract* 2010; **2010** [PMID: 20862346 DOI: 10.1155/2010/618260]
- 11 **Mencin A**, Kluwe J, Schwabe RF. Toll-like receptors as targets in chronic liver diseases. *Gut* 2009; **58**: 704-720 [PMID: 19359436 DOI: 10.1136/gut.2008.156307]
- 12 **Aderem A**, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. *Nature* 2000; **406**: 782-787 [PMID: 10963608 DOI: 10.1038/35021228]
- 13 **Soares JB**, Pimentel-Nunes P, Roncon-Albuquerque R, Leite-Moreira A. The role of lipopolysaccharide/toll-like receptor 4 signaling in chronic liver diseases. *Hepatol Int* 2010; **4**: 659-672 [PMID: 21286336 DOI: 10.1007/s12072-010-9219-x]
- 14 **Carvalho FA**, Aitken JD, Vijay-Kumar M, Gewirtz AT. Toll-like receptor-gut microbiota interactions: perturb at your own risk! *Annu Rev Physiol* 2012; **74**: 177-198 [PMID: 22035346 DOI: 10.1146/annurev-physiol-020911-153330]
- 15 **Szabo G**, Dolganiuc A, Mandrekar P. Pattern recognition receptors: a contemporary view on liver diseases. *Hepatology* 2006; **44**: 287-298 [PMID: 16871558 DOI: 10.1002/hep.21308]
- 16 **Takeuchi O**, Akira S. Pattern recognition receptors and inflammation. *Cell* 2010; **140**: 805-820 [PMID: 20303872 DOI: 10.1016/j.cell.2010.01.022]
- 17 **Yamamoto M**, Takeda K. Current views of toll-like receptor



- signaling pathways. *Gastroenterol Res Pract* 2010; **2010**: 240365 [PMID: 21197425 DOI: 10.1155/2010/240365]
- 18 **Roh YS**, Seki E. Toll-like receptors in alcoholic liver disease, non-alcoholic steatohepatitis and carcinogenesis. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 38-42 [PMID: 23855294 DOI: 10.1111/jgh.12019]
  - 19 **Rakoff-Nahoum S**, Medzhitov R. Role of toll-like receptors in tissue repair and tumorigenesis. *Biochemistry (Mosc)* 2008; **73**: 555-561 [PMID: 18605980 DOI: 10.1134/S0006297908050088]
  - 20 **Iwasaki A**, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 2004; **5**: 987-995 [PMID: 15454922 DOI: 10.1038/ni1112]
  - 21 **Hösel M**, Broxtermann M, Janicki H, Esser K, Arzberger S, Hartmann P, Gillen S, Kleeff J, Stabenow D, Odenthal M, Knolle P, Hallek M, Protzer U, Büning H. Toll-like receptor 2-mediated innate immune response in human nonparenchymal liver cells toward adeno-associated viral vectors. *Hepatology* 2012; **55**: 287-297 [PMID: 21898480 DOI: 10.1002/hep.24625]
  - 22 **Wu J**, Meng Z, Jiang M, Zhang E, Trippler M, Broering R, Bucchi A, Krux F, Dittmer U, Yang D, Roggendorf M, Gerken G, Lu M, Schlaak JF. Toll-like receptor-induced innate immune responses in non-parenchymal liver cells are cell type-specific. *Immunology* 2010; **129**: 363-374 [PMID: 19922426 DOI: 10.1111/j.1365-2567.2009]
  - 23 **Seki E**, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: update. *Hepatology* 2008; **48**: 322-335 [PMID: 18506843 DOI: 10.1002/hep.22306]
  - 24 **Liew FY**, Xu D, Brint EK, O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. *Nat Rev Immunol* 2005; **5**: 446-458 [PMID: 15928677 DOI: 10.1038/nri1630]
  - 25 **Gao B**, Jeong WI, Tian Z. Liver: An organ with predominant innate immunity. *Hepatology* 2008; **47**: 729-736 [PMID: 18167066 DOI: 10.1002/hep.22034]
  - 26 **Hopkins PA**, Sriskandan S. Mammalian Toll-like receptors: to immunity and beyond. *Clin Exp Immunol* 2005; **140**: 395-407 [PMID: 15932500 DOI: 10.1111/j.1365-2249.2005.02801.x]
  - 27 **Pimentel-Nunes P**, Soares JB, Roncon-Albuquerque R, Dinis-Ribeiro M, Leite-Moreira AF. Toll-like receptors as therapeutic targets in gastrointestinal diseases. *Expert Opin Ther Targets* 2010; **14**: 347-368 [PMID: 20146632 DOI: 10.1517/14728221003642027]
  - 28 **Szabo G**, Billiar TR, Machida K, Crispe IN, Seki E. Toll-like receptor signaling in liver diseases. *Gastroenterol Res Pract* 2010; **2010**: 971270 [PMID: 21789039 DOI: 10.1155/2010/971270]
  - 29 **Mohammad Hosseini A**, Majidi J, Baradaran B, Yousefi M. Toll-Like Receptors in the Pathogenesis of Autoimmune Diseases. *Adv Pharm Bull* 2015; **5**: 605-614 [PMID: 26793605 DOI: 10.15171/apb.2015.082]
  - 30 **Akira S**, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006; **124**: 783-801 [PMID: 16497588 DOI: 10.1016/j.cell.2006.02.015]
  - 31 **Beutler B**, Jiang Z, Georgel P, Crozat K, Croker B, Rutschmann S, Du X, Hoebe K. Genetic analysis of host resistance: Toll-like receptor signaling and immunity at large. *Annu Rev Immunol* 2006; **24**: 353-389 [PMID: 16551253 DOI: 10.1146/annurev.immunol.24.021605.090552]
  - 32 **O'Neill LA**, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat Rev Immunol* 2007; **7**: 353-364 [PMID: 17457343]
  - 33 **Chen Y**, Sun R. Toll-like receptors in acute liver injury and regeneration. *Int Immunopharmacol* 2011; **11**: 1433-1441 [PMID: 21601014 DOI: 10.1016/j.intimp.2011.04.023]
  - 34 **Wang Y**, Song E, Bai B, Vanhoutte PM. Toll-like receptors mediating vascular malfunction: Lessons from receptor subtypes. *Pharmacol Ther* 2016; **158**: 91-100 [PMID: 26702901 DOI: 10.1016/j.pharmthera.2015.12.005]
  - 35 **Huebener P**, Schwabe RF. Regulation of wound healing and organ fibrosis by toll-like receptors. *Biochim Biophys Acta* 2013; **1832**: 1005-1017 [PMID: 23220258 DOI: 10.1016/j.bbdis.2012.11.017]
  - 36 **Booth J**, Wilson H, Jimbo S, Mutwiri G. Modulation of B cell responses by Toll-like receptors. *Cell Tissue Res* 2011; **343**: 131-140 [PMID: 20824286 DOI: 10.1007/s00441-010-1031-3]
  - 37 **Lin Q**, Li M, Fang D, Fang J, Su SB. The essential roles of Toll-like receptor signaling pathways in sterile inflammatory diseases. *Int Immunopharmacol* 2011; **11**: 1422-1432 [PMID: 21600309 DOI: 10.1016/j.intimp.2011.04.026]
  - 38 **De Nardo D**. Toll-like receptors: Activation, signalling and transcriptional modulation. *Cytokine* 2015; **74**: 181-189 [PMID: 25846205 DOI: 10.1016/j.cyt.2015.02.025]
  - 39 **Akira S**, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* 2004; **4**: 499-511 [PMID: 15229469 DOI: 10.1038/nri1391]
  - 40 **Medzhitov R**, Preston-Hurlburt P, Janeway CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 1997; **388**: 394-397 [PMID: 9237759 DOI: 10.1038/41131]
  - 41 **Kawai T**, Akira S. TLR signaling. *Cell Death Differ* 2006; **13**: 816-825 [PMID: 16410796 DOI: 10.1038/sj.cdd.4401850]
  - 42 **Barton GM**, Medzhitov R. Control of adaptive immune responses by Toll-like receptors. *Curr Opin Immunol* 2002; **14**: 380-383 [PMID: 11973138 DOI: 10.1016/S0952-7915(02)00343-6]
  - 43 **Kumar H**, Kawai T, Akira S. Toll-like receptors and innate immunity. *Biochem Biophys Res Commun* 2009; **388**: 621-625 [PMID: 19686699 DOI: 10.1016/j.bbrc.2009.08.062]
  - 44 **Babu S**, Blauvelt CP, Kumaraswami V, Nutman TB. Cutting edge: diminished T cell TLR expression and function modulates the immune response in human filarial infection. *J Immunol* 2006; **176**: 3885-3889 [PMID: 16547219 DOI: 10.4049/jimmunol.176.7.3885]
  - 45 **Murad S**. Toll-like receptor 4 in inflammation and angiogenesis: a double-edged sword. *Front Immunol* 2014; **5**: 313 [PMID: 25071774 DOI: 10.3389/fimmu.2014.00313]
  - 46 **Tsan MF**, Gao B. Endogenous ligands of Toll-like receptors. *J Leukoc Biol* 2004; **76**: 514-519 [PMID: 15178705 DOI: 10.1189/jlb.0304127]
  - 47 **Kaisho T**, Akira S. Pleiotropic function of Toll-like receptors. *Microbes Infect* 2004; **6**: 1388-1394 [PMID: 15596125 DOI: 10.1016/j.micinf.2004.08.019]
  - 48 **Takeda K**, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol* 2003; **21**: 335-376 [PMID: 12524386 DOI: 10.1146/annurev.immunol.21.120601.141126]
  - 49 **Yu L**, Wang L, Chen S. Endogenous toll-like receptor ligands and their biological significance. *J Cell Mol Med* 2010; **14**: 2592-2603 [PMID: 20629986 DOI: 10.1111/j.1582-4934.2010.01127.x]
  - 50 **De Creus A**, Abe M, Lau AH, Hackstein H, Raimondi G, Thomson AW. Low TLR4 expression by liver dendritic cells correlates with reduced capacity to activate allogeneic T cells in response to endotoxin. *J Immunol* 2005; **174**: 2037-2045 [PMID: 15699133 DOI: 10.4049/jimmunol.174.4.2037]
  - 51 **Lichtman SN**, Wang J, Lemasters JJ. LPS receptor CD14 participates in release of TNF-alpha in RAW 264.7 and peritoneal cells but not in kupffer cells. *Am J Physiol* 1998; **275**: G39-G46 [PMID: 9655682]
  - 52 **Shu M**, Huang DD, Hung ZA, Hu XR, Zhang S. Inhibition of MAPK and NF-κB signaling pathways alleviate carbon tetrachloride (CCl4)-induced liver fibrosis in Toll-like receptor 5 (TLR5) deficiency mice. *Biochem Biophys Res Commun* 2016; **471**: 233-239 [PMID: 26845355 DOI: 10.1016/j.bbrc.2016.01.119]
  - 53 **Kawai T**, Akira S. TLR signaling. *Semin Immunol* 2007; **19**: 24-32 [PMID: 17275323]
  - 54 **Maeda S**. NF-κB, JNK, and TLR Signaling Pathways in Hepatocarcinogenesis. *Gastroenterol Res Pract* 2010; **2010**: 367694 [PMID: 21151655 DOI: 10.1155/2010/367694]
  - 55 **Chuang T**, Ulevitch RJ. Identification of hTLR10: a novel human Toll-like receptor preferentially expressed in immune cells. *Biochim Biophys Acta* 2001; **1518**: 157-161 [PMID: 11267672 DOI: 10.1016/S0167-4781(00)00289-X]
  - 56 **Palazzo M**, Gariboldi S, Zanobbio L, Dusio GF, Selleri S, Bedoni M, Balsari A, Rumio C. Cross-talk among Toll-like receptors and their ligands. *Int Immunol* 2008; **20**: 709-718 [PMID: 18397908 DOI: 10.1093/intimm/dxn027]
  - 57 **Re F**, Strominger JL. Heterogeneity of TLR-induced responses in dendritic cells: from innate to adaptive immunity. *Immunobiology*

- 2004; **209**: 191-198 [PMID: 15481153 DOI: 10.1016/j.imbio.2004.03.005]
- 58 **Chen R**, Alvero AB, Silasi DA, Mor G. Inflammation, cancer and chemoresistance: taking advantage of the toll-like receptor signaling pathway. *Am J Reprod Immunol* 2007; **57**: 93-107 [PMID: 17217363 DOI: 10.1111/j.1600-0897.2006.00441.x]
- 59 **Zarembek KA**, Godowski PJ. Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. *J Immunol* 2002; **168**: 554-561 [PMID: 11777946 DOI: 10.4049/jimmunol.168.2.554]
- 60 **Matsumura T**, Degawa T, Takii T, Hayashi H, Okamoto T, Inoue J, Onozaki K. TRAF6-NF-kappaB pathway is essential for interleukin-1-induced TLR2 expression and its functional response to TLR2 ligand in murine hepatocytes. *Immunology* 2003; **109**: 127-136 [PMID: 12709026 DOI: 10.1046/j.1365-2567.2003.01627.x]
- 61 **Matsumura T**, Ito A, Takii T, Hayashi H, Onozaki K. Endotoxin and cytokine regulation of toll-like receptor (TLR) 2 and TLR4 gene expression in murine liver and hepatocytes. *J Interferon Cytokine Res* 2000; **20**: 915-921 [PMID: 11054280 DOI: 10.1089/10799900050163299]
- 62 **Bilzer M**, Roggel F, Gerbes AL. Role of Kupffer cells in host defense and liver disease. *Liver Int* 2006; **26**: 1175-1186 [PMID: 17105582 DOI: 10.1111/j.1478-3231.2006.01342.x]
- 63 **Thobe BM**, Frink M, Hildebrand F, Schwacha MG, Hubbard WJ, Choudhry MA, Chaudry IH. The role of MAPK in Kupffer cell toll-like receptor (TLR) 2-, TLR4-, and TLR9-mediated signaling following trauma-hemorrhage. *J Cell Physiol* 2007; **210**: 667-675 [PMID: 17117477 DOI: 10.1002/jcp.20860]
- 64 **Knolle P**, Schlaak J, Uhrig A, Kempf P, Meyer zum Büschenfelde KH, Gerken G. Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. *J Hepatol* 1995; **22**: 226-229 [PMID: 7790711 DOI: 10.1016/0168-8278(95)80433-1]
- 65 **Su GL**, Klein RD, Aminlari A, Zhang HY, Steinstraesser L, Alarcon WH, Remick DG, Wang SC. Kupffer cell activation by lipopolysaccharide in rats: role for lipopolysaccharide binding protein and toll-like receptor 4. *Hepatology* 2000; **31**: 932-936 [PMID: 10733550 DOI: 10.1053/he.2000.5634]
- 66 **Wu J**, Lu M, Meng Z, Trippier M, Broering R, Szczeponek A, Krux F, Dittmer U, Roggendorf M, Gerken G, Schlaak JF. Toll-like receptor-mediated control of HBV replication by nonparenchymal liver cells in mice. *Hepatology* 2007; **46**: 1769-1778 [PMID: 17929296 DOI: 10.1002/hep.21897]
- 67 **Battaller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI24282]
- 68 **Brun P**, Castagliuolo I, Pinzani M, Palù G, Martinez D. Exposure to bacterial cell wall products triggers an inflammatory phenotype in hepatic stellate cells. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G571-G578 [PMID: 15860640 DOI: 10.1152/ajpgi.00537.2004]
- 69 **Gäbele E**, Mühlbauer M, Dorn C, Weiss TS, Froh M, Schnabl B, Wiest R, Schölmerich J, Obermeier F, Hellerbrand C. Role of TLR9 in hepatic stellate cells and experimental liver fibrosis. *Biochem Biophys Res Commun* 2008; **376**: 271-276 [PMID: 18760996 DOI: 10.1016/j.bbrc.2008.08.096]
- 70 **Paik YH**, Schwabe RF, Battaller R, Russo MP, Jobin C, Brenner DA. Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells. *Hepatology* 2003; **37**: 1043-1055 [PMID: 12717385 DOI: 10.1053/jhep.2003.50182]
- 71 **Seki E**, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007; **13**: 1324-1332 [PMID: 17952090 DOI: 10.1038/nm1663]
- 72 **Guo J**, Friedman SL. Toll-like receptor 4 signaling in liver injury and hepatic fibrogenesis. *Fibrogenesis Tissue Repair* 2010; **3**: 21 [PMID: 20964825 DOI: 10.1186/1755-1536-3-21]
- 73 **Watanabe A**, Hashmi A, Gomes DA, Town T, Badou A, Flavell RA, Mehal WZ. Apoptotic hepatocyte DNA inhibits hepatic stellate cell chemotaxis via toll-like receptor 9. *Hepatology* 2007; **46**: 1509-1518 [PMID: 17705260 DOI: 10.1002/hep.21867]
- 74 **Harada K**, Ohira S, Isse K, Ozaki S, Zen Y, Sato Y, Nakanuma Y. Lipopolysaccharide activates nuclear factor-kappaB through toll-like receptors and related molecules in cultured biliary epithelial cells. *Lab Invest* 2003; **83**: 1657-1667 [PMID: 14615419]
- 75 **Harada K**, Isse K, Nakanuma Y. Interferon gamma accelerates NF-kappaB activation of biliary epithelial cells induced by Toll-like receptor and ligand interaction. *J Clin Pathol* 2006; **59**: 184-190 [PMID: 16443736 DOI: 10.1136/jcp.2004.023507]
- 76 **Harada K**, Isse K, Sato Y, Ozaki S, Nakanuma Y. Endotoxin tolerance in human intrahepatic biliary epithelial cells is induced by upregulation of IRAK-M. *Liver Int* 2006; **26**: 935-942 [PMID: 16953833 DOI: 10.1111/j.1478-3231.2006.01325.x]
- 77 **Martin-Armas M**, Simon-Santamaria J, Pettersen I, Moens U, Smedsrød B, Sveinbjørnsson B. Toll-like receptor 9 (TLR9) is present in murine liver sinusoidal endothelial cells (LSECs) and mediates the effect of CpG-oligonucleotides. *J Hepatol* 2006; **44**: 939-946 [PMID: 16458386 DOI: 10.1016/j.jhep.2005.09.020]
- 78 **Uhrig A**, Banafsche R, Kremer M, Hegenbarth S, Hamann A, Neurath M, Gerken G, Limmmer A, Knolle PA. Development and functional consequences of LPS tolerance in sinusoidal endothelial cells of the liver. *J Leukoc Biol* 2005; **77**: 626-633 [PMID: 15860798 DOI: 10.1189/jlb.0604332]
- 79 **Lohse AW**, Knolle PA, Bilo K, Uhrig A, Waldmann C, Ibe M, Schmitt E, Gerken G, Meyer Zum Büschenfelde KH. Antigen-presenting function and B7 expression of murine sinusoidal endothelial cells and Kupffer cells. *Gastroenterology* 1996; **110**: 1175-1181 [PMID: 8613007 DOI: 10.1053/gast.1996.v110.pm8613007]
- 80 **Van Bossuyt H**, De Zanger RB, Wisse E. Cellular and subcellular distribution of injected lipopolysaccharide in rat liver and its inactivation by bile salts. *J Hepatol* 1988; **7**: 325-337 [PMID: 3235801 DOI: 10.1016/S0168-8278(88)80005-9]
- 81 **Mimura Y**, Sakisaka S, Harada M, Sata M, Tanikawa K. Role of hepatocytes in direct clearance of lipopolysaccharide in rats. *Gastroenterology* 1995; **109**: 1969-1976 [PMID: 7498663 DOI: 10.1016/0016-5085(95)90765-3]
- 82 **Hsu W**, Shu SA, Gershwin E, Lian ZX. The current immune function of hepatic dendritic cells. *Cell Mol Immunol* 2007; **4**: 321-328 [PMID: 17976311]
- 83 **Crispe IN**. The liver as a lymphoid organ. *Annu Rev Immunol* 2009; **27**: 147-163 [PMID: 19302037 DOI: 10.1146/annurev.immunol.021908.132629]
- 84 **Edwards AD**, Diebold SS, Slack EM, Tomizawa H, Hemmi H, Kaisho T, Akira S, Reis e Sousa C. Toll-like receptor expression in murine DC subsets: lack of TLR7 expression by CD8 alpha+ DC correlates with unresponsiveness to imidazoquinolines. *Eur J Immunol* 2003; **33**: 827-833 [PMID: 12672047 DOI: 10.1002/eji.200323797]
- 85 **Shu SA**, Lian ZX, Chuang YH, Yang GX, Moritoki Y, Comstock SS, Zhong RQ, Ansari AA, Liu YJ, Gershwin ME. The role of CD11c(+) hepatic dendritic cells in the induction of innate immune responses. *Clin Exp Immunol* 2007; **149**: 335-343 [PMID: 17521321 DOI: 10.1111/j.1365-2249.2007.03419.x]
- 86 **Soares JB**, Pimentel-Nunes P, Afonso L, Rolanda C, Lopes P, Roncon-Albuquerque R, Gonçalves N, Boal-Carvalho I, Pardal F, Lopes S, Macedo G, Lara-Santos L, Henrique R, Moreira-Dias L, Gonçalves R, Dinis-Ribeiro M, Leite-Moreira AF. Increased hepatic expression of TLR2 and TLR4 in the hepatic inflammation-fibrosis-carcinoma sequence. *Innate Immun* 2012; **18**: 700-708 [PMID: 22330637]
- 87 **Yin S**, Gao B. Toll-like receptor 3 in liver diseases. *Gastroenterol Res Pract* 2010; **2010** [PMID: 20936107 DOI: 10.1155/2010/750904]
- 88 **Bedogni G**, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 2005; **42**: 44-52 [PMID: 15895401 DOI: 10.1002/hep.20734]
- 89 **Younossi ZM**, Diehl AM, Ong JP. Nonalcoholic fatty liver disease: an agenda for clinical research. *Hepatology* 2002; **35**: 746-752

- [PMID: 11915019 DOI: 10.1053/jhep.2002.32483]
- 90 **Vaikunthanathan T**, Safinia N, Lombardi G, Lechler RI. Microbiota, immunity and the liver. *Immunol Lett* 2016; **171**: 36-49 [PMID: 26835593 DOI: 10.1016/j.imlet.2016.01.008]
  - 91 **Rivera CA**, Adegboyega P, van Rooijen N, Tagalicud A, Allman M, Wallace M. Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. *J Hepatol* 2007; **47**: 571-579 [PMID: 17644211 DOI: 10.1016/j.jhep.2007.04.019]
  - 92 **Harte AL**, da Silva NF, Creely SJ, McGee KC, Billyard T, Youssef-Elabd EM, Tripathi G, Ashour E, Abdalla MS, Sharada HM, Amin AI, Burt AD, Kumar S, Day CP, McTernan PG. Elevated endotoxin levels in non-alcoholic fatty liver disease. *J Inflamm (Lond)* 2010; **7**: 15 [PMID: 20353583 DOI: 10.1186/1476-9255-7-15]
  - 93 **Thuy S**, Ladurner R, Volynets V, Wagner S, Strahl S, Königsrainer A, Maier KP, Bischoff SC, Bergheim I. Nonalcoholic fatty liver disease in humans is associated with increased plasma endotoxin and plasminogen activator inhibitor 1 concentrations and with fructose intake. *J Nutr* 2008; **138**: 1452-1455 [PMID: 18641190]
  - 94 **Brun P**, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palù G, Martinez D. Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G518-G525 [PMID: 17023554 DOI: 10.1152/ajpgi.00024.2006]
  - 95 **Li Z**, Yang S, Lin H, Huang J, Watkins PA, Moser AB, Desimone C, Song XY, Diehl AM. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* 2003; **37**: 343-350 [PMID: 12540784 DOI: 10.1053/jhep.2003.50048]
  - 96 **Farhadi A**, Gundlapalli S, Shaikh M, Frantzides C, Harrell L, Kwasny MM, Keshavarzian A. Susceptibility to gut leakiness: a possible mechanism for endotoxaemia in non-alcoholic steatohepatitis. *Liver Int* 2008; **28**: 1026-1033 [PMID: 18397235 DOI: 10.1111/j.1478-3231.2008.01723.x]
  - 97 **Cani PD**, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007; **56**: 1761-1772 [PMID: 17456850 DOI: 10.2337/db06-1491]
  - 98 **Miura K**, Ohnishi H. Role of gut microbiota and Toll-like receptors in nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 7381-7391 [PMID: 24966608 DOI: 10.3748/wjg.v20.i23.7381]
  - 99 **Li D**, Wang X, Lan X, Li Y, Liu L, Yi J, Li J, Sun Q, Wang Y, Li H, Zhong N, Holmdahl R, Lu S. Down-regulation of miR-144 elicits proinflammatory cytokine production by targeting toll-like receptor 2 in nonalcoholic steatohepatitis of high-fat-diet-induced metabolic syndrome E3 rats. *Mol Cell Endocrinol* 2015; **402**: 1-12 [PMID: 25534427 DOI: 10.1016/j.mce.2014.12.007]
  - 100 **Miura K**, Seki E, Ohnishi H, Brenner DA. Role of toll-like receptors and their downstream molecules in the development of nonalcoholic Fatty liver disease. *Gastroenterol Res Pract* 2010; **2010**: 362847 [PMID: 21274430 DOI: 10.1155/2010/362847]
  - 101 **Wigg AJ**, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2001; **48**: 206-211 [PMID: 11156641 DOI: 10.1136/gut.48.2.206]
  - 102 **Miele L**, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009; **49**: 1877-1887 [PMID: 19291785 DOI: 10.1002/hep.22848]
  - 103 **Federico A**, Dallio M, Godos J, Loguercio C, Salomone F. Targeting gut-liver axis for the treatment of nonalcoholic steatohepatitis: translational and clinical evidence. *Transl Res* 2016; **167**: 116-124 [PMID: 26318867 DOI: 10.1016/j.trsl.2015.08.002]
  - 104 **Miura K**, Kodama Y, Inokuchi S, Schnabl B, Aoyama T, Ohnishi H, Olefsky JM, Brenner DA, Seki E. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. *Gastroenterology* 2010; **139**: 323-324.e7 [PMID: 20347818 DOI: 10.1053/j.gastro.2010.03.052]
  - 105 **Ye D**, Li FY, Lam KS, Li H, Jia W, Wang Y, Man K, Lo CM, Li X, Xu A. Toll-like receptor-4 mediates obesity-induced non-alcoholic steatohepatitis through activation of X-box binding protein-1 in mice. *Gut* 2012; **61**: 1058-1067 [PMID: 22253482 DOI: 10.1136/gutjnl-2011-300269]
  - 106 **Miura K**, Yang L, van Rooijen N, Brenner DA, Ohnishi H, Seki E. Toll-like receptor 2 and palmitic acid cooperatively contribute to the development of nonalcoholic steatohepatitis through inflammasome activation in mice. *Hepatology* 2013; **57**: 577-589 [PMID: 22987396 DOI: 10.1002/hep.26081]
  - 107 **Broering R**, Lu M, Schlaak JF. Role of Toll-like receptors in liver health and disease. *Clin Sci (Lond)* 2011; **121**: 415-426 [PMID: 21797822 DOI: 10.1042/CS20110065]
  - 108 **Rao R**. Endotoxemia and gut barrier dysfunction in alcoholic liver disease. *Hepatology* 2009; **50**: 638-644 [PMID: 19575462 DOI: 10.1002/hep.23009]
  - 109 **Bjarnason I**, Peters TJ, Wise RJ. The leaky gut of alcoholism: possible route of entry for toxic compounds. *Lancet* 1984; **1**: 179-182 [PMID: 6141332]
  - 110 **Petrasek J**, Mandrekar P, Szabo G. Toll-like receptors in the pathogenesis of alcoholic liver disease. *Gastroenterol Res Pract* 2010; **2010**: 20827314 DOI: 10.1155/2010/710381]
  - 111 **Testro AG**, Visvanathan K. Toll-like receptors and their role in gastrointestinal disease. *J Gastroenterol Hepatol* 2009; **24**: 943-954 [PMID: 19638078 DOI: 10.1111/j.1440-1746.2009.05854.x]
  - 112 **Machida K**. TLRs, Alcohol, HCV, and Tumorigenesis. *Gastroenterol Res Pract* 2010; **2010**: 518674 [PMID: 21331379 DOI: 10.1155/2010/518674]
  - 113 **Gao B**, Seki E, Brenner DA, Friedman S, Cohen JI, Nagy L, Szabo G, Zakhari S. Innate immunity in alcoholic liver disease. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G516-G525 [PMID: 21252049 DOI: 10.1152/ajpgi.00537.2010]
  - 114 **Hritz I**, Mandrekar P, Velayudham A, Catalano D, Dolganiuc A, Kodys K, Kurt-Jones E, Szabo G. The critical role of toll-like receptor (TLR) 4 in alcoholic liver disease is independent of the common TLR adapter MyD88. *Hepatology* 2008; **48**: 1224-1231 [PMID: 18792393 DOI: 10.1002/hep.22470]
  - 115 **Gustot T**, Lemmers A, Moreno C, Nagy N, Quertinmont E, Nicaise C, Franchimont D, Louis H, Devière J, Le Moine O. Differential liver sensitization to toll-like receptor pathways in mice with alcoholic fatty liver. *Hepatology* 2006; **43**: 989-1000 [PMID: 16628628 DOI: 10.1002/hep.21138]
  - 116 **Inokuchi S**, Tsukamoto H, Park E, Liu ZX, Brenner DA, Seki E. Toll-like receptor 4 mediates alcohol-induced steatohepatitis through bone marrow-derived and endogenous liver cells in mice. *Alcohol Clin Exp Res* 2011; **35**: 1509-1518 [PMID: 21463341 DOI: 10.1111/j.1530-0277.2011.01487.x]
  - 117 **Romics L**, Dolganiuc A, Kodys K, Drechsler Y, Oak S, Velayudham A, Mandrekar P, Szabo G. Selective priming to Toll-like receptor 4 (TLR4), not TLR2, ligands by P. acnes involves up-regulation of MD-2 in mice. *Hepatology* 2004; **40**: 555-564 [PMID: 15349893 DOI: 10.1002/hep.20350]
  - 118 **Hernandez-Gea V**, Friedman SL. Pathogenesis of liver fibrosis. *Annu Rev Pathol* 2011; **6**: 425-456 [PMID: 21073339 DOI: 10.1146/annurev-pathol-011110-130246]
  - 119 **Seki E**, Tsutsui H, Nakano H, Tsuji N, Hoshino K, Adachi O, Adachi K, Futatsugi S, Kuida K, Takeuchi O, Okamura H, Fujimoto J, Akira S, Nakanishi K. Lipopolysaccharide-induced IL-18 secretion from murine Kupffer cells independently of myeloid differentiation factor 88 that is critically involved in induction of production of IL-12 and IL-1beta. *J Immunol* 2001; **166**: 2651-2657 [PMID: 11160328 DOI: 10.4049/jimmunol.166.4.2651]
  - 120 **Forbes SJ**, Parola M. Liver fibrogenic cells. *Best Pract Res Clin Gastroenterol* 2011; **25**: 207-217 [PMID: 21497739 DOI: 10.1016/j.bpg.2011.02.006]



- 121 **Isayama F**, Hines IN, Kremer M, Milton RJ, Byrd CL, Perry AW, McKim SE, Parsons C, Rippe RA, Wheeler MD. LPS signaling enhances hepatic fibrogenesis caused by experimental cholestasis in mice. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G1318-G1328 [PMID: 16439470 DOI: 10.1152/ajpgi.00405.2005]
- 122 **Jeong WI**, Park O, Gao B. Abrogation of the antifibrotic effects of natural killer cells/interferon-gamma contributes to alcohol acceleration of liver fibrosis. *Gastroenterology* 2008; **134**: 248-258 [PMID: 18166357 DOI: 10.1053/j.gastro.2007.09.034]
- 123 **Aoyama T**, Paik YH, Seki E. Toll-like receptor signaling and liver fibrosis. *Gastroenterol Res Pract* 2010; Epub 2010 Jul 25 [PMID: 20706677 DOI: 10.1155/2010/192543]
- 124 **Ji L**, Xue R, Tang W, Wu W, Hu T, Liu X, Peng X, Gu J, Chen S, Zhang S. Toll like receptor 2 knock-out attenuates carbon tetrachloride (CCl<sub>4</sub>)-induced liver fibrosis by downregulating MAPK and NF- $\kappa$ B signaling pathways. *FEBS Lett* 2014; **588**: 2095-2100 [PMID: 24815695 DOI: 10.1016/j.febslet.2014.04.042]
- 125 **Lee WM**. Hepatitis B virus infection. *N Engl J Med* 1997; **337**: 1733-1745 [PMID: 9392700 DOI: 10.1056/NEJM199712113372406]
- 126 **McQuillan GM**, Coleman PJ, Kruszon-Moran D, Moyer LA, Lambert SB, Margolis HS. Prevalence of hepatitis B virus infection in the United States: the National Health and Nutrition Examination Surveys, 1976 through 1994. *Am J Public Health* 1999; **89**: 14-18 [PMID: 9987458]
- 127 **Keskinen P**, Nyqvist M, Sarenneva T, Pirhonen J, Melén K, Julkunen I. Impaired antiviral response in human hepatoma cells. *Virology* 1999; **263**: 364-375 [PMID: 10544109 DOI: 10.1006/viro.1999.9983]
- 128 **McClary H**, Koch R, Chisari FV, Guidotti LG. Relative sensitivity of hepatitis B virus and other hepatotropic viruses to the antiviral effects of cytokines. *J Virol* 2000; **74**: 2255-2264 [PMID: 10666256]
- 129 **Isogawa M**, Robek MD, Furuichi Y, Chisari FV. Toll-like receptor signaling inhibits hepatitis B virus replication in vivo. *J Virol* 2005; **79**: 7269-7272 [PMID: 15890966 DOI: 10.1128/JVI.79.11.7269-7272.2005]
- 130 **Xia C**, Lu M, Zhang Z, Meng Z, Zhang Z, Shi C. TLRs antiviral effect on hepatitis B virus in HepG2 cells. *J Appl Microbiol* 2008; **105**: 1720-1727 [PMID: 19149768 DOI: 10.1111/j.1365-2672.2008.03896.x]
- 131 **Dalpke AH**, Lehner MD, Hartung T, Heeg K. Differential effects of CpG-DNA in Toll-like receptor-2/-4/-9 tolerance and cross-tolerance. *Immunology* 2005; **116**: 203-212 [PMID: 16162269 DOI: 10.1111/j.1365-2567.2005.02211.x]
- 132 **Chen Z**, Cheng Y, Xu Y, Liao J, Zhang X, Hu Y, Zhang Q, Wang J, Zhang Z, Shen F, Yuan Z. Expression profiles and function of Toll-like receptors 2 and 4 in peripheral blood mononuclear cells of chronic hepatitis B patients. *Clin Immunol* 2008; **128**: 400-408 [PMID: 18565796 DOI: 10.1016/j.clim.2008.04.006]
- 133 **Visvanathan K**, Skinner NA, Thompson AJ, Riordan SM, Sozzi V, Edwards R, Rodgers S, Kurtovic J, Chang J, Lewin S, Desmond P, Locarnini S. Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. *Hepatology* 2007; **45**: 102-110 [PMID: 17187404 DOI: 10.1002/hep.21482]
- 134 **Wu J**, Meng Z, Jiang M, Pei R, Trippler M, Broering R, Bucchi A, Sowa JP, Dittmer U, Yang D, Roggendorf M, Gerken G, Lu M, Schlaak JF. Hepatitis B virus suppresses toll-like receptor-mediated innate immune responses in murine parenchymal and nonparenchymal liver cells. *Hepatology* 2009; **49**: 1132-1140 [PMID: 19140219 DOI: 10.1002/hep.22751]
- 135 **Wieland S**, Thimme R, Purcell RH, Chisari FV. Genomic analysis of the host response to hepatitis B virus infection. *Proc Natl Acad Sci USA* 2004; **101**: 6669-6674 [PMID: 15100412 DOI: 10.1073/pnas.0401771101]
- 136 **Hösel M**, Quasdorff M, Wiegmann K, Webb D, Zedler U, Broxtermann M, Tedjokusumo R, Esser K, Arzberger S, Kirschning CJ, Langenkamp A, Falk C, Büning H, Rose-John S, Protzer U. Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection. *Hepatology* 2009; **50**: 1773-1782 [PMID: 19937696 DOI: 10.1002/hep.23226]
- 137 **Besinger R**. [Postpartum hemorrhage: perspectives in the United States]. *J Gynecol Obstet Biol Reprod (Paris)* 1997; **26**: 34-38 [PMID: 9410928]
- 138 **Di Bisceglie AM**. Hepatitis C. *Lancet* 1998; **351**: 351-355 [PMID: 9652633 DOI: 10.1016/S0140-6736(97)07361-3]
- 139 **Ishii S**, Koziel MJ. Immune responses during acute and chronic infection with hepatitis C virus. *Clin Immunol* 2008; **128**: 133-147 [PMID: 18514579 DOI: 10.1016/j.clim.2008.03.525]
- 140 **Rehermann B**, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* 2005; **5**: 215-229 [PMID: 15738952 DOI: 10.1038/nri1573]
- 141 **Broering R**, Wu J, Meng Z, Hilgard P, Lu M, Trippler M, Szczeponek A, Gerken G, Schlaak JF. Toll-like receptor-stimulated non-parenchymal liver cells can regulate hepatitis C virus replication. *J Hepatol* 2008; **48**: 914-922 [PMID: 18362039 DOI: 10.1016/j.jhep.2008.01.028]
- 142 **Chang S**, Dolganiuc A, Szabo G. Toll-like receptors 1 and 6 are involved in TLR2-mediated macrophage activation by hepatitis C virus core and NS3 proteins. *J Leukoc Biol* 2007; **82**: 479-487 [PMID: 17595379 DOI: 10.1189/jlb.0207128]
- 143 **Dolganiuc A**, Oak S, Kodys K, Golenbock DT, Finberg RW, Kurt-Jones E, Szabo G. Hepatitis C core and nonstructural 3 proteins trigger toll-like receptor 2-mediated pathways and inflammatory activation. *Gastroenterology* 2004; **127**: 1513-1524 [PMID: 15521019 DOI: 10.1053/j.gastro.2004.08.067]
- 144 **Düesberg U**, von dem Bussche A, Kirschning C, Miyake K, Sauerbruch T, Spengler U. Cell activation by synthetic lipopeptides of the hepatitis C virus (HCV)-core protein is mediated by toll like receptors (TLRs) 2 and 4. *Immunol Lett* 2002; **84**: 89-95 [PMID: 12270544 DOI: 10.1016/S0165-2478(02)00178-5]
- 145 **Machida K**, Cheng KT, Sung VM, Levine AM, Fong S, Lai MM. Hepatitis C virus induces toll-like receptor 4 expression, leading to enhanced production of beta interferon and interleukin-6. *J Virol* 2006; **80**: 866-874 [PMID: 16378988 DOI: 10.1128/JVI.80.2.866-874.2006]
- 146 **Chang S**, Kodys K, Szabo G. Impaired expression and function of toll-like receptor 7 in hepatitis C virus infection in human hepatoma cells. *Hepatology* 2010; **51**: 35-42 [PMID: 19821521 DOI: 10.1002/hep.23256]
- 147 **Dolganiuc A**, Norkina O, Kodys K, Catalano D, Bakis G, Marshall C, Mandrekar P, Szabo G. Viral and host factors induce macrophage activation and loss of toll-like receptor tolerance in chronic HCV infection. *Gastroenterology* 2007; **133**: 1627-1636 [PMID: 17916356 DOI: 10.1053/j.gastro.2007.08.003]
- 148 **Naugler WE**, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 2007; **317**: 121-124 [PMID: 17615358 DOI: 10.1126/science.1140485]
- 149 **Dapito DH**, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, Caviglia JM, Khiabani H, Adeyemi A, Bataller R, Lefkowitz JH, Bower M, Friedman R, Sartor RB, Rabadan R, Schwabe RF. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 2012; **21**: 504-516 [PMID: 22516259 DOI: 10.1016/j.ccr.2012.02.007]
- 150 **Yu LX**, Yan HX, Liu Q, Yang W, Wu HP, Dong W, Tang L, Lin Y, He YQ, Zou SS, Wang C, Zhang HL, Cao GW, Wu MC, Wang HY. Endotoxin accumulation prevents carcinogen-induced apoptosis and promotes liver tumorigenesis in rodents. *Hepatology* 2010; **52**: 1322-1333 [PMID: 20803560 DOI: 10.1002/hep.23845]
- 151 **Maeda S**, Omata M. Inflammation and cancer: role of nuclear factor-kappaB activation. *Cancer Sci* 2008; **99**: 836-842 [PMID: 18294278 DOI: 10.1111/j.1349-7006.2008.00763.x]
- 152 **Hu KQ**. Rationale and feasibility of chemoprevention of hepatocellular carcinoma by cyclooxygenase-2 inhibitors. *J Lab Clin Med* 2002; **139**: 234-243 [PMID: 12024111 DOI: 10.1067/mlc.2002.122281]
- 153 **Karin M**. Nuclear factor-kappaB in cancer development and progression. *Nature* 2006; **441**: 431-436 [PMID: 16724054 DOI: 10.1038/nature04870]



- 154 **Prieto J.** Inflammation, HCC and sex: IL-6 in the centre of the triangle. *J Hepatol* 2008; **48**: 380-381 [PMID: 18093689 DOI: 10.1016/j.jhep.2007.11.007]
- 155 **Sakurai T**, He G, Matsuzawa A, Yu GY, Maeda S, Hardiman G, Karin M. Hepatocyte necrosis induced by oxidative stress and IL-1 alpha release mediate carcinogen-induced compensatory proliferation and liver tumorigenesis. *Cancer Cell* 2008; **14**: 156-165 [PMID: 18691550 DOI: 10.1016/j.ccr.2008.06.016]
- 156 **Liu X**, Xu Q, Chen W, Cao H, Zheng R, Li G. Hepatitis B virus DNA-induced carcinogenesis of human normal liver cells by virtue of nonmethylated CpG DNA. *Oncol Rep* 2009; **21**: 941-947 [PMID: 19287992 DOI: 10.3892/or.00000307]
- 157 **Yoneda K**, Sugimoto K, Shiraki K, Tanaka J, Beppu T, Fuke H, Yamamoto N, Masuya M, Horie R, Uchida K, Takei Y. Dual topology of functional Toll-like receptor 3 expression in human hepatocellular carcinoma: differential signaling mechanisms of TLR3-induced NF-kappaB activation and apoptosis. *Int J Oncol* 2008; **33**: 929-936 [PMID: 18949355 DOI: 10.3892/ijo.00000080]
- 158 **Lopes JA**, Borges-Canha M, Pimentel-Nunes P. Innate immunity and hepatocarcinoma: Can toll-like receptors open the door to oncogenesis? *World J Hepatol* 2016; **8**: 162-182 [PMID: 26839640 DOI: 10.4254/wjh.v8.i3.162]
- 159 **Inokuchi S**, Aoyama T, Miura K, Osterreicher CH, Kodama Y, Miyai K, Akira S, Brenner DA, Seki E. Disruption of TAK1 in hepatocytes causes hepatic injury, inflammation, fibrosis, and carcinogenesis. *Proc Natl Acad Sci USA* 2010; **107**: 844-849 [PMID: 20080763 DOI: 10.1073/pnas.0909781107]
- 160 **Machida K**, Tsukamoto H, Mkrtchyan H, Duan L, Dynnyk A, Liu HM, Asahina K, Govindarajan S, Ray R, Ou JH, Seki E, Deshaies R, Miyake K, Lai MM. Toll-like receptor 4 mediates synergism between alcohol and HCV in hepatic oncogenesis involving stem cell marker Nanog. *Proc Natl Acad Sci USA* 2009; **106**: 1548-1553 [PMID: 19171902 DOI: 10.1073/pnas.0807390106]
- 161 **Zhai Y**, Shen XD, O'Connell R, Gao F, Lassman C, Busuttil RW, Cheng G, Kupiec-Weglinski JW. Cutting edge: TLR4 activation mediates liver ischemia/reperfusion inflammatory response via IFN regulatory factor 3-dependent MyD88-independent pathway. *J Immunol* 2004; **173**: 7115-7119 [PMID: 15585830 DOI: 10.4049/jimmunol.173.12.7115]
- 162 **Tsung A**, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DA, Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. *J Exp Med* 2005; **201**: 1135-1143 [PMID: 15795240 DOI: 10.1084/jem.20042614]
- 163 **Chang WJ**, Toledo-Pereyra LH. Toll-like receptor signaling in liver ischemia and reperfusion. *J Invest Surg* 2012; **25**: 271-277 [PMID: 22853814 DOI: 10.3109/08941939.2012.687802]
- 164 **Evankovich J**, Billiar T, Tsung A. Toll-like receptors in hepatic ischemia/reperfusion and transplantation. *Gastroenterol Res Pract* 2010; Epub 2010 Aug 5 [PMID: 20811615 DOI: 10.1155/2010/537263]
- 165 **Shen XD**, Ke B, Zhai Y, Gao F, Tsuchihashi S, Lassman CR, Busuttil RW, Kupiec-Weglinski JW. Absence of toll-like receptor 4 (TLR4) signaling in the donor organ reduces ischemia and reperfusion injury in a murine liver transplantation model. *Liver Transpl* 2007; **13**: 1435-1443 [PMID: 17902130 DOI: 10.1002/lt.21251]
- 166 **Wang H**, Li ZY, Wu HS, Wang Y, Jiang CF, Zheng QC, Zhang JX. Endogenous danger signals trigger hepatic ischemia/reperfusion injury through toll-like receptor 4/nuclear factor-kappa B pathway. *Chin Med J (Engl)* 2007; **120**: 509-514 [PMID: 17439747]
- 167 **Zhai Y**, Qiao B, Shen XD, Gao F, Busuttil RW, Cheng G, Platt JL, Volk HD, Kupiec-Weglinski JW. Evidence for the pivotal role of endogenous toll-like receptor 4 ligands in liver ischemia and reperfusion injury. *Transplantation* 2008; **85**: 1016-1022 [PMID: 18408583 DOI: 10.1097/TP.0b013e3181684248]
- 168 **King LA**, Toledo AH, Rivera-Chavez FA, Toledo-Pereyra LH. Role of p38 and JNK in liver ischemia and reperfusion. *J Hepatobiliary Pancreat Surg* 2009; **16**: 763-770 [PMID: 19680593 DOI: 10.1007/s00534-009-0155-x]
- 169 **Tsung A**, Hoffman RA, Izuishi K, Critchlow ND, Nakao A, Chan MH, Lotze MT, Geller DA, Billiar TR. Hepatic ischemia/reperfusion injury involves functional TLR4 signaling in nonparenchymal cells. *J Immunol* 2005; **175**: 7661-7668 [PMID: 16301676 DOI: 10.4049/jimmunol.175.11.7661]
- 170 **Liu S**, Salyapongse AN, Geller DA, Vodovotz Y, Billiar TR. Hepatocyte toll-like receptor 2 expression in vivo and in vitro: role of cytokines in induction of rat TLR2 gene expression by lipopolysaccharide. *Shock* 2000; **14**: 361-365 [PMID: 11028557]
- 171 **Negishi H**, Fujita Y, Yanai H, Sakaguchi S, Ouyang X, Shinohara M, Takayanagi H, Ohba Y, Taniguchi T, Honda K. Evidence for licensing of IFN-gamma-induced IFN regulatory factor 1 transcription factor by MyD88 in Toll-like receptor-dependent gene induction program. *Proc Natl Acad Sci USA* 2006; **103**: 15136-15141 [PMID: 17018642 DOI: 10.1073/pnas.0607181103]
- 172 **Ueki S**, Dhupar R, Cardinal J, Tsung A, Yoshida J, Ozaki KS, Klune JR, Murase N, Geller DA. Critical role of interferon regulatory factor-1 in murine liver transplant ischemia reperfusion injury. *Hepatology* 2010; **51**: 1692-1701 [PMID: 20131404 DOI: 10.1002/hep.23501]
- 173 **Zhang JX**, Wu HS, Wang H, Zhang JH, Wang Y, Zheng QC. Protection against hepatic ischemia/reperfusion injury via downregulation of toll-like receptor 2 expression by inhibition of Kupffer cell function. *World J Gastroenterol* 2005; **11**: 4423-4426 [PMID: 16038046 DOI: 10.3748/wjg.v11.i28.4423]
- 174 **Yao XM**, Chen H, Li Y. Protective effect of bicyclol on liver injury induced by hepatic warm ischemia/reperfusion in rats. *Hepatol Res* 2009; **39**: 833-842 [PMID: 19473433 DOI: 10.1111/j.1872-034X.2009.00504.x]
- 175 **Jin X**, Wang L, Wu HS, Zhang L, Wang CY, Tian Y, Zhang JH. N-acetylcysteine inhibits activation of toll-like receptor 2 and 4 gene expression in the liver and lung after partial hepatic ischemia-reperfusion injury in mice. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 284-289 [PMID: 17548252]
- 176 **Bamboot ZM**, Balachandran VP, Ocun LM, Obaid H, Plitas G, DeMatteo RP. Toll-like receptor 9 inhibition confers protection from liver ischemia-reperfusion injury. *Hepatology* 2010; **51**: 621-632 [PMID: 19902481 DOI: 10.1002/hep.23365]
- 177 **Yasuda K**, Yu P, Kirschning CJ, Schlatter B, Schmitz F, Heit A, Bauer S, Hochrein H, Wagner H. Endosomal translocation of vertebrate DNA activates dendritic cells via TLR9-dependent and -independent pathways. *J Immunol* 2005; **174**: 6129-6136 [PMID: 15879108 DOI: 10.4049/jimmunol.174.10.6129]
- 178 **Iimuro Y**, Fujimoto J. TLRs, NF-kB, JNK, and Liver Regeneration. *Gastroenterol Res Pract* 2010; Epub 2010 Sep 26 [PMID: 20936148 DOI: 10.1155/2010/598109]
- 179 **Kluwe J**, Pradere JP, Gwak GY, Mencin A, De Minicis S, Osterreicher CH, Colmenero J, Batailler R, Schwabe RF. Modulation of hepatic fibrosis by c-Jun-N-terminal kinase inhibition. *Gastroenterology* 2010; **138**: 347-359 [PMID: 19782079 DOI: 10.1053/j.gastro.2009.09.015]
- 180 **Seki E**, Tsutsui H, Iimuro Y, Naka T, Son G, Akira S, Kishimoto T, Nakanishi K, Fujimoto J. Contribution of Toll-like receptor/myeloid differentiation factor 88 signaling to murine liver regeneration. *Hepatology* 2005; **41**: 443-450 [PMID: 15723296 DOI: 10.1002/hep.20603]
- 181 **Campbell JS**, Riehle KJ, Brooling JT, Bauer RL, Mitchell C, Fausto N. Proinflammatory cytokine production in liver regeneration is Myd88-dependent, but independent of Cd14, Tlr2, and Tlr4. *J Immunol* 2006; **176**: 2522-2528 [PMID: 16456013 DOI: 10.4049/jimmunol.176.4.2522]
- 182 **Akita K**, Okuno M, Enya M, Imai S, Moriwaki H, Kawada N, Suzuki Y, Kojima S. Impaired liver regeneration in mice by lipopolysaccharide via TNF-alpha/kallikrein-mediated activation of latent TGF-beta. *Gastroenterology* 2002; **123**: 352-364 [PMID: 12105863 DOI: 10.1053/gast.2002.34234]
- 183 **Zorde-Khvaleyevsky E**, Abramovitch R, Barash H, Spivak-Pohis I, Rivkin L, Rachmilewitz J, Galun E, Giladi H. Toll-like receptor 3 signaling attenuates liver regeneration. *Hepatology* 2009; **50**: 198-206 [PMID: 19441101 DOI: 10.1002/hep.22973]
- 184 **Sun R**, Park O, Horiguchi N, Kulkarni S, Jeong WI, Sun HY,

- Radaeva S, Gao B. STAT1 contributes to dsRNA inhibition of liver regeneration after partial hepatectomy in mice. *Hepatology* 2006; **44**: 955-966 [PMID: 17006930 DOI: 10.1002/hep.21344]
- 185 **Washington MK**. Autoimmune liver disease: overlap and outliers. *Mod Pathol* 2007; **20** Suppl 1: S15-S30 [PMID: 17486048 DOI: 10.1038/modpathol.3800684]
- 186 **Lang KS**, Recher M, Junt T, Navarini AA, Harris NL, Freigang S, Odermatt B, Conrad C, Ittner LM, Bauer S, Luther SA, Uematsu S, Akira S, Hengartner H, Zinkernagel RM. Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. *Nat Med* 2005; **11**: 138-145 [PMID: 15654326 DOI: 10.1038/nm1176]
- 187 **Atarashi K**, Nishimura J, Shima T, Umesaki Y, Yamamoto M, Onoue M, Yagita H, Ishii N, Evans R, Honda K, Takeda K. ATP drives lamina propria T(H)17 cell differentiation. *Nature* 2008; **455**: 808-812 [PMID: 18716618 DOI: 10.1038/nature07240]
- 188 **Lebeis SL**, Powell KR, Merlin D, Sherman MA, Kalman D. Interleukin-1 receptor signaling protects mice from lethal intestinal damage caused by the attaching and effacing pathogen *Citrobacter rodentium*. *Infect Immun* 2009; **77**: 604-614 [PMID: 19075023 DOI: 10.1128/IAI.00907-08]
- 189 **Mao TK**, Lian ZX, Selmi C, Ichiki Y, Ashwood P, Ansari AA, Coppel RL, Shimoda S, Ishibashi H, Gershwin ME. Altered monocyte responses to defined TLR ligands in patients with primary biliary cirrhosis. *Hepatology* 2005; **42**: 802-808 [PMID: 16175622 DOI: 10.1002/hep.20859]
- 190 **Sasatomi K**, Noguchi K, Sakisaka S, Sata M, Tanikawa K. Abnormal accumulation of endotoxin in biliary epithelial cells in primary biliary cirrhosis and primary sclerosing cholangitis. *J Hepatol* 1998; **29**: 409-416 [PMID: 9764987 DOI: 10.1016/S0168-8278(98)80058-5]
- 191 **Ballot E**, Bandin O, Chazouilleres O, Johanet C, Poupon R. Immune response to lipopolysaccharide in primary biliary cirrhosis and autoimmune diseases. *J Autoimmun* 2004; **22**: 153-158 [PMID: 14987744 DOI: 10.1016/j.jaut.2003.11.002]
- 192 **Wang AP**, Migita K, Ito M, Takii Y, Daikoku M, Yokoyama T, Komori A, Nakamura M, Yatsuhashi H, Ishibashi H. Hepatic expression of toll-like receptor 4 in primary biliary cirrhosis. *J Autoimmun* 2005; **25**: 85-91 [PMID: 16006099 DOI: 10.1016/j.jaut.2005.05.003]
- 193 **Honda Y**, Yamagiwa S, Matsuda Y, Takamura M, Ichida T, Aoyagi Y. Altered expression of TLR homolog RP105 on monocytes hypersensitive to LPS in patients with primary biliary cirrhosis. *J Hepatol* 2007; **47**: 404-411 [PMID: 17448566 DOI: 10.1016/j.jhep.2007.03.012]
- 194 **Shimoda S**, Harada K, Niino H, Shirabe K, Taketomi A, Maehara Y, Tsuneyama K, Nakanuma Y, Leung P, Ansari AA, Gershwin ME, Akashi K. Interaction between Toll-like receptors and natural killer cells in the destruction of bile ducts in primary biliary cirrhosis. *Hepatology* 2011; **53**: 1270-1281 [PMID: 21400555 DOI: 10.1002/hep.24194]
- 195 **Shimoda S**, Harada K, Niino H, Yoshizumi T, Soejima Y, Taketomi A, Maehara Y, Tsuneyama K, Nakamura M, Komori A, Migita K, Nakanuma Y, Ishibashi H, Selmi C, Gershwin ME. Biliary epithelial cells and primary biliary cirrhosis: the role of liver-infiltrating mononuclear cells. *Hepatology* 2008; **47**: 958-965 [PMID: 18181218 DOI: 10.1002/hep.22102]
- 196 **Takii Y**, Nakamura M, Ito M, Yokoyama T, Komori A, Shimizu-Yoshida Y, Nakao R, Kusumoto K, Nagaoka S, Yano K, Abiru S, Ueki T, Matsumoto T, Daikoku M, Taniguchi K, Fujioka H, Migita K, Yatsuhashi H, Nakashima M, Harada M, Ishibashi H. Enhanced expression of type I interferon and toll-like receptor-3 in primary biliary cirrhosis. *Lab Invest* 2005; **85**: 908-920 [PMID: 15856047 DOI: 10.1038/labinvest.3700285]
- 197 **Kikuchi K**, Lian ZX, Yang GX, Ansari AA, Ikehara S, Kaplan M, Miyakawa H, Coppel RL, Gershwin ME. Bacterial CpG induces hyper-IgM production in CD27(+) memory B cells in primary biliary cirrhosis. *Gastroenterology* 2005; **128**: 304-312 [PMID: 15685542 DOI: 10.1053/j.gastro.2004.11.005]
- 198 **Kikuchi K**, Lian ZX, Kimura Y, Selmi C, Yang GX, Gordon SC, Invernizzi P, Podda M, Coppel RL, Ansari AA, Ikehara S, Miyakawa H, Gershwin ME. Genetic polymorphisms of toll-like receptor 9 influence the immune response to CpG and contribute to hyper-IgM in primary biliary cirrhosis. *J Autoimmun* 2005; **24**: 347-352 [PMID: 15878652 DOI: 10.1016/j.jaut.2005.03.002]
- 199 **Karrar A**, Broomé U, Södergren T, Jaksch M, Bergquist A, Björnstedt M, Sumitran-Holgersson S. Biliary epithelial cell antibodies link adaptive and innate immune responses in primary sclerosing cholangitis. *Gastroenterology* 2007; **132**: 1504-1514 [PMID: 17408653 DOI: 10.1053/j.gastro.2007.01.039]

**P- Reviewer:** Arias J, Balaban YH, Skrypnik IN **S- Editor:** Qiu S

**L- Editor:** A **E- Editor:** Li D



Basic Study

## Changes in cellular proliferation and plasma products are associated with liver failure

Juliana Gil Melgaço, Frederico Marianetti Soriani, Pedro Henrique Ferreira Sucupira, Leonardo Assaf Pinheiro, Yasmine Rangel Vieira, Jaqueline Mendes de Oliveira, Lia Laura Lewis-Ximenez, Cristina Carvalho Vianna Araújo, Lúcio Filgueiras Pacheco-Moreira, Gustavo Batista Menezes, Oswaldo Gonçalves Cruz, Claudia Lamarca Vitral, Marcelo Alves Pinto

Juliana Gil Melgaço, Leonardo Assaf Pinheiro, Claudia Lamarca Vitral, Departamento de Microbiologia e Parasitologia, Universidade Federal Fluminense, Niterói 20550-013, Brazil

Juliana Gil Melgaço, Yasmine Rangel Vieira, Jaqueline Mendes de Oliveira, Marcelo Alves Pinto, Instituto Oswaldo Cruz/Fiocruz, Laboratório de Desenvolvimento Tecnológico em Virologia, Rio de Janeiro 21040-900, Brazil

Frederico Marianetti Soriani, Pedro Henrique Ferreira Sucupira, Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte 31270-901, Brazil

Lia Laura Lewis-Ximenez, Instituto Oswaldo Cruz/Fiocruz, Ambulatório de Hepatites Virais, Rio de Janeiro 21040-900, Brazil

Cristina Carvalho Vianna Araújo, Lúcio Filgueiras Pacheco-Moreira, Hospital Federal de Bonsucesso, Rio de Janeiro 21041-030, Brazil

Gustavo Batista Menezes, Laboratório de Imunofarmacologia, Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte 31270-901, Brazil

Oswaldo Gonçalves Cruz, Fundação Oswaldo Cruz, Programa de Computação Científica, Rio de Janeiro 21040-900, Brazil

**Author contributions:** All authors have seen and approved the content of the manuscript and contributed meaningfully to the work. In summary, Melgaço JG conceived the study, performed the experiments, analyzed the data and wrote the manuscript; Pinheiro LA, Vieira YR, de Oliveira JM and Vitral CL acquired the data for cytokine quantification, serological data and the virological marker assay; de Oliveira JM reviewed the analysis and wrote the manuscript; Lewis-Ximenez LL collected the clinical data and the samples from the self-limited acute hepatitis patients and healthy subjects; Araújo CCV and Pacheco-Moreira

LF collected the clinical data and the samples from the acute liver failure patients; Soriani FM, Sucupira PHF and Menezes GB performed the DNA extraction and quantified the mitochondrial DNA using molecular biology assays; Cruz OG performed the statistical analysis; Vitral CL and Pinto MA participated in the study design and coordination; Pinto MA participated in the analysis of data and the preparation of the manuscript.

**Supported by** Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq, No. 308951/2010-7; and the Fundação de Amparo à Pesquisas no Rio de Janeiro - Faperj, No. E-26/110.848/2013.

**Institutional review board statement:** The study protocol was approved by the National Commission on Ethics in Research (CONEP) and by the institutional review board of the Oswaldo Cruz Foundation, FIOCRUZ (222/03).

**Conflict-of-interest statement:** The authors declare that they have no competing interests.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Juliana Gil Melgaço, PhD, Instituto Oswaldo Cruz/Fiocruz, Laboratório de Desenvolvimento Tecnológico em Virologia, Fundação Oswaldo Cruz/FIOCRUZ, Avenida Brasil, 4365, pavilhão Hélio e Peggy Pereira, Rio de

Janeiro 21040-900, Brazil. juliana.melgaco@gmail.com  
Telephone: +55-21-25621711  
Fax: +55-21-25621898

Received: May 23, 2016  
Peer-review started: May 25, 2016  
First decision: July 20, 2016  
Revised: August 3, 2016  
Accepted: September 13, 2016  
Article in press: September 18, 2016  
Published online: November 18, 2016

## Abstract

### AIM

To study the differences in immune response and cytokine profile between acute liver failure and self-limited acute hepatitis.

### METHODS

Forty-six patients with self-limited acute hepatitis (AH), sixteen patients with acute liver failure (ALF), and twenty-two healthy subjects were involved in this study. The inflammatory and anti-inflammatory products in plasma samples were quantified using commercial enzyme-linked immunoassays and quantitative real-time PCR. The cellular immune responses were measured by proliferation assay using flow cytometry. The groups were divided into viral- and non-viral-induced self-limited AH and ALF. Thus, we worked with five groups: Hepatitis A virus (HAV)-induced self-limited acute hepatitis (HAV-AH), HAV-induced ALF (HAV-ALF), non-viral-induced self-limited acute hepatitis (non-viral AH), non-viral-induced acute liver failure (non-viral ALF), and healthy subjects (HC). Comparisons among HAV and non-viral-induced AH and ALF were performed.

### RESULTS

The levels of mitochondrial DNA (mtDNA) and the cytokines investigated [interleukin (IL)-6, IL-8, IL-10, interferon gamma, and tumor necrosis factor] were significantly increased in ALF patients, independently of etiology ( $P < 0.05$ ). High plasma mtDNA and IL-10 were the best markers associated with ALF [mtDNA: OR = 320.5 (95%CI: 14.42-7123.33),  $P < 0.0001$ ; and IL-10: OR = 18.8 (95%CI: 1.38-257.94),  $P = 0.028$ ] and death [mtDNA: OR = 12.1 (95%CI: 2.57-57.07),  $P = 0.002$ ; and IL-10: OR = 8.01 (95%CI: 1.26-50.97),  $P = 0.027$ ]. In the cellular proliferation assay, NK<sup>bright</sup>, NKT and regulatory T cells (TReg) predominated in virus-specific stimulation in HAV-induced ALF patients with an anergic behavior in the cellular response to mitotic stimulation. Therefore, in non-viral-induced ALF, anergic behavior of activated T cells was not observed after mitotic stimulation, as expected and as described by the literature.

### CONCLUSION

mtDNA and IL-10 may be predictors of ALF and death. TReg cells are involved in immunological disturbance in

HAV-induced ALF.

**Key words:** Acute liver failure; Cytokines; Mitochondrial DNA; Cellular immune response; Hepatitis A virus

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Acute liver diseases induced by viral infections are considered major causes of liver failure and death in Brazil. To better understand this pathogenesis, we investigated in a pioneering way the cellular immune response, inflammatory mediators and mitochondrial products in patients with hepatitis A virus (HAV)-induced acute liver failure (ALF) in comparison to patients with non-virus-induced ALF in a cross-sectional study. The results showed that non-invasive samples could be helpful to assay early prognostic markers that would indicate the necessity for liver transplantation. The contribution of *in vitro* immune response involved in ALF can be helpful to show the necessity of mass vaccination against HAV.

Melgaço JG, Soriani FM, Sucupira PHF, Pinheiro LA, Vieira YR, de Oliveira JM, Lewis-Ximenez LL, Araújo CCV, Pacheco-Moreira LF, Menezes GB, Cruz OG, Vitral CL, Pinto MA. Changes in cellular proliferation and plasma products are associated with liver failure. *World J Hepatol* 2016; 8(32): 1370-1383 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i32/1370.htm> DOI: <http://dx.doi.org/10.4254/wjgh.v8.i32.1370>

## INTRODUCTION

Acute liver failure (ALF) is a rare (0.5%-1% of the acute hepatitis cases) and devastating clinical syndrome resulting from an acute insult that occurs when a high percentage of liver cells are rapidly lost. Liver transplantation is the only effective therapy<sup>[1-4]</sup>. Non-invasive methods have been proposed to evaluate the liver damage<sup>[5-7]</sup> and predict the worst outcome (death)<sup>[8-10]</sup>, with little success. Nevertheless, there are few studies on the systemically released inflammatory products that indicate liver failure or regeneration before liver transplantation, such as cytokine profile or mitochondrial DNA<sup>[11-15]</sup>. Additional early prognostic markers are urgently requested to evaluate the necessity of liver transplantation therapy.

The causes of ALF involve a variety of toxic, viral, metabolic, and vascular liver injuries. The etiology of ALF varies with geography<sup>[16]</sup>, and the hepatitis A virus (HAV) is the major cause of acute hepatitis in Brazil<sup>[17,18]</sup> due to absence of an effective hepatitis A vaccination program. Recent studies have shown high counts of natural killer (NK) cells (NK<sup>bright</sup> and NK<sup>dim</sup>) during self-limited hepatitis A<sup>[19]</sup>. Functionally, NK cells are important components of liver immunology, mediating pro-inflammatory functions, such as IFN $\gamma$  secretion by NK<sup>bright</sup> (CD3<sup>-</sup>CD56<sup>+</sup>CD16<sup>-</sup>) cells, as well as the lysis of target cells by a subset of NK<sup>dim</sup> (CD3<sup>-</sup>CD56<sup>low</sup>CD16<sup>+</sup>) cells<sup>[7,19-22]</sup>.



Perrella *et al.*<sup>[23]</sup> (2008) showed that regulatory T cells (TReg) (CD4<sup>+</sup>CD25<sup>+</sup>) are important factors in acute hepatitis A resolution. Trujillo-Ochoa *et al.*<sup>[14]</sup> showed that serum IL-17 is elevated in children with acute hepatitis A infection; however, the involvement of TReg and helper T cells in ALF caused by hepatitis A is unknown.

The goal of our study was to evaluate plasma levels of inflammatory and anti-inflammatory cytokines and mtDNA in a pilot study with a case series of liver injury patients and their association with ALF and occurrence of death. We quantified the mechanism of viral (HAV) and non-viral liver dysfunction by phenotypically characterizing cytotoxic, helper, and TReg and analyzed the cytokine secretion in a peripheral blood mononuclear cell (PBMC) clonal proliferation assay.

## MATERIALS AND METHODS

### Patients and samples

Eighty-four subjects agreed to participate in this study in Rio de Janeiro, Brazil, from 2009 to 2012: 46 (54.76%) were consecutive outpatients with self-limited acute hepatitis (AH) that were referred to the Viral Hepatitis Clinic of Oswaldo Cruz Institute - Fiocruz; 16 (19.05%) inpatients were admitted to the Bonsucesso Federal Hospital, a referral hospital for patients with ALF requiring transplantation; and 22 (26.19%) were healthy donors.

All samples were assayed for HAV, hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis E virus (HEV) serological markers using commercially available enzyme-linked immunoassays (ELISAs): Anti-HAV IgM (Abbott, United States), Vikia HBsAg (Biomerieux, France), Murex anti-HCV version 4.0 (Diasorin, South Africa), and bioELISA HEV IgM version 3.0 (Biokit, Spain). Blood samples were also assayed using rapid tests for syphilis (DPP<sup>®</sup>, Bio-manguinhos, Brazil), HIV-1/2 (DPP<sup>®</sup>, Bio-manguinhos, Brazil), dengue (SD BIOLINE, Standard Diagnostics, South Korea), and leptospirosis (SD BIOLINE, Standard Diagnostics, South Korea). Other current infections and autoimmune diseases were analyzed with a chemiluminescent ELISA for Epstein-Barr, cytomegalovirus and antinuclear antibodies (ANA). The respective reference levels of  $\geq 20$  U/mL,  $\geq 30$  UA/mL, and  $\geq 1.5$  UI/mL were considered positive. Herpes virus type 1 (HSV-1) and herpes virus type 2 (HSV-2) were investigated using a TaqMan-based multiplex assay as previously described<sup>[24]</sup>. Metabolic disorders were also investigated whether the routine exams (biochemical, hematological, etc.) presented alterations or whether the patient had a family history of metabolic disorders.

AH cases were defined by aminotransferase levels of at least  $10 \times$  the upper normal limit and the onset of jaundice in a previously healthy individual<sup>[25]</sup>. The cases were further categorized according to international normalized ratio (INR) and hepatic encephalopathy grade (HE). Cases with INR  $< 1.5$  and no HE were classified as self-limited AH and those with INR  $\geq 1.5$  and an HE score above II as ALF<sup>[4]</sup>.

The timing of sample collection was based on the

onset of jaundice and liver enzyme levels for self-limited AH patients. In ALF patients, the timing of sample collection was based on ALF diagnosis and hospital admission. In healthy subjects, the sample collection was based on the lack of infection found in their routine exams.

The study population was divided into five groups according to etiology and clinical condition: Group 1: Virus-induced self-limited hepatitis, of which all cases were caused by HAV-AH; group 2: Non-viral-induced self-limited hepatitis, which included drug and indeterminate causes (non-viral AH); group 3: Virus-induced ALF, of which all cases were caused by HAV-ALF; group 4: Non-viral-induced ALF, which included drug and indeterminate causes (non-viral ALF); and group 5: Healthy subjects, as the control group (HC).

To assess the PBMCs, blood samples were collected in the anticoagulant citrate-dextrose solution-A (Greiner Bio-one, Kremsmünster, Austria) and stored at  $-70^{\circ}\text{C}$  (plasma) or in liquid nitrogen (peripheral blood mononuclear cells, PBMCs) until assay. Plasma and PBMC samples used were thawed only once for the different assays.

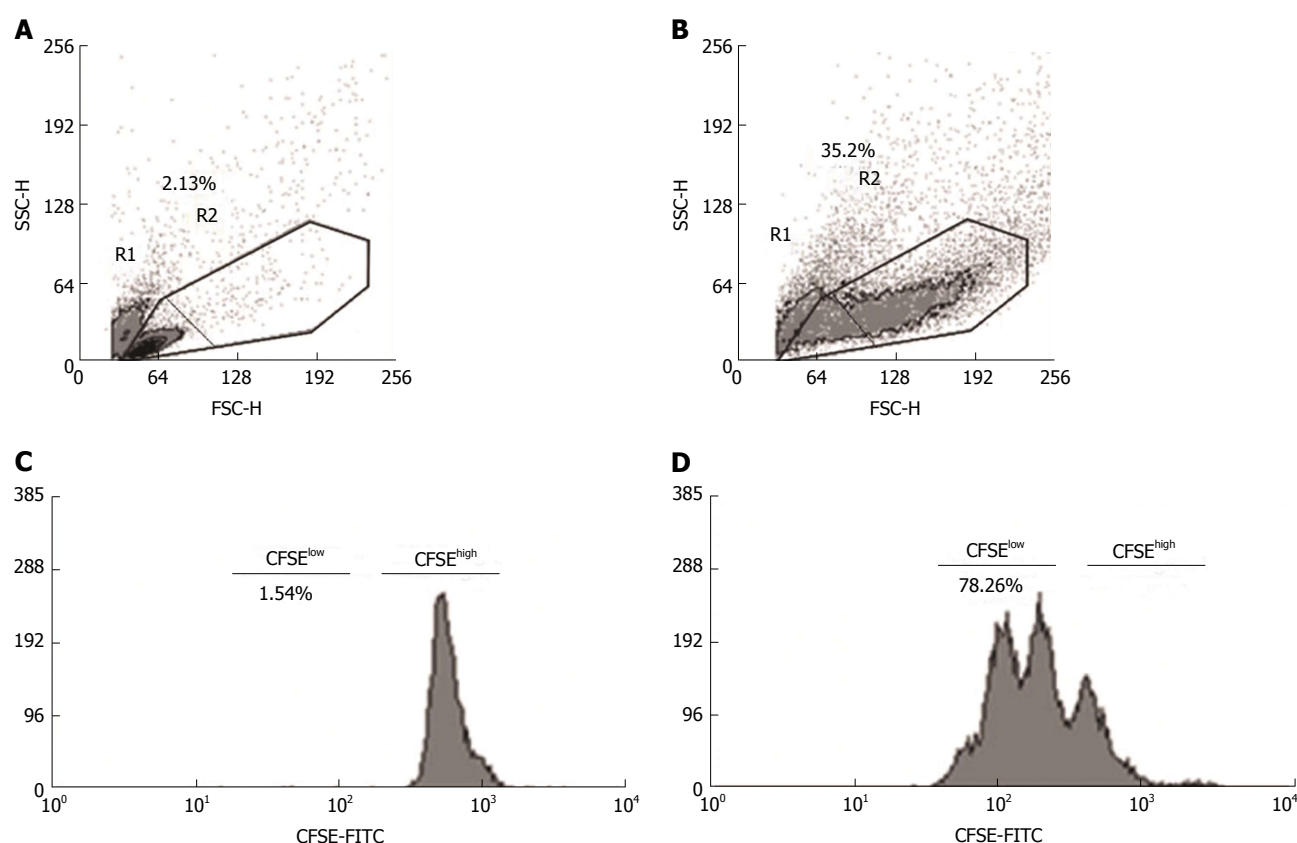
The study protocol was approved by the National Commission on Ethics in Research (CONEP), and by the institutional review board of the Oswaldo Cruz Foundation, FIOCRUZ (222/03). Signed informed consent was obtained from all participants. The study was performed in compliance with the relevant laws and institutional guidelines and in accord with the ethical standards of the Declaration of Helsinki.

### Quantitative detection of cytokines and mitochondrial-derived DNA in ALF, AH and healthy control subjects

To assess the liver inflammatory/anti-inflammatory status, plasma levels of the cytokines IL-6, IL-8, IL-10, IFN $\gamma$  and tumor necrosis factor alpha (TNF $\alpha$ ) were quantified using commercially available Standard ELISA Development kits (Peprotech, United States). To assess hepatocellular damage, the total DNA was purified from the plasma samples using the QIAamp DNA Blood Mini Kit (Qiagen, United States) according to the manufacturer's instructions<sup>[26]</sup>. The mitochondrial DNA (mtDNA) was quantified by real-time PCR as previously reported<sup>[26]</sup> using 3 pairs of primers specific for human cytochrome B (sense 5'atgacccaatacgcacaaat-3' and antisense 5'cgaagtttcacatgcggag3'), human cytochrome C oxidase subunit III (sense 5'atgacccaatacgcacatgc3' and antisense 5'atcacatggctaggccggag3'), and human NADH dehydrogenase (sense 5'ataccatggccaacctct3' and antisense 5'gggcctttgcgtagtgtat3'). The total mtDNA value corresponds to the sum of the individual values from each test. Colorimetric commercial kits were used to assess the levels of liver enzymes and total bilirubin.

### Quantitative evaluation of the clonal proliferation response and cell phenotypes of proliferated PBMCs from ALF and AH patients

Twenty-nine PBMC samples from 62 patients were evaluated for the proliferative cellular immune response: 16



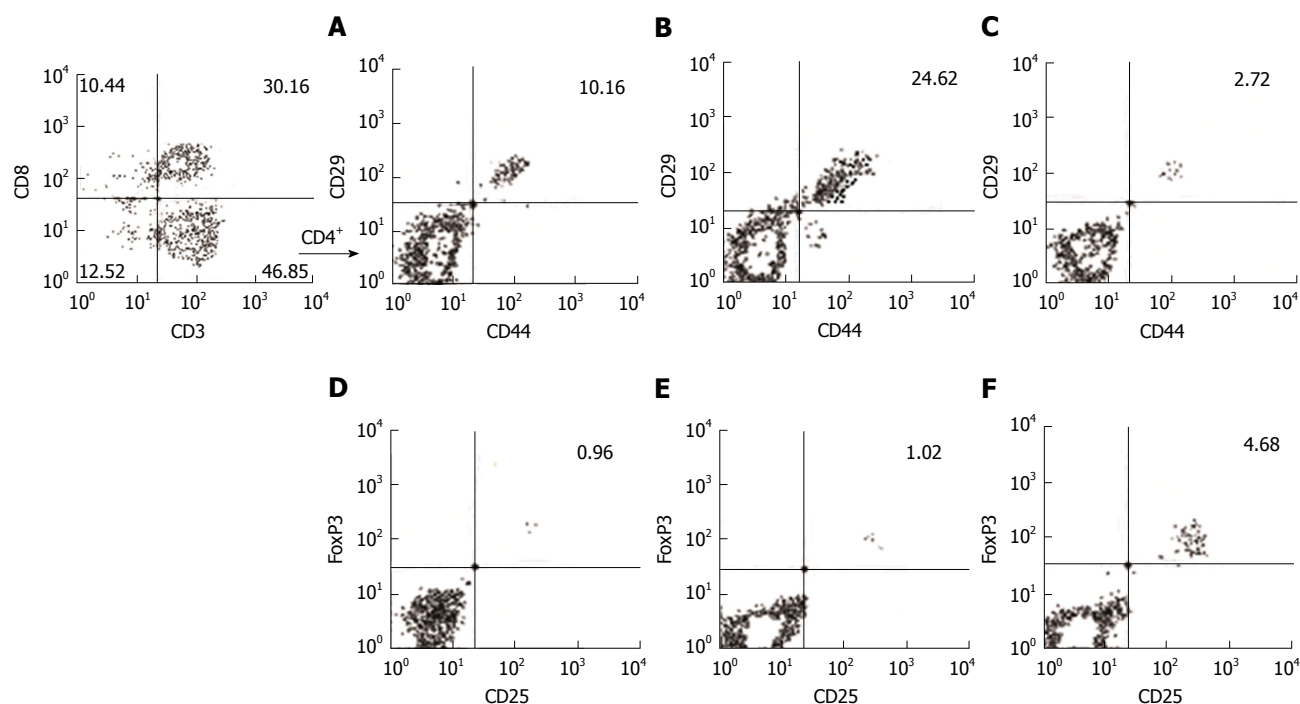
**Figure 1** Flow cytometry analysis of proliferating mononuclear cells in antigenic stimulation. Mononuclear cell populations were gated using forward (FSC) and side (SSC) scatter, and the dot plot identifies the total cells (R1 + R2), resting cells (R1) and blasts (R2). Peripheral blood mononuclear cells, either unstimulated (A and C) or stimulated with antigens (PHA, LPS or HAV Ag) (B and D), were labeled with CFSE. The histograms show the proportion of total (CFSE<sup>low</sup> + CFSE<sup>high</sup>), resting (CFSE<sup>high</sup>) and proliferating cells (CFSE<sup>low</sup>) observed using the Cyan flow cytometer and analyzed using the off-line software Summit version 6.0. CFSE: Carboxyfluorescein succinimidyl ester; PHA: Phytohemagglutinin; LPS: Lipopolysaccharide.

samples from patients with self-limited AH (8 patients diagnosed with HAV-induced hepatitis and 8 with non-viral hepatitis) and 13 samples from patients with ALF (8 patients diagnosed with HAV-induced hepatitis and 5 with non-viral hepatitis). Ten of twenty-two healthy subject samples were included in the cellular response assay.

The PBMCs from each patient were separated on a Ficoll density gradient by centrifugation (30 min at 400 g at 18 °C). The concentration of viable cells was determined by trypan blue exclusion. Samples with less than 80% of viable cells at this stage were excluded. In the proliferation assay, the PBMCs were suspended in RPMI 1640 (Sigma Aldrich, United States) medium at a concentration of  $5 \times 10^6$  cells/mL and mixed with an equal volume of 10 mmol/L carboxyfluorescein succinimidyl ester working solution (CFSE-FITC) (Molecular Probes, Invitrogen, United States) that was diluted 1/1000 for all analyses. Cells that were not labeled with CFSE were used as a negative control for the flow cytometry analysis. The mitogen inducers phytohemagglutinin (PHA) and lipopolysaccharide (LPS) (Sigma Aldrich, United States) were used at final concentrations of 10 µg/mL and 1 ng/mL, respectively, for non-viral proliferation. The HAF-203 strain of HAV was propagated in FRhK-4 cells<sup>[27]</sup> and was used for viral-antigen-specific (HAV Ag) proliferation (viral titer of  $10^6$  HAV-RNA/mL).

Duplicate proliferation cultures were performed with  $5 \times 10^5$  cells/well in 96-well flat bottom culture plates. The plates were incubated at 37 °C in a 5% CO<sub>2</sub> incubator for 72 h with PHA, 24 h with LPS and 96 h with HAV Ag. After incubation, the cells were harvested for the flow cytometry assay.

To assess the cell phenotypes and proliferative response, 20000 live cells were collected from each sample using a Cyan flow cytometer (BD Biosciences, United States) and analyzed using the off-line software Summit version 6.0 (Dako Cytomation, United States) (Figures 1 and 2). PBMCs were labeled and quantified with αCD8-PerCP (clone DK25), αCD25-PE (clone ACT1), αCD56-PE (clone CM55B), αCD16-FITC (clone DJ130c) (all from Dako Cytomation, United States), αCD3-APC (clone OKT3), αCD29-FITC (clone MEM101a), αCD44-PECy7 (clone IM7), αFoxP3-FITC (clone PCH101) and isotypes (eBiosciences, San Diego, CA, United States). The intracellular staining for FoxP3 expression was performed with a Cytofix/Cytoperm<sup>®</sup> kit (BD Biosciences, United States). Total mononuclear cells were electronically gated in R1 *plus* R2 using forward (FSC) and side (SSC) properties; cellular debris and granular cells were excluded (Figure 1A and B). The proliferating cells (R1 + R2) were defined based on their FSC and SSC properties<sup>[28]</sup>. The proliferation index (PI)



**Figure 2** Hepatitis A virus Ag-activated CD4<sup>+</sup> T cells in acute liver disease caused by hepatitis A virus. The data from three subjects selected from our study groups were used to represent the gating strategy to select CD29<sup>+</sup>CD44<sup>+</sup> and CD25<sup>+</sup>FoxP3<sup>+</sup> on CD4<sup>+</sup> cells (CD3<sup>+</sup>CD8<sup>-</sup>). Representative contour plots of the frequency of migratory T helper cells (%) in HAV Ag-activated mononuclear cells from healthy subjects (A), patients with acute hepatitis A (B), patients with acute liver failure with HAV infection (C) and in HAV Ag-activated regulatory T cells from healthy subjects (D), patients with acute hepatitis (E), and patients with acute liver failure (F). HAV: Hepatitis A virus.

was determined by the software program; this index is a measure of the frequency of cells that have gone through more than three divisions (positive proliferation, CFSE<sup>low</sup>) (Figure 1C and D)<sup>[28-30]</sup>. The final PI was determined by calculating the ratio of the average PI for mitogen- or antigen-stimulated cells divided by the average PI of unstimulated cells (Figure 1). The highly expressed surface markers on the T, NK and NKT cell subsets that were activated by antigenic stimulation (R1 + R2) were considered in the off-line software analysis (e.g., Figure 1A and B, and Figure 2). The cell culture supernatants were assayed to quantify IL-6, IL-8, IL-10, IFN $\gamma$  and TNF $\alpha$  using commercially available Standard ELISA Development kits (Peprotech, United States). Human cytokine IL-17/17A was quantified with the commercially available Mini ELISA Development kit (Peprotech, United States).

### Statistical analysis

The data are expressed as the mean  $\pm$  standard deviation (SD) at a 95%CI. The distribution of the data in the groups was initially evaluated by the Kolmogorov-Smirnov test. The correlations were evaluated using the Spearman rank correlation test (R project for Statistical Computing (<http://www.r-project.org/>)). The differences between self-limited AH, ALF, and healthy subjects were evaluated by intergroup comparisons using the Kruskal-Wallis test. If a significant difference was found, a pair of variables in the three groups was assessed with the Mann-Whitney *U*-test. For the plasma samples, receiver

operating characteristic (ROC) curve analysis was used to compare the predictive strength of markers with chance. The area under the curve was used as a measure of the ability of the test to distinguish between the two groups. The software GraphPad Prism 5 for Windows, version 5.01 (San Diego, CA, United States), was used to perform statistical ROC curve analysis. Multivariate logistic regression was applied to select the independent predictors in plasma samples associated with ALF based on cut-off points (90% specificity and with the highest likelihood ratio value) obtained from ROC curve analysis. In the initial logistic model, all variables were tested for predictive strength. The variables showing statistically significant differences were kept in the final model. The logistic regression analyses were performed using SPSS software version 17.0 for Windows (SPSS Inc., Chicago, IL, United States). The significance for all statistical analyses was defined as  $P < 0.05$ .

## RESULTS

### Characterization of the AH and ALF patients

Non-viral ALF cases were caused by  $\alpha$ -methylidopa (1 patient), rifampicin (1), and cryptogenic disease (3). The self-limited AH were caused by NSAIDs (2) and cryptogenic disease (6). HAV infection was the viral etiology found in self-limited AH (38) and ALF (11). The mean  $\pm$  SD of viral load for the HAV was  $1.4 \times 10^6 \pm 8.6 \times 10^5$  HAV-RNA/mL in plasma samples from ALF patients and  $3.6 \times 10^3 \pm 1.8 \times 10^3$  HAV-RNA/mL in

**Table 1 Clinical characteristics of the studied population *n* (%)**

	Acute liver failure ( <i>n</i> = 16)	Acute hepatitis ( <i>n</i> = 46)	Healthy control ( <i>n</i> = 22)
Age (yr)			
Mean ± SD	24.88 ± 21.52	21.21 ± 10.32	24.64 ± 8.79
25%, 75%	9.25, 49	9.1, 29.75	15.2, 47
Gender			
Male	6 (37.50)	25 (54.34)	9 (40.9)
Diagnosis			
Hepatitis A	11 (68.75)	38 (82.60)	0
Drug toxicity	2 (12.50)	2 (4.34)	0
Indeterminate	3 (18.75)	6 (13.04)	0
Liver enzymes			
AST (UI/L)	1095.5 ± 1460	344.5 ± 444.9	21.68 ± 4.87
ALT (UI/L)	806.12 ± 639.11	517.90 ± 884.30	14.36 ± 4.50
Total bilirubin (mg/dL)	21.47 ± 10.48	10.01 ± 6.88	0.85 ± 0.09
Coma grade			
0- I	3 (18.75)	0	0
II-IV	13 (81.25)	0	0
Coagulopathy			
INR (mean ± SD)	4.88 ± 0.99	1.16 ± 0.04	0.98 ± 0.06
Outcome			
Survived	6 (46.15)	46 (100.00)	22 (100)
Died	10 (53.84)	0	0

INR: International normalized ratio; SD: Standard deviation; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

samples from AH self-limited patients (AH).

The time of blood collection in self-limited AH was 1-4 wk in the HAV-AH group and 2-6 wk in the non-viral AH group. In the ALF patient group it was 1-3 wk for HAV-ALF and non-viral ALF. Three patients with acute HAV infection, INR < 1.5 and no coma grade (HE < I) had their samples collected before the evolution to liver failure. They progressed to death before transplant, according to medical records, so they were included in the ALF group. Table 1 shows more information about the study population, including age, gender, coma grade, coagulopathy, liver enzymes, total bilirubin, and outcome.

### **Elevated plasma cytokines and mtDNA are seen in AH and ALF patients compared to healthy controls**

The intensity of the inflammatory status was not associated with etiology ( $P > 0.05$ ). Table 2 compares the systemic inflammatory parameters between clinical conditions. The cytokines IL-6, IL-8, IL-10 and IFN $\gamma$  were significantly raised in the AH and ALF patients compared to the healthy subjects. TNF $\alpha$  was also elevated in the ALF patients compared to the healthy subjects (Table 2). Similarly, total mtDNA was significantly higher in both the AH and ALF groups than in the healthy controls. ALF patients showed a significant elevation in IL-6, IL-10, IFN $\gamma$  and TNF $\alpha$  as well as high levels of mtDNA compared to the AH patients (Table 2).

### **Elevated plasma cytokines and mtDNA are positively correlated with the degree of liver damage, as represented by the presence of HE or coagulopathy**

When we evaluated the correlations between INR and

HE and the plasma cytokine and mtDNA levels, the HE grade showed significant positive correlations with IL-6 ( $P < 0.0001$ ), IL-10 ( $P < 0.0001$ ), TNF $\alpha$  ( $P = 0.0001$ ), and IL-8 ( $P = 0.0034$ ) (Supplementary Figure 1A, C, E and G). The elevated INR values showed significant positive correlations with IL-6 ( $P < 0.0001$ ), IL-10 ( $P = 0.0002$ ), TNF $\alpha$  ( $P = 0.0004$ ) and IFN $\gamma$  ( $P = 0.0057$ ) (Supplementary Figure 1B, D, F and H). A positive correlation was observed between mtDNA and HE ( $P = 0.0002$ ; Supplementary Figure 1I) as well as INR ( $P = 0.0043$ ; Supplementary Figure 1J).

### **Elevated cytokines and mtDNA are correlated with outcome in ALF**

To determine whether the plasma concentrations of the inflammatory or anti-inflammatory cytokines could be used as indicators of liver dysfunction, we used ROC curve analysis, which showed that IL-6 ( $P < 0.0001$ ), IL-10 ( $P < 0.0001$ ), TNF $\alpha$  ( $P < 0.0001$ ), and IFN $\gamma$  ( $P < 0.00104$ ) had the highest diagnostic accuracy for ALF. When we evaluated hepatocyte damage, the ROC curve showed that mtDNA ( $P = 0.0046$ ) had the highest diagnostic accuracy for ALF.

Among the cytokines, elevated IL-10 was the best indicator of ALF ( $P = 0.028$ ). Although the IL-6, IL-8, IFN $\gamma$  and TNF $\alpha$  levels had a positive correlation with hepatic encephalopathy, the association with ALF was not significant (Table 3). Elevated mtDNA ( $P < 0.0001$ ) was associated with ALF diagnosis.

Subsequently, the indicators that were associated with death were investigated in all 62 acute liver disease patients: 52 survived (AH and ALF patients) and 10 died (ALF patients). Figure 3 shows that the mtDNA ( $P$



**Table 2** Systemic inflammatory products in the plasma samples from patients with acute hepatitis or acute liver failure and healthy subjects

Plasma variables	HC (n = 22)	AH (n = 49)	ALF (n = 13)	HC vs AH <sup>a</sup>	HC vs ALF	AH vs ALF
IL-6 (pg/mL)	15.07 ± 25.92 (3.58-26.57) <sup>1</sup>	68.93 ± 109.7 (38.39-99.46)	509.30 ± 678.70 (147.6-870.9)	0.0009	< 0.0001	< 0.0001
IL-8 (pg/mL) <sup>2</sup>	ND	10.50 ± 20.05 (4.92-16.09)	144.70 ± 437.6 (-88.45-377.9)	< 0.001	< 0.0001	ns
IL-10 (pg/mL)	1.81 ± 5.58 (-0.66-4.28)	17.28 ± 51.97 (2.81-31.75)	249.60 ± 379.60 (47.35-451.9)	0.0006	< 0.0001	< 0.0001
IFN $\gamma$ (pg/mL)	4.80 ± 18.00 (-3.18-12.79)	113.0 ± 265.33 (39.1-186.8)	229.70 ± 342.20 (47.37-412.1)	0.0075	< 0.0001	0.0016
TNF $\alpha$ (pg/mL)	1.08 ± 2.38 (0.02-2.13)	27.25 ± 64.05 (9.42-45.08)	179.40 ± 161.40 (93.42-265.4)	ns	< 0.0001	< 0.0001
mtDNA (ng/100 $\mu$ L plasma)	81.79 ± 121.6 (27.88-135.7)	159.6 ± 202.2 (64.99-254.3)	4228.00 ± 4286.0 (1944-6512)	0.0131	< 0.0001	0.0008

<sup>1</sup>Mean ± standard deviation (95%CI); <sup>2</sup>IL-8 levels in the plasma samples were evaluated only by the Kruskal-Wallis test. <sup>a</sup>P < 0.05. The differences between the acute liver failure patients, the self-limited acute hepatitis patients and the healthy controls were evaluated by intergroup comparisons using the Mann-Whitney U-test. IL: Interleukin; IFN $\gamma$ : Interferon gamma; TNF $\alpha$ : Tumor necrosis factor alpha; mtDNA: Total mitochondrial DNA; ND: Not detectable; ns: Not significant; HC: Healthy control; AH: Acute hepatitis (viral plus non-viral etiologies); ALF: Acute liver failure (viral plus non-viral etiologies).

**Table 3** Potential clinical and inflammatory parameters as indicators of acute liver failure syndrome and death

Plasma variables <sup>1</sup>	Cut-off	Adjusted OR	95%CI	P value
IL-6 (pg/mL)	> 197.6	1.36	0.04-40.27	0.856
IL-10 (pg/mL)	> 55.77	18.86	1.38-257.94	0.028
TNF $\alpha$ (pg/mL)	> 122.6	4.42	0.185-105.93	0.359
mtDNA (ng/100 $\mu$ L plasma)	> 174	320.54	14.42-7123.33	0.000
Plasma variables <sup>2</sup>				
IL-6 (pg/mL)	> 473.2	2.27	0.19-26.92	0.515
IL-8 (pg/mL)	> 66.30	10.42	1.54-70.45	0.016
IL-10 (pg/mL)	> 95.71	8.01	1.26-50.97	0.027
TNF $\alpha$ (pg/mL)	> 313.7	0.27	0.03-2.17	0.220
mtDNA (ng/100 $\mu$ L plasma)	> 405.3	12.11	2.57-57.07	0.002
INR	> 2.12	29.88	5.44-164.19	0.000

<sup>1</sup>Multivariate analysis from clinical and inflammatory parameters associated with ALF;

<sup>2</sup>Multivariate analysis from clinical and inflammatory parameters associated with death.

OR: Odds ratio; IL: Interleukin; IFN $\gamma$ : Interferon gamma; TNF $\alpha$ : Tumor necrosis factor alpha; mtDNA: Total mitochondrial DNA; ALF: Acute liver failure; INR: International normalized ratio.

< 0.01) and all investigated cytokines were significantly elevated in the non-surviving patients ( $P < 0.01$ ). The ROC curve analysis showed that elevated INR, IL-6, IL-8, IL-10, TNF $\alpha$ , IFN $\gamma$  and mtDNA in the plasma samples were able to discriminate survivors from non-survivors with a sensitivity and specificity above 70%. The high plasma levels of mtDNA, IL-8, IL-10 and INR were considered predictive factors for poor outcome (death) in patients with acute liver disease (Table 3). Despite the high levels of IL-6, and TNF $\alpha$ , these factors did not predict death (Figure 3 and Table 3).

### Changes in the frequency of mononuclear cell phenotypes and cytokine secretion after the clonal proliferation assay are associated with virus (HAV)-induced AH and ALF syndrome

The panel of phenotypic analyses for PBMC clonal proliferation was composed of activated and migratory T helper cells (CD4<sup>+</sup>CD29<sup>+</sup>CD44<sup>+</sup>), activated and migratory cytotoxic T cells (CD8<sup>+</sup>CD29<sup>+</sup>CD44<sup>+</sup>), activated NK cells [CD3<sup>+</sup>CD56<sup>low</sup>CD16<sup>+</sup> (NK<sup>dim</sup>), CD3<sup>+</sup>CD56<sup>+</sup>CD16<sup>-</sup> (NK<sup>bright</sup>)], and NKT cells (CD3<sup>+</sup>CD16<sup>+</sup>CD56<sup>+</sup>). Mitogens (PHA and LPS) and virus particles (HAV Ag) were used for non-specific and specific PBMC proliferation, respectively.

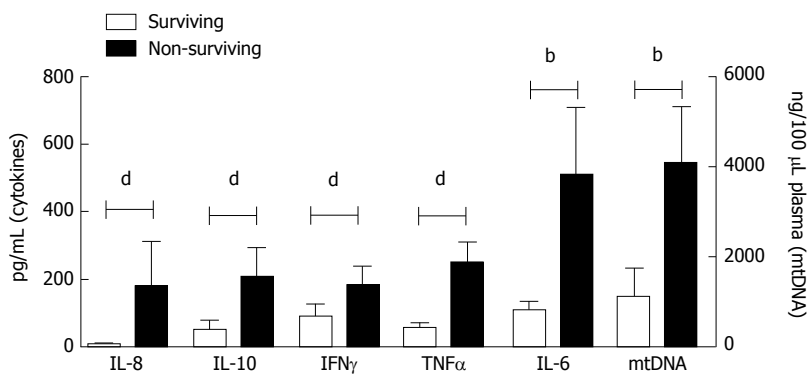
The mitogen stimulation showed a reduced frequency (anergic behavior) in all investigated phenotypes from HAV-induced hepatitis (ALF and AH patients) (Table 4). The same patients, when stimulated with HAV Ag, exhibited positive proliferation of the regulatory (CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup>), NKT (CD3<sup>+</sup>CD16<sup>+</sup>CD56<sup>+</sup>), and NK<sup>bright</sup> (CD3<sup>+</sup>CD56<sup>+</sup>CD16<sup>-</sup>) phenotypes, and only the helper phenotype (CD4<sup>+</sup>CD29<sup>+</sup>CD44<sup>+</sup>) frequency was reduced in HAV-induced ALF patients (Figure 2). In general, the PBMCs from HAV-induced AH showed a tendency toward negative proliferation after mitogen stimulation in all analyzed phenotypes. A significant decrease was detected in the T helper and NKT cells (AH vs HC) (Table 4). The PBMCs showed a significant positive proliferation of the T helper, cytotoxic (CD8<sup>+</sup>CD29<sup>+</sup>CD44<sup>+</sup>), and NKT cells with HAV-specific stimulation.

The secreted cytokines, IL-6, TNF $\alpha$ , IL-8 and IL-17, were reduced in the supernatant of HAV-induced hepatitis PBMCs from ALF patients compared to AH patients during mitogen stimulation. Additionally, IL-10 and IFN $\gamma$  were reduced in ALF patients vs the HC subjects. In patients with AH A, we observed a significant reduction in IL-6 secretion and a general tendency toward a reduced

**Table 4** Variables from the mitogen-stimulated peripheral blood mononuclear cell phenotypes from patients with acute hepatitis A infection and healthy subjects

Phenotypes/cytokines (PHA/LPS)	HC (n = 10)	AH (n = 8)	ALF (n = 8)	HC vs AH	HC vs ALF	AH vs ALF
PI of CD3 <sup>+</sup>	133.1 ± 71.12 (95.19-171.0)	44.4 ± 25.83 (22.80-65.99)	17.48 ± 5.94 (11.24-23.72)	0.0155	0.0021	0.0426
CD4 <sup>+</sup> CD25 <sup>+</sup> FoxP3 <sup>+</sup> (%)	17.23 ± 9.74 (12.03-22.42)	17.08 ± 5.37 (12.59-21.57)	5.99 ± 2.80 (3.65-8.33)	ns	0.0009	0.0003
CD4 <sup>+</sup> CD29 <sup>+</sup> CD44 <sup>+</sup> (%)	38.63 ± 18.37 (28.84-48.42)	20.75 ± 7.82 (14.21-27.29)	10.22 ± 4.74 (6.25-14.18)	0.0062	< 0.0001	0.0047
CD8 <sup>+</sup> CD29 <sup>+</sup> CD44 <sup>+</sup> (%)	39.76 ± 19.91 (29.15-50.36)	37.56 ± 25.01 (16.74-58.57)	9.03 ± 4.59 (5.18-12.88)	ns	0.0002	0.0104
CD3 <sup>+</sup> CD56 <sup>+</sup> CD16 <sup>-</sup> (%)	8.31 ± 6.75 (2.07-14.56)	4.19 ± 2.28 (2.08-6.30)	0.50 ± 0.37 (0.15-0.84)	ns	0.0006	0.0009
CD3 <sup>+</sup> CD56 <sup>low</sup> CD16 <sup>+</sup> (%)	12.70 ± 8.93 (4.44-20.96)	8.52 ± 5.68 (3.26-13.78)	1.11 ± 0.66 (0.50-1.72)	ns	0.0012	0.0018
CD3 <sup>+</sup> CD56 <sup>+</sup> CD16 <sup>+</sup> (%)	13.66 ± 3.54 (11.77-15.55)	7.20 ± 5.28 (2.79-11.63)	1.83 ± 1.06 (0.94-2.72)	0.0117	< 0.0001	0.0070
IL-6 (pg/mL)	2625.33 ± 3320 (856.5-4394)	565.7 ± 313.3 (303.8-827.6)	156.8 ± 173.9 (11.40-302.2)	0.0155	< 0.0001	0.0070
TNFα (pg/mL)	1675.20 ± 623.4 (1343-2007)	1405.0 ± 324.3 (1134-1676)	145.3 ± 107.9 (55.09-235.5)	ns	< 0.0001	0.0002
IL-10 (pg/mL)	528.86 ± 755.1 (126.5-931.2)	269.5 ± 145.8 (147.6-391.5)	188.4 ± 267.1 (-16.88-393.7)	ns	0.0454	ns
IFNγ (pg/mL)	3379.1 ± 1869 (2383-4375)	2467.0 ± 2787 (137.1-4798)	1249.0 ± 2067 (-479.2-2977)	ns	0.0205	ns
IL-8 (pg/mL)	273.9 ± 116.3 (211.9-335.9)	274.2 ± 148.5 (150.0-398.3)	151.0 ± 156.4 (20.21-281.8)	ns	0.0205	0.0379
IL-17 (pg/mL)	73.81 ± 107.0 (16.81-130.8)	31.55 ± 35.58 (1.80-61.30)	4.16 ± 3.20 (1.49-6.84)	ns	0.0029	0.0116

The differences between the hepatitis A-induced acute liver failure (ALF) patients, the self-limited acute hepatitis A (AH) patients, and the healthy control (HC) subjects were evaluated by intergroup comparisons using the Mann-Whitney *U*-test. The significance cutoff for all statistical analyses was defined as *P* < 0.05. IL: Interleukin; IFNγ: Interferon gamma; TNFα: Tumor necrosis factor alpha; ns: Not significant; PI: Proliferation index.



**Figure 3** Differences in inflammatory cytokines (interleukin 6, 8 and 10, interferon gamma, and tumor necrosis factor α) and hepatocyte damage (mitochondrial DNA) parameters between the surviving and non-surviving patients. <sup>b</sup>*P* < 0.01; <sup>d</sup>*P* < 0.001. IL-6: Interleukin 6; IFNγ: Interferon gamma; TNFα: Tumor necrosis factor alpha; mtDNA: Mitochondrial DNA.

secretion of all cytokines, but there were no significant differences (Table 4).

The analysis of the secreted cytokines after HAV Ag stimulation of PBMCs from HAV-induced hepatitis patients showed reduced levels of TNFα and IL-17 when comparing the ALF and AH patients. Reduced levels of secreted TNFα were also observed in the ALF patients compared to the HC subjects. Additionally, we observed elevated levels of secreted IL-10 and IFNγ. The ALF patients presented elevated levels of secreted IL-8 compared to the HC subjects. The levels of secreted IL-10, IFNγ, IL-8 and IL-17 were elevated in cultures

from the AH patients (Table 5).

#### Changes in the frequency of mononuclear cell phenotypes and cytokine secretion after the clonal proliferation assay in non-viral-induced AH and ALF

We observed a tendency toward positive proliferation for the migratory T helper (CD4<sup>+</sup>CD29<sup>+</sup>CD44<sup>+</sup>) and cytotoxic T (CD8<sup>+</sup>CD29<sup>+</sup>CD44<sup>+</sup>) cells for IL-6 and IL-17 release in the ALF patients compared to the AH patients. Significant elevations of NK<sup>dim</sup> (CD3<sup>+</sup>CD56<sup>low</sup>CD16<sup>+</sup>) and NK<sup>bright</sup> (CD3<sup>+</sup>CD56<sup>+</sup>CD16<sup>-</sup>) cell frequencies were associated with high levels of TNFα in the non-viral

**Table 5** Variables from hepatitis A virus Ag-stimulated peripheral blood mononuclear cell phenotypes from patients with acute hepatitis A infection and healthy subjects

Phenotypes/cytokines (HAVAg)	HC (n = 10)	AH (n = 8)	ALF (n = 8)	HC vs AH	HC vs ALF	AH vs ALF
PI of CD3 <sup>+</sup>	1.09 ± 0.85 (0.64-1.55)	3.15 ± 1.92 (1.54-4.76)	3.34 ± 2.29 (1.42-5.25)	0.0053	0.0044	ns
CD4 <sup>+</sup> CD25 <sup>+</sup> FoxP3 <sup>+</sup> (%)	0.85 ± 0.96 (0.34-1.37)	1.0 ± 0.97 (0.18-1.81)	3.19 ± 1.49 (1.95-4.44)	ns	0.0011	0.0070
CD4 <sup>+</sup> CD29 <sup>+</sup> CD44 <sup>+</sup> (%)	11.99 ± 6.43 (8.55-15.42)	27.93 ± 8.16 (21.1-34.76)	5.46 ± 5.92 (0.50-10.42)	0.0008	0.0077	0.0006
CD8 <sup>+</sup> CD29 <sup>+</sup> CD44 <sup>+</sup> (%)	12.65 ± 4.31 (10.35-14.95)	30.13 ± 6.74 (24.49-35.77)	36.05 ± 10.59 (27.19-44.90)	0.0001	0.0001	ns
CD3 <sup>+</sup> CD56 <sup>+</sup> CD16 <sup>-</sup> (%)	0.19 ± 0.20 (0.05-0.34)	0.30 ± 0.19 (0.13-0.46)	1.33 ± 0.85 (0.62-2.04)	ns	0.0009	0.0005
CD3 <sup>+</sup> CD56 <sup>low</sup> CD16 <sup>+</sup> (%)	4.28 ± 2.22 (2.70-5.87)	10.09 ± 8.94 (2.61-17.5)	14.24 ± 11.81 (4.36-24.12)	ns	ns	ns
CD3 <sup>+</sup> CD56 <sup>+</sup> CD16 <sup>+</sup> (%)	1.67 ± 2.71 (0.22-3.12)	4.25 ± 4.06 (0.85-7.65)	15.06 ± 7.74 (8.58-21.53)	0.0110	0.0003	0.0019
IL-6 (pg/mL)	50.49 ± 76.14 (9.92-91.06)	76.41 ± 93.18 (-1.46-154.3)	139.7 ± 165.9 (0.98-278.4)	ns	ns	ns
TNFα (pg/mL)	92.49 ± 133.4 (21.42-163.6)	23.96 ± 28.92 (-0.21-48.14)	1.63 ± 1.01 (0.78-2.48)	ns	0.0089	0.0098
IL-10 (pg/mL)	10.39 ± 13.97 (2.94-17.84)	52.78 ± 62.06 (0.89-104.7)	164.3 ± 75.56 (101.1-227.5)	0.0297	0.0001	0.0148
IFNγ (pg/mL)	0.88 ± 1.08 (0.30-1.46)	106.6 ± 183.9 (-47.14-260.4)	1095 ± 1962 (-546-2735)	0.0035	0.0001	0.0499
IL-8 (pg/mL)	88.64 ± 45.40 (64.44-112.8)	148.9 ± 54.77 (103.1-194.7)	150.2 ± 72.19 (89.84-210.5)	0.0131	0.0110	ns
IL-17 (pg/mL)	3.36 ± 3.75 (1.36-5.36)	32.61 ± 38.30 (0.59-64.64)	7.58 ± 5.43 (3.05-12.13)	0.0008	ns	0.0499

The differences between the hepatitis A-induced acute liver failure (ALF) patients, the self-limited acute hepatitis A (AH) patients and the healthy control (HC) subjects were evaluated by intergroup comparisons using the Mann-Whitney *U*-test. The significance cutoff for all statistical analyses was defined as *P* < 0.05. IL: Interleukin; IFNγ: Interferon gamma; TNFα: Tumor necrosis factor alpha; ns: Not significant; PI: Proliferation index.

ALF patients compared to the AH patients and the HC subjects. The IL-8 levels were also significantly elevated in the ALF patients compared to the HC subjects (Table 5). In general, the comparison between the PBMCs from non-viral AH patients and the HC subjects showed a tendency toward a negative proliferation of all phenotypes investigated and the secreted cytokines IL-6, IL-10, IFNγ and IL-17 (Table 6).

#### **Evidence of effects on the TReg and migratory T helper cells obtained from viral and non-viral AH and ALF patients**

To understand the influence of the TReg in AH and ALF, we evaluated the balance between the frequency of TReg with the innate and adaptive immune cells studied. Tables 5 and 6 reveal the change in the frequencies of TReg (CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup>) and migratory T helper frequencies (CD4<sup>+</sup>CD29<sup>+</sup>CD44<sup>+</sup>) in viral ALF after HAV stimulation and in non-viral ALF after mitogen stimulation. Figure 4A shows an elevated TReg-to-T-helper ratio in the HAV-induced ALF patients after HAV stimulation compared to the AH patients and the HC subjects. No changes in the ratios between the TReg and the other phenotypes were observed. After mitogen stimulation, the imbalance between the TReg-to-T helper ratio was significantly reduced in the non-viral ALF patients compared to the AH patients (Figure 4B). For the other investigated phenotypes, the alterations in this

ratio were not significant.

## **DISCUSSION**

Acute viral hepatitis was represented by hepatitis A cases in our study. There are a large number of outbreaks of hepatitis A in Brazil; HAV infection is the major etiology of AH and ALF<sup>[17,31,32]</sup>. Here, we introduced plasma mtDNA level as a new predictor for HAV-induced ALF syndrome. In our opinion, the gross elevation of mtDNA in the ALF patients resulted from massive liver necrosis, as expected. mtDNA, inflammatory and anti-inflammatory cytokines and effector cells are involved in drug-induced liver failure in murine models and in patients<sup>[5,8,33]</sup>.

Increased levels of cytokines and chemokines have been observed in all ALF and non-surviving patients, as described by other authors investigating both drug- and viral-induced ALF<sup>[5,34-36]</sup>. In our study, the high levels of IL-8 and IL-10 were predictive markers of death in acute liver disease.

Additionally, the imbalance between IL-10 and IL-12 levels has been noted in HBV-induced ALF<sup>[37]</sup>, indicating an ineffective attempt to activate the anti-inflammatory pathway<sup>[38-40]</sup>. The elevated plasma levels of IL-8 that were detected in all cases of ALF are also described in patients with drug-induced ALF and are correlated with granulocyte migration into the liver parenchyma<sup>[5,41]</sup>. The elevated levels of circulating IL-6 and TNFα, also described by others<sup>[42,43]</sup>, have been related to attempts

**Table 6** Variables from mitogen-stimulated peripheral blood mononuclear cells from non-viral acute hepatitis patients and healthy control subjects

Phenotypes/cytokines (PHA/LPS)	HC (n = 10)	AH (n = 8)	ALF (n = 5)	HC vs AH	HC vs ALF	AH vs ALF
PI of CD3 <sup>+</sup>	133.1 ± 71.12 (95.19-171.0)	173.3 ± 91.84 (96.51-250.1)	268.4 ± 101.6 (142.3-394.5)	ns	ns	ns
CD4 <sup>+</sup> CD25 <sup>+</sup> FoxP3 <sup>+</sup> (%)	17.23 ± 9.74 (12.03-22.42)	15.82 ± 8.13 (9.02-22.62)	6.84 ± 5.12 (0.48-13.2)	ns	ns	ns
CD4 <sup>+</sup> CD29 <sup>+</sup> CD44 <sup>+</sup> (%)	38.63 ± 18.37 (28.84-48.42)	21.84 ± 7.50 (15.56-28.11)	36.67 ± 14.54 (18.61-54.73)	ns	ns	ns
CD8 <sup>+</sup> CD29 <sup>+</sup> CD44 <sup>+</sup> (%)	39.76 ± 19.91 (29.15-50.36)	22.26 ± 11.16 (12.92-31.59)	29.59 ± 15.21 (10.71-48.47)	ns	ns	ns
CD3 <sup>+</sup> CD56 <sup>+</sup> CD16 <sup>-</sup> (%)	8.31 ± 6.75 (2.07-14.56)	6.33 ± 4.13 (2.88-9.79)	17.22 ± 4.94 (13.09-21.35)	ns	0.0289	0.0030
CD3 <sup>+</sup> CD56 <sup>low</sup> CD16 <sup>+</sup> (%)	12.70 ± 8.93 (4.44-20.96)	11.38 ± 4.67 (7.05-15.71)	27.66 ± 3.49 (22.11-33.21)	ns	0.0061	0.007
CD3 <sup>+</sup> CD56 <sup>+</sup> CD16 <sup>+</sup> (%)	13.66 ± 3.54 (11.77-15.55)	11.63 ± 3.01 (9.11-14.15)	8.52 ± 4.97 (2.35-14.70)	ns	ns	ns
IL-6 (pg/mL)	2625.33 ± 3320 (856.5-4394)	966 ± 622.6 (445.5-1486.0)	1309 ± 851.6 (251.60-2366)	ns	ns	ns
TNFα (pg/mL)	1675.20 ± 623.4 (1343-2007)	1497 ± 219.8 (1313-1681)	3217 ± 991.5 (1986-4448)	ns	0.0044	0.0016
IL-10 (pg/mL)	528.86 ± 755.1 (126.5-931.2)	217.1 ± 159.1 (84.12-350.2)	152.1 ± 126.4 (-4.89-309.0)	ns	ns	ns
IFNγ (pg/mL)	3379.1 ± 1869 (2383-4375)	2257 ± 2872 (-143.3-4658)	1378 ± 2533 (-1767-4524)	ns	ns	ns
IL-8 (pg/mL)	273.9 ± 116.3 (211.9-335.9)	293.9 ± 120.2 (193.4-394.4)	733.1 ± 404.8 (230.4-1236)	ns	0.0267	ns
IL-17 (pg/mL)	73.81 ± 107.0 (16.81-130.8)	36.31 ± 34.62 (7.36-65.25)	62.73 ± 5.78 (55.55-69.91)	ns	ns	ns

The differences between the non-viral acute liver failure (ALF) patients, the self-limited acute hepatitis (AH) patients, and the healthy control (HC) subjects were evaluated by intergroup comparisons using the Mann-Whitney *U*-test. The significance cutoff for all statistical analyses was defined as *P* < 0.05. IL: Interleukin; IFNγ: Interferon gamma; TNFα: Tumor necrosis factor alpha; PI: Proliferation index; HC: Healthy control; ns: Not significant.

at liver regeneration<sup>[44,45]</sup> and liver injury<sup>[46]</sup>, respectively. Therapeutic approaches targeting the clearance of inflammatory/toxic products (plasmapheresis, hemodiafiltration, and bioartificial livers) from the liver or anti-cytokine therapy are currently being considered<sup>[42,47-49]</sup> despite contradictory clinical results<sup>[50,51]</sup>.

Even though the profile of monocytes was not explored here, several studies showed the important role of these cells in association with their activation, migration to the liver, and differentiation into hepatic macrophages induced by growth-factor β and IL-10 in humans<sup>[52,53]</sup> and experimental animal models<sup>[54]</sup>. Production of the inflammatory cytokines TNF, IL1-β, IL-6, IL-8 and MCP-1 by hepatic macrophages has been associated with cytokine storm in liver injury<sup>[52,53]</sup>. These findings could explain the biological relevance of high levels of circulating IL-6, IL-8 and IL-10 in ALF patients with the worst outcomes, which were produced by activated monocytes/macrophages, by antigen presentation, and by T cell proliferation.

When we evaluated the linear correlation between coagulopathy/encephalopathy and the plasma variables studied, we observed that the INR and HE scores increased in ALF cases. mtDNA, IL-6, IL-10, IFNγ, TNFα and IL-8 were also significantly elevated and were positively correlated with the elevated INR and/or HE scores observed in severe liver disease. Thus, this study also showed that elevated mtDNA and

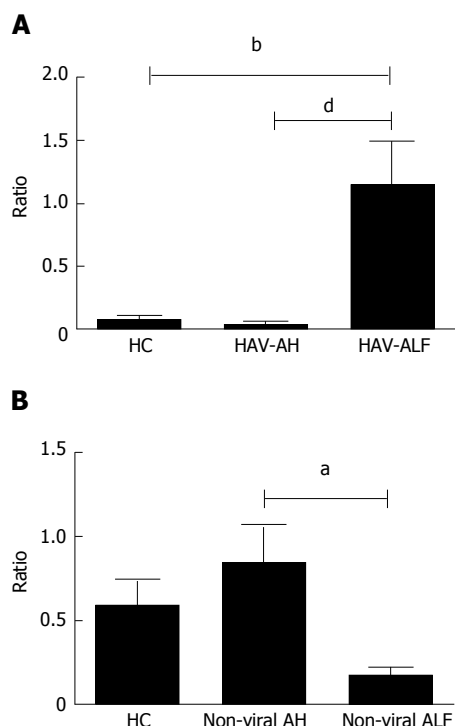
IL-10 are positively associated with the risk of ALF and mortality. Other authors described IL-10 as an important immunosuppressive cytokine that is released by TReg and is strongly expressed in HBV-induced acute-on-chronic liver failure<sup>[38,55,56]</sup>.

Indeed, the most puzzling fact revealed here was the anergic behavior of the PBMCs from HAV-induced AH and ALF after *in vitro* mitotic stimulation. This fact may be explained by PBMC clonal exhaustion<sup>[57-59]</sup> or may suggest that the TReg influence HAV Ag-primed PBMCs *in vivo* during AH and ALF syndrome<sup>[23]</sup>. In addition, when the TReg cells have been previously primed by a specific antigen (e.g., viral antigen), they may develop a non-specific suppressor activity, as described by others<sup>[60]</sup>.

Here, the impairment of the PBMC response was associated with liver dysfunction in patients with AH A. The high TReg cell frequencies in HAV-induced ALF and the increase in IL-10 after HAV Ag stimulation were consistent with the reduced frequency found for the Th17 migratory phenotype (CD4<sup>+</sup>CD29<sup>+</sup>CD44<sup>+</sup> and IL-17 secretion) and the modulation of the T lymphocyte (CD3<sup>+</sup>) and cytotoxic T cell (CD8<sup>+</sup>CD29<sup>+</sup>CD44<sup>+</sup>) phenotypes.

Our results suggest that the negative regulation of the TReg cells attempts to control liver inflammation and disease progression by reducing the Th17 migration to the liver tissue in patients with HAV-induced ALF. A similar profile of antigen-specific and unspecific stimulation was





**Figure 4** Imbalance between peripheral CD4<sup>+</sup> regulatory T cells and migratory T helper cells in viral and non-viral acute hepatitis cases. A: Comparison of the ratio of CD4<sup>+</sup> TReg-to-Thelper in HAV-induced acute liver disease (ALF and AH) and healthy controls (HC); B: Comparison of the ratio of CD4<sup>+</sup> TReg-to-Thelper cells in non-viral-induced acute liver disease (ALF and AH). <sup>a</sup>*P* < 0.05; <sup>b</sup>*P* < 0.01; <sup>d</sup>*P* < 0.001. HAV: Hepatitis A virus; AH: Acute hepatitis; ALF: Acute liver failure.

observed in patients with HEV-induced AH and ALF<sup>[59]</sup> and in chronic hepatitis B infection after anti-CD3/CD28 (unspecific) stimulation<sup>[56]</sup>. Our results did not confirm the TReg influence in non-viral ALF patients, which corroborates other results<sup>[61-63]</sup>. The expansion of T helper cells (Th17) and the suppression TReg cell production are involved in the mechanisms of liver damage in drug-induced liver disease<sup>[61,63]</sup>.

In HAV-AH, T helper cell proliferation was increased after HAV Ag stimulation and was reduced after mitogen stimulation. The scarce literature available describes defects in cell signaling in CD4<sup>+</sup> T cells that are secondary to ALF<sup>[59]</sup>. Other authors reported that an increase in TReg cells and a decrease in Th17 cells are associated with the survival of HBV-related acute-on-chronic liver failure patients<sup>[56]</sup>, although contradictory opinions have been reported<sup>[38,64]</sup>. In our study, a similar profile was exhibited by migratory cytotoxic T cells (CD8<sup>+</sup>CD29<sup>+</sup>CD44<sup>+</sup>) for both antigens (viral and mitogen) in HAV-induced AH and ALF. Impaired proliferation was also demonstrated with HEV Ag (pORF3), which was dependent on ERK activation (a member of mitogen-activated protein kinase) and involved in cell proliferation through the TCR/CD3 complex<sup>[65]</sup>.

A linear reduction of NK<sup>bright</sup>, NK<sup>dim</sup> and NKT cell reactivity occurred after mitogen stimulation in patients with HAV-induced AH and ALF, which was reversed

by HAV Ag-stimulation. The loss of NK<sup>dim</sup> reactivity in our ALF patients corroborated the suppressor function of the TReg cells, as described above, which appears to modulate the NK-mediated liver injury. A marked elevation in the frequency of NK<sup>bright</sup> and NKT cells in patients with HAV-induced ALF reinforces the importance of these cells in liver injury<sup>[19,66-69]</sup>.

The significant reduction in the secreted TNF $\alpha$  levels following the HAV Ag-stimulation of patients with HAV-induced AH and ALF shown here was also observed by other authors in HEV-induced AH and ALF<sup>[59]</sup>. However, TNF $\alpha$ , IL-17 and T helper cell reactivity are positively correlated with the progression to chronic liver disease and acute-on-chronic liver failure in hepatitis B infection<sup>[39,64]</sup>. In addition, Zhou *et al.*<sup>[70]</sup> (2012) observed a reduced frequency of the CD4<sup>+</sup>IL-2<sup>+</sup>IFN $\gamma$ <sup>+</sup>TNF<sup>+</sup> population after resolution of hepatitis A, suggesting an increased risk of hepatitis relapse. We observed that the frequency of T cells was not reduced in mitogen stimulated non-viral-induced AH and ALF. The NK<sup>bright</sup> and NK<sup>dim</sup> cells with TNF $\alpha$  and IL-8 secretion were significantly elevated in patients with ALF compared to patients with AH and the HC subjects, as expected. The literature describes that the NK cells have an important role in liver damage during non-viral-induced liver diseases and contribute to ALF progression<sup>[7,33]</sup>.

The relative weaknesses of our study included the variance in the plasma cytokine levels, the age of the patients, and the timing of sampling during the evolution of the disease. To minimize the effect of time on our analysis, the blood collection was performed considering the clinical manifestations in self-limited AH and the time of liver failure diagnosis and hospital admission for ALF patients. The sample size was small because the participants who were in the acute symptomatic phase (including pain and malaise) had to agree to the collection of additional samples for cellular immune response investigation; many patients did not return to the ambulatory clinic after resolution of their infection, hindering longitudinal assessment.

In conclusion, The increase of systemically released inflammatory and anti-inflammatory products is associated with AH and ALF. mtDNA and IL-10 may be useful clinical markers as part of a panel to indicate viral (HAV) and non-viral liver disease outcome. These markers, along with IL-8, may be useful to predict death. The anergic behavior of mononuclear cells in fulminant hepatitis A may, in part, be a consequence of the predominant TReg influence that is exclusively detected in HAV infection. Taken together, our results provide additional information to understand the complex immunological disturbances presented during ALF syndrome. Additional efforts are necessary to clarify the anergy mechanism in HAV infection.

## ACKNOWLEDGMENTS

The authors thank the blood donors and patients who

participated in this study, and Renata Tourinho for standardizing the molecular assay for detecting the HAV-RNA in biological samples.

## COMMENTS

### Background

The immune response can induce gross inflammation and consequently liver damage in acute liver diseases, independently of etiology. The role of immune cells in inducing acute liver failure (ALF) in hepatitis A infection is still unknown. High levels of systemic inflammatory products and *in vitro* immune response can be helpful markers to evaluate the necessity for liver transplantation, mainly in hepatitis A patients. Additionally, to minimize the effects of liver failure caused by hepatitis A, universal vaccination should be improved in developing countries such as Brazil.

### Research frontiers

Circulating cytokines have been associated with liver failure. Imbalance between peripheral regulatory T cells and helper T cells has been correlated with the worst outcome in hepatitis B-induced liver failure, a disease preventable by vaccination.

### Innovations and breakthroughs

This is the first study evaluating biological markers to show the necessity of liver transplantation, particularly in hepatitis A patients. The role of antigen-specific T cells during ALF caused by hepatitis A virus was investigated in a pioneering way in comparison to non-viral etiologies.

### Applications

Non-invasive samples as early prognostic markers are urgently needed to determine the necessity of liver transplantation. These findings can be helpful to highlight the development of facilities for laboratory diagnostics in acute liver diseases progression. This study supports the mass vaccination against hepatitis A in developing countries.

### Peer-review

The authors describe interesting findings in the circulating cytokines, mitochondrial damage and cell proliferation when comparing different clinical statuses in acute liver diseases (self-limited acute hepatitis and ALF) and healthy controls. The correlation of these factors with the severity of liver disease and outcome is also interesting. This study evaluated accurate markers to predict the necessity for liver transplantation, which is very important for guiding clinical work. Data from T cells in the hepatitis A cohort with liver failure, as the authors note, have not been reported.

## REFERENCES

- 1 Lee HS, Choi GH, Joo DJ, Kim MS, Kim SI, Han KH, Ahn SH, Kim DY, Park JY, Choi JS. Prognostic value of model for end-stage liver disease scores in patients with fulminant hepatic failure. *Transplant Proc* 2013; **45**: 2992-2994 [PMID: 24157020 DOI: 10.1016/j.transproceed.2013.08.036]
- 2 Jayakumar S, Chowdhury R, Ye C, Karvellas CJ. Fulminant viral hepatitis. *Crit Care Clin* 2013; **29**: 677-697 [PMID: 23830658 DOI: 10.1016/j.ccc.2013.03.013]
- 3 Soundravalu R, Narayanan P, Bhat BV, Soundraragavan J, Setia S. Fulminant hepatic failure in an infant with severe dengue infection. *Indian J Pediatr* 2010; **77**: 435-437 [PMID: 20140763 DOI: 10.1007/s12098-010-0027-z]
- 4 Sugawara K, Nakayama N, Mochida S. Acute liver failure in Japan: definition, classification, and prediction of the outcome. *J Gastroenterol* 2012; **47**: 849-861 [PMID: 22825549 DOI: 10.1007/s00535-012-0624-x]
- 5 Marques PE, Amaral SS, Pires DA, Nogueira LL, Soriani FM, Lima BH, Lopes GA, Russo RC, Avila TV, Melgaço JG, Oliveira AG, Pinto MA, Lima CX, De Paula AM, Cara DC, Leite MF, Teixeira MM, Menezes GB. Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure. *Hepatology* 2012; **56**: 1971-1982 [PMID: 22532075 DOI: 10.1002/hep.25801]
- 6 McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, Jaeschke H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. *J Clin Invest* 2012; **122**: 1574-1583 [PMID: 22378043 DOI: 10.1172/JCI59755]
- 7 Foureau DM, Walling TL, Maddukuri V, Anderson W, Culbreath K, Kleiner DE, Ahrens WA, Jacobs C, Watkins PB, Fontana RJ, Chalasani N, Talwalkar J, Lee WM, Stolz A, Serrano J, Bonkovsky HL. Comparative analysis of portal hepatic infiltrating leucocytes in acute drug-induced liver injury, idiopathic autoimmune and viral hepatitis. *Clin Exp Immunol* 2015; **180**: 40-51 [PMID: 25418487 DOI: 10.1111/cei.12558]
- 8 McGill MR, Staggs VS, Sharpe MR, Lee WM, Jaeschke H. Serum mitochondrial biomarkers and damage-associated molecular patterns are higher in acetaminophen overdose patients with poor outcome. *Hepatology* 2014; **60**: 1336-1345 [PMID: 24923598 DOI: 10.1002/hep.27265]
- 9 Azhar N, Ziraldo C, Barclay D, Rudnick DA, Squires RH, Vodovotz Y. Analysis of serum inflammatory mediators identifies unique dynamic networks associated with death and spontaneous survival in pediatric acute liver failure. *PLoS One* 2013; **8**: e78202 [PMID: 24244295 DOI: 10.1371/journal.pone.0078202]
- 10 Bucuvalas J, Filipovich L, Yazigi N, Narkewicz MR, Ng V, Belle SH, Zhang S, Squires RH. Immunophenotype predicts outcome in pediatric acute liver failure. *J Pediatr Gastroenterol Nutr* 2013; **56**: 311-315 [PMID: 23111765 DOI: 10.1097/MPG.0b013e31827a78b2]
- 11 Llovet JM, Peña CE, Lathia CD, Shan M, Meinhardt G, Bruix J. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2012; **18**: 2290-2300 [PMID: 22374331 DOI: 10.1158/1078-0432.CCR-11-2175]
- 12 Kasztelan-Szczerbinska B, Surdacka A, Slomka M, Rolinski J, Celinski K, Cichoz-Lach H, Madro A, Szczerbinski M. Angiogenesis-related biomarkers in patients with alcoholic liver disease: their association with liver disease complications and outcome. *Mediators Inflamm* 2014; **2014**: 673032 [PMID: 24959006 DOI: 10.1155/2014/673032]
- 13 Baquerizo A, Anselmo D, Shackleton C, Chen TW, Cao C, Weaver M, Gornbein J, Geevarghese S, Nissen N, Farmer D, Demetriou A, Busuttil RW. Phosphorus ans an early predictive factor in patients with acute liver failure. *Transplantation* 2003; **75**: 2007-2014 [PMID: 12829902 DOI: 10.1097/01.TP.0000063219.21313.32]
- 14 Trujillo-Ochoa JL, Corral-Jara KF, Escobedo-Meléndez G, Realpe M, Panduro A, Roman S, Fierro NA. T-helper 17-related cytokines and IgE antibodies during hepatitis A virus infection in children. *Mem Inst Oswaldo Cruz* 2015; **110**: 263-266 [PMID: 25946253 DOI: 10.1590/0074-02760140309]
- 15 Castro-García FP, Corral-Jara KF, Escobedo-Melendez G, Sandoval-Hernandez MA, Rosenstein Y, Roman S, Panduro A, Fierro NA. Conjugated bilirubin affects cytokine profiles in hepatitis A virus infection by modulating function of signal transducer and activator of transcription factors. *Immunology* 2014; **143**: 578-587 [PMID: 24943111 DOI: 10.1111/imm.12336]
- 16 Wang DW, Yin YM, Yao YM. Advances in the management of acute liver failure. *World J Gastroenterol* 2013; **19**: 7069-7077 [PMID: 24222950 DOI: 10.3748/wjg.v19.i41.7069]
- 17 Vitral CL, Souto FJ, Gaspar AM. Changing epidemiology of hepatitis A in Brazil: reassessing immunization policy. *J Viral Hepat* 2008; **15** Suppl 2: 22-25 [PMID: 18837829 DOI: 10.1111/j.1365-2893.2008.01024.x]
- 18 Vitral CL, da Silva-Nunes M, Pinto MA, de Oliveira JM, Gaspar AM, Pereira RC, Ferreira MU. Hepatitis A and E seroprevalence and associated risk factors: a community-based cross-sectional survey in rural Amazonia. *BMC Infect Dis* 2014; **14**: 458 [PMID: 25149658 DOI: 10.1186/1471-2334-14-458]
- 19 Cho H. Phenotypic characteristics of natural killer cells in acute

- hepatitis. *J Microbiol* 2013; **51**: 247-251 [PMID: 23625228 DOI: 10.1007/s12275-013-2522-1]
- 20 **Cooper MA**, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001; **22**: 633-640 [PMID: 11698225]
- 21 **Björkström NK**, Ljunggren HG, Sandberg JK. CD56 negative NK cells: origin, function, and role in chronic viral disease. *Trends Immunol* 2010; **31**: 401-406 [PMID: 20829113 DOI: 10.1016/j.it.2010.08.003]
- 22 **Lugli E**, Marcenaro E, Mavilio D. NK Cell Subset Redistribution during the Course of Viral Infections. *Front Immunol* 2014; **5**: 390 [PMID: 25177322 DOI: 10.3389/fimmu.2014.00390]
- 23 **Perrella A**, Vitiello L, Atripaldi L, Sbreglia C, Grattacaso S, Bellopede P, Patarino T, Morelli G, Altamura S, Racioppi L, Perrella O. Impaired function of CD4<sup>+</sup>/CD25<sup>+</sup> T regulatory lymphocytes characterizes the self-limited hepatitis A virus infection. *J Gastroenterol Hepatol* 2008; **23**: e105-e110 [PMID: 17645467 DOI: 10.1111/j.1440-1746.2007.05008.x]
- 24 **Weidmann M**, Armbruster K, Hufert FT. Challenges in designing a Taqman-based multiplex assay for the simultaneous detection of Herpes simplex virus types 1 and 2 and Varicella-zoster virus. *J Clin Virol* 2008; **42**: 326-334 [PMID: 18439871 DOI: 10.1016/j.jcv.2008.03.005]
- 25 **Motta-Castro AR**, Yoshida CF, Lemos ER, Oliveira JM, Cunha RV, Lewis-Ximenez LL, Cabello PH, Lima KM, Martins RM. Seroprevalence of Hepatitis B virus infection among an Afro-descendant community in Brazil. *Mem Inst Oswaldo Cruz* 2003; **98**: 13-17 [PMID: 12700856 DOI: 10.1590/S0074-02762003000100002]
- 26 **Zhang Q**, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 2010; **464**: 104-107 [PMID: 20203610 DOI: 10.1038/nature08780]
- 27 **Gaspar AM**, Vitral CL, Yoshida CF, Schatzmayr HG. Primary isolation of a Brazilian strain of hepatitis A virus (HAF-203) and growth in a primate cell line (FRhK-4). *Braz J Med Biol Res* 1992; **25**: 697-705 [PMID: 1342600]
- 28 **Carollo M**, Palazzo R, Bianco M, Smits K, Mascart F, Ausiello CM. Antigen-specific responses assessment for the evaluation of Bordetella pertussis T cell immunity in humans. *Vaccine* 2012; **30**: 1667-1674 [PMID: 22230582 DOI: 10.1016/j.vaccine.2011.12.104]
- 29 **Dalgaard TS**, Norup LR, Rubbenstroth D, Watrang E, Juul-Madsen HR. Flow cytometric assessment of antigen-specific proliferation in peripheral chicken T cells by CFSE dilution. *Vet Immunol Immunopathol* 2010; **138**: 85-94 [PMID: 20739071 DOI: 10.1016/j.vetimm.2010.07.010]
- 30 **Moore SM**, Wilkerson MJ, Davis RD, Wyatt CR, Briggs DJ. Detection of cellular immunity to rabies antigens in human vaccinees. *J Clin Immunol* 2006; **26**: 533-545 [PMID: 16964551 DOI: 10.1007/s10875-006-9044-0]
- 31 **Santos DC**, Martinho JM, Pacheco-Moreira LF, Araújo CC, Oliveira BC, Lago BV, Pinto MA, Paula VS. Fulminant hepatitis failure in adults and children from a Public Hospital in Rio de Janeiro, Brazil. *Braz J Infect Dis* 2009; **13**: 323-329 [PMID: 20428629 DOI: 10.1590/S1413-86702009000500002]
- 32 **Lima LR**, De Almeida AJ, Tourinho Rdos S, Hasselmann B, Ximenez LL, De Paula VS. Evidence of hepatitis A virus person-to-person transmission in household outbreaks. *PLoS One* 2014; **9**: e102925 [PMID: 25050760 DOI: 10.1371/journal.pone.0102925]
- 33 **dos Santos DC**, Neves PC, Azeredo EL, Pelajo-Machado M, Martinho JM, Pacheco-Moreira LF, Araújo CC, Cruz OG, de Oliveira JM, Pinto MA. Activated lymphocytes and high liver expression of IFN- $\gamma$  are associated with fulminant hepatic failure in patients. *Liver Int* 2012; **32**: 147-157 [PMID: 22098464 DOI: 10.1111/j.1478-3231.2011.02654.x]
- 34 **Sekiyama KD**, Yoshida M, Thomson AW. Circulating proinflammatory cytokines (IL-1  $\beta$ , TNF- $\alpha$ , and IL-6) and IL-1 receptor antagonist (IL-1Ra) in fulminant hepatic failure and acute hepatitis. *Clin Exp Immunol* 1994; **98**: 71-77 [PMID: 7923888 DOI: 10.1111/j.1365-2249.1994.tb06609.x]
- 35 **Steuerwald NM**, Foureau DM, Norton HJ, Zhou J, Parsons JC, Chalasani N, Fontana RJ, Watkins PB, Lee WM, Reddy KR, Stolz A, Talwalkar J, Davern T, Saha D, Bell LN, Barnhart H, Gu J, Serrano J, Bonkovsky HL. Profiles of serum cytokines in acute drug-induced liver injury and their prognostic significance. *PLoS One* 2013; **8**: e81974 [PMID: 24386086 DOI: 10.1371/journal.pone.0081974]
- 36 **Wang JY**, Wang XL, Liu P. Detection of serum TNF- $\alpha$ , IFN- $\beta$ , IL-6 and IL-8 in patients with hepatitis B. *World J Gastroenterol* 1999; **5**: 38-40 [PMID: 11819382 DOI: 10.3748/wjg.v5.i1.38]
- 37 **Leifeld L**, Cheng S, Ramakers J, Dumoulin FL, Trautwein C, Sauerbruch T, Spengler U. Imbalanced intrahepatic expression of interleukin 12, interferon gamma, and interleukin 10 in fulminant hepatitis B. *Hepatology* 2002; **36**: 1001-1008 [PMID: 12297850 DOI: 10.1053/jhep.2002.35532]
- 38 **Wang Q**, Zheng Y, Huang Z, Tian Y, Zhou J, Mao Q, Wu Y, Ni B. Activated IL-23/IL-17 pathway closely correlates with increased Foxp3 expression in livers of chronic hepatitis B patients. *BMC Immunol* 2011; **12**: 25 [PMID: 21489307 DOI: 10.1186/1471-2172-12-25]
- 39 **Ye Y**, Xie X, Yu J, Zhou L, Xie H, Jiang G, Yu X, Zhang W, Wu J, Zheng S. Involvement of Th17 and Th1 effector responses in patients with Hepatitis B. *J Clin Immunol* 2010; **30**: 546-555 [PMID: 20393789 DOI: 10.1007/s10875-010-9416-3]
- 40 **Xu D**, Fu J, Jin L, Zhang H, Zhou C, Zou Z, Zhao JM, Zhang B, Shi M, Ding X, Tang Z, Fu YX, Wang FS. Circulating and liver resident CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. *J Immunol* 2006; **177**: 739-747 [PMID: 16785573 DOI: 10.4049/jimmunol.177.1.739]
- 41 **dos Santos DC**, da Silva Gomes Martinho JM, Pacheco-Moreira LF, Carvalho Viana de Araújo C, Caroli-Bottino A, Pannain VL, Soares Trinta K, Gandini M, da Costa Neves PC, de Souza Matos DC, Gonçalves Caputo LF, Pelajo-Machado M, Alves Pinto M. Eosinophils involved in fulminant hepatic failure are associated with high interleukin-6 expression and absence of interleukin-5 in liver and peripheral blood. *Liver Int* 2009; **29**: 544-551 [PMID: 19323781 DOI: 10.1111/j.1478-3231.2008.01872.x]
- 42 **Topuria D**, Kakabadze Z, Lobdjanidze N, Chavchanidze N. Anti-hepato-cytotoxic serum treatment results in acute liver failure. *Georgian Med News* 2006; **(130)**: 111-115 [PMID: 16510928]
- 43 **Yamada K**, Yamamoto Y, Uchiyama A, Ito R, Aoki Y, Uchida Y, Nagasawa H, Kimura H, Ichiyama T, Fukao T, Kohno Y. Successful treatment of neonatal herpes simplex-type 1 infection complicated by hemophagocytic lymphohistiocytosis and acute liver failure. *Tohoku J Exp Med* 2008; **214**: 1-5 [PMID: 18212481 DOI: 10.1620/tjem.214.1]
- 44 **Tiberio GA**, Tiberio L, Benetti A, Cervi E, Montani N, Dreano M, Garotta G, Cerea K, Steinberg N, Pandolfo G, Ferrari-Bravo A, Mazzoleni G, Giulini SM, Schiaffonati L. IL-6 Promotes compensatory liver regeneration in cirrhotic rat after partial hepatectomy. *Cytokine* 2008; **42**: 372-378 [PMID: 18455423 DOI: 10.1016/j.cyt.2008.03.012]
- 45 **Best DH**, Butz GM, Coleman WB. Cytokine-dependent activation of small hepatocyte-like progenitor cells in retrorsine-induced rat liver injury. *Exp Mol Pathol* 2010; **88**: 7-14 [PMID: 19874816 DOI: 10.1016/j.yexmp.2009.10.009]
- 46 **Jia B**, Guo M, Li G, Yu D, Zhang X, Lan K, Deng Q. Hepatitis B virus core protein sensitizes hepatocytes to tumor necrosis factor-induced apoptosis by suppression of the phosphorylation of mitogen-activated protein kinase 7. *J Virol* 2015; **89**: 2041-2051 [PMID: 25428880 DOI: 10.1128/JVI.03106-14]
- 47 **Brenndörfer ED**, Weiland M, Frelin L, Derk E, Ahlén G, Jiao J, Bode JG, Sällberg M. Anti-tumor necrosis factor  $\alpha$  treatment promotes apoptosis and prevents liver regeneration in a transgenic mouse model of chronic hepatitis C. *Hepatology* 2010; **52**: 1553-1563 [PMID: 20886569 DOI: 10.1002/hep.23870]
- 48 **Akdogan M**, Camci C, Gurakar A, Gilcher R, Alamian S, Wright H, Nour B, Sebastian A. The effect of total plasma exchange on fulminant hepatic failure. *J Clin Apher* 2006; **21**: 96-99 [PMID:

- 16142721 DOI: 10.1002/jca.20064]
- 49 **Ash SR**. Powdered sorbent liver dialysis and pheresis in treatment of hepatic failure. *Ther Apher* 2001; **5**: 404-416 [PMID: 11778927 DOI: 10.1046/j.1526-0968.2001.00384.x]
- 50 **Laszikova E**, Prazak J, Ryska O, Koblihova E, Tyll T, Ryska M. Fractionated plasmatic separation and adsorption does not alter haemodynamic parameters in experimental acute liver failure. *Neuro Endocrinol Lett* 2014; **35**: 280-284 [PMID: 25038598]
- 51 **Cisneros-Garza LE**, Muñoz-Ramírez Mdel R, Muñoz-Espinoza LE, Ruiz Velasco JA, Moreno-Alcántar R, Marín-López E, Méndez-Sánchez N. The molecular adsorbent recirculating system as a liver support system: summary of Mexican experience. *Ann Hepatol* 2014; **13**: 240-247 [PMID: 24552866]
- 52 **Liaskou E**, Zimmermann HW, Li KK, Oo YH, Suresh S, Stamataki Z, Qureshi O, Lalor PF, Shaw J, Syn WK, Curbishley SM, Adams DH. Monocyte subsets in human liver disease show distinct phenotypic and functional characteristics. *Hepatology* 2013; **57**: 385-398 [PMID: 22911542 DOI: 10.1002/hep.26016]
- 53 **Antoniades CG**, Quaglia A, Taams LS, Mitry RR, Hussain M, Abeles R, Possamai LA, Bruce M, McPhail M, Starling C, Wagner B, Barnardo A, Pomplun S, Auzinger G, Bernal W, Heaton N, Vergani D, Thursz MR, Wendon J. Source and characterization of hepatic macrophages in acetaminophen-induced acute liver failure in humans. *Hepatology* 2012; **56**: 735-746 [PMID: 22334567 DOI: 10.1002/hep.25657]
- 54 **Zimmermann HW**, Trautwein C, Tacke F. Functional role of monocytes and macrophages for the inflammatory response in acute liver injury. *Front Physiol* 2012; **3**: 56 [PMID: 23091461 DOI: 10.3389/fphys.2012.00056]
- 55 **Dunn C**, Peppas D, Khanna P, Nebbia G, Jones M, Brendish N, Lascar RM, Brown D, Gilson RJ, Tedder RJ, Dusheiko GM, Jacobs M, Klennerman P, Maini MK. Temporal analysis of early immune responses in patients with acute hepatitis B virus infection. *Gastroenterology* 2009; **137**: 1289-1300 [PMID: 19591831 DOI: 10.1053/j.gastro.2009.06.054]
- 56 **Liang XS**, Li CZ, Zhou Y, Yin W, Liu YY, Fan WH. Changes in circulating Foxp3(+) regulatory T cells and interleukin-17-producing T helper cells during HBV-related acute-on-chronic liver failure. *World J Gastroenterol* 2014; **20**: 8558-8571 [PMID: 25024610 DOI: 10.3748/wjg.v20.i26.8558]
- 57 **Azeredo EL**, Neves-Souza PC, Alvarenga AR, Reis SR, Torrentes-Carvalho A, Zagne SM, Nogueira RM, Oliveira-Pinto LM, Kubelka CF. Differential regulation of toll-like receptor-2, toll-like receptor-4, CD16 and human leucocyte antigen-DR on peripheral blood monocytes during mild and severe dengue fever. *Immunology* 2010; **130**: 202-216 [PMID: 20113369 DOI: 10.1111/j.1365-2567.2009.03224.x]
- 58 **Srivastava R**, Aggarwal R, Jameel S, Puri P, Gupta VK, Ramesh VS, Bhatia S, Naik S. Cellular immune responses in acute hepatitis E virus infection to the viral open reading frame 2 protein. *Viral Immunol* 2007; **20**: 56-65 [PMID: 17425421 DOI: 10.1089/vim.2006.0053]
- 59 **Srivastava R**, Aggarwal R, Sachdeva S, Alam MI, Jameel S, Naik S. Adaptive immune responses during acute uncomplicated and fulminant hepatitis E. *J Gastroenterol Hepatol* 2011; **26**: 306-311 [PMID: 21143520 DOI: 10.1111/j.1440-1746.2010.06356.x]
- 60 **Thornton AM**, Shevach EM. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. *J Immunol* 2000; **164**: 183-190 [PMID: 10605010 DOI: 10.4049/jimmunol.164.1.183]
- 61 **Wang X**, Zhang L, Jiang Z. T-helper cell-mediated factors in drug-induced liver injury. *J Appl Toxicol* 2015; **35**: 695-700 [PMID: 25752261 DOI: 10.1002/jat.3115]
- 62 **Masubuchi Y**, Sugiyama S, Horie T. Th1/Th2 cytokine balance as a determinant of acetaminophen-induced liver injury. *Chem Biol Interact* 2009; **179**: 273-279 [PMID: 19014921 DOI: 10.1016/j.cbi.2008.10.028]
- 63 **Wang X**, Jiang Z, Cao W, Yuan Z, Sun L, Zhang L. Th17/Treg imbalance in triptolide-induced liver injury. *Fitoterapia* 2014; **93**: 245-251 [PMID: 24444892 DOI: 10.1016/j.fitote.2014.01.006]
- 64 **Dong X**, Gong Y, Zeng H, Hao Y, Wang X, Hou J, Wang J, Li J, Zhu Y, Liu H, Han J, Zhou H, Shen L, Gao T, Zhou T, Yang S, Li S, Chen Y, Meng Q, Li H. Imbalance between circulating CD4+ regulatory T and conventional T lymphocytes in patients with HBV-related acute-on-chronic liver failure. *Liver Int* 2013; **33**: 1517-1526 [PMID: 23869954 DOI: 10.1111/liv.12248]
- 65 **Kar-Roy A**, Korkaya H, Oberoi R, Lal SK, Jameel S. The hepatitis E virus open reading frame 3 protein activates ERK through binding and inhibition of the MAPK phosphatase. *J Biol Chem* 2004; **279**: 28345-28357 [PMID: 15096509 DOI: 10.1074/jbc.M400457200]
- 66 **Ding L**, Chen T, Wang XJ, Zhou L, Shi AC, Ning Q. CD69+NK cells contribute to the murine hepatitis virus strain 3-induced murine hepatitis. *J Huazhong Univ Sci Technolog Med Sci* 2013; **33**: 505-510 [PMID: 23904369 DOI: 10.1007/s11596-013-1150-7]
- 67 **Bonorino P**, Ramzan M, Camous X, Dufeu-Duchesne T, Thélou MA, Sturm N, Dariz A, Guillermet C, Pernollet M, Zarski JP, Marche PN, Leroy V, Jouvin-Marche E. Fine characterization of intrahepatic NK cells expressing natural killer receptors in chronic hepatitis B and C. *J Hepatol* 2009; **51**: 458-467 [PMID: 19596474 DOI: 10.1016/j.jhep.2009.05.030]
- 68 **Golden-Mason L**, Castelblanco N, O'Farrelly C, Rosen HR. Phenotypic and functional changes of cytotoxic CD56pos natural T cells determine outcome of acute hepatitis C virus infection. *J Virol* 2007; **81**: 9292-9298 [PMID: 17553896 DOI: 10.1128/JVI.00834-07]
- 69 **Meier UC**, Owen RE, Taylor E, Worth A, Naoumov N, Willberg C, Tang K, Newton P, Pellegrino P, Williams I, Klennerman P, Borrow P. Shared alterations in NK cell frequency, phenotype, and function in chronic human immunodeficiency virus and hepatitis C virus infections. *J Virol* 2005; **79**: 12365-12374 [PMID: 16160163 DOI: 10.1128/JVI.79.19.12365-12374.2005]
- 70 **Zhou Y**, Callendret B, Xu D, Brasky KM, Feng Z, Hensley LL, Guedj J, Perelson AS, Lemon SM, Lanford RE, Walker CM. Dominance of the CD4(+) T helper cell response during acute resolving hepatitis A virus infection. *J Exp Med* 2012; **209**: 1481-1492 [PMID: 22753925 DOI: 10.1084/jem.20111906]

**P- Reviewer:** Kulkarni S, Kumar R, Zhao YR **S- Editor:** Gong ZM

**L- Editor:** A **E- Editor:** Li D





## Retrospective Study

# Systemic-to-pulmonary artery pressure ratio as a predictor of patient outcome following liver transplantation

Annette Rebel, Dung Nguyen, Brooke Bauer, Paul A Sloan, Amy DiLorenzo, Zaki-Udin Hassan

Annette Rebel, Dung Nguyen, Brooke Bauer, Paul A Sloan, Amy DiLorenzo, Zaki-Udin Hassan, Department of Anesthesiology and Surgery, Medical Center N 202, University of Kentucky, Lexington, KY 40536, United States

**Author contributions:** Rebel A and Bauer B collected and analyzed the data, and drafted the manuscript; Nguyen D, Sloan PA, DiLorenzo A and Hassan ZU provided analytical oversight and supervised the study; all authors contributed and revised the manuscript for importance; all authors have read and approved the final version to be published.

**Institutional review board statement:** The need for informed consent of study participants was waived by the Institutional Review Board since the de-identified data review demonstrated minimal risk to patient population.

**Informed consent statement:** The need for informed consent of study participants was waived by the Institutional Review Board.

**Conflict-of-interest statement:** The authors have no conflict of interest to report.

**Data sharing statement:** Technical appendix, statistical code, and dataset are available from the corresponding author at arebe2@email.uky.edu.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Annette Rebel, MD, Associate Professor, Department of Anesthesiology and Surgery, Medical Center N 202, University of Kentucky, 800 Rose Street, Lexington, KY 40536, United States. arebe2@uky.edu

Telephone: +1-859-3235956  
 Fax: +1-859-3231080

Received: June 22, 2016  
 Peer-review started: June 28, 2016  
 First decision: September 5, 2016  
 Revised: September 23, 2016  
 Accepted: October 22, 2016  
 Article in press: October 24, 2016  
 Published online: November 18, 2016

## Abstract

### AIM

To assess the value of the mean systemic-to-pulmonary artery pressure (MAP/mPAP) ratio for predicting outcomes following orthotopic liver transplant (OLT).

### METHODS

A retrospective data analysis was performed and data (mean arterial blood pressure, mean pulmonary artery pressure and Cardiac Index) were collected at several points during OLT. Outcomes evaluated were duration of postoperative endotracheal intubation [ET; minutes after intensive care unit (ICU) arrival], length of ICU stay, total hospitalization and frequency of immediate postoperative complications. A total of 91 patients were included in the data analysis. Based on the intraoperative course of the MAP/mPAP ratio, 2 hemodynamic responses were identified: Group 1 (MAP/mPAP ratio increase during anhepatic period with postreperfusion recovery,  $n = 66$ ); and Group 2 (MAP/mPAP ratio with no change during anhepatic period or decreased without recovery,  $n = 25$ ).

### RESULTS

The main finding was that the lack of increased MAP/mPAP ratio in the anhepatic period was associated with: (1) longer intubation times; and (2) prolonged ICU

stays and total hospitalization time, when compared to patients with an increase in MAP/mPAP ratio during the anhepatic period.

### CONCLUSION

The data from this retrospective study should raise awareness to the mean systemic to pulmonary artery pressure ratio as a potential indicator for poor outcome after OLT. Further prospective studies are needed for validation.

**Key words:** Anesthesiology; Liver transplantation; Right heart function; Outcome; Morbidity

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The aim of this study was to assess the value of the mean systemic-to-pulmonary artery pressure (MAP/mPAP) ratio for predicting outcomes following orthotopic liver transplant. The intraoperative pattern of this ratio has not been previously described. Performing a retrospective analysis we identified 2 different MAP/mPAP patterns: Group 1 (MAP/mPAP ratio increase during anhepatic period with postreperfusion recovery,  $n = 66$ ); and Group 2 (MAP/mPAP ratio with no change during anhepatic period or decreased without recovery,  $n = 25$ ). The main finding was that the lack of increased MAP/mPAP ratio in the anhepatic period was associated with longer intubation times, and prolonged hospitalization time.

Rebel A, Nguyen D, Bauer B, Sloan PA, DiLorenzo A, Hassan ZU. Systemic-to-pulmonary artery pressure ratio as a predictor of patient outcome following liver transplantation. *World J Hepatol* 2016; 8(32): 1384-1391 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i32/1384.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i32.1384>

### INTRODUCTION

The mean systemic-to-pulmonary artery pressure ratio (MAP/mPAP) has been shown to be a valuable predictor of outcomes following cardiac surgery. Previous studies documented that the MAP/mPAP ratio was easy to obtain during cardiac surgery, and correlated with the development of pulmonary hypertension and diastolic dysfunction<sup>[1-4]</sup>. Outcomes following orthotopic liver transplant (OLT) are dependent on the ability of the patient's cardiovascular system to compensate for the physiological stress related to OLT. While detailed and expensive cardiac evaluation is routinely performed on patients before OLT, the extent of cirrhotic cardiomyopathy and biventricular dysfunction is often underestimated<sup>[5,6]</sup>. Intraoperatively, due to circumstances related to advanced multi-organ disease, limited cardiac reserve and procedural related stressors including blood loss, fluid shifts, acidosis, or hypothermia,

the patient may present more hemodynamic challenges than anticipated<sup>[7]</sup>.

The MAP/mPAP ratio as a predictor of patient outcome following OLT has not been investigated. We hypothesized that the pattern of the MAP/mPAP ratio during the different stages of OLT may predict the ability of the circulatory system to compensate for the surgery related stress. If the MAP/mPAP pattern indicates sufficient cardiac reserve, the patient should have a better outcome than patients with a MAP/mPAP ratio that is less favorable. In order to more reliably risk stratify these patients undergoing OLT, we performed a retrospective data analysis to explore the feasibility of obtaining useable data during OLT. With desirable outcomes defined as less morbidity/mortality, decreased need for mechanical ventilation and shorter length of stay, the aim of this study was to assess the value of the MAP/mPAP ratio for predicting desirable outcomes following OLT.

### MATERIALS AND METHODS

The Institutional Review Board (IRB) reviewed the study protocol and gave approval access to an institutional database to retrieve patient information. The IRB waived the need for informed consent since the de-identified data review demonstrated minimal risk to patient population.

The retrospective data analysis was performed on patients undergoing OLT for end-stage liver disease in the time period from October 2011 through October 2014 at a single University Hospital. All patients undergoing OLT during this time period regardless of underlying liver disease, model for end-stage liver disease (MELD) score and age were included. Exclusion criteria were patients undergoing OLT as a combined procedure with kidney transplant, redo-OLT or OLT as a treatment for acute liver failure. Patient records with incomplete intraoperative information were excluded from the data analysis.

The selected time period was based on the absence of changes in surgical approach or staff (transplant surgeon/anesthesiology). All patients underwent cardiac evaluation with transthoracic echocardiography prior to listing for liver transplant. None of the included patients were diagnosed with or had signs of pulmonary hypertension prior to OLT.

All OLT were performed using the end-to-end inferior vena cava (IVC) anastomosis technique requiring total IVC cross-clamp during the anhepatic period. Using this technique, the anhepatic period was less than 60 min for all OLTs in this study period. Intraoperative anesthesia care for all patients followed a standardized protocol for anesthesia induction, intravenous access, monitoring and vasoactive medications. All patients remained intubated at the conclusion of their surgical procedure and were transported to a dedicated intensive care unit (ICU) for anesthesia emergence and recovery.

Patient demographic data were collected including preoperative creatinine level, comorbidities, MELD score,

**Table 1 Patient demographics and surgical characteristics**

	Age (yr)	Gender (F:M ratio)	MELD (score)	OR duration (min)	Crystalloid (mL)	Colloid (mL)	PRBC (units)	FFP (units)
Group 1 ( <i>n</i> = 66)	55.6 ± 8.6	24:42 (36%)	18.7 ± 8.4	400.9 ± 43.5	5804 ± 2824	1440 ± 972	3.3 ± 3.7	2.8 ± 3.0
Group 2 ( <i>n</i> = 25)	58.9 ± 5.4	7:18 (28%)	16.3 ± 6.9	427.9 ± 63.3	6086 ± 3424	1607 ± 626	3.9 ± 3.1	4.0 ± 4.6
<i>P</i> -value	0.081	0.189	0.204	0.081	0.690	0.428	0.473	0.148

Data are shown as mean ± SD. *P* value was obtained using paired *t*-test for all parameters except gender. The 1-tail exact binomial calculation with probability value of 0.5. Group 1 (*n* = 66): Patients with MAP/mPAP ratio increase during anhepatic period; Group 2 (*n* = 25): Patients with MAP/mPAP ratio decrease or no change during anhepatic period. MELD: Model For End-Stage Liver Disease score; OR duration: Anesthesia time from induction to ICU transfer; Crystalloid: Amount of intraoperative normal saline; Colloid: Amount of intraoperative 5% Albumin; PRBC: Packed red blood concentrate; FFP: Fresh frozen plasma; MAP/mPAP: Mean systemic-to-pulmonary artery pressure.

age and gender. Basic intraoperative information such as procedure time, intraoperative intravenous fluids and blood component therapy were extracted from the surgical records. During the retrospective chart review, the following intraoperative hemodynamic data were gathered: Mean arterial blood pressure, mean pulmonary artery pressure and Cardiac Index (CI). These hemodynamic parameters were collected at several time points during the surgical procedure: Baseline (30 min after incision), pre-anhepatic (1 h before IVC cross-clamp), anhepatic (15 min before reperfusion), neo-hepatic (15 min after reperfusion), and 1 h neo-hepatic (1 h after reperfusion).

#### MAP/mPAP patterns

Based on pilot observations, the MAP/mPAP ratio was expected to increase during the anhepatic phase. During the data analysis, patients indicating an increase in MAP/mPAP ratio by  $\geq 1$  from baseline to anhepatic phase were categorized into Group 1. Patients showing a decrease in MAP/mPAP ratio of  $\geq -1$  or no change ( $< 1$  to  $> -1$ ) from baseline to anhepatic phase were categorized into Group 2. We chose the anhepatic period as comparison to the baseline value because this surgical stage is characterized by a single hemodynamic alteration (preload reduction) and for a prolonged duration. In our institution, the anhepatic period is approximately 45-55 min. Therefore, all patients received a similar type of cardiac stress. To account for fluctuations in this anhepatic period we chose a measurement point at 15 min before reperfusion (IVC cross-clamp release) to allow sufficient equilibration time for reduction in cardiac preload caused by the IVC flow interruption.

#### Vasopressor use

Further chart review was performed regarding the use of vasoactive agents in the anhepatic phase of the operation. The following medications are available intraoperatively: Norepinephrine (NE), Epinephrine (EPI), Vasopressin (V), Dopamine (DOP) and Phenylephrine (PHE). Intraoperative documentation allowed the investigators to identify the frequency and dosing of vasoactive medication at 30 to 15 min before reperfusion (anhepatic measurement of MAP/mPAP ratio). The patients were sorted into three categories: No vasoactive medication use, low dose vasoactive medication use (NE

$< 0.05$   $\mu\text{g/kg}$  per minute, EPI  $< 0.03$   $\mu\text{g/kg}$  per minute, V  $< 0.03$  units/min, or PHE  $< 0.1$   $\mu\text{g/kg}$  per minute) and high dose vasoactive medication use (NE  $\geq 0.05$   $\mu\text{g/kg}$  per minute, EPI  $\geq 0.03$   $\mu\text{g/kg}$  per minute, V  $\geq 0.03$  units/min or any vasopressor combination).

#### Outcomes

The patient outcomes evaluated were duration of post-operative endotracheal intubation (ET, minutes after ICU arrival), length of ICU stay (LOS ICU, days post OLT) and length of hospital stay (LOS Total, days post OLT to hospital discharge). Postoperative complications were recorded if they occurred in the first 14 postoperative days after OLT. The frequency of reintubation within 48 h post extubation, need for renal replacement therapy and the need for ICU readmission were recorded. Mortality  $< 1$  mo post OLT was also recorded.

Data is reported as mean ± SD. Statistical analysis was performed by the paired *t*-test or  $\chi^2$  test. A *P* value of  $< 0.05$  was used to identify statistical significance.

## RESULTS

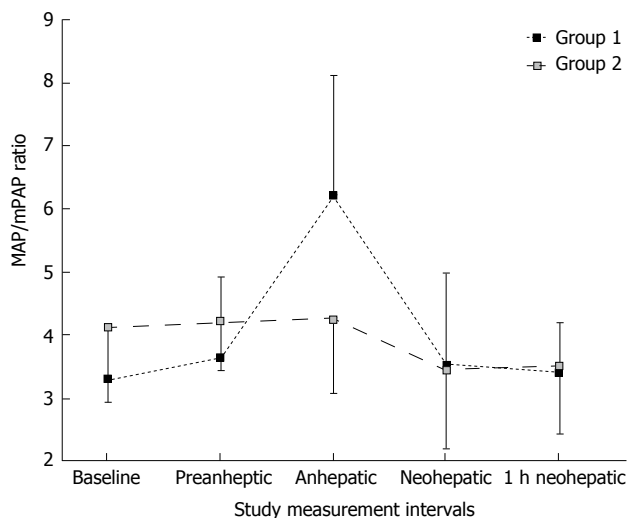
A total of 100 patients were included in the study. Due to incomplete data recordings, 9 patients were excluded from data analysis. Thus, data from a total of 91 patients was included in this analysis. The demographic patient characteristics and characterization of intraoperative course are shown in Table 1. Age, gender and MELD scores were equally distributed in both groups. The most common causes for end-stage liver disease in our patient collective were Hepatitis C related liver cirrhosis (48 patients, Group 1: 35 patients; Group 2: 13 patients), NASH related cirrhosis (21 patients, Group 1: 16 patients; Group 2: 5 patients) and alcohol induced liver cirrhosis (25 patients, Group 1: 16 patients; Group 2: 9 patients). Primary sclerosing cholangitis related liver cirrhosis was the leading diagnosis in 5 patients (all in Group 1). Other rare OLT indications were autoimmune hepatitis or alpha-trypsin 2 deficiency (one patient each, all Group 1). Two patients had hepato-pulmonary syndrome prior to OLT (both in Group 1).

Pre-OLT Creatinine was not different between the two groups (Group 1:  $1.26 \pm 0.67$  mg/dL, Group 2:  $1.30 \pm 0.82$  mg/dL). There were no significant differences in surgical duration, fluid requirements or use of blood

**Table 2** Mean systemic-to-pulmonary artery pressure ratio and cardiac index, measured at several times during the liver transplant: Baseline (30 min after incision), preanhepatic (1 h before inferior vena cava cross-clamp), anhepatic (15 min before reperfusion), neohepatic (15 min after reperfusion), and 1 h neohepatic (1 h after reperfusion)

	Group 1 MAP/mPAP increase during anhepatic period ( <i>n</i> = 66)	Group 2 MAP/mPAP no change or decrease during anhepatic period ( <i>n</i> = 25)	<i>P</i> -value
MAP/mPAP baseline	3.32 ± 0.734	4.13 ± 1.191	0.185
% change preanhepatic	0.278 ± 1.276	0.184 ± 1.268	0.754
% change anhepatic	2.892 ± 1.827	-0.088 ± 1.137	< 0.01 <sup>a</sup>
% change neohepatic	0.206 ± 1.411	-0.688 ± 1.325	< 0.01 <sup>a</sup>
% change 1 h neohepatic	0.098 ± 0.828	-0.611 ± 1.373	< 0.01 <sup>a</sup>
CI baseline	4.111 ± 1.229	4.214 ± 0.961	0.879
% change preanhepatic	0.039 ± 0.790	-0.204 ± 0.832	0.200
% change anhepatic	-1.606 ± 1.018	-1.188 ± 1.082	0.089
% change neohepatic	-0.327 ± 1.316	-0.058 ± 1.231	0.378
% change 1 h neohepatic	0.335 ± 1.106	0.454 ± 1.038	0.643
ET			
min	967 ± 1361	1719 ± 1933	0.040 <sup>a</sup>
h	16.1 ± 22.7	28.7 ± 32.2	
Median (min)	540	1045	
LOS ICU (d)	3.9 ± 4.4	12.1 ± 19.2	< 0.01 <sup>a</sup>
Median (d)	2	6	
LOS hospital (d)	12.0 ± 12.5	26.3 ± 33.2	< 0.01 <sup>a</sup>
Median (d)	8	11	

% change is calculated as difference from value to baseline value; data are shown as mean ± SD, and median value for ET and LOS. *P* value was obtained using paired *t*-test. <sup>a</sup>*P* < 0.05. ET (duration of postoperative endotracheal intubation): Minutes from ICU arrival to extubation to supplemental oxygen; LOS ICU: Length of stay in intensive care unit; LOS total: Time from ICU arrival to hospital discharge; MAP/mPAP: Mean systemic-to-pulmonary artery pressure ratio; CI: Cardiac index.



**Figure 1** Mean systemic-to-pulmonary artery pressure ratio. MAP/mPAP = Mean systemic-to-pulmonary artery pressure ratio, measured at several times during the liver transplant: Baseline (30 min after incision), preanhepatic (1 h before IVC cross-clamp), anhepatic (15 min before reperfusion), neohepatic (15 min after reperfusion), and 1 h neohepatic (1 h after reperfusion). Group 1 (*n* = 66): Patients with MAP/mPAP ratio increase during anhepatic period; Group 2 (*n* = 25): Patients with MAP/mPAP ratio decrease or no change during anhepatic period. Data are shown as mean ± SD. *P* value was obtained using paired *t*-test. IVC: Inferior vena cava; MAP/mPAP: Mean systemic-to-pulmonary artery pressure.

component therapy between the patient collectives (Table 1).

The intraoperative MAP/mPAP values for each group at the different measurement points are shown in Figure 1. MAP/mPAP values at baseline and at the prean-

hepatic period were slightly higher in Group 2 than in Group 1 (Table 2). However, the difference was not statistically significant. Patients in Group 1 demonstrated a significant increase in MAP/mPAP during the anhepatic period. The MAP/mPAP ratio pattern in Group 2 showed less variability throughout the surgical procedure (Figure 1). The analysis of the absolute MAP/mPAP values indicated that MAP/mPAP recovered to baseline range after reperfusion in both groups. However, the percent change of MAP/mPAP ratio at baseline to the different measurement points indicated that MAP/mPAP recovered to baseline after reperfusion in Group 1. The percent change of MAP/mPAP ratio was significantly decreased in Group 2 after reperfusion, lasting up to 1 h post reperfusion (Table 2). In Group 1 the MAP/mPAP ratio increased by 2.9 ± 1.8 during the anhepatic period, while MAP/mPAP ratio in Group 2 only changed by 0.1 ± 1.1 at this observation point. CI measurements did not parallel the hemodynamic patterns shown by the MAP/mPAP ratio (Table 2). There were no significant changes in CI between both groups at any observation points during the surgical procedure.

The majority of patients required vasoactive medication during the anhepatic phase and the presence of these medications may have influenced the hemodynamic measurements at the anhepatic measurement point (Table 3). Approximately 80% of patients in both groups received some vasopressor assistance during the anhepatic phase. Phenylephrine, Vasopressin and Norepinephrine were the most commonly used vaso-pressors. While approximately half of patients required some low-dose vasoactive medication, the frequency



**Table 3** Vasopressor use

Vasopressor use frequency	Group 1 MAP/mPAP increase during anhepatic period ( <i>n</i> = 66)	Group 2 MAP/mPAP no change or decrease during anhepatic period ( <i>n</i> = 25)	<i>P</i> -value
No vasopressor use	14	5	0.624
Low dose vasopressor use	39	12	0.341
High dose vasopressor use	13	8	0.214

Following medications are intraoperatively available: Norepinephrine (NE), Epinephrine (EPI), Vasopressin (V), Dopamine or Phenylephrine (PHE). Based on intraoperative documentation at 15 min before reperfusion (anhepatic measurement of MAP/mPAP ratio). The patients were sorted into three different categories: No vasoactive medication use; low dose vasoactive medication use (NE < 0.05 mcg/kg per minute or EPI < 0.03 mcg/kg per minute or V < 0.03 units/min or PHE < 0.1 µg/kg per minute) and high dose vasoactive medication use (NE ≥ 0.05 µg/kg per minute, EPI ≥ 0.03 µg/kg per minute, V ≥ 0.03 units/min or any vasopressor combination). Data are shown as patient number receiving vasopressor therapy at the anhepatic measurement point (15 min before reperfusion). *P* value was obtained using  $\chi^2$  test. A *P*-value for < 0.05 was set for statistical significance. MAP/mPAP: Mean systemic-to-pulmonary artery pressure.

and severity of vasopressor support was not different between Group 1 and 2.

There were significant differences in duration of postoperative intubation, length of stay in the ICU and length of hospital stay between Group 1 and Group 2 (Table 2). Patients in Group 1 extubated on an average of 12 h earlier than the patients in Group 2 (16.1 ± 22.7 h vs 28.7 ± 32.2 h, respectively; *P* = 0.04). The duration of ICU stay was reduced by almost 8 d (Group 1: 3.9 ± 4.4 d, Group 2: 12.1 ± 19.2; *P* < 0.01). Total length of hospitalization was approximately 2 wk less in Group 1 than in Group 2 (12.0 ± 12.5 d vs 26.3 ± 33.2 d, respectively; *P* < 0.01).

In the immediate post OLT period (up to 30 d postoperatively) no mortality was reported in either group. In Group 1 four out of 66 patients (6%) required reintubation and five out of 66 patients (8%) received RRT; no other complications were reported in this group. In Group 2 three out of 25 patients (12%) required reintubation and seven out of 25 patients (28%) received RRT post OLT. One patient was readmitted to ICU and one patient developed seizures.

## DISCUSSION

The main finding of this study was that despite having similar preoperative pathophysiology, one quarter of patients undergoing OLT did not display the expected increase of MAP/mPAP ratio during the anhepatic phase. This lack of increased MAP/mPAP ratio in the anhepatic period by > 1 compared to baseline values was associated with: (1) longer duration of postoperative intubation; and (2) prolonged ICU stay and total hospitalization time when compared to patients with an increase in MAP/mPAP ratio during the anhepatic period. The changes in MAP/mPAP ratio were not mirrored by CI, thus the intraoperative pattern of MAP/mPAP may be predictive of patient clinical outcomes after liver transplantation. To our knowledge, this is the first study to describe the intraoperative pattern of MAP/mPAP ratio during liver transplant and its possible relationship with patient

outcomes following OLT.

Advanced liver disease has been known to affect other organ functions, most importantly the cardiovascular and renal systems<sup>[8,9]</sup>. The connection between liver cirrhosis and cardiac dysfunction has been previously recognized<sup>[8,10-12]</sup> as recent studies have documented that cirrhosis is associated with biventricular systolic dysfunction<sup>[5,6]</sup>. It is standard practice for patients to undergo an extensive preoperative cardiac evaluation to risk stratify them prior to listing for liver transplantation. The echocardiographic assessment and stress testing is usually centered on left ventricular function. While right ventricular systolic pressure and or Tricuspid annular plane systolic excursion is usually measured to evaluate the patient for right heart function and pulmonary hypertension; neither of these parameters correlates well with right systolic function<sup>[5]</sup>. Advanced echocardiography techniques (strain analysis/right ventricle relative area change) are rarely included in the standard pre OLT echocardiography assessment<sup>[5,13]</sup>.

Cardiologists commonly use the MAP/mPAP ratio to stratify the severity of pulmonary hypertension because it describes the close relationship between systemic and pulmonary circulations<sup>[14]</sup>. Preoperative value of MAP/mPAP < 4 would be of concern for cardiologists for possible pulmonary hypertension, and MAP/mPAP values < 4 have been correlated with lower survival rates after cardiac surgery<sup>[2,15]</sup>. We used the MAP/mPAP ratio in this study to assess its value to predict outcomes after liver transplantation, since this parameter has shown to be a useful predictor of hemodynamic complications after cardiac surgery<sup>[2]</sup>. Our data confirm the previous findings of the use of MAP/mPAP to identify patients at risk for adverse events after high-risk surgery. In this study, the intraoperative hemodynamic MAP/mPAP pattern of patients undergoing OLT indicated that an increase in MAP/mPAP during the anhepatic phase is associated with better outcomes. A novelty of this report is the analysis of the MAP/mPAP ratio intraoperative pattern and correlating it with postoperative outcomes.

While our reported data are truly observational, it is

not well established what significance the MAP/mPAP ratio represents, especially in patients with advanced liver disease. Cardiologists have set a normal value of  $> 4$ <sup>[14]</sup>. The observation that the majority of the included patients in our study had values  $< 4$  in absence of pulmonary hypertension may be due to advanced liver disease or the effect of anesthetic medications, since the baseline measurements were taken post induction of anesthesia. Previous studies indicated only minor effect of anesthesia on the MAP/mPAP ratio<sup>[2]</sup>. However, patients with advanced liver disease may have lower numbers due to systemic vasodilation and may have an exaggerated vascular response to anesthetics.

A recent study from Bushyhead *et al.*<sup>[16]</sup> investigated preoperative data of liver transplant recipients and found that the pulmonary artery systolic pressure correlates with posttransplant outcome and therefore emphasized the importance of right ventricular assessment and pulmonary vascular resistance for the morbidity and mortality associated with the procedure. However, the publication did not assess the value of the MAP/mPAP ratio for preoperative risk stratification. While our study did not obtain preoperative MAP/mPAP values prior to anesthesia induction and did not include patients with pre-existing pulmonary hypertension defined by elevated pulmonary artery pressures, the findings of our study support the need for more thorough assessment of right heart function and cardiac reserve prior to liver transplantation. With the scarcity of acceptable donor organs, the best surgical candidate with the least likelihood for postoperative complication needs to be identified. Including MAP/mPAP ratio into the preoperative assessment may provide useful information.

Minimizing the importance of a single measurement and focusing on the intraoperative patterns of the MAP/mPAP ratio, the trend of the parameter may be interpreted as an indication of contractility reserve. The hemodynamic hallmark of the anhepatic phase during liver transplantation is characterized by significant reduction in IVC flow and therefore blood return to the heart. The behavior of MAP/mPAP ratio during the anhepatic phase therefore may indicate the systemic and pulmonary circulatory response during reduced cardiac preload. An increase in MAP/mPAP ratio may suggest that circulatory systems are able to adjust to stress and hypovolemia by vasoconstriction and inotropic compensation. Therefore, the ability to increase the MAP/mPAP ratio in the anhepatic phase observed in Group 1 indicates better cardiovascular reserve than the lack of increase or decrease as observed in Group 2.

We chose to use the MAP/mPAP ratio difference between baseline and anhepatic phase because the anhepatic stage primarily represents a single hemodynamic alteration (preload reduction). Therefore, all patients received a similar type of cardiac stress and the MAP/mPAP ratio may be more representative for the cardiac ability to compensate for the preload reduction. Although hepatic reperfusion can cause significant cardiac

strain, the cardiac response to reperfusion depends on multiple factors and some of them may be due to the donor organ. The duration of reperfusion is usually short and therefore changes in MAP/mPAP may be not reflecting the cardiac response to the changes in preload, cardiac contractility or afterload. Our study used single MAP/mPAP ratio measurements at predetermined measurement point representing the hemodynamic situation during the surgical stage. Due to the fluctuating nature of all hemodynamic parameters during OLT, a continuous assessment of MAP/mPAP ratio throughout the entire surgical procedure may be more desirable in future studies to describe the cardiac reserve.

In our study, vasoactive medications were not controlled during the surgical procedure, and were titrated to effect by the anesthesia provider to ensure hemodynamic stability. Variable doses of vasoactive medications were given in both study groups. However, drug selection and dosing did not appear to influence the MAP/mPAP pattern since there were no statistical differences in distribution between both groups.

In previous studies on cardiac patients without pre-existing pulmonary hypertension, a low MAP/mPAP ratio was found to be an independent predictor of difficult separation of cardiopulmonary bypass and right heart failure<sup>[2,17]</sup>. Robitaille *et al.*<sup>[2]</sup> found that patients with lower MAP/mPAP ratios had more hemodynamic complications after cardiac surgery defined as cardiac arrest, vasopressor therapy  $> 24$  h postop, and/or use of intra-aortic balloon pump postop. These findings are in agreement with our interpretation of MAP/mPAP ratio as a predictor of the ability of the cardiovascular system to provide hemodynamic compensation. If a MAP/mPAP increase during the anhepatic phase is interpreted as a positive cardiovascular response to stressors, the lack of such compensation would explain why the patients without such a response would have less favorable outcomes.

Robitaille *et al.*<sup>[2]</sup> correlated the preoperative MAP/mPAP ratio with surgical outcome after cardiac surgery and reported that the preoperative MAP/mPAP ratio was significantly higher in survivors ( $3.9 \pm 1.4$ ) than in those who died ( $3.2 \pm 1.4$ ). Since the surgical procedure during liver transplantation has more complexity and varying hemodynamic challenges specific to each surgical phase, we chose (per expert consensus) observation points to describe the MAP/mPAP pattern throughout different surgical stages of the procedure. Our pattern analysis confirms the findings of the single preoperative measurement in the previous study<sup>[2]</sup>. However, our observation and current understanding of the ratio is that it is not a static parameter and, per our data, large ratio fluctuations can occur and should alert the clinician to initiate an adjustment in the treatment plan.

Our study has several limitations. First, all data was gathered as a retrospective study from only one institution without randomization or blinding. The normal hemodynamic response was defined by the authors

based on preliminary observations and understanding of hemodynamic response to the IVC flow alterations during the anhepatic phase. However, this categorization may be oversimplified to demonstrate the variety of possible dynamic responses in the anhepatic period. The study was also limited by data analysis. The data was not measured continuously, as we selected the ratio of mean systemic to pulmonary artery pressure at defined time points during the liver transplant. Our endpoint data (time to extubation in ICU, LOS ICU, *etc.*) could have also been affected by variability in ICU provider practice. Certain providers may not have been as aggressive as other providers in extubating patients. In addition, there are other factors that affect duration of endotracheal intubation, such as failure to extubate due to opioid induced apnea. All these variables are not factored into this study.

If future prospective trials confirm the value of intra-operative MAP/mPAP ratio patterns for postoperative outcome prediction, the question will arise if MAP/mPAP ratio manipulation may be able to alter the outcome after major surgery. Increasing MAP with vasopressor/inotropic medications or lowering mPAP with pulmonary vasodilators could be beneficial.

In conclusion, the data of this retrospective study raises awareness of the mean systemic to mean pulmonary artery pressure ratio during surgery as a potential indicator for poor patient outcome following OLT. To further delineate the significance of this parameter, a multi-center, randomized, blinded prospective study with more frequent measurement points is needed for validation.

## ACKNOWLEDGMENTS

The project was presented in part at the Annual Meeting of the International Anesthesia Research Society in May, 2016.

## COMMENTS

### Background

This study provides relevant information to identify patients at risk for complications after orthotopic liver transplantation.

### Research frontiers

With the scarcity of available livers for transplantation, it is crucial that patients are properly selected and every effort is made to have the best possible outcome after the procedure.

### Innovations and breakthroughs

The study is offering useful information for patient selection; the next step would be (after validation) to assess if intraoperative manipulation of this parameter would optimize patient outcomes.

### Applications

This study should be of interest to any care provider involved with the care of potential liver transplant recipients.

### Peer-review

Rebel *et al* described that the systemic to pulmonary artery pressure ratio can

be a predictor of survival after liver transplantation.

## REFERENCES

- 1 Tuman KJ, McCarthy RJ, March RJ, Najafi H, Ivankovich AD. Morbidity and Duration of ICU stay after Cardiac Surgery. A Model for Preoperative Risk Assessment. *Chest* 1992; **102**: 36-44 [PMID: 1623792]
- 2 Robitaille A, Denault AY, Couture P, Belisle S, Fortier A, Guertin MC, Carrier M, Martineau R. Importance of relative pulmonary hypertension in cardiac surgery: the mean systemic to pulmonary artery pressure ratio. *J Cardiothor Vasc Anesth* 2006; **20**: 331-339 [PMID: 16750732 DOI: 10.1053/j.jvca.2005.11.018]
- 3 Kaw R, Pasupuleti V, Deshpande A, Hamieh T, Walker E, Minai OA. Pulmonary hypertension: An important predictor of outcomes in patients undergoing non-cardiac surgery. *Respir Med* 2011; **105**: 619-624 [PMID: 21195595 DOI: 10.1016/j.rmed.2010.12.006]
- 4 Ramakrishna G, Sprung J, Barugur BS, Chandrasekaran K, McGoon MD. Impact of pulmonary hypertension on the outcomes of noncardiac surgery. *J Am Coll Cardiol* 2005; **45**: 1691-1699 [PMID: 15893189 DOI: 10.1016/j.jacc.2005.02.055]
- 5 Chen Y, Chan AC, Chan SC, Chok SH, Sharr W, Fung J, Liu JH, Zhen Z, Sin WC, Lo CM, Tse HF, Yiu KH. A detailed evaluation of cardiac function in cirrhotic patients and its alteration with or without liver transplantation. *J Cardiol* 2016; **67**: 140-146 [PMID: 26304615 DOI: 10.1016/j.jcc.2015.08.001]
- 6 Kia L, Shah SJ, Wang E, Sharma D, Selvaraj S, Medina C, Cahan J, Mahon H, Levitsky J. Role of pretransplant echocardiographic evaluation in predicting outcomes following liver transplantation. *Am J Transplant* 2013; **13**: 2395-2401 [PMID: 23915391 DOI: 10.1111/ajt.12385]
- 7 Fukazawa K, Yamada Y, Gologorsky E, Arheart K, Pretto EA. Hemodynamic Recovery Following Postreperfusion Syndrome in Liver Transplantation. *J Cardiothor Vasc Anesth* 2014; **28**: 994-1002 [PMID: 25107717 DOI: 10.1053/j.jvca.2014.02.017]
- 8 Ruiz-del-Arbol L, Serradilla R. Cirrhotic cardiomyopathy. *World J Gastroenterol* 2015; **21**: 11502-11521 [PMID: 26556983 DOI: 10.3748/wjg.v21.i41.11502]
- 9 Schrier RW, Shchekochikhin D, Gines P. Renal failure in cirrhosis: prerenal azotemia, hepatorenal syndrome and acute tubular necrosis. *Nephrol Dial Transplant* 2012; **27**: 2625-2628 [PMID: 22492830 DOI: 10.1093/ndt/gfs067]
- 10 Van Wagner LB, Lapin B, Levitsky J, Wilkins JT, Abecassis MM, Skaro AI, Lloyd-Jones DM. High early cardiovascular mortality after liver transplantation. *Liver Transpl* 2014; **20**: 1306-1316 [PMID: 25044256 DOI: 10.1002/lt.23950]
- 11 Van Wagner LB, Serper M, Kang R, Levitsky J, Hohmann S, Abecassis M, Skaro A, Lloyd-Jones DM. Factors associated with major adverse cardiovascular events after liver transplantation among a national sample. *Am J Transplant* 2016; **16**: 2684-2694 [PMID: 26946333 DOI: 10.1111/ajt.13779]
- 12 Piazza NA, Singal AK. Frequency of Cardiovascular Events and Effect on Survival in Liver Transplant Recipients for Cirrhosis Due to Alcoholic or Nonalcoholic Steatohepatitis. *Exp Clin Transplant* 2016; **14**: 79-85 [PMID: 26581602 DOI: 10.6002/ect.2015.0089]
- 13 Waxman AB, Farber HW. Using Clinical Trial End Points to Risk Stratify Patients with Pulmonary Arterial Hypertension. *Circulation* 2015; **132**: 2152-2161 [PMID: 26621638 DOI: 10.1161/CIRCULATIONAHA.114.012328]
- 14 Therrien J, Dore A, Gersony W, Iserin L, Liberthson R, Meijboom F, Colman JM, Oechslin E, Taylor D, Perloff J, Somerville J, Webb GD; Canadian Cardiovascular Society. CCA Consensus Conference 2001 update: Recommendations for the management of adults with congenital heart disease. *Can J Cardiol* 2001; **17**: 940-959 [PMID: 11586386]
- 15 Gomez CM, Palazzo MG. Pulmonary artery catheterization in anaesthesia and intensive care. *Br J Anaesth* 1998; **81**: 945-956 [PMID: 10211024]
- 16 Bushyhead D, Kirkpatrick JN, Goldberg D. Pretransplant Echocardiographic Parameters as Markers of Posttransplant Outcomes in Liver Transplant Recipients. *Liver Transpl* 2016; **22**: 316-323

[PMID: 26609681 DOI: 10.1002/lt.24375]

- 17 **Carricart M**, Denault AY, Couture P, Limoges P, Babin D, Levesque S, Fortier A, Pellerin M, Tardif JC, Buithieu J. Incidence

and significance of abnormal hepatic venous Doppler flow velocities before cardiac surgery. *J Cardiothorac Vasc Anesth* 2005; **19**: 751-758 [PMID: 16326300 DOI: 10.1053/j.jvca.2004.11.052]

**P- Reviewer:** Baddour N, Hilmi I, Sugawara Y, Tanaka N  
**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D





Observational Study

## Novel non-invasive biological predictive index for liver fibrosis in hepatitis C virus genotype 4 patients

Mahmoud Khattab, Mohamed Amin Sakr, Mohamed Abdel Fattah, Youssef Mousa, Elwy Soliman, Ashraf Breedy, Mona Fathi, Salwa Gaber, Ahmed Altaweil, Ashraf Osman, Ahmed Hassouna, Ibrahim Motawea

Mahmoud Khattab, Mohamed Abdel Fattah, Youssef Mousa, Elwy Soliman, Ibrahim Motawea, Department of Internal Medicine, Minia University, Minia 61111, Egypt

Mohamed Amin Sakr, Ashraf Breedy, Department of Tropical Medicine, Ain Shams University, Cairo 11566, Egypt

Mona Fathi, Department of Clinical Pathology, Ain Shams University, Cairo 11566, Egypt

Salwa Gaber, Department of Pathology, Minia University, Minia 61111, Egypt

Ahmed Altaweil, Department of Pathology, Ain Shams University, Cairo 11566, Egypt

Ashraf Osman, Department of Clinical Pathology, Minia University, Minia 61111, Egypt

Ahmed Hassouna, Department of Cardiothoracic Surgery, Ain Shams University, Cairo 11566, Egypt

**Author contributions:** Khattab M proposed study design and supervised all stages of the research; Sakr MA, Fattah MA, Mousa Y, Soliman E, Breedy A and Motawea I shared patient recruitment, physical assessments and data interpretation; Fathi M and Osman A performed the laboratory investigations; Gaber S and Altaweil A assessed histopathological specimens of liver biopsies; Hassouna A processed the statistical analysis of our data.

**Supported by** The Governmental Foundation Scientific and Technology Development Foundation (STDF), Egypt, Project ID: 1538.

**Institutional review board statement:** The study protocol was approved by the Institutional ethics committee of Minia School of medicine in Egypt. The study was conducted in accordance with the ethical guidelines of the 1975 Helsinki declaration.

**Informed consent statement:** All patients signed informed consent to participate in the study.

**Conflict-of-interest statement:** The authors declare that they have no competing interests.

**Data sharing statement:** Participants gave informed consent to share in the study anonymously and risk of identification is low.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Mahmoud Khattab, MD, PhD, Professor of Medicine, Head of Liver Unit, Department of Internal Medicine, Minia University, Cornish Al Nile Road, Minia 61111, Egypt. [mkhattabmed@hotmail.com](mailto:mkhattabmed@hotmail.com)  
Telephone: +20-25-197818  
Fax: +20-86-2156056

**Received:** June 26, 2016

**Peer-review started:** June 26, 2016

**First decision:** September 2, 2016

**Revised:** September 9, 2016

**Accepted:** October 5, 2016

**Article in press:** October 9, 2016

**Published online:** November 18, 2016

## Abstract

### AIM

To investigate the diagnostic ability of a non-invasive biological marker to predict liver fibrosis in hepatitis C genotype 4 patients with high accuracy.

## METHODS

A cohort of 332 patients infected with hepatitis C genotype 4 was included in this cross-sectional study. Fasting plasma glucose, insulin, C-peptide, and angiotensin-converting enzyme serum levels were measured. Insulin resistance was mathematically calculated using the homeostasis model of insulin resistance (HOMA-IR).

## RESULTS

Fibrosis stages were distributed based on Metavir score as follows: F0 = 43, F1 = 136, F2 = 64, F3 = 45 and F4 = 44. Statistical analysis relied upon reclassification of fibrosis stages into mild fibrosis (F0-F1) = 179, moderate fibrosis (F2) = 64, and advanced fibrosis (F3-F4) = 89. Univariate analysis indicated that age, log aspartate amino transaminase, log HOMA-IR and log platelet count were independent predictors of liver fibrosis stage ( $P < 0.0001$ ). A stepwise multivariate discriminant functional analysis was used to drive a discriminative model for liver fibrosis. Our index used cut-off values of  $\geq 0.86$  and  $\leq -0.31$  to diagnose advanced and mild fibrosis, respectively, with receiving operating characteristics of 0.91 and 0.88, respectively. The sensitivity, specificity, positive predictive value, negative predictive value and positive likelihood ratio were: 73%, 91%, 75%, 90% and 8.0 respectively for advanced fibrosis, and 67%, 88%, 84%, 70% and 4.9, respectively, for mild fibrosis.

## CONCLUSION

Our predictive model is easily available and reproducible, and predicted liver fibrosis with acceptable accuracy.

**Key words:** Liver fibrosis; Insulin resistance; Aspartate amino transaminase; Platelets; Age

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This observational study included a cohort of 332 recruited patients with hepatitis C virus (HCV) genotype 4 infections. The study assessed the status of demographic and biological variables at different stages of liver fibrosis. Liver biopsy with Metavir scoring was the reference standard used to classify patients into five stages of liver fibrosis (F0-F4). Patient regrouping to include three levels of fibrosis, mild (F0-F1), moderate (F2), and advanced (F3-F4), was performed to conform with practical guidelines for the management and follow-up of HCV patients. Age, aspartate transaminase enzyme (AST), insulin resistance (HOMA-IR), and platelet count were significant predictors of liver fibrosis as shown on univariate analysis. Log AST, log HOMA-IR, log platelet count and age were introduced into stepwise multivariate discriminative analysis, and a model for the prediction of liver fibrosis level was derived. Our predictive index exhibited an area under the curve (AUC) of 0.91 for the diagnosis of advanced stages of fibrosis and an AUC of 0.88 for the diagnosis of mild stages of fibrosis. The index exhibited a lower AUC of 0.64 in the diagnosis of moderate stages of fibrosis.

Khattab M, Sakr MA, Fattah MA, Mousa Y, Soliman E, Breedy A, Fathi M, Gaber S, Altaweil A, Osman A, Hassouna A, Motawea I. Novel non-invasive biological predictive index for liver fibrosis in hepatitis C virus genotype 4 patients. *World J Hepatol* 2016; 8(32): 1392-1401 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i32/1392.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i32.1392>

## INTRODUCTION

Hepatitis C virus (HCV) infection exhibits worldwide distribution with a global prevalence of 2.35%, and it affects 160-170 millions of chronically infected individuals<sup>[1]</sup>. Approximately three to four million peoples are infected annually<sup>[2]</sup>. Egypt has one of the highest prevalence rates worldwide, 14.9%, as estimated by the Egypt Demographic and Health Survey. HCV genotype 4 is the most common genotype in Egypt<sup>[3]</sup>. Liver fibrosis is the essential pathophysiological consequence of chronic liver injury regardless of injurious agent because it is the pathological outcome of chronic HCV infections<sup>[4]</sup>.

Hepatic stellate cells (HSCs) are the major fibrogenic cells in the liver. Apoptotic HSCs regulate the balance between the synthesis and degradation of the extracellular matrix<sup>[5]</sup>. HCV-induced bioactive transforming growth beta 1 is critical for the induction of  $\alpha$ -smooth muscle actin and type-1 collagen, which are markers for HSC activation and proliferation<sup>[6]</sup>.

The assessment of liver fibrosis level (stage) is a major issue for the management and follows-up of patients with chronic hepatitis C infection. Liver biopsy is the gold standard for the assessment of fibrosis and grade of necro-inflammation and histological staging is based on semi-quantitative scoring systems (e.g., Metavir and Ishak Scores)<sup>[7]</sup>.

However, liver biopsy exhibits certain drawbacks, including sampling error, invasiveness with potentiality adverse effects, complications, such as haemorrhage in 0.3% of cases, pain in 30% of cases and mortality in 0.01% of cases, and inter and intra observer variability in the reading of biopsy specimens<sup>[8]</sup>. Therefore, liver biopsy is not a perfect assessment of liver fibrosis and there is a growing need to identify surrogate non-invasive markers of liver injury with its clinical consequences and future events.

HCV chronic infections are associated with insulin resistance and type 2 diabetes mellitus, which are more frequently observed in HCV infections compared with healthy controls and liver diseases of other aetiology. HCV infection promotes insulin resistance primarily via increased TNF- $\alpha$  production and enhanced suppressor of cytokine, which block PI3K and Akt phosphorylation<sup>[9]</sup>. Insulin resistance and geographical origin (Egyptian) are the major predictors of liver fibrosis and response to therapy in HCV-genotype 4<sup>[10]</sup>.

Physiological hepatic angiogenesis occurs during liver regeneration and leads to the formation of new functional

sinusoids. However pathological angiogenesis occurs in fibrosis, and it is characterized by the appearance of capillaries vascular structures<sup>[11]</sup>. The resulting hypoxia in liver injury induces activation of the renin-angiotensin system (RAS), which plays a role in the pathogenesis of fibrosis in the heart, kidney, lung and liver<sup>[12]</sup>.

Multiple markers using non-invasive methods to determine liver fibrosis are available. No single non-invasive test or model can match the information obtained from actual perfect histology, and there is a need to develop further tests or models that alleviate or that reduce the need for invasive liver biopsy.

We used simple biological parameters that are related to the development and progression of liver fibrosis, to obtain a model of acceptable accuracy that predicted levels of liver fibrosis in HCV-genotype 4 patients.

## MATERIALS AND METHODS

This cross-sectional observational study included a cohort of 352 recruited patients with chronic hepatitis C infection. Patients were attending liver clinics at Minia University, Egypt, from June 2011 to July 2013. Data from twenty patients were excluded because eight patients were not genotype 4, five patients had a small core of liver biopsy that required correct assessment, four patients were diabetic, and three patients failed to follow-up. Only data of 332 patients were subjected to statistical analyses. Included patients had HCV-genotype 4 infection. HCV infection was defined as positive second generation anti-HCV antibodies and detection of HCV RNA in serum using quantitative reverse transcription polymerase chain reaction during the study period (Abbott M 2000, United States; -lower limit of detection 12 IU/mL). HCV genotyping was performed using line probe assay or reverse hybridisation and commercially available kits (Innolipa, Innogenetic, and Genetics, Belgium).

Exclusion criteria included co-infection with hepatitis B virus, human immunodeficiency virus or schistosomal infections, regular alcohol intake greater than 10 g/d, previous interferon therapy, other aetiologies of liver disease such as immune-mediated liver diseases, clinical evidence of liver decompensation and use of drugs that may alter insulin resistance, such as insulin sensitizers. Obesity determined as body mass index > 30 [body mass index (BMI) > 30] and frank diabetes mellitus diagnosed according to the American Diabetes Association diagnosis criteria<sup>[13]</sup> were exclusion criteria from the study because these conditions may confound the results. Associated lung disease was also excluded because it may confound angiotensin converting enzyme (ACE) levels.

### Informed consent

The Institutional Ethics Committee of participating units approved the study protocol, and all patients signed informed consent. The study was conducted in accordance with the ethical guidelines of the 1975

Helsinki Declaration.

### Liver histopathology

Sonographic-guided liver biopsy was performed on the second day of blood withdrawal for tests using disposable true cut needles (14 gauge) to obtain a sufficient liver tissue core. Liver biopsy specimens not less than 15 mm in length or the presence of at least 10 complete portal tracts were required for data inclusion.

Liver biopsy specimens were fixed and paraffin embedded, stained with the routinary haematoxylin and eosin (H and E) and mason trichrome stain to define fibrosis in combination with Prussian blue for iron staining. A single experienced pathologist who was blinded to clinical and laboratory data examined liver biopsy specimens.

Fibrosis staging and necroinflammatory grading were scored according to Metavir scores, which scores fibrosis as F0 (absent), F1 (portal fibrosis), F2 (portal fibrosis with few septa), F3 (septal fibrosis) and F4 (cirrhosis). Necroinflammatory activity was graded as A0 (absent), A1 (mild), A2 (moderate) and A3 (severe)<sup>[14]</sup>.

### Demographic and laboratory assessment

The following data were collected from all patients at baseline: Age, sex, weight (W) in kilograms, height (H) in meters, waist and hip circumferences in centimeters, and BMI calculated as W/H<sup>2</sup>, and Waist/Hip ratio. Venous blood was withdrawn after an 8-h overnight fast and was analysed for fasting plasma glucose.

Other sample of venous blood was withdrawn after a 12-h overnight fast and collected in three tubes, one of which contained EDTA-K3 for haemogram assessment. Serum from the other two tubes was distributed as follows: One sample was frozen in a -70 °C refrigerator for later assessments of insulin, C-peptide and ACE. Serum from the third tube was analysed on the same day for, cholesterol, triglycerides, and liver biochemical and renal profiles.

### Laboratory methods

Serum insulin and C-peptide were assayed using a sandwich ELISA technique and kits from Monobind Inc (Lake Forest, CA, United States); Serum ACE was assayed using kits from R and D systems (R and D Systems, Inc. United States and Canada) that employ a quantitative sandwich immunoassay technique; Liver function tests [serum total and conjugated bilirubin, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphates, total proteins and albumin], kidney function tests (urea and creatinine), and total cholesterol and triglycerides were performed using a Synchron CX<sup>9</sup> auto-analyser using Beckman reagents (Beckman Instruments; Scientific Instruments Division, Fullerton, CA, United States); Complete blood count was performed on using Coulter Counter T 660 (Beckman Coulter, Inc., Harbor Blvd., Fullerton, CA, United States); Prothrombin time was assessed on an STA-Stago Compact CT autoanalyser (Diagnostic Stago,

Inc., Parsippany, NJ, United States) using reagents from Dade Behring (Dade Behring Holdings Inc., IL, United States); hepatitis B surface antigen and C-antibody were measured using Roche Cobase 411 (Roche Diagnostic GmbH); insulin resistance (IR) was determined using the homeostasis model assessment for insulin resistance (HOMA-IR) method and the following equation:  $\text{HOMA-IR} = \text{Fasting insulin (mU/mL)} \times \text{Fasting plasma glucose (mmol/L)} / 22.5$ . Insulin resistance as calculated using this method correlates closely with the gold standard hyperinsulinemic/euglycemic clamp method in diabetic and non-diabetic subjects<sup>[15,16]</sup>.

### Statistical analysis

Qualitative data are presented as numbers, (%). Normally distributed variables are presented as the means  $\pm$  SD and non-parametric data are presented as the medians and interquartile range. The distribution of qualitative variables was evaluated using the  $\chi^2$  test or Fisher's exact test, as indicated. The means were compared between groups using the non-parametric independent-samples Kruskal-Wallis test, and the level of significance following pairwise comparisons was adjusted for the number of comparisons made.

Fibrosis stages based on Metavir scores were distributed into 5 classes: F0, F1, F2, F3 and F4. Patients were further regrouped into 3 stages of mild (F0-F1), moderate (F2) and advanced fibrosis (F3-F4) for statistical analyses. Univariate analyses identified patient's age, AST and platelet count added to HOMA-IR as significantly different between the 3 levels of fibrosis in overall and pairwise comparisons. All variable were introduced in a stepwise discriminative functional analysis model for the three levels of fibrosis after normalising HOMA-IR, AST and platelet count into their  $\log^{10}$  values. Diagnostic accuracy is expressed as area under the curve of receiving operating characteristic (AUROC) (asymptomatic 95%CI), sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios. All tests were bilateral, and a *P* value of 0.05 was the limit of statistical significance. Statistical analyses were performed using the IBM SPSS statistical software package for MAC version 22.

## RESULTS

A total of 332 HCV-genotype 4 Egyptian patients were included to statistical analysis. Patients exhibited a mean age of  $42 \pm 10.7$  years and male to female ratio of 180/146 (65/44%). Gender showed no statistically significant difference between levels of liver fibrosis. Mean BMI and Waist/Hip ratio were  $26.7 \pm 4.4$  and  $0.89 \pm 0.08$ , respectively, which indicates that none of our patients was obese. None of the study patients consumed alcohol or had history of drug abuse. A total of 69.6% were non-smokers, 19% were moderate smokers and 11.4% were heavy smokers. The Metavir scoring system identified F0 = 43, F1 = 136, F2 = 64, F3 = 45 and F4 = 44 patients.

Table 1 presents quantitative variables such as the

mean, SD, median and quartile range in the five stages of liver fibrosis. Table 2 presents pairwise comparisons of significant variables between the three levels of fibrosis. Table 3 presents the overall significant variables using independent - samples Kruskal-Wallis tests which indicated that age, ACE, blood glucose, ALT, AST, platelet count, fasting serum insulin, serum creatinine, total and direct bilirubin, and serum albumin were significant predictors of liver fibrosis stage. Viral load showed no statistically significant difference among stages and levels of liver fibrosis.

Statistically significant variables that discriminated between the 3 levels of fibrosis on univariate analysis, namely AST, platelet count and age and HOMA-IR were introduced to a stepwise multivariate discriminant analysis. This analysis requires a normal distribution of the dependent variables and equality of variance. Therefore; HOMA-IR, AST and platelet count were transformed into  $\log^{10}$  values.

Table 4 indicates that all variables were statistically significant before being introduced in the model. These variables were introduced into a model that significantly predicted liver fibrosis. Stepwise analysis derived the following equation.

$\text{Outcome} = 0.514 (\text{age}) + 0.373 (\text{Log HOMA-IR}) + 0.49 (\text{Log AST}) + (-0.532) \text{Log platelet count}$ .

The interpretation of outcome is dependent on the functions of group centroids as: (1) mild fibrosis if outcome is  $\leq -0.31$  or more negative; (2) moderate fibrosis if outcome is  $> -0.31$  (more positive) and up to  $+0.86$ ; and (3) advance fibrosis if outcome is  $> 0.86$ .

Table 5 presents accuracy indices of the model in the discrimination of fibrosis stages. In mild fibrosis and at a cut-off value  $-0.31$  or more negative, AUC was 0.88 with sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratios and negative likelihood ratios were 67.2%, 86.3%, 83.6%, 69.5%, 4.9 and 0.38, respectively, Figure 1. In advanced fibrosis and at a cut-off value  $> 0.86$ , AUC was 0.91 with sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratios and negative likelihood ratios were 73%, 90.9%, 74.4%, 90.1%, 8.0 and 0.3, respectively (Figure 2). While, in moderate fibrosis and at a cut-off value  $> -0.31$  up to  $+0.86$ , AUC was 0.64 with sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratios and negative likelihood ratios were 53.1%, 74.1%, 33%, 86.8%, 2.0 and 0.63, respectively, Figure 3.

The obtained model was validated by applying the model to the selected studied groups. Table 6 shows the results of this validation which indicated that two-thirds of the cases were correctly classified by the model (66.1%). This sensitivity increased to 67.2% and 73% in mild and advanced fibrosis, respectively, but dropped to 53.1% in moderate fibrosis.

## DISCUSSION

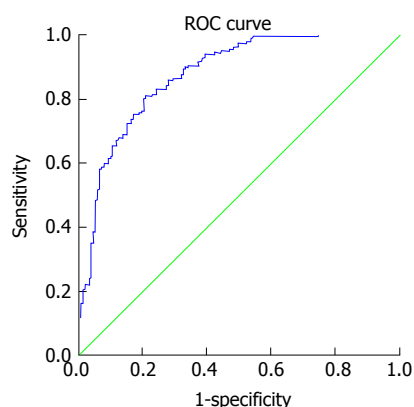
The prediction of liver fibrosis is a major issue for management and follow-up of patients with chronic



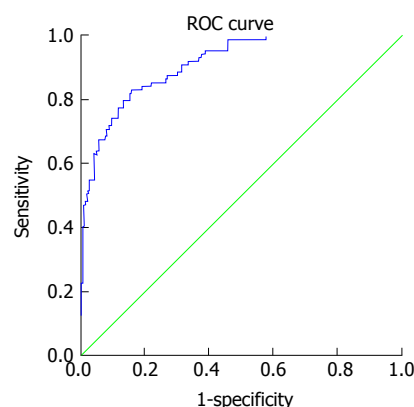
**Table 1** Presentation of quantitative variable as means, standard deviation, median and quartile range by stages of fibrosis

Variant	Total (n = 332)	F0 (n = 43)	F1 (n = 136)	F2 (n = 64)	F3 (n = 45)	F4 (n = 44)
Age (yr)						
Mean ± SD	42 ± 9.8	31.6 ± 7.4	41.1 ± 9.2	42 ± 7.4	48.5 ± 8.9	49.7 ± 7.5
Median ± QR	42 ± 15	31 ± 11	40.5 ± 15	42 ± 10	50 ± 11	49 ± 13
Gender (male)						
n (%)	184 (55.4)	24 (55.8)	77 (56.6)	40 (62.5)	22 (48.9)	21 (47.7)
HOMA-IR						
Mean ± SD	3.1 ± 1.3	2.4 ± 0.9	2.7 ± 1	3.4 ± 1.2	4.1 ± 1.7	4.0 ± 1.2
Median ± QR	2.9 ± 1.6	2.4 ± 1.8	2.7 ± 1.4	3.4 ± 1.4	4.1 ± 2.5	4.2 ± 2
ACE (U/mL)						
Mean ± SD	286.7 ± 132.9	248.9 ± 122.3	277.3 ± 129	287.2 ± 122.5	325.3 ± 173.3	320.4 ± 122.4
Median ± QR	260 ± 180	235 ± 127.5	260 ± 195	300 ± 190	275 ± 171.3	285 ± 138.8
Glucose (mmol)						
Mean ± SD	5.1 ± 0.9	5 ± 0.6	5.1 ± 0.9	4.8 ± 0.8	5.3 ± 0.9	5.7 ± 1.1
Median ± QR	5.1 ± 1.22	5.1 ± 1.1	4.9 ± 1.1	4.7 ± 1.2	5.3 ± 1.6	5.4 ± 1.7
ALT (U/L)						
Mean ± SD	58.4 ± 36.9	37 ± 16.6	53.6 ± 37.7	55.5 ± 30.4	79.8 ± 42.6	82 ± 33.9
Median ± QR	44 ± 52	36 ± 24.3	43 ± 43	47 ± 53	81.5 ± 57.5	90 ± 41.5
AST (U/L)						
Mean ± SD	53.2 ± 37.6	27.5 ± 10.9	41.1 ± 21.3	55 ± 35.4	87.2 ± 53.9	88.6 ± 42.5
Median ± QR	36 ± 43	23.5 ± 18.3	34 ± 25.8	36 ± 39	89 ± 61.3	85 ± 67.3
Platelet (× 10 <sup>9</sup> /L)						
Mean ± SD	213.6 ± 70	225.8 ± 49.4	240 ± 65.4	207.1 ± 70.2	164.8 ± 73.8	158.3 ± 36.2
Median ± QR	215 ± 105	221.5 ± 77.5	233 ± 82.5	226 ± 120	150.5 ± 90.3	162.5 ± 38.8
BMI						
Mean ± SD	27.4 ± 4.5	25.7 ± 4.3	27.9 ± 5.1	27.8 ± 4.3	27.2 ± 3.6	26.6 ± 3.1
Median ± QR	27.7 ± 5.8	25.9 ± 7.5	28.3 ± 7.8	27.7 ± 5.3	27.6 ± 4.6	27.8 ± 4.2
Waist: Hip ratio						
Mean ± SD	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Median ± QR	0.9 ± 0.1	0.9 ± 0.04	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Insulin (uU/mL)						
Mean ± SD	13.9 ± 5.3	11.1 ± 4.2	12.2 ± 4.5	15.8 ± 4.9	17.7 ± 7.1	15.7 ± 3.3
Median ± QR	13.6 ± 6.7	12.6 ± 7.2	12.3 ± 6.3	14.6 ± 3.4	16.7 ± 10.2	16.6 ± 5.8
Albumin (g/dL)						
Mean ± SD	4.2 ± 0.5	4.2 ± 0.3	4.4 ± 0.5	4 ± 0.8	4 ± 0.5	4 ± 0.3
Median ± QR	4.2 ± 0.6	4.1 ± 0.5	4.4 ± 0.5	4.2 ± 0.8	3.9 ± 0.7	4 ± 0.6
Viral load (IU/mL)						
Mean ± SD	372826.7 ± 902784.9	338113.1 ± 624770.4	409890.1 ± 941388.1	283586 ± 858939.26	264338.4 ± 452377.9	119830.8 ± 162200.8
Median ± QR	78000 ± 280088	117466.5 ± 358614	59112 ± 310055	156797 ± 251419	47546 ± 278956	97133 ± 167712

SD: Standard deviation; QR: Quartile range; HOMA-IR: Homeostasis model for insulin resistance, a mathematically calculated formula; ACE: Angiotensin converting enzyme; AST: Aspartate transaminase enzyme; ALT: Alanine amino transferase; BMI: Body mass index.



**Figure 1** Receiving operating characteristic curve for discriminating mild fibrosis. At cut off value: -0.31 or more negative: AUC 0.88, 95%CI: 0.84-0.91, sensitivity 67.2%, specificity 6.3%, PPV 83.6%, NPV 69.5%, PLR 4.9 and NLR 0.38. AUC: Area under the curve; PPV: Positive predictive value; NPV: Negative predictive value; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio; ROC: Receiving operating characteristic.



**Figure 2** Receiving operating characteristic curve for discriminating advanced fibrosis. At cut off value > 0.86: AUC 0.91, 95%CI: 0.88-0.94, sensitivity 73%, specificity 90.9%, PPV 74.4%, NPV 90.1%, PLR 8.0 and NLR 0.3. PPV: Positive predictive value; NPV: Negative predictive value; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio; ROC: Receiving operating characteristic.

**Table 2** Presentation of quantitative variables and their significance among three levels of fibrosis and significance between groups

Variant	Mild fibrosis (F0-F1) (n = 179)	Moderate fibrosis (F2) n = 64)	Advanced fibrosis (F3-F4) (n = 89)	P1	P2	P3
Age (yr)						
Mean ± SD	39.1 ± 9.6	42 ± 7.4	49 ± 8.2	0.001	0.001	0.001
Median ± QR	38.5 ± 16	42 ± 10	50 ± 11			
Gender (male)						
n (%)	101 (56)	40 (62)	43 (48)	0.100	0.060	0.832
HOMA-IR						
Mean ± SD	2.6 ± 0.9	3.4 ± 1.2	4.1 ± 1.5	0.027	0.001	0.008
Median ± QR	2.5 ± 1.4	3.4 ± 1.4	4.1 ± 2.2			
ACE (U/mL)						
Mean ± SD	271.5 ± 127.3	287.2 ± 122.5	323.2 ± 150.9	0.051	0.001	0.022
Median ± QR	255 ± 187.5	300 ± 190	275 ± 142.5			
Glucose (mmol)						
Mean ± SD	5.1 ± 0.9	4.8 ± 0.8	5.4 ± 1	0.629	0.013	0.017
Median ± QR	5 ± 1	4.6 ± 1.1	5.3 ± 1.5			
ALT (U/L)						
Mean ± SD	50.2 ± 34.9	55.5 ± 30.3	80.7 ± 38.4	0.004	0.001	0.022
Median ± QR	40 ± 43	47 ± 53	85.5 ± 53.3			
AST (U/L)						
Mean ± SD	38.3 ± 20.3	55 ± 35.4	87.8 ± 48.5	0.001	0.001	0.001
Median ± QR	34 ± 20.5	36 ± 39	87 ± 61			
Platelet (× 10 <sup>9</sup> /L)						
Mean ± SD	237.1 ± 62.4	207.1 ± 70.2	162 ± 59.8	0.003	0.001	0.001
Median ± QR	231 ± 81.3	226 ± 120	160.5 ± 64.3			
Insulin (uU/mL)						
Mean ± SD	11.9 ± 4.4	15.8 ± 4.9	16.8 ± 5.8	0.016	0.001	0.071
Median ± QR	12.4 ± 6.2	14.6 ± 3.4	16.7 ± 6.4			
Creatinine (mg/dL)						
Mean ± SD	0.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.2	0.999	0.009	0.039
Median ± QR	0.7 ± 0.2	0.6 ± 0.3	0.6 ± 0.3			
Albumin (g/dL)						
Mean ± SD	4.3 ± 0.43	4 ± 0.8	4 ± 0.41	0.044	0.001	0.005
Median ± QR	4.4 ± 0.54	4.2 ± 0.84	4 ± 0.67			
Viral load <sup>1</sup>						
Range	45979 (2.47-6570282)	36355.5 (2.46-6403601)	55000 (6.00-5600790)	0.37	0.96	0.52
Mean ± SD	358316.6 ± 909311.81	283586 ± 858939.26	331799.1 ± 863675.2			

<sup>1</sup>Comparison was done using non-parametric Mann-Whitney test. SD: Standard deviation; QR: Quartile range; HOMA-IR: Homeostasis model for insulin resistance, a mathematically calculated formula; ACE: Angiotensin converting enzyme; AST: Aspartate transaminase enzyme; ALT: Alanine amino transferase; P1: Significance between mild and moderate fibrosis; P2: Significance between mild and advanced fibrosis; P3: Significance between moderate and advanced fibrosis.

**Table 3** The overall significant variables among the studied variables: Using independent-samples Kruskal-Wallis test

Variables	P value
Age (yr)	0.001
HOMA-IR	0.001
ACE (U/mL)	0.001
Glucose (mmol)	0.021
ALT (U/L)	0.001
AST (U/L)	0.001
Platelet (× 10 <sup>9</sup> /L)	0.001
Insulin (uU/mL)	0.001
Creatinine (mg/dL)	0.024
Total Bilirubin (mg/dL)	0.001
Direct Bilirubin (mg/dL)	0.002
Albumin (mg/dL)	0.001
Portal vein diameter	0.004
Splenic diameter	0.001

HOMA-IR: Homeostasis model for insulin resistance, a mathematically calculated formula; ACE: Angiotensin converting enzyme; AST: Aspartate transaminase enzyme; ALT: Alanine amino transferase.

hepatitis C. Liver biopsy provides the best for evaluation

**Table 4** Multivariate discriminant functional analysis among the significant predictive variables

Variable	Statistic	P value
Log AST	61.295	0.001
Log platelet	44.331	0.001
Age (yr)	39.635	0.001
Log HOMA-IR	33.682	0.001

HOMA-IR: Homeostasis model for insulin resistance, a mathematically calculated formula; AST: Aspartate transaminase enzyme.

of liver fibrosis stages<sup>[17]</sup>, but this technique has its drawbacks. Liver biopsy is not the perfect tool for follow up assessments of fibrosis in patients with chronic hepatitis C with or without virological cure<sup>[18]</sup>.

The limitations of liver biopsy disclosed the need for the development of non-invasive tests to assess liver fibrosis. Currently available methods to predict liver fibrosis rely on two different but complementary approaches: (1) a biological approach based on measurements of serum levels of biological markers that

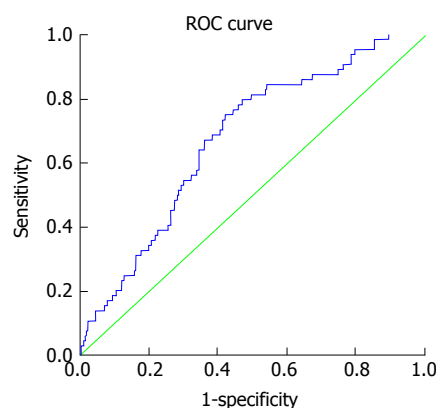
**Table 5** Accuracy indices of the discriminant score in the prediction of fibrosis

Stage of fibrosis	Cut-off value	AUC	95%CI	Sens	Specific	PPV	NPV	PLR	NLR
Mild fibrosis (F0-F1)	-0.31 or more negative	0.88	0.84-0.91	67.2%	86.3%	83.6%	69.5%	4.9	0.38
Moderate fibrosis (F2)	> -0.31 up to +0.86	0.64	0.61-0.74	53.1%	74.1%	33%	86.8%	2.0	0.63
Advanced fibrosis (F3-F4)	> 0.86	0.91	0.88-0.94	73%	90.9%	74.4%	90.1%	8.0	0.3

Sens: Sensitivity; Specific: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio; AUC: Area under the curve.

**Table 6** Validation results done on the studied selected groups

Stage of fibrosis	Predicted group membership			Total
	Mild fibrosis	Moderate fibrosis	Advanced fibrosis	
Count				
Mild fibrosis	119	50	8	177
Moderate fibrosis	16	34	14	64
Advanced fibrosis	5	19	65	89
Percent				
Mild fibrosis	67.2	28.2	4.5	100.0
Moderate fibrosis	25.0	53.1	21.9	100.0
Advanced fibrosis	5.6	21.3	73.0	100.0



**Figure 3** Receiving operating characteristic curve for discriminating moderate fibrosis. At cut off value > -0.31 up to +0.86: AUC 0.64, 95%CI: 0.61-0.74, sensitivity 53.1%, specificity 74.1%, PPV 33%, NPV 86.8%, PLR 2.0 and NLR 0.63. AUC: Area under the curve; PPV: Positive predictive value; NPV: Negative predictive value; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio; ROC: Receiving operating characteristic.

are independent predictors of liver fibrosis<sup>[19]</sup>; and (2) a “physical” approach based on the measurement of liver stiffness using transient elastography or other recent radiological tools<sup>[20]</sup>.

Many biomarkers have been developed and validated, but none of these markers provide the perfect test. This result may be due to the relatively reduced accuracy of otherwise the sophisticated techniques and the high costs of these tests<sup>[21]</sup>. We developed a non-invasive biomarker using variables that are biologically relevant to the development and progression of liver fibrosis, because of limitations of the available methods of non-invasive markers for assessment of liver fibrosis<sup>[22]</sup>.

Our study demonstrated on univariate analysis that age significantly ( $P < 0.001$ ) correlated with the stage of liver fibrosis. Age is used with some of the current biomarkers as an independent determinant of liver fibrosis, such as Forn's Index<sup>[23]</sup> and Fib 4<sup>[24]</sup>.

The results of univariate and multivariate analyses demonstrated that AST was a highly significant ( $P < 0.0001$ ) independent predictor of liver fibrosis stage. AST is used in many available biomarker tests as an independent predictor for liver fibrosis, such as Fib-4<sup>[24]</sup>, APRI test<sup>[25]</sup>, Fibro index<sup>[26]</sup>, and Fibrometere<sup>[27]</sup>.

Our results indicated that platelets were significantly negatively correlated with the advancement of liver fibrosis stage ( $P < 0.0001$ ). Platelet count was reported previously to progressively decrease with the progression of liver fibrosis<sup>[28]</sup>, which makes it to be included in some currently available biomarker evaluations of liver fibrosis stage including Forn's index<sup>[23]</sup>, and Fib-4<sup>[24]</sup>, APRI test<sup>[25]</sup>, and Fibro index<sup>[26]</sup>.

Our univariate analysis results demonstrated a significantly increasing level of HOMA-IR with the progression of liver fibrosis stage ( $P < 0.0001$ ). Insulin resistance is a powerful promoter of fibrogenesis *via* direct HSC

stimulation, TNF- $\alpha$ , connective growth factor production and ductular reaction induction<sup>[29]</sup>. However, only the Sud index included insulin resistance as a variable to evaluate liver fibrosis<sup>[30]</sup>.

Our study is the first report of the correlation of the progressive rise of serum ACE levels with the advancement of liver fibrosis stage ( $P < 0.0001$ ). Multivariate analysis of ACE serum level significantly predicted the stage of liver fibrosis ( $P < 0.001$ ).

ACE is the key rate-limiting enzyme for activation of the RAS, which results in the production of angiotensin II. Angiotensin II induces the contraction and proliferation of the human HSCs that are responsible for hepatic fibrogenesis<sup>[31]</sup>. However, it was excluded from our discriminating analysis to avoid the possible confounding effect of some disease states that may alter ACE serum level.

Stepwise multiple discriminative functional analysis indicated that platelets, age, AST, and HOMA-IR variables, in this order of frequency, were independent predictors of liver fibrosis with highly significant values ( $P < 0.0001$ ).

Log AST, log platelet count, log HOMA-IR and age were introduced in a stepwise discriminant analysis model. Our discriminating index for the prediction of liver fibrosis was processed into three levels based on 2014 EASL recommendations for the management of HCV patients to discriminate fibrosis in chronic hepatitis C: 1-No to Mild Fibrosis = F0-F1. 2-Moderate fibrosis = F2 3-Advanced fibrosis = F3-F4 according to Metavir staging

score.

All variables were statistically significant before introduction into the model. The following discriminative outcome was obtained using multiple stepwise analysis: Outcome = 0.514 (age) + 0.373 (Log HOMA-IR) + 0.49 (Log AST) + (-0.532) Log platelet count.

Where the level of fibrosis was predicted using the following cut-off values: (1) mild fibrosis = -0.31 or more negative; (2) moderate fibrosis if outcome > -0.31 (more positive) and up to +0.86; and (3) advance fibrosis if outcome > 0.86.

Our index with a cut-off value  $\geq 0.86$  exhibited an AUROC of 0.91 for predicting advanced stages of liver fibrosis (F3-F4) with a sensitivity, specificity, positive predictive value, negative predictive value and positive likelihood ratio of 73%, 90.9%, 74.7%, 90.0% and 8.0, respectively. The diagnostic accuracy of our index for the predicting of advanced liver fibrosis (F3-F4) was more effective than other scores such as the Fibrotest, APRI, Fibrometere, Hepascore. Degos *et al.*<sup>[32]</sup> performed a large study ( $n = 1307$ ) that compared transient elastography with patented and non-patented biomarkers (e.g., Fibrotest, Fibrometere, Hepascore and APRI) compared to liver biopsy. They reported an AUROCs of 0.76 for transient elastography, which did not differ from the AUROCs of the serum markers (0.72-0.78) for the diagnosing of significant fibrosis (F2-F3). However, they reported an AUROC of 0.90 for transient elastography compared to 0.82, 0.86, 0.77 and 0.86 for the Fibrotest, Fibrometere, APRI and Hepascore respectively, for the diagnosing of F4.

Our discriminating index using a cut-off value < 0.31 exhibited an AUROC of 0.88 in the diagnosing of no or mild fibrosis (F0-F2) with a sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio of 67.2%, 86.3%, 83.6%, 69.5% and 4.9, respectively, which indicates high diagnostic performance in the diagnosing of this group of patients. Most currently available scores did not diagnose this group of patients. Poynard *et al.*<sup>[33]</sup> performed a meta-analysis of 30 studies that assessed the diagnostic value of the Fibrotest compared to liver biopsy and found that the AUROC for Fibrotest in the diagnosing of adjacent stages of fibrosis (F1 vs F2) was 0.77 (0.75-0.79), and the AUROC was 0.83 (0.81-0.85) for advanced fibrosis (F3-F4). These figures for Fibrotest in the diagnosing of mild and advanced fibrosis are lower in performance than in our index.

Koda *et al.*<sup>[26]</sup> formulated their Fibroindex and reported its accuracy compared to APRI and Forns indices. Their data indicated that the AUROCs of APRI, Forns index, and Fibronectin were 0.78, 0.78 and 0.83, respectively, in discriminating mild degrees of fibrosis (F0-F1) vs significant stages of fibrosis (F2-F3), but the AUROCs were 0.81, 0.83 and 0.85, respectively, for discriminating F3-F4.

These data indicate that our index exhibited higher AUROCs for predicting advanced and mild stages of fibrosis than the currently available scores with higher

performance accuracy.

Attallah *et al.*<sup>[34]</sup> reported the Fibronectin discriminant score (FDS), using fibronectin, APRI and albumin. FDS exhibited an AUROC of 0.91 in discriminating F0-F1 vs F2-F4 and an AUROC of 0.86 in discriminating F0-F2 vs F3-F4. These data are nearly equal to the values of our discriminating index.

However, one limitation of our index is the low performance in the diagnosing of F2. The AUROCs for the diagnosing of F2 was 0.64 with cut-off values of  $\geq -0.31$  up to +0.86 with a sensitivity, specificity, positive predictive value, negative predictive value, and a positive likelihood ratio of 53.1%, 74.1%, 33.0%, 86.8% and 2.0, respectively.

Crisan *et al.*<sup>[35]</sup> validated the performance of six blood scores (APRI, Forns, Fib-4, Hepascore, Fibrotest and Fibrometere) using transient elastography compared to liver biopsy. Their data indicated that significant fibrosis  $F > 2$  was predicted with AUROCs of 0.727, 0.680, 0.714, 0.778, 0.688, 0.797 and 0.751 for APRI, Forns, Fib-4, Fibrotest, Hepascore, Fibrometere and transient elastography, respectively, and AUROCs were 0.741, 0.737, 0.767, 0.705, 0.811, 0.782 and 0.809 in the diagnosis of severe fibrosis (F3-F4). These data provide further support to the higher performance of our index compared to these six serum scores.

Chisti *et al.*<sup>[36]</sup> performed a prospective study to validate three biological scores (Fibrotest, Fibrometere and Hepascore) and reported AUROCs for the predicting of mild to moderate fibrosis of 0.81, 0.85, and 0.77, respectively and AUROCs for the diagnosing of F4 of 0.84, 0.92 and 0.88 respectively. These figures approximate our discriminating scores in the predicting of mild and advanced stages of fibrosis.

Our discriminating index was validated *via* application to originally selected patients. The results indicated that the model correctly classified two-thirds of the cases (66.1%). This sensitivity increased to 67.2% and 73% in mild and advanced fibrosis, respectively, but dropped to 53.1% in moderate fibrosis.

Our discriminating score exhibited higher performance in the diagnosing of mild or no fibrosis and advanced stages of liver fibrosis than the currently available blood tests, but our study and others evaluations of biological scores used liver biopsy as the reference standard.

Poynard *et al.*<sup>[37]</sup> investigated the performance of liver biopsy itself compared to two non-invasive tests (Fibrotest and Fibroscan) in the absence of the gold standard. The authors reported a relatively lower level of performance for liver biopsy even with the use of 20 mm length for the diagnosis of significant fibrosis (F2-F3). The specificity and sensitivity were 0.67 and 0.63, respectively, for liver biopsy compared to 0.93 and 70 and 0.95 and 0.50 for the Fibrotest and Fibroscan, respectively. These reported data suggested that the discordance between a non-invasive blood test and liver biopsy may be due to the lower diagnostic efficiency of the liver biopsy itself.

The end point of treatment of patients with HCV infections is virus eradication, improvements in liver



histology and prevention of the development of complications. Lee *et al.*<sup>[38]</sup> recently reported on the regression, maintenance and progression of liver fibrosis after virological cure. Pyonard *et al.*<sup>[39]</sup> validated the use of non-invasive markers (Fibrotest and Fibroscan) in a prospective longitudinal study for the prediction of fibrosis regression and development of complications.

The current policy is to follow-up with hepatitis C patients even if these patients are cured virologically. Here, the availability of an easily assessed, less expensive, reproducible blood test with high performance may alleviate or reduce the need for liver biopsy.

In conclusion, our discriminating index for liver fibrosis in hepatitis C genotype 4 patients is a simple, easily reproducible test with accepted accuracy. The index is based on biomarkers that are related to the development and progression of liver fibrosis.

### Limitation of the study

The lack of external validation of the obtained discriminating index is a limitation of this study. Our index is a candidate for multicenter external validation. This index may also be subjected to longitudinal studies to validate its prediction of future complications in HCV patients. Other limitations are the lack of two pathological observers for each specimen and the lack of determination of elastin connective tissue added to collagens.

## COMMENTS

### Background

Hepatitis C virus (HCV) induces liver fibrosis through transforming hepatic stellate cells and other intrahepatic cells to fibrous tissue laying cells. The severity of liver fibrosis is related to multiple host and viral factors. These factors are reflected on changes on biological variables. Studying levels of serum levels of some of these biological markers may provide a non-invasive test that can predict the liver fibrosis stage.

### Research frontiers

Multiple studies have reported about the increased insulin resistance in HCV infections, possibly as a part of HCV-induced metabolic syndrome. Also, there are available data about the impact of increased activity of hepatitis on the development and progression of liver fibrosis. The authors studied multiple biological and host factors in a cohort of genotype 4 Egyptian patients to assess the predictive ability of these variables in diagnosis of liver fibrosis stage.

### Innovations and breakthroughs

This study identified insulin resistance as estimated by homeostasis model of insulin resistance, aspartate transaminase enzyme, platelet count, and age as significant predictors of liver fibrosis stage. A model could be obtained utilizing these markers that could predict liver fibrosis stage with accuracy performance higher than available biological tests. The index is easily applicable and with low expenses.

### Applications

The non-invasive test for diagnosis of liver fibrosis stage can alleviate or reduce the need of the invasive liver biopsy to determine the level of liver fibrosis at basal level before starting antiviral treatment. Because liver biopsy cannot be done sequentially to follow-up HCV patients with or without virus cure, the non-invasive test may provide acceptable tool to do this task.

### Terminology

HOMA-IR: Homeostasis model for insulin resistance, a mathematically calculated

formula; ACE: Angiotensin converting enzyme; AST: Aspartate transaminase enzyme; BMI: Body mass index; W/H: Waist/hip ratio.

### Peer-review

The study is interesting and shows a good bibliographic study.

## REFERENCES

- 1 **Lavanchy D.** Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011; **17**: 107-115 [PMID: 21091831 DOI: 10.1111/j.1469-0691.2010.03432.x]
- 2 **Mohd Hanafiah K,** Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]
- 3 **Sievert W,** Altraif I, Razavi HA, Abdo A, Ahmed EA, Alomair A, Amarapurkar D, Chen CH, Dou X, El Khayat H, Elshazly M, Esmat G, Guan R, Han KH, Koike K, Lagen A, McCaughan G, Mogawer S, Monis A, Nawaz A, Piratvisuth T, Sanai FM, Sharara AI, Sibbel S, Sood A, Suh DJ, Wallace C, Young K, Negro F. A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. *Liver Int* 2011; **31** Suppl 2: 61-80 [PMID: 21651703 DOI: 10.1111/j.1478-3231.2011.02540.x]
- 4 **Moreira RK.** Hepatic stellate cells and liver fibrosis. *Arch Pathol Lab Med* 2007; **131**: 1728-1734 [PMID: 17979495]
- 5 **Friedman SL.** Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; **134**: 1655-1669 [PMID: 18471545 DOI: 10.1053/j.gastro.2008.03.003]
- 6 **Presser LD,** McRae S, Waris G. Activation of TGF- $\beta$ 1 promoter by hepatitis C virus-induced AP-1 and Sp1: role of TGF- $\beta$ 1 in hepatic stellate cell activation and invasion. *PLoS One* 2013; **8**: e56367 [PMID: 23437118 DOI: 10.1371/journal.pone.0056367]
- 7 **Franciscus A.** HCV diagnostic tools: grading and staging a liver biopsy. 2010
- 8 **Seeff LB,** Everson GT, Morgan TR, Curto TM, Lee WM, Ghany MG, Shiffman ML, Fontana RJ, Di Bisceglie AM, Bonkovsky HL, Dienstag JL. Complication rate of percutaneous liver biopsies among persons with advanced chronic liver disease in the HALT-C trial. *Clin Gastroenterol Hepatol* 2010; **8**: 877-883 [PMID: 20362695 DOI: 10.1016/j.cgh.2010.03.025]
- 9 **Basaranoglu M,** Basaranoglu G. Pathophysiology of insulin resistance and steatosis in patients with chronic viral hepatitis. *World J Gastroenterol* 2011; **17**: 4055-4062 [PMID: 22039318 DOI: 10.3748/wjg.v17.i36.4055]
- 10 **Moucari R,** Ripault MP, Martinot-Peignoux M, Voitot H, Cardoso AC, Stern C, Boyer N, Maylin S, Nicolas-Chanoine MH, Vidaud M, Valla D, Bedossa P, Marcellin P. Insulin resistance and geographical origin: major predictors of liver fibrosis and response to peginterferon and ribavirin in HCV-4. *Gut* 2009; **58**: 1662-1669 [PMID: 19671541 DOI: 10.1136/gut.2009.185074]
- 11 **Medina J,** Arroyo AG, Sánchez-Madrid F, Moreno-Otero R. Angiogenesis in chronic inflammatory liver disease. *Hepatology* 2004; **39**: 1185-1195 [PMID: 15122744 DOI: 10.1002/hep.20193]
- 12 **Oruç N,** Lamb J, Whitcomb DJ, Sass DA. The ACE gene I/D polymorphism does not affect the susceptibility to or prognosis of PBC. *Turk J Gastroenterol* 2008; **19**: 250-253 [PMID: 19119484]
- 13 **American Diabetes Association.** Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2004; **27** Suppl 1: S5-S10 [PMID: 14693921 DOI: 10.2337/diacare.27.2007.S5]
- 14 **Bedossa P,** Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293 [PMID: 8690394 DOI: 10.1002/hep.510240201]
- 15 **Bonora E,** Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 2000; **23**: 57-63 [PMID: 10857969 DOI: 10.2337/diacare.23.1.57]
- 16 **Eslam M,** Kawaguchi T, Del Campo JA, Sata M, Khattab MA, Romero-Gomez M. Use of HOMA-IR in hepatitis C. *J Viral Hepat* 2011; **18**: 675-684 [PMID: 21914084 DOI: 10.1111/j.1365-2893.

- 2011.01474.x]
- 17 **Bedossa P**, Carrat F. Liver biopsy: the best, not the gold standard. *J Hepatol* 2009; **50**: 1-3 [PMID: 19017551 DOI: 10.1016/j.jhep.2008.10.014]
- 18 **Castera L**, Bedossa P. How to assess liver fibrosis in chronic hepatitis C: serum markers or transient elastography vs. liver biopsy? *Liver Int* 2011; **31** Suppl 1: 13-17 [PMID: 21205132 DOI: 10.1111/j.1478-3231.2010.02380.x]
- 19 **Pinzani M**, Vizzutti F, Arena U, Marra F. Technology Insight: non-invasive assessment of liver fibrosis by biochemical scores and elastography. *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 95-106 [PMID: 18253138 DOI: 10.1038/ncpgasthep1025]
- 20 **Lupsor Platon M**, Stefanescu H, Feier D, Maniu A, Badea R. Performance of unidimensional transient elastography in staging chronic hepatitis C. Results from a cohort of 1,202 biopsied patients from one single center. *J Gastrointest Liver Dis* 2013; **22**: 157-166 [PMID: 23799214]
- 21 **Poynard T**, Ngo Y, Munteanu M, Thabut D, Massard J, Moussalli J, Varaud A, Benhamou Y, Ratziu V. Biomarkers of liver injury for hepatitis clinical trials: a meta-analysis of longitudinal studies. *Antivir Ther* 2010; **15**: 617-631 [PMID: 20587855 DOI: 10.3851/IMP1570]
- 22 **Castera L**. Non-invasive assessment of liver fibrosis in chronic hepatitis C. *Hepatol Int* 2011; **5**: 625-634 [PMID: 21484142 DOI: 10.1007/s12072-010-9240-0]
- 23 **Forns X**, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM, Rodés J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986-992 [PMID: 12297848 DOI: 10.1053/jhep.2002.36128]
- 24 **Sterling RK**, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325 [PMID: 16729309 DOI: 10.1002/hep.21178]
- 25 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]
- 26 **Koda M**, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. *Hepatology* 2007; **45**: 297-306 [PMID: 17256741 DOI: 10.1002/hep.21520]
- 27 **Halfon P**, Bacq Y, De Muret A, Penaranda G, Bourliere M, Ouzan D, Tran A, Botta D, Renou C, Bréchet MC, Degott C, Paradis V. Comparison of test performance profile for blood tests of liver fibrosis in chronic hepatitis C. *J Hepatol* 2007; **46**: 395-402 [PMID: 17156890 DOI: 10.1016/j.jhep.2006.09.020]
- 28 **Kawasaki T**, Takeshita A, Souda K, Kobayashi Y, Kikuyama M, Suzuki F, Kageyama F, Sasada Y, Shimizu E, Murohisa G, Koide S, Yoshimi T, Nakamura H, Ohno R. Serum thrombopoietin levels in patients with chronic hepatitis and liver cirrhosis. *Am J Gastroenterol* 1999; **94**: 1918-1922 [PMID: 10406260 DOI: 10.1111/j.1572-0241.1999.01231.x]
- 29 **Macaluso FS**, Maida M, Minissale MG, Li Vigni T, Attardo S, Orlando E, Petta S. Metabolic factors and chronic hepatitis C: a complex interplay. *Biomed Res Int* 2013; **2013**: 564645 [PMID: 23956991 DOI: 10.1155/2013/564645]
- 30 **Sud A**, Hui JM, Farrell GC, Bandara P, Kench JG, Fung C, Lin R, Samarasinghe D, Liddle C, McCaughan GW, George J. Improved prediction of fibrosis in chronic hepatitis C using measures of insulin resistance in a probability index. *Hepatology* 2004; **39**: 1239-1247 [PMID: 15122752 DOI: 10.1002/hep.20207]
- 31 **Bataller R**, Ginès P, Nicolás JM, Görgbig MN, Garcia-Ramallo E, Gasull X, Bosch J, Arroyo V, Rodés J. Angiotensin II induces contraction and proliferation of human hepatic stellate cells. *Gastroenterology* 2000; **118**: 1149-1156 [PMID: 10833490 DOI: 10.1016/S0016-5085(00)70368-4]
- 32 **Degos F**, Perez P, Roche B, Mahmoudi A, Asselineau J, Voitot H, Bedossa P. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC study). *J Hepatol* 2010; **53**: 1013-1021 [PMID: 20850886 DOI: 10.1016/j.jhep.2010.05.035]
- 33 **Poynard T**, Morra R, Halfon P, Castera L, Ratziu V, Imbert-Bismut F, Naveau S, Thabut D, Lebre C, Zoulim F, Bourliere M, Cacoub P, Messous D, Munteanu M, de Ledinghen V. Meta-analyses of FibroTest diagnostic value in chronic liver disease. *BMC Gastroenterol* 2007; **7**: 40 [PMID: 17937811 DOI: 10.1186/1471-230X-7-40]
- 34 **Attallah AM**, Abdallah SO, Attallah AA, Omran MM, Farid K, Nasif WA, Shiha GE, Abdel-Aziz AA, Rasafy N, Shaker YM. Diagnostic value of fibronectin discriminant score for predicting liver fibrosis stages in chronic hepatitis C virus patients. *Ann Hepatol* 2013; **12**: 44-53 [PMID: 23293193]
- 35 **Crisan D**, Radu C, Lupsor M, Sparchez Z, Grigorescu MD, Grigorescu M. Two or more synchronous combination of non-invasive tests to increase accuracy of liver fibrosis assessment in chronic hepatitis C; results from a cohort of 446 patients. *Hepat Mon* 2012; **12**: 177-184 [PMID: 22550525 DOI: 10.5812/hepatmon.853]
- 36 **Chisti MJ**, Graham SM, Duke T, Ahmed T, Ashraf H, Faruque AS, La Vincente S, Banu S, Raqib R, Salam MA. A prospective study of the prevalence of tuberculosis and bacteraemia in Bangladeshi children with severe malnutrition and pneumonia including an evaluation of Xpert MTB/RIF assay. *PLoS One* 2014; **9**: e93776 [PMID: 24695758 DOI: 10.1371/journal.pone.0093776]
- 37 **Poynard T**, de Ledinghen V, Zarski JP, Stanciu C, Munteanu M, Vergniol J, France J, Trifan A, Le Naour G, Vaillant JC, Ratziu V, Charlotte F. Relative performances of FibroTest, Fibroscan, and biopsy for the assessment of the stage of liver fibrosis in patients with chronic hepatitis C: a step toward the truth in the absence of a gold standard. *J Hepatol* 2012; **56**: 541-548 [PMID: 21889468 DOI: 10.1016/j.jhep.2011.08.007]
- 38 **Lee YA**, Friedman SL. Reversal, maintenance or progression: what happens to the liver after a virologic cure of hepatitis C? *Antiviral Res* 2014; **107**: 23-30 [PMID: 24726738 DOI: 10.1016/j.antiviral.2014.03.012]
- 39 **Poynard T**, Vergniol J, Ngo Y, Foucher J, Munteanu M, Merrouche W, Colombo M, Thibault V, Schiff E, Brass CA, Albrecht JK, Rudler M, Deckmyn O, Lebray P, Thabut D, Ratziu V, de Ledinghen V. Staging chronic hepatitis C in seven categories using fibrosis biomarker (FibroTest™) and transient elastography (FibroScan®). *J Hepatol* 2014; **60**: 706-714 [PMID: 24291240 DOI: 10.1016/j.jhep.2013.11.016]

**P- Reviewer:** Cabibi D, Wang Y **S- Editor:** Ji FF **L- Editor:** A  
**E- Editor:** Li D



Randomized Clinical Trial

# Telbivudine vs tenofovir in hepatitis B e antigen-negative chronic hepatitis B patients: OPTIMA roadmap study

Zahari Krastev, Diana Petrova, Iskren Kotzev, Mustafa Kemal Celen, Meryl Mendelson, Richa Chandra, Priti Pandey, Kamal Hamed

Zahari Krastev, Clinic of Gastroenterology, St. Ivan Rilsky University Hospital, Medical University, Sofia 1606, Bulgaria

Diana Petrova, Department of Gastroenterology, University Hospital Alexandrovska, Sofia 1431, Bulgaria

Iskren Kotzev, Clinic of Hepatogastroenterology, University Hospital St Marina, Varna 9010, Bulgaria

Mustafa Kemal Celen, Infectious Disease Clinic, Dicle University, 21280 Diyarbakir, Turkey

Meryl Mendelson, Richa Chandra, Kamal Hamed, Novartis Pharmaceuticals Corporation, East Hanover, NJ 07936, United States

Priti Pandey, Novartis Healthcare Pvt. Ltd., Hyderabad 500081, India

**Author contributions:** All authors were involved in study conduct, data interpretation and defining the content for the manuscript; all authors had full access to data in the study, discussed the results, critically reviewed the draft manuscript and agreed on the final version.

Supported by Novartis Pharma AG.

**Institutional review board statement:** The study received approval from the Ethik-Kommission der Medizinischen Universität Wien und des Allgemeinen Krankenhauses der Stadt in Austria; Ethics Committee for Multicentre Trials in Bulgaria; RF MoHSD, Department of State Regulation of Circulation of Medicines, Ethics Council in Russia; National Ethics Committee for Clinical Trials in Greece; Comitato Etico Azienda Ospedaliera Universitaria Policlinico P. Giaccone in Italy; Institut Municipal D'Investigació mèdica in Spain; Ethik-Kommission der Albert-Ludwigs-Universität Freiburg in Germany; and Ege University Medical Faculty Clinical Research Ethics Committee in Turkey.

**Informed consent statement:** This study was conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines. Written informed consent was obtained from

each patient before enrolment.

**Conflict-of-interest statement:** Krastev Z received fees for serving as a member of advisory board of Gilead, as well as research funding from Abbvie, Applied Clinical Pharmacology Services, Centocor, Comac Medical, Gilead, GSK, Idenix, Johnson and Johnson, Millennium Pharmaceuticals, MSD, Norgine, Novartis, Roche, Receptos and Schwabe; Petrova D received research funding from Aventis, Centocor, Gilead, GSK, Idenix, Johnson and Johnson, Norgine, Novartis and Roche; Kotzev I received lecture fees from Novartis; Celen MK has nothing to declare; Mendelson M, Chandra R and Hamed K are employees of Novartis Pharmaceuticals Corporation; Pandey P is an employee of Novartis Healthcare Pvt. Ltd.

**Data sharing statement:** No data were created, so no data are available.

**Open-Access:** This article is an open-access article, which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Kamal Hamed, MD, MPH, Sr Worldwide Medical Director, Novartis Pharmaceuticals Corporation, 1 Health Plaza, East Hanover, NJ 07936, United States. [kamal.hamed@novartis.com](mailto:kamal.hamed@novartis.com)  
**Telephone:** +1-862-7781371  
**Fax:** +1-973-7817153

**Received:** March 16, 2016  
**Peer-review started:** March 18, 2016  
**First decision:** April 19, 2016  
**Revised:** May 6, 2016  
**Accepted:** July 14, 2016  
**Article in press:** July 18, 2016

Published online: November 18, 2016

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

## Abstract

### AIM

To make efficacy and safety comparison of telbivudine-roadmap and tenofovir-roadmap in hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) patients.

### METHODS

This was the first prospective, randomised, two-arm, open-label, non-inferiority study in HBeAg-negative CHB patients that compared telbivudine and tenofovir administered as per roadmap concept. Patients were treated up to 24 wk and, depending on virologic response, continued the same therapy or received add-on therapy up to 104 wk. Eligible patients received an additional 52 wk of treatment in the extension period (*i.e.*, up to 156 wk). Patients who developed virologic breakthrough (VB) while on monotherapy also received add-on therapy. The primary efficacy endpoint was the rate of patients achieving hepatitis B virus (HBV) DNA < 300 copies/mL at week 52. Secondary efficacy endpoints included the rates of HBV DNA < 300 and < 169 copies/mL, HBV DNA change from baseline, alanine aminotransferase normalisation, hepatitis B surface antigen (HBsAg) loss, HBsAg seroconversion, VB, and emergence of resistance at various timepoints throughout the study. Safety and estimated glomerular filtration rate (eGFR) were also analysed.

### RESULTS

A total of 241 patients were randomised. Non-inferiority of telbivudine arm to tenofovir arm was demonstrated at week 52 ( $\pm 7$  d window), with over 91% of patients in each treatment arm achieving HBV DNA level < 300 copies/mL. Both arms were similar in terms of key secondary efficacy variables at weeks 104 and 156. The percentage of patients achieving HBV DNA < 300 copies/mL remained high and was similar in the telbivudine and tenofovir arms at both weeks 104 and 156. Over 82% of patients in both arms achieved alanine aminotransferase normalisation at week 52, and this percentage remained high at weeks 104 and 156. Telbivudine treatment progressively reduced serum HBsAg levels from baseline while no change was reported in quantitative HBsAg during therapy with tenofovir. Both treatments showed acceptable safety profiles. The telbivudine arm showed eGFR improvement unlike the tenofovir arm.

### CONCLUSION

Efficacy was shown for both telbivudine-roadmap and tenofovir-roadmap regimens in HBeAg-negative CHB patients over 156 wk. Telbivudine arm was associated with renal improvement.

**Key words:** Chronic hepatitis B; Glomerular filtration rate; Telbivudine; Tenofovir; Roadmap concept

**Core tip:** This was the first prospective, randomised, non-inferiority study in hepatitis B e antigen-negative chronic hepatitis B patients that compared telbivudine and tenofovir administered as per roadmap concept. Both treatments based on the roadmap approach were effective over a 156 wk treatment period. Non-inferiority of telbivudine arm to tenofovir arm was demonstrated at week 52, with over 91% of patients in each treatment arm achieving hepatitis B virus DNA level < 300 copies/mL. Both treatments showed acceptable safety profiles. Moreover, telbivudine showed an improvement in estimated glomerular filtration rate from baseline.

Krastev Z, Petrova D, Kotzev I, Celen MK, Mendelson M, Chandra R, Pandey P, Hamed K. Telbivudine vs tenofovir in hepatitis B e antigen-negative chronic hepatitis B patients: OPTIMA roadmap study. *World J Hepatol* 2016; 8(32): 1402-1413 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i32/1402.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i32.1402>

## INTRODUCTION

Approximately 240-400 million people worldwide are chronically infected with hepatitis B virus (HBV), with a wide variation of prevalence among countries, and more than 780000 people die every year due to acute or chronic hepatitis B (CHB)<sup>[1-3]</sup>. Although CHB may be treated with interferon or nucleos(t)ide analogue (NA) antivirals, emergence of resistance due to prolonged NA therapy or incomplete suppression of HBV still remains an important concern<sup>[4]</sup>. Several studies have suggested that the use of response-guided add-on therapy is associated with a higher rate of virologic response and reduced antiviral resistance as compared to sequential monotherapy<sup>[5,6]</sup>.

Early virologic response has been used as a guide to predict better outcomes and to reduce the risk of antiviral resistance<sup>[7,8]</sup>. As previously reported<sup>[9,10]</sup>, the roadmap concept uses early virologic response at week 24 to individualize ongoing management of CHB patients. Patients with a complete response at week 24 can remain on their initial therapy, whereas treatment modification that may include the addition of a second drug is done for those with an inadequate virologic response. This strategy is relevant mainly in patients receiving NA with a low genetic barrier to resistance (clevudine, emtricitabine, lamivudine, telbivudine)<sup>[10]</sup>. In hepatitis B e antigen (HBeAg)-positive CHB patients treated with telbivudine, a response-guided treatment optimization strategy with telbivudine based on the roadmap concept has been demonstrated to improve the clinical outcomes of patients with a suboptimal antiviral response<sup>[11,12]</sup>.

The aim of this study, OPTIMA, was to assess the efficacy and safety of telbivudine and tenofovir regimens,



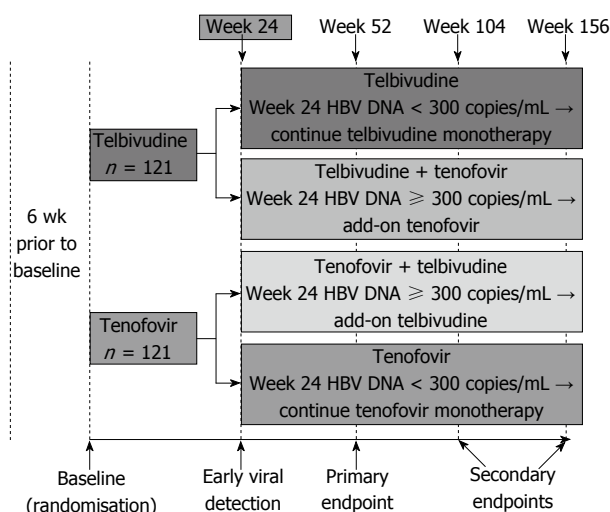


Figure 1 Study design. HBV: Hepatitis B virus.

when administered using the roadmap concept, in HBeAg-negative patients with CHB. This was the first study that compared efficacy of the 2 regimens in a prospective manner. The safety of the combination of telbivudine and tenofovir, for which limited data are currently available, was also evaluated.

## MATERIALS AND METHODS

### Study design and conduct

OPTIMA was a prospective, randomised, 2-arm, open-label study (ClinicalTrials.gov ID: NCT01379508) that enrolled patients between February 2011 and October 2012 in 8 countries (Austria, Bulgaria, Germany, Greece, Italy, Russia, Spain and Turkey). This study was approved by the Institutional Review Board at each participating centre, and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from each patient before enrolment.

Eligible patients were randomised *via* an interactive voice response system in a 1:1 ratio to either telbivudine arm (600 mg/d) or tenofovir arm (300 mg/d) (Figure 1). Randomisation was stratified by the screening HBV DNA level ( $< 7 \log_{10}$  copies/mL or  $\geq 7 \log_{10}$  copies/mL) and alanine aminotransferase (ALT) level [ $< 3 \times$  upper limit of normal (ULN) or  $\geq 3 \times$  ULN].

This study used the response-guided add-on strategy (roadmap concept). For patients with HBV DNA  $\geq 300$  copies/mL ( $\geq 51$  IU/mL) at week 24, tenofovir was added to telbivudine by week 26 in the telbivudine arm, and telbivudine was added to tenofovir by week 26 in the tenofovir arm. For patients with HBV DNA  $< 300$  copies/mL at week 24, telbivudine and tenofovir monotherapies in the respective arms were continued. Patients who developed virologic breakthrough (VB) while on monotherapy received add-on therapy. However, patients who developed VB after week 24 while on combination therapy were discontinued from the study.

### Patients

Eligible patients were male or female  $\geq 18$  years of age, with detectable hepatitis B surface antigen (HBsAg) for  $\geq 6$  mo, HBeAg-negative with positive hepatitis B e antibody, available liver histology report within 12 mo before screening compatible with CHB (patients without evaluable liver histology were eligible if they had clinical evidence of compensated liver cirrhosis or non-invasive methods that support the diagnosis of moderate to severe liver inflammation and/or fibrosis), serum HBV DNA  $> 2000$  IU/mL, and serum ALT level  $> 1 \times$  ULN and  $< 10 \times$  ULN at the screening visit. Patients with ALT  $\leq 1 \times$  ULN at screening were eligible if they had at least moderate liver inflammation or fibrosis, clinical evidence of compensated cirrhosis, or ALT level  $> 1 \times$  ULN within the last 6 mo.

Main exclusion criteria included co-infection with hepatitis C virus, hepatitis D virus or human immunodeficiency virus; hepatic decompensation; liver disease other than CHB; any nucleos(t)ide or interferon/immunomodulator treatment in the previous 6 mo; chronic renal insufficiency or serum creatinine clearance  $< 50$  mL/min; history of myopathy, myositis, or persistent muscle weakness; pregnant or nursing (lactating) women; or history of malignancy of any organ system (other than localized basal cell carcinoma of the skin).

Patients were allowed to receive an additional 52 wk of treatment in the extension period (*i.e.*, up to 156 wk) if they had HBV DNA  $< 300$  copies/mL at both weeks 92 and 104, and serum creatinine clearance  $\geq 50$  mL/min at two consecutive visits including week 104.

### Efficacy and safety analyses

The primary efficacy endpoint was the rate of patients achieving HBV DNA  $< 300$  copies/mL (51 IU/mL) at week 52. Secondary efficacy endpoints included the rates of patients with HBV DNA  $< 300$  copies/mL at weeks 104 and 156, and HBV DNA  $< 169$  copies/mL (29 IU/mL) (lower limit of detection) at weeks 24, 52, 104 and 156; change from baseline in HBV DNA; ALT normalisation at weeks 52, 104 and 156; HBsAg loss and HBsAg seroconversion; VB; and emergence of resistance. In addition, subgroup analyses were performed for secondary efficacy endpoints by baseline HBV DNA (*i.e.*,  $< 7 \log_{10}$  copies/mL or  $\geq 7 \log_{10}$  copies/mL).

VB was defined as an increase of HBV DNA by at least  $1 \log_{10}$  copies/mL (or  $1 \log_{10}$  IU/mL) above nadir on 2 consecutive visits, or at the last on-treatment visit in patients who did not have a primary non-response. Emergence of resistance was assessed as the rate of confirmed treatment-emergent genotypic resistance and was assessed at the time of confirmed VB and at week 24 in patients with viral load  $\geq 300$  copies/mL, it was calculated cumulatively at weeks 52, 104 and 156.

HBV DNA detection and quantification were performed at a central laboratory using the COBAS TaqMan real-time polymerase chain reaction assay (Roche Molecular Systems, Branchburg, NJ, United States).

Safety assessments included monitoring of adverse events (AEs), vital signs, and graded laboratory abnormalities. Estimated glomerular filtration rate (eGFR), calculated by the modification of diet in renal disease formula was recorded. AEs of special interest (muscle and renal function related events) were also reported.

### Statistical analysis

For the primary efficacy analysis, study treatments were compared for non-inferiority.

Based on the assumptions of 96% and 97% HBV DNA < 300 copies/mL at week 52 in the telbivudine arm and the tenofovir arm, respectively, and an approximately 10% dropout rate, it was estimated that 120 randomised patients per arm would provide 87% power for the non-inferiority testing on the primary analysis. Non-inferiority in efficacy of telbivudine arm to tenofovir arm was to be claimed if the lower limit of the 2-sided confidence interval (CI) for the difference was above the pre-determined non-inferiority margin (-10%).

A weighted Cochran-Mantel-Haenszel method, adjusting for randomisation strata [HBV DNA (< or  $\geq 7 \log_{10}$  copies/mL) and ALT (< or  $\geq 3 \times$  ULN) levels], was used to assess comparative therapeutic response rates.

For continuous variables, summary statistics of absolute value and of change from baseline, including mean, standard deviation (SD), median, minimum, and maximum were used. For dichotomous endpoints, statistical summaries included count and percentage of patients with a positive response (response rate) and also 95%CI for the response rate.

The intent-to-treat (ITT) population consisted of all patients who received at least one dose of study drug and had at least one post-baseline assessment of serum HBV DNA. The roadmap ITT (rITT) population consisted of all patients who did not discontinue before week 24 and did not deviate from the protocol defined rules of receiving add-on at week 24 (*i.e.*, patients who received the add-on therapy at week 24 if they had HBV DNA  $\geq 300$  copies/mL, or did not receive the add-on at week 24 if they had HBV DNA < 300 copies/mL). The modified ITT (mITT) population consisted of all patients in the ITT population who were eligible and enrolled in the extension period beyond week 104. The per-protocol population consisted of all patients in the ITT population who had no major protocol deviations.

All efficacy observations on or after censoring date were treated as missing. A patient's censoring date was the date of the first occurrence of: One day after the last dose of the study drug, the start of first prohibited CHB-related medication, pregnancy, or a specific major protocol deviation. To assess the robustness of the results due to missing data, the analysis of primary and all secondary efficacy endpoints were performed based on the rITT and ITT analysis populations. The mITT population was used only for the week 156 analysis.

The primary efficacy endpoint (week 52) analysis was performed on the rITT population. The analyses presented include: (1) assessments within the  $\pm 7$  d protocol-pre-

specified visit window around the scheduled week 52 date; (2) missing data at week 52 treated as failure; (3) missing data imputed using the earliest available assessment within the 28 d window starting from the scheduled week 52 date; and (4) missing data imputed using the last observation carried forward (LOCF).

Secondary efficacy parameters including HBV DNA, ALT normalisation, HBsAg loss, and HBsAg seroconversion were analysed using two imputation methods for missing data: (1) missing data treated as failure; and (2) missing data imputed using the earliest available assessment within the 28 d window starting from the scheduled visit for weeks 52 (except HBV DNA < 300 copies/mL), 104 and 156. VB and eGFR were analysed using the LOCF imputation method for missing data. Treatment-emergent genotypic resistance was analysed using cumulative imputation method for missing data. Missing eGFR assessments were imputed using the LOCF method.

Analyses of endpoints using LOCF imputation at weeks 104 and 156 are presented for the rITT and mITT populations, respectively.

## RESULTS

### Study patients

A total of 241 patients (121 in the telbivudine arm and 120 in the tenofovir arm) were randomised in this study. A total of 22 (18.2%) patients in the telbivudine arm and 13 (10.8%) patients in the tenofovir arm discontinued prematurely from the study. The most common reasons for discontinuation in the telbivudine arm were consent withdrawal ( $n = 7$ ), lost to follow-up ( $n = 5$ ), and administrative reasons ( $n = 4$ ). In the tenofovir arm, the most common reasons for discontinuation were AEs ( $n = 5$ ), consent withdrawal ( $n = 4$ ), and lost to follow-up ( $n = 3$ ).

Major protocol deviations were reported in 11 (9.1%) patients in the telbivudine arm and 8 (6.7%) patients in the tenofovir arm. The most commonly reported major deviations were patients on monotherapy with confirmed VB not starting add-on therapy within 2 wk of laboratory confirmation of VB ( $n = 9$ ), patients with a positive HBeAg result ( $n = 6$ ), and patients not completing 3 wk of treatment before the third visit ( $n = 4$ ).

The safety population comprised 120 patients in each of the 2 treatment arms. One patient in the telbivudine arm was excluded from the safety population as this patient did not receive any study treatment. Of the 241 randomized patients, 235 patients were included in the ITT population, with 117 (96.7%) in the telbivudine arm and 118 (98.3%) in the tenofovir arm. Six patients were excluded from the ITT population (4 patients in the telbivudine arm due to no post-baseline HBV DNA assessments, non-compliance with the study conduct, or no study treatment received; and 2 patients in the tenofovir arm because of no post-baseline HBV DNA assessments and viral resistance at baseline). A total of 113 (93.4%) patients in the telbivudine arm and 117 (97.5%) patients in the tenofovir arm comprised the

**Table 1** Demographic and baseline characteristics, randomised population

Patients characteristics	Telbivudine ( <i>n</i> = 121)	Tenofovir ( <i>n</i> = 120)
Age, mean (SD), yr	42.1 (11.5)	43.3 (12.6)
Median (min-max)	42.0 (19-70)	44.0 (18-73)
Male gender, <i>n</i> (%)	86 (71.1)	82 (68.3)
Race, Caucasian, <i>n</i> (%)	117 (96.7)	118 (98.3)
Body mass index, mean (SD), kg/m <sup>2</sup>	25.8 (4.1)	25.7 (4.0)
Median (min-max)	25.6 (16.5-40.4)	25.2 (18.4-39.8)
Genotype, <i>n</i> (%)		
A	6 (5.0)	2 (1.7)
B	1 (0.8)	0 (0.0)
C	0 (0.0)	1 (0.8)
D	104 (86.0)	110 (91.7)
G	1 (0.8)	0 (0.0)
Other	1 (0.8)	0 (0.0)
Unknown	8 (6.6)	7 (5.8)
HBV DNA, mean (SD), log <sub>10</sub> copies/mL	6.2 (1.5)	6.0 (1.4)
Median (min-max)	6.1 (3.2-9.5)	5.9 (2.5-9.9)
< 7 log <sub>10</sub> , <i>n</i> (%)	85 (70.2)	86 (71.7)
≥ 7 log <sub>10</sub> , <i>n</i> (%)	36 (29.8)	34 (28.3)
Serum alanine aminotransferase, mean (SD), IU/L	79.8 (84.1)	78.2 (86.1)
Median (min-max)	53.0 (13-494)	49.0 (5-568)
Serum aspartate aminotransferase, mean (SD), IU/L	54.0 (52.8)	52.5 (47.1)
Median (min-max)	35.0 (13-347)	35.0 (13-322)
Creatine phosphokinase, mean (SD), IU/L	118.6 (64.4)	160.1 (299.3)
Median (min-max)	104.0 (35-430)	111.0 (36-2976)
eGFR <sup>1</sup> , mean (SD), (mL/min per 1.73 m <sup>2</sup> )	97.4 (17.9)	95.8 (16.4)
Median (min-max)	96.6 (60.9-147.1)	94.2 (60.5-138.4)

<sup>1</sup>eGFR: Estimated glomerular filtration rate (modification of diet in renal disease formula). HBV: Hepatitis B virus; SD: Standard deviation.

rITT population. Five patients (4 in the telbivudine arm and 1 in the tenofovir arm) that were included in the ITT population were excluded from the rITT population because they discontinued before week 24 and were not eligible for or enrolled into the roadmap concept period (weeks 24 to 104).

The per-protocol population consisted of 107 (88.4%) patients in the telbivudine arm and 111 (92.5%) patients in the tenofovir arm. A total of 17 patients (10 in the telbivudine arm and 7 in the tenofovir arm) were included in the ITT and rITT populations but were excluded from the per-protocol population because of major protocol deviations. The mITT population consisted of 79 (65.3%) patients in the telbivudine arm and 89 (74.2%) patients in the tenofovir arm.

Treatment arms were balanced with respect to demographics and baseline characteristics, with no clinically meaningful differences between the telbivudine and tenofovir arms (Table 1). Most (86.0% telbivudine, 91.7% tenofovir) patients were infected with HBV genotype D, and the mean HBV DNA at baseline was 6.2 log<sub>10</sub> copies/mL in the telbivudine arm and 6.0 log<sub>10</sub> copies/mL in the tenofovir arm, with 70.2% and 71.7% of patients, respectively, having a baseline HBV DNA < 7 log<sub>10</sub> copies/mL.

### Primary efficacy endpoint

Virologic response (HBV DNA < 300 copies/mL) at week 52 was achieved in more than 91% of patients in each treatment arm (Figure 2A). The primary endpoint

analysis showed that the antiviral efficacy of telbivudine-roadmap was non-inferior to that of tenofovir-roadmap application at week 52 in the rITT population; the lower bound of the 95%CI for the difference between the 2 treatment arms was above the non-inferiority margin of -10%: -9.4% (utilizing assessments within the ±7 d protocol-prespecified visit window); -8.3% for the 28 d window imputation; and -7.9% for the LOCF imputation. Using missing data as treatment failure, non-inferiority was not demonstrated (lower bound of the 95%CI: -10.5%, just below the protocol defined non-inferiority margin) (Table 2). In this analysis, HBV DNA samples from 6 patients (4 in the telbivudine arm and 2 in the tenofovir arm), although resulted in < 300 copies/mL, were considered as missing because they were not obtained at the week 52 visit date itself (*i.e.*, patients were counted as treatment failures).

The primary endpoint analysis at week 52 in the per-protocol population supported the non-inferiority of the telbivudine arm to the tenofovir arm (98.0% in the telbivudine arm and 99.0% in the tenofovir arm, lower bound of the 95%CI: -4.3%).

### Secondary efficacy endpoints

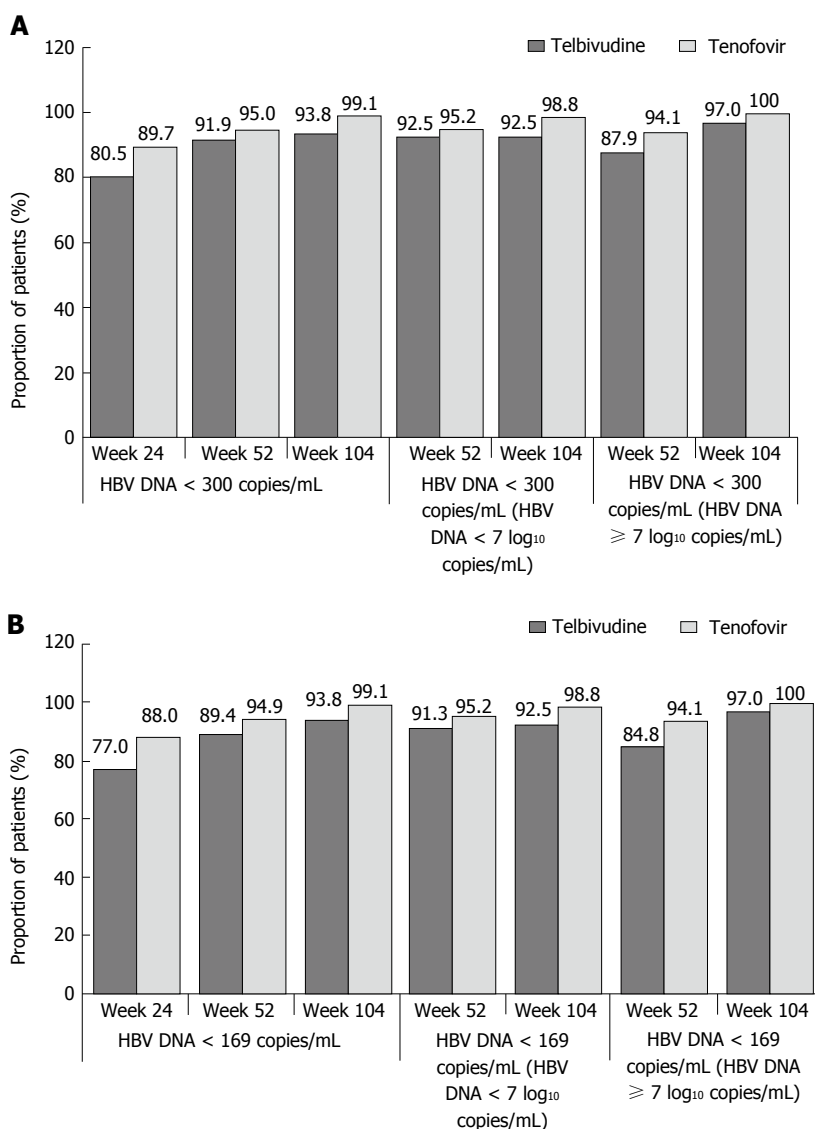
**Virologic responses:** Percentage of patients achieving HBV DNA < 300 copies/mL (51 IU/mL) at weeks 24 and 104, and by baseline viral load at weeks 24, 52 and 104 in the rITT population: The percentage of patients achieving HBV DNA < 300 copies/mL in the telbivudine and tenofovir arms at week 24 was 80.5% and 89.7%,

**Table 2** Virologic response, roadmap intent-to-treat population

Parameters	Telbivudine ( <i>n</i> = 113)	Tenofovir ( <i>n</i> = 117)	Difference between arms and 95%CI
Patients achieving HBV DNA < 300 copies/mL (51 IU/mL) at week 52, <i>n</i> (%)			
± 7 d protocol-prespecified visit window	104 (91.9)	111 (95.0)	-3.1% (-9.4%, 3.1%) <sup>1</sup>
Treating missing as failure	103 (91.0)	111 (95.0)	-4.0% (-10.5%, 2.5%) <sup>1</sup>
28 d imputation	105 (92.7)	111 (95.0)	-2.3% (-8.3%, 3.8%) <sup>1</sup>
Last observation carried forward	108 (95.4)	116 (99.2)	-3.8% (-7.9%, 0.4%) <sup>1</sup>
Change from baseline in HBV DNA levels (log <sub>10</sub> copies/mL) by visit, mean (SD)			<i>P</i> -value
Week 24	-4.001 (1.256)	-4.122 (1.165)	<i>P</i> < 0.0001 <sup>2</sup>
Week 52	-4.356 (1.473)	-4.305 (1.343)	<i>P</i> < 0.0001 <sup>2</sup>
Week 104	-4.281 (1.753)	-4.349 (1.382)	<i>P</i> < 0.0001 <sup>2</sup>

<sup>1</sup>Percentages and 95%CI were calculated using Mantel-Haenszel weighted estimates stratified by baseline HBV DNA and alanine aminotransferase levels;

<sup>2</sup>*P*-values were calculated using paired *t*-test comparing post-baseline timepoints to baseline timepoints. CI: Confidence interval; HBV: Hepatitis B virus; SD: Standard deviation.



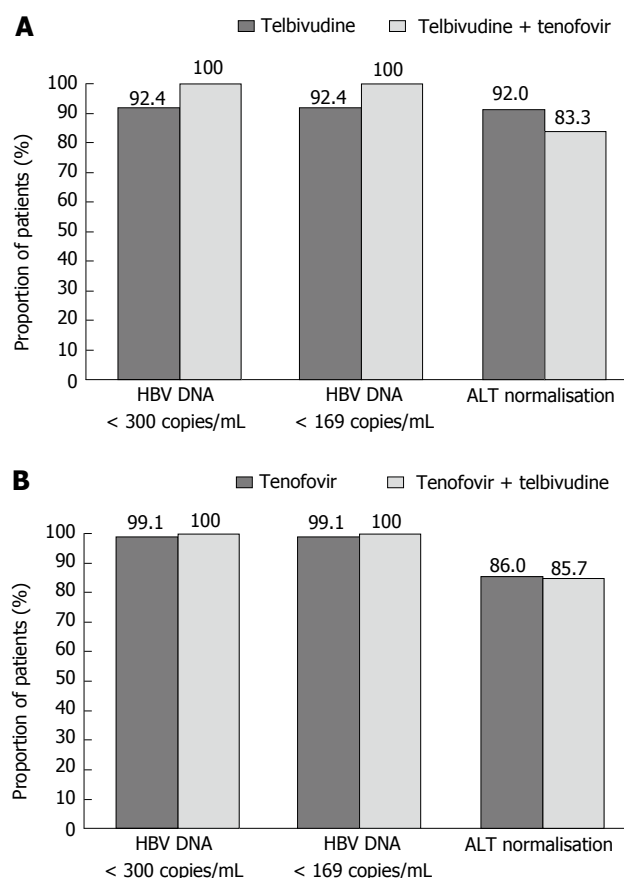
**Figure 2** Proportions of patients achieving hepatitis B virus DNA < 300 (A) or < 169 copies/mL (B), by visit and by baseline hepatitis B virus DNA levels (< 7 or ≥ 7 log<sub>10</sub> copies/mL), roadmap intent-to-treat population. HBV: Hepatitis B virus.

and at week 104, 93.8% and 99.1%, respectively (Figure 2A).

In patients with lower baseline viral load (HBV DNA level < 7 log<sub>10</sub> copies/mL) at week 24, telbivudine and

tenofovir regimens were similar in terms of viral load reduction with 93.8% and 95.2% of patients achieving HBV DNA levels < 300 copies/mL in the telbivudine and tenofovir arms, respectively. At weeks 52 and





**Figure 3** Intensification with tenofovir (A) or telbivudine (B), virologic response and aminotransferase normalisation at week 104, roadmap intent-to-treat population. ALT: Alanine aminotransferase; HBV: Hepatitis B virus.

104, telbivudine and tenofovir regimens seemed to be similar in terms of viral load reduction, with over 92% of patients achieving HBV DNA levels < 300 copies/mL at weeks 52 and 104 (Figure 2A). The proportion of patients in each arm with higher baseline viral load ( $\geq 7 \log_{10}$  copies/mL) was relatively small to make any meaningful interpretation.

Change from baseline in HBV DNA levels from week 24 to week 104 in the rITT population: A statistically significant ( $P < 0.0001$ ) reduction in HBV DNA levels vs baseline was achieved in both treatment arms at week 24 and was sustained through week 104 (Table 2).

Intensification with tenofovir or telbivudine for HBV DNA  $\geq 300$  copies/mL at week 24 or for VB post week 24 through week 104 in the rITT population; response at week 104 (HBV DNA < 300 copies/mL) according to the requirement for add-on therapy at week 24: A greater number of patients in the telbivudine arm required add-on therapy compared with the tenofovir arm (35 patients in the telbivudine arm including 22 patients requiring add-on therapy at week 24 and 13 requiring add-on therapy post week 24 vs 11 patients in the tenofovir arm, all requiring add-on therapy at week 24).

The proportion of patients in the telbivudine arm achieving HBV DNA < 300 copies/mL at week 104 was greater in those who required tenofovir add-on therapy at week 24 (100%, 21/21 patients) than patients who

were in the telbivudine monotherapy group following the week 24 visit (92.4%, 85/92 patients) (Figure 3A).

The proportion of patients in the tenofovir arm achieving HBV DNA < 300 copies/mL at week 104 was similar in those who required telbivudine add-on therapy at week 24 (100%, 11/11 patients) to those who were in the tenofovir monotherapy group following the week 24 visit (99.1%, 105/106 patients) (Figure 3B).

Percentage of patients achieving HBV DNA < 169 copies/mL (29 IU/mL) at weeks 24, 52 and 104 in the rITT population: The rate of patients achieving HBV DNA < 169 copies/mL at weeks 24, 52 and 104 was consistent with that observed for the endpoint of HBV DNA < 300 copies/mL (Figure 2B).

Percentage of patients achieving HBV DNA < 169 copies/mL at week 104 in the rITT population according to the requirement for add-on therapy at week 24: The proportion of patients in the telbivudine and tenofovir arms achieving HBV DNA < 169 copies/mL at week 104 and receiving add-on therapy were 7.6 and 0.9 percentage points greater, respectively, than patients who received monotherapy (Figure 3).

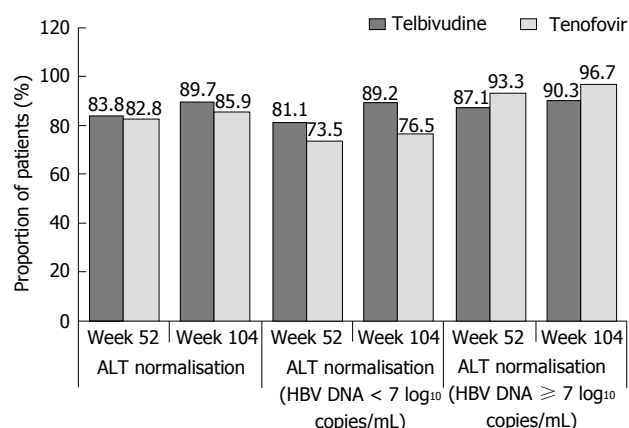
Maintained virologic responses at week 156 in the mITT population: The percentage of patients who maintained HBV DNA < 300 copies/mL at week 156 was similar in the telbivudine and tenofovir arms: 91.1% (72/79 patients) and 100% (89/89 patients), respectively, using LOCF imputation. Similar results were found in patients maintaining HBV DNA < 169 copies/mL [91.1% (72/79 patients) and 96.6% (86/89 patients), respectively].

**HBsAg loss and HBsAg seroconversion:** HBsAg loss and HBsAg seroconversion were not observed in any patient from either treatment arm at weeks 52, 104 or 156. Telbivudine treatment progressively reduced serum HBsAg levels (mean  $\pm$  SD) from baseline in the rITT population [ $-0.116 \pm 0.581 \log_{10}$  IU/mL at week 52 ( $P = 0.0368$ ) and  $-0.179 \pm 0.633 \log_{10}$  IU/mL at week 104 ( $P = 0.0032$ )]. In contrast, no change was reported in quantitative HBsAg during therapy with tenofovir [ $-0.038 \pm 0.349 \log_{10}$  IU/mL at week 52 ( $P = 0.2399$ ) and  $-0.030 \pm 0.385 \log_{10}$  IU/mL at week 104 ( $P = 0.4063$ )]. At week 156, change from baseline in HBsAg levels in the mITT population was  $-0.204 \pm 0.759 \log_{10}$  IU/mL ( $P = 0.0193$ ) in the telbivudine arm and  $-0.031 \pm 0.412 \log_{10}$  IU/mL ( $P = 0.4760$ ) in the tenofovir arm.

**Biochemical response:** ALT normalisation at weeks 52 and 104 in the rITT population: ALT levels significantly improved vs baseline in both treatment arms, with over 82% of patients in both arms achieving ALT normalisation at week 52 that was sustained up until week 104 (89.7% and 85.9% in the telbivudine and tenofovir arms, respectively) (Figure 4).

The results at week 104 by baseline viral load are presented in Figure 4.

ALT normalisation at week 104 in the rITT population according to the requirement for add-on therapy at week 24: The proportion of patients who achieved ALT



**Figure 4** Proportions of patients achieving aminotransferase normalisation, by visit and by baseline hepatitis B virus DNA levels (< 7 or ≥ 7 log<sub>10</sub> copies/mL), roadmap intent-to-treat population. ALT: Alanine aminotransferase; HBV: Hepatitis B virus.

normalization at week 24 was higher (telbivudine arm) or similar (tenofovir arm) in patients who received add-on therapy (Figure 3).

Maintained biochemical response at week 156 in the mITT population: ALT normalisation was maintained in 92.0% of patients in the telbivudine arm and 91.1% of patients in the tenofovir arm.

#### Patients experiencing VB and emergence of resistance in the rITT and mITT populations:

At weeks 52 and 104, respectively, in the rITT population, cumulative rates of VB were reported in 2.7% (3/113) and 9.7% (11/113) of patients in the telbivudine arm (3.3% and 12.4% in the monotherapy group, none in the add-on treatment group). In the tenofovir arm, no patients developed VB cumulatively at week 52 and 1.7% (2/117) of patients developed VB cumulatively at week 104.

At week 52, cumulative emergence of resistance was reported in 2.7% (3/113) of patients in the telbivudine arm (3.3% in the monotherapy group, none in the add-on treatment group) and no treatment-emergent resistance was observed in the tenofovir arm. At week 104, cumulative emergence of resistance was reported in 7.4% (8/108) of patients in the telbivudine arm (9.2% in the monotherapy group, none in the add-on treatment group) and none in the tenofovir arm.

In the telbivudine arm, 10 patients experienced VB and 5 had emergence of resistance between weeks 104 and 156 in the mITT population. In the tenofovir arm, only 1 patient had VB and none developed viral resistance. The cumulative rate of VB at week 156 was 16.5% (13/79) in the telbivudine arm, and 1.1% (1/89) in the tenofovir arm. Cumulative rates of resistance were 10.8% (8/74) in the telbivudine arm (14.0% in the monotherapy group, none in the add-on treatment group) and none in the tenofovir arm.

#### Safety

No patients died or experienced ALT flare during the

study. The overall incidence of serious AEs (SAEs) was similar in the telbivudine arm and in the tenofovir arm [11 (9.2%) patients and 13 (10.8%) patients, respectively]. One patient in the tenofovir arm reported drug-related SAEs [moderately increased blood creatine phosphokinase (CPK), mild arthralgia, and moderate fatigue], which led to temporary interruption of the study drug (Table 3). There were no cases of myositis or myopathy.

Two patients in the telbivudine arm and 5 patients in the tenofovir arm discontinued due to AEs [myalgia and hepatocellular carcinoma (HCC) for telbivudine; headache, HCC, hepatic cirrhosis, cholestatic jaundice, and breast cancer for tenofovir], which were assessed by the investigator as unrelated to the study drugs. Most AEs were mild to moderate in severity. The proportion of patients reporting at least 1 AE, regardless of study drug relationship, was similar for telbivudine and tenofovir arms. The overall incidence of AEs suspected to be related to study drug was somewhat higher in the telbivudine arm compared with the tenofovir arm. The most frequent (≥ 2%) drug-related AEs reported in both arms are described in Table 3. Increased blood CPK levels [31 (25.8%) patients], myalgia [8 (6.7%) patients, and nausea 8 (6.7%) patients] were the drug-related AEs that were observed more frequently in the telbivudine arm compared with the tenofovir arm [16 (13.3%), 0, and 2 (1.7%) patients, respectively]. AEs of special interest were observed in 46 (38.3%) patients in the telbivudine arm and 27 (22.5%) patients in the tenofovir arm. These included elevated blood CPK and myalgia as the most commonly reported AEs in the telbivudine arm, and elevated blood CPK and ALT as the most commonly reported AEs in the tenofovir arm. Myalgia suspected to be drug related was reported in the telbivudine arm. The number of patients experiencing at least 1 muscle event along with 1 new-onset abnormal CPK episode during the study was greater in the telbivudine arm (Table 3).

The telbivudine arm showed a higher incidence of Grade 3/4 CPK elevations during the study than the tenofovir arm [19 (15.8%) patients vs 5 (4.2%) patients, respectively]. All Grade 3/4 CPK elevations were resolved (Table 3).

Telbivudine monotherapy (as of week 24) was associated with a significant improvement in eGFR as compared with tenofovir monotherapy (as of week 24). At week 24, the telbivudine monotherapy showed a statistically significant ( $P = 0.0798$ ) improvement from baseline in eGFR compared to worsening with tenofovir monotherapy, with least squares mean percentage changes from baseline of 2.46% vs -1.17%, respectively. Further improvement in eGFR in the telbivudine monotherapy group (as of week 24) was observed at weeks 52 (4.90% vs -2.68% with tenofovir,  $P = 0.0098$ ), 104 (5.54% vs -5.36%,  $P < 0.0001$ , respectively), and 156 (9.55% vs -6.23%,  $P < 0.0001$ , respectively) (Figure 5).

There was no significant change in vital signs from baseline for either treatment arm.

**Table 3** Summary of safety results, safety population *n* (%)

Safety parameters	Telbivudine			Tenofovir		
	Monotherapy ( <i>n</i> = 98)	Intensification with tenofovir ( <i>n</i> = 22)	Overall ( <i>n</i> = 120)	Monotherapy ( <i>n</i> = 109)	Intensification with telbivudine ( <i>n</i> = 11)	Overall ( <i>n</i> = 120)
Any AE	69 (70.4)	17 (77.3)	86 (71.7)	75 (68.8)	8 (72.7)	83 (69.2)
AE related to drug	36 (36.7)	11 (50.0)	47 (39.2)	21 (19.3)	6 (54.5)	27 (22.5)
AE leading to drug discontinuation	2 (2.0)	0 (0.0)	2 (1.7)	5 (4.6)	0 (0.0)	5 (4.2)
Any SAE	6 (6.1)	5 (22.7)	11 (9.2)	11 (10.1)	2 (18.2)	13 (10.8)
SAE related to drug	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	1 (0.8)
Death	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AEs related to drug occurring in $\geq 2\%$ of patients in any treatment arm						
Blood CPK increased	23 (23.5)	8 (36.4)	31 (25.8)	13 (11.9)	3 (27.3)	16 (13.3)
Nausea	6 (6.1)	2 (9.1)	8 (6.7)	0 (0.0)	2 (18.2)	2 (1.7)
Myalgia	7 (7.1)	1 (4.5)	8 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)
Alanine aminotransferase increased	2 (2.0)	0 (0.0)	2 (1.7)	3 (2.8)	1 (9.1)	4 (3.3)
Proteinuria	2 (2.0)	0 (0.0)	2 (1.7)	4 (3.7)	0 (0.0)	4 (3.3)
Aspartate aminotransferase increased	3 (3.1)	0 (0.0)	3 (2.5)	2 (1.8)	0 (0.0)	2 (1.7)
Any AE of special interest	35 (35.7)	11 (50.0)	46 (38.3)	23 (21.1)	4 (36.4)	27 (22.5)
AEs of special interest occurring in $\geq 2\%$ of patients in any treatment arm						
Blood CPK increased	24 (24.5)	10 (45.5)	34 (28.3)	17 (15.6)	3 (27.3)	20 (16.7)
Myalgia	10 (10.2)	2 (9.1)	12 (10.0)	2 (1.8)	1 (9.1)	3 (2.5)
Alanine aminotransferase increased	5 (5.1)	0 (0.0)	5 (4.2)	5 (4.6)	1 (9.1)	6 (5.0)
Proteinuria	3 (3.1)	0 (0.0)	3 (2.5)	4 (3.7)	0 (0.0)	4 (3.3)
Any patient with muscle event	12 (12.2)	2 (9.1)	14 (11.7)	2 (1.8)	1 (9.1)	3 (2.5)
Experiencing new-onset Grade 3/4 abnormal CPK within the study	4 (4.1)	1 (4.5)	5 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)
Experiencing new-onset Grade 1/2 abnormal CPK within the study	6 (6.1)	1 (4.5)	7 (5.8)	1 (0.9)	1 (9.1)	2 (1.7)
Any patient with new-onset Grade 3/4 CPK episode within the study	17 (17.3)	2 (9.1)	19 (15.8)	3 (2.8)	2 (18.2)	5 (4.2)
Episode not resolved	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

AE: Adverse event; CPK: Creatine phosphokinase; SAE: Serious adverse event.

## DISCUSSION

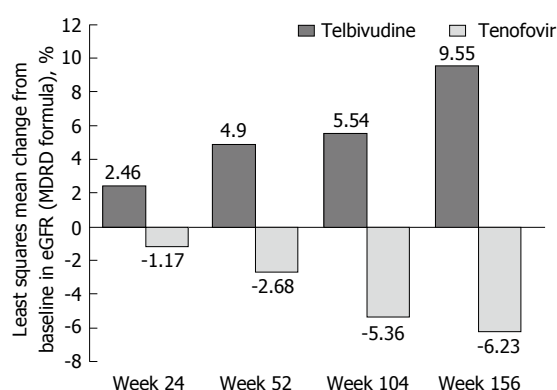
NAs given as a single daily oral dose are considered the mainstay of CHB treatment<sup>[13]</sup>. In clinical practice, attaining optimal efficacy with a low emergence of drug resistance remains an important goal<sup>[14]</sup>. The roadmap concept utilizing add-on therapy for patients who do not achieve HBV DNA < 300 copies/mL at week 24 (in particular for agents with lower barriers to resistance) has been identified as a strategy to achieve this goal. This study was the first prospective, randomised clinical trial using the roadmap concept in HBeAg-negative CHB patients comparing efficacy and safety of telbivudine with tenofovir. As previously reported<sup>[15]</sup>, early detection of virologic response may be a useful guide to individualize CHB treatment. This study confirmed that monitoring virologic response at week 24 is a strong predictor of the treatment response by week 104<sup>[16]</sup>. These data were consistent with an earlier study comparing telbivudine with lamivudine<sup>[15]</sup>.

In the real-world setting, use of the roadmap concept may offer several advantages such as early identification of patients with suboptimal responses to initiate an appropriate change in therapy<sup>[10,11]</sup>, and to provide clinicians with options for individualized treatment decisions<sup>[5]</sup>. Although emergence of resistance had been identified as

an issue for HBeAg-negative CHB patients treated with telbivudine monotherapy<sup>[15,17]</sup>, the data from our study suggest that the risk for resistance is lower if telbivudine is administered using the roadmap concept, as compared to the GLOBE trial showing higher rates of resistance<sup>[15]</sup>. Moreover, despite a somewhat higher percentage of patients requiring add-on therapy in the telbivudine arm, the overall efficacy profile of the 2 roadmap approach arms was comparable, as assessed by the percentages of patients achieving HBV DNA levels < 300 or < 169 copies/mL, and ALT normalisation at weeks 52, 104 and 156. Moreover, telbivudine treatment resulted in a statistically significant reduction in serum HBsAg levels from baseline while no change was reported in quantitative HBsAg during therapy with tenofovir.

Overall, both treatments based on the roadmap concept were well tolerated over the 156 wk treatment period in HBeAg-negative patients. Although myalgia and elevated blood CPK levels were reported in a higher number of patients in the telbivudine arm, the rates were consistent with the findings reported earlier in the literature<sup>[12,15,18,19]</sup>. It is recommended that serum CPK levels should be monitored closely during treatment with telbivudine<sup>[20]</sup>.

Renal safety issues with oral NAs have been well-documented<sup>[21-23]</sup>. Particularly, adefovir is considered to



**Figure 5** Changes in estimated glomerular filtration rate over time with telbivudine and tenofovir, safety population. eGFR: Estimated glomerular filtration rate; MDRD: Modification of diet in renal disease.

have high potential for nephrotoxicity and tenofovir has been associated with this risk<sup>[24]</sup>. In our study, telbivudine was associated with improvement in eGFR from baseline to week 156 compared to the increasing deterioration over time with tenofovir. The finding of improvement in eGFR with telbivudine treatment was consistent with that reported in previous studies where telbivudine significantly improved while adefovir and lamivudine worsened renal function<sup>[25,26]</sup>. CHB patients with impaired renal function at baseline have also shown an eGFR improvement after 1 year<sup>[27]</sup> and 2 years of treatment with telbivudine<sup>[11,28]</sup>. Similar results for telbivudine have also been reported in patients with cirrhosis, patients with compensated cirrhosis, or patients with no cirrhosis<sup>[29,30]</sup>. These findings imply that telbivudine may offer benefit in patients with known or at risk of renal impairment. Although telbivudine improves renal function, the mechanism of this renal protective effect remains to be determined<sup>[31]</sup>.

The main limitations of the study are related to its design (open-label) and the relatively small sample size.

In conclusion, this study was the first prospective, randomised, comparative study of telbivudine-roadmap vs tenofovir-roadmap concept in HBeAg-negative patients with CHB. Both treatments based on the roadmap concept were effective over the 156 wk treatment period. Moreover, telbivudine showed an improvement in eGFR from baseline while a deterioration was observed with tenofovir; this could be an important consideration for long term therapy in CHB patients especially in those with a high risk for renal impairment.

## ACKNOWLEDGMENTS

The authors acknowledge the work of the OPTIMA investigators and participating institutions located in various countries. The investigators included Peter Ferenci and Wolfgang Vogel (Austria); Rozalina Balabanska, Jordan Genov, and Krum Katarov (Bulgaria); Thomas Berg, Peter Buggisch, Heinz Hartmann, Hartwig Klinker, Jens Rasenack, Hans Wedemeyer, and Stefan Zeuzem

(Germany); Evangelos Akriviadis, Alexandra Alexopoulou, Ioannis Elefsiniotis, and Konstantinos Mimidis (Greece); Evangelista Sagnelli (Italy); Djamal Abdurakhmanov, Pavel Bogomolov, Vladimir Chulanov, Marina Maevskaya, Maria Matsievich, Igor Nikitin, Olga Znoiko, and Konstantin Zhdanov (Russia); Maria Buti Ferret, Jose Luis Calleja, Albert Pardo, and Ricard Sola Lamoglia (Spain); Ulus Akarca, Iftihar Koksak, and Fehmi Tabak (Turkey). The authors would like to thank Krassimir Antonov, Deian Jeleu, Lyudmila Mateva, and Dimitar Popov (Bulgaria) for their technical assistance. Medical writing support was provided by Farid Khalfi (Novartis Ireland Ltd., Dublin, Ireland).

## COMMENTS

### Background

Hepatitis B virus (HBV) infection is the major cause of chronic hepatitis worldwide. Emergence of resistance due to prolonged nucleos(t)ide analogue use or incomplete suppression of HBV still remains an important concern. Therefore, early virologic response at week 24 of therapy has been used to predict better outcomes and to reduce the risk of antiviral resistance.

### Research frontiers

This study used the response-guided add-on strategy (roadmap concept). For patients with HBV DNA  $\geq 300$  copies/mL ( $\geq 51$  IU/mL) at week 24, tenofovir was added to telbivudine by week 26 in the telbivudine arm, and telbivudine was added to tenofovir by week 26 in the tenofovir arm. For patients with HBV DNA  $< 300$  copies/mL at week 24, telbivudine and tenofovir monotherapies in the respective arms were continued.

### Innovations and breakthroughs

This was the first prospective, randomised, 2-arm, open-label, non-inferiority study in hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) patients that compared telbivudine and tenofovir administered as per the roadmap concept. The safety of the combination of telbivudine and tenofovir, for which limited data are currently available, was also evaluated.

### Applications

Efficacy was shown for both telbivudine-roadmap and tenofovir-roadmap regimens in HBeAg-negative CHB patients over 156 wk. Both treatments showed acceptable safety profiles. In addition, the telbivudine arm was associated with renal improvement.

### Peer-review

This is an extensive randomised study to compare the roadmap treatment strategy between telbivudine and tenofovir in patients with HBeAg-negative CHB patients. As antiviral treatment may be life-long, renal protection becomes an important consideration. The current manuscript should be of benefit to the hepatologists and liver transplantation specialists worldwide.

## REFERENCES

- Schweitzer A**, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet* 2015; **386**: 1546-1555 [PMID: 26231459 DOI: 10.1016/s0140-6736(15)61412-x]
- Tang CM**, Yau TO, Yu J. Management of chronic hepatitis B infection: current treatment guidelines, challenges, and new developments. *World J Gastroenterol* 2014; **20**: 6262-6278 [PMID: 24876747 DOI: 10.3748/wjg.v20.i20.6262]
- World Health Organization**. Hepatitis B Fact Sheet No. 204. July 2015. Available from: URL: <http://www.who.int/mediacentre/>



- factsheets/fs204/en/
- 4 **Zoulim F**, Locarnini S. Optimal management of chronic hepatitis B patients with treatment failure and antiviral drug resistance. *Liver Int* 2013; **33** Suppl 1: 116-124 [PMID: 23286855 DOI: 10.1111/liv.12069]
- 5 **Gu EL**, Yu YQ, Wang JL, Ji YY, Ma XY, Xie Q, Pan HY, Wu SM, Li J, Chen CW, Xu XW, Wang YE, Yao GB, Wang H, Zhang WH. Response-guided treatment of cirrhotic chronic hepatitis B patients: multicenter prospective study. *World J Gastroenterol* 2015; **21**: 653-660 [PMID: 25605989 DOI: 10.3748/wjg.v21.i2.653]
- 6 **Ryu HJ**, Lee JM, Ahn SH, Kim do Y, Lee MH, Han KH, Chon CY, Park JY. Efficacy of adefovir add-on lamivudine rescue therapy compared with switching to entecavir monotherapy in patients with lamivudine-resistant chronic hepatitis B. *J Med Virol* 2010; **82**: 1835-1842 [PMID: 20872709 DOI: 10.1002/jmv.21898]
- 7 **Yang YJ**, Shim JH, Kim KM, Lim YS, Lee HC. Assessment of current criteria for primary nonresponse in chronic hepatitis B patients receiving entecavir therapy. *Hepatology* 2014; **59**: 1303-1310 [PMID: 24170683 DOI: 10.1002/hep.26910]
- 8 **Shin JW**, Jung SW, Park BR, Kim CJ, Eum JB, Kim BG, Du Jeong I, Bang SJ, Park NH. HBV DNA level at 24 weeks is the best predictor of virological response to adefovir add-on therapy in patients with lamivudine resistance. *Antivir Ther* 2012; **17**: 387-394 [PMID: 22293395 DOI: 10.3851/imp1945]
- 9 **Lo AO**, Wong GL. Current developments in nucleoside/nucleotide analogues for hepatitis B. *Expert Rev Gastroenterol Hepatol* 2014; **8**: 607-622 [PMID: 24787673 DOI: 10.1586/17474124.2014.909724]
- 10 **Gane EJ**. The Roadmap concept: using early on-treatment virologic responses to optimize long-term outcomes for patients with chronic hepatitis B. *Hepatol Int* 2008; **2**: 304-307 [PMID: 19669258 DOI: 10.1007/s12072-008-9083-0]
- 11 **Sun J**, Xie Q, Tan D, Ning Q, Niu J, Bai X, Fan R, Chen S, Cheng J, Yu Y, Wang H, Xu M, Shi G, Wan M, Chen X, Tang H, Sheng J, Dou X, Shi J, Ren H, Wang M, Zhang H, Gao Z, Chen C, Ma H, Jia J, Hou J. The 104-week efficacy and safety of telbivudine-based optimization strategy in chronic hepatitis B patients: a randomized, controlled study. *Hepatology* 2014; **59**: 1283-1292 [PMID: 24382690 DOI: 10.1002/hep.26885]
- 12 **Piratvisuth T**, Komolmit P, Tanwandee T, Sukeepaisarnjaroen W, Chan HL, Pessoa MG, Fassio E, Ono SK, Bessone F, Daruich J, Zeuzem S, Cheinquer H, Pathan R, Dong Y, Trylesinski A. 52-week efficacy and safety of telbivudine with conditional tenofovir intensification at week 24 in HBsAg-positive chronic hepatitis B. *PLoS One* 2013; **8**: e54279 [PMID: 23390496 DOI: 10.1371/journal.pone.0054279]
- 13 **Liu F**, Wang X, Wei F, Hu H, Zhang D, Hu P, Ren H. Efficacy and resistance in de novo combination lamivudine and adefovir dipivoxil therapy versus entecavir monotherapy for the treatment-naïve patients with chronic hepatitis B: a meta-analysis. *Virol J* 2014; **11**: 59 [PMID: 24673792 DOI: 10.1186/1743-422x-11-59]
- 14 **Yu HC**, Lin KH, Hsu PI, Tsay FW, Wang HM, Tsai TJ, Lai KH. Real-world application of the roadmap model in chronic hepatitis B patients with telbivudine therapy. *Clin Ther* 2013; **35**: 1386-1399 [PMID: 24054706 DOI: 10.1016/j.clinthera.2013.07.329]
- 15 **Liaw YF**, Gane E, Leung N, Zeuzem S, Wang Y, Lai CL, Heathcote EJ, Manns M, Bzowej N, Niu J, Han SH, Hwang SG, Cakaloglu Y, Tong MJ, Papatheodoridis G, Chen Y, Brown NA, Albanis E, Galil K, Naoumov NV. 2-Year GLOBE trial results: telbivudine is superior to lamivudine in patients with chronic hepatitis B. *Gastroenterology* 2009; **136**: 486-495 [PMID: 19027013 DOI: 10.1053/j.gastro.2008.10.026]
- 16 **Zeuzem S**, Gane E, Liaw YF, Lim SG, DiBisceglie A, Buti M, Chutaputti A, Rasenack J, Hou J, O'Brien C, Nguyen TT, Jia J, Poynard T, Belanger B, Bao W, Naoumov NV. Baseline characteristics and early on-treatment response predict the outcomes of 2 years of telbivudine treatment of chronic hepatitis B. *J Hepatol* 2009; **51**: 11-20 [PMID: 19345439 DOI: 10.1016/j.jhep.2008.12.019]
- 17 **Wang Y**, Thongsawat S, Gane EJ, Liaw YF, Jia J, Hou J, Chan HL, Papatheodoridis G, Wan M, Niu J, Bao W, Trylesinski A, Naoumov NV. Efficacy and safety of continuous 4-year telbivudine treatment in patients with chronic hepatitis B. *J Viral Hepat* 2013; **20**: e37-e46 [PMID: 23490388 DOI: 10.1111/jvh.12025]
- 18 **Lai CL**, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, Chen Y, Heathcote EJ, Rasenack J, Bzowej N, Naoumov NV, Di Bisceglie AM, Zeuzem S, Moon YM, Goodman Z, Chao G, Constance BF, Brown NA. Telbivudine versus lamivudine in patients with chronic hepatitis B. *N Engl J Med* 2007; **357**: 2576-2588 [PMID: 18094378 DOI: 10.1056/NEJMoa066422]
- 19 **Seto WK**, Lai CL, Fung J, Wong DK, Yuen JC, Hung IF, Yuen MF. Significance of HBV DNA levels at 12 weeks of telbivudine treatment and the 3 years treatment outcome. *J Hepatol* 2011; **55**: 522-528 [PMID: 21147187 DOI: 10.1016/j.jhep.2010.11.018]
- 20 **Wang YH**, Wu BQ, Liu H. Continuous venovenous hemodiafiltration for hyperlactatemia caused by telbivudine in a patient with chronic hepatitis B: a case report and update review. *J Dig Dis* 2015; **16**: 164-167 [PMID: 25043654 DOI: 10.1111/1751-2980.12173]
- 21 **Chan HL**, Chen YC, Gane EJ, Sarin SK, Suh DJ, Piratvisuth T, Prabhakar B, Hwang SG, Choudhuri G, Safadi R, Tanwandee T, Chutaputti A, Yurdaydin C, Bao W, Avila C, Trylesinski A. Randomized clinical trial: efficacy and safety of telbivudine and lamivudine in treatment-naïve patients with HBV-related decompensated cirrhosis. *J Viral Hepat* 2012; **19**: 732-743 [PMID: 22967105 DOI: 10.1111/j.1365-2893.2012.01600.x]
- 22 **Liaw YF**, Raptopoulou-Gigi M, Cheinquer H, Sarin SK, Tanwandee T, Leung N, Peng CY, Myers RP, Brown RS, Jr., Jeffers L, Tsai N, Bialkowska J, Tang S, Beebe S, Cooney E. Efficacy and safety of entecavir versus adefovir in chronic hepatitis B patients with hepatic decompensation: a randomized, open-label study. *Hepatology* (Baltimore, Md) 2011; **54**: 91-100 [PMID: 21503940 DOI: 10.1002/hep.24361]
- 23 **Mallet V**, Schwarzsinger M, Vallet-Pichard A, Fontaine H, Corouge M, Sogni P, Pol S. Effect of nucleoside and nucleotide analogues on renal function in patients with chronic hepatitis B virus mono-infection. *Clin Gastroenterol Hepatol* 2015; **13**: 1181-1188. e1181 [PMID: 25460550 DOI: 10.1016/j.cgh.2014.11.021]
- 24 **EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]**
- 25 **Qi X**, Wang JY, Mao RC, Zhang JM. Impact of nucleos(t)ide analogues on the estimated glomerular filtration rate in patients with chronic hepatitis B: a prospective cohort study in China. *J Viral Hepat* 2015; **22**: 46-54 [PMID: 25402626 DOI: 10.1111/jvh.12229]
- 26 **Gane EJ**, Deray G, Liaw YF, Lim SG, Lai CL, Rasenack J, Wang Y, Papatheodoridis G, Di Bisceglie A, Buti M, Samuel D, Uddin A, Bosset S, Trylesinski A. Telbivudine improves renal function in patients with chronic hepatitis B. *Gastroenterology* 2014; **146**: 138-146. e135 [PMID: 24067879 DOI: 10.1053/j.gastro.2013.09.031]
- 27 **Tsai MC**, Chen CH, Hung CH, Lee CM, Chiu KW, Wang JH, Lu SN, Tseng PL, Chang KC, Yen YH, Hu TH. A comparison of efficacy and safety of 2-year telbivudine and entecavir treatment in patients with chronic hepatitis B: a match-control study. *Clin Microbiol Infect* 2014; **20**: O90-O100 [PMID: 23659493 DOI: 10.1111/1469-0691.12220]
- 28 **Lee M**, Oh S, Lee HJ, Yeum TS, Lee JH, Yu SJ, Kim HY, Yoon JH, Lee HS, Kim YJ. Telbivudine protects renal function in patients with chronic hepatitis B infection in conjunction with adefovir-based combination therapy. *J Viral Hepat* 2014; **21**: 873-881 [PMID: 24351112 DOI: 10.1111/jvh.12217]
- 29 **Amarapurkar DN**, Patel N. Increased eGFR with telbivudine in combination therapy of chronic hepatitis B infection. *Indian J Gastroenterol* 2014; **33**: 89-91 [PMID: 23512213 DOI: 10.1007/s12664-013-0325-2]
- 30 **Tsai MC**, Yu HC, Hung CH, Lee CM, Chiu KW, Lin MT, Tseng PL, Chang KC, Yen YH, Chen CH, Hu TH. Comparing the efficacy and clinical outcome of telbivudine and entecavir naïve patients with hepatitis B virus-related compensated cirrhosis. *J Gastroenterol Hepatol* 2014; **29**: 568-575 [PMID: 24716215]

- 31 **Liang KH**, Chen YC, Hsu CW, Chang ML, Yeh CT. Decrease of serum Angiotensin converting enzyme levels upon telbivudine treatment for chronic hepatitis B virus infection and negative correlations between the enzyme levels and estimated glomerular filtration rates. *Hepat Mon* 2014; **14**: e15074 [PMID: 24596580 DOI: 10.5812/hepatmon.15074]

**P- Reviewer:** Balaban YH, Chiu KW, Cholongitas EC, Chuang QL, Gong ZJ, Montasser MF, Romero MR, Wong GLH, Zhu Z  
**S- Editor:** Qiu S **L- Editor:** A **E- Editor:** Li D



## Spontaneous liver rupture as first sign of polyarteritis nodosa

Irene Gómez-Luque, Felipe Alconchel, Rubén Ciria, M Dolores Ayllón, Antonio Luque, Marina Sánchez, Pedro López-Cillero, Javier Briceño

Irene Gómez-Luque, General Surgery, Reina Sofia University Hospital, 14004 Córdoba, Spain

Felipe Alconchel, Department of Surgery, Faculty of Medicine and Nursing, University of Córdoba, 14071 Córdoba, Spain

Rubén Ciria, M Dolores Ayllón, Antonio Luque, Pedro López-Cillero, Javier Briceño, Unit of Hepatobiliary Surgery and Liver Transplant, Reina Sofia University Hospital, 14004 Córdoba, Spain

Marina Sánchez, Department of Pathology, Reina Sofia University Hospital, 14004 Córdoba, Spain

**Author contributions:** Gómez-Luque I and Alconchel F contributed equally to this work; Ciria R, López-Cillero P and Briceño J designed the research; Gómez-Luque I, Alconchel F, Ayllón MD and Luque A performed the research; Sánchez M performed the pathological analyses; Gómez-Luque I, Alconchel F and Ayllón MD wrote the paper.

**Institutional review board statement:** The following manuscript is in accordance with the ethics standards in our Hospital. It has been reviewed and approved by all the authors and by the Institute of Biomedical Research of Cordoba (IMIBIC).

**Informed consent statement:** The following manuscript has been written and produced after full approval from the patient, who, in the informed consent, gave the authorization to use the data and images derived from his clinical condition.

**Conflict-of-interest statement:** Authors declare have no conflicts of interest. The present study did not receive any financial support.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Rubén Ciria, MD, PhD, Unit of Hepatobiliary Surgery and Liver Transplant, Reina Sofia University Hospital, Avda, Menéndez Pidal s/n, 14004 Córdoba, Spain. [rubenciria@hotmail.com](mailto:rubenciria@hotmail.com)  
 Telephone: +34-600-890079  
 Fax: +34-95-7010949

**Received:** March 1, 2016

**Peer-review started:** March 2, 2016

**First decision:** March 22, 2016

**Revised:** September 13, 2016

**Accepted:** October 5, 2016

**Article in press:** October 9, 2016

**Published online:** November 18, 2016

### Abstract

Polyarteritis nodosa (PAN) is one of the systemic vasculitis that affects the media wall of arteries of small and medium diameter. Diagnosis proves difficult due to the unspecific symptoms that dominate the clinical profile. Liver involvement is very diverse, ranging from the development of cirrhotic liver disease to acute abdomen presentation that requires surgery because of liver rupture. The management of these patients requires an expert multidisciplinary team. There are several cases in the literature that describe a sudden liver rupture as the first manifestation of a PAN. In this paper we present the case of a 75 years old patient without any previous disease, who is subjected to major hepatic resection for spontaneous liver rupture.

**Key words:** Polyarteritis nodosa; Spontaneous liver rupture; Liver surgery; Vasculitis; Rheumatology

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Spontaneous liver rupture is a rare entity with very few cases in the literature reviewed; even when it has an autoimmune disease such etiology and with no previous trauma. We present our experience managing an urgent abdominal hemorrhage caused by a liver rupture as a first manifestation of Polyarteritis in a 75-year-old woman.

Gómez-Luque I, Alconchel F, Ciria R, Ayllón MD, Luque A, Sánchez M, López-Cillero P, Briceño J. Spontaneous liver rupture as first sign of polyarteritis nodosa. *World J Hepatol* 2016; 8(32): 1414-1418 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i32/1414.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i32.1414>

## INTRODUCTION

The first International Chapel Hill Consensus Conference of Rheumatological Diseases defined polyarteritis nodosa (PAN) as a systemic necrotizing vasculitis of arterial tunica media of small and medium sized arteries without the presence of any glomerulonephritis or vasculitis in arterioles, venules and capillaries or without association with anti-cytoplasmic neutrophil antibodies positivity<sup>[1]</sup>.

PAN is a common systemic vasculitis generally involving several organs such as kidneys, skin, central and peripheral nervous system and the gastrointestinal tract. The certain diagnosis is a complex task because of nonspecific laboratory tests and clinical features; therefore it must be based on histopathological analysis by biopsies.

The "American College of Rheumatology: Defined the criteria for the diagnosis of PAN in 1990<sup>[2]</sup>. To diagnose a PAN the patient must have 3 characteristics out of a list of 10 features, estimating a diagnostic sensitivity of 82.2% with a specificity of 86.6% (Table 1).

Liver involvement in this disease is uncommon and difficult to diagnose. Hepatomegaly (21%), jaundice (12%) and alteration of biochemical liver-function markers (6%) have been reported in the literature in different publications<sup>[3]</sup>. The development of this type of autoimmune disease has been associated to positive hepatitis B surface antigen (HBsAg), although the exact etiology is unclear. It is reported that HBsAg positive patients may have a better response to treatment and therefore better prognosis<sup>[4]</sup>. In addition, PAN may cause aneurysm development due to fibrotic arterial lumen occlusion and necrosis, including organs such as liver, spleen and kidneys. These may derive in chronic abdominal pain, gastrointestinal bleeding, stroke, intestinal perforation and even pancreatitis. In some cases infarction and hemorrhage may occur, causing hemoperitoneum bearing a poor prognosis.

Very few cases have been published to date in which spontaneous hepatic rupture would be the first clinical manifestation; even when it has an autoimmune disease such etiology and with no previous trauma.

We present our experience managing an urgent abdominal hemorrhage caused by a liver rupture as a first manifestation of PAN in a 75-year-old woman.

## CASE REPORT

A 75-year-old woman with no previous medical history except chronic anemia and well-controlled arterial hypertension with outpatient follow-up. She is referred for transfusion from another hospital because of a severe anemia. The patient reported feeling general malaise with unmeasured fever several days before. No nausea or gastrointestinal symptoms were noted.

On examination the patient's blood pressure was near the low end of normality without tachycardia. There was no neurological deficit, paraesthesia or loss of motor reflexes. The abdomen palpation proved pain predominantly in right quadrants, with some upper quadrant abdominal defense.

Blood tests found hemoglobin 6.7 g/dL with a hematocrit of 19.2% and white blood cell count of 18000 mm<sup>3</sup> (neutrophilia 70%). Coagulation tests showed an INR of 1.34 with a prothrombin activity of 54%. Liver function enzymes showed altered cytolysis enzymes (AST/ALT: 273/275 U/L) and cholestatic enzymes (GGT/FA: 90/159 U/L); bilirubin was within normal range. Other inflammatory parameters reflected reactive-C protein of 227.5 mg/dL.

With this scenario an abdominal computed tomography scan was performed (Figure 1) where liver damage was reported in the form of a right hepatic lobe lesion with poorly defined and confluent contours, heterogeneous density and hypodense predominance. There was also a heterogeneous subcapsular and subhepatic collection, with some hyperdense areas that could be a hematoma, along with a moderate amount of intraabdominal free fluid.

In this context an exploratory laparotomy was performed. There was a bleeding liver injury involving the inferior segments of the right hepatic lobe (segments V-VI), the source of this bleeding was difficult to identify. Hemoperitoneum was present in all abdominal quadrants. An urgent right hepatectomy was executed. The patient received four red cell concentrates in the perioperative care and no vasoactive drugs were necessary.

The postoperative was otherwise uneventful with only a persistent leukocytosis outstanding, without any other signs of sepsis. White blood cell count was normal at the time of discharge.

The pathology report described the existence of an inflammatory and haemorrhagic abscess with a volume of 10 cm × 7 cm × 2 cm as the cause of the hemoperitoneum. The remaining liver parenchyma appeared normal.

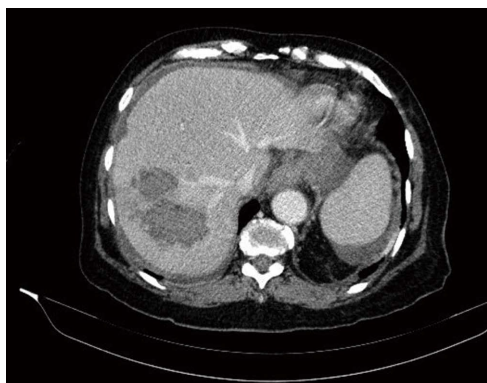
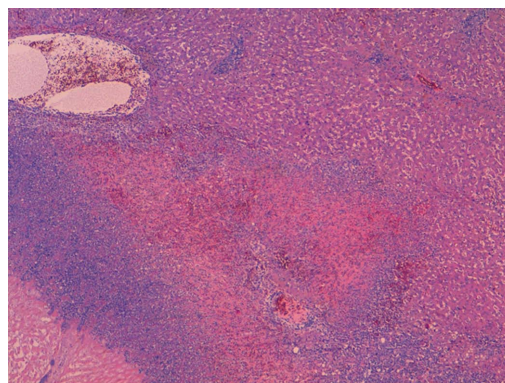
In the microscopic study, the liver specimen was compatible with nongranulomatous acute necrotizing vasculitis (Figure 2). The gallbladder sample (Figures 3 and 4) showed acute vasculitis with fibrinoid necrosis in



**Table 1** For classification purposes, a patient shall be said to have polyarteritis nodosa if at least 3 of these 10 criteria are present

Criteria diagnosis of polyarteritis nodosa	
Weight loss	Loss of 4 kg or more of body weight since illness began, not due to dieting or other factors
Livedo reticularis	Mottled reticular pattern over the skin or portions of the extremities or torso
Testicular pain or tenderness	Pain or tenderness of the testicles, not due to infection, trauma, or other causes
Myalgias, weakness or leg tenderness	Diffuse myalgias (excluding shoulder and hip girdle)
Mononeuropathy or polyneuropathy	Development of mononeuropathy, multiple mononeuropathies, or polyneuropathy
Diastolic BP > 90 mmHg	Development of hypertension with diastolic BP higher than 90 mmHg
Elevated BUN or creatinine	Elevation of BUN > 40 mg/dL or creatinine > 1.5 mg/dL, not due to dehydration or obstruction
Hepatitis B virus	Presence of hepatitis B surface antigen or antibody in serum
Arteriographic abnormality	Arteriogram showing aneurysms or occlusions of the visceral arteries, not due to arteriosclerosis, fibromuscular dysplasia, or other noninflammatory causes
Biopsy of small or medium-sized artery containing PMN	Histologic changes showing the presence of granulocytes or granulocytes and mononuclear leukocytes in the artery wall

Available from: Lightfoot RW Jr, Michel BA, Bloch DA, Hunder GG, Zvaifler NJ, McShane DJ, *et al.* The American College of Rheumatology: 1990 criteria for the classification of polyarteritis nodosa. *Arthritis Rheum* 1990; 33: 1088-1093. BP: Blood pressure; BUN: Blood urea nitrogen; PMN: Polymorphonuclear neutrophils.

**Figure 1** Computed tomography scan of the liver.**Figure 2** Liver (hematoxylin and eosin; × 40 original magnification): Bleeding, abscesses and avascular necrosis.

muscular arteries of parietal medium caliber. All these findings are compatible with PAN type vasculitis.

In the year of follow-up after the surgery, the patient has been treated with prednisone and cyclophosphamide with good results. Outpatient blood tests were negative for HBsAg and the autoimmunity study revealed positive antinuclear antibodies. After two months cyclophosphamide was discontinued because of pancytopenia, with the patient reaching a full recovery after drug suspension. Currently, one year after diagnosis, treatment consists of 10 mg of prednisone once a day, the patient is asymptomatic.

## DISCUSSION

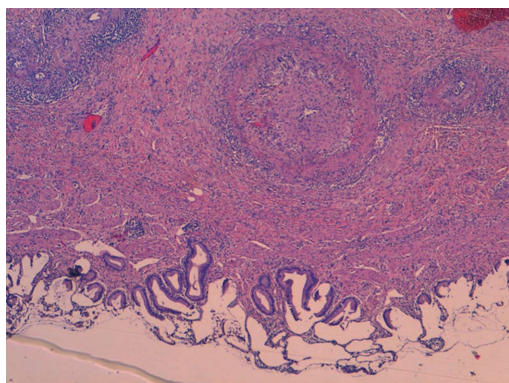
The diagnosis of PAN is presented as a challenge for the clinical practice. This is because it has mainly nonspecific symptoms, which may involve more than one organ and the absence of specific serological tests for this disease. PAN usually appears as a chronic disease with periods of remission and deterioration<sup>[5]</sup>.

Hepatic involvement may be of a PAN clinical profile. Different clinical entities have been described in the literature ranging from chronic liver failure and cirrhosis to acute hepatitis, hepatic regeneration

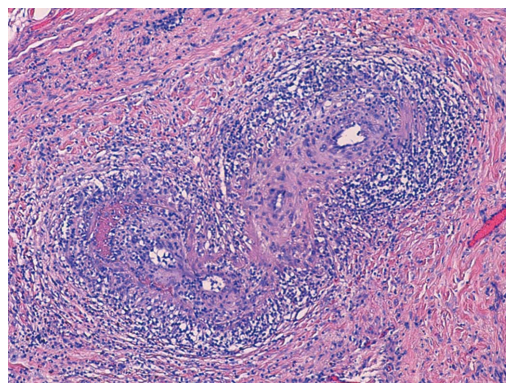
nodules and vascular and bile-duct complications. In most cases the pathogenesis involves immune complex depositions leading to obstruction in hepatic blood flow and obliteration of small vasculature resulting in aneurysm formation (13%-60%)<sup>[6-8]</sup>. These may cause complications of difficult diagnosis. Most patients are asymptomatic at the moment of diagnosis and only 10%-15% of patients with hepatic artery aneurysms have presenting symptoms, such as abdominal pain, gastrointestinal bleeding or cholestasis<sup>[9]</sup>.

Spontaneous intrahepatic hemorrhage caused by the rupture of a hepatic artery aneurysm is a rare complication of PAN, however it has a high mortality<sup>[10,11]</sup>. There are about fifteen reported cases in which the diagnosis of PAN was learned due to hemodynamic instability secondary to spontaneous hepatic rupture that required an urgent laparotomy, as in the case presented.

There are different options when deciding how to manage these patients. Some of the reported cases resolved the bleeding using vascular radiology techniques with selective artery embolization<sup>[12,13]</sup>. In other patients with hemodynamic stability expectant attitude was decided, administering blood transfusion after corticosteroid and immunosuppressive treatment<sup>[12,14-16]</sup>.



**Figure 3** Gallbladder (hematoxylin and eosin; × 40 original magnification): Acute vasculitis.



**Figure 4** Gallbladder (hematoxylin and eosin; × 100 original magnification): Acute vasculitis with fibrinoid necrosis in muscular arteries of parietal medium caliber.

In those cases in which surgical management was decided, most did not do any type of liver resection, opting for hepatic parenchyma hemostasis and packing<sup>[4,5]</sup> with high mortality rate.

In one case reported<sup>[17]</sup> a right hepatectomy surgery was performed on a 20-year-old with severe bleeding. This patient died after 12 wk because of several complications after surgery.

This article presents the first case in which an urgent major hepatectomy for treatment of a liver rupture secondary to a previously unknown PAN is performed. In this case the patient is still alive after approximately one year has passed without any kind of complication.

Early and proper diagnosis is decisive in this disease when it is not presented acutely as in the case presented. For this reason, a whole and exhaustive history is required. To confirm the diagnosis of PAN a pathological study is crucial. The biopsy can be obtained from muscle tissue. In cases where the biopsy can not be performed or it is assumed that it would be negative, arteriography is mandatory to confirm the presence of aneurysms<sup>[18]</sup>. After that, treatment with steroids and immunosuppressive drugs has been found to eliminate all clinical manifestations of the disease. Decrease of the aneurysm size and its risk of rupture has been described with this treatment<sup>[12]</sup>.

## ACKNOWLEDGMENTS

The paper was supported by Reina Sofía University Hospital, 14004 Córdoba, Spain (Andalusia Public Health Service).

## COMMENTS

### Case characteristics

The patient reported feeling general malaise with unmeasured fever and she feels pain predominantly in right quadrants on the abdomen.

### Clinical diagnosis

The most frequent at the beginning of disease symptoms are fever, weight loss, muscles pain, peripheral neuropathy, gastrointestinal disorders and skin lesions.

### Differential diagnosis

For diagnosis, the criteria established by the American College of Rheumatology are often used, a high clinical suspicion, a biopsy showing vasculitis or arteriography showing aneurysms.

### Laboratory diagnosis

The increased erythrocyte sedimentation rate and C-reactive protein is practically constant during the active phase of the disease. Other common findings are leukocytosis, eosinophilia, and normochromic anemia.

### Imaging diagnosis

Is useful to perform an arteriography or reconstructions high-quality computed tomography (CT)-scan imaging that showing the presence of aneurysms or occlusions of visceral arteries not display due to arteriosclerosis?

### Pathological diagnosis

Histological alterations show granulocytes or granulocyte and mononuclear leukocytes into the arterial wall of medium diameter.

### Treatment

The treatment has undergone major changes in recent years although cyclophosphamide remains the cornerstone despite its side effects, there are promising new therapies such as biologic therapies.

### Related reports

There are several cases in the literature related to liver rupture due to polyarteritis nodosa (PAN) them out various treatment are carried with different results. There is so far an established treatment for this type of clinical presentation. The decision is based on the clinical condition of the patient and therapeutics means available in the hospital.

### Term explanation

The patient had low suspicion of vasculitis, but had a history of hypertension, the presence of aneurysms in the CT-scan and a PAN conclusive biopsy. The hepatitis B surface antigen was negative and antinuclear antibodies were positive. It shows the difficult diagnosis of this disease and the need for a broad differential diagnosis.

### Experiences and lessons

In this case report, the authors show an uncommon PAN debuts with a spontaneous liver rupture as first symptom that requires urgent liver major resection in a patient without previous clinical manifestations.

### Peer-review

This case report describes spontaneous liver rupture due to polyarteritis nodosa

which was treated by surgical intervention. The paper is well written.

## REFERENCES

- 1 **Jennette JC**. Overview of the 2012 revised International Chapel Hill Consensus Conference nomenclature of vasculitides. *Clin Exp Nephrol* 2013; **17**: 603-606 [PMID: 24072416 DOI: 10.1007/s10157-013-0869-6]
- 2 **Cowan RE**, Mallinson CN, Thomas GE, Thomson AD. Polyarteritis nodosa of the liver: a report of two cases. *Postgrad Med J* 1977; **53**: 89-93 [PMID: 17854 DOI: 10.1136/pgmj.53.616.89]
- 3 **Mills PR**, Sturrock RD. Clinical associations between arthritis and liver disease. *Ann Rheum Dis* 1982; **41**: 295-307 [PMID: 6124216 DOI: 10.1136/ard.41.3.295]
- 4 **Leung VK**, Lam CY, Chan CC, Ng WL, Loke TK, Luk IS, Chau TN, Wu AH, Fong WN, Lam SH. Spontaneous intra-hepatic haemorrhage in a patient with fever of unknown origin. *Hong Kong Med J* 2007; **13**: 319-322 [PMID: 17664537]
- 5 **Travers RL**, Allison DJ, Brett RP, Hughes GR. Polyarteritis nodosa: a clinical and angiographic analysis of 17 cases. *Semin Arthritis Rheum* 1979; **8**: 184-199 [PMID: 34221 DOI: 10.1016/S0049-0172(79)80007-4]
- 6 **Sellar RJ**, Mackay IG, Buist TA. The incidence of microaneurysms in polyarteritis nodosa. *Cardiovasc Intervent Radiol* 1986; **9**: 123-126 [PMID: 2873891 DOI: 10.1007/BF02577919]
- 7 **Ewald EA**, Griffin D, McCune WJ. Correlation of angiographic abnormalities with disease manifestations and disease severity in polyarteritis nodosa. *J Rheumatol* 1987; **14**: 952-956 [PMID: 2892931]
- 8 **Kanai R**, Nakamura M, Tomisato K, Fukuhara T, Kondo A, Nakamura S, Matsukawa S, Yabutani A, Kobashikawa K, Nakayoshi T, Uchima N, Kosuge N, Yoshimi N. Cholangitis as an initial manifestation of polyarteritis nodosa. *Intern Med* 2014; **53**: 2307-2312 [PMID: 25318793 DOI: 10.2169/internalmedicine.53.2508]
- 9 **Schröder W**, Brandstetter K, Vogelsang H, Nathrath W, Siewert JR. Massive intrahepatic hemorrhage as first manifestation of polyarteritis nodosa. *Hepatogastroenterology* 1997; **44**: 148-152 [PMID: 9058134]
- 10 **Choy CW**, Smith PA, Frazer C, Jeffrey GP. Ruptured hepatic artery aneurysm in polyarteritis nodosa: a case report and literature review. *Aust N Z J Surg* 1997; **67**: 904-906 [PMID: 9451353 DOI: 10.1111/j.1445-2197.1997.tb07624.x]
- 11 **Battula N**, Tsapralis D, Morgan M, Mirza D. Spontaneous liver haemorrhage and haemobilia as initial presentation of undiagnosed polyarteritis nodosa. *Ann R Coll Surg Engl* 2012; **94**: e163-e165 [PMID: 22613289 DOI: 10.1308/003588412X13171221590737]
- 12 **Kühn JP**, Hegenscheid K, Puls R. [Ruptured visceral artery aneurysm as the initial symptomatic manifestation of panarteritis nodosa]. *Rofo* 2008; **180**: 922-924 [PMID: 19238643]
- 13 **Senaati S**, Cekirge S, Akhan O, Balkanci F. Spontaneous perirenal and hepatic hemorrhage in periarteritis nodosa. *Can Assoc Radiol J* 1993; **44**: 49-51 [PMID: 8093852]
- 14 **Alleman MJ**, Janssens AR, Spoelstra P, Kroon HM. Spontaneous intrahepatic hemorrhages in polyarteritis nodosa. *Ann Intern Med* 1986; **105**: 712-713 [PMID: 2876668 DOI: 10.7326/0003-4819-105-5-712]
- 15 **Bonomo L**, De Pascale A, Di Giandomenico E, Gidaro G. Hepatic hematoma in polyarteritis nodosa. *Rays* 1988; **13**: 19-21 [PMID: 2908007]
- 16 **Dzwonczyk J**, Serlin O, Skerrett PV. Spontaneous rupture of the liver; report of a case secondary to polyarteritis nodosa. *Ann Surg* 1959; **150**: 327-330 [PMID: 13670600 DOI: 10.1097/0000658-195908000-00018]
- 17 **Li AK**, Rhodes JM, Valentine AR. Spontaneous liver rupture in polyarteritis nodosa. *Br J Surg* 1979; **66**: 251-252 [PMID: 36958 DOI: 10.1002/bjs.1800660410]
- 18 **Gumà M**, Lorenzo-Zúñiga V, Olivé A, Perendreu J, Bechini J, Doménech E, Planas R. Occult liver involvement by polyarteritis nodosa. *Clin Rheumatol* 2002; **21**: 184-186 [PMID: 12086174 DOI: 10.1007/s10067-002-8280-9]

**P- Reviewer:** Aseni P, Kubota K, Rothschild BM **S- Editor:** Ji FF

**L- Editor:** A **E- Editor:** Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>





# World Journal of *Hepatology*

*World J Hepatol* 2016 November 28; 8(33): 1419-1488





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*



**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*



Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**REVIEW**

- 1419 Recent advances in the diagnosis and treatment of primary biliary cholangitis  
*Huang YQ*

**ORIGINAL ARTICLE****Basic Study**

- 1442 Performance of cold-preserved rat liver Microorgans as the biological component of a simplified prototype model of bioartificial liver  
*Pizarro MD, Mediavilla MG, Quintana AB, Scandizzi AL, Rodriguez JV, Mamprin ME*

**Case Control Study**

- 1452 Pancreatic hyperechogenicity associated with hypoadiponectinemia and insulin resistance: A Japanese population study  
*Makino N, Shirahata N, Honda T, Ando Y, Matsuda A, Ikeda Y, Ito M, Nishise Y, Saito T, Ueno Y, Kawata S*

**Observational Study**

- 1459 Neglected features of lifestyle: Their relevance in non-alcoholic fatty liver disease  
*Trovato FM, Martines GF, Brischetto D, Trovato G, Catalano D*

**Prospective Study**

- 1466 Can platelet count/spleen diameter ratio be used for cirrhotic children to predict esophageal varices?  
*Sezer OB, Çelik D, Tutar N, Özçay F*
- 1471 Elevation of serum urokinase plasminogen activator receptor and liver stiffness in postoperative biliary atresia  
*Udomsinprasert W, Honsawek S, Jirathanathornnukul N, Chongsrisawat V, Poovorawan Y*

**SYSTEMATIC REVIEWS**

- 1478 Bibliometric analysis of top 100 cited articles in nonalcoholic fatty liver disease research  
*Zhang TS, Qin HL, Wang T, Li HT, Li H, Xia SH, Xiang XH*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, D Nageshwar Reddy, FACP, FASGE, MD, Chief Doctor, Director, Department of Gastroenterology, Asian Institute of Gastroenterology, Hyderabad, Andhra Pradesh 500082, India

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xin-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Department of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL BOARD MEMBERS  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

EDITORIAL OFFICE  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
November 28, 2016

COPYRIGHT  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS  
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION  
<http://www.wjgnet.com/esps/>

## Recent advances in the diagnosis and treatment of primary biliary cholangitis

Ying-Qiu Huang

Ying-Qiu Huang, Department of Gastroenterology, General Hospital of Benxi Steel and Iron (Group) Co., LTD, Fifth Clinical College of China Medical University, Benxi 117000, Liaoning Province, China

**Author contributions:** Huang YQ independently wrote the manuscript.

**Conflict-of-interest statement:** The author declares no conflict of interest.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Ying-Qiu Huang, Professor of Medicine, Chief Physician, Department of Gastroenterology, General Hospital of Benxi Steel and Iron (Group) Co., LTD, Fifth Clinical College of China Medical University, 29 Renmin Road, Pingshan District, Benxi 117000, Liaoning Province, China. [huangyingqiu\\_bx@126.com](mailto:huangyingqiu_bx@126.com)  
Telephone: +86-24-42215137  
Fax: +86-24-42215087

Received: March 31, 2016  
Peer-review started: April 5, 2016  
First decision: June 12, 2016  
Revised: July 26, 2016  
Accepted: August 27, 2016  
Article in press: August 29, 2016  
Published online: November 28, 2016

### Abstract

Primary biliary cholangitis (PBC), formerly referred to

as primary biliary cirrhosis, is an infrequent progressive intrahepatic cholestatic autoimmune illness that can evolve into hepatic fibrosis, hepatic cirrhosis, hepatic failure, and, in some cases, hepatocellular carcinoma. The disease itself is characterized by T-lymphocyte-mediated chronic non-suppurative destructive cholangitis and elevated serum levels of extremely specific anti-mitochondrial autoantibodies (AMAs). In this article, we will not only review epidemiology, risk factors, natural history, predictive scores, radiologic approaches (*e.g.*, acoustic radiation force impulse imaging, vibration controlled transient elastography, and magnetic resonance elastography), clinical features, serological characteristics covering biochemical markers, immunoglobulins, infections markers, biomarkers, predictive fibrosis marker, specific antibodies (including AMAs such as AMA-M2), anti-nuclear autoantibodies [such as anti-multiple nuclear dot autoantibodies (anti-sp100, PML, NDP52, anti-sp140), anti-rim-like/membranous anti-nuclear autoantibodies (anti-gp210, anti-p62), anti-centromere autoantibodies, and some of the novel autoantibodies], histopathological characteristics of PBC, diagnostic advances, and anti-diastole of PBC. Furthermore, this review emphasizes the recent advances in research of PBC in terms of therapies, including ursodeoxycholic acid, budesonide, methotrexate, obeticholic acid, cyclosporine A, fibrates such as bezafibrate and fenofibrate, rituximab, mesenchymal stem cells transplant, and hepatic transplant. Currently, hepatic transplant remains the only optimal choice with acknowledged treatment efficiency for end-stage PBC patients.

**Key words:** Autoimmune liver diseases; Primary biliary cholangitis; Primary biliary cirrhosis; Diagnosis; Therapy

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Primary biliary cholangitis (PBC), previously called primary biliary cirrhosis, is an autoimmune non-suppurative inflammatory disease of the bile duct



that is usually complicated by intrahepatic cholestasis and intrahepatic bile ductule damage, and eventually leads to liver fibrosis and cirrhosis. This review will focus on the clinical, serological and histopathological characteristics of PBC, as well as the advances in the diagnosis and treatment of the disease.

Huang YQ. Recent advances in the diagnosis and treatment of primary biliary cholangitis. *World J Hepatol* 2016; 8(33): 1419-1441 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i33/1419.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i33.1419>

## INTRODUCTION

Primary biliary cholangitis (PBC)<sup>[1-8]</sup> is a relatively rare chronic intrahepatic cholestatic illness characterized by a T-lymphocyte-mediated attack on small intralobular biliary ducts and the presence of elevated plasma concentrations of specific anti-mitochondrial antibodies (AMAs), resulting in hepatic fibrosis and, ultimately, hepatic cirrhosis or hepatic failure, with the potential for hepatic cellular carcinoma *via* complications<sup>[9-11]</sup>. PBC predominantly affects women, at a ratio of approximately 12:1 of women to men, who are normally diagnosed at middle-age, primarily in an initial symptomless early stage<sup>[9-12]</sup>. There is positive association between the national incidence of PBC and socioeconomic status, as estimated by the Human Development Index (HDI)<sup>[13]</sup>. Moreover, in less-developed countries, the incidence of PBC might be less common<sup>[13]</sup>. Fatigue and pruritus are incipient clinical manifestations that appear in approximately 20% of PBC patients<sup>[14]</sup>. Although the clinical presentation and natural disease history of PBC patients have progressively improved over the years due to the recognition of earlier widespread use of ursodeoxycholic acid (UDCA), about 1/3 of PBC patients display suboptimal biochemical responses to UDCA and a poor prognosis<sup>[9-12]</sup>. At present, hepatic transplant remains the most beneficial therapeutic modality for patients with end-stage PBC<sup>[9-12]</sup>. This article will focus on the epidemiology, risk factors, clinicopathologic characteristics, serological features, histopathological characteristics, radiologic evaluation approaches, diagnosis, and differential diagnosis, as well as recent advances in the therapy of PBC.

## ALTERED TERMINOLOGY FOR PBC: FROM “PRIMARY BILIARY CIRRHOSIS” TO “PRIMARY BILIARY CHOLANGITIS”

The disorder generally referred to currently as “primary biliary cirrhosis” was primitively depicted in 1851, but not formally named until 1950<sup>[1-8]</sup>. However, it was later rightly recognized that the application of the terminology “primary biliary cirrhosis” is for a catachresis

in patients in the presence of early-stage disease and histopathological characteristics of non-suppurative destructive cholangitis that are usually complicated with intrahepatic cholestasis and intrahepatic bile ductule damage. In recent decades, the prognosis of PBC patients has been observably ameliorated since the disease entity was first described more than 150 years ago due to the application of UDCA. Since a great number of PBC patients do not suffer from hepatic cirrhosis, this tag has perceptibly disrupted many PBC patients, who strive for more accurate nomenclature<sup>[1-8]</sup>. At the second European Association for the Study of the Liver (EASL) monothematic conference on “primary biliary cirrhosis” in 2014, representatives of multitudinous patient cohorts from a variety of countries worldwide requested altering the eponym “cirrhosis” to another that would more precisely represent the characteristics of the disorder<sup>[1-8]</sup>. From the point of view of the patient, the eponym “cirrhosis” is misdirecting in some ways, and may result in stigmatization and confusion with alcoholic cirrhosis, as well as a shortage of transparency with regards to the stage and prognosis of the disease. From the physician’s perspective, misapplication of the terminology “cirrhosis” is counter-productive to their job. In order to assist and cure patients both within and without the hospital setting who are trying to balance their private lives with their medical demands, it is vital that the term “cirrhosis” be changed<sup>[1-8]</sup>. The suggested change of “cirrhosis” to “cholangitis” was ratified by the EASL in November 2014, by the American Association for the Study of Liver Diseases in April 2015, and by the AGA in July 2015, respectively<sup>[1-8]</sup>. In order to inform more people worldwide regarding this change, an article was published in 2015 titled “Changing nomenclature for PBC: From “cirrhosis” to “cholangitis” ” in various well-known international medical journals, such as *Gastroenterology*, *Am J Gastroenterol*, *Gut*, *Hepatology*, *J Hepatol*, *Dig Liver Dis*, *Clin Res Hepatol Gastroenterol*, and *Clin Gastroenterol Hepatol*<sup>[1-8]</sup>. Adopting the terminology “primary biliary cholangitis” for the illness known by the acronym PBC is therefore long overdue, so as to bring it into correspondence with a very recent global consensus.

## Epidemiology

Epidemiology provides significant clues towards our comprehension of the unsearchable etiopathogenesis of PBC. In the past two decades, there have been a chain of epidemiological retrospective investigations concerning patients with PBC<sup>[15-22]</sup>. The epidemiology of PBC has not only changed significantly over the past twenty years, with a trend towards increasing prevalence in many places around the world<sup>[15-22]</sup>, but is also positively correlated with the national HDI<sup>[13]</sup>. There is a positive, but not significant, correlation between PBC incidence and HDI on a global level ( $r = 0.348$ ,  $P = 0.082$ )<sup>[13]</sup>. However, in Europe, a significantly positive correlation exists between PBC incidence and HDI ( $r = 0.455$ ,  $P = 0.044$ )<sup>[13]</sup>. Moreover, the PBC incidence

is positively related to the health index ( $r = 0.422$ ,  $P = 0.036$ ), but negatively related to the education index ( $r = -0.650$ ,  $P < 0.01$ )<sup>[13]</sup>. The prevalence and incidence rates of PBC patients have been reportedly augmenting annually worldwide, making changing the name “cirrhosis” vital<sup>[15-22]</sup>. A study in the United States showed that, during the period of 1975-1995, the overall age and sex-adjusted incidence rate of PBC was 27/1000000 per year, with the incidence in female and male populations being 45/1000000 and 7/1000000 per year, respectively<sup>[15]</sup>. In 1995, the age- and sex-adjusted prevalence was 654/1000000 for women, 121/1000000 for men, and 402/1000000 overall<sup>[15]</sup>. A study in Canada revealed that, from 1996 to 2002, the overall age and sex-adjusted incidence rate of PBC was 30.3/1000000 per year (female: 48.4/1000000, male: 10.4/1000000); the prevalence was 100/1000000 in 1996 and 227/1000000 in 2002<sup>[16]</sup>. A study in Lombardy, Italy and in Denmark suggested that during 2000-2009, the overall age and sex-adjusted incidence rate of PBC in Lombardy was 16.7/1000000 per year (female-to-male ratio 2.3:1), the point prevalence in Lombardy was 160/1000000 in 2009, the incidence of PBC in Denmark was 11.4/1000000 per year (female-to-male ratio 4.2:1), and the point prevalence in Denmark was 115/1000000 in 2009<sup>[17]</sup>. A study in Crete, Greece showed that, from 1990 to 2010, the incidence of PBC was 20.88/1000000, and the prevalence was 365/1000000<sup>[18]</sup>. A study in the Netherlands demonstrated that, between 2000 and 2008, the incidence of PBC was 11/1000000 (3/1000000 in men and 19/1000000 in women) and the point prevalence in 2008 was 132/1000000<sup>[19]</sup>. A study in Iceland indicated that the point prevalence in 2010 was 383/1000000, while the age-standardized rate of incidence for female patients in the 1<sup>st</sup> (1991-2000) and 2<sup>nd</sup> phases (2001-2010) were 34/1000000 and 41/1000000, respectively<sup>[20]</sup>. Overall incidence rates in the 1<sup>st</sup> and 2<sup>nd</sup> phases were 20/1000000 and 25/1000000, respectively<sup>[20]</sup>. Although the prevalence of PBC was higher in some regions of North America and northern Europe, it was rarely seen in Australia. A study in Australia demonstrated that the age-adjusted prevalence rate of PBC was 51/1000000<sup>[21]</sup>. A study in South Korea revealed that, between 2009 and 2013, the age and sex-adjusted incidence of PBC from 2011 to 2013 was 8.57/1000000 per year (ratio of female to male was 6.2:1), while the age and sex-adjusted prevalence rate from 2009 to 2013 was 47.50/1000000<sup>[22]</sup>. At the time of writing, there is still a notable lack of large, nationwide population-based solid epidemiological information concerning PBC in China. One study in China indicated that the point prevalence rate of adult PBC patients who received a health examination in southern China was 492/1000000, with the prevalence in women over 40 years old being up to 1558/1000000 (ELISA method)<sup>[23]</sup>. The overall prevalence of PBC reported by Liu *et al*<sup>[23]</sup> was much higher than previously reported in the literature, which was likely due to the methodology used in the study.

In general, the different prevalence and incidence rates of PBC reported in the aforementioned literature were mainly due to differences in assay methods, gender and age distributions of population groups, and geographic regions. There is presently no precise epidemiologic data on the prevalence of PBC in Africa, but it is speculated to be one of the lowest in the world.

## RISK FACTORS

To date, the pathogenesis of PBC remains largely unknown, although geographical distributions, genetic susceptibility, and environmental factors may be some potential risk factors for the disease<sup>[9-10]</sup>. Both familial clustering and monozygotic twins with an identical DNA sequence provided good evidence for its genetic susceptibility and high degree of consistency<sup>[11]</sup>. Environmental factors, such as smoking, drug abuse, and microbiome complexities, may play a vital role in breaking the immune tolerance of individuals with genetic susceptibility<sup>[9-11]</sup>. Furthermore, recent novel hypotheses on latent environmental triggers, such as chemical xenobiotics, which result in the breaking of self-tolerance within the unparalleled immunological environment of the liver, have also been suggested<sup>[11,12]</sup>. As PBC overwhelmingly affects females, factors such as major defects in sex chromosomes, abnormal genetic architecture, and epigenetic abnormalities strongly suggest an effect of genetic and epigenetic factors in the triggering and perpetuation of autoimmune aggression in PBC<sup>[9-12]</sup>. Several human leukocyte antigen (HLA) risk loci that provide prognostic information and a few non-HLA risk loci associated with the development of PBC have recently been confirmed by means of genome-wide association studies (GWAS)<sup>[24-31]</sup>. GWAS showed that *HLA-DQB1* (\*0402), *HLA-DRB1* genes (\*08,\*14), and *HLA-DPB1* gene (\*03:01) were predisposing risk alleles for PBC susceptibility<sup>[24,25]</sup>. Aside from the HLA locus, a number of non-HLA genes including *IL-12A* (*rs6441286*, *rs574808*), *IL-12RB2* (*rs3790567*), *STAT4*, *CD80*, *DENND1B*, *CXCR5*, *IL-7R*, *TNFRSF1A*, *NFKB1* and *CLEC16A* were also closely related to PBC susceptibility<sup>[24,26]</sup>. These data demonstrate not only that there are extraordinary associations between PBC and the usual heritable aberrance at HLA class II, *IL12A*, and *IL12RB2* loci, but also that the IL-12 immunoregulatory signaling axis plays an outstanding role in the physiopathology of PBC. Several recent GWAS have shown that some non-HLA genes, such as *STAT4* SNPs (*rs10168266*, *rs11889341*, *rs7574865*, *rs8179673*, *rs10181656*)<sup>[27]</sup>, *ESR2* *rs1256030* T allele<sup>[28]</sup>, *CLEC16A*, *SOC1*, *SPIB* and *SIAE* genes<sup>[29]</sup>, may also be significant risk factors for the progression of PBC. One study demonstrated that the downregulated expression of *IL-12A* in lymphoblastoid cell lines obtained from Han Chinese were markedly associated with the risk alleles of *rs4679868* and *rs6441286* ( $P = 0.0031$  and  $0.0073$ , respectively)<sup>[30]</sup>. Furthermore, the risk alleles of the 2 SNPs were observably related to a decreased expression

of *SCHIP1* gene that is 91.5 kb, located upstream of IL-12A and associated with susceptibility to celiac disease<sup>[30]</sup>. These data have disclosed the IL-12/JAK-STAT signaling pathway as a pivotal etiologic factor for PBC. In addition, the allele of rs79267778 was observably relevant to PBC<sup>[31]</sup>. The amino acid at position 1904 (NM\_001037335) from threonine (ACG) had been changed to methionine (ATG)<sup>[31]</sup>. This gene locus was exceedingly conservative in mammals and estimated to have the potential risk score of 0.469 by PolyPhen-2 (bioinformatics tools)<sup>[31]</sup>. PBC and gene expression were related to allele-specific transcription factor binding to usual and infrequent geno-variation<sup>[32]</sup>. DNA methylation analysis of the X chromosome exposes abnormal demethylation on CXCR3 promoter in PBC<sup>[33]</sup>. Furthermore, other associated risk factors include concurrent autoimmune disease, lifestyle factors (e.g., cigarette smoking), urinary tract infection, vaginal infection, and environmental influences (e.g., toxic and chemical exposure to such substances as nail polish and hair dye)<sup>[34-36]</sup>. In general, the close link between environment factors and genetic susceptibility may play a vital part in the epigenetic mechanisms of PBC.

### Clinical characteristics

Common clinical symptoms of PBC include fatigue, pruritus, weakness, daytime sleepiness, loss of weight, xanthelasma palpebrarum, jaundice, skin hyperpigmentation, upper abdominal discomfort, hepatosplenomegaly, osteodystrophy, osteoporosis, cholelithiasis, malabsorption syndrome, and extrahepatic manifestations of an autoimmune nature, although roughly 50% of PBC patients are asymptomatic at diagnosis<sup>[9-12]</sup>. Patients with PBC normally suffer from itching and fatigue, regardless of disease severity<sup>[14,37,38]</sup>. Serum fat-soluble vitamin D deficiency may be detected, particularly in advanced PBC patients<sup>[39,40]</sup>. Metabolic bone disease includes osteoporosis and, more rarely, osteomalacia, which have been considered important complications of PBC<sup>[41,42]</sup>. The extremely infrequent PBC complication of tubulointerstitial nephritis with Fanconi syndrome should be highly suspected in adult PBC patients in the presence of agnogenic haliteresis, even without the presence of abnormal liver function<sup>[41]</sup>. The serum levels of sclerostin were found to be observably increased in PBC patients in comparison with controls ( $P < 0.001$ ), while the hepatic mRNA overexpression of sclerostin and elevated serum levels of sclerostin were inversely related to osteogenesis and reabsorption biological markers<sup>[42]</sup>. In addition, liver sclerostin was mainly distributed in the bile ducts, was relevant to the seriousness of cholangitis ( $P = 0.02$ ), and was indirectly related to the extent of inflammation in the hepatic lobule ( $P = 0.03$ )<sup>[42]</sup>. These results indicated that sclerostin overexpression in the bile duct of patients with PBC in the presence of chronic intrahepatic cholestasis may affect metabolic osteopathia in PBC<sup>[42]</sup>. In general, although the main target organ is the liver, multiple systems may also be involved, such as interstitial lung

disease (ILD)-related pulmonary hypertension (PH) and esophageal dysfunction. PBC is also often accompanied by nephritis, connective tissue diseases (CTDs), hepatocellular carcinoma, and other rare diseases. The concurrence of these rare diseases often augments the difficulty in establishing an exact diagnosis. Specific clinical features are as follows.

### Fatigue

Fatigue is not an uncommon complaint of PBC patients, and is related to a lower quality of life<sup>[9-11]</sup>. In recent years, PBC is mainly diagnosed in the majority of patients who are asymptomatic<sup>[9-11]</sup>. However, fatigue is a significant problem in approximately 50% of PBC patients, with 20% of all PBC patients experiencing significant or life-altering fatigue<sup>[9-11]</sup>. The pathogenesis of fatigue in PBC has not been fully elucidated, although it isn't relevant to the seriousness of the underlying illness and is unresponsive to UDCA<sup>[9-11]</sup>. As the symptom of fatigue is non-specific, multifactorial, and potentially incapacitating, conditions such as anemia, diabetes, hypothyroidism, and depression should be considered and excluded<sup>[9-11]</sup>. Fatigue is typically identified with a subset of PBC patients who are predominantly young women who have particularly active illness, a suboptimal response to UDCA therapy, and are more likely to develop hepatic cirrhosis and its complications<sup>[37]</sup>. At present, there is no special drug therapy for the management of PBC-related fatigue and no significant improvement following liver transplantation<sup>[9-11]</sup>. The clinical efficacy of modafinil in the treatment of PBC-related fatigue for 12 wk has been proved to be secure and reasonably well-tolerated in randomized, placebo-controlled, phase II clinical trials<sup>[38]</sup>. Nevertheless, it did not give rise to an advantageous impact on fatigue when compared to a placebo-treated group<sup>[38]</sup>.

### Pruritus

Pruritus is a pre-eminent symptom in PBC patients with chronic cholestasis and is variably reported in PBC characterized by cholestasis<sup>[9-11,14]</sup>. More than two-thirds of PBC patients experience pruritus during the process of the illness<sup>[9-11]</sup>. Compared to asymptomatic PBC patients without pruritus, symptomatic PBC patients with pruritus more frequently suffer from hepatic cirrhosis and its related complications ( $P = 0.004$ )<sup>[37]</sup>, and are less likely to respond to UDCA treatment ( $P = 0.006$ ). The pathogenesis of cholestatic pruritus remains largely elusive<sup>[9-11]</sup>; its natural history, related pathogenesis, and molecular mechanisms are under continued investigation<sup>[9-11]</sup>. The autotaxin (ATX)-lysophosphatidic acid signaling axis may play a vital part in the nosogenesis of pruritus, and has lately has been connected with pruritus in PBC<sup>[14]</sup>. Several pieces of evidence have showed that a circulating pruritogen will take responsibility for it, but identification of the small molecule has yet to be ultimately identified<sup>[14]</sup>. In comparison, plasma ATX activity is observably associated with pruritus in PBC,

suggesting a new molecular targeting therapy<sup>[14]</sup>.

## FAT-SOLUBLE VITAMIN DEFICIENCY

Malabsorption, steatorrhea, and fat-soluble vitamin D deficiency are uncommon, except in cases of advanced liver disease and long-standing, severe cholestasis<sup>[9,10,39,40,43]</sup>. In addition to vitamin D deficiency, as luminal bile acid levels in severe cholestasis are below the critical concentration required for micelle formation and subsequent lipid absorption, clinically-relevant fat-soluble vitamin (vitamin A, E and K) deficiencies may also exist in PBC<sup>[43]</sup>. Deficiencies in fat-soluble vitamins A, D, E and K have been reported in 33.5%, 13.2%, 1.9% and 7.8% of PBC patients, respectively<sup>[43]</sup>. Vitamin A deficiency appears to be markedly associated with advanced PBC stage, decreased cholesterol, and increased Mayo risk score<sup>[43]</sup>. High Mayo risk score, low serum albumin level, and elevated total bilirubin have been shown to be independently related to vitamin D deficiency<sup>[43]</sup>. Baseline vitamin D deficiency was associated with severity of disease and response to UDCA treatment<sup>[39]</sup>. Serum 25(OH)D concentrations reduced with elevating histological grading of stage ( $P = 0.029$ ) and were inversely associated with serum bilirubin and alkaline phosphatase (ALP) concentrations in PBC<sup>[39]</sup>. Serum 25(OH)D concentration at baseline was observably reduced in non-responders to UDCA ( $P = 0.005$ )<sup>[39]</sup>. Baseline vitamin D deficiency was related to an elevated risk of an inappropriate response with no relationship to advanced histological stages ( $P = 0.047$ )<sup>[39]</sup>. Mean serum concentrations of vitamin D were observably reduced among PBC patients compared to the control group ( $P = 0.029$ ) and vitamin D deficiency ( $\leq 10$  ng/mL) was observed in 33% of PBC patients vs 7% of the control group ( $P < 0.0001$ )<sup>[40]</sup>. Vitamin D concentrations were negatively associated with advanced hepatic injury, as well as the existence of accompanying autoimmune disorders<sup>[40]</sup>. The potential role of vitamin D in PBC may involve genetic and cell signaling mechanisms. Relatively few PBC patients have vitamin E or K deficiencies<sup>[43]</sup>.

### **PBC complicated with portal-venous hypertension**

Portal-venous hypertension is not an uncommon aftermath of PBC, and may even occur before cirrhosis develops in PBC patients<sup>[9-11]</sup>; approximately 10% of PBC patients presented with characteristics of portal hypertension as an initial clinical symptom<sup>[9-11]</sup>. Gastroesophageal varices may occur in any of the different histological phases of PBC<sup>[9-11]</sup>. Signs of portal hypertension should therefore be carefully observed for in PBC patients at the moment of diagnosis, as well as during the observation period<sup>[9-11]</sup>. The pathogenesis of portal hypertension in PBC is still unclear<sup>[9,10]</sup>. A recent study suggested sinusoidal blockage as a potential physiopathology mechanism during the early phases of PBC, which was verified by the obvious intrahepatic portal vein in 3 non-cirrhotic PBC patients, with intrahepatic portal vein hepatica interflow being responsible for relieving the hepatic venous pressure

gradient<sup>[44]</sup>. Another study indicates that angiogenetic and fibrotic responses are presumably induced by aquaporin-1 (AQP-1), resulting in the enhanced perfusion of arterial blood flow to the sinusoids<sup>[45]</sup>. The result demonstrates that AQP-1 is related to arterial capillary wall proliferation and hepatic sinusoidal transformation facilitating portal-venous hypertension in PBC<sup>[45]</sup>. Esophageal varicosities (EV) can be found in PBC patients with early histological stages<sup>[46,47]</sup>. A study revealed that 6% (8/127) of early histological stage PBC patients suffered from EV and 95% of PBC patients in the presence of varices were required to meet at least one of the following criteria: Male sex, hypoalbuminemia ( $< 3.5$  g/dL), hyperbilirubinemia ( $\geq 1.2$  mg/dL), and/or prolonged prothrombin time (PT) ( $\geq 12.9$  s)<sup>[46]</sup>. Therefore, these parameters that include male sex, hypoalbuminemia, hyperbilirubinemia, and/or PT can be used as a tool for non-invasive prediction of EV<sup>[46]</sup>. A study demonstrated that among 256 cases of PBC with early histological stage, 22 cases suffered from EV at the time of diagnosis, with elevated serum ALP levels and decreasing platelet counts being markedly related to the presence of EV in early histological stage PBC<sup>[47]</sup>. The prominent relationship between these two factors with the development of EV was also disclosed, and PBC with early-stage and elevated ALP ratios  $\geq 1.9$  had an observably high risk of progressing EV<sup>[47]</sup>. In addition, another study has shown that quantitative parameters in the diagnosis of hepatic fibrosis in portal, septal and fibrillar areas may accurately predict gastroesophageal varices in PBC; the diagnostic specificity and sensitivity in PBC was 75% and 100%, respectively<sup>[48]</sup>.

### **PBC complicated with PH**

PH is generally complicated by heart or lung disorders, but it is also known to be related to PBC<sup>[49]</sup>. PH that suggests poor prognosis as a complication of PBC is not only common, but is closely related to portal-venous hypertension and immunological dysregulation<sup>[49]</sup>. PH is significantly more frequent than was previously assessed in PBC patients with portal hypertension<sup>[49]</sup>. The risk of progressing PH could be enhanced with the persistent time of portal hypertension without any explicit relationship with the degree of portal hypertension, liver failure, or amount of blood shunted<sup>[49]</sup>. The prevalence rate of PH in PBC patients with portal hypertension has been reported by McDonnell *et al*<sup>[50]</sup> as 0.61% in a clinical series of 2459 PBC patients with biopsy-proved cirrhosis of the liver. PH associated with PBC without portal hypertension is very infrequent; among 178 PBC patients, 21 (11.8%) suffered from PH<sup>[49]</sup>. Four cases (19.0%) suffered from medium to severe PH and one died of right ventricular dysfunction rather than hepatic dysfunction<sup>[49]</sup>.

### **PBC complicated with esophageal dysfunction**

Esophageal dysmotility can exist in some PBC patients<sup>[51]</sup>, particularly in those with scleroderma or Sjögren's syndrome in the absence of scleroderma<sup>[51]</sup>. As a result, some



esophageal motor disturbances could be considered associated with Sjögren's syndrome<sup>[51]</sup>. Esophageal motor dysfunction is by no means uncommon in Sjögren's syndrome or scleroderma; however, whether any esophageal dysmotility also exists in PBC without Sjögren's syndrome or scleroderma is still controversial. A recent study showed that, among 37 PBC patients, 17 (45.9%) had esophageal dysmotility (10 cases of non-specific esophageal motor disorder, 5 cases of esophageal hypomotility, 1 case of nutcracker esophagus, and 1 case of hypertensive lower esophageal sphincter)<sup>[51]</sup>. These results demonstrate that sub-clinical esophageal motor dysfunction is common in PBC patients<sup>[51]</sup>.

### **PBC complicated with ILD**

ILD is a frequent and major complication of PBC<sup>[52,53]</sup>. PBC patients who suffer from Raynaud's phenomenon and other CTDs were considered to have the greater possibility of developing ILD<sup>[52,53]</sup>. PBC with concomitant Sjögren's syndrome was considered to have a higher risk of developing ILD and presenting a poor prognosis<sup>[53]</sup>. A study showed that, among 178 PBC patients, 28 (15.7%) suffered from ILD, with 53.6% said patients suffering from difficult breathing and tussis, and 88.2% demonstrating restrictive and diffusing ventilation impairment by means of pulmonary function test<sup>[52]</sup>. Patients with PBC in the presence of ILD were older in age and displayed higher serum levels of sedimentation rate of erythrocyte compared to those without ILD ( $P < 0.05$ )<sup>[52]</sup>. Raynaud's phenomenon, as well as the coexistence of PBC and CTDs, were considered to be risk factors for PBC patients developing ILD ( $P = 0.04$ , OR = 3.12 and  $P = 0.01$ , OR = 3.18, respectively), although 42.9% of patients with PBC in the presence of ILD had not suffered from other CTDs<sup>[52]</sup>. There was much higher incidence rate of ILD in PBC patients with concomitant Sjögren's syndrome compared to those without the syndrome ( $P = 0.005$ )<sup>[53]</sup>. In some instances, ILD can even appear to precede PBC<sup>[54]</sup>.

### **PBC complicated with nephritis**

Symptomless distal renal tubular acidosis should be considered the main feature of PBC-related kidney damage, and can appear in approximately 1/3 of PBC patients<sup>[55]</sup>. However, various rarer methods of PBC-associated kidney damage have also been described in the literature, including: Fanconi syndrome, microscopic polyangiitis, membranous nephropathy, membranous glomerulonephritis, tubulointerstitial nephritis, fibrillary glomerulonephritis, interstitial nephritis, Goodpasture syndrome, anti-neutrophil cytoplasmic autoantibody (ANCA)-associated rapidly progressive glomerulonephritis, and focal segmental glomerulosclerosis<sup>[41,56-64]</sup>.

### **PBC complicated by CTDs**

PBC can be complicated by CTDs, more specifically systemic lupus erythematosus (SLE), systemic sclerosis (SSc), rheumatoid arthritis (RA), Sjögren's syndrome

(SS), polymyositis (PM), and dermatomyositis<sup>[65]</sup>. Moreover, combined PBC and CTDs often enhance the difficulty in making an exact diagnosis and treatment of PBC<sup>[65]</sup>. One study showed that, among 322 patients with PBC, 150 cases (46.6%) suffered from CTDs, of which 11 cases (3.4%) suffered from two or more CTDs<sup>[65]</sup>. SS should be considered the most common CTD (122 cases, 36.2%)<sup>[65]</sup>. Other CTDs in this group of patients, in order of rarity from high to low, were as follow: 12 cases of SLE (3.7%), 10 cases of PM (3.1%), 9 cases of SSc (2.8%), and 9 cases of RA (2.8%)<sup>[65]</sup>.

### **PBC complicated by hepatocellular carcinoma**

It is by no means uncommon for PBC patients to suffer from hepatocellular carcinoma (HCC)<sup>[66-69]</sup>. Of two retrospective studies in China, one demonstrated an incidence of HCC in PBC patients of 4.13% (52/1255)<sup>[66]</sup>, while the other found it to be 3.75% (70/1865)<sup>[67]</sup>; this incidence was observably higher in men (9.52%) than in women (3.31%)<sup>[66]</sup>. Risk factors for PBC-related HCC in China for the two studies were found to be body mass index (BMI)  $\geq 25$ , male sex and a history of drinking alcohol for the first study<sup>[66]</sup> and age  $> 54$  years, male sex, co-existence of diabetes, and previous hepatitis B virus (HBV) infection for the second study<sup>[67]</sup>. A retrospective Japanese study found the incidence of HCC in PBC patients to be 5.2% (11/210), with the only risk factor for PBC-associated HCC being associated with advanced histological stage<sup>[68]</sup>. Recently, a multicenter international study demonstrated that incidence rates of HCC in PBC patients were 2.69% (123/4565), with markedly higher rates in male PBC patients compared to female patients ( $P < 0.0001$ ). Univariate analysis of potential risk factors in establishing diagnosis of PBC related to HCC progression were: Male sex ( $P < 0.0001$ ), increased aspartate aminotransferase (AST) ( $P < 0.0001$ ), progressing liver illness ( $P = 0.022$ ), platelet decline ( $P < 0.0001$ ), and decompensated hepatic function ( $P < 0.0001$ )<sup>[69]</sup>. According to the Paris-I criteria, one year stratification by inappropriate biochemical response with UDCA therapy was markedly related to risk factors of progressing HCC ( $P < 0.0001$ )<sup>[69]</sup>. Biochemical non-response to UDCA therapy predicted future trends of HCC in early stage PBC (stages I - II) ( $P = 0.005$ ) and advanced stage PBC (stages III - IV) ( $P = 0.02$ )<sup>[69]</sup>. The international multicenter study clearly demonstrates that one year biochemical non-response to UDCA is related to incremental future risk factors of progressing HCC in PBC<sup>[69]</sup>. In addition, another study showed that repeat liver resection for recurrent HCC complicating PBC is an option and may provide and improved outcome<sup>[70]</sup>. In general, PBC with hepatic cirrhosis or non-response to one year of UDCA therapy are at incremental risk of HCC.

### **PBC with concurrent viral hepatitis**

The difficulty in identifying hepatitis C virus (HCV) and/or HBV infections in PBC patients is such that an accurate diagnosis of PBC is usually observably delayed in this particular patient cohort<sup>[71]</sup>. In PBC patients with

accompanying HCV infection, impact therapy might be approved in consideration of the relevant and more serious cirrhosis<sup>[72]</sup>. A retrospective Greek study showed that, among 1493 HBV and 526 HCV patients, 17 were confirmed as having a coexistence of viral hepatitis and PBC (8 cases of HCV and 9 cases of HBV)<sup>[71]</sup>. It is very difficult to make an exact diagnosis of PBC in HBV or HCV-infected patients, meaning that a precise diagnosis is usually delayed<sup>[71]</sup>. Cholestasis should therefore be an important indication of PBC for physicians<sup>[71]</sup>. A study in Taiwan showed that, among 76 patients with PBC, 9 cases were confirmed as having a coexistence of HCV infection and PBC, and suffered from more serious hepatic cirrhosis on the basis of Child-Pugh ( $P = 0.019$ ) and the Model for End-Stage Liver Disease (MELD) ( $P = 0.01$ ) scores<sup>[72]</sup>. One case report showed that a patient with chronic HBV infection was later found to have active, asymptomatic PBC due to a persistently elevated ALP level after optimal HBV DNA suppression on antiviral therapy<sup>[73]</sup>. This report emphasizes the significance of keeping a high clinical index of suspicion for potential PBC, even after a patient with HBV has been successfully treated for a primary liver condition<sup>[73]</sup>. Clinical vigilance, particularly in atypical clinical manifestations, can result in earlier accurate diagnosis and prompt treatment of PBC<sup>[73]</sup>.

#### **PBC with concurrent rare diseases**

Although uncommon, the coexistence of PBC and some rare diseases are frequently believed to enhance the difficulty in making an exact diagnosis of PBC, as well as its treatment, due to the very complicated clinical manifestation of diseases with coexistence conditions. PBC is occasionally associated with some rare diseases, including Guillain-Barré syndrome, warm autoimmune hemolytic anemia, primary hepatic mucosa-associated lymphoid tissue lymphoma, ANCA-associated vasculitis, pseudolymphoma, hereditary hemorrhagic telangiectasia, generalized morphea, myasthenia gravis, hepatic inflammatory pseudotumor, idiopathic retroperitoneal fibrosis, celiac disease, Wilson's disease, bullous pemphigoid, idiopathic granulomatous hepatitis, CREST syndrome, Crohn's disease, hepatic sarcoidosis, Evans syndrome, and Hürthle cell adenoma<sup>[74-92]</sup>.

## **SEROLOGICAL FEATURES**

#### **Serum antibody specific for PBC**

**AMAs:** AMAs, including AMAs-M2, are a specific and sensitive marker for the diagnosis of PBC<sup>[9,10,93,94]</sup>. The existing evidence shows that AMAs and AMAs-M2 have excellent diagnostic value, with high specificity and sensitivity for PBC<sup>[93]</sup>. Compared with AMAs-M2, AMAs is a faster and more comprehensive diagnostic marker<sup>[93]</sup>. AMAs consist of nine subtypes, four of which are associated with PBC: AMA-M2, AMA-M4, AMA-M8, and AMA-M9<sup>[9-11]</sup>. Although these four AMA subtypes have comparatively specific diagnostic value for PBC, AMA-M2

remains the foremost subtype applied as a routine diagnostic marker for PBC<sup>[9-11]</sup>. AMAs are present in 95% of PBC patients; however, 5% of patients with PBC are still AMA-negative<sup>[94]</sup>. AMA-negative PBC patients had an observably worse prognosis in comparison with AMA-positive PBC patients<sup>[94]</sup>; however, an obvious distinction between positive and negative PBC AMAs should not have been found on the basis of clinical manifestation, serum biochemical features, histopathological characteristics, disease process, or response to UDCA treatment<sup>[94]</sup>. Notably, AMA-negative PBC patients had an observably decreased free survival of liver-associated complications covering liver transplant and death in comparison with AMA-positive PBC patients ( $P = 0.0182$ )<sup>[94]</sup>.

**Anti-nuclear antibodies:** Besides AMAs, PBC patient serum is able to demonstrate other PBC-related autoantibodies, especially anti-nuclear antibodies (ANAs) covering anti-multiple nuclear dot autoantibodies (anti-sp100, PML, NDP52, anti-sp140), anti-nuclear envelope protein autoantibodies (lamin, lamin B receptor), and anti-rim-like/membranous anti-nuclear autoantibodies (anti-gp210, anti-p62)<sup>[95-103]</sup>. Determination of AMAs and PBC-specific ANAs identified them as being associated with PBC severity<sup>[9,10]</sup>. Elevated serum concentrations of ANAs should be found in approximately 50% of PBC patients and 85% of AMA-negative PBC patients<sup>[96]</sup>. In short, 44% of PBC patients had anti-sp100, 15.1% had PML, 25% had anti-gp210 and 25% had ACAs<sup>[97-100]</sup>. AMAs and ANAs (anti-gp210, anti-sp100, ACAs) are particularly prevalent in PBC<sup>[101]</sup>. Although changes in most autoantibodies that occur naturally with the passage of time do not appear to associate with clinical results in PBC, changes in serum anti-sp100 antibody levels can be used as an evaluation of prognostic factors with regard to the progress of liver fibrosis diagnosed via hepatic biopsy<sup>[102]</sup>. Sp140L is the phylogenetically nearest family member to anti-sp100 protein, and serves as an autologous antigen in PBC patients<sup>[103]</sup>. The polymerization of anti-p62 is significantly augmented in the impaired biliary ducts of PBC and may reflect the inappropriate autophagy and subsequent senescence of biliary ducts cells in the etiopathogenesis of biliary duct injury in PBC<sup>[104]</sup>. In clinical practice, it is vital to detect these autoantibodies in order to establish PBC diagnosis, assess disease severity, determine the PBC clinical phenotype, and calculate the long-term outcome<sup>[101]</sup>. Positive anti-gp210 antibody and elevated vanishing biliary duct score were observable risk factors for elevated ALP predicted worsened response<sup>[105]</sup>. Positive anti-gp210 antibody and elevated hepatitis score were observable risk factors for elevated IgM predicted worsened response<sup>[105]</sup>. Elevation of ALP and IgM worsened response were observable risk factors for development to end-stage liver illness in the absence of jaundice<sup>[105]</sup>. Therefore, in the classical or typical form of PBC, characterized by the chronic progressive disappearance of small intrahepatic biliary ducts with a simultaneous augment in hepatic fibrosis, anti-gp210 autoantibodies are a powerful risk factor for development

to icterus and liver failure<sup>[101,105]</sup>. Age, positive anti-gp210 antibody, and positive ACAs were observable risk factors for elevated of alanine aminotransferase (ALT) worsened response<sup>[105]</sup>. Elevation of ALT worsened response was an observable risk factor for development to end-stage hepatic illness with persistent icterus<sup>[105]</sup>. Of PBC patients with ACAs positivity, 30% had serious bile duct damage and portal hypertension<sup>[106]</sup>. Therefore, the presence of ACAs is a risk factor for development to hepatic cirrhosis and portal-venous hypertension<sup>[101,105,106]</sup>. Biochemical response to UDCA therapy at two years, which is affected by the serum autoantibody status of ACAs, anti-gp210, and histological and morphometric variables at baseline, may predict long-term clinical results in PBC patients<sup>[105]</sup>. By contrast, another study showed that continuous variations of anti-sp100 titers, rather than anti-gp210 titers, might be effective for the surveillance of disease procession and UDCA treatment outcome<sup>[107]</sup>. The study revealed a reduced rate of eGFR, an elevated possibility of chronic kidney disease (CKD), and an elevated rate of annual eGFR decline in PBC patients with ACAs compared to those without ACAs ( $P < 0.05$ , separately)<sup>[108]</sup>. ACAs may serve as an independent predictor for CKD in patients with PBC<sup>[108]</sup>; therefore, it is important to assess ACAs and renal function in order to deter CKD evolution in PBC<sup>[108]</sup>.

**New autoantibodies:** The recognition of novel autoantibodies as a non-invasive serum hallmark is still an important area of PBC research<sup>[109]</sup>. Hu *et al*<sup>[109]</sup> created a PBC-focused microarray with 21 of these recently affirmed alternatives, as well as 9 supererogatory familiar PBC autoantigens<sup>[109]</sup>. By screening the PBC-focused microarrays with PBC patients, 6 proteins were identified as new PBC autoantigens in the presence of high specificities and sensitivities, covering hexokinase-1 (HK 1, and isoforms I and II), Kelch-like protein 7 (KLHL7), KLHL12, zinc finger, BTB domain-containing protein 2, and eukaryotic translation initiation factor 2C, subunit 1<sup>[109]</sup>. In addition, both anti-KLHL12 and anti-HK1 antibodies with higher specificity and sensitivity were more likely to be detected in PBC in comparison with controls without PBC ( $P < 0.001$ )<sup>[110]</sup>. Anti-HK1 in combination with anti-KLHL12 in the presence of usable signs (*i.e.*, MIT3, gp210 and sp100), improved the sensitivity of PBC diagnosis<sup>[110]</sup>. Importantly, both anti-KLHL12 and anti-HK1 autoantibodies had been detected in 10% to 35% of AMA-negative patients with PBC, and increasing both biomarkers in routine PBC tests significantly increased the sensitivity in AMA-negative patients with PBC from 55% to 75% by means of immunoblot and from 48.3% to 68.5% with the ELISA method<sup>[110]</sup>. Supplementing both anti-KLHL12 and anti-HK1 autoantibodies with highly specific assays for AMAs and ANA serological tests observably enhanced the serological surveillance effect and PBC diagnosis, particularly for AMA-negative patients<sup>[110]</sup>.

**Serum biochemical sign in PBC:** The enhanced serum activity of ALP, gamma-glutamyltransferase

( $\gamma$ -GT), ALT, AST, total bilirubin (TBIL), and bile acids can be detected in most patients with PBC<sup>[9,10,47,111-113]</sup>. Evidence from several studies has shown that the presence of elevated serum activity of ALP is not only an obvious guidepost of intrahepatic cholestasis, but also a pronounced succedaneous hallmark PBC severity<sup>[111-113]</sup>. A Japanese study showed that elevated serum ALP levels were not only markedly related to the presence of esophageal varicosities in PBC patients with early histological stage, but also associated with the progression of esophageal varicosities during the follow-up period<sup>[47]</sup>. In addition, a meta-analysis of individual patient information from 4845 PBC cases covering 15 European and North American countries demonstrated that serum levels of ALP and TBIL detected at research enrollment and every year for 5 years were significantly related to clinical results<sup>[111]</sup>. The study result showed that serum levels of ALP and TBIL may predict clinical results (*i.e.*, hepatic transplant or dying) of PBC patients, and could serve as alternative terminal points in treatment tests<sup>[111]</sup>. In addition, a study in China showed that, among serum biolabeling in PBC patients, the serum concentrations of bile acids were augmented with the development of PBC, while the concentrations of carnitines were reduced with the development of PBC<sup>[113]</sup>; these factors, high serum levels of ALP, TBIL, and bile acids, are markedly associated with progressive PBC and worsened outcomes.

**Serum immunoglobulins in PBC:** PBC patients characteristically show elevated serum levels of IgM<sup>[9,10]</sup>. Environmental factors, but not genetic ones, are considered to play an important role in the pathogenesis of high serum IgM in PBC<sup>[114]</sup>. In addition, serum IgG2 and IgG3 levels were most prominently increased in PBC<sup>[115]</sup>. However, evidence of decreased serum levels of IgA, IgM, and IgG in a PBC patient seems to demonstrate that immunoglobulin-mediated etiopathogenesis may be unessential for the development of PBC<sup>[116]</sup>.

**Serum markers of infection in PBC:** As a screening test, serum from 69 PBC patients were detected for IgG-antibodies against *Toxoplasma gondii* (anti-*T. gondii*), *Helicobacter pylori* (anti-*H. pylori*), Epstein-Barr virus (anti-EBV), cytomegalovirus (anti-CMV), anti-HBV and anti-HCV<sup>[117]</sup>. The results demonstrated that the prevalence rates of 4 anti-infectious agent antibodies: Scilicet anti-*T. gondii* ( $P < 0.0001$ ), anti-*H. pylori* ( $P < 0.01$ ), EBV early antigen ( $P < 0.0001$ ), and anti-CMV ( $P < 0.05$ ) in PBC patients was observably higher than in the controls<sup>[117]</sup>. The coexistence of the 4 anti-infectious agent antibodies was comparatively ordinary in PBC, but the infection burden was infrequent in normal controls ( $P < 0.0001$ )<sup>[117]</sup>. In addition, peculiar contagion reciprocities that potentially accelerate PBC patient risk were also pointed out. Seropositivity of ammodytotoxin A was negatively related to hepatic cirrhosis among patients with PBC ( $P < 0.05$ )<sup>[117]</sup>.

**Serum biomarkers in PBC:** Serum microRNAs (miRNAs), which are sufficiently steady and control RNase-mediated



degeneration in body fluids, have been used as novel potential biomarkers for many illnesses. However, the expression spectrum of serum miRNAs in patients with PBC is poorly understood. Recently, a miRNA panel (hsa-miR-122-5p, hsa-miR-141-3p, and hsa-miR-26b-5p) was confirmed to have prominent diagnostic accuracy for PBC (sensitivity = 80.5%, specificity = 88.3%)<sup>[118]</sup>. There was a remarkable difference between expression profiles of the miRNA panel, those of serum ALP ( $P < 0.001$ ), and those of serum ANAs ( $P = 0.0282$ )<sup>[118]</sup>. Seventeen miRNAs were confirmed to be distinctively expressed in peripheral blood mononuclear cells from PBC patients<sup>[119]</sup>. In addition, the downregulated expression of hsa-miR-505-3p and miR-197-3p can be used as biological markers of PBC<sup>[120]</sup>. Functional bioinformatics analysis showed prediction of microRNA target genes involved in multiple signaling passageways and biological processes<sup>[119]</sup>. In general, serum biological markers for inchoate diagnosis of PBC are a new subject of ongoing research.

**Serum predictive marker in PBC:** Non-invasive predictive markers of hepatic fibrosis in PBC patients should be used for predicting illness development. The Wisteria floribunda agglutinin-positive Mac-2-binding protein [WFA (+)-M2BP] could serve as an effortless and dependable non-invasive succedaneous serum glycol-biomarker for the diagnosis of hepatic fibrosis in PBC<sup>[121]</sup>. Serum WFA (+)-M2BP was not only considered to be better than the other non-invasive markers in determining the important and serious fibrosis stages of PBC, but was also forcefully and separately related to clinical result<sup>[121]</sup>. Serum FGF19 is related to hepatic illness severity, and can also be used as a potential predictive marker of chronic cholestatic hepatic lesion in PBC<sup>[122]</sup>. Serum ANAs, total cholesterol, and bile acids are predictors of liver failure in PBC<sup>[123]</sup>. Elevated serum levels of fractalkine in patients with PBC could serve as predictive markers of cholangitis activity at early stages<sup>[124]</sup>. Comparative proteomics analysis demonstrated not only obvious elevated serum levels of vitronectin in AMA-negative PBC patients compared to those of AMA-positive PBC ( $P < 0.01$ ), but also a potential association with the more serious bile duct destruction found in this group<sup>[125]</sup>. Serum hyaluronan is considered a hopeful hallmark for the estimation of hepatic fibrosis in PBC<sup>[126]</sup>. In addition, serum cartilage oligomeric matrix protein might be a novel non-invasive biomarker for estimating PBC and the risk of HCC<sup>[127]</sup>.

## PREDICTIVE SCORES IN PBC

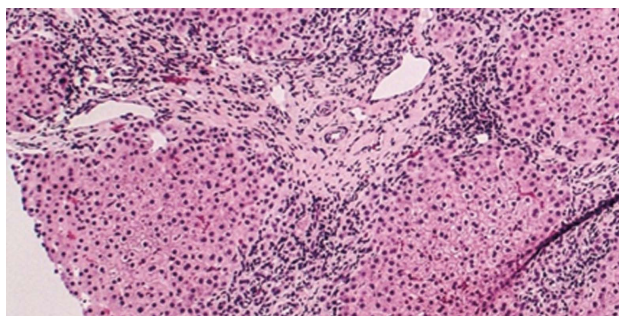
A retrospective analysis showed that PBC predictive scores, covering the European and Yale model, MELD score, and Child-Pugh score, should be interpreted prudently, with the Mayo Risk Score being deemed beneficial in predicting a helpful result<sup>[128]</sup>. As current approaches for risk stratification of PBC patients are limited and single-center-based, as well as often

dichotomous, the novel prognostic tool of GLOBE score was recently proposed by the Global PBC Study Group on the basis of an international meta-analysis of 4119 PBC patients receiving UDCA<sup>[129]</sup>. A GLOBE score to forecast transplant-free survival of PBC patients receiving UDCA therapy within 1 year was formulated and confirmed by means of clinical and biochemical variables, and the prognostic capacity of the GLOBE score was assessed along with those of the Paris-1, Barcelona, Toronto, Rotterdam, and Paris-2 criteria<sup>[129]</sup>. Serum levels of ALP, albumin, and hematoidin, as well as blood platelet counts and age, were all independently related to patient mortality or hepatic transplant<sup>[129]</sup>. There were significantly reduced times of transplant-free survival in patients with risk scores  $> 0.30$  compared to matched normal subjects ( $P < 0.0001$ )<sup>[129]</sup>. The 5-year and 10-year survival rates for patients with positive predictive values verified by the GLOBE score were 98% and 88%, separately<sup>[129]</sup>. The GLOBE score can therefore not only be considered predictive of the transplant-free survival of PBC patients treated with UDCA, but may also be used to choose the therapy and nursing scheme<sup>[129]</sup>.

## RADIOLOGIC APPROACHES TO ASSESSING FIBROSIS IN PBC

At present, there are three proposed important radiologic prediction approaches for assessing hepatic fibrosis: Acoustic radiation force impulse (ARFI), vibration controlled transient elastography (VCTE), and magnetic resonance elastography (MRE)<sup>[130-132]</sup>. The diagnostic value of the degree of liver fibrosis by means of ARFI together with the 4 serum prediction markers of hepatic fibrosis covering laminin, hyaluronan (HA), type III collagen, and type IV collagen is of a satisfying effect and has significant practical value<sup>[130]</sup>. ARFI elastography correlated observably with hepatic histological stage ( $r = 0.74$ ,  $P < 0.001$ ) in PBC patients<sup>[131]</sup>. The area under the receiver operating curve of ARFI elastography for predicting histological stage equal to or higher than II or III and equal to IV were 0.83, 0.93 and 0.91, respectively<sup>[131]</sup>. The optimal cut-off values of ARFI elastography were 1.51 m/s, 1.79 m/s, and 2.01 m/s for PBC stage equal to or higher than II or III and equal to IV, respectively<sup>[131]</sup>. ARFI elastography is therefore an acceptable and powerful technique for the quantitative assessment of PBC stage<sup>[131]</sup>. Dependable VCTE consequences can exclude advanced hepatic fibrosis and avoid the need for biopsy in the lowest 45% of patients<sup>[132]</sup>. A recent prospective study in the United States has demonstrated that three-dimensional (3D)-MRE at 40 Hz has supreme diagnostic precision in diagnosing advanced hepatic fibrosis<sup>[133]</sup>. Both 2D-MRE and 3D-MRE at 60 Hz, the standard shear-wave frequency, are also reasonably precise in diagnosing advanced hepatic fibrosis<sup>[133]</sup>. MRE has obvious diagnostic precision in advanced hepatic fibrosis and cirrhosis in hepatic transplantation receivers, independent of the extent of inflammation





**Figure 1** Histology of primary biliary cholangitis (hematoxylin and eosin staining; × 200 liver biopsy). An absence/paucity of bile ducts is seen with focal chronic inflammation in a portal area consistent with late-stage primary biliary cholangitis<sup>[9]</sup>.

and BMI<sup>[134]</sup>. Magnetic resonance imaging (MRI) has potential diagnostic value for PBC, and the periportal halo sign and signal strength contribute to assessing the extent of hepatic fibrosis<sup>[135]</sup>. In addition, Gd-EOB-DTPA-enhanced MRI might offer beneficial detection approaches for hepatopathy in PBC patients<sup>[136]</sup>. In general, none of the radiologic approaches have perfect accuracy in any published study to date; however, VCTE outperformed all other non-invasive current surrogate markers of hepatic fibrosis in PBC. Due to its high acceptability and ability to predict hepatic decompensation, VCTE could be a useful tool in allocating PBC patients into different categories of risk.

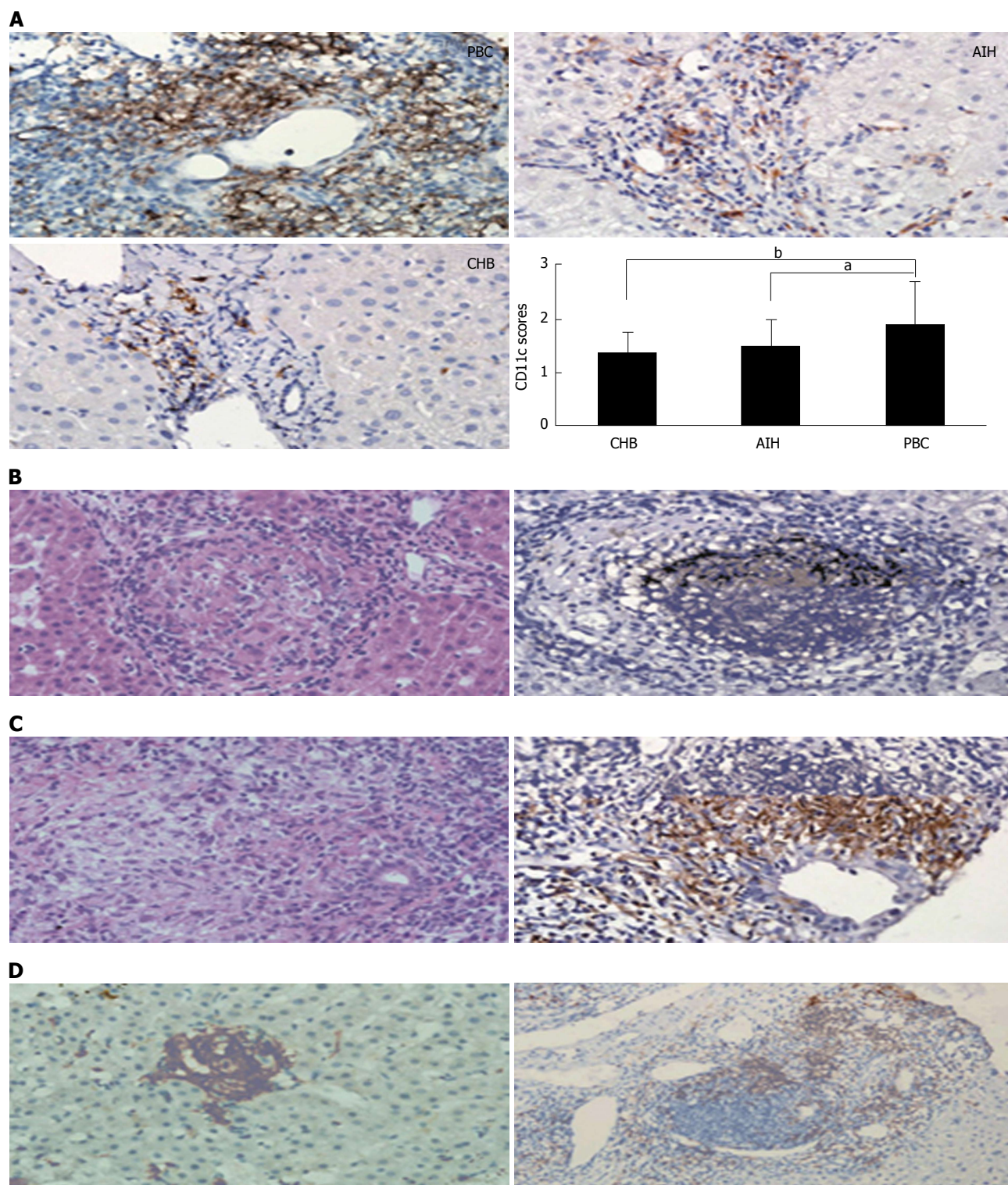
## HISTOPATHOLOGICAL FEATURES

Histopathologically, PBC is not only characterized by predominantly different stages of hepatic fibrosis that eventually result in cirrhosis of the liver or hepatic failure, but also as a granulomatous lymphocytic cholangitis that consequently leads to such small bile duct loss such as vanishing bile duct syndrome and cholestasis (Figures 1 and 2)<sup>[9,137]</sup>. The typical dendritic-cellular CD11c marker has markedly effective expression and significant sensitivity compared with classical hematoxylin and eosin (H and E) staining in discovering hepatic granulomatous lesions related to PBC and other illnesses<sup>[137]</sup>. There are significantly elevated serum concentrations of IgM and earlier stages of illness in PBC patients in the presence of CD11c-positive expression hepatic granulomas<sup>[137]</sup>. There are hallmarks of immature dendritic cells, namely CD11b, decreased expression of MHC II, IL23, CD83 and CCR7, and increased expression of C1q in granulomatous lesions from PBC and other illnesses<sup>[137]</sup>. PBC-related granulomatous lesions largely represented by B lymphocytes and IgM-positive plasmacytes together with macrophages<sup>[137]</sup>. Put simply, dendritic cells play a pivotal role in the etiopathogenesis of granulomas, regardless of their origin<sup>[137]</sup>. More specifically, hepatic granulomas may be caused by the reciprocities between IgM and immature dendritic cells in PBC<sup>[137]</sup>. In addition, spleen tissue samples from PBC demonstrated accumu-

lation of IgM-positive cells, along with CXCL13-positive cells, in CD21-positive lymph follicles<sup>[138]</sup>. CXCL13-positive follicular dendritic cells might be conducive to the production of excess IgM from the spleen<sup>[138]</sup>. The deviant expression of mitochondrial autoantigens and subsequent autoimmune mechanism in PBC may be closely associated with deregulated autophagy and the following cellular senescence in biliary epithelial cells (BECs)<sup>[139]</sup>. Activated NKT cells may prompt BEC death, leading to the development of PBC<sup>[140]</sup>. A novel hepatic histological grading system for PBC, proposed by Japanese scholars, includes the degree of chronic cholangitis activity (CA 0-3), which is associated with clinic-laboratory characteristics of cholangitis, and hepatitides activity (HA 0-3), which is related to the progression of cirrhosis-related conditions<sup>[141]</sup>. French scholars proposed another novel histological scoring system for PBC, which covers assessment of hepatic fibrosis, leukomonocyte interface hepatitides, and absence of biliary ducts<sup>[142]</sup>. Abnormal expression of K-7 in hepatic cells may serve as an accessional hallmark for predicting rapid development to hepatic failure in diagnosed asymptomatic patients with PBC<sup>[143]</sup>. In other words, the histological features of PBC, in addition to typical non-suppurative destructive cholangitis and hepatic granulomatous lesions, include portal inflammation, chronic cholestasis, hepatic changes (interface hepatitides or lobular hepatitides), and bile duct loss. The two histological classifications by Ludwig's and Scheuer's stages have been used globally for PBC staging since the 1960s, and are based on the histopathological findings of PBC. In addition, two novel histological scoring systems for PBC have been proposed by Japanese and French scholars, respectively.

## DIAGNOSIS

Cholestasis, which is a general clinical manifestation in hepatic illnesses that gives rise to reactive hyperplasia of the bile ducts, is the main complication in PBC patients. PBC diagnosis can be made in a patient *via* high serum AMAs in the presence of significantly elevated serum ALP, after ruling out other common or rare causes of cholestasis, such as viral hepatitides, drug-induced hepatic damage, alcoholic liver disease, intrahepatic cholestasis of pregnancy, progressive familial intrahepatic cholestasis, autoimmune hepatitides (AIH), primary sclerosing cholangitis (PSC), immunoglobulin G4-associated sclerosing cholangitis (IgG4-SC), and autoimmune hepatic illnesses overlap syndrome (PBC/AIH, PSC/AIH, PBC/PSC, PBC/IgG4-SC), as well as biliary obstructions such as biliary calculi, biliary ascariasis, biliary tract inflammation, postoperative bile duct benign stricture, pancreatic pseudocyst, cholangiocarcinoma, and pancreatic head carcinoma. PBC diagnosis requires two of the three following objective criteria: (1) biochemical proof of intrahepatic cholestasis based primarily on elevated levels of serum ALP greater than or equal to 1.5 times the upper limit of normal (ULN) for more than



**Figure 2** Histology (hematoxylin and eosin staining) and immunochemical staining of primary biliary cholangitis. A: Magnification  $\times 400$ ; B: Magnification  $\times 400$ ; C, D: Magnification  $\times 400$  (left); Magnification  $\times 200$  (right). PBC livers demonstrated observably stronger portal area immunostain for CD11c, the position of CD11c sedimentation scored on a 0-4 scale to be compared among PBC, AIH and CHB patients ( $^aP < 0.05$ ,  $^bP < 0.01$ ) (A); PBC hepatic granulomatous lesions were classically situated within portal areas, generally near or around the impaired bile duct (B); Hepatic granulomatous lesions were also occasionally detected in the liver lobule or close to the germinal center (C)<sup>[137]</sup>. PBC: Primary biliary cholangitis; AIH: Autoimmune hepatitis; CHB: Chronic hepatitis B.

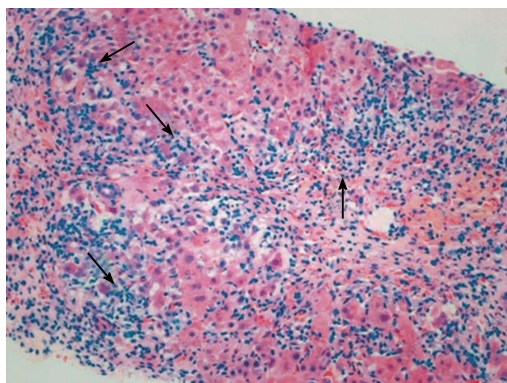
24 wk; (2) presence of serum titers of AMAs greater than or equal to 1:40; and (3) liver histology characterized by non-suppurative cholangitis and granulomatous destruction of interlobular bile ducts<sup>[9,10,144]</sup>. Furthermore, patients with PBC frequently have elevated serum levels

of ALT, AST and IgM<sup>[144]</sup>.

## DIFFERENTIAL DIAGNOSIS OF PBC

Autoimmune liver diseases (AILDs) cover PBC, PSC,





**Figure 3** Histology characteristic of autoimmune hepatitis (hematoxylin and eosin staining). The inflammatory cell infiltration feature of autoimmune hepatitis is composed of leukomonocytes, monocytes/macrophagocytes, and plasmacytes (interface hepatitis, arrows) in the portal and periportal areas<sup>[145]</sup>.

IgG4-SC and AIH; PBC should therefore be distinguished from AMA-positive AIH, PSC, or IgG4-SC. In addition, some AILDs patients present with features of PBC or PSC and AIH or IgG4-SC, either simultaneously or consecutively. They are traditionally deemed as obvious entities, although shared modes in so-called “overlap syndrome” have been recognized across the spectrum. The diagnosis of such overlap syndromes as PBC/AIH, PSC/AIH, PBC/PSC, and PBC/IgG4-SC is still challenging, but it is indispensable to diagnosis due to its rapid progression to cirrhosis and liver failure. Overlap syndromes should be considered in AIH patients with cholestatic findings, concurrent inflammatory bowel disease (IBD), or steroid-refractory disease. Clinical, biochemical, immunological, histological, and bile duct imaging characteristics contribute to the diagnosis of AILD overlap syndrome.

#### AMA-positive AIH

AIH is an immune-mediated severe hepatopathy characterized by elevated serum levels of ALT, AST and IgG, a high percentage of circulating non-organ-specific autoantibodies, and histologically with interface hepatitis (Figure 3)<sup>[145]</sup>. The incidence rates of AIH display a positive correlation with the national HDI ( $r = 0.638$ ,  $P = 0.014$ ) and the income index ( $r = 0.649$ ,  $P = 0.012$ )<sup>[13]</sup>. AIH is divided into type 1, which is defined according to the seropositivity of smooth muscle autoantibody (SMA) and/or ANAs, and type 2, which is defined according to the seropositivity of liver-kidney microsome type 1 and/or liver cytosol type 1<sup>[145]</sup>. The non-classical clinical phenotypes of AIH, particularly AMA-positive AIH, should be distinguished from PBC. In general, AMAs-M2 antibody is specific to PBC patients, but may also be occasionally discovered in certain AIH patients<sup>[146]</sup>. Efficient means of discriminating between AIH and PBC are required, due to the fact that their clinical process and treatment are disparate<sup>[146]</sup>. One recent study has shown that antibodies to filamentous-actin (anti-F-actin) protein can not only be considered the serological marker of type 1 AIH, but may also predict AIH recurrence<sup>[147]</sup>.

Furthermore, the application of repetition hepatic biopsy is an efficient method for AIH diagnosing comorbid liver conditions<sup>[148]</sup>. Although certain AIH patients were detected to be AMAs-M2 (+), the titers were markedly reduced compared to PBC patients<sup>[146]</sup>. During the follow-up period, the serum titers of AMAs-M2 were reduced in AIH patients<sup>[146]</sup>.

#### PSC

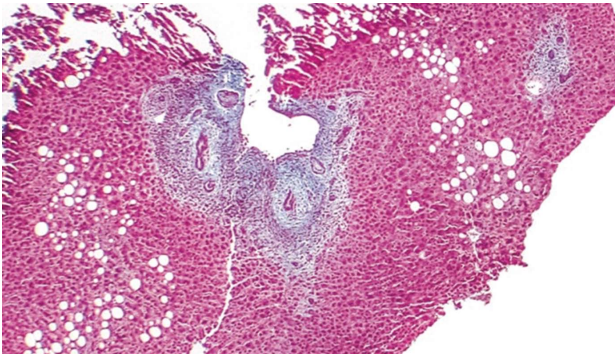
PSC is an immune-mediated chronic idiopathic cholestatic hepatobiliary illness characterized by progressive fibrosis and the stricturing of medium and large-sized extrahepatic and/or intrahepatic bile ducts (Figure 4)<sup>[9]</sup>. PSC is related to an elevated risk of cholangiocarcinoma and when IBD is present for colorectal carcinoma. Approximately 75% of PSC patients suffer from IBD; primarily ulcerative colitis (UC)<sup>[9]</sup>. Although no statistical correlation between PSC incidence and HDI was discovered ( $r = 0.116$ ,  $P = 0.706$ ), the income index was positively related to PSC incidence ( $r = 0.599$ ,  $P = 0.031$ )<sup>[13]</sup>. PSC classically evolves slowly over 10 to 15 years, eventually leading to biliary cirrhosis and premature death due to decompensated hepatic illness in the majority of patients<sup>[149]</sup>. Additional complications of PSC include hepatic osteodystrophy, dominant bile duct stenosis, recurrent cholangitis, and such disease-related malignancies as hepatobiliary (especially cholangiocarcinoma), pancreatic, and colorectal (especially with IBD) carcinoma<sup>[149]</sup>. In one recent study, 65% of patients with long-term IBD had subclinical PSC related to progressive IBD, with no biochemical anomalousness and mild illness, based on magnetic resonance cholangiography findings<sup>[150]</sup>. There is currently no specific biomarker for PSC, although the prevalence of p-ANCA has been reported to range from 33% to 85% in patients with PSC<sup>[149]</sup>. Other non-specific autoantibodies in PSC cover ANAs and SMA<sup>[149]</sup>.

#### IgG4-SC

IgG4-SC is an immune-mediated peculiar sclerosing cholangitis of unknown etiopathogenesis that is frequently related to autoimmune pancreatitis (AIP)<sup>[151]</sup>. The diagnosis of IgG4-SC is performed on the basis of a combination of the following four standards: (1) distinctive cholangiography features; (2) elevated serum levels of IgG4; (3) concurrence of IgG4-associated illnesses, excluding those of the bile duct; and (4) typical histopathological characteristics<sup>[151,152]</sup>. Moreover, the efficiency of corticosteroid treatment is a selectable additional diagnostic standard to affirm a precise diagnosis of IgG4-SC<sup>[151,152]</sup>. Typical characteristics of IgG4-SC may be divided into four types on the basis of the stenosis regions disclosed by endoscopic retrograde cholangiography and anti-diastole (Figure 5)<sup>[152]</sup>.

#### PBC/AIH overlap syndromes

PBC/AIH overlap syndrome patients, including both characteristics of PBC and AIH, were diagnosed based on the Paris diagnostic criteria proposed by Chazouillères



**Figure 4** Histology of primary sclerosing cholangitis. Trichrome  $\times 40$ , liver biopsy. Low power view demonstrating a focal lesion typical for primary sclerosing cholangitis. Periductular layered fibrosis (featuring “onion skin” pattern) is found with edema and inflammation around the interlobular bile ducts in the center of the field<sup>[9]</sup>.

*et al*<sup>[153]</sup>. Characteristics of PBC were the following: (1) serum ALP levels more than twice the ULN value and/or  $\gamma$ -GT five or more times the ULN value; (2) serum AMA positivity; and (3) histopathological evidence of bile duct damage<sup>[153]</sup>. Characteristics of AIH were: (1) ALT elevation at a minimum of five times the ULN value; (2) levels of IgG at a minimum of twice the ULN value and/or SMA positivity; and (3) hepatic biopsy revealed interface hepatitis in the presence of moderate to serious periportal lymphocyte infiltration<sup>[153]</sup>. Diagnosis of PBC/AIH overlap syndrome was considered with the presence of 2/3 of the criteria<sup>[153]</sup>. According to the Paris diagnostic criteria: (1) PBC/AIH overlap syndromes are uncommon; (2) flares of AIH can appear either voluntarily or under UDCA; and (3) a combination of corticosteroids and UDCA is requested in the majority of patients in order to achieve the best efficient biochemical response<sup>[153]</sup>. In addition, the revised and simplified diagnostic criteria for AIH were established by the International Autoimmune Hepatitis Group (IAIHG) in 1999<sup>[154]</sup> and 2008, respectively<sup>[155]</sup>. The latter is constructed on the basis of four clinical components that appear to be more peculiar in PBC/AIH patients<sup>[156]</sup>. The simplified diagnostic criteria seem to be more effective in comparison with the Paris diagnostic criteria and revised diagnostic criteria for patients with PBC/AIH overlap syndrome<sup>[157]</sup>. However, the IAIHG’s position statement on this controversial issue suggests that patients with AILDs should be classified on the basis of their dominant characteristics as PBC, AIH or PSC/small duct PSC, and that those with overlapping characteristics should not be referred to as unique diagnostic entities<sup>[158]</sup>. Combination treatment with budesonide and UDCA was more efficacious than UDCA monotherapy for PBC/AIH overlap syndrome<sup>[159]</sup>. Furthermore, combination treatment with immunosuppression and UDCA offered better short-term responses in PBC/AIH overlap syndromes<sup>[160]</sup>.

#### **PSC/AIH overlap syndrome**

PSC/AIH overlap syndrome is a comparatively infrequent variant of PSC<sup>[161]</sup>. There were remarkable distinctions

in the below listed arguments, such as mean age ( $P < 0.01$ ), serum levels of AST ( $P < 0.005$ ), ALT ( $P < 0.005$ ), and IgG ( $P < 0.0001$ ) in PSC/AIH overlap syndromes compared with “typical” PSC patients<sup>[161]</sup>; the former seemingly profits from combination treatment with UDCA and immunosuppression, while survival is distinctly superior in the latter<sup>[161]</sup>. In addition, the clinical course of PSC/AIH overlap syndrome appears to be superior to typical PSC, suggesting that immunosuppression likely has an active efficacy on the development of PSC composition<sup>[162]</sup>.

#### **PBC/PSC overlap syndromes**

PBC/PSC overlap syndromes demonstrating the clinical manifestations of both PBC and PSC are an exceedingly uncommon condition that has been reported in a mere eight published cases, including the previously mentioned two cases<sup>[163]</sup>.

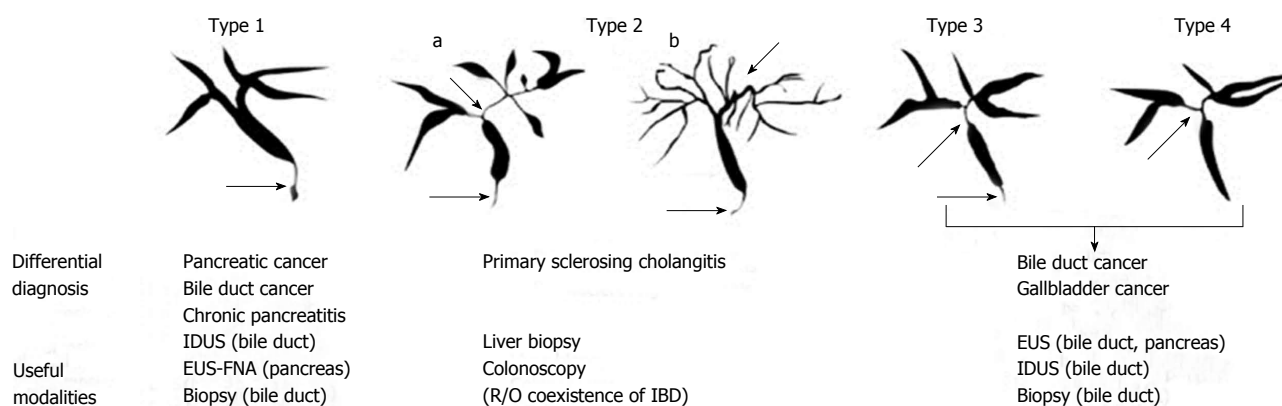
#### **PBC/IgG4-SC overlap syndromes**

IgG4-SC and PBC are two distinct autoimmune liver diseases. Approximately 90% of IgG4-SC patients have AIP, so therefore the presence of AIP may contribute to the diagnosis of IgG4-SC<sup>[151,152]</sup>. Nevertheless, PBC/IgG4-SC overlap syndrome is an extremely rare condition that has been reported in very few published cases to date, with the diagnosis of PBC/IgG4-SC overlap syndromes without the coexistence of AIP being particularly difficult<sup>[164]</sup>. Serum IgG4 concentrations may be worthwhile detecting in patients with PBC intractable to routine therapy<sup>[164]</sup>.

### **NATURAL HISTORY OF PBC**

The natural history pattern of PBC has observably changed over the past 20 years due to earlier diagnosis and the introduction of UDCA treatment. However, little is known about the natural history of PBC patients without efficient therapy. Hence, an epidemiological survey of the natural history of PBC patients in the absence of treatment might contribute to a greater understanding of the natural history of patients with UDCA-resistant PBC and in developing criteria for estimating UDCA response. A recent study demonstrated greatly reduced serum levels of ALP and very slight fluctuations in the other biochemical parameters of PBC patients treated with placebo at the 2 year follow-up period<sup>[165]</sup>. There was histological development in 39.4% of patients treated with placebo and a mild worsening of histological grade after 2 years of research<sup>[165]</sup>. In the meantime, histological progression was observed in 39.4% of the placebo-treated patients, with a moderate deterioration in histological scores noted after 2 years. Furthermore, the pooled 2 year rates of death, transplant, and progression of varicosities were 11.4%, 8.7% and 10.6%, respectively, in patients treated with placebo<sup>[165]</sup>. The natural history of PBC patients with AIH characteristics significantly differs from those without AIH characteristics<sup>[160]</sup>. In addition, although considered to possess a higher prevalence





**Figure 5** Endoscopic retrograde cholangiography classification of IgG4-SC and anti-diastole. Stricture is distributed only in the distal choledoch in type 1; stricture is widely spread throughout in the intrahepatic and extrahepatic biliary ducts in type 2. Type 2 is once more divided into two. Expanded stenosis of the intrahepatic biliary ducts in the presence of pre-stenotic expansion is diffusely spread all over in type 2a. Stenosis of the intrahepatic biliary ducts in the absence of pre-stenotic expansion and decreased biliary tree are diffusely spread throughout in type 2b; stricture is found in both porta hepatis damage and the distal choledoch in type 3; stenosis of the biliary ducts are found only in the porta hepatis damage in type 4. IDUS: Intraductal ultra-sonography; EUS-FNA: Endoscopic ultrasonography-guided fine-needle aspiration<sup>[152]</sup>; IBD: Inflammatory bowel disease.

rate of AMAs, first-degree relatives of PBC patients have a lower risk of developing PBC over time, especially in those without baseline biochemical test evidence of intrahepatic cholestasis<sup>[166]</sup>.

## THERAPY OF PBC

### UDCA

The optimal dosage for UDCA of 13-15 mg/kg per day is the standardized treatment for PBC<sup>[9,10]</sup>, as it can postpone its development, improve long-term clinical outcomes, and is extremely safe and well-tolerated. Therefore, reliable identification of so-called treatment non-response to UDCA is very important, not only for selecting PBC patients who could benefit from new therapeutic approaches, but also for discerning those who are at low risk of developing end-stage PBC. The biochemical response to UDCA after 1 year of treatment in PBC has been deemed to be a powerful predictive indicator of long-term clinical outcomes and thus facilitate the rapid recognition of patients requiring novel treatment methods. However, another study demonstrated that, in comparison with biochemical responses assessed after 12 mo of UDCA treatment, biochemical responses at the 6 mo mark showed higher positive predictive value and negative predictive value, as well as lower negative likelihood ratio according to all criteria used in the Paris, Toronto, Barcelona, and Ehime definitions<sup>[167]</sup>. Therefore, the biochemical responses at the 6<sup>th</sup> month may be served as a new standard of prediction substitute for those assessed after 12 mo of UDCA treatment<sup>[167]</sup>. In addition, the UK-PBC risk scores (composed of baseline albumin, bilirubin, platelet count, ALT, AST and ALP) after 1 year of UDCA treatment might not only be available for identification in higher risks patients for rigorous surveillance and 2<sup>nd</sup>-line treatments, as well as lower risks patients who could possibly be tracked after observation during initial treatment, but the 5-, 12- and 15-year risk scores might also be considered extremely precise<sup>[168]</sup>.

### Budesonide

Budesonide is a corticosteroids receptor/pregnane X receptor (PXR) agonist<sup>[9,10]</sup>. Treble treatment with budesonide (6 mg/d), UDCA (13-15 mg/kg per day), and mycophenolate mofetil (1.5 g/d) may afford an advantage in non-cirrhotic PBC patients with characteristics of serious illness without biochemical response to UDCA<sup>[169]</sup>. Combination therapy of budesonide (6 mg/d) and UDCA (15 mg/kg per day) was able to ameliorate the plasma biochemical index of hepatic function and hepatic histology, particularly in PBC patients with hepatic fibrosis (grade I - III), whereas the treatment effectiveness of UDCA alone was principally on lab results<sup>[170]</sup>. Although larger studies are still required, the preparatory results of agents targeting PXR, such as budesonide, have been encouraging, particularly in subsets of patients with PBC, and may mark a new therapeutic era<sup>[169,170]</sup>.

### Methotrexate

The immunosuppressive agent methotrexate (MTX) has a long history in the treatment of PBC, however little is known about its action mechanisms and roles, if any<sup>[9,10]</sup>. MTX was assessed for PBC treatment, which is currently recommended only in patients for whom PBC failed to respond adequately to UDCA and in AIH/PBC overlap syndromes<sup>[171,172]</sup>. PBC patients with characteristics of AIH should be considered for immunosuppressive therapy<sup>[158]</sup>, with the therapeutic goal being to attain normal serum aminotransferase levels and histological improvement<sup>[158]</sup>. In patients who responded improperly to UDCA, MTX observably improved hepatic enzyme tests and hepatic histology<sup>[171]</sup>. Combination therapy of UDCA and MTX brought about continuous clinical anesis in a subgroup of PBC patients, and the response to a combination of MTX and UDCA appears to be more time-proof<sup>[172]</sup>.

### FXR agonist

FXR is the receptor for primary bile acids expressed in enterohepatic tissues, where it regulates bile acid uptake, metabolism, and disposal, and has been considered

a significant target for intrahepatic cholestatic illness therapy<sup>[173-176]</sup>. Obeticholic acid (OCA) is a semi-synthetic bile acid analogue for 6 $\alpha$ -ethyl-chenodeoxycholic acid that is nearly 100-fold more potent than chenodeoxycholic acid (CDCA) and is a powerful, first class alternative FXR agonist derived from primary human bile acid CDCA, the natural endogenous FXR agonist<sup>[173]</sup>. OCA is being developed by Intercept Pharmaceuticals for the treatment of a variety of intrahepatic cholestatic illnesses, and has lately been permitted expedited approval in the United States for the treatment of PBC in combination with UDCA in adults with inappropriate response to UDCA or as monotherapy in adults unable to tolerate UDCA<sup>[174]</sup>; OCA (Ocaliva<sup>TM</sup>) is in preregistration for this function in the European Union<sup>[174]</sup>. A randomized controlled clinical trial showed that treatment with OCA (10-50 mg/d) observably decreased the serum concentrations of  $\gamma$ -GT, ALP and ALT in PBC patients with inappropriate response to UDCA, in comparison with placebo<sup>[175]</sup>. Furthermore, PBC patients treated with OCA (10 mg/d) had the lowest incidence rates and seriousness of itching<sup>[175]</sup>. Clinical trials demonstrated the treatment effectiveness of OCA in PBC without biochemical response to UDCA, as evidenced by changes in laboratory parameters substituted for long-term clinical outcomes<sup>[176]</sup>. Dose-dependent itching is a usual side-effect of OCA, but can be overcome *via* dose-titration<sup>[176]</sup>. Furthermore, INT-767, which is another steroidal semi-synthetic bile acid analogue, has been testified to be able to modulate the activity of monocytes and macrophages, decrease inflammation through the inactivation of NF- $\kappa$ B *via* a protein kinase A dependent pattern, and decrease hepatic damage by promoting biliary bicarbonate excretion as a dual FXR and TGR5 agonist<sup>[173]</sup>.

### Cyclosporine A

Recurrence of PBC after hepatic transplant has been proven to adversely influence transplant and patient survival. Protective potencies of cyclosporine A (CyA) against PBC recurrence after hepatic transplant have been reported<sup>[177]</sup>. Changing from tacrolimus to CyA was possible without sequelae, with no patients demonstrating recurrence of PBC<sup>[177]</sup>. Therefore, CyA might be serviceable for the prevention of PBC recurrence after living-donor hepatic transplant<sup>[177]</sup>. However, a retrospective multicenter study in Japan showed that although there was no influence on patient survival, original immunosuppression with CyA was considered to be major risk for PBC recurrence after hepatic transplant<sup>[178]</sup>. However, in subset analysis, switching from tacrolimus to CyA within 12 mo reduced recurrence<sup>[178]</sup>.

### Fibrates

Fibrates, including bezafibrate and fenofibrate, may be useful for treating asymptomatic patients with PBC who exhibit inappropriate response to UDCA<sup>[179-183]</sup>. A nationwide retrospective survey in Japan demonstrated that normalizing serum ALT concentrations with accessional

bezafibrate therapy observably reduced the occurrence of hepatic illness-associated clinical signs in symptomless patients, with PBC responding incompletely to UDCA<sup>[179]</sup>. Moreover, long-term combined bezafibrate and UDCA treatment in PBC not only observably ameliorated the Mayo risk score and serum concentrations of ALP, but also observably elevated the serum concentrations of creatinine<sup>[180]</sup>. Hence, it is very important to consider adverse drug reaction associated with long-term combination treatment<sup>[180]</sup>. Although the treatment effectiveness of fenofibrate has been confirmed to be related to obvious improvement in ALP, decompensation amelioration, and hepatic transplantation-free survival in patients with PBC who reveal inappropriate responses to UDCA, fenofibrate should be more prudently applied in PBC, with frequent supervision for biochemical/clinical maladjustment<sup>[181]</sup>. Long-term fenofibrate therapy as a second-line auxiliary drug in PBC patients without appropriate response to UDCA was considered to be safe and efficient in ameliorating ALP, but did not markedly decrease the evaluated possibility of hepatic-associated death or demand for hepatic transplant<sup>[182]</sup>. In addition, the optimal dosage for fenofibrate (100-200 mg/d) seems to be efficient for assistant treatment in PBC patients without optimal biochemical response to UDCA<sup>[183]</sup>.

### Rituximab

Rituximab, an anti-CD20 monoclonal antibody that selectively depletes B cells, which are precursors of the autoantibody-producing plasmocytes, may be successfully used in autoimmune-mediated hepatic illnesses<sup>[184]</sup>. Selective depletion of B-cells with rituximab was safe and related to an obvious reduction in autoantibody product, but had limited biochemical effect in PBC patients without optimal biochemical response to UDCA<sup>[184]</sup>. The efficacy of B-cell depletion with rituximab therapy and the significant improvement in both biochemical and immunologic markers that it provides has been found in PBC patients with inappropriate biochemical response to UDCA<sup>[185]</sup>. The results of these studies demonstrate that depletion of B-lymphocyte affects the induciveness, maintenance, and activation of both B- and T-lymphocytes, and offers an underlying principle for the treatment of PBC patients with incomplete response to UDCA<sup>[184,185]</sup>.

### Mesenchymal stem cells

Mesenchymal stem cell (MSC) transplantation is considered to be safe, and has been diffusely tested in autoimmune hepatic disease clinical trials with encouraging results<sup>[186,187]</sup>. MSC transplantation could modulate the systemic immune response and promote recovery in hepatic inflammation of PBC<sup>[186,187]</sup>. One single-arm clinical trial has shown that umbilical cord-derived MSC (UC-MSC) transplantation is viable and well-tolerance in patients with PBC, who response only partly to UDCA therapy, hence the need for a new treatment method for PBC patients in this subset<sup>[186]</sup>. However, the exact effect of UC-MSC transplantation in patients with PBC

still requires confirmation by a larger placebo-controlled randomized clinical trial<sup>[186]</sup>. In addition, allogeneic bone marrow MSC transplantation has been confirmed to safely improve histologic fibrosis and hepatic function in UDCA-resistant PBC patients<sup>[187]</sup>.

### Liver transplantation

At present, liver transplantation (LT) is still a lifesaving approach with outstanding results for end-stage PBC patients<sup>[177,178]</sup>. Although the 15 year survival of PBC patients was confirmed as 52.6% after LT, regrettably, the recurrent rates of PBC were 21%-37% and 43% at 10 and 15 years after LT, respectively<sup>[178,188]</sup>. Though there is still no specific treatment for recurrent PBC (rPBC), cyclosporine A and UDCA may be useful for the prevention of rPBC after LT<sup>[177,188,189]</sup>. Furthermore, the expression of mitochondrial proteins in small biliary ducts may be a beneficial diagnostic hallmark for both end-stage PBC and rPBC after LT<sup>[190]</sup>.

## CONCLUSION

In the past decade, recent advances in PBC have attempted to improve the accuracy of the disease's diagnosis and prognosis, as well as affording the chance to refine therapeutic methods. Promising novel therapies, including budesonide, fibrates, and rituximab, are being tested in PBC patients on the basis of understanding thoroughly the cellular and molecular mechanisms touched upon in all histological stages of PBC, from the early autoimmune-mediated bile duct epithelial cell damage to the destructive and illness-persistent influences of intrahepatic cholestasis, and finally giving rise to hepatic fibrosis and hepatic cirrhosis progression. Although much progress has been seen in the last 5 to 10 years, including in the diagnosis and treatment of PBC, the ultimate challenge for physicians is reducing UDCA non-responders and recurrent PBC after liver transplantation. Some novel therapeutic agents, including FXR agonists like OCA, FXR/TGR5 agonists like as INT-767, and PPAR-alpha have been identified as novel targets for drug development, with further investigation in PBC-related clinical trials still being implemented. Results of ongoing clinical trials and burgeoning therapeutic paradigms for PBC patients will likely further improve medical management and stride toward accurate treatment in the near foreseeable future.

## REFERENCES

- 1 **Beuers U**, Gershwin ME, Gish RG, Invernizzi P, Jones DE, Lindor K, Ma X, Mackay IR, Parés A, Tanaka A, Vierling JM, Poupon R. Changing nomenclature for PBC: from 'cirrhosis' to 'cholangitis'. *Gastroenterology* 2015; **149**: 1627-1629 [PMID: 26385706 DOI: 10.1053/j.gastro.2015.08.031]
- 2 **Beuers U**, Gershwin ME, Gish RG, Invernizzi P, Jones DE, Lindor K, Ma X, Mackay IR, Parés A, Tanaka A, Vierling JM, Poupon R. Changing Nomenclature for PBC: From 'Cirrhosis' to 'Cholangitis'. *Am J Gastroenterol* 2015; **110**: 1536-1538 [PMID: 26416194 DOI: 10.1038/ajg.2015.312]
- 3 **Beuers U**, Gershwin ME, Gish RG, Invernizzi P, Jones DE, Lindor K, Ma X, Mackay IR, Parés A, Tanaka A, Vierling JM, Poupon R. Changing nomenclature for PBC: from 'cirrhosis' to 'cholangitis'. *Gut* 2015; **64**: 1671-1672 [PMID: 26374822 DOI: 10.1136/gutjnl-2015-310593]
- 4 **Beuers U**, Gershwin ME, Gish RG, Invernizzi P, Jones DE, Lindor K, Ma X, Mackay IR, Parés A, Tanaka A, Vierling JM, Poupon R. Changing nomenclature for PBC: From 'cirrhosis' to 'cholangitis'. *Hepatology* 2015; **62**: 1620-1622 [PMID: 26372460 DOI: 10.1002/hep.28140]
- 5 **Beuers U**, Gershwin ME, Gish RG, Invernizzi P, Jones DE, Lindor K, Ma X, Mackay IR, Parés A, Tanaka A, Vierling JM, Poupon R. Changing nomenclature for PBC: From 'cirrhosis' to 'cholangitis'. *J Hepatol* 2015; **63**: 1285-1287 [PMID: 26385765 DOI: 10.1016/j.jhep.2015.06.031]
- 6 **Beuers U**, Gershwin ME, Gish RG, Invernizzi P, Jones DE, Lindor K, Ma X, Mackay IR, Parés A, Tanaka A, Vierling JM, Poupon R. Changing nomenclature for PBC: From 'cirrhosis' to 'cholangitis'. *Dig Liver Dis* 2015; **47**: 924-926 [PMID: 26419788 DOI: 10.1016/j.dld.2015.08.007]
- 7 **Beuers U**, Gershwin ME, Gish RG, Invernizzi P, Jones DE, Lindor K, Ma X, Mackay IR, Parés A, Tanaka A, Vierling JM, Poupon R. Changing nomenclature for PBC: From 'cirrhosis' to 'cholangitis'. *Clin Res Hepatol Gastroenterol* 2015; **39**: e57-e59 [PMID: 26433440 DOI: 10.1016/j.clinre.2015.08.001]
- 8 **Beuers U**, Gershwin ME, Gish RG, Invernizzi P, Jones DE, Lindor K, Ma X, Mackay IR, Parés A, Tanaka A, Vierling JM, Poupon R. Changing Nomenclature for PBC: From 'Cirrhosis' to 'Cholangitis'. *Clin Gastroenterol Hepatol* 2015; **13**: 1867-1869 [PMID: 26386643 DOI: 10.1016/j.cgh.2015.08.025]
- 9 **Lindor KD**, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ. Primary biliary cirrhosis. *Hepatology* 2009; **50**: 291-308 [PMID: 19554543 DOI: 10.1002/hep.22906]
- 10 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. *J Hepatol* 2009; **51**: 237-267 [PMID: 19501929 DOI: 10.1016/j.jhep.2009.04.009]
- 11 **Carey EJ**, Ali AH, Lindor KD. Primary biliary cirrhosis. *Lancet* 2015; **386**: 1565-1575 [PMID: 26364546 DOI: 10.1016/S0140-6736(15)00154-3]
- 12 **Floreani A**, Franceschet I, Perini L, Cazzagon N, Gershwin ME, Bowlus CL. New therapies for primary biliary cirrhosis. *Clin Rev Allergy Immunol* 2015; **48**: 263-272 [PMID: 25331740 DOI: 10.1007/s12016-014-8456-5]
- 13 **Pan HY**, Dai YN, Zheng JN, Shi KQ, Van Poucke S, Zou H, Zheng MH. National incidence of autoimmune liver diseases and its relationship with the human development index. *Oncotarget* 2016; Epub ahead of print [PMID: 27323833 DOI: 10.18632/oncotarget.10090]
- 14 **Sun Y**, Zhang W, Evans JF, Floreani A, Zou Z, Nishio Y, Qi R, Leung PS, Bowlus CL, Gershwin ME. Autotaxin, Pruritus and Primary Biliary Cholangitis (PBC). *Autoimmun Rev* 2016; **15**: 795-800 [PMID: 27019050 DOI: 10.1016/j.autrev.2016.03.019]
- 15 **Kim WR**, Lindor KD, Locke GR, Therneau TM, Homburger HA, Batts KP, Yawn BP, Petz JL, Melton LJ, Dickson ER. Epidemiology and natural history of primary biliary cirrhosis in a US community. *Gastroenterology* 2000; **119**: 1631-1636 [PMID: 11113084]
- 16 **Myers RP**, Shaheen AA, Fong A, Burak KW, Wan A, Swain MG, Hilsden RJ, Sutherland L, Quan H. Epidemiology and natural history of primary biliary cirrhosis in a Canadian health region: a population-based study. *Hepatology* 2009; **50**: 1884-1892 [PMID: 19821525 DOI: 10.1002/hep.23210]
- 17 **Leo A**, Jepsen P, Morenghi E, Carbone M, Moroni L, Battezzati PM, Podda M, Mackay IR, Gershwin ME, Invernizzi P. Evolving Trends in Female to Male Incidence and Male Mortality of Primary Biliary Cholangitis. *Sci Rep* 2016; **6**: 25906 [PMID: 27192935 DOI: 10.1038/srep25906]
- 18 **Koulentaki M**, Mantaka A, Sifaki-Pistolla D, Thalassinou E, Tzanakis N, Kouroumalis E. Geoeidemiology and space-time



- analysis of Primary biliary cirrhosis in Crete, Greece. *Liver Int* 2014; **34**: e200-e207 [PMID: 24502439 DOI: 10.1111/liv.12479]
- 19 **Boonstra K**, Kunst AE, Stadhouders PH, Tuynman HA, Poen AC, van Nieuwkerk KM, Witteman EM, Hamann D, Witteman BJ, Beuers U, Ponsioen CY. Rising incidence and prevalence of primary biliary cirrhosis: a large population-based study. *Liver Int* 2014; **34**: e31-e38 [PMID: 24387641 DOI: 10.1111/liv.12434]
  - 20 **Baldursdottir TR**, Bergmann OM, Jonasson JG, Ludviksson BR, Axelsson TA, Björnsson ES. The epidemiology and natural history of primary biliary cirrhosis: a nationwide population-based study. *Eur J Gastroenterol Hepatol* 2012; **24**: 824-830 [PMID: 22562114 DOI: 10.1097/MEG.0b013e328353753d]
  - 21 **Sood S**, Gow PJ, Christie JM, Angus PW. Epidemiology of primary biliary cirrhosis in Victoria, Australia: high prevalence in migrant populations. *Gastroenterology* 2004; **127**: 470-475 [PMID: 15300579]
  - 22 **Kim KA**, Ki M, Choi HY, Kim BH, Jang ES, Jeong SH. Population-based epidemiology of primary biliary cirrhosis in South Korea. *Aliment Pharmacol Ther* 2016; **43**: 154-162 [PMID: 26526639 DOI: 10.1111/apt.13448]
  - 23 **Liu H**, Liu Y, Wang L, Xu D, Lin B, Zhong R, Gong S, Podda M, Invernizzi P. Prevalence of primary biliary cirrhosis in adults referring hospital for annual health check-up in Southern China. *BMC Gastroenterol* 2010; **10**: 100 [PMID: 20815889 DOI: 10.1186/1471-230X-10-100]
  - 24 **Hirschfield GM**, Liu X, Xu C, Lu Y, Xie G, Lu Y, Gu X, Walker EJ, Jing K, Juran BD, Mason AL, Myers RP, Peltekian KM, Ghent CN, Coltescu C, Atkinson EJ, Heathcote EJ, Lazaridis KN, Amos CI, Siminovitch KA. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *N Engl J Med* 2009; **360**: 2544-2555 [PMID: 19458352 DOI: 10.1056/NEJMoa0810440]
  - 25 **Invernizzi P**, Ransom M, Raychaudhuri S, Kosoy R, Lleo A, Shigeta R, Franke A, Bossa F, Amos CI, Gregersen PK, Siminovitch KA, Cusi D, de Bakker PI, Podda M, Gershwin ME, Seldin MF. Classical HLA-DRB1 and DPB1 alleles account for HLA associations with primary biliary cirrhosis. *Genes Immun* 2012; **13**: 461-468 [PMID: 22573116 DOI: 10.1038/gene.2012.17]
  - 26 **Mells GF**, Floyd JA, Morley KI, Cordell HJ, Franklin CS, Shin SY, Heneghan MA, Neuberger JM, Donaldson PT, Day DB, Ducker SJ, Muriithi AW, Wheeler EF, Hammond CJ, Dawwas MF, Jones DE, Peltonen L, Alexander GJ, Sandford RN, Anderson CA. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. *Nat Genet* 2011; **43**: 329-332 [PMID: 21399635 DOI: 10.1038/ng.789]
  - 27 **Joshita S**, Umemura T, Nakamura M, Katsuyama Y, Shibata S, Kimura T, Morita S, Komatsu M, Matsumoto A, Yoshizawa K, Ishibashi H, Tanaka E, Ota M. STAT4 gene polymorphisms are associated with susceptibility and ANA status in primary biliary cirrhosis. *Dis Markers* 2014; **2014**: 727393 [PMID: 24648611 DOI: 10.1155/2014/727393]
  - 28 **Yang L**, Zhang H, Jiang YF, Jin QL, Zhang P, Li X, Gao PJ, Niu JQ. Association of Estrogen Receptor Gene Polymorphisms and Primary Biliary Cirrhosis in a Chinese Population: A Case-Control Study. *Chin Med J (Engl)* 2015; **128**: 3008-3014 [PMID: 26608979 DOI: 10.4103/0366-6999.168964]
  - 29 **Hirschfield GM**, Xie G, Lu E, Sun Y, Juran BD, Chellappa V, Coltescu C, Mason AL, Milkiewicz P, Myers RP, Odin JA, Luketic VA, Bacon B, Bodenheimer H, Liakina V, Vincent C, Levy C, Pillai S, Lazaridis KN, Amos CI, Siminovitch KA. Association of primary biliary cirrhosis with variants in the CLEC16A, SOCS1, SPIB and SIAE immunomodulatory genes. *Genes Immun* 2012; **13**: 328-335 [PMID: 22257840 DOI: 10.1038/gene.2011.89]
  - 30 **Li P**, Lu G, Cui Y, Wu Z, Chen S, Li J, Wen X, Zhang H, Mu S, Zhang F, Li Y. Association of IL12A Expression Quantitative Trait Loci (eQTL) With Primary Biliary Cirrhosis in a Chinese Han Population. *Medicine (Baltimore)* 2016; **95**: e3665 [PMID: 27175695 DOI: 10.1097/MD.00000000000003665]
  - 31 **Li P**, Lu G, Wang L, Cui Y, Wu Z, Chen S, Li J, Wen X, Zhang H, Mu S, Zhang F, Li Y. A rare nonsynonymous variant in the lipid metabolic gene HELZ2 related to primary biliary cirrhosis in Chinese Han. *Allergy Asthma Clin Immunol* 2016; **12**: 14 [PMID: 27047549 DOI: 10.1186/s13223-016-0120-6]
  - 32 **Cavalli M**, Pan G, Nord H, Wallerman O, Wallén Arzt E, Berggren O, Elvers I, Eloranta ML, Rönnblom L, Lindblad Toh K, Wadelius C. Allele-specific transcription factor binding to common and rare variants associated with disease and gene expression. *Hum Genet* 2016; **135**: 485-497 [PMID: 26993500 DOI: 10.1007/s00439-016-1654-x]
  - 33 **Lleo A**, Zhang W, Zhao M, Tan Y, Bernuzzi F, Zhu B, Liu Q, Tan Q, Malinverno F, Valenti L, Jiang T, Tan L, Liao W, Coppel R, Invernizzi P, Lu Q, Adams DH, Gershwin ME. DNA methylation profiling of the X chromosome reveals an aberrant demethylation on CXCR3 promoter in primary biliary cirrhosis. *Clin Epigenetics* 2015; **7**: 61 [PMID: 26150899 DOI: 10.1186/s13148-015-0098-9]
  - 34 **Lammert C**, Nguyen DL, Juran BD, Schlicht E, Larson JJ, Atkinson EJ, Lazaridis KN. Questionnaire based assessment of risk factors for primary biliary cirrhosis. *Dig Liver Dis* 2013; **45**: 589-594 [PMID: 23490343 DOI: 10.1016/j.dld.2013.01.028]
  - 35 **Smyk D**, Rigopoulou EI, Bizzaro N, Bogdanos DP. Hair dyes as a risk for autoimmunity: from systemic lupus erythematosus to primary biliary cirrhosis. *Auto Immun Highlights* 2013; **4**: 1-9 [PMID: 26000137 DOI: 10.1007/s13317-011-0027-7]
  - 36 **Gershwin ME**, Selmi C, Worman HJ, Gold EB, Watnik M, Utts J, Lindor KD, Kaplan MM, Vierling JM. Risk factors and comorbidities in primary biliary cirrhosis: a controlled interview-based study of 1032 patients. *Hepatology* 2005; **42**: 1194-1202 [PMID: 16250040 DOI: 10.1002/hep.20907]
  - 37 **Quarneti C**, Muratori P, Lalanne C, Fabbri A, Menichella R, Granito A, Masi C, Lenzi M, Cassani F, Pappas G, Muratori L. Fatigue and pruritus at onset identify a more aggressive subset of primary biliary cirrhosis. *Liver Int* 2015; **35**: 636-641 [PMID: 24698666 DOI: 10.1111/liv.12560]
  - 38 **Silveira MG**, Gossard AA, Stahler AC, Jorgensen RA, Petz JL, Ali AH, Lindor KD. A Randomized, Placebo-Controlled Clinical Trial of Efficacy and Safety: Modafinil in the Treatment of Fatigue in Patients With Primary Biliary Cirrhosis. *Am J Ther* 2016; Epub ahead of print [PMID: 27148676 DOI: 10.1097/MJT.0000000000000387]
  - 39 **Guo GY**, Shi YQ, Wang L, Ren X, Han ZY, Guo CC, Cui LN, Wang JB, Zhu J, Wang N, Zhang J, Cai Y, Han Y, Zhou XM, Fan DM. Serum vitamin D level is associated with disease severity and response to ursodeoxycholic acid in primary biliary cirrhosis. *Aliment Pharmacol Ther* 2015; **42**: 221-230 [PMID: 25982180 DOI: 10.1111/apt.13244]
  - 40 **Agmon-Levin N**, Kopilov R, Selmi C, Nussinovitch U, Sánchez-Castañón M, López-Hoyos M, Amit H, Kivity S, Gershwin EM, Shoenfeld Y. Vitamin D in primary biliary cirrhosis, a plausible marker of advanced disease. *Immunol Res* 2015; **61**: 141-146 [PMID: 25424577 DOI: 10.1007/s12026-014-8594-0]
  - 41 **Yamaguchi S**, Maruyama T, Wakino S, Tokuyama H, Hashiguchi A, Tada S, Homma K, Monkawa T, Thomas J, Miyashita K, Kurihara I, Yoshida T, Konishi K, Hayashi K, Hayashi M, Itoh H. A case of severe osteomalacia caused by Tubulointerstitial nephritis with Fanconi syndrome in asymptomatic primary biliary cirrhosis. *BMC Nephrol* 2015; **16**: 187 [PMID: 26554665 DOI: 10.1186/s12882-015-0184-4]
  - 42 **Guañabens N**, Ruiz-Gaspà S, Gifre L, Miquel R, Peris P, Monegal A, Dubrueil M, Arias A, Parés A. Sclerostin Expression in Bile Ducts of Patients with Chronic Cholestasis May Influence the Bone Disease in Primary Biliary Cirrhosis. *J Bone Miner Res* 2016; **31**: 1725-1733 [PMID: 27019303 DOI: 10.1002/jbmr.2845]
  - 43 **Phillips JR**, Angulo P, Petterson T, Lindor KD. Fat-soluble vitamin levels in patients with primary biliary cirrhosis. *Am J Gastroenterol* 2001; **96**: 2745-2750 [PMID: 11569705 DOI: 10.1111/j.1572-0241.2001.04134.x]
  - 44 **Maruyama H**, Kondo T, Sekimoto T, Takahashi M, Fujiwara K, Imazeki F, Yokosuka O. Retrograde detection of the intrahepatic portal vein in primary biliary cirrhosis: is sinusoidal blockage the underlying pathophysiology? *Eur J Gastroenterol Hepatol* 2015; **27**: 321-327 [PMID: 25563140 DOI: 10.1097/



- MEG.0000000000000268]
- 45 **Iguchi H**, Oda M, Yamazaki H, Yoshimura K, Ando W, Yokomori H. Aquaporin-1 is associated with arterial capillary proliferation and hepatic sinusoidal transformation contributing to portal hypertension in primary biliary cirrhosis. *Med Mol Morphol* 2014; **47**: 90-99 [PMID: 23949237 DOI: 10.1007/s00795-013-0048-6]
  - 46 **Ali AH**, Sinakos E, Silveira MG, Jorgensen RA, Angulo P, Lindor KD. Varices in early histological stage primary biliary cirrhosis. *J Clin Gastroenterol* 2011; **45**: e66-e71 [PMID: 20856137 DOI: 10.1097/MCG.0b013e3181f18c4e]
  - 47 **Ikeda F**, Okamoto R, Baba N, Fujioka S, Shoji B, Yabushita K, Ando M, Matsumura S, Kubota J, Yasunaka T, Miyake Y, Iwasaki Y, Kobashi H, Okada H, Yamamoto K. Prevalence and associated factors with esophageal varices in early primary biliary cirrhosis. *J Gastroenterol Hepatol* 2012; **27**: 1320-1328 [PMID: 22414162 DOI: 10.1111/j.1440-1746.2012.07114.x]
  - 48 **Wu QM**, Zhao XY, You H. Quantitative fibrosis parameters highly predict esophageal-gastro varices in primary biliary cirrhosis. *Eur Rev Med Pharmacol Sci* 2016; **20**: 1037-1043 [PMID: 27049254]
  - 49 **Shen M**, Zhang F, Zhang X. Pulmonary hypertension in primary biliary cirrhosis: a prospective study in 178 patients. *Scand J Gastroenterol* 2009; **44**: 219-223 [PMID: 18821172 DOI: 10.1080/00365520802400883]
  - 50 **McDonnell PJ**, Toye PA, Hutchins GM. Primary pulmonary hypertension and cirrhosis: are they related? *Am Rev Respir Dis* 1983; **127**: 437-441 [PMID: 6838050]
  - 51 **Bektas M**, Seven G, Idilman R, Yakut M, Doğanay B, Kabacam G, Ustun Y, Korkut E, Kalkan Ç, Sahin G, Cetinkaya H, Bozkaya H, Yurdaydin C, Bahar K, Cinar K, Soykan I. Manometric assessment of esophageal motor function in patients with primary biliary cirrhosis. *Eur J Intern Med* 2014; **25**: 230-234 [PMID: 24534163 DOI: 10.1016/j.ejim.2014.01.008]
  - 52 **Shen M**, Zhang F, Zhang X. Primary biliary cirrhosis complicated with interstitial lung disease: a prospective study in 178 patients. *J Clin Gastroenterol* 2009; **43**: 676-679 [PMID: 19247207 DOI: 10.1097/MCG.0b013e31818aa11e]
  - 53 **Chen CT**, Tseng YC, Yang CW, Lin HH, Chen PJ, Huang TY, Shih YL, Chang WK, Hsieh TY, Chu HC. Increased Risks of Spontaneous Bacterial Peritonitis and Interstitial Lung Disease in Primary Biliary Cirrhosis Patients With Concomitant Sjögren Syndrome. *Medicine* (Baltimore) 2016; **95**: e2537 [PMID: 26765478 DOI: 10.1097/MD.0000000000002537]
  - 54 **Franco I**, Dubini A, Picciocchi S, Casoni G, Poletti V. Interstitial lung disease preceding primary biliary cirrhosis in a male patient. *Rev Port Pneumol* (2006) 2015; **21**: 214-217 [PMID: 25998779 DOI: 10.1016/j.rppnen.2015.02.008]
  - 55 **Parés A**, Rimola A, Bruguera M, Mas E, Rodés J. Renal tubular acidosis in primary biliary cirrhosis. *Gastroenterology* 1981; **80**: 681-686 [PMID: 7202940]
  - 56 **Iannone F**, Falappone P, Pannarale G, Gentile A, Grattagliano V, Covelli M, Lapadula G. Microscopic polyangiitis associated with primary biliary cirrhosis. *J Rheumatol* 2003; **30**: 2710-2712 [PMID: 14719218]
  - 57 **Sakamaki Y**, Hayashi M, Wakino S, Fukuda S, Konishi K, Hashiguchi A, Hayashi K, Itoh H. A case of membranous nephropathy with primary biliary cirrhosis and cyclosporine-induced remission. *Intern Med* 2011; **50**: 233-238 [PMID: 21297326 DOI: 10.2169/internalmedicine.50.4020]
  - 58 **Goto T**, Komatsu M, Fujii T, Ohshima S, Nakane K, Yoneyama K, Shibuya T, Meng XW, Masamune O, Imai H. Primary biliary cirrhosis associated with membranous glomerulonephritis. *Intern Med* 1999; **38**: 22-26 [PMID: 10052737 DOI: 10.2169/internalmedicine.38.22]
  - 59 **Iwakura T**, Fujigaki Y, Matsuyama T, Fujikura T, Ohashi N, Yasuda H, Kato A, Baba S. Tubulointerstitial nephritis and primary biliary cirrhosis with a T cell-dominant profile of infiltrating cells and granulomas in both organs. *Intern Med* 2013; **52**: 467-471 [PMID: 23411703 DOI: 10.2169/internalmedicine.52.9003]
  - 60 **Kornblihtt LI**, Vassallu PS, Heller PG, Lago NR, Alvarez CL, Molinas FC. Primary myelofibrosis in a patient who developed primary biliary cirrhosis, autoimmune hemolytic anemia and fibrillary glomerulonephritis. *Ann Hematol* 2008; **87**: 1019-1020 [PMID: 18575863 DOI: 10.1007/s00277-008-0516-6]
  - 61 **Macdougall IC**, Isles CG, Whitworth JA, More IA, MacSween RN. Interstitial nephritis and primary biliary cirrhosis: a new association? *Clin Nephrol* 1987; **27**: 36-40 [PMID: 3815907]
  - 62 **Komatsu T**, Utsunomiya K, Oyaizu T. Goodpasture's syndrome associated with primary biliary cirrhosis. *Intern Med* 1998; **37**: 611-613 [PMID: 9711889 DOI: 10.2169/internalmedicine.37.611]
  - 63 **Nakamura T**, Kawagoe Y, Ueda Y, Koide H. Antineutrophil cytoplasmic autoantibody-associated rapidly progressive glomerulonephritis in a patient with primary biliary cirrhosis. *Am J Med Sci* 2004; **328**: 176-179 [PMID: 15367878 DOI: 10.1097/00000441-200409000-00009]
  - 64 **Azak A**, Koçak G, Huddam B, Koçak E, Ergül B, Duranay M. Focal segmental glomerulosclerosis associated with primary biliary cirrhosis. *Ren Fail* 2011; **33**: 1052-1053 [PMID: 22013944 DOI: 10.3109/0886022X.2011.618971]
  - 65 **Wang L**, Zhang FC, Chen H, Zhang X, Xu D, Li YZ, Wang Q, Gao LX, Yang YJ, Kong F, Wang K. Connective tissue diseases in primary biliary cirrhosis: a population-based cohort study. *World J Gastroenterol* 2013; **19**: 5131-5137 [PMID: 23964148 DOI: 10.3748/wjg.v19.i31.5131]
  - 66 **Zhang XX**, Wang LF, Jin L, Li YY, Hao SL, Shi YC, Zeng QL, Li ZW, Zhang Z, Lau GK, Wang FS. Primary biliary cirrhosis-associated hepatocellular carcinoma in Chinese patients: incidence and risk factors. *World J Gastroenterol* 2015; **21**: 3554-3563 [PMID: 25834320 DOI: 10.3748/wjg.v21.i12.3554]
  - 67 **Rong G**, Wang H, Bowlus CL, Wang C, Lu Y, Zeng Z, Qu J, Lou M, Chen Y, An L, Yang Y, Gershwin ME. Incidence and risk factors for hepatocellular carcinoma in primary biliary cirrhosis. *Clin Rev Allergy Immunol* 2015; **48**: 132-141 [PMID: 25762349 DOI: 10.1007/s12016-015-8483-x]
  - 68 **Tomiyama Y**, Takenaka K, Kodama T, Kawanaka M, Sasaki K, Nishina S, Yoshioka N, Hara Y, Hino K. Risk factors for survival and the development of hepatocellular carcinoma in patients with primary biliary cirrhosis. *Intern Med* 2013; **52**: 1553-1559 [PMID: 23857086]
  - 69 **Trivedi PJ**, Lammers WJ, van Buuren HR, Parés A, Floreani A, Janssen HL, Invernizzi P, Battezzati PM, Ponsioen CY, Corpechot C, Poupon R, Mayo MJ, Burroughs AK, Nevens F, Mason AL, Kowdley KV, Lleo A, Caballeria L, Lindor KD, Hansen BE, Hirschfield GM. Stratification of hepatocellular carcinoma risk in primary biliary cirrhosis: a multicentre international study. *Gut* 2016; **65**: 321-329 [PMID: 25567117 DOI: 10.1136/gutjnl-2014-308351]
  - 70 **Mochizuki S**, Nakayama H, Higaki T, Okubo T, Midorikawa Y, Moriguchi M, Aramaki O, Yamazaki S, Sugitani M, Takayama T. Repeat liver resection for hepatocellular carcinoma complicating primary biliary cirrhosis. *Int Surg* 2013; **98**: 424-427 [PMID: 24229035 DOI: 10.9738/INTSURG-D-13-00082.1]
  - 71 **Rigopoulou EI**, Zachou K, Gatselis NK, Papadamou G, Koukoulis GK, Dalekos GN. Primary biliary cirrhosis in HBV and HCV patients: Clinical characteristics and outcome. *World J Hepatol* 2013; **5**: 577-583 [PMID: 24179617 DOI: 10.4254/wjh.v5.i10.577]
  - 72 **Chen HW**, Huang HH, Lai CH, Chang WE, Shih YL, Chang WK, Hsieh TY, Chu HC. Hepatitis C virus infection in patients with primary biliary cirrhosis. *Ann Hepatol* 2013; **12**: 78-84 [PMID: 23293197]
  - 73 **Javadi A**, Poongkunran M, Allard FD, Kyaw W, Maung HH, Lau D. Subtle presentation of active primary biliary cirrhosis in chronic hepatitis B: a case report. *Gastroenterol Rep (Oxf)* 2016; Epub ahead of print [PMID: 26893441 DOI: 10.1093/gastro/gov064]
  - 74 **Munday WR**, DiCapua D, Vortmeyer A, Gomez JL, Guillain-Barré syndrome mimics primary biliary cirrhosis-related myopathy. *Oxf Med Case Reports* 2015; **2015**: 272-275 [PMID: 26634144 DOI: 10.1093/omcr/omv033]
  - 75 **Gonzalez-Moreno EI**, Martinez-Cabriaes SA, Cruz-Moreno MA, Borjas-Almaguer OD, Cortez-Hernandez CA, Bosques-Padilla FJ, Garza AA, Gonzalez-Gonzalez JA, Garcia-Compean D, Ocampo-

- Candiani J, Maldonado-Garza HJ. Primary biliary cholangitis associated with warm autoimmune hemolytic anemia. *J Dig Dis* 2016; **17**: 128-131 [PMID: 26630456 DOI: 10.1111/1751-2980.12303]
- 76 **Nakayama S**, Yokote T, Kobayashi K, Hirata Y, Akioka T, Miyoshi T, Oka S, Hiraoka N, Iwaki K, Takayama A, Fukui H, Tsuda Y, Takubo T, Tsuji M, Higuchi K, Hanafusa T. Primary hepatic MALT lymphoma associated with primary biliary cirrhosis. *Leuk Res* 2010; **34**: e17-e20 [PMID: 19679352 DOI: 10.1016/j.leukres.2009.07.031]
- 77 **Yamashita H**, Suzuki A, Takahashi Y, Kaneko H, Kano T, Mimori A. Anti-neutrophil Cytoplasmic Antibody (ANCA)-associated Vasculitis Associated with Primary Biliary Cirrhosis: A Case Report and Literature Review. *Intern Med* 2015; **54**: 1303-1308 [PMID: 25986275 DOI: 10.2169/internalmedicine.54.3678]
- 78 **Calvo J**, Carbonell N, Scatton O, Marzac C, Ganne-Carrie N, Wendum D. Hepatic nodular lymphoid lesion with increased IgG4-positive plasma cells associated with primary biliary cirrhosis: a report of two cases. *Virchows Arch* 2015; **467**: 613-617 [PMID: 26358058 DOI: 10.1007/s00428-015-1841-5]
- 79 **Macaluso FS**, Maida M, Alessi N, Cabibbo G, Cabibi D. Primary biliary cirrhosis and hereditary hemorrhagic telangiectasia: When two rare diseases coexist. *World J Hepatol* 2013; **5**: 288-291 [PMID: 23717740 DOI: 10.4254/wjh.v5.i5.288]
- 80 **Iga N**, Otsuka A, Iwata M, Ueda Y, Kabashima K. Generalized morphea with preceding severe pain and coexistent early primary biliary cirrhosis. *Eur J Dermatol* 2015; **25**: 365-366 [PMID: 26055733 DOI: 10.1684/ejd.2015.2588]
- 81 **Taddy H**, Yoshida EM, Gibson G, Chatur N. Acetylcholine receptor antibody positive generalized myasthenia gravis in association with primary biliary cirrhosis. *Ann Hepatol* 2010; **9**: 471-472 [PMID: 21057170]
- 82 **Koide H**, Sato K, Fukusato T, Kashiwabara K, Sunaga N, Tsuchiya T, Morino S, Sohara N, Kakizaki S, Takagi H, Mori M. Spontaneous regression of hepatic inflammatory pseudotumor with primary biliary cirrhosis: case report and literature review. *World J Gastroenterol* 2006; **12**: 1645-1648 [PMID: 16570364 DOI: 10.3748/wjg.v12.i10.1645]
- 83 **Tang KH**, Schofield JB, Powell-Jackson PR. Primary biliary cirrhosis and idiopathic retroperitoneal fibrosis: a rare association. *Eur J Gastroenterol Hepatol* 2002; **14**: 783-786 [PMID: 12169990 DOI: 10.1097/00042737-200207000-00013]
- 84 **Volta U**, Caio G, Tovoli F, De Giorgio R. Gut-liver axis: an immune link between celiac disease and primary biliary cirrhosis. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 253-261 [PMID: 23445234 DOI: 10.1586/egh.13.5]
- 85 **Zhao SX**, Zhang YG, Wang RQ, Li WC, Kong LB, Kong L, Nan YM. A Patient With Primary Biliary Cirrhosis Accompanied by Wilson's Disease. *Hepat Mon* 2016; **16**: e29077 [PMID: 27148382 DOI: 10.5812/hepatmon.29077]
- 86 **Guerra-Urbe NB**, González-Huezo MS. Bullous pemphigoid and primary biliary cirrhosis, an infrequent association: A case report. *Rev Gastroenterol Mex* 2016; **81**: 174-176 [PMID: 26949192 DOI: 10.1016/j.rgm.2015.08.004]
- 87 **Paul S**, Sepehr GJ, Weinstein B, Roper J. Co-occurrence of idiopathic granulomatous hepatitis and primary biliary cirrhosis. *Dig Dis Sci* 2014; **59**: 2831-2835 [PMID: 25108519 DOI: 10.1007/s10620-014-3216-1]
- 88 **Riviere E**, Vergnol J, Reffet A, Lipa N, Le Bail B, de Ledinghen V. Gastric variceal bleeding uncovering a rare association of CREST syndrome, primary biliary cirrhosis, nodular regenerative hyperplasia and pulmonary hypertension. *Eur J Gastroenterol Hepatol* 2010; **22**: 1145-1148 [PMID: 20485183 DOI: 10.1097/MEG.0b013e32833ab83a]
- 89 **Triantafyllidis JK**, Durakis S, Merikas E. Crohn's disease of the small bowel, complicated by primary biliary cirrhosis, Hashimoto thyroiditis, and Raynaud's phenomenon: favorable response of all disorders to adalimumab treatment. *Gastroenterol Hepatol Bed Bench* 2013; **6**: 101-105 [PMID: 24834253]
- 90 **Alempijević T**, Sokić-Milutinović A, Tončev L, Pavlović-Marković A, Djuranović S, Tomanović N, Drulović J. Primary biliary cirrhosis and hepatic sarcoidosis--a case report. *Vojnosanit Pregl* 2014; **71**: 83-86 [PMID: 24516996]
- 91 **Korkmaz H**, Bugdaci MS, Temel T, Dagli M, Karabagli P. Autoimmune hepatitis-primary biliary cirrhosis overlap syndrome concomitant with immune hemolytic anemia and immune thrombocytopenic purpura (Evans syndrome). *Clin Res Hepatol Gastroenterol* 2013; **37**: e45-e50 [PMID: 23273499 DOI: 10.1016/j.clinre.2012.11.001]
- 92 **Yaşar DG**, Ozenirler S, Doğan M. A patient with primary biliary cirrhosis accompanied by Graves disease and Hürthle cell adenoma. *Turk J Gastroenterol* 2007; **18**: 198-200 [PMID: 17891696]
- 93 **Hu S**, Zhao F, Wang Q, Chen WX. The accuracy of the anti-mitochondrial antibody and the M2 subtype test for diagnosis of primary biliary cirrhosis: a meta-analysis. *Clin Chem Lab Med* 2014; **52**: 1533-1542 [PMID: 24501161 DOI: 10.1515/cclm-2013-0926]
- 94 **Juliusson G**, Imam M, Björnsson ES, Talwalkar JA, Lindor KD. Long-term outcomes in antimitochondrial antibody negative primary biliary cirrhosis. *Scand J Gastroenterol* 2016; **51**: 745-752 [PMID: 26776319 DOI: 10.3109/00365521.2015.1132337]
- 95 **Cancado EL**, Harritz M. The Importance of Autoantibody Detection in Primary Biliary Cirrhosis. *Front Immunol* 2015; **6**: 309 [PMID: 26157439 DOI: 10.3389/fimmu.2015.00309]
- 96 **Yamagiwa S**, Kamimura H, Takamura M, Aoyagi Y. Auto-antibodies in primary biliary cirrhosis: recent progress in research on the pathogenetic and clinical significance. *World J Gastroenterol* 2014; **20**: 2606-2612 [PMID: 24627596 DOI: 10.3748/wjg.v20.i10.2606]
- 97 **Bauer A**, Habior A, Kraszewska E. Detection of anti-SP100 antibodies in primary biliary cirrhosis. Comparison of ELISA and immunofluorescence. *J Immunoassay Immunochem* 2013; **34**: 346-355 [PMID: 23859785 DOI: 10.1080/15321819.2012.741088]
- 98 **Villalta D**, Sorrentino MC, Girolami E, Tampoaia M, Alessio MG, Brusca I, Daves M, Porcelli B, Barberio G, Bizzaro N. Auto-antibody profiling of patients with primary biliary cirrhosis using a multiplexed line-blot assay. *Clin Chim Acta* 2015; **438**: 135-138 [PMID: 25172039 DOI: 10.1016/j.cca.2014.08.024]
- 99 **Valour F**, Durupt S, Khenifer S, Durieu I. Diagnostic value of anti-gp210 antibodies in primary biliary cirrhosis: a case-based review. *BMJ Case Rep* 2013; **2013**: pii: bcr2013009803 [PMID: 23814122 DOI: 10.1136/bcr-2013-009803]
- 100 **Himoto T**, Tanaka N, Saito A, Muro Y, Sugiura K, Tani J, Miyoshi H, Morishita A, Yoneyama H, Haba R, Masaki T. Diversity of humoral responses to the centromere proteins among HCV-related chronic liver disease, PBC and AIH patients. *Clin Res Hepatol Gastroenterol* 2015; **39**: 222-229 [PMID: 25220385 DOI: 10.1016/j.clinre.2014.08.004]
- 101 **Nakamura M**. Clinical significance of autoantibodies in primary biliary cirrhosis. *Semin Liver Dis* 2014; **34**: 334-340 [PMID: 25057956 DOI: 10.1055/s-0034-1383732]
- 102 **Tana MM**, Shums Z, Milo J, Norman GL, Leung PS, Gershwin ME, Nouredin M, Kleiner DE, Zhao X, Heller T, Hoofnagle JH. The Significance of Autoantibody Changes Over Time in Primary Biliary Cirrhosis. *Am J Clin Pathol* 2015; **144**: 601-606 [PMID: 26386081 DOI: 10.1309/AJCPQV4A7QAEEFEV]
- 103 **Saare M**, Hämarik U, Venta R, Panarina M, Zucchelli C, Pihlap M, Remm A, Kisand K, Toots U, Möll K, Salupere R, Musco G, Uibo R, Peterson P. SP140L, an Evolutionarily Recent Member of the SP100 Family, Is an Autoantigen in Primary Biliary Cirrhosis. *J Immunol Res* 2015; **2015**: 526518 [PMID: 26347895 DOI: 10.1155/2015/526518]
- 104 **Sasaki M**, Miyakoshi M, Sato Y, Nakanuma Y. A possible involvement of p62/sequestosome-1 in the process of biliary epithelial autophagy and senescence in primary biliary cirrhosis. *Liver Int* 2012; **32**: 487-499 [PMID: 22098537 DOI: 10.1111/j.1478-3231.2011.02656.x]
- 105 **Nakamura M**, Kondo H, Tanaka A, Komori A, Ito M, Yamamoto K, Ohira H, Zeniya M, Hashimoto E, Honda M, Kaneko S, Ueno Y, Kikuchi K, Shimoda S, Harada K, Arai K, Miyake Y, Abe M, Taniai M, Saibara T, Sakisaka S, Takikawa H, Onji M, Tsubouchi H, Nakanuma Y, Ishibashi H. Autoantibody status and histological

- variables influence biochemical response to treatment and long-term outcomes in Japanese patients with primary biliary cirrhosis. *Hepatol Res* 2015; **45**: 846-855 [PMID: 25220608 DOI: 10.1111/hepr.12423]
- 106 **Liberal R**, Grant CR, Sakkas L, Bizzaro N, Bogdanos DP. Diagnostic and clinical significance of anti-centromere antibodies in primary biliary cirrhosis. *Clin Res Hepatol Gastroenterol* 2013; **37**: 572-585 [PMID: 23876351 DOI: 10.1016/j.clinre.2013.04.005]
  - 107 **Gatselis NK**, Zachou K, Norman GL, Gabeta S, Papamichalis P, Koukoulis GK, Dalekos GN. Clinical significance of the fluctuation of primary biliary cirrhosis-related autoantibodies during the course of the disease. *Autoimmunity* 2013; **46**: 471-479 [PMID: 23777462 DOI: 10.3109/08916934.2013.801461]
  - 108 **Mandai S**, Kanda E, Arai Y, Hirasawa S, Hirai T, Aki S, Inaba N, Aoyagi M, Tanaka H, Ikeda T, Tamura T, Sasaki S. Anti-centromere antibody is an independent risk factor for chronic kidney disease in patients with primary biliary cirrhosis. *Clin Exp Nephrol* 2013; **17**: 405-410 [PMID: 23268283 DOI: 10.1007/s10157-012-0724-1]
  - 109 **Hu CJ**, Song G, Huang W, Liu GZ, Deng CW, Zeng HP, Wang L, Zhang FC, Zhang X, Jeong JS, Blackshaw S, Jiang LZ, Zhu H, Wu L, Li YZ. Identification of new autoantigens for primary biliary cirrhosis using human proteome microarrays. *Mol Cell Proteomics* 2012; **11**: 669-680 [PMID: 22647870 DOI: 10.1074/mcp.M111.015529]
  - 110 **Norman GL**, Yang CY, Ostendorff HP, Shums Z, Lim MJ, Wang J, Awad A, Hirschfield GM, Milkiewicz P, Bloch DB, Rothschild KJ, Bowlus CL, Adamopoulos IE, Leung PS, Janssen HJ, Cheung AC, Coltescu C, Gershwin ME. Anti-kelch-like 12 and anti-hexokinase 1: novel autoantibodies in primary biliary cirrhosis. *Liver Int* 2015; **35**: 642-651 [PMID: 25243383 DOI: 10.1111/liv.12690]
  - 111 **Lammers WJ**, van Buuren HR, Hirschfield GM, Janssen HL, Invernizzi P, Mason AL, Ponsioen CY, Floreani A, Corpechot C, Mayo MJ, Battezzati PM, Parés A, Nevens F, Burroughs AK, Kowdley KV, Trivedi PJ, Kumagi T, Cheung A, Lleo A, Imam MH, Boonstra K, Cazzagon N, Franceschet I, Poupon R, Caballeria L, Pieri G, Kanwar PS, Lindor KD, Hansen BE. Levels of alkaline phosphatase and bilirubin are surrogate end points of outcomes of patients with primary biliary cirrhosis: an international follow-up study. *Gastroenterology* 2014; **147**: 1338-1349.e5; quiz e15 [PMID: 25160979 DOI: 10.1053/j.gastro.2014.08.029]
  - 112 **Giljaca V**, Stimac D, Gluud C. Are levels of alkaline phosphatases and bilirubin surrogate markers of outcomes of patients with primary biliary cirrhosis? *Gastroenterology* 2015; **148**: 860 [PMID: 25726742 DOI: 10.1053/j.gastro.2014.11.050]
  - 113 **Tang YM**, Wang JP, Bao WM, Yang JH, Ma LK, Yang J, Chen H, Xu Y, Yang LH, Li W, Zhu YP, Cheng JB. Urine and serum metabolomic profiling reveals that bile acids and carnitine may be potential biomarkers of primary biliary cirrhosis. *Int J Mol Med* 2015; **36**: 377-385 [PMID: 26046127 DOI: 10.3892/ijmm.2015.2233]
  - 114 **Lleo A**, Liao J, Invernizzi P, Zhao M, Bernuzzi F, Ma L, Lanzi G, Ansari AA, Coppel RL, Zhang P, Li Y, Zhou Z, Lu Q, Gershwin ME. Immunoglobulin M levels inversely correlate with CD40 ligand promoter methylation in patients with primary biliary cirrhosis. *Hepatology* 2012; **55**: 153-160 [PMID: 21898485 DOI: 10.1002/hep.24630]
  - 115 **Zhang H**, Li P, Wu D, Xu D, Hou Y, Wang Q, Li M, Li Y, Zeng X, Zhang F, Shi Q. Serum IgG subclasses in autoimmune diseases. *Medicine (Baltimore)* 2015; **94**: e387 [PMID: 25590841 DOI: 10.1097/MD.0000000000000387]
  - 116 **Kawaguchi T**, Tanaka T, Hashiguchi M, Miyoshi H, Akiba J, Kage M, Yano H, Ohshima K, Okamura T, Sata M. Decreased serum levels of immunoglobulin A, immunoglobulin M and immunoglobulin G in a patient with primary biliary cirrhosis: A case report. *Hepatol Res* 2014; **44**: E261-E266 [PMID: 23890027 DOI: 10.1111/hepr.12211]
  - 117 **Shapira Y**, Agmon-Levin N, Renaudineau Y, Porat-Katz BS, Barzilai O, Ram M, Youinou P, Shoenfeld Y. Serum markers of infections in patients with primary biliary cirrhosis: evidence of infection burden. *Exp Mol Pathol* 2012; **93**: 386-390 [PMID: 23022373 DOI: 10.1016/j.yexmp.2012.09.012]
  - 118 **Tan Y**, Pan T, Ye Y, Ge G, Chen L, Wen D, Zou S. Serum micro-RNAs as potential biomarkers of primary biliary cirrhosis. *PLoS One* 2014; **9**: e111424 [PMID: 25347847 DOI: 10.1371/journal.pone.0111424]
  - 119 **Qin B**, Huang F, Liang Y, Yang Z, Zhong R. Analysis of altered microRNA expression profiles in peripheral blood mononuclear cells from patients with primary biliary cirrhosis. *J Gastroenterol Hepatol* 2013; **28**: 543-550 [PMID: 23173724 DOI: 10.1111/jgh.12040]
  - 120 **Ninomiya M**, Kondo Y, Funayama R, Nagashima T, Kogure T, Kakazu E, Kimura O, Ueno Y, Nakayama K, Shimosegawa T. Distinct microRNAs expression profile in primary biliary cirrhosis and evaluation of miR-505-3p and miR197-3p as novel biomarkers. *PLoS One* 2013; **8**: e66086 [PMID: 23776611 DOI: 10.1371/journal.pone.0066086]
  - 121 **Umamura T**, Joshita S, Sekiguchi T, Usami Y, Shibata S, Kimura T, Komatsu M, Matsumoto A, Ota M, Tanaka E. Serum Wisteria floribunda Agglutinin-Positive Mac-2-Binding Protein Level Predicts Liver Fibrosis and Prognosis in Primary Biliary Cirrhosis. *Am J Gastroenterol* 2015; **110**: 857-864 [PMID: 25916223 DOI: 10.1038/ajg.2015.118]
  - 122 **Wunsch E**, Milkiewicz M, Wasik U, Trottier J, Kempńska-Podhorodecka A, Elias E, Barbier O, Milkiewicz P. Expression of hepatic Fibroblast Growth Factor 19 is enhanced in Primary Biliary Cirrhosis and correlates with severity of the disease. *Sci Rep* 2015; **5**: 13462 [PMID: 26293907 DOI: 10.1038/srep13462]
  - 123 **Zhao P**, Liu WW, Li JF, Wang CY, Wang H, Xu J, Wang RF, Yang HZ, Jin C, Wei ZM. Predictors of liver failure in primary biliary cirrhosis. *Ups J Med Sci* 2015; **120**: 47-51 [PMID: 25430562 DOI: 10.3109/03009734.2014.985763]
  - 124 **Harada K**, Kakuda Y, Nakamura M, Shimoda S, Nakanuma Y. Clinicopathological significance of serum fractalkine in primary biliary cirrhosis. *Dig Dis Sci* 2013; **58**: 3037-3043 [PMID: 23765258 DOI: 10.1007/s10620-013-2734-6]
  - 125 **Deng C**, Hu C, Wang L, Zhang S, Li P, Wu Z, Chen S, Zhang F, Li Y. Serological comparative proteomics analysis of mitochondrial autoantibody-negative and -positive primary biliary cirrhosis. *Electrophoresis* 2015; **36**: 1588-1595 [PMID: 25875855 DOI: 10.1002/elps.201400342]
  - 126 **Voumvouraki A**, Koulentaki M, Notas G, Sfakianaki O, Kouroumalis E. Serum surrogate markers of liver fibrosis in primary biliary cirrhosis. *Eur J Intern Med* 2011; **22**: 77-83 [PMID: 21238899 DOI: 10.1016/j.ejim.2010.10.002]
  - 127 **Norman GL**, Gatselis NK, Shums Z, Liaskos C, Bogdanos DP, Koukoulis GK, Dalekos GN. Cartilage oligomeric matrix protein: A novel non-invasive marker for assessing cirrhosis and risk of hepatocellular carcinoma. *World J Hepatol* 2015; **7**: 1875-1883 [PMID: 26207169 DOI: 10.4254/wjh.v7.i14.1875]
  - 128 **Weinmann A**, Sattler T, Unold HP, Grambihler A, Teufel A, Koch S, Schuchmann M, Biesterfeld S, Wörns MA, Galle PR, Schulze-Bergkamen H. Predictive scores in primary biliary cirrhosis: a retrospective single center analysis of 204 patients. *J Clin Gastroenterol* 2015; **49**: 438-447 [PMID: 25014239 DOI: 10.1097/MCG.0000000000000176]
  - 129 **Lammers WJ**, Hirschfield GM, Corpechot C, Nevens F, Lindor KD, Janssen HL, Floreani A, Ponsioen CY, Mayo MJ, Invernizzi P, Battezzati PM, Parés A, Burroughs AK, Mason AL, Kowdley KV, Kumagi T, Harms MH, Trivedi PJ, Poupon R, Cheung A, Lleo A, Caballeria L, Hansen BE, van Buuren HR. Development and Validation of a Scoring System to Predict Outcomes of Patients With Primary Biliary Cirrhosis Receiving Ursodeoxycholic Acid Therapy. *Gastroenterology* 2015; **149**: 1804-1812.e4 [PMID: 26261009 DOI: 10.1053/j.gastro.2015.07.061]
  - 130 **Zhang HC**, Hu RF, Zhu T, Tong L, Zhang QQ. Primary biliary cirrhosis degree assessment by acoustic radiation force impulse imaging and hepatic fibrosis indicators. *World J Gastroenterol* 2016; **22**: 5276-5284 [PMID: 27298571 DOI: 10.3748/wjg.v22.i22.5276]
  - 131 **Zhang DK**, Chen M, Liu Y, Wang RF, Liu LP, Li M. Acoustic



- radiation force impulse elastography for non-invasive assessment of disease stage in patients with primary biliary cirrhosis: A preliminary study. *Clin Radiol* 2014; **69**: 836-840 [PMID: 24837697 DOI: 10.1016/j.crad.2014.03.019]
- 132 **Tapper EB**, Challies T, Nasser I, Afdhal NH, Lai M. The Performance of Vibration Controlled Transient Elastography in a US Cohort of Patients With Nonalcoholic Fatty Liver Disease. *Am J Gastroenterol* 2016; **111**: 677-684 [PMID: 26977758 DOI: 10.1038/ajg.2016.49]
  - 133 **Loomba R**, Cui J, Wolfson T, Haufe W, Hooker J, Szevenenyi N, Ang B, Bhatt A, Wang K, Aryafar H, Behling C, Valasek MA, Lin GY, Gamst A, Brenner DA, Yin M, Glaser KJ, Ehman RL, Sirlin CB. Novel 3D Magnetic Resonance Elastography for the Noninvasive Diagnosis of Advanced Fibrosis in NAFLD: A Prospective Study. *Am J Gastroenterol* 2016; **111**: 986-994 [PMID: 27002798 DOI: 10.1038/ajg.2016.65]
  - 134 **Singh S**, Venkatesh SK, Keaveny A, Adam S, Miller FH, Asbach P, Asbach P, Godfrey EM, Silva AC, Wang Z, Murad MH, Asrani SK, Lomas DJ, Ehman RL. Diagnostic accuracy of magnetic resonance elastography in liver transplant recipients: A pooled analysis. *Ann Hepatol* 2016; **15**: 363-376 [PMID: 27049490 DOI: 10.5604/1665-2681.1198808]
  - 135 **Meng Y**, Liang Y, Liu M. The value of MRI in the diagnosis of primary biliary cirrhosis and assessment of liver fibrosis. *PLoS One* 2015; **10**: e0120110 [PMID: 25781184 DOI: 10.1371/journal.pone.0120110]
  - 136 **Takeyama Y**, Tsuchiya N, Kunimoto H, Fukunaga A, Sakurai K, Hirano G, Yokoyama K, Morihara D, Anan A, Irie M, Shakado S, Sohda T, Sakisaka S. Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging as a useful detection method for advanced primary biliary cirrhosis. *Hepatol Res* 2015; **45**: E108-E114 [PMID: 25560223 DOI: 10.1111/hepr.12470]
  - 137 **You Z**, Wang Q, Bian Z, Liu Y, Han X, Peng Y, Shen L, Chen X, Qiu D, Selmi C, Gershwin ME, Ma X. The immunopathology of liver granulomas in primary biliary cirrhosis. *J Autoimmun* 2012; **39**: 216-221 [PMID: 22727562 DOI: 10.1016/j.jaut.2012.05.022]
  - 138 **Kikuchi K**, Tsuneyama K, Yamada H, Kajiyama Y, Matsumoto K, Tsunashima H, Yamashita R, Takai A, Negishi M, Hara M, Moritoki Y, Miyakawa H. Splenic lymph follicles generate immunoglobulin M-producing B cells in primary biliary cirrhosis. *Hepatol Res* 2014; **44**: E253-E256 [PMID: 24033874 DOI: 10.1111/hepr.12231]
  - 139 **Sasaki M**, Yoshimura-Miyakoshi M, Sato Y, Nakanuma Y. A possible involvement of endoplasmic reticulum stress in biliary epithelial autophagy and senescence in primary biliary cirrhosis. *J Gastroenterol* 2015; **50**: 984-995 [PMID: 25552342 DOI: 10.1007/s00535-014-1033-0]
  - 140 **Aso-Ishimoto Y**, Yamagiwa S, Ichida T, Miyakawa R, Tomiyama C, Sato Y, Watanabe H, Aoyagi Y. Increased activated natural killer T cells in the liver of patients with advanced stage primary biliary cirrhosis. *Biomed Res* 2014; **35**: 161-169 [PMID: 24759184]
  - 141 **Harada K**, Hsu M, Ikeda H, Zeniya M, Nakanuma Y. Application and validation of a new histologic staging and grading system for primary biliary cirrhosis. *J Clin Gastroenterol* 2013; **47**: 174-181 [PMID: 23269312 DOI: 10.1097/MCG.0b013e31827234e4]
  - 142 **Wendum D**, Boëlle PY, Bedossa P, Zafrani ES, Charlotte F, Saint-Paul MC, Michalak S, Chazouillères O, Corpechot C. Primary biliary cirrhosis: proposal for a new simple histological scoring system. *Liver Int* 2015; **35**: 652-659 [PMID: 24939754 DOI: 10.1111/liv.12620]
  - 143 **Seki H**, Ikeda F, Nanba S, Moritou Y, Takeuchi Y, Yasunaka T, Onishi H, Miyake Y, Takaki A, Nouse K, Iwasaki Y, Nakamura M, Yamamoto K. Aberrant Expression of Keratin 7 in Hepatocytes as a Predictive Marker of Rapid Progression to Hepatic Failure in Asymptomatic Primary Biliary Cirrhosis. *Acta Med Okayama* 2015; **69**: 137-144 [PMID: 26101189]
  - 144 **Bowlus CL**, Gershwin ME. The diagnosis of primary biliary cirrhosis. *Autoimmun Rev* 2014; **13**: 441-444 [PMID: 24424173]
  - 145 **Liberal R**, Vergani D, Mieli-Vergani G. Update on Autoimmune Hepatitis. *J Clin Transl Hepatol* 2015; **3**: 42-52 [PMID: 26357634 DOI: 10.14218/JCTH.2014.00032]
  - 146 **Tomizawa M**, Shinozaki F, Fugo K, Motoyoshi Y, Sugiyama T, Yamamoto S, Kishimoto T, Ishige N. Anti-mitochondrial M2 antibody-positive autoimmune hepatitis. *Exp Ther Med* 2015; **10**: 1419-1422 [PMID: 26622500]
  - 147 **Himoto T**, Fujita K, Nomura T, Tani J, Miyoshi H, Morishita A, Yoneyama H, Haba R, Masaki T. Diagnostic Dilemma in the Detection of Antibodies to Filamentous Actin. *Clin Lab* 2016; **62**: 839-847 [PMID: 27349009]
  - 148 **Putra J**, Toor A, Suriawinata AA. The utility of repeat liver biopsy in autoimmune hepatitis: a series of 20 consecutive cases. *Pathology* 2016; **48**: 449-453 [PMID: 27306577 DOI: 10.1016/j.pathol.2016.05.001]
  - 149 **Schulze K**, Weismüller TJ, Bubenheim M, Huebener P, Zenouzi R, Lenzen H, Rupp C, Gotthardt D, de Leuw P, Teufel A, Zimmer V, Reiter FP, Rust C, Tharun L, Quas A, Weidemann SA, Lammert F, Sarrazin C, Manns MP, Lohse AW, Schramm C. Criteria Used in Clinical Practice to Guide Immunosuppressive Treatment in Patients with Primary Sclerosing Cholangitis. *PLoS One* 2015; **10**: e0140525 [PMID: 26489083 DOI: 10.1371/journal.pone.0140525]
  - 150 **Lunder AK**, Hov JR, Borthne A, Gleditsch J, Johannesen G, Tveit K, Viktil E, Henriksen M, Hovde Ø, Huppertz-Hauss G, Høie O, Lie Høivik M, Monstad I, Solberg IC, Jahnsen J, Karlsen TH, Moum B, Vatn M, Negård A. Prevalence of Sclerosing Cholangitis, Detected by Magnetic Resonance Cholangiography, in Patients with Long-term Inflammatory Bowel Disease. *Gastroenterology* 2016; **151**: 660-669.e4 [PMID: 27342213 DOI: 10.1053/j.gastro.2016.06.021]
  - 151 **Zen Y**, Kawakami H, Kim JH. IgG4-related sclerosing cholangitis: all we need to know. *J Gastroenterol* 2016; **51**: 295-312 [PMID: 26817943 DOI: 10.1007/s00535-016-1163-7]
  - 152 **Ohara H**, Okazaki K, Tsubouchi H, Inui K, Kawa S, Kamisawa T, Tazuma S, Uchida K, Hirano K, Yoshida H, Nishino T, Ko SB, Mizuno N, Hamano H, Kanno A, Notohara K, Hasebe O, Nakazawa T, Nakanuma Y, Takikawa H. Clinical diagnostic criteria of IgG4-related sclerosing cholangitis 2012. *J Hepatobiliary Pancreat Sci* 2012; **19**: 536-542 [PMID: 22717980 DOI: 10.1007/s00534-012-0521-y]
  - 153 **Chazouillères O**, Wendum D, Serfaty L, Montembault S, Rosmorduc O, Poupon R. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy. *Hepatology* 1998; **28**: 296-301 [PMID: 9695990 DOI: 10.1002/hep.510280203]
  - 154 **Alvarez F**, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, Chapman RW, Cooksley WG, Czaja AJ, Desmet VJ, Donaldson PT, Eddleston AL, Fainboim L, Heathcote J, Homberg JC, Hoofnagle JH, Kakumu S, Krawitt EL, Mackay IR, MacSween RN, Maddrey WC, Manns MP, McFarlane IG, Meyer zum Büschenfelde KH, Zeniya M. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; **31**: 929-938 [PMID: 10580593]
  - 155 **Hennes EM**, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL, Bittencourt PL, Porta G, Boberg KM, Hofer H, Bianchi FB, Shibata M, Schramm C, Eisenmann de Torres B, Galle PR, McFarlane I, Dienes HP, Lohse AW. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008; **48**: 169-176 [PMID: 18537184 DOI: 10.1002/hep.22322]
  - 156 **Neuhauser M**, Björnsson E, Treeprasertsuk S, Enders F, Silveira M, Talwalkar J, Lindor K. Autoimmune hepatitis-PBC overlap syndrome: a simplified scoring system may assist in the diagnosis. *Am J Gastroenterol* 2010; **105**: 345-353 [PMID: 19888204 DOI: 10.1038/ajg.2009.616]
  - 157 **Liu F**, Pan ZG, Ye J, Xu D, Guo H, Li GP, Xu KS, Hou XH, Song YH. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: simplified criteria may be effective in the diagnosis in Chinese patients. *J Dig Dis* 2014; **15**: 660-668 [PMID: 25236944 DOI: 10.1111/1751-2980.12196]
  - 158 **Boberg KM**, Chapman RW, Hirschfield GM, Lohse AW, Manns MP, Schrumph E. Overlap syndromes: the International



- Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. *J Hepatol* 2011; **54**: 374-385 [PMID: 21067838 DOI: 10.1016/j.jhep.2010.09.002]
- 159 **Zhang H**, Yang J, Zhu R, Zheng Y, Zhou Y, Dai W, Wang F, Chen K, Li J, Wang C, Li S, Liu T, Abudumijiti H, Zhou Z, Wang J, Lu W, Wang J, Xia Y, Zhou Y, Lu J, Guo C. Combination therapy of ursodeoxycholic acid and budesonide for PBC-AIH overlap syndrome: a meta-analysis. *Drug Des Devel Ther* 2015; **9**: 567-574 [PMID: 25632224 DOI: 10.2147/DDDT.S74515]
  - 160 **Yang F**, Wang Q, Wang Z, Miao Q, Xiao X, Tang R, Chen X, Bian Z, Zhang H, Yang Y, Sheng L, Fang J, Qiu D, Krawitt EL, Gershwin ME, Ma X. The Natural History and Prognosis of Primary Biliary Cirrhosis with Clinical Features of Autoimmune Hepatitis. *Clin Rev Allergy Immunol* 2016; **50**: 114-123 [PMID: 26411425 DOI: 10.1007/s12016-015-8516-5]
  - 161 **Floreani A**, Rizzotto ER, Ferrara F, Carderi I, Caroli D, Blasone L, Baldo V. Clinical course and outcome of autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome. *Am J Gastroenterol* 2005; **100**: 1516-1522 [PMID: 15984974]
  - 162 **Zenouzi R**, Lohse AW. Long-term outcome in PSC/AIH "overlap syndrome": does immunosuppression also treat the PSC component? *J Hepatol* 2014; **61**: 1189-1191 [PMID: 25111172 DOI: 10.1016/j.jhep.2014.08.002]
  - 163 **Floreani A**, Motta R, Cazzagon N, Franceschet I, Roncalli M, Del Ross T, Rosina F, Lleo A, Mescoli C, Colloredo G, Invernizzi P. The overlap syndrome between primary biliary cirrhosis and primary sclerosing cholangitis. *Dig Liver Dis* 2015; **47**: 432-435 [PMID: 25747115 DOI: 10.1016/j.dld.2015.02.002]
  - 164 **Takemoto R**, Miyake Y, Harada K, Nakanuma Y, Moriya A, Ando M, Hirohata M, Yamamoto K. Overlap of IgG4-related sclerosing cholangitis and primary biliary cirrhosis. *Intern Med* 2014; **53**: 1429-1433 [PMID: 24990335]
  - 165 **Xu P**, Li L, Li G, Yu C, Li Y. Insight into the natural history of primary biliary cirrhosis: A systemic review of data from placebo-controlled clinical trials. *Turk J Gastroenterol* 2016; **27**: 342-348 [PMID: 27458850 DOI: 10.5152/tjg.2016.15535]
  - 166 **Gulamhusein AF**, Juran BD, Atkinson EJ, McCauley B, Schlicht E, Lazaridis KN. Low incidence of primary biliary cirrhosis (PBC) in the first-degree relatives of PBC probands after 8 years of follow-up. *Liver Int* 2016; **36**: 1378-1382 [PMID: 27062298 DOI: 10.1111/liv.13143]
  - 167 **Zhang LN**, Shi TY, Shi XH, Wang L, Yang YJ, Liu B, Gao LX, Shuai ZW, Kong F, Chen H, Han W, Han SM, Fei YY, Cui QC, Wang Q, Shen M, Xu D, Zheng WJ, Li YZ, Zhang W, Zhang X, Zhang FC. Early biochemical response to ursodeoxycholic acid and long-term prognosis of primary biliary cirrhosis: results of a 14-year cohort study. *Hepatology* 2013; **58**: 264-272 [PMID: 23408380 DOI: 10.1002/hep.26322]
  - 168 **Carbone M**, Sharp SJ, Flack S, Paximadas D, Spiess K, Adgey C, Griffiths L, Lim R, Trembling P, Williamson K, Wareham NJ, Aldersley M, Bathgate A, Burroughs AK, Heneghan MA, Neuberger JM, Thorburn D, Hirschfield GM, Cordell HJ, Alexander GJ, Jones DE, Sandford RN, Mells GF. The UK-PBC risk scores: Derivation and validation of a scoring system for long-term prediction of end-stage liver disease in primary biliary cholangitis. *Hepatology* 2016; **63**: 930-950 [PMID: 26223498 DOI: 10.1002/hep.28017]
  - 169 **Rabahi N**, Chrétien Y, Gaouar F, Wendum D, Serfaty L, Chazouillères O, Corpechot C, Poupon R. Triple therapy with ursodeoxycholic acid, budesonide and mycophenolate mofetil in patients with features of severe primary biliary cirrhosis not responding to ursodeoxycholic acid alone. *Gastroenterol Clin Biol* 2010; **34**: 283-287 [PMID: 20417047 DOI: 10.1016/j.gcb.2010.02.004]
  - 170 **Rautiainen H**, Kärkkäinen P, Karvonen AL, Nurmi H, Pikkarainen P, Nuutinen H, Färkkilä M. Budesonide combined with UDCA to improve liver histology in primary biliary cirrhosis: a three-year randomized trial. *Hepatology* 2005; **41**: 747-752 [PMID: 15754377 DOI: 10.1002/hep.20646]
  - 171 **Kaplan MM**, Bonder A, Ruthazer R, Bonis PA. Methotrexate in patients with primary biliary cirrhosis who respond incompletely to treatment with ursodeoxycholic acid. *Dig Dis Sci* 2010; **55**: 3207-3217 [PMID: 20559727 DOI: 10.1007/s10620-010-1291-5]
  - 172 **Leung J**, Bonis PA, Kaplan MM. Colchicine or methotrexate, with ursodiol, are effective after 20 years in a subset of patients with primary biliary cirrhosis. *Clin Gastroenterol Hepatol* 2011; **9**: 776-780 [PMID: 21699802 DOI: 10.1016/j.cgh.2011.05.010]
  - 173 **Ali AH**, Carey EJ, Lindor KD. Recent advances in the development of farnesoid X receptor agonists. *Ann Transl Med* 2015; **3**: 5 [PMID: 25705637 DOI: 10.3978/j.issn.2305-5839.2014.12.06]
  - 174 **Markham A**, Keam SJ. Obeticholic Acid: First Global Approval. *Drugs* 2016; **76**: 1221-1226 [PMID: 27406083 DOI: 10.1007/s40265-016-0616-x]
  - 175 **Hirschfield GM**, Mason A, Luketic V, Lindor K, Gordon SC, Mayo M, Kowdley KV, Vincent C, Bodhenheimer HC, Parés A, Trauner M, Marshall HU, Adorini L, Sciacca C, Beecher-Jones T, Castellote E, Böhm O, Shapiro D. Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid. *Gastroenterology* 2015; **148**: 751-761.e8 [PMID: 25500425 DOI: 10.1053/j.gastro.2014.12.005]
  - 176 **Trivedi PJ**, Hirschfield GM, Gershwin ME. Obeticholic acid for the treatment of primary biliary cirrhosis. *Expert Rev Clin Pharmacol* 2016; **9**: 13-26 [PMID: 26549695 DOI: 10.1586/17512433.2015.1092381]
  - 177 **Shiba H**, Wakiyama S, Futagawa Y, Gocho T, Ito R, Furukawa K, Ishida Y, Misawa T, Yanaga K. Switching from tacrolimus to cyclosporine A to prevent primary biliary cirrhosis recurrence after living-donor liver transplantation. *Int Surg* 2013; **98**: 156-159 [PMID: 23701152 DOI: 10.9738/CC188]
  - 178 **Egawa H**, Sakisaka S, Teramukai S, Sakabayashi S, Yamamoto M, Umeshita K, Uemoto S. Long-Term Outcomes of Living-Donor Liver Transplantation for Primary Biliary Cirrhosis: A Japanese Multicenter Study. *Am J Transplant* 2016; **16**: 1248-1257 [PMID: 26731039 DOI: 10.1111/ajt.13583]
  - 179 **Tanaka A**, Hirohara J, Nakanuma Y, Tsubouchi H, Takikawa H. Biochemical responses to bezafibrate improve long-term outcome in asymptomatic patients with primary biliary cirrhosis refractory to UDCA. *J Gastroenterol* 2015; **50**: 675-682 [PMID: 25239675 DOI: 10.1007/s00535-014-0998-z]
  - 180 **Hosonuma K**, Sato K, Yamazaki Y, Yanagisawa M, Hashizume H, Horiguchi N, Kakizaki S, Kusano M, Yamada M. A prospective randomized controlled study of long-term combination therapy using ursodeoxycholic acid and bezafibrate in patients with primary biliary cirrhosis and dyslipidemia. *Am J Gastroenterol* 2015; **110**: 423-431 [PMID: 25732417 DOI: 10.1038/ajg.2015.20]
  - 181 **Cheung AC**, Lapointe-Shaw L, Kowgier M, Meza-Cardona J, Hirschfield GM, Janssen HL, Feld JJ. Combined ursodeoxycholic acid (UDCA) and fenofibrate in primary biliary cholangitis patients with incomplete UDCA response may improve outcomes. *Aliment Pharmacol Ther* 2016; **43**: 283-293 [PMID: 26559762 DOI: 10.1111/apt.13465]
  - 182 **Hegade VS**, Khanna A, Walker LJ, Wong LL, Dyson JK, Jones DE. Long-Term Fenofibrate Treatment in Primary Biliary Cholangitis Improves Biochemistry but Not the UK-PBC Risk Score. *Dig Dis Sci* 2016; **61**: 3037-3044 [PMID: 27435324 DOI: 10.1007/s10620-016-4250-y]
  - 183 **Grigorian AY**, Mardini HE, Corpechot C, Poupon R, Levy C. Fenofibrate is effective adjunctive therapy in the treatment of primary biliary cirrhosis: A meta-analysis. *Clin Res Hepatol Gastroenterol* 2015; **39**: 296-306 [PMID: 25882906 DOI: 10.1016/j.clinre.2015.02.011]
  - 184 **Myers RP**, Swain MG, Lee SS, Shaheen AA, Burak KW. B-cell depletion with rituximab in patients with primary biliary cirrhosis refractory to ursodeoxycholic acid. *Am J Gastroenterol* 2013; **108**: 933-941 [PMID: 23649186 DOI: 10.1038/ajg.2013.51]
  - 185 **Tsuda M**, Moritoki Y, Lian ZX, Zhang W, Yoshida K, Wakabayashi K, Yang GX, Nakatani T, Vierling J, Lindor K, Gershwin ME, Bowlus CL. Biochemical and immunologic effects of rituximab in patients with primary biliary cirrhosis and an incomplete response to ursodeoxycholic acid. *Hepatology* 2012; **55**: 512-521 [PMID: 22006563 DOI: 10.1002/hep.24748]
  - 186 **Wang L**, Li J, Liu H, Li Y, Fu J, Sun Y, Xu R, Lin H, Wang S, Lv

- S, Chen L, Zou Z, Li B, Shi M, Zhang Z, Wang FS. Pilot study of umbilical cord-derived mesenchymal stem cell transfusion in patients with primary biliary cirrhosis. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 85-92 [PMID: 23855301 DOI: 10.1111/jgh.12029]
- 187 **Wang L**, Han Q, Chen H, Wang K, Shan GL, Kong F, Yang YJ, Li YZ, Zhang X, Dong F, Wang Q, Xu D, Hu ZJ, Wang SH, Keating A, Bi YL, Zhang FC, Zhao RC. Allogeneic bone marrow mesenchymal stem cell transplantation in patients with UDCA-resistant primary biliary cirrhosis. *Stem Cells Dev* 2014; **23**: 2482-2489 [PMID: 24835895 DOI: 10.1089/scd.2013.0500]
- 188 **Raczyńska J**, Habior A, Pączek L, Foroniewicz B, Paweł A, Mucha K. Primary biliary cirrhosis in the era of liver transplantation. *Ann Transplant* 2014; **19**: 488-493 [PMID: 25262831 DOI: 10.12659/AOT.890753]
- 189 **Bosch A**, Dumortier J, Maucort-Boulch D, Scoazec JY, Wendum D, Conti F, Morard I, Rubbia-Brandt L, Terris B, Radenne S, Abenavoli L, Poupon R, Chazouillères O, Calmus Y, Boillot O, Giostra E, Corpechot C. Preventive administration of UDCA after liver transplantation for primary biliary cirrhosis is associated with a lower risk of disease recurrence. *J Hepatol* 2015; **63**: 1449-1458 [PMID: 26282232 DOI: 10.1016/j.jhep.2015.07.038]
- 190 **Sasaki M**, Hsu M, Yeh MM, Nakanuma Y. In recurrent primary biliary cirrhosis after liver transplantation, biliary epithelial cells show increased expression of mitochondrial proteins. *Virchows Arch* 2015; **467**: 417-425 [PMID: 26259963 DOI: 10.1007/s00428-015-1819-3]

**P- Reviewer:** Invernizzi P, Lalor P, Licinio R **S- Editor:** Qiu S

**L- Editor:** Rutherford A **E- Editor:** Li D



Basic Study

# Performance of cold-preserved rat liver Microorgans as the biological component of a simplified prototype model of bioartificial liver

María Dolores Pizarro, María Gabriela Mediavilla, Alejandra Beatriz Quintana, Ángel Luis Scandizzi, Joaquín Valentín Rodríguez, María Eugenia Mamprin

María Dolores Pizarro, Joaquín Valentín Rodríguez, Centro Binacional de Criobiología Clínica y Aplicada, Rosario 2000, Argentina

María Gabriela Mediavilla, Instituto de Biología Molecular y Celular de Rosario, Rosario 2000, Argentina

María Gabriela Mediavilla, Joaquín Valentín Rodríguez, María Eugenia Mamprin, Consejo Nacional de Investigaciones Científicas y Técnicas, Caba C1033AAJ, Argentina

Alejandra Beatriz Quintana, Morfología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Rosario 2000, Argentina

Ángel Luis Scandizzi, María Eugenia Mamprin, Área Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario S2002 LRK, Argentina

**Author contributions:** Pizarro MD performed the majority of experiments and analyzed the data; Mediavilla MG and Mamprin ME have designed research, performed research, contributed new reagents, analyzed data, wrote the manuscript; all the authors were involved in reviewing the literature for latest contributions in the field, writing, and edition of the manuscript; Mediavilla MG and Mamprin ME have equally contributed to this work.

**Supported by** Universidad Nacional de Rosario (UNR), No. 677/2013.

**Institutional review board statement:** The study was reviewed and approved by the National University of Rosario Institutional Review Board (Resol. C.S., No. 677/2013).

**Institutional animal care and use committee statement:** All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Faculty of Biochemical and Pharmaceutical Sciences-UNR (Resol. No. 139/2011).

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** María Eugenia Mamprin, PhD, Professor of Pharmacology, Área Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, Rosario S2002 LRK, Argentina. [mmamprin@fbioyf.unr.edu.ar](mailto:mmamprin@fbioyf.unr.edu.ar)  
 Telephone: +54-341-4393400

**Received:** May 31, 2016

**Peer-review started:** June 1, 2016

**First decision:** July 20, 2016

**Revised:** July 28, 2016

**Accepted:** September 13, 2016

**Article in press:** September 18, 2016

**Published online:** November 28, 2016

## Abstract

### AIM

To develop a simplified bioartificial liver (BAL) device prototype, suitable to use freshly and preserved liver Microorgans (LMOs) as biological component.

### METHODS

The system consists of 140 capillary fibers through which goat blood is pumped. The evolution of hema-

tocrit, plasma and extra-fiber fluid osmolality was evaluated without any biological component, to characterize the prototype. LMOs were cut and cold stored 48 h in BG35 and ViaSpan® solutions. Fresh LMOs were used as controls. After preservation, LMOs were loaded into the BAL and an ammonia overload was added. To assess LMOs viability and functionality, samples were taken to determine lactate dehydrogenase (LDH) release and ammonia detoxification capacity.

## RESULTS

The concentrations of ammonia and glucose, and the fluids osmolalities were matched after the first hour of perfusion, showing a proper exchange between blood and the biological compartment in the minibioreactor. After 120 min of perfusion, LMOs cold preserved in BG35 and ViaSpan® were able to detoxify  $52.9\% \pm 6.5\%$  and  $53.6\% \pm 6.0\%$ , respectively, of the initial ammonia overload. No significant differences were found with Controls ( $49.3\% \pm 8.8\%$ ,  $P < 0.05$ ). LDH release was  $6.0\% \pm 2.3\%$  for control LMOs, and  $6.2\% \pm 1.7\%$  and  $14.3\% \pm 1.1\%$  for BG35 and ViaSpan® cold preserved LMOs, respectively ( $n = 6$ ,  $P < 0.05$ ).

## CONCLUSION

This prototype relied on a simple design and excellent performance. It's a practical tool to evaluate the detoxification ability of LMOs subjected to different preservation protocols.

**Key words:** Rat liver Microorgans; Cold preservation; BG35 preservation solution; Bioartificial liver device; Acute liver failure

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** This work describes the development of a simplified bioartificial liver prototype (BAL, suitable to use rat liver Microorgans (LMOs) as biological component, and the evaluation of these tissue slices performance in this new model. We demonstrate that the minibioreactor constructed allows a good performance of fresh and cold preserved LMOs, showing the importance of architecture and model configuration on these devices design. Besides its application as BAL, this minibioreactor could serve as a suitable laboratory tool to evaluate the behavior and functionality of LMOs subjected to different preservation protocols due to its simple design and the utilization of standard materials.

Pizarro MD, Mediavilla MG, Quintana AB, Scandizzi ÁL, Rodríguez JV, Mamprin ME. Performance of cold-preserved rat liver Microorgans as the biological component of a simplified prototype model of bioartificial liver. *World J Hepatol* 2016; 8(33): 1442-1451 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i33/1442.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i33.1442>

## INTRODUCTION

To date, acute liver failure continues to be a defeating syndrome in the clinical practice due to its rapid development and its high risk of mortality. Patients always require a multidisciplinary approach for adequate management and subsequent organ transplantation. Unfortunately, the scarcity of donor organs often limits liver transplantation in time. Among the different approaches that have been tested to maintain the patients until transplantation and/or to facilitate self-regeneration of the damaged liver is the bioartificial liver (BAL)<sup>[1]</sup>. In BAL devices, the plasma of the patient is treated by its circulation through a bioreactor that accommodates a biologically active component which performs the diminished or lacking hepatic metabolic functions. Ammonia detoxification is one key task this biological component must carry out because increased blood levels of this metabolite are toxic to the central nervous system<sup>[2]</sup>.

Investigations concerning the development of BAL devices containing normal hepatocytes are still being conducted<sup>[3,4]</sup>. Some researchers have chosen to employ immortalized hepatocytes<sup>[5]</sup> while others have focused their efforts in preparing bioreactors housing isolated hepatocyte with or without extra-cellular matrix and structural components<sup>[6,7]</sup>.

Our group has already reported the construction of a minibioreactor (MBR) consisting in a hollow fiber based cartridge with blood flowing through the fiber lumens. Rat isolated hepatocytes were used as the biological component, showing an effective ammonia depuration rate<sup>[8]</sup>. Since it is thought that the "ideal" biological component for a BAL should contain all the constituents present in a liver lobule in order to obtain maximal function, we became interested in evaluating the performance of rat liver Microorgans (LMOs). These are thin fragments of tissue that retain the basic micro-architecture of the liver lobe, including cell to cell contact and cell to cell communication<sup>[9,10]</sup>.

On the other hand, in order to become a useful clinical tool, any BAL device must be ready to use when a patient needs it. This means the biological component should be not only available but viable and functional. In a previous work we have presented BG35 [Bis-Gluconate-Polyethyleneglycol (PEG) 35 kDa], a novel preservation solution, that exhibited an efficacy similar to that of the ViaSpan® to give protection to LMOs against injury produced by the ischemia followed by reoxygenation suffered as a consequence of cold preservation<sup>[11]</sup>.

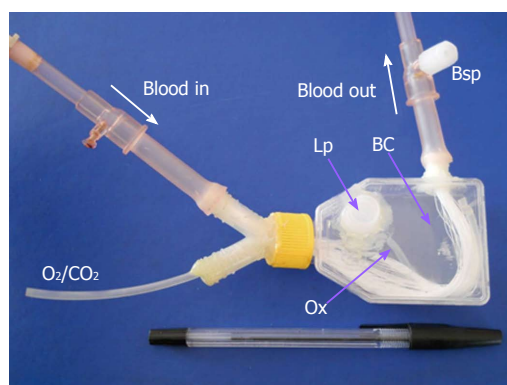
The objectives of this work were to develop a simplified prototype BAL suitable to use LMOs as biological component, and to evaluate the performance of fresh and cold preserved rat LMOs in this model.

## MATERIALS AND METHODS

### MBR

The MBR (Figure 1) was constructed using a 25 cm<sup>2</sup>





**Figure 1 Minibioreactor device.** BC: Biological compartment; Lp: Loading port; O<sub>2</sub>/CO<sub>2</sub>: Carbogen supply line; Ox: Silicone tube oxygenator; Bsp: Blood sample port.

culture flask, adding a loading port (Lp) on top, a Y-polypropylene connector (Nalgene cat. 6152-0375) onto its lid and a simple connector at one side. One hundred and forty Polyamix™ hollow fibers (Gambro, Hechingen, Germany) are assembled to these two connectors and sealed with epoxy glue. The diverse parts that made up the MBR can be appreciated in Figure 1.

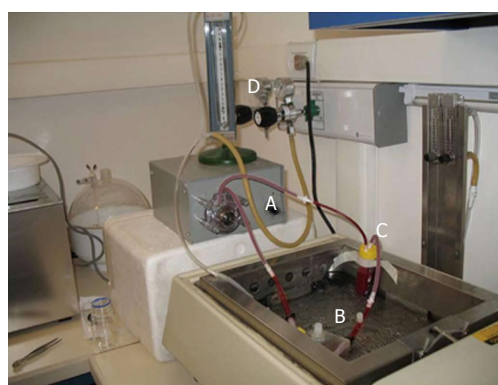
In the MBR, two main compartments can be distinguished: The hollow fibers internal lumen constitutes the blood compartment, while the biological compartment (BC) comprises the space outside the hollow fibers (total volume of 50 cm<sup>3</sup>). A silicone tube (Ox, oxygenator) enters to the BC through the Y connector and allows the oxygenation of the BC fluid. The LMOs were placed in the biological compartment through the Lp port and released on the flat surface of the device. This allows a homogeneous distribution of LMOs and a better oxygenation and exchange of solutes.

### Components of the perfusion system and its manipulation

The components of the perfusion system used are detailed in Figure 2. The blood reservoir, that contains a clot filter, and the MBR are immersed in a water bath at 37 °C. The peristaltic pump (model 7554-60, Cole Parmer, United States) allows the recirculation of heparinized goat blood (total volume: 35 mL) through all the system at a constant flow of 9 mL/min.

In all the experiments performed, we first filled the system with goat blood *via* the inlet tube and then inoculated 1 g of LMOs into the BC (or Krebs-Henseleit Reoxygenation media (KHR) alone, in the experiments done to characterize system operation, which composition is shown in page 11). The silicone tube was used to oxygenate the BC compartment with carbogen gas (95% O<sub>2</sub>/5% CO<sub>2</sub>) at a stable pressure of 85 mmHg. The blood pH was kept at 7.40 ± 0.50 adding 8.4% sodium bicarbonate if necessary.

To test ammonia detoxification capability of the rat LMOs, we added an aliquot of an ammonium chloride solution (approximate concentration: 350 mmol/L) to



**Figure 2 In vitro perfusion system.** A: Peristaltic pump; B: Minibioreactor; C: Blood reservoir; D: External oxygen supply.

the blood in order to achieve an initial ammonia plasma concentration of 1.06 ± 0.12 mmol/L, *n* = 6 (blood sample *t* = 0). Then, we initiated blood perfusion and took blood and BC fluid samples after 60 and 120 min of operation to perform the different assays detailed below.

### Characterization of the MBR perfusion system

In order to characterize the operation of the system “*in vitro*”, *i.e.*, without LMOs, different MBR were perfused for 120 min with only KHR solution inside the BC compartment and the following parameters were evaluated:

Hematocrit, to determine the probable rupture of some fibers with the concomitant passage of blood to the BC, and to study the possible hemolytic action of the peristaltic pump. Blood samples were taken from blood sample port at different perfusion times and were centrifuged (1000 × *g* - 3 min, Rolco CH24 centrifuge). The hematocrit was calculated using the next equation:

Hematocrit (%) = red blood cells volume/blood total volume.

Plasma and extra-fiber fluid osmolality, were measured in order to monitor the correct transfer of fluids between blood and the BC, using a freezing point osmometer (Osmomat 030, Gonotec, GmbH, Berlin, Germany).

Protein analysis using fast protein liquid chromatography (FPLC), in order to study the diffusive properties of the hollow fibers used in the construction of the MBR and to determine the possible passage of plasmatic proteins towards the BC, especially those belonging to the immune system that could damage the biological component (described below).

Metabolite concentration in both compartments, such as glucose and ammonia, to determine their correct distribution in the MBR (described below).

### Hemolysis determination

Samples of plasma were taken after 0, 60 and 120 min of perfusion and hemoglobin concentration was determined using the oxyhemoglobin method<sup>[12]</sup>.

**Table 1** Composition of the preservation solutions ViaSpan® and BG35

	ViaSpan®	BG35
Impermeants (mmol/L)		
Lactobionate	100	
Gluconate		100
Raffinose	30	
Buffers (mmol/L)		
KH <sub>2</sub> PO <sub>4</sub>	25	2.5
BES		50
Substrates (mmol/L)		
Allopurinol	1	1
Glutathione	3	3
Adenosine	5	5
Glycine		15
MgSO <sub>4</sub>	5	5
Colloids (g/L)		
HES	50	
PEG 35000		40
pH	7.40	7.40
Osm (mOsm/kg water)	320 ± 4	339 ± 4

Dexamethasone 16 mg/L, insulin 40 UI/L and penicillin G 200000 UI/L were added to ViaSpan® before use. Streptomycin 0.25 mg/mL and penicillin G 10 UI/mL were added to BG35 before use. All the solutions were bubbled with 100 % N<sub>2</sub> for 45 min at 0 °C before use. BES: N, N-bis (2-hydroxyethyl)-2-aminoethanesulfonic acid; HES: Hydroxyethyl starch; PEG: Polyethyleneglycol.

To calculate the percentage of hemolysis, we used the following equation, described by Arnaud<sup>[13]</sup>:

$$\text{Hemolysis (\%)} = 100 \times \{[\text{Hbs} \times (1 - \text{Ht})] \div \text{Hbr}\}$$

where Hbs is the hemoglobin content, expressed in g/100 mL, of the different samples; Hbr is the total hemoglobin content (in whole blood), and Ht is the hematocrit value measured after 0, 60 or 120 min of perfusion.

### FPLC analysis

Samples of basal plasma and BC fluid were taken after 60 and 120 min of perfusion and analyzed by Gel Filtration Chromatography. They were centrifuged (12100 × *g* - 5 min), filtered and 100 µL were seeded in a Tricorn Superdex-200 column (30 × 1 cm, GE Healthcare, Sweden), equilibrated with 50 mmol/L Tris, 150 mmol/L NaCl buffer, pH 7.00, previously degassed by vacuum filtration. The column was manipulated using an ÄKTA-Prime equipment (GE Healthcare, Sweden), at a constant flow of 0.5 mL/min. Each sample was analyzed in duplicate. Chromatograms were registered measuring absorbance at 280 nm and, to determine the protein molecular weight, a standard calibration curve was made using a "Molecular Weights 29000-700000" kit, following the supplier's instructions (Sigma-Aldrich, St Louis, Missouri, United States).

### Animals

The livers were obtained from male Wistar rats weighing 250-300 g. Animals had access to regular laboratory food for rodents and water *ad libitum*. Animals were cared in conformity with the principles and recommendations for

the care and utilization of laboratory animals, suggested by the National Academy of Sciences. The rats were adapted to experimental laboratory environment for fourteen days before to experimentation. All experimental procedures were authorized by the School of Biochemical and Pharmaceutical Sciences Institutional Animal Care and Use Committee (Res No. 139/2011).

### Preparation of rat LMOs

LMOs were manually cut from rat livers into slices of 338 ± 27 µm thickness, *n* = 25. They were cut using a microtome blade attached to a plastic handle. We performed all the manipulations on ice (at 0 °C) to decrease tissue injury, and on top of a paper filter to avoid the pieces of livers from sliding what could impede the correct cutting of the tissue<sup>[14]</sup>.

Subsequently, LMOs were allocated in various solutions. Control group (non-preserved or fresh) LMOs were suspended in KHR and directly put in the MBR perfusion. KHR buffer was composed as follows: 114 mmol/L NaCl, 25 mmol/L NaHCO<sub>3</sub>, 1.2 mmol/L KH<sub>2</sub>PO<sub>4</sub>, 1.2 mmol/L MgSO<sub>4</sub>, 4.8 mmol/L KCl, 1.5 mmol/L CaCl<sub>2</sub>, 10 mmol/L HEPES, 25 mmol/L glucose, 5 mmol/L fructose, 1 mmol/L allopurinol, 3 mmol/L glycine, 10 µmol/L adenosine, 6 mmol/L ornithine, 10 mmol/L sodium lactate; pH 7.40, 328 ± 7 mOsm/kg water (*n* = 6)<sup>[15]</sup>. Preserved LMOs were stored 48 h in BG35 and ViaSpan® solutions (Table 1) before MBR perfusion as explained in the next section.

### Preservation of LMOs

As it was stated, in the case of the preserved groups, LMOs were stored in two different preservation solutions. Fifty LMOs were preserved during 48 h at 0 °C<sup>[11]</sup> in a crystal flask immerse in 50 mL of one of these preservation solutions: (1) ViaSpan® (Bristol-Myers Squibb Pharmaceutical Limited; ViaSpan® group); and (2) BG35 (Bes-Gluconate plus 4% PEG 35 kDa; BG35 group).

The composition of the preservation solutions used are shown in Table 1. A period of 48 h of preservation was selected since in initial investigations (data not exposed) the viability evaluated by lactate dehydrogenase (LDH) leakage was modestly changed by 1 d of cold ischemia, but a pronounced increase was observed after 2 d.

After 48 h of cold preservation, LMOs were completely rinsed with a flush solution earlier reported by our group<sup>[16]</sup> to fully eliminate residual cold preservation solution. After that, LMOs were placed into the MBR.

### LDH release

Viability of LMOs was tested by LDH release. LDH activity was determined in the BC fluid and the slices as earlier explained<sup>[17]</sup>. Data are shown as the percentage of the total enzyme activity released into the incubation medium.

### Measurement of plasma and BC fluid ammonia concentrations

Samples of blood and BC fluid were taken at different

**Table 2** Time course evolution of minibioreactor functional parameters during 120 min of perfusion

Perfusion time	(Osm) <sub>B</sub> /((Osm) <sub>BC</sub>	Hto (%)	Hemolysis (%)	(Glucose) <sub>B</sub> /((Glucose) <sub>BC</sub>	(NH <sub>4</sub> <sup>+</sup> ) <sub>B</sub> /((NH <sub>4</sub> <sup>+</sup> ) <sub>BC</sub>	QNH <sub>4</sub> <sup>+</sup> (μmol)
0 min	0.94 ± 0.02	47 ± 3	0.27 ± 0.09	0.09 ± 0.04	52.8 ± 4.0	36.3 ± 1.6
60 min	1.00 ± 0.02	44 ± 5	0.59 ± 0.10	0.77 ± 0.07	1.1 ± 0.1	36.1 ± 1.6
120 min	1.00 ± 0.01	45 ± 3	0.79 ± 0.12	0.90 ± 0.06	1.2 ± 0.3	36.1 ± 1.5

B: Blood; BC: Biological compartment.

periods of time (0, 60 and 120 min), blood samples were centrifuged (12000 × *g*, 3 min) and all samples were conserved in liquid nitrogen until the determinations were performed. Ammonia was measured using the van Anken enzymatic determination in a volume of 0.8 mL consisting of 66.7 mmol/L phosphate buffer, pH 8.30, 0.14 mmol/L, NADPH, 6.5 mmol/L sodium-ketoglutarate, 2.5 mmol/L ADP, 120 UI/mL glutamate dehydrogenase (cat. #G2626, Sigma Aldrich St. Louis, MO, United States)<sup>[18]</sup>.

The following equations were then used to calculate ammonia mass balance:

$$Q_{B,t} = [(A)_{B,t} \times V_{B,t}] - [(A)_{B,Bas} \times V_{B,t}]$$

$$Q_{BC,t} = [(A)_{BC,t} \times V_{BC,t}] - [(A)_{BC,Bas} \times V_{BC,t}]$$

$$Q_{T,t} = Q_{B,t} + Q_{BC,t}$$

Where:  $Q_{B,t}$  and  $Q_{BC,t}$  represent the ammonia mass at time *t* in blood and the BC fluid respectively;  $(A)_{B,t}$  and  $(A)_{BC,t}$  are the ammonia concentrations in blood and BC fluid at different times;  $(A)_{B,Bas}$  is basal blood ammonia concentration;  $V_{B,t}$  and  $V_{BC,t}$  are the blood and BC fluid volumes, and  $Q_{T,t}$  is the total ammonia mass at different times.

The ammonia detoxification capacity is expressed as the % of the initial dose detoxified at different times and was calculated using the following equation.

$$\% \text{ Dose} = 100 - [(Q_{T,t} \times 100)/Q_{T,0}]$$

Where  $Q_{T,0}$  is total ammonia mass at time 0.

### Determination of plasma and BC fluid glucose concentrations

Glucose was determined using a commercial kit ("Glicemia Enzimática AA", Wiener Laboratories, Rosario, Argentina) and following the manufacturer's instructions.

### Histology

Samples of livers from all experimental groups were fixed in 10% formaldehyde, dehydrated, embedded in paraffin, sectioned with a micrometer, stained with hematoxylin-eosin and mounted. Sections were microscopically analyzed and some aspects of the hepatic parenchyma were taken into consideration: Hepatic cell plate organization, the form of endothelial cells and hepatocytes, presence of necrotic areas and blebs in the plasmatic membrane of the hepatocytes. To perform the analyses, we used a light field microscope (Olympus Co, LTD. Model U-MDOB), equipped with a digital camera (Olympus model D-360 Zoom-3.2 megapixels of resolution).

### Materials

Chemicals were purchased from Sigma (St. Louis,

Missouri, United States) and were analytical grade pure.

### Statistical analysis

Results are presented as mean ± SD. We performed a one-way or multifactor analysis of variance with Scheffé's multiple range test as post-test to establish the statistical significance of the differences between means. *P* values smaller than 0.05 were taken as statistically significant. The statistical review of the study was performed by a biomedical statistician.

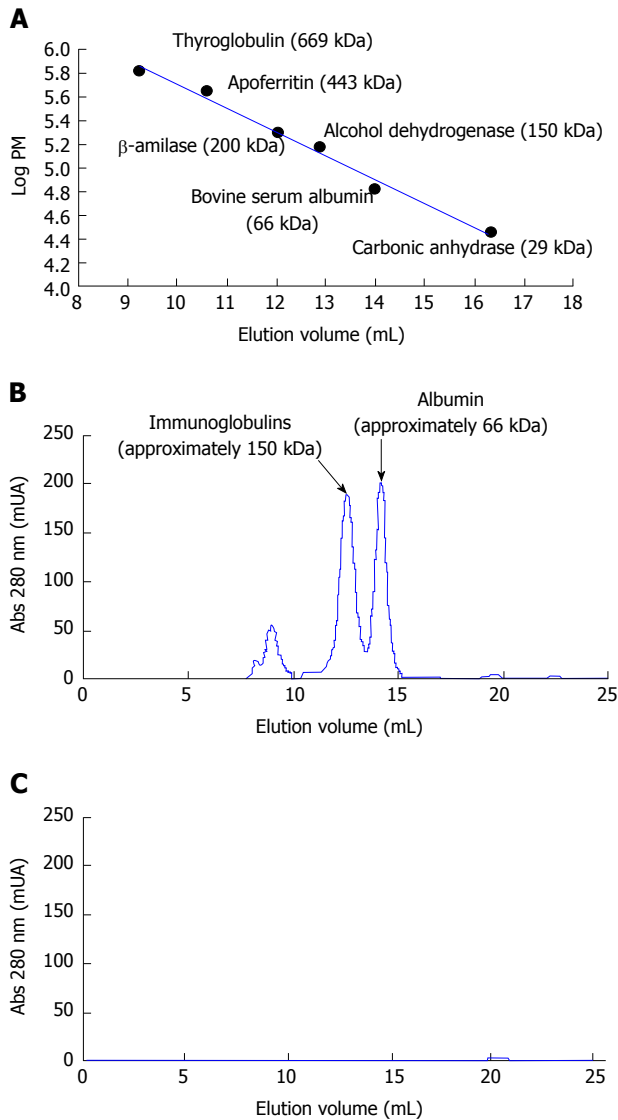
## RESULTS

### Time course evolution of MBR functional parameters during 120 min of perfusion

In order to characterize the "in vitro system" operation, different MBR were perfused for 120 min, without any biological component. The mean data of six individual runs are shown in Table 2. The plasma/BC relationship did not change during the experiments. Plasma and KHR solution osmolalities were arrived to equilibrium after the first hour of perfusion, demonstrating a proper exchange of solutes between the two compartments. No significant variation of the hematocrits was observed during the function of the system, but a minimum breakup of the erythrocytes was generated after 120 min of perfusion by the activity of the peristaltic pump. Ammonia concentration became equal in both compartments after the first hour of perfusion and the total mass (*Q*) of this metabolite remained constant during the whole experiment, indicating that no loss or interactions with any system component occurred. Similar behavior was observed for glucose distribution.

### FPLC analysis

The protein analysis by gel filtration chromatography is shown in Figure 3. In the chromatogram obtained for a sample of basal plasma (Figure 3B) we can observe the presence of two main peaks. Based on the calibration curve obtained (Figure 3A), they can be assigned to the major plasma proteins: Albumin [elution volume (*Ve*) = 14.15 mL] and immunoglobulins (mainly IgG, *Ve* = 12.52 mL). Two minor peaks are also appreciated (*Ve* < 9 mL) that correspond to proteins of high molecular weight (*MW* > 700 kDa). These could be α2-macroglobulin (*MW* = 725 kDa, *Ve* = 8.92 mL) and the pentameric form of IgM (*MW* = 950 kDa, *Ve* = 8.22 mL). Figure 3C shows the chromatogram obtained for a sample of the BC fluid after 120 min of blood perfusion (the same result was obtained after 60 min). It can be noticed that none of the

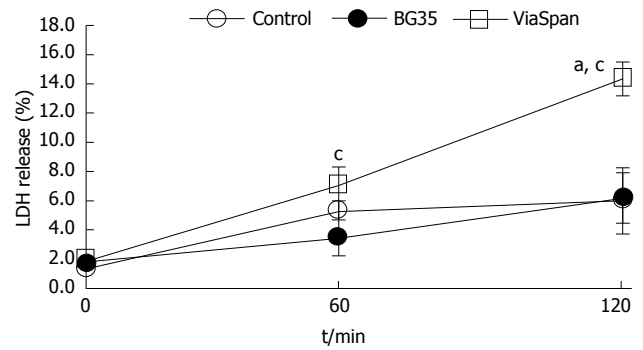


**Figure 3 Protein analysis by gel filtration chromatography.** A: Calibration curve carried out with molecular weight markers; B: Chromatogram obtained for a sample of basal plasma; C: Chromatogram obtained for a sample of biological compartment, taken after 120 min of perfusion  $n = 5$ .

plasma proteins was capable of crossing the membrane of the hollow fibers used in the construction of the MBR.

#### Evolution of the amount of LDH released by fresh LMOs and LMOs cold preserved in BG35 and ViaSpan® solutions after two hours of MBR perfusion

Figure 4 exposes the time changes in LMOs viability (determined by LDH release) throughout the two hours of the experiments performed. One gram of fresh LMOs (controls) or LMOs cold preserved in BG35 and ViaSpan® solutions was loaded into the BC and the MBR was then perfused during 120 min. The amount of enzyme released by fresh LMOs and LMOs cold preserved in BG35 showed a minor increase after two hours of perfusion. However, LMOs preserved in ViaSpan® solution showed a statistically significant raise in this parameter as perfusion time increased. The values of LDH release reached after 120 min in the MBR were:  $6.0\% \pm 2.3\%$  for controls;



**Figure 4 Time course of lactate dehydrogenase release during 120 min of minibioreactor perfusion determined for fresh and cold preserved liver microorgans in BG35 and ViaSpan® solutions.** Data are expressed as mean  $\pm$  SD for 6 liver microorgans (LMOs) preparations. Different from control,  $^aP < 0.05$ ; Different from all the other reoxygenation times,  $^cP < 0.05$ ,  $n = 6$  LMOs independent preparations for each condition. LDH: Lactate dehydrogenase.

$6.2\% \pm 1.7\%$  for LMOs cold preserved in BG35 and  $14.3\% \pm 1.1\%$  for the group cold preserved in ViaSpan®, ( $P < 0.05$ ,  $n = 6$ ).

#### Evolution of ammonia detoxification for fresh LMOs and LMOs cold preserved in BG35 and ViaSpan® solutions

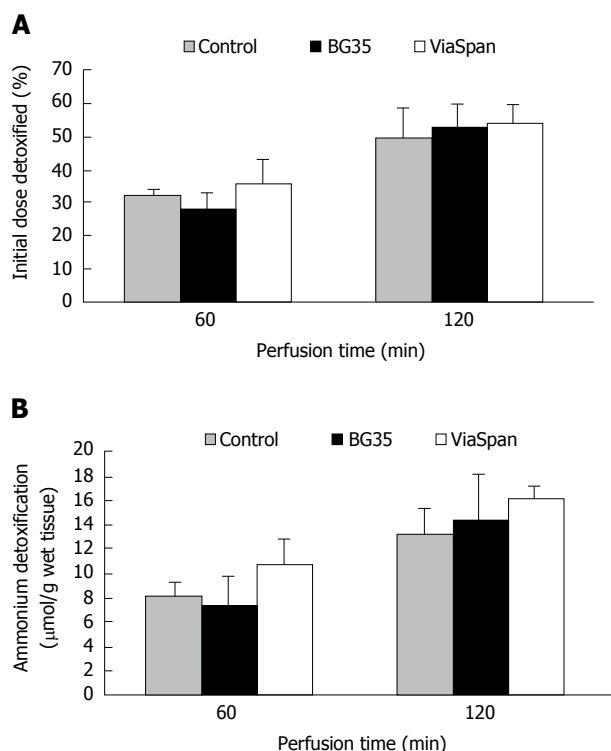
The ammonia detoxification capability of the device was evaluated by measuring the time course evolution of plasma ammonia concentration: An ammonia overload was added to the blood to obtain an ammonia plasma concentration of  $1.06 \pm 0.12$  mmol/L,  $n = 6$ . We determined the ammonia content in blood and BC fluid samples obtained before initiating the perfusion (time 0) and after the first and second hour of operation (time 60 and 120 min, respectively). In Figure 5A it can be appreciated the LMOs ammonia detoxification capacity during two hours of MBR functioning. It can be observed that both preserved groups were able to detoxify a percentage of ammonium initial doses similar to control group, during the whole experiment. After two hours, the percentage of the initial dose detoxified (Figure 5A) was  $49.3\% \pm 8.8\%$  for controls LMOs;  $52.9 \pm 6.5$  for BG35 and  $53.6 \pm 6.0$  for ViaSpan® preserved LMOs ( $n = 6$ ). To get a better knowledge about the amount of ammonia that LMOs were able to metabolize in the MBR, Figure 5B shows the  $\mu$ mol of this compound detoxified per gram of wet tissue. The values reached at the end of the perfusion period were: Control:  $13.2 \pm 2.2$ ; BG35:  $14.2 \pm 3.8$ , and ViaSpan®  $16.0 \pm 1.1$   $\mu$ mol of  $\text{NH}_4^+$  detoxified/g wet tissue ( $n = 6$ ).

#### Histology

Control and cold preserved LMOs (48 h in BG35 and ViaSpan® solutions) were morphologically analyzed to assess hepatic tissue integrity, at the beginning and after 2 h of perfusion in the MBR.

Control LMOs showed normal hepatocyte cords with fusiform endothelial cells attached to the extracellular matrix of perisinusoidal space (EMPS), both at 0 min and at 120 min of perfusion period in the MBR (Figure





**Figure 5** Evolution of (A) initial dose of ammonium detoxified (%) (B) detoxification of ammonia ( $\mu\text{mol/g}$  wet tissue) for fresh cold preserved liver Microorgans in BG35 and ViaSpan® solutions used as a biological component in the minibioreactor. Data are expressed as mean  $\pm$  SD,  $n = 6$  liver Microorgans independent preparations for each condition.

6A and B).

LMOs preserved in BG35 had organized hepatocyte cords with sinusoids slightly dilated and endothelial cells with two different morphology patterns: Fusiform or rounded, both attached to EMPS (Figure 6D) at 0 min. After 120 min, morphological features changed. Hepatocyte cords continued to be organized but sinusoids were dilated with abundant rounded endothelial cells either attached to EMPS or seen inside sinusoidal lumen (Figure 6E).

At 0 min, LMOs preserved in ViaSpan® solution showed balonized hepatocytes and abundant rounded endothelial cells. Endothelial cells were attached to EMPS and sinusoidal lumen was dilated (Figure 6D). At 120 min LMOs had abundant blebs and areas of disrupted hepatocyte cords (Figure 6E).

## DISCUSSION

The goal of this study was the development of a simplified BAL prototype suitable to use LMOs as biological component, and the evaluation of fresh and cold preserved rat LMOs performance in this model.

Our simple hollow fiber MBR was constructed to enable the control of LMOs performance (*i.e.*, viability and detoxification, but also suitable for the measurement of other parameters such as synthesis functions specific of liver) and sampling of blood and BC fluid during operation. In a first stage, we characterized this simplified prototype by setting different functional para-

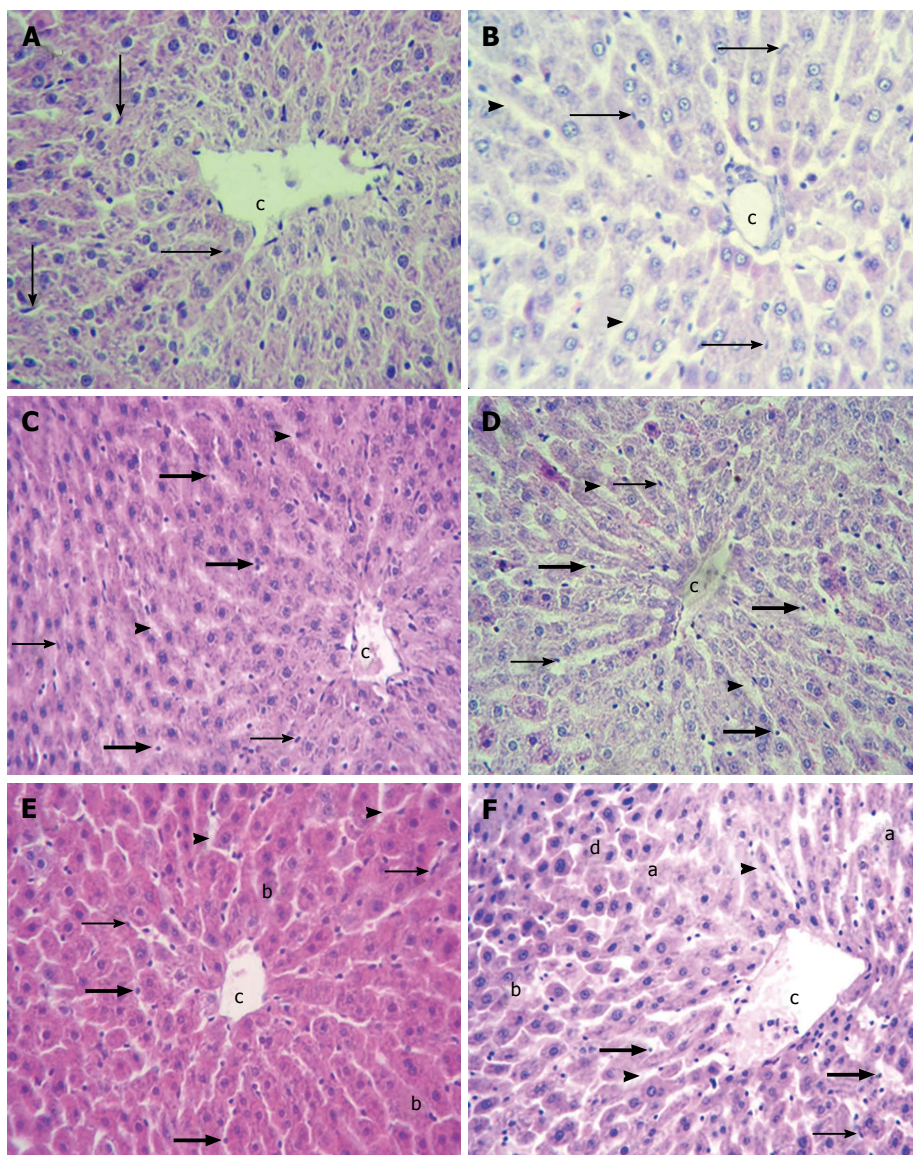
meters without the biological component. We observed an optimum exchange of fluids and metabolites. The Polyamix™ hollow fibers used allow adequate diffusive and convective mass transport. In order to evaluate the performance of these fibers against large size molecules we determined their permeability to plasma proteins. The experiments using FPLC showed that the pore size of the membranes used, with a cutoff value of 50 kDa, blocks the transfer of plasma proteins into the BC thus preventing damage of LMOs by the hypothetical patient's immune system proteins (antibodies, complement system).

After checking the system operation without any biological component, as a final step of the "*in vitro*" characterization of our BAL model, a validation step was performed, evaluating the performance of control and preserved LMOs in the MBR designed. The architecture chosen for the BAL we present here was not trivial; the BAL system in use in our laboratory, with isolated hepatocytes as biological component, was not suitable for LMOs which almost did not detoxify ammonia when applied to it (data not shown). As LMOs detoxification of ammonia on flat plates<sup>[11]</sup> was satisfactory, we decided to construct a "flat bottom" BAL to allow accommodating the tissue slices in a less crowded manner. In BAL devices designed to use LMOs, it is essential that the bioreactor architecture ensures a good viability of this biological component during the blood detoxification performance.

We observed that LDH releasing from LMOs cold preserved in Viaspan® was increasing with the perfusion time and this phenomenon was not observed for LMOs preserved in BG35 solution or controls. This fact can be attributed to a protective effect exerted by PEG 35000 kDa (key component of BG35 solution) on cell membranes<sup>[14,19,20]</sup>.

Observation of ammonia depuration is an evidence of hepatic synthetic function and is an important feature to propose the device we present here for clinical application<sup>[21,22]</sup>. When this MBR was challenged with an ammonia overload it showed an effective detoxification of this detrimental metabolite, either when cold preserved or fresh LMOs were examined. LMOs cold preserved in both preservation solutions were able to detoxify a similar percentage of the initial dose as compared to the control group. Although LMOs cold preserved in ViaSpan® showed higher levels of LDH release after 120 min of reperfusion they were able to detoxify an ammonium overload as well as control and cold preserved in BG35 solution LMOs did. Our group had already shown that, immediately after 48 h of cold preservation, ATP levels were severely decreased but they were actively replenished during reperfusion<sup>[23,24]</sup>. This fact can explain the good ammonium detoxification performance observed and constitute an indication of LMOs conserved mitochondrial function after cold preservation. Histological evaluation of LMOs showed that although BG35 protect hepatic morphology better than ViaSpan® solution, both cold preservation solutions proved to be useful to preserve the biological component integrity in our flat-plate model of MBR.

To provide a clear idea of the amount of ammonia that



**Figure 6 Liver microorgans histology.** Hematoxylin-eosin. Samples were taken from control and preserved groups at the beginning (A, C and E) and at the end ( $t = 120$  min) (B, D and F) of the experiment. Controls (A and B) showed normal hepatic parenchyma with fusiform endothelial cells (arrows) attached to perisinusoidal extracellular matrix and conserved hepatocyte cords. Sinusoids appeared dilated (bold arrow head) after 120 min. Liver microorgans (LMOs) preserved in BG35 (C and D) presented conserved hepatic architecture with fusiform (arrows) and rounded (bold arrows) endothelial cells. Sinusoids were dilated (bold arrow heads). LMOs preserved in ViaSpan® (E and F) had fusiform (arrows) and rounded (bold arrows) endothelial cells, dilated sinusoids (bold arrow head), and balonized hepatocytes (b) at the beginning of the experiment. F: After 120 min, blebs (a) and areas of hepatocyte trabecular disruption (d) were also found. Central Vein (CV). Magnification  $\times 200$ .  $n = 6$  LMOs independent preparations for each condition. c: Central vein.

LMOs were able to metabolize, we also determined the amount ( $\mu\text{mol}$ ) of this compound detoxified per gram of wet tissue during reperfusion. Once again we found similar levels of ammonium detoxification between control LMOs and LMOs cold preserved in Viaspan® or BG35 solution. The ammonium concentration in blood of patients with acute liver failure (ALF) could be greater than  $0.2 \text{ mmol/L}$  and it should be considered that also there is a continuous infusion of this metabolite to blood flow. In our *in vitro* experiments we used a higher concentration ( $1 \text{ mmol/L}$ ) since we worked with a single initial dose of ammonium. In addition, Calligaris *et al.*<sup>[25]</sup> showed that neither cell viability nor ammonium detoxification capacity of freshly isolated hepatocyte suspensions were affected by the

concentration of the initial ammonium overload.

It is important to consider that in this work we tested two preservation solutions: ViaSpan® which is the gold standard in liver preservation<sup>[26,27]</sup> and BG35 that was design by our group specifically to suit cold preservation of LMOs, and the entire liver in the future. The use of BG35 solution for the cold storage of LMOs may facilitate liver research since one litter of ViaSpan® is about 3 time more expensive than the same volume of BG35<sup>[11,14]</sup>.

The experimental MBR presented in this study relied on a simple design and was constructed using standard materials available in most laboratories. Due to these facts we foresee its employment as a useful tool to study the performance of LMOs submitted either



to preservation protocols or any other treatment or condition. Taking into account all the results previously shown, we have demonstrated that LMOs could be used as the biological component of the MBR designed, showing an adequate capacity to detoxify ammonia. We have also optimized the techniques to cold preserve this biocomponent to ensure its continuous availability, which is essential for any BAL to become a useful therapeutic tool for patients with ALF. As future prospects, these results encourage us to study other important liver functions, as transcription of albumin and clotting factors during reperfusion and to challenge it to treat acute liver failure of small animal models which will allow the measurement of bilirubin conjugation, blood clotting functions or intracranial pressure all important clinical prognostic predictors for ALF patients<sup>[28-30]</sup>. Also, to scale this MBR up and evaluate it in big animal models of ALF such as pigs.

## ACKNOWLEDGMENTS

The authors would like to thanks Dr. German Rosano for his technical support in FPLC analysis.

## COMMENTS

### Background

Acute liver failure is a condition that sometimes is resolved spontaneously but in most cases requires liver transplantation. The regeneration capacity of the liver seems to be behind the cases were, after the first insult has disappeared, the organ recovers by itself. This has been shown to be a consequence of the amount of viable mass remaining in terms of tissue capacity to cope with the detoxification of harmful metabolites produced by the damage and to provide the needed quantities of essential liver produced molecules and factors. This is why many attempts have been pursued to help the patient's liver to transit this acute failure and either recover or extend the time frame for a liver transplantation to be practical. In this sense, bioartificial liver (BAL) is thought as the better choice to accomplish this job but till now it is only performed by medical care teams that are able to obtain the biological component in the same unit making the practice limited to very few centers in the world.

### Research frontiers

A choice for the optimal biological component for BAL devices, as looking forward to develop a tool ready to use worldwide, is not straightforward. Hepatic derived cell lines, whole animal livers (even "humanized" organs) and primary human or animal hepatocytes have been proposed and tested but none have proven to be easily translatable to health centers reality. The work presented here proposes the use of tissue slices [liver Microorgans (LMOs)] and their preservation for at least 48 h in a preservation solution designed by their group. The obtainment of this biological component presents much less technical difficulty than isolation of viable hepatocytes and it bares all the cellular types and a conserved micro-architecture compared to the liver itself. The authors also show the extension of the period of use of these LMOs from few hours to 2 d and they are certain that it could be increased more by tuning the composition of their BG35 solution further.

### Innovations and breakthroughs

To date the reports found in the literature inform the use of isolated cells, either primary hepatocytes or continuous cell lines, or even whole pig livers and attempts have been made to cultivate the cellular component on artificial scaffolds mimicking extracellular matrices and micro-architecture. This biological components are used either fresh isolated or obtained directly by *in vitro* culturing. To the best of our knowledge, the authors are the only group using and combining tissue slices and cold preservation techniques to

successfully apply these LMOs onto BAL devices. The authors are still working with the dimensions of a mini-prototype that should be scaled up to be used for human patients and this is the future challenge the authors have to undertake.

## Applications

It follows that the application of their results would be the design of a BAL accessible on demand at low cost in health care centers for the treatment of patients, with either acute or chronic liver failure, for their recovery, or as a support until organ transplantation, and to ameliorate their quality of life in the process.

## Peer-review

This is an *in vitro* study for demonstration of the bio-artificial liver with detoxification. An interesting study for research design and innovation of the device.

## REFERENCES

- 1 Wang Y, Susando T, Lei X, Anene-Nzulu C, Zhou H, Liang LH, Yu H. Current development of bioreactors for extracorporeal bioartificial liver (Review). *Biointerphases* 2010; **5**: FA116-FA131 [PMID: 21171705 DOI: 10.1116/1.3521520]
- 2 Strain AJ, Neuberger JM. A bioartificial liver--state of the art. *Science* 2002; **295**: 1005-1009 [PMID: 11834813 DOI: 10.1126/science.1068660]
- 3 Han B, Shi XL, Zhang Y, Chu XH, Gu JY, Xiao JQ, Ren HZ, Tan JJ, Gu ZZ, Ding YT. Microbiological safety of a novel bio-artificial liver support system based on porcine hepatocytes: a experimental study. *Eur J Med Res* 2012; **17**: 13 [PMID: 22632261 DOI: 10.1186/2047-783X-17-13]
- 4 Giri S, Acikgöz A, Bader A. Isolation and Expansion of Hepatic Stem-like Cells from a Healthy Rat Liver and their Efficient Hepatic Differentiation of under Well-defined Vivo Hepatic like Micro-environment in a Multiwell Bioreactor. *J Clin Exp Hepatol* 2015; **5**: 107-122 [PMID: 26155038 DOI: 10.1016/j.jceh.2015.03.003]
- 5 Pan X, Wang Y, Yu X, Li J, Zhou N, Du W, Zhang Y, Cao H, Zhu D, Chen Y, Li L. Establishment and characterization of an immortalized human hepatic stellate cell line for applications in co-culturing with immortalized human hepatocytes. *Int J Med Sci* 2015; **12**: 248-255 [PMID: 25678842 DOI: 10.7150/ijms.11002]
- 6 Kostadinova R, Boess F, Applegate D, Suter L, Weiser T, Singer T, Naughton B, Roth A. A long-term three dimensional liver co-culture system for improved prediction of clinically relevant drug-induced hepatotoxicity. *Toxicol Appl Pharmacol* 2013; **268**: 1-16 [PMID: 23352505 DOI: 10.1016/j.taap.2013.01.012]
- 7 Ebrahimkhani MR, Neiman JA, Raredon MS, Hughes DJ, Griffith LG. Bioreactor technologies to support liver function in vitro. *Adv Drug Deliv Rev* 2014; **69-70**: 132-157 [PMID: 24607703 DOI: 10.1016/j.addr.2014.02.011]
- 8 Rodriguez JV, Pizarro MD, Scandizzi AL, Guibert EE, Almada LL, Mamprin ME. Construction and performance of a minibioreactor suitable as experimental bioartificial liver. *Artif Organs* 2008; **32**: 323-328 [PMID: 18370948 DOI: 10.1111/j.1525-1594.2007.00435.x]
- 9 Gershonowitz A, Itach EG, Shouval D, Mitrani D, Ilan Y, Mitrani E. Development of a scaled up liver device incorporating cryo-preserved pig liver micro-organs. *J Hepatol* 2004; **41**: 950-956 [PMID: 15582128 DOI: 10.1016/j.jhep.2004.08.016]
- 10 Guan N, Blomsma SA, van Midwoud PM, Fahy GM, Groothuis GM, de Graaf IA. Effects of cryoprotectant addition and washout methods on the viability of precision-cut liver slices. *Cryobiology* 2012; **65**: 179-187 [PMID: 22722061 DOI: 10.1016/j.cryobiol.2012.05.011]
- 11 Pizarro MD, Mediavilla MG, Berardi F, Tiribelli C, Rodríguez JV, Mamprin ME. Cold storage of liver microorgans in ViaSpan and BG35 solutions: study of ammonia metabolism during normothermic reoxygenation. *Ann Hepatol* 1979; **13**: 256-264 [PMID: 24552868]
- 12 Rodkey FL, Hill TA, Pitts LL, Robertson RF. Spectrophotometric measurement of carboxyhemoglobin and methemoglobin in blood. *Clin Chem* 1979; **25**: 1388-1393 [PMID: 455674]
- 13 Arnaud FG, Khirabadi BS, Fahy GM. Normothermic blood perfusion of isolated rabbit kidneys. III. In vitro physiology of kidneys after perfusion with Euro-Collins solution or 7.5 M cryoprotectant

- (VS4). *Transpl Int* 2002; **15**: 278-289 [PMID: 12072898 DOI: 10.1111/j.1432-2277.2002.tb00166.x]
- 14 **Mandolino C**, Pizarro MD, Quintana AB, Rodríguez JV, Mamprin ME. Hypothermic preservation of rat liver microorgans (LMOs) in bes-gluconate solution. Protective effects of polyethyleneglycol (PEG) on total water content and functional viability. *Ann Hepatol* 2011; **10**: 196-206 [PMID: 21502682]
- 15 **Mamprin ME**, Vega F, Rodríguez JV. Adenosine 5'triphosphate transport and accumulation during the cold preservation of rat hepatocytes in University of Wisconsin solution. *World J Gastroenterol* 2005; **11**: 1957-1964 [PMID: 15800986 DOI: 10.3748/wjg.v11.i13.1957]
- 16 **Mamprin ME**, Guibert EE, Rodríguez JV. Glutathione content during the rinsing and rewarming process of rat hepatocytes preserved in University of Wisconsin solution. *Cryobiology* 2000; **40**: 270-276 [PMID: 10860626 DOI: 10.1006/cryo.2000.2242]
- 17 **Olinga P**, Merema MT, Hof IH, De Jager MH, De Jong KP, Slooff MJ, Meijer DK, Groothuis GM. Effect of cold and warm ischaemia on drug metabolism in isolated hepatocytes and slices from human and monkey liver. *Xenobiotica* 1998; **28**: 349-360 [PMID: 9604299 DOI: 10.1080/004982598239461]
- 18 **van Anken HC**, Schiphorst ME. A kinetic determination of ammonia in plasma. *Clin Chim Acta* 1974; **56**: 151-157 [PMID: 4154813 DOI: 10.1016/0009-8981(74)90223-X]
- 19 **Faure JP**, Hauet T, Han Z, Goujon JM, Petit I, Maucou G, Eugene M, Carretier M, Papadopoulos V. Polyethylene glycol reduces early and long-term cold ischemia-reperfusion and renal medulla injury. *J Pharmacol Exp Ther* 2002; **302**: 861-870 [PMID: 12183641 DOI: 10.1124/jpet.102.033688]
- 20 **Giraud S**, Bon D, Neuzillet Y, Thuillier R, Eugene M, Hauet T, Barrou B. Concentration and chain length of polyethylene glycol in islet isolation solution: evaluation in a pancreatic islet transplantation model. *Cell Transplant* 2012; **21**: 2079-2088 [PMID: 22507302 DOI: 10.3727/096368912X638928]
- 21 **Shi XL**, Gao Y, Yan Y, Ma H, Sun L, Huang P, Ni X, Zhang L, Zhao X, Ren H, Hu D, Zhou Y, Tian F, Ji Y, Cheng X, Pan G, Ding YT, Hui L. Improved survival of porcine acute liver failure by a bioartificial liver device implanted with induced human functional hepatocytes. *Cell Res* 2016; **26**: 206-216 [PMID: 26768767 DOI: 10.1038/cr.2016.6]
- 22 **Gerlach JC**. Development of a hybrid liver support system: a review. *Int J Artif Organs* 1996; **19**: 645-654 [PMID: 8970832]
- 23 **Mamprin ME**, Petrocelli S, Guibert E, Rodríguez J. A novel BES-gluconate-sucrose (BGS) solution for cold storage of isolated hepatocytes. *Cryo Letters* 2008; **29**: 121-33 [PMID: 18516342]
- 24 **Miszcuk G**, Mediavilla MG, Pizarro MD, Tiribelli C, Rodríguez J, Mamprin ME. Expression and distribution of aquaporin 8 in rat hepatocytes cold stored 72 hours in modified University of Wisconsin and bes-gluconate-sucrose solutions. Study of their correlation with water content. *Cryo Letters* 2012; **33**: 75-85 [PMID: 22434125]
- 25 **Calligaris SD**, Almada LL, Guibert EE, Tiribelli C, Rodríguez JV. Ammonium detoxifying activity is maintained after 72 hours of cold preservation of rat hepatocytes in University of Wisconsin (UW) solution. *Cryo Letters* 2002; **23**: 245-254 [PMID: 12391485]
- 26 **Belzer FO**, Southard JH. Principles of solid-organ preservation by cold storage. *Transplantation* 1988; **45**: 673-676 [PMID: 3282347 DOI: 10.1097/00007890-198804000-00001]
- 27 **Southard JH**, Belzer FO. Control of canine kidney cortex slice volume and ion distribution at hypothermia by impermeable anions. *Cryobiology* 1980; **17**: 540-548 [PMID: 7471786 DOI: 10.1016/0011-2240(80)90068-1]
- 28 **Hoekstra R**, Nibourg GA, van der Hoeven TV, Plomer G, Seppen J, Ackermans MT, Camus S, Kulik W, van Gulik TM, Elferink RP, Chamuleau RA. Phase 1 and phase 2 drug metabolism and bile acid production of HepaRG cells in a bioartificial liver in absence of dimethyl sulfoxide. *Drug Metab Dispos* 2013; **41**: 562-567 [PMID: 23238784 DOI: 10.1124/dmd.112.049098]
- 29 **Hochleitner B**, Hengster P, Bucher H, Ladurner R, Schneeberger S, Krismer A, Kleinsasser A, Barnas U, Klima G, Margreiter R. Significant survival prolongation in pigs with fulminant hepatic failure treated with a novel microgravity-based bioartificial liver. *Artif Organs* 2006; **30**: 906-914 [PMID: 17181831 DOI: 10.1111/j.1525-1594.2006.00323.x]
- 30 **Selden C**, Spearman CW, Kahn D, Miller M, Figaji A, Erro E, Bundy J, Massie I, Chalmers SA, Arendse H, Gautier A, Sharratt P, Fuller B, Hodgson H. Evaluation of encapsulated liver cell spheroids in a fluidised-bed bioartificial liver for treatment of ischaemic acute liver failure in pigs in a translational setting. *PLoS One* 2013; **8**: e82312 [PMID: 24367515 DOI: 10.1371/journal.pone.0082312]

P- Reviewer: Chiu KW S- Editor: Qi Y L- Editor: A  
E- Editor: Li D





Case Control Study

## Pancreatic hyperechogenicity associated with hypoadiponectinemia and insulin resistance: A Japanese population study

Naohiko Makino, Nakao Shirahata, Teiichiro Honda, Yoshiaki Ando, Akiko Matsuda, Yushi Ikeda, Miho Ito, Yuko Nishise, Takafumi Saito, Yoshiyuki Ueno, Sumio Kawata

Naohiko Makino, Nakao Shirahata, Teiichiro Honda, Yoshiaki Ando, Akiko Matsuda, Yushi Ikeda, Miho Ito, Yuko Nishise, Takafumi Saito, Yoshiyuki Ueno, Department of Gastroenterology, Faculty of Medicine, Yamagata University, Yamagata 990-9585, Japan

Sumio Kawata, Department of Internal Medicine, Hyogo Prefectural Nishinomiya Hospital, Hyogo 662-0918, Japan

**Author contributions:** Makino N and Kawata S designed the research; Shirahata N, Honda T, Ando Y, Matsuda A, Ikeda Y, Ito M, Nishise Y, Saito T and Ueno Y performed the research; Makino N wrote the paper.

**Institutional review board statement:** The study was reviewed and approved by the Ethics Committee of Yamagata University Faculty of Medicine.

**Informed consent statement:** All participants provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** The authors declare that they have no conflict of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Naohiko Makino, MD, PhD, Associate

Professor, Department of Gastroenterology, Faculty of Medicine, Yamagata University, 2-2-2 Iida-Nishi, Yamagata 990-9585, Japan. [namakino@med.id.yamagata-u.ac.jp](mailto:namakino@med.id.yamagata-u.ac.jp)  
 Telephone: +81-23-6285307  
 Fax: +81-23-6285311

Received: June 27, 2016  
 Peer-review started: June 28, 2016  
 First decision: August 22, 2016  
 Revised: September 8, 2016  
 Accepted: October 17, 2016  
 Article in press: October 18, 2016  
 Published online: November 28, 2016

### Abstract

#### AIM

To examine the relationship between pancreatic hyperechogenicity and risk factors for metabolic syndrome.

#### METHODS

A general population-based survey of lifestyle-related diseases was conducted from 2005 to 2006 in Japan. The study involved 551 participants older than 40 year of age. Data for 472 non-diabetic adults were included in the analysis. The measures included the demographic factors, blood parameters, results of a 75 g oral glucose tolerance test, and abdominal ultrasonography. The echogenicity of the pancreas and liver was compared, and then the subjects were separated into two groups: cases with pancreatic hyperechogenicity ( $n = 208$ ) and cases without (controls,  $n = 264$ ). The differences between both groups were compared using an unpaired  $t$ -test or Fisher's exact test. Multiple logistic regression analysis was used to determine the relationship between the pancreatic hyperechogenicity and clinical and bio-

chemical parameters.

## RESULTS

Subjects with pancreatic hyperechogenicity had decreased serum adiponectin concentration compared to control subjects [8.9 (6.5, 12.8) *vs* 11.1 (7.8, 15.9),  $P < 0.001$ ] and more frequently exhibited features of metabolic syndrome. Logistic regression analysis showed that the following variables were significantly and independently associated with pancreatic hyperechogenicity: Presence of hypoadiponectinemia, increased body mass index (BMI), higher homeostasis model assessment of insulin resistance (HOMA-IR) score, and presence of fatty liver. Similar associations were also observed in subjects with pancreatic hyperechogenicity without fatty liver. Multivariate association analysis of data from participants without fatty liver showed that hypoadiponectinemia was significantly associated with pancreatic hyperechogenicity (OR = 0.93, 95%CI: 0.90 - 0.97,  $P < 0.001$ ). This association was independent of other confounding variables. Additionally, an increased BMI and higher HOMA-IR score were significantly associated with pancreatic hyperechogenicity.

## CONCLUSION

Pancreatic hyperechogenicity is independently associated with increased BMI, insulin resistance, and hypoadiponectinemia in the general population.

**Key words:** Pancreatic hyperechogenicity; Metabolic syndrome; Obesity; Adiponectin; The Takahata study

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Pancreatic hyperechogenicity is related to aging. Several recent studies have reported that hepatic steatosis and increased body mass index (BMI) are predictors of a hyperechogenic pancreas. In the present study, fatty liver was also significantly associated with pancreatic hyperechogenicity. We performed additional analyses excluding participants with fatty liver in order to account for the effect of this condition. Our analyses showed that an increased BMI, higher homeostasis model assessment of insulin resistance score, and decreased adiponectin were also significantly associated with pancreatic hyperechogenicity.

Makino N, Shirahata N, Honda T, Ando Y, Matsuda A, Ikeda Y, Ito M, Nishise Y, Saito T, Ueno Y, Kawata S. Pancreatic hyperechogenicity associated with hypoadiponectinemia and insulin resistance: A Japanese population study. *World J Hepatol* 2016; 8(33): 1452-1458 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i33/1452.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i33.1452>

## INTRODUCTION

Pancreatic hyperechogenicity may be related to the

aging process<sup>[1-3]</sup>. Previous studies have reported that increased echogenicity of the pancreas is associated with pancreatic lipomatosis<sup>[4,5]</sup>. Additionally, recent ultrasound studies have demonstrated pancreatic hyperechogenicity is correlated with obesity, hepatic steatosis<sup>[6,7]</sup>, and insulin resistance<sup>[8]</sup>. To our knowledge, there are a limited number of reports examining the relationship between pancreatic hyperechogenicity and lifestyle-related risk factors.

Recent studies have demonstrated that adipose tissue not only stores fat but also functions as an endocrine organ by producing various adipocytokines such as adiponectin. Adiponectin is a peptide hormone that plays a key role in the development of insulin resistance associated with metabolic syndrome<sup>[9]</sup>. Adiponectin levels are correlated directly with insulin sensitivity and are decreased in obese individuals and patients with type 2 diabetes<sup>[10,11]</sup>. Recent studies have shown that there is an association between low concentrations of adiponectin and cancer development<sup>[12,13]</sup>. However, no report has examined the relationship between adiponectin concentration and pancreatic hyperechogenicity.

The aim of the present study was to examine the associations between pancreatic hyperechogenicity and risk factors for metabolic syndrome, including adiponectin concentration, in the general Japanese population.

## MATERIALS AND METHODS

### Study population

The study was part of an ongoing molecular epidemiological project utilizing the regional characteristics of the 21<sup>st</sup> Century Centers of Excellence program in Japan, which has been previously described<sup>[14]</sup>. The surveyed population was the entire population of adults aged over 40 year in the town of Takahata, Yamagata prefecture, located in northeastern Japan. There were 551 participants enrolled in the study between 2005 and 2006. All participants received a physical examination, blood tests, a 75-g oral glucose tolerance test (OGTT), and abdominal ultrasonography (US). We excluded 79 of the 551 participants for the following reasons: Poor US images of the pancreas (43 individuals); blood samples collected in a non-fasting state (27 individuals); or OGTT-diagnosed diabetes mellitus based on the American Diabetes Association criteria (9 individuals)<sup>[15]</sup>. Exclusion criteria included a history of diabetes or pancreatic disease. However, no participants met the exclusion criteria. The data collected from 472 subjects (201 males and 271 females) were included in the final analysis.

### Measurements

We obtained information on current medication and lifestyle-related characteristics from all participants using a questionnaire. Trained study staff measured height, weight and systolic and diastolic blood pressure (BP) using standard methods. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters squared). Insulin resistance was evaluated using

the homeostasis model assessment of insulin resistance (HOMA-IR) method with the following equation:  $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting glucose (mg/dL)} / 405$ . Venous blood was drawn the morning after an overnight fast. The serum and plasma were separated immediately and stored at  $-80^\circ\text{C}$  until analysis. The serum adiponectin concentrations were measured by an enzyme-linked immunosorbent assay as described previously<sup>[9]</sup>. The other biochemical blood parameters were determined by standard laboratory procedures in the General Laboratory of BML, Inc. (Saitama, Japan). All laboratory personnel were blinded to the status of the samples.

All participants underwent US and an OGTT during a single visit at the research center and within 2 mo of the physical examination and initial blood collection. Blood samples were obtained to determine plasma glucose in the basal period and 120 min after an oral glucose load in the morning following an overnight fast.

The participants fasted overnight for the US study, and the scans were performed by one of four experienced operators using either a Toshiba Nemio™ scanner or an Aloka SSD-3500 scanner with a 3.5-MHz convex transducer. The participants were scanned while lying supine. The images were recorded on a standard computer hard disk drive. The recorded images were analyzed and judged simultaneously by four experienced physicians blinded to the details of the subjects, as described in a previous report<sup>[5]</sup>. The echogenicity of the pancreatic body was compared with the liver. The subjects were separated into cases with pancreatic echogenicity higher than hepatic echogenicity ( $n = 208$ ) and controls whose pancreatic echogenicity was equal to or lower than the liver ( $n = 264$ ). The presence of fatty liver was defined as a US pattern consistent with evidence of increased ultrasonographic contrast between the hepatic and renal parenchyma, vessel blurring, and narrowing of the lumen of the hepatic veins. We used these criteria to divide the subjects into cases with ( $n = 206$ ) or without ( $n = 266$ ) fatty liver.

### Statistical analysis

The distribution of the continuous variables was assessed for normality. If a normal distribution was evident, then the data were expressed as the means  $\pm$  standard deviation. The data with a non-normal distribution were log-transformed for analysis and expressed as the median with 25<sup>th</sup>/75<sup>th</sup> percentiles. The case and control groups were compared using the unpaired *t* test or Fisher's exact test. The relationship between pancreatic hyperechogenicity and clinical and biochemical parameters was determined using multiple logistic regression analyses with the backward elimination method. The odds ratio (OR) and 95%CI were then calculated.

The parameters in the multiple logistic regression analysis were categorized using the following cutoff values: BMI ( $< 25 \text{ kg/m}^2$ ,  $\geq 25 \text{ kg/m}^2$ ), systolic BP ( $< 130 \text{ mmHg}$ ,  $\geq 130 \text{ mmHg}$ ), diastolic BP ( $< 85 \text{ mmHg}$ ,  $\geq 85 \text{ mmHg}$ ), fasting plasma glucose ( $< 110 \text{ mg/dL}$ ,

$\geq 110 \text{ mg/dL}$ ), HOMA-IR score ( $< 2.0$ ,  $\geq 2.0$ ), high-density lipoprotein (HDL) cholesterol ( $< 40 \text{ mg/dL}$ ,  $\geq 40 \text{ mg/dL}$ ), triglyceride ( $< 150 \text{ mg/dL}$ ,  $\geq 150 \text{ mg/dL}$ ), glutamic pyruvic transaminase (GPT) ( $< 35 \text{ IU/L}$ ,  $\geq 35 \text{ IU/L}$ ), pre-load plasma glucose in the OGTT ( $< 110 \text{ mg/dL}$ ,  $\geq 110 \text{ mg/dL}$ ) and post-load 2-h plasma glucose in the OGTT ( $< 140 \text{ mg/dL}$ ,  $\geq 140 \text{ mg/dL}$ ). The median values for serum insulin and pancreatic isoamylase were used as the cutoff points. Serum adiponectin was analyzed as a continuous variable. All data were analyzed using SPSS software (version 15.0, SPSS Inc., Chicago, IL, United States). All differences with  $P < 0.05$  were considered statistically significant. The statistical methods of this study were reviewed by Yuko Nishise from the Faculty of Medicine, Yamagata University.

## RESULTS

The baseline clinical and biochemical data for the cases and controls are shown in Table 1. The serum adiponectin levels were markedly lower in the cases than in the controls [8.9 (6.5-12.8) vs 11.1 (7.8-15.9),  $P < 0.001$ ]. In addition, there were significant differences between the cases and controls for the following variables: Age, presence of fatty liver, weight, BMI, systolic BP, diastolic BP, serum insulin, fasting plasma glucose, HOMA-IR score, HDL cholesterol, total cholesterol, triglyceride, pancreatic isoamylase, GPT, pre-load plasma glucose in the OGTT, and 2-h plasma glucose.

Each parameter was dichotomized according to the cutoff points to further explore the relationship between pancreatic hyperechogenicity and other parameters. We then conducted multiple logistic regression analyses with the backward elimination method.

We first used an age-adjusted model to exclude the influence of aging. The results indicate that there was a significant negative association between decreased adiponectin levels and pancreatic hyperechogenicity (OR = 0.92, 95%CI: 0.88-0.95,  $P < 0.001$ ). In addition, the presence of fatty liver, higher values of BMI, systolic BP, diastolic BP, serum insulin, HOMA-IR score, 2-h plasma glucose and lower pancreatic isoamylase levels were significantly associated with pancreatic hyperechogenicity (Table 2).

We next performed the analysis after adjustment for age, presence of fatty liver, BMI, systolic and diastolic BP, adiponectin, serum insulin, HOMA-IR, triglyceride, pancreatic isoamylase, GPT, and 2-h plasma glucose. The analysis showed that hypoadiponectinemia was significantly associated with pancreatic hyperechogenicity (OR = 0.9, 95%CI: 0.91-0.98,  $P = 0.004$ ), independent of the other confounding variables. The presence of fatty liver, increased BMI, and higher HOMA-IR score were also significantly associated with pancreatic hyperechogenicity. In addition, decreased pancreatic isoamylase showed a weak relationship with pancreatic hyperechogenicity (Table 2).

We then performed further analyses to exclude the influence of fatty liver. The baseline clinical and

**Table 1 Comparison of baseline characteristics between the pancreatic hyperechogenicity and control groups**

Clinical parameters	Pancreatic hyperechogenicity ( <i>n</i> = 208)	Controls ( <i>n</i> = 264)	<i>P</i> value
Age (yr)	60.8 ± 9.4	56.9 ± 9.8	< 0.001
Fatty liver (fatty/non-fatty)	113/95	93/171	< 0.001
Sex (male/female)	86/122	115/149	0.640
Height (cm)	157.9 ± 8.4	159.3 ± 8.8	0.085
Weight (kg)	61.2 ± 9.4	56.2 ± 9.3	< 0.001
BMI (kg/m <sup>2</sup> )	24.4 ± 2.6	22.1 ± 2.7	< 0.001
Systolic BP (mmHg)	135.9 ± 15.8	129.6 ± 15.7	< 0.001
Diastolic BP (mmHg)	83.1 ± 10.2	79.6 ± 10.2	< 0.001
Adiponectin (μg/mL)	8.9 (6.5-12.8)	11.1 (7.8-15.9)	< 0.001
Serum insulin (μU/mL)	4.7 (3.4-6.8)	3.6 (2.7-5.0)	< 0.001
Fasting plasma glucose (mg/dL)	94.5 ± 9.2	90.8 ± 9.7	< 0.001
HOMA-IR	1.1 (0.7-1.6)	0.8 (0.6-1.1)	< 0.001
High-density lipoprotein cholesterol (mg/dL)	58.4 ± 14.2	63.2 ± 15.4	0.001
Low-density lipoprotein cholesterol (mg/dL)	127.1 ± 36.1	123.8 ± 32.4	0.310
Total cholesterol (mg/dL)	205.9 ± 33.1	199.8 ± 34.4	0.049
Triglyceride (mg/dL)	96 (71-135)	81 (63-112)	< 0.001
Pancreatic isoamylase (U/L)	28 (23-34)	30 (25-37)	0.014
Glutamic oxaloacetic transaminase (IU/L)	23 (20-27)	22 (19-28)	0.706
Glutamic pyruvic transaminase (IU/L)	21 (17-28)	20 (15-26)	0.011
γ-glutamyl transpeptidase (IU/L)	24 (17-42)	22 (15-33)	0.070
Preload plasma glucose (OGTT) (mg/dL)	97.4 ± 9.9	93.1 ± 10.2	< 0.001
2-h plasma glucose (OGTT) (mg/dL)	114.6 ± 29.7	101.1 ± 26.7	< 0.001

Data are expressed as means ± SD, or median (25<sup>th</sup>; 75<sup>th</sup> percentiles). Unpaired *t* test or Fisher's exact test were used to compare the two groups. BMI: Body mass index; BP: Blood pressure; HOMA-IR: Homeostasis model assessment of insulin resistance; OGTT: Oral glucose tolerance test.

**Table 2 Age-adjusted and multivariate odds ratios for pancreatic hyperechogenicity**

Confounding factor	Pancreatic hyperechogenicity ( <i>n</i> )	Controls ( <i>n</i> )	Age-adjusted		Multivariate <sup>3</sup>	
			Odds ratio (95%CI)	<i>P</i> value	Odds ratio (95%CI)	<i>P</i> value
Non-fatty liver/fatty liver	95/113	171/93	2.6 (1.8-3.9)	< 0.001	1.77 (1.15-2.72)	0.009
BMI (kg/m <sup>2</sup> ), < 25/≥ 25	125/83	229/35	5.0 (3.1-8.0)	< 0.001	3.56 (2.17-5.83)	< 0.001
Systolic BP (mmHg), < 130/≥ 130	60/148	119/145	1.6 (1.1-2.5)	0.016		
Diastolic BP (mmHg), < 85/≥ 85	110/98	180/84	1.9 (1.3-2.7)	0.001		
Adiponectin (μg/mL) <sup>1</sup>			0.92 (0.88-0.95)	< 0.001	0.9 (0.91-0.98)	0.004
Serum insulin (μU/mL) <sup>2</sup> , ≤ 4.0/> 4.0	83/125	153/111	2.2 (1.5-3.2)	< 0.001		
Fasting plasma glucose (mg/dL), < 110/≥ 110	198/10	251/13	0.8 (0.3-1.9)	0.593		
HOMA-IR, < 2.0/≥ 2.0	177/31	253/11	4.4 (2.1-9.1)	< 0.001	2.4 (1.1-5.1)	0.032
HDL cholesterol (mg/dL), ≥ 40/< 40	194/14	253/11	1.9 (0.8-4.5)	0.119		
Triglyceride (mg/dL), < 150/≥ 150	173/35	234/30	1.7 (1.0-2.9)	0.055		
Pancreatic isoamylase (U/L) <sup>2</sup> , ≥ 30/< 30	89/119	143/121	1.7 (1.2-2.5)	0.004	2.08 (0.95-4.57)	0.068
GPT (IU/L), < 35/≥ 35	177/31	234/30	1.7 (1.0-2.9)	0.069		
Preload plasma glucose (OGTT) (mg/dL), < 110/≥ 110	189/19	247/17	1.1 (0.6-2.3)	0.710		
2-h plasma glucose (OGTT) (mg/dL), < 140/≥ 140	170/38	244/20	2.4 (1.3-4.3)	0.003		

<sup>1</sup>Serum adiponectin was analyzed as a continuous variable; <sup>2</sup>The median values for serum insulin and pancreatic isoamylase were used as the cutoff points;

<sup>3</sup>Adjusted for the age, presence of fatty liver, BMI, systolic BP, diastolic BP, adiponectin, serum insulin, HOMA-IR, triglyceride, pancreatic isoamylase, GPT and 2-h plasma glucose. Odds ratios and 95%CI were estimated using the multiple logistic regression model with backward elimination. BMI: Body mass index; BP: Blood pressure; HOMA-IR: Homeostasis model assessment of insulin resistance; HDL: High-density lipoprotein; GPT: Glutamic pyruvic transaminase; OGTT: Oral glucose tolerance test.

biochemical data for cases and controls without fatty liver are shown in Table 3. The serum adiponectin levels were lower in cases than in controls [10.3 (7.6-14.6) vs 12.0 (8.6-17.0), *P* = 0.022]. Furthermore, there were significant differences between cases and controls for the following parameters: Age, weight, BMI, serum insulin, fasting plasma glucose, HOMA-IR score, HDL cholesterol, triglycerides, and preload plasma glucose in the OGTT.

We next performed multivariate association analyses of data from participants without fatty liver. The multi-

variate analysis showed that hypoadiponectinemia was significantly associated with pancreatic hyperechogenicity (OR = 0.93, 95%CI: 0.90-0.97, *P* < 0.001), independent of the other confounding variables. Additionally, an increased BMI and higher HOMA-IR score were also significantly associated with pancreatic hyperechogenicity (Table 4).

## DISCUSSION

Studies examining digestive organ disease and altered



**Table 3** Comparison of baseline characteristics between the pancreatic hyperechogenicity and control groups excluding participants with fatty liver

Clinical parameters	Pancreatic hyperechogenicity (n = 95)	Control (n = 171)	P value
Age (yr)	62 ± 9	58 ± 10	0.001
Sex (male/female)	44/51	76/95	0.769
Height (cm)	158.0 ± 8.0	158.1 ± 9.0	0.960
Weight (kg)	59.5 ± 9.4	53.9 ± 8.5	< 0.001
BMI (kg/m <sup>2</sup> )	23.7 ± 2.6	21.5 ± 2.5	< 0.001
Systolic BP (mmHg)	133 ± 17	130 ± 16	0.135
Diastolic BP (mmHg)	82 ± 11	80 ± 10	0.180
Adiponectin (μg/mL)	10.3 (7.6-14.6)	12.0 (8.6-17.0)	0.022
Serum insulin (μU/mL)	4.0 (2.7-5.4)	3.3 (2.5-4.5)	0.004
Fasting plasma glucose (mg/dL)	93 ± 8	90 ± 10	0.026
HOMA-IR	0.9 (0.59-1.28)	0.71 (0.54-1.02)	0.002
High-density lipoprotein cholesterol (mg/dL)	60 ± 15	66 ± 15	0.001
Low-density lipoprotein cholesterol (mg/dL)	124 ± 35	122 ± 33	0.708
Total cholesterol (mg/dL)	202 ± 33	199 ± 36	0.464
Triglyceride (mg/dL)	87 (66-133)	76 (59-96)	0.005
Pancreatic isoamylase (U/L)	29 (24-35)	31 (25-37)	0.054
Glutamic oxaloacetic transaminase (IU/L)	22 (19-25)	23 (19-28)	0.189
Glutamic pyruvic transaminase (IU/L)	19 (16-23)	20 (15-25)	0.937
γ-glutamyl transpeptidase (IU/L)	21 (16-40)	21 (15-32)	0.266
Preload plasma glucose (OGTT) (mg/dL)	96 ± 9	92 ± 10	0.009
2-h plasma glucose (OGTT) (mg/dL)	107 ± 29	100 ± 28	0.063

Data are expressed as means ± SD, or median (25<sup>th</sup>; 75<sup>th</sup> percentiles). Unpaired *t* test or Fisher's exact test were used to compare the two groups. BMI: Body mass index; BP: Blood pressure; HOMA-IR: Homeostasis model assessment of insulin resistance; OGTT: Oral glucose tolerance test.

**Table 4** Multivariate association analysis of clinical parameters for pancreatic hyperechogenicity excluding participants with fatty liver

Confounding factor	Multivariate <sup>2</sup>	
	Odds ratio (95%CI)	P value
BMI (kg/m <sup>2</sup> )	3.89 (2.39-6.35)	< 0.001
Adiponectin (μg/mL) <sup>1</sup>	0.93 (0.90-0.97)	< 0.001
HOMA-IR	2.23 (1.02-4.89)	0.045

<sup>1</sup>Serum adiponectin was analyzed as a continuous variable; <sup>2</sup>Adjusted for age, BMI, diastolic BP, adiponectin, serum insulin, HOMA-IR, HDL cholesterol, triglyceride and pancreatic isoamylase. Odds ratios and 95%CI were estimated using the multiple logistic regression model with backward elimination. CI: Confidence interval; BMI: Body mass index; HOMA-IR: Homeostasis model assessment of insulin resistance.

secretion of adipocytokines caused by metabolic syndrome will improve our understanding of the mechanisms involved in pathophysiological conditions. It is possible that such investigations might lead to the development of preventive measures for diseases linked to metabolic syndrome.

Pancreatic hyperechogenicity is thought to be related to the aging process<sup>[1-3]</sup>. Our data showed that several features of metabolic syndrome, such as higher BMI, increased HOMA-IR score, and hypoadiponectinemia, were also independently associated with pancreatic hyperechogenicity.

This is the first study conducted in a general population that investigated the relationship between pancreatic hyperechogenicity and risk factors of metabolic syndrome, such as insulin resistance and serum adiponectin concentration. The main study findings were: (1)

that serum adiponectin concentrations were markedly lower in subjects with pancreatic hyperechogenicity than in controls [8.9 (6.5-12.8) vs 11.1 (7.8-15.9), *P* < 0.001]; and (2) that decreased adiponectin levels were associated independently with pancreatic hyperechogenicity (OR = 0.9, 95%CI: 0.91-0.98, *P* = 0.004). Adiponectin is produced by adipose tissue, and a low adiponectin concentration is considered to be a key factor in the development of insulin resistance underlying metabolic syndrome<sup>[9-11]</sup>.

Several studies have reported that increased echogenicity of the pancreas is related to lipomatosis of the pancreatic parenchyma<sup>[4,5]</sup>. Pancreatic lipomatosis is the most common histological change in the pancreas associated with age, obesity, and insulin resistance<sup>[6-8,16-19]</sup>. Recently, Raeder *et al.*<sup>[5]</sup> evaluated the pancreatic fat content using US and magnetic resonance imaging and showed that pancreatic lipomatosis may reflect early events involved in the pathogenesis of diabetes and exocrine pancreatic dysfunction in non-diabetic children with mutations in carboxyl-ester lipase. In addition, Tushuizen and co-workers<sup>[20]</sup> measured the pancreatic fat content using proton magnetic resonance spectroscopy and found that pancreatic fat was inversely associated with β-cell function parameters in non-diabetic men. However, there was no association in their diabetic counterparts. The authors suggested that pancreatic fat content may contribute to β-cell dysfunction<sup>[20]</sup>. In our logistic regression analysis, we adjusted for age and pancreatic hyperechogenicity, which is a potential marker of lipomatosis. We found that these parameters were associated with higher serum insulin, HOMA-IR, and 2-h plasma glucose levels in the OGTT. There was

no association with fasting and preload plasma glucose concentrations in the OGTT (Table 2). It is possible that pancreatic hyperechogenicity with insulin resistance precedes the development of diabetes in the non-diabetic general population. Thus, further large-scale prospective studies are necessary to investigate whether pancreatic hyperechogenicity is an early pathological event in the diabetes disease process.

Several recent studies have reported that hepatic steatosis and increased BMI are predictors of a hyperechogenic pancreas<sup>[6,7]</sup>. In the present study, fatty liver was also significantly associated with pancreatic hyperechogenicity (OR = 1.77, 95%CI: 1.15-2.72, *P* = 0.009) (Table 2). Therefore, we performed additional analyses excluding participants with fatty liver in order to account for the effect of this condition in our results. The adjusted analysis showed that increased BMI, higher HOMA-IR score, and decreased adiponectin were also significantly associated with pancreatic hyperechogenicity (Table 4). This is the first study investigating the relationship between pancreatic hyperechogenicity and risk factors for metabolic syndrome by excluding the influence of fatty liver.

In this study, we used a simple and traditional method of assessing the severity of pancreatic hyperechogenicity, which was the comparison of echogenicity between the pancreatic body and the liver. However, this approach can potentially result in misdiagnosis of pancreatic hyperechogenicity if the extent of fatty liver is severe. We also performed additional analyses to exclude the influence of fatty liver. As shown in Tables 2 and 4, the results of our analyses that either included or excluded fatty liver, respectively, were similar and both showed increased BMI, higher HOMA-IR scores, and decreased adiponectin levels. However, our study had a limitation: No histological confirmation of pancreatic fat was possible.

There may be unknown factors that may cause the histological changes associated with obesity in addition to fat accumulation, fibrosis and functional changes in the exocrine pancreas. We recently demonstrated that intra-lobular fat accumulates in exocrine pancreatic tissue and that lipid droplets in acinar cells increase in Zucker diabetic fatty rats, which is an animal model of type 2 diabetes caused by the chronic intake of a high-fat diet. These conditions appear cause acinar cell injury and fibrosis<sup>[21]</sup>. Thus, additional clinical and experimental studies of the interrelationships between diabetes, metabolic syndrome and pancreatic injury should be conducted to clarify the pathogenesis of "non-alcoholic fatty pancreatic disease".

In conclusion, our study of a non-diabetic general population showed that pancreatic hyperechogenicity was independently associated with increased BMI, insulin resistance and hypoadiponectinemia.

## ACKNOWLEDGMENTS

We are grateful to all the participants and volunteers who enrolled in this study. We also thank Ms. Miho Ishii

and Dr. Mitsuru Emi for helpful advice.

## COMMENTS

### Background

Pancreatic hyperechogenicity is thought to be related to the aging process. However, little is known about the association between pancreatic hyperechogenicity and other life-style related risk factors.

### Research frontiers

Prior studies have reported that increased echogenicity of the pancreas is associated with pancreatic lipomatosis. Recent ultrasound studies have shown that pancreatic hyperechogenicity is correlated with obesity, hepatic steatosis, and insulin resistance.

### Innovations and breakthroughs

This is the first study investigating the relationship between pancreatic hyperechogenicity and risk factors for metabolic syndrome by excluding the influence of fatty liver.

### Applications

Pancreatic hyperechogenicity is independently associated with increased body mass index, insulin resistance, and hypoadiponectinemia in the general population. Pancreatic hyperechogenicity could be a useful marker of the metabolic syndrome.

### Peer-review

Accept the manuscript for publication without significant corrections.

## REFERENCES

- 1 **Worthen NJ**, Beabeau D. Normal pancreatic echogenicity: relation to age and body fat. *AJR Am J Roentgenol* 1982; **139**: 1095-1098 [PMID: 6983252 DOI: 10.2214/ajr.139.6.1095]
- 2 **Glaser J**, Stienecker K. Pancreas and aging: a study using ultrasonography. *Gerontology* 2000; **46**: 93-96 [PMID: 10671806 DOI: 10.1159/000022141]
- 3 **Silva ME**, Vezozzo DP, Ursich MJ, Rocha DM, Cerri GG, Wajchenberg BL. Ultrasonographic abnormalities of the pancreas in IDDM and NIDDM patients. *Diabetes Care* 1993; **16**: 1296-1297 [PMID: 8404436 DOI: 10.2337/diacare.16.9.1296]
- 4 **Marks WM**, Filly RA, Callen PW. Ultrasonic evaluation of normal pancreatic echogenicity and its relationship to fat deposition. *Radiology* 1980; **137**: 475-479 [PMID: 7433680 DOI: 10.1148/radiology.137.2.7433680]
- 5 **Raeder H**, Haldorsen IS, Ersland L, Gr ner R, Taxt T, S vik O, Molven A, N l stad PR. Pancreatic lipomatosis is a structural marker in nondiabetic children with mutations in carboxyl-ester lipase. *Diabetes* 2007; **56**: 444-449 [PMID: 17259390 DOI: 10.2337/db06-0859]
- 6 **Al-Haddad M**, Khashab M, Zyromski N, Pungpapong S, Wallace MB, Scolapio J, Woodward T, Noh K, Raimondo M. Risk factors for hyperechogenic pancreas on endoscopic ultrasound: a case-control study. *Pancreas* 2009; **38**: 672-675 [PMID: 19506531 DOI: 10.1097/MPA.0b013e3181a9d5af]
- 7 **Sepe PS**, Ohri A, Sanaka S, Berzin TM, Sekhon S, Bennett G, Mehta G, Chuttani R, Kane R, Pleskow D, Sawhney MS. A prospective evaluation of fatty pancreas by using EUS. *Gastrointest Endosc* 2011; **73**: 987-993 [PMID: 21521567 DOI: 10.1016/j.gie.2011.01.015]
- 8 **Lee JS**, Kim SH, Jun DW, Han JH, Jang EC, Park JY, Son BK, Kim SH, Jo YJ, Park YS, Kim YS. Clinical implications of fatty pancreas: correlations between fatty pancreas and metabolic syndrome. *World J Gastroenterol* 2009; **15**: 1869-1875 [PMID: 19370785 DOI: 10.3748/wjg.15.1869]
- 9 **Matsuzawa Y**. The metabolic syndrome and adipocytokines.

- FEBS Lett* 2006; **580**: 2917-2921 [PMID: 16674947 DOI: 10.1016/j.febslet.2006.04.028]
- 10 **Arita Y**, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; **257**: 79-83 [PMID: 10092513 DOI: 10.1006/bbrc.1999.0255]
  - 11 **Hotta K**, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1595-1599 [PMID: 10845877 DOI: 10.1161/01.ATV.20.6.1595]
  - 12 **Otake S**, Takeda H, Suzuki Y, Fukui T, Watanabe S, Ishihama K, Saito T, Togashi H, Nakamura T, Matsuzawa Y, Kawata S. Association of visceral fat accumulation and plasma adiponectin with colorectal adenoma: evidence for participation of insulin resistance. *Clin Cancer Res* 2005; **11**: 3642-3646 [PMID: 15897559 DOI: 10.1158/1078-0432.CCR-04-1868]
  - 13 **Otake S**, Takeda H, Fujishima S, Fukui T, Orii T, Sato T, Sasaki Y, Nishise S, Kawata S. Decreased levels of plasma adiponectin associated with increased risk of colorectal cancer. *World J Gastroenterol* 2010; **16**: 1252-1257 [PMID: 20222170 DOI: 10.3748/WJG.v16.i10.1252]
  - 14 **Konta T**, Hao Z, Abiko H, Ishikawa M, Takahashi T, Ikeda A, Ichikawa K, Takasaki S, Kubota I. Prevalence and risk factor analysis of microalbuminuria in Japanese general population: the Takahata study. *Kidney Int* 2006; **70**: 751-756 [PMID: 16807548 DOI: 10.1038/sj.ki.5001504]
  - 15 **Genuth S**, Alberti KG, Bennett P, Buse J, DeFronzo R, Kahn R, Kitzmiller J, Knowler WC, Lebovitz H, Lernmark A, Nathan D, Palmer J, Rizza R, Saudek C, Shaw J, Steffes M, Stern M, Tuomilehto J, Zimmet P. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003; **26**: 3160-3167 [PMID: 14578255 DOI: 10.2337/diacare.26.11.3160]
  - 16 **Olsen TS**. Lipomatosis of the pancreas in autopsy material and its relation to age and overweight. *Acta Pathol Microbiol Scand A* 1978; **86A**: 367-373 [PMID: 716899 DOI: 10.1111/j.1699-0463.1978.tb02058.x]
  - 17 **Schmitz-Moormann P**, Pittner PM, Heinze W. Lipomatosis of the pancreas. A morphometrical investigation. *Pathol Res Pract* 1981; **173**: 45-53 [PMID: 7335549 DOI: 10.1016/S0344-0338(81)80006-4]
  - 18 **Matsumoto S**, Mori H, Miyake H, Takaki H, Maeda T, Yamada Y, Oga M. Uneven fatty replacement of the pancreas: evaluation with CT. *Radiology* 1995; **194**: 453-458 [PMID: 7824726 DOI: 10.1148/radiology.194.2.7824726]
  - 19 **Lingvay I**, Esser V, Legendre JL, Price AL, Wertz KM, Adams-Huet B, Zhang S, Unger RH, Szczepaniak LS. Noninvasive quantification of pancreatic fat in humans. *J Clin Endocrinol Metab* 2009; **94**: 4070-4076 [PMID: 19773401 DOI: 10.1210/jc.2009-0584]
  - 20 **Tushuizen ME**, Bunck MC, Pouwels PJ, Bontemps S, van Waesberghe JH, Schindhelm RK, Mari A, Heine RJ, Diamant M. Pancreatic fat content and beta-cell function in men with and without type 2 diabetes. *Diabetes Care* 2007; **30**: 2916-2921 [PMID: 17666465 DOI: 10.2337/dc07-0326]
  - 21 **Matsuda A**, Makino N, Tozawa T, Shirahata N, Honda T, Ikeda Y, Sato H, Ito M, Kakizaki Y, Akamatsu M, Ueno Y, Kawata S. Pancreatic fat accumulation, fibrosis, and acinar cell injury in the Zucker diabetic fatty rat fed a chronic high-fat diet. *Pancreas* 2014; **43**: 735-743 [PMID: 24717823 DOI: 10.1097/MPA.0000000000000129]

P- Reviewer: Tretjakovs P S- Editor: Qi Y L- Editor: A  
E- Editor: Li D



Observational Study

## Neglected features of lifestyle: Their relevance in non-alcoholic fatty liver disease

Francesca M Trovato, Giuseppe Fabio Martines, Daniela Brischetto, Guglielmo Trovato, Daniela Catalano

Francesca M Trovato, Giuseppe Fabio Martines, Daniela Brischetto, Daniela Catalano, Department of Clinical and Experimental Medicine, Postgraduate School of Clinical Echography, the University of Catania, 95100 Catania, Italy

Guglielmo Trovato, Clinical Research and Innovation Project Planning Unit, the School of Medicine and AOU Policlinico, the University of Catania, 95100 Catania, Italy

Daniela Catalano, Department of Medicine, the School of Medicine of the University of Catania, 95100 Catania, Italy

**Author contributions:** The article was written by the authors stated.

**Institutional review board statement:** The study and the manuscript were approved by the Institutional Review Board of the Project Office.

**Informed consent statement:** Written informed consent was obtained from each patient prior to the clinical data recording and before the US procedure, allowing the use of information for teaching and clinical research.

**Conflict-of-interest statement:** No conflict of interest is declared in this invited manuscript.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Daniela Catalano, MD, Department of Medicine, the School of Medicine of the University of Catania,

Policlinico, Via Santa Sofia 78, 95100 Catania, Italy. [danielacatalano@unict.it](mailto:danielacatalano@unict.it)  
 Telephone: +39-953-781535

Received: May 30, 2016

Peer-review started: May 31, 2016

First decision: July 20, 2016

Revised: August 4, 2016

Accepted: October 22, 2016

Article in press: October 24, 2016

Published online: November 28, 2016

### Abstract

#### AIM

To investigate in non-alcoholic-fatty-liver-disease (NAFLD), with ultrasound (US)-detected fatty liver, and in a group of non-alcoholic and otherwise healthy subjects, relationship of neglected features of lifestyle with NAFLD and obesity.

#### METHODS

Five hundred and thirty-two NAFLD and 667 non-NAFLD healthy subjects, age 21-60 years were studied. Severity of liver steatosis was assessed by US bright liver score. The adherence to mediterranean diet score (AMDS) was assessed on the basis of a 1-wk recall computerized questionnaire which included a detailed physical activity reports (Baecke questionnaire). The western dietary profile score, as a simplified paradigm of unhealthy diet, a questionnaire quantifying sun exposure score and a sleep habits questionnaires provided a further comprehensive lifestyle assessment.

#### RESULTS

Body mass index (BMI), insulin resistance (HOMA), and triglycerides, poorer adherence to a mediterranean diet profile, sedentary habits, minor sun exposure and use of "western diet" foods are greater in NAFLD. Multiple



linear regression analysis, weighted by years of age, displays BMI, HOMA and AMDS as the most powerful independent predictors of fatty liver severity; however, also the physical activity score, the western diet habit and the sun exposure score are acting inside the model with significant independent effects.

### CONCLUSION

Articulated clinical intervention, according to our results, are justified in NAFLD and can be pursued addressing by focused intervention nutritional profile, physical exercise mainly in open-air subsets for enhancing sun exposure and healthier sleep duration and rhythm.

**Key words:** Fatty liver; Ultrasound; Diet; Malnutrition; Sleep; Clinical risk management; Health psychology; Sun exposure; Obesity

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Non-alcoholic-fatty-liver-disease (NAFLD) is a multifactorial condition associated with malnutrition and, mainly, with obesity, sedentary life and insulin resistance; some neglected factor, such as sleep and sun exposure curtailment, along with D vitamin deficiency, are associated with NAFLD; articulated clinical intervention, according to our results, is justified in NAFLD and can be pursued addressing by focused intervention nutritional profile, open-air physical exercise for enhancing sun exposure and healthy behaviour targeted to improved sleep duration and rhythm.

Trovato FM, Martines GF, Brischetto D, Trovato G, Catalano D. Neglected features of lifestyle: Their relevance in non-alcoholic fatty liver disease. *World J Hepatol* 2016; 8(33): 1459-1465 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i33/1459.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i33.1459>

## INTRODUCTION

Liver diseases, already in the past, were considered at least partly a consequence of unhealthy lifestyles and adverse environmental conditions, a concept that was very well addressed also by pathologists<sup>[1]</sup>. Lifestyle regards the use of the body functions related to physical exercise, exerted in work, love, leisure or sport, the quality and quantity of food, the sleep and rest rhythms, the exposure to hostile or unhealthy environmental factors, and other aspects, including fashion, clothing and non-sport leisure activity<sup>[2,3]</sup>. As in the past, the impact of the fashions and of beliefs based on alleged scientific statements and commercial information, namely publicity, is the key factor<sup>[4]</sup>. This framework, also by conditioning different lifestyles, reasonably affects the "establishment and maintenance of several diseases, including liver disease"<sup>[5]</sup>. In a very simplified manner today we tend to describe the lifestyles in medicine especially in terms

of diet and physical inactivity or sedentary life, with a synergistic effect on body size - obesity - and on disease related with excessive food intake (atherosclerosis and liver disease)<sup>[6]</sup>. Marketing strategies focus much on some related aspects that have an influence on nutrition and physical activity, but also with trade repercussions, while neglecting and avoiding other modes of social behavior. Some of these factors, such as sleep duration<sup>[7,8]</sup>, the sleeping patterns<sup>[9-12]</sup>, including shift-work related effects<sup>[7]</sup>, exposure to noise<sup>[13,14]</sup>, the level of social alarm about events or situations<sup>[15]</sup>, the possibility of urban mobility<sup>[16,17]</sup>, may have determinant effects on nutritional profiles and exercise implementation. Communication and perception of risks, as traditionally recognized, are flanked by communication and induction of apparently neutral behavior that can behave as true risk factors for disease. The strong pressure towards practices aimed at optimizing physical fitness and dietary methods aimed at healthy foods often involves forms of orthorexia<sup>[18]</sup>; such strategies are widely used to gain and maintain niches of food and fitness markets. All this would be irrelevant, except that, as in the case of prevention of obesity and fatty liver, and probably also in the field of atherosclerotic, neurodegenerative and cancer diseases, dietary caloric intake and a sedentary lifestyle are not the only factors exerting independent synergistic effects<sup>[6]</sup>. In fact, even the dietary profiles<sup>[19]</sup>, methods of exercise implementation<sup>[20,21]</sup>, and other related factors, such as sleep deprivation<sup>[4]</sup>, D vitamin deficiency and exposure to sunlight<sup>[22]</sup>, environmental noise<sup>[16]</sup>, and reasonably also others, seem to be part of an interrelated group of neglected risk factors, which only now are beginning to be studied more methodically.

Aim of our research is to investigate if some of the above mentioned neglected behavioural factors, concurrently with nutritional and physical exercise profile, may be associated or contribute independently as factors related to fatty liver in a group of non-alcoholic and otherwise healthy subjects with ultrasound (US)-detected fatty liver.

## MATERIALS AND METHODS

### Patients

Five hundred thirty-two non-alcoholic-fatty-liver-disease (NAFLD) and 667 non-NAFLD subjects (women 749, men 450, total 1199), age 21-60 years, without relevant acute or chronic disease, as below detailed in the exclusion criteria, were studied. These patients were consecutively referred to the same out-patients public medical unit (day-hospital) for lifestyle-nutritional prescription addressed to the management of minor digestive disease (mainly gastro-esophageal reflux syndrome or irritable bowel syndrome), overweight or obesity. The subjects were enrolled throughout January 2008-December 2015, were all patients first-time visitors, had not had previous referral or intervention in our unit, and were studied by a comprehensive US assessment (liver-abdomen, heart, thyroid and lung),

according to our current practice<sup>[3]</sup>. Exclusion criteria: (1) all patients with signs of moderate-severe congestive heart failure, previous myocardial infarction, idiopathic cardiomyopathy, pericarditis, malignancies; (2) severe chronic liver disease, apart from the lone finding of bright liver; abnormal aminotransferase levels at the beginning of this study, defined as alanine transaminase (ALT) > 30 IU/L in men and ALT > 19 IU/L in women; acute or chronic virus hepatitis, which were excluded by concurrent laboratory assays, as below detailed; (3) any history of diabetes mellitus (fasting glucose  $\geq$  126 mg/dL or HbA1c  $\geq$  6.5%) or drug intake of anti-diabetic drugs, particularly metformin; (4) extreme obesity [body mass index (BMI)  $\geq$  40] and underweight bad-nourished profile (BMI < 18.5 or serum albumin < 3 g/dL); (5) acute and/or chronic infectious, rheumatic or autoimmune disease; and (6) alcohol abuse (exceeding 20 g/d on a weekly base); renal insufficiency, *i.e.*, glomerular filtration rate < 90 mL/min per 1.73 m<sup>2</sup> and/or proteinuria > 0.10 g/d. According to these exclusion criteria 1508 further subjects, potentially but only partially eligible, are excluded by this study.

#### Laboratory/imaging methods

The severity of liver steatosis was assessed by US bright liver score (BLS), graded 1-3: grade 0 was the absence of bright liver, *i.e.*, a normal pattern<sup>[23]</sup>, BLS was and previously validated by US-guided fine needle aspirate biopsy by 20 Gauge Menghini's needles<sup>[3]</sup>; GE echo color Doppler machines (GE Logiq 5/Vivid7 Expert US manufactured by GE Medical Systems, Milwaukee, WI, United States), high resolution, equipped with real-time convex, phased array and linear scan transducers, were used throughout this study.

Routine laboratory tests included virus hepatitis (hepatitis A, B and C virus, *i.e.*, HAV, HBV and HCV) and cancer biomarkers (Alpha-fetoprotein, CEA, Ca125, Ca 19-9, Ca15-3), thyroid hormones FT3 FT4, thyroid-stimulating hormone, aspartate aminotransferase, ALT,  $\gamma$ -glutamyl transpeptidase, ferritin, total protein, and albumin. Mediterranean diet adherence profile was assessed by the adherence to mediterranean diet score (AMDS) on the basis of a 1-wk recall computerized questionnaire<sup>[3,5]</sup> using a pictogram-based method of visualizing dietary intake, descriptive also of the size of the single portion; pictograms includes also items for the quantification of physical activity, which is otherwise quantified by detailed physical activity reports (Baecke questionnaire)<sup>[5]</sup>. The Western Dietary Profile score, as a simplified paradigm of unhealthy diet, was assessed submitting a specific questionnaire, which is reported in Appendix; also the Baecke's physical activity questionnaire is briefly described in appendix, and subsequently the total score was studied by statistical analysis. The questionnaires submitted for quantifying sun exposure score, used mainly as an index of the open air activity and sleep habits questionnaires are routinely included within the context of a comprehensive lifestyle

assessment, and detailed in appendix. The study and the manuscript were approved by the institutional review board of the project office. No conflict of interest is to be declared for this invited manuscript. Written informed consent was obtained from each patient prior to the clinical data recording and before the US procedure, allowing the use of information for teaching and clinical research. Detail that might disclose the identity of the subjects under study is carefully omitted in any part of the study.

#### Statistical analysis

Comparison of data between the two groups of patients, NAFLD vs controls, is reported and differences assessed by Student's *t* test. Subsequently: (1) receiver operating characteristic (ROC) curve analysis of data of controls vs NAFLD subjects is used for defining the optimal cut-offs which may distinct the two group. The performance of each measure in prediction of NAFLD was evaluated by ROC curve. The area under the ROC curve and the 95%CI were used as indexes of accuracy. The optimal cut-off value was determined with maximum sum of sensitivity and specificity. For the purpose of identifying such thresholds, the measures used were BMI, HOMA, AMDS, western diet score (WDS), Physical activity Baecke's total score, sun exposure score, and sleep daily hours, calculated on a weekly base; (2) contingency tables and odds ratio of NAFLS vs non-NAFLD were calculated, according to each defined cut-off; and (3) MLR analysis, weighted by age, using BMI, HOMA, AMDS, WDS, physical activity baecke's total score, Sun exposure score, sleep hours vs BLS score of fatty liver was at last performed.

## RESULTS

The two groups of patients were comparable for age (Table 1), while other measures, such as BMI, HOMA and Triglycerides are greater in NAFLD. Comparison of data between the two groups of patients, NAFLD vs controls, is reported in detail (Table 1): A poorer adherence to a mediterranean diet profile, greater sedentary habits and greater use of "western diet" foods are the main differences. Moreover, liver size and, obviously, detection of fatty liver are the main US feature distinctive of the two groups. The ROC curve analysis graph of the data of controls vs NAFLD subjects for BMI, HOMA, HDL Cholesterol is displayed in Figure 1.

The most suitable thresholds distinctive of NAFLD vs controls are, in our population: BMI  $\geq$  26.40, HOMA  $\geq$  1.87, HDL < 54.50, TGL  $\geq$  94, AMDS < 34, WDS  $\geq$  15.5, physical activity Baecke's total score < 41.5, Sun exposure score SES < 34.5, and sleep daily hours, calculated on a weekly base sleep hours < 8.0. Contingency tables and Odds ratio were calculated for NAFLD vs controls, according to above defined thresholds. Greater prevalence of overweight-obesity, insulin resistance, increased triglycerides and low HDL cholesterol, poor adherence

**Table 1** Differences between non-alcoholic-fatty-liver-disease and control patients

	NAFLD (n = 532)	Controls (n = 667)	P value
Age, yr	48.11 ± 9.00	48.60 ± 8.70	0.343
Systolic blood pressure (mmHg)	124.53 ± 9.71	121.21 ± 10.80	< 0.0001
Diastolic blood pressure (mmHg)	78.84 ± 6.72	76.50 ± 6.73	< 0.0001
BMI, kg/m <sup>2</sup>	30.49 ± 5.55	24.44 ± 3.72	< 0.0001
HOMA	2.89 ± 1.76	1.80 ± 1.28	< 0.0001
eGFR	82.49 ± 14.15	82.15 ± 17.44	0.714
Total cholesterol, mg/dL	205.17 ± 37.16	207.09 ± 38.82	0.387
HDL cholesterol, mg/dL	51.67 ± 15.85	61.45 ± 16.41	< 0.0001
Triglycerides, mg/dL	109.08 ± 42.41	95.23 ± 58.59	< 0.0001
LDL cholesterol, mg/dL	131.98 ± 33.45	126.59 ± 37.29	0.009
γ-GT (U/L)	33.24 ± 29.40	26.03 ± 21.95	< 0.0001
AST (U/L)	20.77 ± 5.91	21.01 ± 7.10	0.530
ALT (U/L)	15.65 ± 4.60	10.40 ± 4.88	< 0.0001
Alkaline phosphatase (U/L)	68.37 ± 18.49	72.75 ± 43.42	0.030
Serum albumin (g/dL)	4.62 ± 0.39	4.53 ± 0.40	< 0.0001
Lifestyle items			
AMDS	32.21 ± 0.91	34.91 ± 0.61	< 0.0001
Baecke - physical activity	39.82 ± 3.60	41.43 ± 3.32	< 0.0001
total score			
Western diet score	22.84 ± 7.87	12.73 ± 2.48	< 0.0001
Sun exposure score	31.43 ± 3.89	35.73 ± 5.25	< 0.0001
Sleep hours	7.86 ± 1.31	7.90 ± 1.23	0.552

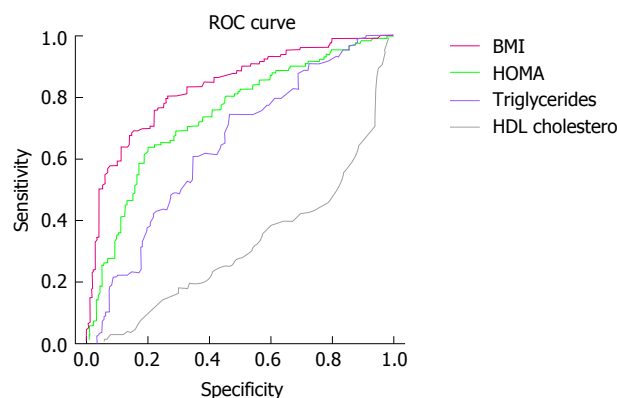
BMI: Body mass index; HOMA-IR: Homoeostasis model insulin resistance; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; γ-GT: γ-glutamyl transpeptidase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AMDS: Adherence mediterranean diet score; NAFLD: Non-alcoholic-fatty-liver-disease; eGFR: Estimated glomerular filtration rate.

to mediterranean diet profile, greater use of Western diet food, greater sedentary life habits and minor sun exposure, open air time were observed (Table 2).

Multiple Linear regression analysis (Table 3), weighted by years of age for avoiding age as a potential confounding factor, using the same items as predictors of the severity of fatty liver, assessed by US as BLS, confirms the significance of the chosen model, displaying BMI, HOMA and AMDS as the most powerful predictors of fatty liver severity; also the physical activity score, the western diet habit and the sun exposure score are still inside the model, with significant independent effects. The number of sleep hours does not show any significant linear effect in the model. Nonetheless, in a separate analysis, sleep hours display a U shaped behaviour, showing a greater relationship with more severe fatty liver at the two extremes of the curve: Few and many hours of sleep are both associated with more severe fatty liver.

## DISCUSSION

Currently, overweight and obesity are the most established associated factors of NAFLD, and are considered, even with some limitation, actual risk factors and putative, indirect causative factors<sup>[2,3]</sup>. Nonetheless, other and quite neglected factors were and are studied: Most of



**Figure 1** Receiver operating characteristic curves of body mass index, homoeostasis model insulin resistance, Triglycerides and high-density lipoprotein - cholesterol. The performance of each measure in the prediction of NAFLD is evaluated by the receiver operating characteristic (ROC) curve. The area under the ROC curve (AUROC) and the 95%CI are used as indexes of accuracy. The optimal cutoff value is determined as the maximum sum of sensitivity and specificity. Accordingly, BMI displays the greater accuracy for predicting NAFLD in comparison of HOMA, Triglycerides and HDL-Cholesterol. The cutoffs are used as thresholds for the calculation of odds of NAFLD, as reported in Table 2. BMI: Body mass index; HOMA: Homoeostasis; HDL: High-density lipoprotein; NAFLD: Non-alcoholic-fatty-liver-disease.

them are related to behaviour, such as physical activity<sup>[5]</sup>, sleep habits<sup>[4]</sup> and Sun exposure, this last with a likely effects on vitamin D status<sup>[22]</sup>. Nutrition has a qualitative profile, and not only a quantitative one, *i.e.*, not only caloric intake, so that the association of unhealthy dietary habits, apart the abuse of alcohol, is associated with unhealthy liver and, notably, NAFLD. This is confirmed in our study in which we observe that, apart the greater BMI, also a poorer adherence to mediterranean diet profile<sup>[5]</sup>, widely and since several years used as a proxy of healthy diet, strongly predicts the occurrence of NAFLD, independently from overweight. Also the almost reciprocal western diet profile displays an unfavourable relationship for the occurrence of NAFLD. This is confirmed in our study by the significant difference of averages, with a greater WDS in NAFLD (Table 1), by the greater odds of NAFLD associated with greater BMI and western diet habits, and with lower adherence to mediterranean diet (Table 2). Moreover, by a model of multivariate analysis (Table 3) the effects of BMI, mediterranean diet and western diet are independently operating, addressing clearly to the opposite effects of mediterranean diet (favourable) and of western diet and overweight (detrimental). Concurrently with nutritional profiles and BMI, sedentary life, assessed quantitatively as physical activity score, displays the same effects: A better physical exercise profile is associated with a lower prevalence (Table 2) and severity of bright liver score (Table 3), as assessed in NAFLD by liver US. Physical activity score is overall poorer in NAFLD vs controls (Table 1). The same association is observed for the sun exposure score, which is greater in controls (Table 1) and which may indicate, apart a greater open air life, also a better D vitamin status, important because vitamin D deficiency is associated with NAFLD<sup>[22]</sup>. Differently from

**Table 2** Pearson's  $\chi^2$  and odds ratio

	NAFLD	Controls	$\chi^2$	P value	OR	95%CI
BMI $\geq$ 26.40	408	167	316.385 <sup>1</sup>	< 0.0001	9.851	7.546-12.861
BMI < 26.40	124	500				
HOMA $\geq$ 1.87	368	211	167.011 <sup>1</sup>	< 0.0001	4.849	3.792-6.202
HOMA < 1.87	164	456				
HDL $\geq$ 54.50	204	400	55.358 <sup>1</sup>	< 0.0001	0.415	0.329-0.524
HDL < 54.50	328	267				
TGL $\geq$ 94	324	240	73.775 <sup>1</sup>	< 0.0001	2.771	2.191-3.506
TGL < 94	208	427				
AMDS $\geq$ 34	32	650	1008.831 <sup>1</sup>	< 0.0001	0.002	0.001-0.003
AMDS < 34	500	17				
BAECKE $\geq$ 41.5	181	354	43.468 <sup>1</sup>	< 0.0001	0.456	0.360-0.577
BAECKE < 41.5	351	313				
WDS $\geq$ 15.5	399	97	445.981 <sup>1</sup>	< 0.0001	17.629	13.174-23.590
WDS < 15.5	133	570				
SES $\geq$ 34.5	111	348	122.788 <sup>1</sup>	< 0.0001	0.242	0.187-0.313
SES < 34.5	421	319				
Sleep hours $\geq$ 8	319	370	2.592 <sup>1</sup>	0.107	1.210	0.959-1.527
Sleep hours < 8	208	292				

<sup>1</sup>Indicates the thresholds calculated by ROC analysis used as cut-offs for comparison between groups with lower measures (BMI, HOMA, AMDS, WDS, SES, BAECKE) *vs* groups with greater measures. BMI: Body mass index; HOMA-IR: Homoeostasis model insulin resistance; HDL: High-density lipoprotein; TGL: Triglycerides; AMDS: Adherence to mediterranean diet score; WDS: Western diet score; SES: Sun Exposure Score; BAECKE: Baecke's physical activity questionnaire total score.

**Table 3** Multiple linear regression of variables

Predictors	R	R <sup>2</sup>	F	Sig.	$\beta$	P value
	<b>0.965</b>	<b>0.932</b>	<b>2309.1</b>	<b>&lt; 0.0001</b>		
BMI, kg/m <sup>2</sup>					-0.448	< 0.0001
HOMA					-0.393	< 0.0001
AMDS					-1.398	< 0.0001
Baecke					-0.074	< 0.0001
WDS					0.069	< 0.0001
Sun exposure score					-0.044	< 0.0001
Sleep hours					-0.008	0.296

Weighted Least Squares Regression - Weighted by Age. Baecke's physical activity questionnaire total score and sleep hours *vs* the severity of NAFLD (included in this analysis as a categorical variable with all 3 severity grades), assessed by ultrasound as bright liver score. BMI: Body mass index; HOMA-IR: Homoeostasis model insulin resistance; AMDS: Adherence to mediterranean diet score; WDS: Western diet score; NAFLD: Non-alcoholic-fatty-liver-disease.

the observation reported in youngsters<sup>[4]</sup>, sleep hours do not show any significant relationship with NAFLD.

We must acknowledge several limitations of our study. First, the overall, comparison between NAFLD patients and controls (Table 1) does not display extreme differences, even if they are statistically significant, when considering sleep hours, sun exposure, AMDS and physical activity. There are very different features considering the greater score of Western Diet profile pattern in NAFLD. These even small differences between NAFLD and controls become more relevant within the model that takes into account all the co-variates, so that we must still consider them as relatively important features regarding NAFLD, even envisaging a size effect in the group studied.

Second limitation is that our eligibility criteria were rather strict, resulting in a population without significant co-morbidities, since all patients with diabetes and/or even minimally elevated ALT levels were excluded. It is possible that the analyzed lifestyle measures might work differently in a more comprehensive NAFLD cohort that includes other associated diseases. Scope of the study was to investigate NAFLD as an almost-isolated disease, and even with these restrictions association of recognized and neglected aspects of lifestyle are seemingly operating.

Modification over the time of healthier nutritional and behavioural profiles is a very articulated topic of investigation, which includes also the need of assessing the process of erosion of traditionally cohesive family and community relationships<sup>[24]</sup> with effects on health and mortality. Such studies have a counterpart in the current societal efforts aimed at the preservation of traditional habits, and even clinical conditions, such as high hemoglobin levels<sup>[25]</sup> which often are credited as healthier. Many animal models have been studied in which dietary variations produce liver injury, and by extrapolation, malnutrition, particularly deficiencies of protein and vitamins has long been considered an important factor in human cirrhosis when no evidence existed for another aetiology; by contrast, weight reduction through low-calorie diets or starvation reduces the steatosis resulting from obesity<sup>[1]</sup>. Malnutrition was in the last century, and now again, the key of many disease and, notably of liver disease, with its paradigm of fatty liver evolving toward fibrosis. Apart the pioneering studies on lifestyle changes<sup>[26]</sup> we are still on the starting blocks because each aspect of lifestyle is studied, and thereafter assessed and managed as



an individual factor. Despite the great attention which is devoted in Europe to healthier environment and to urban mobility, using the paradigm of smart city, few or no research are at the moment published and available, even if elsewhere there is already a move in this sense also by comprehensive approach focused to clinical risk assessment and management<sup>[2]</sup>. The important most recent reviews appropriately address benefits of healthy diet and exercise on NAFLD<sup>[27]</sup> both in adults<sup>[28]</sup> and in children<sup>[29]</sup>, even if other factors, genetic<sup>[30]</sup>, behavioural and environmental should not be neglected<sup>[31,32]</sup>. The opportunity for the medicine are relevant since articulated clinical intervention, which, according to our results, are justified, can be pursued with a focus on nutritional profile, physical exercise mainly open-air for enhancing sun exposure and improving sleep duration and rhythm<sup>[33]</sup>, cultural and traditional medicine issues and, comprehensively, the quality of life<sup>[34-39]</sup>. The pre-requisite is that both medical doctor and patient should not be mucking around in search of the magic bullet, and instead try to take seriously and with a strategy the road of lasting lifestyle change. Individual, professional and societal benefits are the outcomes that can be reached<sup>[2]</sup>.

## COMMENTS

### Background

In a very simplified manner today the authors tend to describe the lifestyles in medicine especially in terms of diet and physical inactivity or sedentary life, with a synergistic effect on body size - obesity - and on disease related with excessive food intake (atherosclerosis and liver disease).

### Research frontiers

Many animal models have been studied in which dietary variations produce liver injury, and by extrapolation, malnutrition; particularly deficiencies of protein and vitamins has long been considered an important factor in human cirrhosis when no evidence existed for another aetiology; by contrast, weight reduction through low-calorie diets or starvation reduces the steatosis resulting from obesity.

### Innovations and breakthroughs

This is confirmed in their study in which they observe that, apart the greater BMI, also a poorer adherence to mediterranean diet profile, widely and since several years used as a proxy of healthy diet, strongly predicts the occurrence of non-alcoholic-fatty-liver-disease (NAFLD), independently from overweight. Also the almost reciprocal western diet profile displays an unfavourable relationship for the occurrence of NAFLD. This is confirmed in our study by the significant difference of averages, with a greater western diet score in NAFLD, by the greater odds of NAFLD associated with greater body mass index and western diet habits, and with lower adherence to mediterranean diet.

### Applications

The opportunity for the medicine is relevant since articulated clinical intervention, which, according to their results, are justified, can be pursued with a focus on nutritional profile, physical exercise mainly open-air for enhancing sun exposure and improving sleep duration and rhythm, cultural and traditional medicine issues and, comprehensively, the quality of life. The pre-requisite is that both medical doctor and patient should not be mucking around in search of the magic bullet, and instead try to take seriously and with a strategy the road of lasting lifestyle change. Individual, professional and societal benefits are the outcomes that can be reached.

### Peer-review

The manuscript of "Neglected features of lifestyle: Their relevance in non-

alcoholic fatty liver disease" is very interesting.

## REFERENCES

- 1 **Popper H**, Schaffner F. Nutritional cirrhosis in man? *N Engl J Med* 1971; **285**: 577-578 [PMID: 5560571 DOI: 10.1056/NEJM197109022851010]
- 2 **Trovato FM**, Catalano D, Musumeci G, Trovato GM. 4Ps medicine of the fatty liver: the research model of predictive, preventive, personalized and participatory medicine-recommendations for facing obesity, fatty liver and fibrosis epidemics. *EPMA J* 2014; **5**: 21 [PMID: 25937854 DOI: 10.1186/1878-5085-5-21]
- 3 **Catalano D**, Trovato GM, Martines GF, Randazzo M, Tonzuso A. Bright liver, body composition and insulin resistance changes with nutritional intervention: a follow-up study. *Liver Int* 2008; **28**: 1280-1287 [PMID: 18435716 DOI: 10.1111/j.1478-3231.2008.01742.x]
- 4 **Trovato FM**, Martines GF, Brischetto D, Catalano D, Musumeci G, Trovato GM. Fatty liver disease and lifestyle in youngsters: diet, food intake frequency, exercise, sleep shortage and fashion. *Liver Int* 2016; **36**: 427-433 [PMID: 26346413 DOI: 10.1111/liv.12957]
- 5 **Trovato FM**, Catalano D, Martines GF, Pace P, Trovato GM. Mediterranean diet and non-alcoholic fatty liver disease: the need of extended and comprehensive interventions. *Clin Nutr* 2015; **34**: 86-88 [PMID: 24529325 DOI: 10.1016/j.clnu.2014.01.018]
- 6 **Trovato GM**. Clinical research and methodology. The paradigm of fatty liver and atherosclerosis behind the chicken or the egg dilemma. *Atherosclerosis* 2016; **249**: 228-229 [PMID: 27012655 DOI: 10.1016/j.atherosclerosis.2016.02.031]
- 7 **Kim CW**, Yun KE, Jung HS, Chang Y, Choi ES, Kwon MJ, Lee EH, Woo EJ, Kim NH, Shin H, Ryu S. Sleep duration and quality in relation to non-alcoholic fatty liver disease in middle-aged workers and their spouses. *J Hepatol* 2013; **59**: 351-357 [PMID: 23578884 DOI: 10.1016/j.jhep.2013.03.035]
- 8 **Imaizumi H**, Takahashi A, Tanji N, Abe K, Sato Y, Anzai Y, Watanabe H, Ohira H. The Association between Sleep Duration and Non-Alcoholic Fatty Liver Disease among Japanese Men and Women. *Obes Facts* 2015; **8**: 234-242 [PMID: 26138724 DOI: 10.1159/000436997]
- 9 **Bernsmeier C**, Weisskopf DM, Pflueger MO, Mosimann J, Campana B, Terracciano L, Beglinger C, Heim MH, Cajochen C. Sleep Disruption and Daytime Sleepiness Correlating with Disease Severity and Insulin Resistance in Non-Alcoholic Fatty Liver Disease: A Comparison with Healthy Controls. *PLoS One* 2015; **10**: e0143293 [PMID: 26576055 DOI: 10.1371/journal.pone.0143293]
- 10 **Yu JH**, Ahn JH, Yoo HJ, Seo JA, Kim SG, Choi KM, Baik SH, Choi DS, Shin C, Kim NH. Obstructive sleep apnea with excessive daytime sleepiness is associated with non-alcoholic fatty liver disease regardless of visceral fat. *Korean J Intern Med* 2015; **30**: 846-855 [PMID: 26552460 DOI: 10.3904/kjim.2015.30.6.846]
- 11 **Miyake T**, Kumagi T, Furukawa S, Hirooka M, Kawasaki K, Koizumi M, Todo Y, Yamamoto S, Tokumoto Y, Ikeda Y, Abe M, Kitai K, Matsuura B, Hiasa Y. Short sleep duration reduces the risk of nonalcoholic fatty liver disease onset in men: a community-based longitudinal cohort study. *J Gastroenterol* 2015; **50**: 583-589 [PMID: 25120172 DOI: 10.1007/s00535-014-0989-0]
- 12 **Nobili V**, Cutrera R, Liccardo D, Pavone M, Devito R, Giorgio V, Verrillo E, Baviera G, Musso G. Obstructive sleep apnea syndrome affects liver histology and inflammatory cell activation in pediatric nonalcoholic fatty liver disease, regardless of obesity/insulin resistance. *Am J Respir Crit Care Med* 2014; **189**: 66-76 [PMID: 24256086 DOI: 10.1164/rccm.201307-1339OC]
- 13 **Oliveira MJ**, Freitas D, Carvalho AP, Guimarães L, Pinto A, Águas AP. Exposure to industrial wideband noise increases connective tissue in the rat liver. *Noise Health* 2012; **14**: 227-229 [PMID: 23117537 DOI: 10.4103/1463-1741.102959]
- 14 **Xi YP**. [Histologic and ultrastructural changes in the liver in ageing rats and the effects due to food restriction and noise]. *Zhonghua*

- Bing Li Xue Za Zhi 1989; **18**: 118-120 [PMID: 2582548]
- 15 **Trovato G**, Pace P, Martinez GF, Brischetto D. Mala-movida: late bed-timing and wake-up induce malnutrition and underweight in youngsters. *Chronobiol Int* 2014; **31**: 945-946 [PMID: 24963991 DOI: 10.3109/07420528.2014.931414]
  - 16 **Trovato G**, Brischetto D, Martinez GF. Teens' obesity, noise and sleep deprivation: a perverse liaison. Let's move beyond "movida". *Obesity* (Silver Spring) 2014; **22**: 1209 [PMID: 24470382 DOI: 10.1002/oby.20712]
  - 17 **Trovato G**, Brischetto D, Pace P, Fabio Martinez G. Perceived body weight status of youngsters interferes with headache in obese and non-obese subjects. *Headache* 2014; **54**: 1062-1063 [PMID: 24916593 DOI: 10.1111/head.12374]
  - 18 **Musolino C**, Warin M, Wade T, Gilchrist P. 'Healthy anorexia': The complexity of care in disordered eating. *Soc Sci Med* 2015; **139**: 18-25 [PMID: 26150064 DOI: 10.1016/j.socscimed.2015.06.030]
  - 19 **Trovato GM**, Catalano D, Martinez GF, Pirri C, Trovato FM. Western dietary pattern and sedentary life: independent effects of diet and physical exercise intensity on NAFLD. *Am J Gastroenterol* 2013; **108**: 1932-1933 [PMID: 24300872 DOI: 10.1038/ajg.2013.356]
  - 20 **Shamsoddini A**, Sobhani V, Ghamar Chehreh ME, Alavian SM, Zaree A. Effect of Aerobic and Resistance Exercise Training on Liver Enzymes and Hepatic Fat in Iranian Men With Nonalcoholic Fatty Liver Disease. *Hepat Mon* 2015; **15**: e31434 [PMID: 26587039 DOI: 10.5812/hepatmon.31434]
  - 21 **Whitsett M**, VanWagner LB. Physical activity as a treatment of non-alcoholic fatty liver disease: A systematic review. *World J Hepatol* 2015; **7**: 2041-2052 [PMID: 26261693 DOI: 10.4254/wjh.v7.i16.2041]
  - 22 **Lee SM**, Jun DW, Cho YK, Jang KS. Vitamin D deficiency in non-alcoholic fatty liver disease: The chicken or the egg? *Clin Nutr* 2015; Epub ahead of print [PMID: 26615912 DOI: 10.1016/j.clnu.2015.10.017]
  - 23 **Mathiesen UL**, Franzén LE, Aselius H, Resjö M, Jacobsson L, Foberg U, Frydén A, Bodemar G. Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. *Dig Liver Dis* 2002; **34**: 516-522 [PMID: 12236486 DOI: 10.1016/S1590-8658(02)80111-6]
  - 24 **Egolf B**, Lasker J, Wolf S, Potvin L. The Roseto effect: a 50-year comparison of mortality rates. *Am J Public Health* 1992; **82**: 1089-1092 [PMID: 1636828 DOI: 10.2105/AJPH.82.8.1089]
  - 25 **Tanoglu A**, Kara M. Nonalcoholic fatty liver disease-related cardiovascular risk: Is there an association with blood hemoglobin levels? *Eur J Gastroenterol Hepatol* 2015; **27**: 1126-1129 [PMID: 26193051 DOI: 10.1097/MEG.0000000000000434]
  - 26 **Bruhn JG**, Philips BU, Wolf S. Social readjustment and illness patterns: comparisons between first, second and third generation Italian-Americans living in the same community. *J Psychosom Res* 1972; **16**: 387-394 [PMID: 4666655 DOI: 10.1016/0022-3999(72)90063-3]
  - 27 **Fan M**, Sun J, Zhou B, Chen M. The Smart Health Initiative in China: The Case of Wuhan, Hubei Province. *J Med Syst* 2016; **40**: 62 [PMID: 26667820 DOI: 10.1007/s10916-015-0416-y]
  - 28 **Hannah WN**, Harrison SA. Lifestyle and Dietary Interventions in the Management of Nonalcoholic Fatty Liver Disease. *Dig Dis Sci* 2016; **61**: 1365-1374 [PMID: 27052013 DOI: 10.1007/s10620-016-4153-y]
  - 29 **Africa JA**, Newton KP, Schwimmer JB. Lifestyle Interventions Including Nutrition, Exercise, and Supplements for Nonalcoholic Fatty Liver Disease in Children. *Dig Dis Sci* 2016; **61**: 1375-1386 [PMID: 27041377 DOI: 10.1007/s10620-016-4126-1]
  - 30 **Younossi Z**, Henry L. Contribution of Alcoholic and Nonalcoholic Fatty Liver Disease to the Burden of Liver-Related Morbidity and Mortality. *Gastroenterology* 2016; **150**: 1778-1785 [PMID: 26980624 DOI: 10.1053/j.gastro.2016.03.005]
  - 31 **Karrar A**, Stepanova M, Alaparthi L, Lingam S, Younoszai Z, Zheng L, Malik KS, Younossi E, Monge F, Hunt SL, Goodman Z, Younossi ZM. Anti-adipocyte antibody response in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2015; **30**: 900-908 [PMID: 25469790 DOI: 10.1111/jgh.12856]
  - 32 **Estep JM**, Goodman Z, Sharma H, Younossi E, Elarainy H, Baranova A, Younossi Z. Adipocytokine expression associated with miRNA regulation and diagnosis of NASH in obese patients with NAFLD. *Liver Int* 2015; **35**: 1367-1372 [PMID: 24684403 DOI: 10.1111/liv.12555]
  - 33 **Mir HM**, Stepanova M, Afendy H, Cable R, Younossi ZM. Association of Sleep Disorders with Nonalcoholic Fatty Liver Disease (NAFLD): A Population-based Study. *J Clin Exp Hepatol* 2013; **3**: 181-185 [PMID: 25755498 DOI: 10.1016/j.jceh.2013.06.004]
  - 34 **Golabi P**, Otgonsuren M, Cable R, Felix S, Koenig A, Sayiner M, Younossi ZM. Non-alcoholic Fatty Liver Disease (NAFLD) is associated with impairment of Health Related Quality of Life (HRQOL). *Health Qual Life Outcomes* 2016; **14**: 18 [PMID: 26860700 DOI: 10.1186/s12955-016-0420-z]
  - 35 **Kim MS**, Ong M, Qu X. Optimal management for alcoholic liver disease: Conventional medications, natural therapy or combination? *World J Gastroenterol* 2016; **22**: 8-23 [PMID: 26755857 DOI: 10.3748/wjg.v22.i1.8]
  - 36 **Yao H**, Qiao YJ, Zhao YL, Tao XF, Xu LN, Yin LH, Qi Y, Peng JY. Herbal medicines and nonalcoholic fatty liver disease. *World J Gastroenterol* 2016; **22**: 6890-6905 [PMID: 27570425 DOI: 10.3748/wjg.v22.i30.6890]
  - 37 **Danielsson J**, Kangastupa P, Laatikainen T, Aalto M, Niemelä O. Impacts of common factors of life style on serum liver enzymes. *World J Gastroenterol* 2014; **20**: 11743-11752 [PMID: 25206278 DOI: 10.3748/wjg.v20.i33.11743]
  - 38 **Nseir W**, Hellou E, Assy N. Role of diet and lifestyle changes in nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 9338-9344 [PMID: 25071328 DOI: 10.3748/wjg.v20.i28.9338]
  - 39 **Thomas EL**, Brynes AE, Hamilton G, Patel N, Spong A, Goldin RD, Frost G, Bell JD, Taylor-Robinson SD. Effect of nutritional counselling on hepatic, muscle and adipose tissue fat content and distribution in non-alcoholic fatty liver disease. *World J Gastroenterol* 2006; **12**: 5813-5819 [PMID: 17007047 DOI: 10.3748/wjg.v12.i36.5813]

**P- Reviewer:** Tanoglu A, Zielinski J **S- Editor:** Qi Y **L- Editor:** A  
**E- Editor:** Li D



Prospective Study

## Can platelet count/spleen diameter ratio be used for cirrhotic children to predict esophageal varices?

Oya Balci Sezer, Deniz Çelik, Nihal Tutar, Figen Özçay

Oya Balci Sezer, Figen Özçay, Department of Pediatric Gastroenterology, Hepatology and Nutrition, Baskent University, 06540 Ankara, Turkey

Deniz Çelik, Department of Child Health and Diseases, Baskent University, 06540 Ankara, Turkey

Nihal Tutar, Department of Radiology, Baskent University, 06540 Ankara, Turkey

**Author contributions:** Sezer OB and Özçay F designed the manuscript; Sezer OB, Çelik D and Özçay F collected the data; Sezer OB and Özçay F substantially contributed to the conception or design; Tutar N performed all of the ultrasonographic examinations; Sezer OB wrote the manuscript; Sezer OB and Özçay F gave final approval and agreed to be accountable for all aspects of the work, ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Institutional review board statement:** This study was reviewed and approved by the Ethics Committee of Baskent University.

**Clinical trial registration statement:** Study registration information number of our study is KA11/11252.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** None of authors has any conflict of interest to disclose.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

**Correspondence to:** Oya Balci Sezer, MD, Department of Pediatric Gastroenterology, Hepatology and Nutrition, Baskent University, Baglica Kampusu Eskisehir Yolu, 20 km Baglica, 06540 Ankara, Turkey. [oyabalci@yahoo.com](mailto:oyabalci@yahoo.com)  
Telephone: +90-533-6915083  
Fax: +90-312-3569002

Received: July 19, 2016

Peer-review started: July 21, 2016

First decision: September 2, 2016

Revised: September 10, 2016

Accepted: October 5, 2016

Article in press: October 9, 2016

Published online: November 28, 2016

### Abstract

#### AIM

To determine the laboratory and radiologic parameters, including the platelet count (PC)-to-spleen diameter (SD) ratio as a non-invasive marker that may predict the presence of esophageal varices (EV) in children with cirrhosis.

#### METHODS

Eighty-nine patients with cirrhosis, but without a history of variceal bleeding were prospectively included. The children were grouped into 6-12 and 12-18 years of age groups. These groups were also divided into 2 subgroups (presence and absence of EV). All of the patients underwent a complete biochemical and radiologic evaluation. The PC (n/mm<sup>3</sup>)-to-SD (mm) ratio was calculated for each patient.

#### RESULTS

Sixty-nine of 98 (70.4%) patients had EV. The presence of ascites in all age groups was significantly associated

with the presence of EV. There were no differences in serum albumin levels, PC, SD and the PC-to-SD ratio between the presence and absence of EV groups in both age groups ( $P > 0.05$ ).

## CONCLUSION

Laboratory and radiologic parameters, including the PC-to-SD ratio as a non-invasive marker (except for the presence of ascites), was inappropriate for detecting EV in children with cirrhosis.

**Key words:** Esophageal varices; Variceal bleeding; Platelet count-to-spleen diameter ratio; Children

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Laboratory and radiologic parameters, including the platelet count (PC)-to-spleen diameter (SD) ratio were investigated in children with cirrhosis as a non-invasive marker that may predict the presence of esophageal varices (EV). This study is the first study to assess the PC-to-SD ratio in children with cirrhosis for detecting EV according to age groups. This study demonstrated that the parameters, other than the presence of ascites, were inappropriate for detecting EV in children with cirrhosis.

Sezer OB, Çelik D, Tutar N, Özçay F. Can platelet count/spleen diameter ratio be used for cirrhotic children to predict esophageal varices? *World J Hepatol* 2016; 8(33): 1466-1470 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i33/1466.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i33.1466>

## INTRODUCTION

Esophageal variceal bleeding is among the most serious consequences of chronic liver disease<sup>[1]</sup>. Approximately two-thirds of children with cirrhosis have esophageal varices (EV), and the mortality associated with a variceal bleeding episode is 20%-35%<sup>[1-4]</sup>. Prevention of bleeding from a ruptured EV has become one of the main goals in the follow-up of these patients. Although a consensus has been reached for adults, there is no formal recommendation for endoscopic screening in children with cirrhosis<sup>[5]</sup>.

Esophagogastroduodenoscopy (EGD) is the present reference standard diagnostic test for EV. Nevertheless, only 50%-70% of cirrhotic patients have varices on the first EGD and < 30% have large varices and/or the red wale sign (high-risk EV for bleeding) in adults and children<sup>[6-8]</sup>. Because of the relatively low prevalence of varices that require primary prophylaxis, the cost, inconvenience, and morbidity associated with endoscopic surveillance may not be justified for all patients with cirrhosis. To reduce the increasing burden on endoscopy units and prevent unnecessary harm to patients, researchers have attempted to identify parameters for non-invasive

prediction of EV<sup>[9]</sup>. Several reports have identified non-invasive variables that may predict the presence of EV in childhood and have shown predictive factors for bleeding risk, such as hypoalbuminemia, the Child-Pugh score, an increased spleen diameter (SD), a low platelet count (PC), the PC-to-SD ratio, the clinical prediction rule, and the aspartate aminotransferase-to-platelet ratio index<sup>[6,7,10]</sup>. For this purpose, the PC-to-SD ratio was investigated to predict the presence of EV in adult patients with cirrhosis<sup>[11-14]</sup>. Chawla *et al*<sup>[15]</sup> concluded that the PC-to-SD ratio is elegant, simple and inexpensive, and it may become a helpful tool to limit the number of endoscopies for primary prophylaxis in adult patients with portal hypertension. Therefore, we conducted this study to investigate laboratory and radiologic parameters, including the PC-to-SD ratio, as predictors of EV in children with cirrhosis.

## MATERIALS AND METHODS

All children (6-18 years of age) who had been diagnosed with cirrhosis in the outpatient clinics of the Paediatric Gastroenterology Hepatology and Nutrition at Baskent University, Ankara, Turkey, were included in this prospective study. The diagnosis of cirrhosis was made based on laboratory, radiologic, and physical examination findings or by liver histology in the absence of clear clinical signs of liver cirrhosis. Demographic characteristics (age, gender and underlying disease), blood chemistry evaluations, international normalized ratio, and Child-Pugh scores were recorded for each patient.

Patients with a clinical history of upper digestive hemorrhage, band ligation, sclerosing therapy, transjugular intrahepatic portosystemic stent shunt, surgery for portal hypertension, hepatic encephalopathy, and use of beta-blockers or other vasoactive drugs were excluded from the study.

The children were further grouped into 6-12 and 12-18 year age groups. These groups were divided into two sub-groups (EV-present and -absent) based on the EGD. The EGD was performed by the same paediatric endoscopists in our endoscopy unit using a video endoscope (Olympus GIF-XP 240; Tokyo, Japan or Fujinon EG 590W videoendoscopy; Tokyo, Japan). EV were classified according to the Baveno IV criteria<sup>[16,17]</sup> and American Association for the Study of Liver Diseases practice guidelines<sup>[18]</sup> as no, small, and large varices. EV were also classified according to the bleeding risk as high risk and non-high risk using varices diameters and red sign parameters.

The spleen bipolar diameter and presence of ascites were evaluated by ultrasonography (Siemens Sonoline Antares 4.1 MHz or 9.4 MHz probe; Siemens Medical Solutions United States, Inc., Issaquah, WA, United States) by the same radiologist.

The study design was approved by the Ethics Committee of our hospital (Study No. KA11/11252). Before enrollment, written informed consent was obtained from the primary caretaker of each patient.

We used SPSS software (version 16.0; SPSS, Inc.,



**Table 1** Laboratory and ultrasonographic data in the age group of 6-12 years

	Patient with varices (n = 29)	Control (n = 13)	P value
Mean age (yr)	9.7 ± 2.0	10.0 ± 1.9	0.595
Gender (% female)	45	38.5	0.384
INR	1.5 ± 0.5	1.4 ± 0.5	0.860
ALT (IU/L)	57.3 ± 50.8	44.3 ± 29.3	0.210
AST (IU/L)	79.1 ± 71.5	53.1 ± 32.9	0.241
Total bilirubin (mg/dL)	3.5 ± 6.0	2.5 ± 4.7	0.618
Albumin (mg/dL)	3.9 ± 0.6	4.2 ± 0.6	0.231
Ultrasonographic ascites (%)	27.5%	0%	0.037
Spleen diameter (mm)	167.3 ± 39.1	151.3 ± 32.4	0.206
Platelet count (thousand/mm <sup>3</sup> )	129000 ± 53519	153000 ± 97798	0.312
Platelet count/spleen diameter	976.6 ± 793.5	1062.4 ± 718.0	0.741
Child-Pugh score	6.3 ± 1.5	5.7 ± 1.4	0.193

INR: International normalized ratio; ALT: Alanine transaminase; AST: Aspartate transaminase.

**Table 2** Laboratory and ultrasonographic data in the age group of 12-18 years

	Patient with varices (n = 40)	Control (n = 16)	P value
Mean age (yr)	14.2 ± 1.7	13.5 ± 1.2	0.161
Gender (% female)	48	56	0.591
INR	1.3 ± 0.3	1.4 ± 0.6	0.347
ALT (IU/L)	86.5 ± 76.1	60.5 ± 67.2	0.250
AST (IU/L)	115.2 ± 124.2	105.5 ± 231.8	0.842
Total bilirubin (mg/dL)	5.0 ± 10.4	5.3 ± 12.5	0.931
Albumin (mg/dL)	3.8 ± 0.7	3.9 ± 0.7	0.757
Ultrasonographic ascites (%)	35%	6%	0.028
Spleen diameter (mm)	181.4 ± 37.2	150.3 ± 34.2	0.389
Platelet count (thousand/mm <sup>3</sup> )	103000 ± 55867	115000 ± 65472	0.499
Platelet count/spleen diameter	733.9 ± 737.4	830.78 ± 553.5	0.637
Child-Pugh score	6.9 ± 1.9	6.2 ± 1.8	0.214

INR: International normalized ratio; ALT: Alanine transaminase; AST: Aspartate transaminase.

Chicago, IL, United States) for statistical analysis. Data are expressed as the mean and standard deviation and proportions. For comparison of categorical variables, Fisher's exact test or a  $\chi^2$  test was used. Differences between numeric variables were tested with a Mann-Whitney *U*-test. Values of  $P < 0.05$  were considered to indicate statistically significant differences.

## RESULTS

Ninety-eight children with cirrhosis were included in this study. The ages of the children ranged from 6-18 years (median age, 12.16 ± 2.70 years). Forty-six children were females (46.9%) and 52 were males (53.1%).

The etiology of cirrhosis was cryptogenic cirrhosis ( $n = 40$ ), Wilson's disease ( $n = 35$ ), progressive familial intrahepatic cholestasis (2), sclerosing cholangitis ( $n = 4$ ), Budd-Chiari syndrome ( $n = 4$ ), tyrosinemia ( $n = 3$ ), glycogen storage disease ( $n = 3$ ), autoimmune hepatitis ( $n = 2$ ), hepatitis B infection ( $n = 2$ ), Allagille syndrome ( $n = 2$ ), and alfa1-antitrypsin deficiency ( $n = 1$ ). Sixty-one patients were Child-Pugh class A, 29 were class B, and 8 were class C.

In this study, 69 children (70.4%) were shown to have EV based on the first EGD and 29 children (29.6%) were shown not to have EV.

Fifty-five of the 69 patients had small EV (79.7%) and 14 patients (20.3%) had large EV. There were 11 children (15.9%) with red wale signs (seven children had large EV and four children had small EV). Therefore, 18 of the 69 patients with EV (26.1%) had high-risk EV for bleeding according to the presence of large varices and/or red sign (six patients in the 6-12 year age group, and 12 patients in the 12-18 year age group).

There were no differences in age and gender between the EV-present and -absent sub groups in both age groups ( $P > 0.05$ ). In the two age groups, a higher

percentage of ascites was observed among the EV-present group than the EV-absent group (Tables 1 and 2). We did not find a statistically significant difference in the PC-to-SD ratio between patients with large and small varices ( $636.9 \pm 256.5$  and  $894.1 \pm 844.4$ , respectively;  $P = 0.89$ ).

We did not find a significant difference for serum albumin, PC, SD and the PC-to-SD ratio between the EV-present and -absent varices sub-groups in both age groups ( $P > 0.05$ ; Tables 1 and 2).

## DISCUSSION

Despite advances in diagnosis and treatment, bleeding from EV is one of the major causes of morbidity and mortality among patients with cirrhosis. Hence, preventing the first episode of variceal bleeding may reduce mortality and morbidity.

In this prospective study involving children 6-18 years of age with cirrhosis, we found that only the presence of ascites is associated with the presence of EV. There have been several studies identifying non-invasive variables that may predict the presence of EV in children<sup>[6,7,10]</sup>. The first study, in which the predictive risk factors were evaluated by Fagundes *et al*<sup>[6]</sup> in a pediatric group [median age at the time of first EGD was 6 years (age range, 0.7-17.6 years)], showed that children with cirrhosis and splenomegaly were nearly 15-fold more likely to have EV compared with children with cirrhosis but without splenomegaly. Fagundes *et al*<sup>[6]</sup> concluded that hypoalbuminemia, splenomegaly, and a PC < 130000/mm<sup>3</sup> were predictors for the presence of EV, spleen size was not measured by ultrasonography. The second study, conducted by Gana *et al*<sup>[7]</sup>, derived a non-invasive clinical prediction rule capable of identifying children with EV. In this study, 17 of 51 children (< 18 years of age) with liver disease or portal vein thromboses were shown

to have EV, and hypoalbuminemia was shown to be an independent variable for the presence of EV. In the same study<sup>[7]</sup>, a higher percentage of ascites, increased spleen length, and lower PC (cut-off value = 115000/mm<sup>3</sup>) were reported among children with EV. Further, the PC-to-spleen length-for age Z score ratio was significantly lower among the EV-present group<sup>[7]</sup>.

Fagundes *et al.*<sup>[6]</sup> and Gana *et al.*<sup>[7]</sup> reported lower albumin levels among children with EV. Our results were not in agreement with the findings of these two studies. A possible explanation may be the difference in etiologic factors in our patients.

A recent study involving 103 patients with a diagnosis of chronic liver disease or extrahepatic portal vein obstruction (mean age, 8.9 ± 4.7 years) showed a significantly higher spleen length and lower PC (cut-off value = 115000/mm<sup>3</sup>) among children with EV than children without EV<sup>[10]</sup>. In the same study, it was reported that a PC-to-spleen size (cm) ratio < 1.0 discriminated between patients with and without EV, despite a lack of statistical significance based on logistic regression. The authors suggested that the lack of statistical significance was explained by the age and gender differences in spleen size.

Based on the findings of these three studies<sup>[6,7,10]</sup>, low PC and increased spleen length are logical parameters by which to determine EV in children with cirrhosis. In addition, Gana *et al.*<sup>[7]</sup> and Adami *et al.*<sup>[10]</sup> reported that PC (cut-off value = 115000/mm<sup>3</sup>) was the best predictor of EV.

In the current study, we did not find a significant difference for PC, SD and the PC-to-SD ratio between the EV-present and -absent sub-groups in both age groups. A possible explanation is the heterogeneity of patients studied. Another explanation is the lack of children with portal vein thromboses in the current study. The three studies investigating risk factors for EV included children with cirrhosis and portal vein thromboses<sup>[6,7,10]</sup>. It is well-known that portal vein thrombosis is a risk factor for splenomegaly and thrombocytopenia. The PC loses discriminatory power because of multi-causality (such as autoimmune events, myelotoxic effects of viruses, or reduced synthesis of thrombopoietin) as a consequence of progressive liver dysfunction; however, in children with portal vein thromboses, thrombocytopenia is directly related to portal hypertension, as well as the development of varices<sup>[19]</sup>.

One of the most important limitations of our study was the small number of patients; however, this study was the first study to assess the PC-to-SD ratio in children in two age groups with cirrhosis as a means to detect EV. We consider the PC, SD and PC-to-SD ratio to lack suitability as non-invasive markers for detecting EV in children with cirrhosis. Further studies on this subject with larger sample sizes are required to assess the importance of the PC, SD and PC-to-SD ratio in cirrhotic children with or without portal vein thrombosis.

## COMMENTS

### Background

Esophageal variceal (EV) bleeding is among the most serious consequences of chronic liver disease. Approximately two-thirds of children with cirrhosis have EV and the mortality associated with a variceal bleeding episode is 20%-35%. Identification of children with cirrhosis who are at high risk for EV using a non-invasive test is important to reduce the need for endoscopy. The authors' goal was to investigate laboratory and radiologic parameters, including the platelet count (PC)-to-spleen diameter (SD) ratio to predict the presence of EV in children with cirrhosis.

### Research frontiers

To reduce the increasing burden on endoscopy units and prevent unnecessary harm to patients with cirrhosis, researchers have attempted to identify parameters for the non-invasive prediction of EV.

### Innovations and breakthroughs

A few studies have shown that a low PC and PC-to-SD ratio may predict the presence of EV in patients with cirrhosis. In their study, the authors did not find a significant difference in the PC, SD and PC-to-SD ratio between the EV-present and -absent sub-groups in both age groups of children.

### Applications

The PC-to-SD ratio is not an appropriate index with which to predict EV in children with cirrhosis. This may indicate that endoscopy remains the ideal choice for detecting EV in children with cirrhosis.

### Terminology

Esophageal varices are abnormal, enlarged veins which generally occur in patients with serious liver diseases. The vessels can leak blood, or even rupture, thus causing life-threatening bleeding.

### Peer-review

It is helpful for clinical doctors to perform endoscopic examination promptly.

## REFERENCES

- 1 Jensen DM. Endoscopic screening for varices in cirrhosis: findings, implications, and outcomes. *Gastroenterology* 2002; **122**: 1620-1630 [PMID: 12016427 DOI: 10.1053/gast.2002.33419]
- 2 Gürakan F, Eren M, Koçak N, Yüce A, Ozen H, Temizel IN, Demir H. Extrahepatic portal vein thrombosis in children: etiology and long-term follow-up. *J Clin Gastroenterol* 2004; **38**: 368-372 [PMID: 15087698 DOI: 10.1097/00004836-200404000-00013]
- 3 Lykavieiris P, Gauthier F, Hadchouel P, Duche M, Bernard O. Risk of gastrointestinal bleeding during adolescence and early adulthood in children with portal vein obstruction. *J Pediatr* 2000; **136**: 805-808 [PMID: 10839880 DOI: 10.1016/S0022-3476(00)09680-3]
- 4 Gonçalves ME, Cardoso SR, Maksoud JG. Prophylactic sclerotherapy in children with esophageal varices: long-term results of a controlled prospective randomized trial. *J Pediatr Surg* 2000; **35**: 401-405 [PMID: 10726678 DOI: 10.1016/S0022-3468(00)90203-3]
- 5 Molleston JP. Variceal bleeding in children. *J Pediatr Gastroenterol Nutr* 2003; **37**: 538-545 [PMID: 14581793 DOI: 10.1097/0005176-200311000-00006]
- 6 Fagundes ED, Ferreira AR, Roquete ML, Penna FJ, Goulart EM, Figueiredo Filho PP, Bittencourt PF, Carvalho SD, Albuquerque W. Clinical and laboratory predictors of esophageal varices in children and adolescents with portal hypertension syndrome. *J Pediatr Gastroenterol Nutr* 2008; **46**: 178-183 [PMID: 18223377 DOI: 10.1097/MPG.0b013e318156ff07]
- 7 Gana JC, Turner D, Roberts EA, Ling SC. Derivation of a clinical prediction rule for the noninvasive diagnosis of varices in children. *J Pediatr Gastroenterol Nutr* 2010; **50**: 188-193 [PMID: 19966576]

- DOI: 10.1097/MPG.0b013e3181b64437]
- 8 **Barrera F**, Riquelme A, Soza A, Contreras A, Barrios G, Padilla O, Viviani P, Pérez-Ayuso RM. Platelet count/spleen diameter ratio for non-invasive prediction of high risk esophageal varices in cirrhotic patients. *Ann Hepatol* 2009; **8**: 325-330 [PMID: 20009131]
  - 9 **Leffler DA**, Kheraj R, Garud S, Neeman N, Nathanson LA, Kelly CP, Sawhney M, Landon B, Doyle R, Rosenberg S, Aronson M. The incidence and cost of unexpected hospital use after scheduled outpatient endoscopy. *Arch Intern Med* 2010; **170**: 1752-1757 [PMID: 20975024 DOI: 10.1001/archinternmed.2010.373]
  - 10 **Adami MR**, Ferreira CT, Kieling CO, Hirakata V, Vieira SM. Noninvasive methods for prediction of esophageal varices in pediatric patients with portal hypertension. *World J Gastroenterol* 2013; **19**: 2053-2059 [PMID: 23599624 DOI: 10.3748/wjg.v19.i13.2053]
  - 11 **Giannini EG**, Botta F, Borro P, Dulbecco P, Testa E, Mansi C, Savarino V, Testa R. Application of the platelet count/spleen diameter ratio to rule out the presence of oesophageal varices in patients with cirrhosis: a validation study based on follow-up. *Dig Liver Dis* 2005; **37**: 779-785 [PMID: 15996912 DOI: 10.1016/j.dld.2005.05.007]
  - 12 **Sarangapani A**, Shanmugam C, Kalyanasundaram M, Rangachari B, Thangavelu P, Subbarayan JK. Noninvasive prediction of large esophageal varices in chronic liver disease patients. *Saudi J Gastroenterol* 2010; **16**: 38-42 [PMID: 20065573 DOI: 10.4103/1319-3767.58767]
  - 13 **Schwarzenberger E**, Meyer T, Golla V, Sahdala NP, Min AD. Utilization of platelet count spleen diameter ratio in predicting the presence of esophageal varices in patients with cirrhosis. *J Clin Gastroenterol* 2010; **44**: 146-150 [PMID: 19593164 DOI: 10.1097/MCG.0b013e3181a745ff]
  - 14 **Mattos AZ**, Mattos AA, Vianna FF, Musskopf MI, Pereira-Lima JC, Maciel AC. Platelet count/spleen diameter ratio: analysis of its capacity as a predictor of the existence of esophageal varices. *Arg Gastroenterol* 2010; **47**: 275-278 [PMID: 21140089 DOI: 10.1590/S0004-28032010000300012]
  - 15 **Chawla S**, Katz A, Attar BM, Gupta A, Sandhu DS, Agarwal R. Platelet count/spleen diameter ratio to predict the presence of esophageal varices in patients with cirrhosis: a systematic review. *Eur J Gastroenterol Hepatol* 2012; **24**: 431-436 [PMID: 22410714 DOI: 10.1097/MEG.0b013e3283505015]
  - 16 **de Franchis R**. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2010; **53**: 762-768 [PMID: 20638742 DOI: 10.1016/j.jhep.2010.06.004]
  - 17 **Shneider B**, Emre S, Groszmann R, Karani J, McKiernan P, Sarin S, Shashidhar H, Squires R, Superina R, de Ville de Goyet J, de Franchis R. Expert pediatric opinion on the Report of the Baveno IV consensus workshop on methodology of diagnosis and therapy in portal hypertension. *Pediatr Transplant* 2006; **10**: 893-907 [PMID: 17096755 DOI: 10.1111/j.1399-3046.2006.00597.x]
  - 18 **Garcia-Tsao G**, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology* 2007; **46**: 922-938 [PMID: 17879356 DOI: 10.1002/hep.21907]
  - 19 **Shneider BL**. Portal hypertension. In: Suchy FJ, Sokal RJ, Balistreri WF (eds). *Liver Disease in Children*. Philadelphia: Lippincott Williams & Wilkins, 2001: 130-151

**P- Reviewer:** Guo XZ, Wei XQ **S- Editor:** Ji FF **L- Editor:** A  
**E- Editor:** Li D



Prospective Study

## Elevation of serum urokinase plasminogen activator receptor and liver stiffness in postoperative biliary atresia

Wanvisa Udomsinprasert, Sittisak Honsawek, Napaphat Jirathanathornnukul, Voranush Chongsrisawat, Yong Poovorawan

Wanvisa Udomsinprasert, Sittisak Honsawek, Napaphat Jirathanathornnukul, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok 10330, Thailand

Voranush Chongsrisawat, Yong Poovorawan, Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok 10330, Thailand

**Author contributions:** Honsawek S designed the study; Udomsinprasert W, Honsawek S and Jirathanathornnukul N performed the research; Udomsinprasert W, Honsawek S, Jirathanathornnukul N, Chongsrisawat V and Poovorawan Y analyzed the data; Chongsrisawat V and Poovorawan Y examined all the patients and collected the clinical data; Udomsinprasert W and Honsawek S wrote the paper; Honsawek S revised the manuscript for final submission.

**Institutional review board statement:** The study was reviewed and approved by the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** All the authors have no conflicts of interests to declare.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at [sittisak.h@chula.ac.th](mailto:sittisak.h@chula.ac.th). Participants gave informed consent for data sharing.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this

work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Sittisak Honsawek, MD, PhD, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, 1873 Rama IV Road, Patumwan, Bangkok 10330, Thailand. [sittisak.h@chula.ac.th](mailto:sittisak.h@chula.ac.th)  
Telephone: +662-256-4482  
Fax: +662-256-4482

**Received:** June 5, 2016

**Peer-review started:** June 7, 2016

**First decision:** August 10, 2016

**Revised:** August 21, 2016

**Accepted:** October 22, 2016

**Article in press:** October 24, 2016

**Published online:** November 28, 2016

### Abstract

#### AIM

To investigate serum urokinase-type plasminogen activator receptor (uPAR) and liver stiffness in biliary atresia (BA) and examine the correlation of circulating uPAR, liver stiffness, and clinical outcomes in postoperative BA children.

#### METHODS

Eighty-five postKasai BA children and 24 control subjects were registered. Circulating uPAR was measured using enzyme-linked immunosorbent assay. Liver stiffness was analyzed using transient elastography.

#### RESULTS

BA children had significantly greater circulating uPAR and



liver stiffness scores than control subjects ( $P < 0.001$ ). Circulating uPAR and liver stiffness were substantially higher in jaundiced BA children than non-jaundiced BA children ( $P < 0.001$ ). In addition, circulating uPAR was positively associated with serum aspartate aminotransferase ( $r = 0.507$ ,  $P < 0.001$ ), alanine aminotransferase ( $r = 0.364$ ,  $P < 0.001$ ), total bilirubin ( $r = 0.559$ ,  $P < 0.001$ ), alkaline phosphatase ( $r = 0.325$ ,  $P < 0.001$ ), and liver stiffness scores ( $r = 0.508$ ,  $P < 0.001$ ).

### CONCLUSION

Circulating uPAR and liver stiffness values were greater in BA children than healthy controls. The increased circulating uPAR was associated with liver dysfunction in BA. As a consequence, serum uPAR and liver stiffness may be used as noninvasive biomarkers indicating the progression of liver fibrosis in postKasai BA.

**Key words:** Biliary atresia; Jaundice; Liver stiffness; Severity; Urokinase plasminogen activator receptor

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Urokinase plasminogen activator receptor (uPAR) is known to be a substantial factor in the etiopathogenesis of hepatic inflammation and liver fibrogenesis. This study is the first to show that circulating uPAR is more elevated in biliary atresia (BA) children than in control subjects, and that circulating uPAR is correlated with the degree of jaundice and liver fibrosis in biliary atresia. Elevated serum uPAR is positively correlated with the severity of liver stiffness in postKasai BA children. Hence, serum uPAR could be used as a biological parameter indicating the progression and prognosis of liver fibrosis in BA children.

Udomsinprasert W, Honsawek S, Jirathanathornnukul N, Chongsrisawat V, Poovorawan Y. Elevation of serum urokinase plasminogen activator receptor and liver stiffness in postoperative biliary atresia. *World J Hepatol* 2016; 8(33): 1471-1477 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i33/1471.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i33.1471>

## INTRODUCTION

Biliary atresia (BA) is a severe chronic cholestatic liver disease of unknown etiology in young infants. The estimated incidence of BA varies from 1 in 8000 to 1 in 20000 live births, with a high frequency in Asians<sup>[1]</sup>. Affected newborns exhibit evidence of biliary obstruction within the first few months of life. BA is manifested by impaired liver function and fibroinflammatory obliterative cholangiopathy of both intrahepatic and extrahepatic bile ducts<sup>[2,3]</sup>. Extrahepatic BA is the most common form of ductal cholestasis. BA patients initially develop neonatal jaundice due to hepatic cholestasis and progress to

hepatic fibrosis, which result in biliary cirrhosis<sup>[1-3]</sup>. Even though no medical therapies exist, sequential treatment strategy involving surgical Kasai portoenterostomy and liver transplantation is the only option for the most affected children. Nonetheless the precise pathogenesis of BA has yet to be determined, a number of theories regarding the etiology of BA include toxin exposure, virus-mediated inflammation, abnormal inflammatory response, defective morphogenesis, genetic mutation, and immunological dysregulation<sup>[4]</sup>.

Urokinase-type plasminogen activator receptor (uPAR, CD87) is a cellular membrane receptor that attaches to urokinase-type plasminogen activator (uPA) with high affinity, through promoting the pericellular activation of plasminogen<sup>[5]</sup>. The involvement of uPA, its receptor (uPAR), and plasminogen activator inhibitor-1 (PAI-1) in regulation of cell adhesion, migration, proliferation, differentiation, and cell survival has recently demonstrated<sup>[6]</sup>. uPAR is expressed by a wide range of immune cells and endothelial cells, which contribute to the etiopathogenesis of hepatic inflammation and liver fibrogenesis<sup>[7,8]</sup>. Once inflammation is activated, uPAR is released from the cell membrane by proteolytic enzymes to produce soluble uPAR<sup>[9]</sup>. In recent years, previous studies have investigated that elevated circulating uPAR levels have been observed in acute liver failure, chronic liver diseases, and nonalcoholic fatty liver diseases<sup>[10-12]</sup>.

It has been previously shown that certain cytokines and growth factors play possible parts in the etiopathology of biliary atresia<sup>[13-16]</sup>. The measurements on circulating uPAR and liver stiffness of BA have never been documented. We hypothesized that circulating uPAR and liver stiffness could be more elevated in BA patients than in control subjects and circulating uPAR would be associated with the disease severity and clinical outcomes in postKasai biliary atresia. Hence, the purpose of the current research is to determine circulating uPAR and liver stiffness measurements and to investigate the plausible correlation of circulating uPAR, liver stiffness, and clinical outcomes in postoperative biliary atresia children.

## MATERIALS AND METHODS

The present study was approved by the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University, and was conducted in compliance with the ethical guidelines of the Declaration of Helsinki. All parents of children were informed of the study's purpose and of any interventions involved in the current study. Written informed consent was derived from the parents prior to the subjects entering the study.

### Study population

Eighty-five BA children (39 girls and 46 boys with mean age of  $9.0 \pm 0.6$  years) and 24 normal control subjects (11 girls and 13 boys with mean age of  $8.5 \pm 0.5$  years) were enrolled in the study. None of them had undergone

liver transplantation. Healthy controls attending the Well Baby Clinic at our institution for vaccination had normal physical findings and no underlying disease. BA children were classified into two groups according to their serum total bilirubin (TB): Non-jaundiced BA children (TB < 2 mg/dL,  $n = 46$ ) and persistent jaundiced BA children (TB  $\geq 2$  mg/dL,  $n = 39$ ).

### Laboratory methods

Samples of peripheral venous blood were collected from every participant, and were kept at  $-80^{\circ}\text{C}$  for subsequent measurement. The quantitative assessment of serum uPAR was performed by using commercially available enzyme-linked immunosorbent assay (Quantikine, R and D Systems, Minneapolis, MN, United States). According to the manufacturer's protocol, recombinant human uPAR standards and serum samples were added into each well, which has been pre-coated with specific antibody to uPAR. After incubating for 2 h at room temperature, every well was washed thoroughly with wash buffer. Then, uPAR conjugate was pipetted into each well and incubated for 2 h at room temperature. After 4 washes, substrate solution was added into the wells and the microplate was incubated for 30 min at room temperature with protection from light. Lastly, the reaction was stopped by the stop solution and the optical density was determined using an automated microplate reader at 450 nm. A standard optical density-concentration curve was drawn for the determination of uPAR concentration. The liver function tests including serum albumin, TB, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured using a Hitachi 912 (Roche Diagnostics, Basel, Switzerland) automated machine at the central laboratory of our hospital.

### Liver stiffness measurement

Transient elastography (Fibroscan, Echosens, Paris, France) measured the liver stiffness between 25 to 65 mm from the skin surface, which is approximately equivalent to the volume of a cylinder of 1 cm diameter and 4 cm length. The measurements were performed by placing a transducer probe of Fibroscan on the intercostal space at the area of the right lobe of the liver with patients lying in a dorsal decubitus position with maximum abduction of the right arm. The target location for measurement was a liver portion that was at least 6 cm thick, and devoid of major vascular structures. The measurements were performed until 10 validated results had been obtained with a success rate of at least 80%. The median value of 10 validated scores was considered the elastic modulus of the liver, and it was expressed in kilopascals (kPa).

### Statistical analysis

Statistical analysis was executed by using the SPSS version 22.0 statistical software package (SPSS Inc., Chicago, IL, United States). Comparisons of demographic

and clinical outcomes between groups were performed using  $\chi^2$  and Student's unpaired  $t$ -test when appropriate. Correlation between numerical data was obtained using Pearson's correlation coefficient ( $r$ ). Data were presented as mean  $\pm$  SEM of the mean. A two-tailed  $P$ -value of less than 0.05 was taken to indicate statistical significance.

## RESULTS

### Comparison between BA children and control subjects

Eighty-five postoperative biliary atresia children and 24 ethnically matched unaffected volunteers were prospectively recruited in the current work. The baseline features of BA children and control subjects are presented in Table 1. There was no significant difference of age and gender between case and control groups. However, circulating uPAR values were substantially greater in BA children than in control subjects ( $6085.9 \pm 400.7$  pg/mL vs  $4754.5 \pm 294.9$  pg/mL,  $P = 0.01$ ) (Figure 1). Moreover, BA group had notably greater liver stiffness values than control group ( $28.7 \pm 2.7$  kPa vs  $4.1 \pm 0.2$  kPa,  $P < 0.001$ ).

### Differences between jaundiced group and non-jaundiced group of BA children

BA children were subdivided into jaundiced group ( $n = 39$ ) and non-jaundiced group ( $n = 46$ ). The clinical characteristics and biochemical features of patients according to jaundice status are illustrated in Table 2. Jaundiced BA children exhibited remarkably greater serum uPAR levels than non-jaundiced BA children ( $7373.5 \pm 684.6$  pg/mL vs  $4994.2 \pm 400.9$  pg/mL,  $P = 0.003$ ) (Figure 2). Furthermore, mean liver stiffness measurement of jaundiced BA group was greatly increased compared with that of non-jaundiced BA group ( $46.2 \pm 3.7$  kPa vs  $13.9 \pm 2.0$  kPa,  $P < 0.001$ ).

Subsequent investigation revealed that circulating uPAR was directly associated with serum AST ( $r = 0.507$ ,  $P < 0.001$ ), ALT ( $r = 0.364$ ,  $P < 0.001$ ), TB ( $r = 0.559$ ,  $P < 0.001$ ), ALP ( $r = 0.325$ ,  $P < 0.001$ ), and liver stiffness values ( $r = 0.508$ ,  $P < 0.001$ ) in BA children (Figure 3). However, circulating uPAR concentration was negatively associated with serum albumin level ( $r = -0.666$ ,  $P < 0.001$ ) (Figure 3).

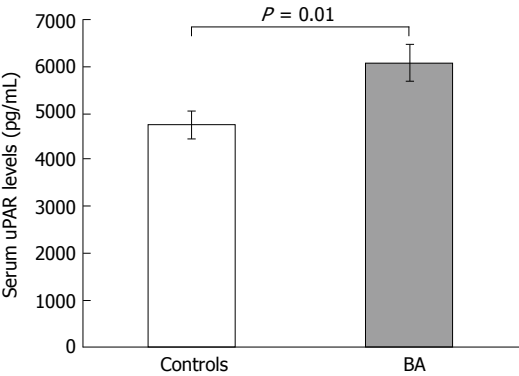
## DISCUSSION

Biliary atresia is a chronic progressive fibroinflammatory liver disorder with mysterious etiology. The etiopathology of BA currently remains elusive and it seems that multiple factors may contribute to the development of BA. Yet today, Kasai operation has been proved as the most effective option of surgical treatment. Without surgery, children with biliary atresia will finally die due to biliary cirrhosis and liver failure<sup>[1]</sup>. Recently, circulating uPAR levels have been shown to be involved in chronic liver disorders, including chronic hepatitis B and C, liver cirrhosis, and hepatocellular carcinoma<sup>[17-20]</sup>. Based on

**Table 1** Demographic data, biochemical characteristics, and liver stiffness scores of biliary atresia patients and healthy controls

Variables	BA (n = 85)	Controls (n = 24)	P value
Age (yr)	9.0 ± 0.6	8.5 ± 0.5	0.2
Gender (female:male)	39:46	11:13	0.4
Albumin (g/dL)	4.2 ± 0.1	-	NA
Total bilirubin (mg/dL)	2.7 ± 0.4	-	NA
Direct bilirubin (mg/dL)	2.3 ± 0.4	-	NA
AST (IU/L)	143.7 ± 11.9	-	NA
ALT (IU/L)	137.1 ± 12.5	-	NA
ALP (IU/L)	449.2 ± 34.0	-	NA
Liver stiffness (kPa)	28.7 ± 2.7	4.1 ± 0.2	< 0.001
uPAR (pg/mL)	6085.9 ± 400.7	4754.5 ± 294.9	0.01

The data was expressed as mean ± SEM. BA: Biliary atresia; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; uPAR: Urokinase-type plasminogen activator receptor; NA: Not applicable.



**Figure 1** Comparison of serum urokinase-type plasminogen activator receptor levels in biliary atresia patients and healthy controls. uPAR: Urokinase-type plasminogen activator receptor; BA: Biliary atresia.

our experience, there is no report about circulating uPAR and hepatic fibrosis in various degrees of postoperative biliary atresia.

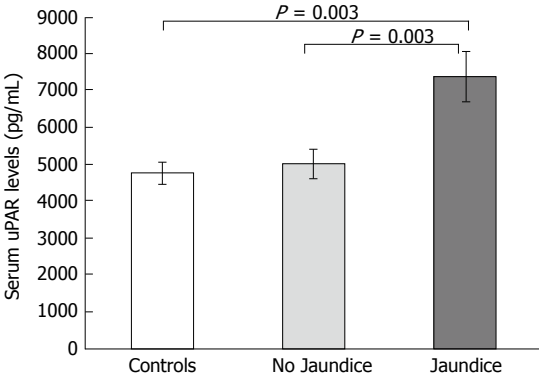
The present study is the first to show that circulating uPAR and liver fibrosis values were significantly higher in children suffering from BA than in control subjects. Additionally, circulating uPAR in jaundiced BA children was markedly increased with respect to that in non-jaundiced BA children. Elevated circulating uPAR levels were directly associated with total bilirubin, AST, ALT, ALP in post Kasai BA children, suggesting that circulating uPAR is related to degree of jaundice BA children. Furthermore, the degree of jaundice is possibly linked to the severity of intrahepatic biliary obliteration. Both AST and ALT are extensively used as biochemical parameters of hepatic abnormality indicating liver cell injury. Hence, the findings imply that uPAR could have a plausible role in the mechanism of liver cell injury in postoperative biliary atresia, and it would be associated with the severity of bile duct obliteration.

The present investigation demonstrated that circulating uPAR was more pronounced in biliary atresia children than control subjects. In accordance with this

**Table 2** Comparison of biliary atresia patients without and with jaundice

Variables	BA patients with jaundice (n = 39)	BA patients without jaundice (n = 46)	P-value
Age (yr)	9.5 ± 0.9	8.6 ± 0.9	0.4
Gender (female:male)	18:21	21:25	0.5
Albumin (g/dL)	3.8 ± 0.1	4.5 ± 0.1	< 0.001
Total bilirubin (mg/dL)	5.1 ± 0.7	0.5 ± 0.1	< 0.001
Direct bilirubin (mg/dL)	4.5 ± 0.6	0.2 ± 0.1	< 0.001
AST (IU/L)	210.4 ± 17.2	84.7 ± 10.2	< 0.001
ALT (IU/L)	195.9 ± 19.9	85.1 ± 10.7	< 0.001
ALP (IU/L)	599.7 ± 52.8	313.0 ± 32.0	< 0.001
Liver stiffness (kPa)	46.2 ± 3.7	13.9 ± 2.0	< 0.001
uPAR (pg/mL)	7373.5 ± 684.6	4994.2 ± 400.9	0.003

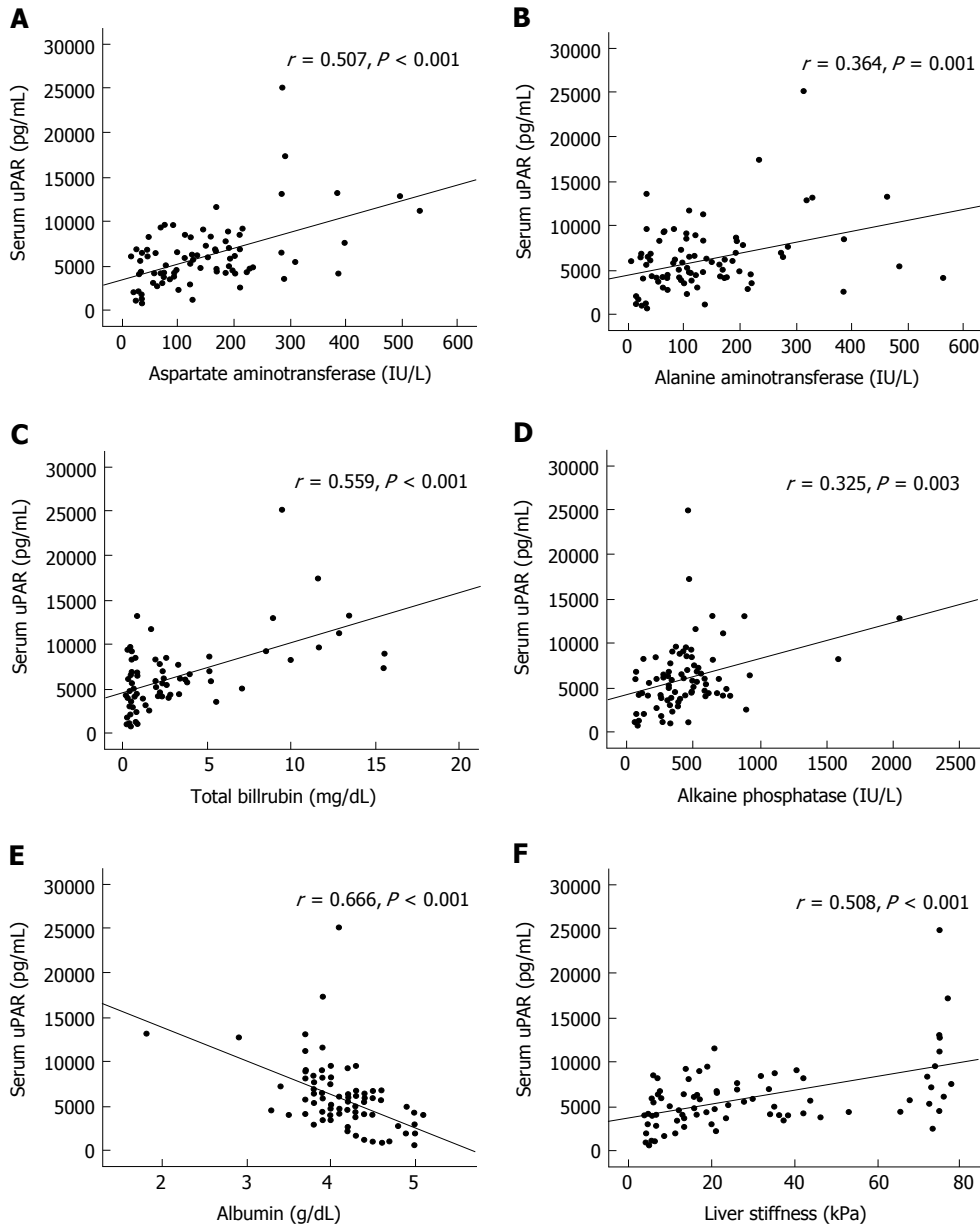
The data are expressed as mean ± SEM. BA: Biliary atresia; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; uPAR: Urokinase-type plasminogen activator receptor; NA: Not applicable.



**Figure 2** Comparison of serum urokinase-type plasminogen activator receptor levels in biliary atresia patients without jaundice and with jaundice. uPAR: Urokinase-type plasminogen activator receptor.

observation, Sjöwall *et al*<sup>[10]</sup> reported that circulating uPAR was increased in subjects with non-alcoholic fatty liver disease and associated with the severity of fibrosis. Moreover, uPAR expressions in liver tissue samples have been documented in subjects with hepatocellular carcinoma as shown by Morita *et al*<sup>[21]</sup>. In addition, Zimmermann *et al*<sup>[12]</sup> reported that circulating uPAR was substantially elevated in subjects with chronic liver diseases compared with controls and were closely correlated with liver function and fibrosis.

In light of our findings, certain hypotheses could explain high circulating uPAR in jaundiced biliary atresia children. Firstly, the release of uPAR in the injured liver could be accountable for the increased circulating uPAR. Secondly, the elevation of circulating uPAR may be ascribed to the unbalance between uPAR synthesis and uPAR clearance. The reduction of uPAR destruction in BA children with liver fibrosis may lead to the elevated circulating uPAR. Decreased pre-systemic hepatic metabolism might explain the increased uPAR levels in serum BA children with hepatic dysfunction. Besides, other tissues outside the liver could synthesize and



**Figure 3** Scatter diagram and regression analysis in biliary atresia patients. uPAR levels are correlated with (A) serum aspartate aminotransferase (B) serum alanine aminotransferase (C) serum total bilirubin (D) serum alkaline phosphatase (E) serum albumin and (F) liver stiffness. uPAR: Urokinase-type plasminogen activator receptor.

release uPAR into the blood. The rising serum level of uPAR is likely attributable to the results of hepatocellular injury and further liver fibrosis. Whether increment of serum uPAR in BA children indicates low destruction, high production, or both remain obscure. Additional research will be needed to clarify the molecular basis leading to increased circulating uPAR.

Several caveats need to be acknowledged in this study. First, relatively small sample size of enrolled subjects limits the statistical power of our findings. Second, the cross-sectional study precludes definite information regarding causal relationships. In addition, inadequate assessment of various confounders such as comorbidity must be considered. To address these challenges, future studies should collect prospective measurements of these data

to preclude bias and reverse causation. Moreover, the present investigation was restricted to the subjects under follow-up at our institution. Accordingly, our results may not be generalized across different populations. Finally, hepatic expression of uPAR has not been investigated. Further studies on immunohistochemistry of uPAR from liver tissues might provide better knowledge on molecular mechanisms of uPAR in biliary atresia.

To sum up, our study illustrated that circulating uPAR and liver stiffness measurement were markedly higher in biliary atresia children than in control subjects. Circulating uPAR was more elevated in jaundiced BA children compared to non-jaundiced BA children. Furthermore, elevated serum uPAR was correlated with hepatic dysfunction and outcome parameters. Circulating uPAR



and liver stiffness values might be used as noninvasive biological markers indicating the progression and prognosis of hepatic fibrosis in postoperative biliary atresia children. Although underlying mechanisms of the cause and effect relationships remain elusive, there is abundant room for the definite role of uPAR in the etiopathogenesis of hepatic fibrosis in BA.

## ACKNOWLEDGMENTS

The authors thank the Thailand Research Fund (RSA5880019), the Research Chair Grant from the National Science and Technology Development Agency, and the 100<sup>th</sup> Anniversary Chulalongkorn University Fund for Doctoral Scholarship to WU, National Research University Project, through the Ageing Cluster (NRU59-056-AS), Chulalongkorn University.

## COMMENTS

### Background

Biliary atresia (BA) is a severe chronic cholestatic liver disease of unknown etiology in young infants. The exact pathogenesis of BA remains a matter of debate. Circulating urokinase plasminogen activator receptor (uPAR) has arisen as a promising biochemical marker of certain disorders, such as liver injury and fibrosis. Although recent reports suggest a potential applicability for the measurement of circulating uPAR in liver fibrosis, the assessments on circulating uPAR and liver stiffness of BA have never been documented.

### Research frontiers

Recent evidences demonstrate the significance of urokinase plasminogen activator receptor in hepatitis, liver fibrosis, and liver failure. The current study shows that circulating uPAR levels are more elevated in BA children than in control subjects. Moreover, uPAR level is correlated with liver stiffness, and clinical outcomes in postoperative BA.

### Innovations and breakthroughs

BA children exhibited significantly higher circulating uPAR and liver stiffness values than control subjects. Circulating uPAR and liver stiffness values were more pronounced in jaundiced BA children than in non-jaundiced BA children. Additionally, elevated circulating uPAR levels were associated with hepatic dysfunction and clinical outcomes.

### Applications

Increased circulating uPAR and liver stiffness values were associated with hepatocellular dysfunction in postKasai children affected with BA. As a consequence, circulating uPAR and liver stiffness measurements could be used as noninvasive biological markers indicating the progression and prognosis of liver fibrogenesis in BA children.

### Terminology

uPAR also known as CD87, is a multidomain membrane protein that has a role in the regulation of cell migration, proliferation, and survival and is expressed by diverse immune cells and endothelial cells, which contribute to the etiopathogenesis of hepatic inflammation and liver fibrogenesis.

### Peer-review

Great paper that needs to be published. uPAR is known to be a substantial factor in the etiopathogenesis of hepatic inflammation and liver fibrogenesis.

## REFERENCES

1 Hartley JL, Davenport M, Kelly DA. Biliary atresia. *Lancet*

- 2009; **374**: 1704-1713 [PMID: 19914515 DOI: 10.1016/S0140-6736(09)60946-6]
- 2 Bassett MD, Murray KF. Biliary atresia: recent progress. *J Clin Gastroenterol* 2008; **42**: 720-729 [PMID: 18496390 DOI: 10.1097/MCG.0b013e3181646730]
- 3 Erlichman J, Hohlweg K, Haber BA. Biliary atresia: how medical complications and therapies impact outcome. *Expert Rev Gastroenterol Hepatol* 2009; **3**: 425-434 [PMID: 19673629 DOI: 10.1586/egh.09.30]
- 4 A-Kader HH, Abdel-Hameed A, Al-Shabrawi M, Mohsen N, El-Karaksy H, Hassanein B, Elsayed B, Abdel-Khalik MK, Karjoo M. Is biliary atresia an autoimmune disease? *Eur J Gastroenterol Hepatol* 2003; **15**: 447 [PMID: 12655270 DOI: 10.1097/01.meg.0000050021.68425.6c]
- 5 Dear AE, Medcalf RL. The urokinase-type-plasminogen-activator receptor (CD87) is a pleiotropic molecule. *Eur J Biochem* 1998; **252**: 185-193 [PMID: 9523687]
- 6 Blasi F. uPA, uPAR, PAI-1: key intersection of proteolytic, adhesive and chemotactic highways? *Immunol Today* 1997; **18**: 415-417 [PMID: 9293155]
- 7 Donadello K, Scolletta S, Covajes C, Vincent JL. suPAR as a prognostic biomarker in sepsis. *BMC Med* 2012; **10**: 2 [PMID: 22221662 DOI: 10.1186/1741-7015-10-2]
- 8 Zhang LP, Takahara T, Yata Y, Furui K, Jin B, Kawada N, Watanabe A. Increased expression of plasminogen activator and plasminogen activator inhibitor during liver fibrogenesis of rats: role of stellate cells. *J Hepatol* 1999; **31**: 703-711 [PMID: 10551395]
- 9 Zimmermann HW, Reuken PA, Koch A, Bartneck M, Adams DH, Trautwein C, Stallmach A, Tacke F, Bruns T. Soluble urokinase plasminogen activator receptor is compartmentally regulated in decompensated cirrhosis and indicates immune activation and short-term mortality. *J Intern Med* 2013; **274**: 86-100 [PMID: 23432143 DOI: 10.1111/joim.12054]
- 10 Sjöwall C, Martinsson K, Cardell K, Ekstedt M, Kechagias S. Soluble urokinase plasminogen activator receptor levels are associated with severity of fibrosis in nonalcoholic fatty liver disease. *Transl Res* 2015; **165**: 658-666 [PMID: 25445207 DOI: 10.1016/j.trsl.2014.09.007]
- 11 Koch A, Zimmermann HW, Gassler N, Jochum C, Weiskirchen R, Bruensing J, Buendgens L, Dückers H, Bruns T, Gerken G, Neumann UP, Adams DH, Trautwein C, Canbay A, Tacke F. Clinical relevance and cellular source of elevated soluble urokinase plasminogen activator receptor (suPAR) in acute liver failure. *Liver Int* 2014; **34**: 1330-1339 [PMID: 24575897 DOI: 10.1111/liv.12512]
- 12 Zimmermann HW, Koch A, Seidler S, Trautwein C, Tacke F. Circulating soluble urokinase plasminogen activator is elevated in patients with chronic liver disease, discriminates stage and aetiology of cirrhosis and predicts prognosis. *Liver Int* 2012; **32**: 500-509 [PMID: 22098627 DOI: 10.1111/j.1478-3231.2011.02665.x]
- 13 Udomsinprasert W, Honsawek S, Anomasiri W, Chongsrisawat V, Vejchapipat P, Poovorawan Y. Elevated adiponectin is associated with poor outcome in children with biliary atresia. *Asian Biomedicine* 2012; **6**: 369-376 [DOI: 10.5372/1905-7415.0603.068]
- 14 Honsawek S, Chayanupatkul M, Chongsrisawat V, Vejchapipat P, Poovorawan Y. Increased osteopontin and liver stiffness measurement by transient elastography in biliary atresia. *World J Gastroenterol* 2010; **16**: 5467-5473 [PMID: 21086566]
- 15 Chayanupatkul M, Honsawek S, Vejchapipat P, Chongsrisawat V, Poovorawan Y. Elevated serum bone morphogenetic protein 7 levels and clinical outcome in children with biliary atresia. *Eur J Pediatr Surg* 2009; **19**: 246-250 [PMID: 19387926 DOI: 10.1055/s-0029-1216378]
- 16 Honsawek S, Chongsrisawat V, Vejchapipat P, Thawornsuk N, Poovorawan Y. Association of serum levels of tissue inhibitors of metalloproteinase-1 with clinical outcome in children with biliary atresia. *Asian Pac J Allergy Immunol* 2006; **24**: 161-166 [PMID: 17136882]
- 17 Berres ML, Schlosser B, Berg T, Trautwein C, Wasmuth HE. Soluble urokinase plasminogen activator receptor is associated with progressive liver fibrosis in hepatitis C infection. *J Clin*

- Gastroenterol* 2012; **46**: 334-338 [PMID: 21934527 DOI: 10.1097/MCG.0b013e31822da19d]
- 18 **Filik L.** Soluble urokinase plasminogen activator receptor in chronic hepatitis due to hepatitis C virus. *J Clin Gastroenterol* 2012; **46**: 346-347 [PMID: 22186743 DOI: 10.1097/MCG.0b013e31823a86f5]
  - 19 **Chounta A,** Ellinas C, Tzanetakou V, Pliarhopoulou F, Mplani V, Oikonomou A, Leventogiannis K, Giamarellos-Bourboulis EJ. Serum soluble urokinase plasminogen activator receptor as a screening test for the early diagnosis of hepatocellular carcinoma. *Liver Int* 2015; **35**: 601-607 [PMID: 25348952 DOI: 10.1111/liv.12705]
  - 20 **Sevgi DY,** Bayraktar B, Gündüz A, Özgüven BY, Togay A, Bulut E, Uzun N, Dökmetaş İ. Serum soluble urokinase-type plasminogen activator receptor and interferon- $\gamma$ -induced protein 10 levels correlate with significant fibrosis in chronic hepatitis B. *Wien Klin Wochenschr* 2016; **128**: 28-33 [PMID: 26546355 DOI: 10.1007/s00508-015-0886-4]
  - 21 **Morita Y,** Hayashi Y, Wang Y, Kanamaru T, Suzuki S, Kawasaki K, Ohta K, Yamamoto M, Saitoh Y, Itoh H, Doe WF. Expression of urokinase-type plasminogen activator receptor in hepatocellular carcinoma. *Hepatology* 1997; **25**: 856-861 [PMID: 9096588 DOI: 10.1002/hep.510250412]

**P- Reviewer:** Fernandez-Pineda I   **S- Editor:** Qi Y   **L- Editor:** A  
**E- Editor:** Li D



## Bibliometric analysis of top 100 cited articles in nonalcoholic fatty liver disease research

Tong-Shuo Zhang, Hua-Lei Qin, Tong Wang, Hai-Tao Li, Hai Li, Shi-Hai Xia, Xiao-Hui Xiang

Tong-Shuo Zhang, Hua-Lei Qin, Tong Wang, Hai-Tao Li, Hai Li, Shi-Hai Xia, Xiao-Hui Xiang, Department of Hepatopancreatobiliary and Splenic Medicine, Affiliated Hospital, Logistics University of People's Armed Police Force, Tianjin 300162, China

**Author contributions:** Zhang TS, Qin HL, Wang T, Li HT, Li H and Xiang XH prepared the manuscript; Xia SH and Xiang XH contributed to the conception of this work, revised and approved the manuscript.

**Supported by** The National Natural Science Foundation of China, No. 81173393; the Natural Science Foundation of Tianjin City, No. 12JCZDJC25500; and the Innovation Team Program from Logistics University of People's Armed Police Force, No. WHTD201310.

**Conflict-of-interest statement:** No conflicts of interest, financial or otherwise, are declared by the authors.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Xiao-Hui Xiang, Department of Hepatopancreatobiliary and Splenic Medicine, Affiliated Hospital, Logistics University of People's Armed Police Force, 220 Chenglin Road, Hedong District, Tianjin 300162, China. [xiaohuixiang@163.com](mailto:xiaohuixiang@163.com)  
 Telephone: +86-22-60578765  
 Fax: +86-22-24370605

Received: June 10, 2016

Peer-review started: June 15, 2016

First decision: July 20, 2016

Revised: August 10, 2016

Accepted: September 21, 2016

Article in press: September 22, 2016

Published online: November 28, 2016

### Abstract

#### AIM

To identify and assess the research situation of top 100 cited articles in nonalcoholic fatty liver disease (NAFLD).

#### METHODS

The global scientific research articles in the Science Citation Index-Expanded relevant to NAFLD were retrieved and listed according to their citation times from the most to the least. The 100 most frequently cited original articles were selected to systematically evaluate their bibliometric parameters including times cited, publication year, journals, subject categories, and the highly related concepts of NAFLD, which reflected the history and current situation, publication distribution of leading countries and institutes as well as the research hotspots of NAFLD.

#### RESULTS

Top 100 cited articles in NAFLD were published from 1965 to 2015 with a citation ranging of 227 to 2151 times since publication, in which the United States was the most predominant country and Mayo Clin was the most productive institution. The majority of the top 100 cited articles were concentrated in SCI subject category of Gastroenterology and Hepatology. Hepatology and Gastroenterology is the top journal that published over half 100 top-cited articles. The significant peak of top cited articles present in the first half of the 2000s while the highest mean number of citation presents in first half of the 1980s. In addition, concepts related to pathology characteristics, epidemiology and medicalization, metabolic syndrome and its combination of symptoms including insulin resistance, biomarkers

of lipid metabolism and obesity are listed as the highly related concepts.

### CONCLUSION

The 100 top-cited articles marked with the leading countries, institutions, journals, hotspots and development trend in NAFLD field that could provide the foundation for further investigations.

**Key words:** Bibliometrics; Top-cited articles; Metabolic syndrome; Prevalence; Medicalization; Nonalcoholic fatty liver disease

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Bibliometrics was used to quantitatively analyze top 100 cited articles from the database of the Science Citation Index Expanded to reveal the global publication trends about nonalcoholic fatty liver disease (NAFLD). This study is the first global look at the history and current situation of NAFLD research to assess the performances of leading countries/territories and institutes and research hotspots of this disease. The performances and research hotspots are related to the potential pathogenesis of NAFLD. Incidence and prevalence as well as treatment progress for NAFLD were systematically reviewed, and their relationships with global performances results were also discussed.

Zhang TS, Qin HL, Wang T, Li HT, Li H, Xia SH, Xiang XH. Bibliometric analysis of top 100 cited articles in nonalcoholic fatty liver disease research. *World J Hepatol* 2016; 8(33): 1478-1488 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i33/1478.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i33.1478>

### INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is defined by liver fat deposition with a concentration of hepatic triglycerides exceeding 5% of liver weight in the absence of excessive alcohol intake. NAFLD is an umbrella term used to describe a histological spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). NASH is virtually indistinguishable histologically from alcoholic steatohepatitis, which is designated the disease with inflammation and liver cell injury in some NAFLD patients<sup>[1]</sup>. It was thought that hepatic fatty change was a kind of benign lesions previously. However, the recent research showed that about 10%-30% of NAFLD could evolve into NASH, accompanying by fibrosis, cirrhosis, liver failure and even hepatocellular carcinoma<sup>[2]</sup>. NAFLD patients are more likely to be accompanied with obesity, diabetes, cardiovascular and cerebrovascular diseases to increase death and disability rate. Owing to the high morbidity rate of obesity and metabolic syndrome worldwide, NAFLD has become the leading cause of chronic liver disease<sup>[1]</sup>. It is time to identify and evaluate

the high citation articles to get insight into history and current situation of NAFLD research.

Citation rank list has been often used in medicine to characterize works with the remarkable intellectual influence<sup>[3]</sup>. Many highly cited articles have stimulated further standard-breaking investigations and discussions<sup>[4]</sup>. However, the bibliometric analysis of the most influential articles in NAFLD field remains unexploited. As the most frequently used source database for a broad review of scientific value in a specific research field, Science Citation Index-Expanded (SCI-Expanded) from Thomson Reuters is a highly effective research tool for evaluating scientific performance and tracking evolution trends. In this study, bibliometric method was applied to analyze the citation times, publication year, countries and institutes, journals, subspecialty, and key words of the 100 most cited articles in NAFLD field in SCI-Expanded from 1965 to 2015.

### MATERIALS AND METHODS

The data were obtained from the SCI-Expanded from the Institute for Scientific Information, which indexed 8618 major journals with citation references across 176 categories in science edition in 2015. The keywords for bibliography retrieval in database consisted of "nonalcoholic steatohepatitis", "nonalcoholic fatty liver disease", and their heteromorphic form and abbreviation limited in liver or hepatology fields. Papers were listed according to their citation times from the most to the least. Only the top 100 original articles from the most citation list were included for further analysis. The retrieve process of the top 100 cited articles was shown in Figure 1. In detail, the retrieved data for statistical process were imported to Excel 2010. According to JCR in 2014 (available in June 2015), the reported impact factor (IF) of each journal was referred. The 100 top cited articles were assessed by decreasing orders of articles and citation. Bibliometric parameters including publication productions of countries and institutes with five indexes including total, independent, collaborative, first author, and corresponding author articles; distribution of journals and subspecialties; top 10 of most cited articles were assessed.

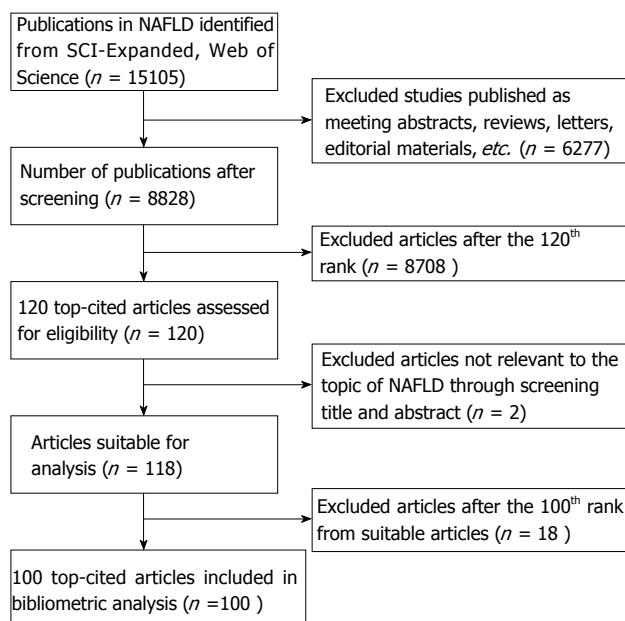
Furthermore, the most frequent key words and concepts were also discussed. Part of concepts such as "NAFLD" and "NASH" were abandoned since they completely overlap with the study content. Highly related concepts including all concepts from the Gene Ontology (GO) and the Medical Subject Headings (MeSH) were categorized by semantic search technology using GoPubMed® search engine (<http://www.gopubmed.org/web/gopubmed/>).

### RESULTS

#### Publication year

After screening, 8828 meaningful articles related to NAFLD were retrieved in the period of 1965 to 2015. It





**Figure 1** Flow chart of the selection process for the top 100 cited in nonalcoholic fatty liver disease. NAFLD: Nonalcoholic fatty liver disease.

can be seen that the number of total articles increased at an exponential rate, which entered an exponential growth phase since 2004 (Figure 2). A power exponential function can describe the growth curve:  $Y = 1 \times 10^{-233} e^{0.2701x}$ ,  $R^2 = 0.9668$ .

The publication years of the top 100 cited articles in NAFLD field spanned from 1980 to 2012 with a citation ranging from 227 to 2151 times since publication. The majority of top 100 cited articles (74%) were concentrated in the 2000s (Figure 3). The most cited article published by Kleiner DE (National Cancer Institute, United States) in 2005 was cited 2151 times according to the SCI-Expanded database (Table 1).

### Publication distribution of countries and institutes

The top 100 cited articles were originated from 19 countries. The most productive country was the United States (55), followed sequentially by Italy (20), Australia (14), France (9), United Kingdom (7). The rest of the countries had less than four publications (Table 2). The numbers in the brackets refer to the publication number (similarly hereinafter).

Twelve institutions published more than 4 top cited articles. Mayo Clin (12) ranked the first place in NAFLD research, followed by University of Bologna (9), University of Turin (9), The University of Sydney (7) and University of California, San Diego (6). And the rest of the Institutes such as University of Texas, Saint Louis University and Virginia Commonwealth University contributed five each to the top 100 cited articles (Table 3).

### Subspecialties and journals

According to the JCR in 2014, the top 100 articles of NAFLD were scattered in 13 SCI subject categories (Table 4).

These main subspecialties were Gastroenterology and Hepatology (71), Endocrinology and Metabolism (7), General and Internal Medicine (6), Research and Experimental Medicine (4) and Science and Technology (4).

The top 100 articles were distributed in 25 journals including professional journals and other disciplines journals. Eleven (44%) journals published 2 or more articles (Table 5), among which the most productive journal was *Hepatology* (42), followed by *Gastroenterology* (16), *Am J Gastroenterol* (5), *J Hepatol* (5), *J Clin Invest* (4), *Proc Natl Acad Sci USA* (3) and *J Clin Endocrinol Metab* (3).

### The most frequently cited articles

As elaboration of all the top 100 cited articles is difficult, the top 10 citation articles were further discussed instead. United States (7), Italy (2) and Australia (1) respectively published the top 10 most frequently cited articles (Table 1). Three in ten focused on epidemiological subjects to investigate the regional and ethnic differences and explore the genetic mechanism implied in NAFLD, which were published respectively in the year of 1990 (864 citations), 2004 (1320 citations) and 2005 (974 citations) (Table 1). Other three articles discussed the pathogenic role of metabolic syndrome where insulin resistance and obesity were repeatedly mentioned. The rest of articles analyzed NAFLD from the clinical and histological aspect, among which two were about the histological grading and staging of NAFLD.

### Highly related concepts

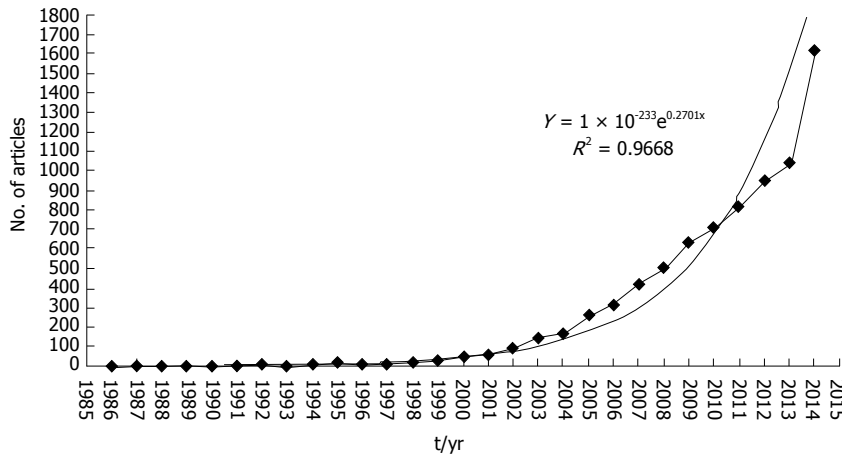
Highly related concepts of the top 100 cited papers from GO and MeSH with frequency more than 10 times were listed in Table 6. The analysis indicated that multi-system metabolic syndrome and its related key words (obesity, insulin resistance, etc.) occupied a majority of proportion. Some key words discussed histological and pathology characteristics of NAFLD including hepatic steatosis, fibrosis, biopsies, etc. Noteworthy, the topic of epidemiology covering prevalence, male/men, female/women, middle aged and adolescent was also involved in frequent concepts (Table 7).

## DISCUSSION

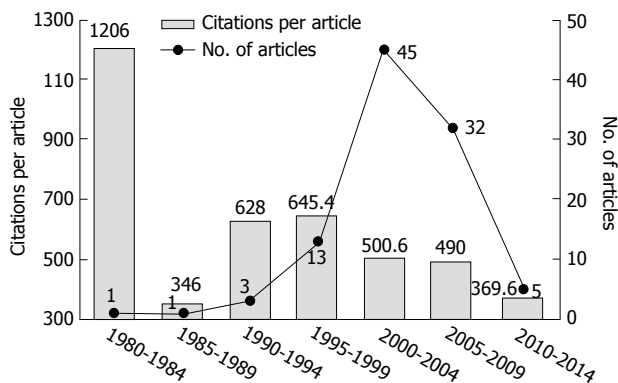
This paper used bibliometrics method to evaluate top 100 cited articles to reveal the global publication performance of NAFLD. The high citation articles can reflect the development evolution direction and scientific level in the NAFLD research field to a certain extent.

### Publication trends and distribution of NAFLD-related literature

In recent five decades, exponential increase of published articles reflects the globally development trend of NAFLD. In line with the increased prevalence of obesity, diabetes, and hyperlipemia, NAFLD has been increasing worldwide over recent half century<sup>[5]</sup>. As a result of modern



**Figure 2** Number of global SCI Journal articles varies with time. Remarks: Fitting equation during 1985-2015 is:  $Y = 1 \times 10^{-233} e^{0.2701x}$ ,  $R^2 = 0.9668$ . In the equation, Y is the number of accumulation articles and X is the sequence number of year. It indicated that research on NAFLD entered an exponential growth phase since 2004. NAFLD: Nonalcoholic fatty liver disease.



**Figure 3** Number of the top 100 cited papers in nonalcoholic fatty liver disease per five year and the mean of the citation of the top cited paper with five years bin.

sedentary and over-nutrition lifestyle which makes a very large population fall risk of NAFLD, research on NAFLD would develop more rapidly in the near future<sup>[6]</sup>.

East Asian countries/territories such as Japan, China (mainland), South Korea and Taiwan occupied an important place in NAFLD research and their importance tended to be more and more obvious. This might owe to the rising prevalence of NAFLD in Asia recently as well as the growth of economic power and the advance of scientific research which prompted these countries/territories to invest more in research to prevent and control NAFLD<sup>[6]</sup>. A global scientific review covered total articles relevant to NAFLD from 1986 to 2013 were performed to analyze distribution of publication number and found that Japan, China (mainland) and South Korea ranked second, fourth and ninth respectively among the most productive country/territories<sup>[7]</sup>. However, only six of top 100 cited papers originate these countries/territories. It shows that the quality and influence of research in NAFLD need to improve for East Asian countries.

It was found that most of the 100 most cited papers

were published in 2000s (74 articles), while the most of high citation times per articles distributed in 1990s. These distributions suggested that the older paper had the more citation times<sup>[8]</sup>. The opinions in 1990s and 2000s were neither too old to be outdated nor too nearly to be cited. Actually, academic community has recognized that the real importance and influence of a work often can't be precisely assessed for at least 2 decades after it is published<sup>[9]</sup>.

### The research hotspots of NAFLD

Highly related concepts and top keywords could partly reflect the profile of hotspots in NAFLD research. GoPubMed® search engine connect text (abstracts from the MEDLINE database) to background knowledge in the form of semantic networks of concept categories, which is done by meaning and not by keywords only. These results are approximately consistent with our contemporaneous bibliometric analysis in high frequency keywords that covered total articles relevant to NAFLD<sup>[7]</sup>.

**Potential pathogenesis:** According to highly related concepts list, a cluster of pathogenesis related keywords occupied a majority of high frequency words mentioned by NAFLD researches. The research hotspots extracted using bibliometrics analysis informs the underlying pathogenesis of NAFLD. The results indicated that multisystem metabolic syndrome and its combination of symptoms including insulin resistance, obesity as well as oxidative stress and dyslipoproteinemia played a vital role in the pathogenesis of NAFLD. In fact, although pathogenesis of NAFLD remains elusive, the severity of NAFLD seems to increase in parallel with the features of metabolic syndrome<sup>[10-12]</sup>. NAFLD/NASH is increasingly regarded as a hepatic manifestation of metabolic syndrome. However, considering that not all patients with NAFLD/NASH suffer from one of these conditions<sup>[1]</sup>, still uncertain pathogenesis of NAFLD might hinder the people and needs to be explored<sup>[13]</sup>.

**Table 1** The information of top 100 cited articles in nonalcoholic fatty liver disease

Rank	Title of article	Journal	First author/institute	Year	Times cited
1	Design and validation of a histological scoring system for nonalcoholic fatty liver disease	<i>Hepatology</i>	Kleiner DE/NCI, United States	2005	2151
2	Nonalcoholic steatohepatitis: A proposal for grading and staging the histological lesions	<i>Am J Gastroenterol</i>	Brunt EM/Saint Louis University, United States	1999	1609
3	Nonalcoholic fatty liver disease: A spectrum of clinical and pathological severity	<i>Gastroenterology</i>	Matteoni CA/Cleveland Clin Fdn, United States	1999	1506
4	Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity	<i>Hepatology</i>	Browning JD/Univ Texas, United States	2004	1320
5	Non-alcoholic steatohepatitis - Mayo-Clinic experiences with A hitherto unnamed disease	<i>Mayo Clin Proc</i>	Ludwig J/Mayo Clin, United States	1980	1206
6	Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome	<i>Hepatology</i>	Marchesini G/Università di Bologna, Bologna, Italy	2003	1134
7	Nonalcoholic fatty liver disease - a feature of the metabolic syndrome	<i>Diabetes</i>	Marchesini G/Univ Bologna, Italy	2001	1072
8	The natural history of nonalcoholic fatty liver disease: A population-based cohort study	<i>Gastroenterology</i>	Adams LA/Mayo Clin, United States	2005	974
9	Nonalcoholic steatohepatitis: Association of insulin resistance and mitochondrial abnormalities	<i>Gastroenterology</i>	Sanyal AJ/Virginia Commonwealth Univ, United States	2001	935
10	The natural-history of nonalcoholic steatohepatitis - a follow-up-study of 42 patients for up to 21 yr	<i>Hepatology</i>	Powell EE/University of Queensland, Australia	1990	864
11	Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis	<i>Hepatology</i>	Angulo P/Mayo Clin, United States	1999	802
12	Sources of fatty acids stored in liver and secreted <i>via</i> lipoproteins in patients with nonalcoholic fatty liver disease	<i>J Clin Invest</i>	Donnelly KL/Univ Minnesota, United States	2005	801
13	Nonalcoholic steatohepatitis - an expanded clinical entity	<i>Gastroenterology</i>	Bacon BR/St. Louis UNIV, United States	1994	756
14	Association of nonalcoholic fatty liver disease with insulin resistance	<i>Am J Med</i>	Marchesini G/Univ Bologna, United States	1999	736
15	Long-term follow-up of patients with NAFLD and elevated liver enzymes	<i>Hepatology</i>	Ekstedt M/Linköping Univ Hosp, Sweden	2006	719
16	Expanding the natural history from cryptogenic cirrhosis to of nonalcoholic steatohepatitis: Hepatocellular carcinoma	<i>Gastroenterology</i>	Bugianesi E/Univ Turin, Italy	2002	712
17	The utility of radiological imaging in nonalcoholic fatty liver disease	<i>Gastroenterology</i>	Saadeh S/Inova Fairfax Hosp, United States	2002	708
18	The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice	<i>J Clin Invest</i>	Xu AM/Univ Auckland, China	2003	696
19	Nonalcoholic fatty liver disease: Predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese	<i>Gastroenterology</i>	Dixon JB/Monash Univ, Australia	2001	666
20	A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis	<i>N Engl J Med</i>	Belfort R/Univ Texas, Italy	2006	662
21	Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease	<i>Nature Genet</i>	Romeo S/Univ Texas, United States	2008	614
22	NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome	<i>Hepatology</i>	Chitturi S/Univ Sydney, Australia	2002	610
23	Sampling variability of liver biopsy in nonalcoholic fatty liver disease	<i>Gastroenterology</i>	Ratziu V/Grp Hosp Pitie Salpetriere, France	2005	572
24	Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men	<i>J Clin Endocrinol Metab</i>	Seppala-Lindroos A/Univ Helsinki, Finland	2002	563
25	Beyond insulin resistance in NASH: TNF-alpha or adiponectin?	<i>Hepatology</i>	Hui JM/Westmead Hosp, Australia	2004	552
26	Magnetic resonance spectroscopy to measure hepatic triglyceride content: Prevalence of hepatic steatosis in the general population	<i>Am J Physiol -Endocrinol Metab</i>	Szczepaniak, LS/Univ Texas, United States	2005	551
27	Pioglitazone, Vitamin E or Placebo for Nonalcoholic Steatohepatitis	<i>N Engl J Med</i>	Sanyal AJ/Virginia Commonwealth Univ, United States	2010	550
28	The natural history of nonalcoholic fatty liver: A follow-up study	<i>Hepatology</i>	Teli MR/Univ Newcastle, United Kingdom	1995	544
29	Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease	<i>J. Biol. Chem.</i>	Samuel VT/Yale Univ, Australia	2004	537
30	Obesity increases sensitivity to endotoxin liver injury: Implications for the pathogenesis of steatohepatitis	<i>Proc Natl Acad Sci USA</i>	Yang SQ/Johns Hopkins Univ, United States	1997	504
31	Prevalence of fatty liver in children and adolescents	<i>Pediatrics</i>	Schwimmer JB/Univ Calif San Diego, United States	2006	454
32	Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma	<i>Gastroenterology</i>	El-Serag HB/Houston Dept Vet Affairs Med Ctr, United States	2004	452

33	Hepatocyte apoptosis and Fas expression are prominent features of human nonalcoholic steatohepatitis	<i>Gastroenterology</i>	Feldstein AE/Mayo Clin, United States	2003	451
34	Prevalence of and risk factors for nonalcoholic fatty liver disease: The Dionysos Nutrition and Liver Study	<i>Hepatology</i>	Bedogni G/Fondo Studio Malattie Fegato ONLUS, Italy	2005	449
35	CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis	<i>J Clin Invest</i>	Leclercq IA/Univ Sydney, United States	2000	435
36	Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease	<i>Hepatology</i>	Li ZP/Johns Hopkins Univ, United States	2003	433
37	Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis	<i>Gastroenterology</i>	George DK/Royal Brisbane Hosp, Australia	1998	431
38	Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values	<i>Hepatology</i>	Mofrad P/Virginia Commonwealth Univ, U United States	2003	427
39	The NAFLD fibrosis score: A noninvasive system that identifies liver fibrosis in patients with NAFLD	<i>Hepatology</i>	Angulo P/Mayo Clin, United Kingdom	2007	425
40	A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis	<i>Hepatology</i>	Promrat K/NIDDK, United States	2004	410
41	Improved nonalcoholic steatohepatitis after 48 wk of treatment with the PPAR-gamma ligand rosiglitazone	<i>Hepatology</i>	Neuschwander-Tetri BA/St. Louis Univ, United States	2003	406
42	Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity	<i>Nature</i>	Henao-Mejia J/Yale Univ, United States	2012	399
43	Liver pathology and the metabolic syndrome X in severe obesity	<i>J Clin Endocrinol Metab</i>	Marceau P/SUNY Hlth Sci Ctr, Canada	1999	389
44	The metabolic syndrome as a predictor of nonalcoholic fatty liver disease	<i>Ann Intern Med</i>	Hamaguchi M/Asahi Univ, Japan	2005	387
45	Metformin in non-alcoholic steatohepatitis	<i>Lancet</i>	Marchesini G/Univ Bologna, Italy	2001	376
46	Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: Further evidence for an etiologic association	<i>Hepatology</i>	Pagano G/Univ Turin, Italy	2002	373
47	Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice	<i>Proc Natl Acad Sci USA</i>	Dumas ME/Univ London Imperial Coll Sci Technol & Med, United Kingdom	2006	361
48	Hepatic cytochrome p450 2E1 is increased in patients with nonalcoholic steatohepatitis	<i>Hepatology</i>	Weltman MD/Westmead Hosp, Sweden	1998	355
49	The histological course of nonalcoholic fatty liver disease: A longitudinal study of 103 patients with sequential liver biopsies	<i>J Hepatol</i>	Adams LA/Mayo Clin, United States	2005	349
50	Nonalcoholic steatohepatitis - A study of 49 patients	<i>Hum Pathol</i>	Lee RG/Oregon Health Sciences University, United States	1989	346
51	Prevalence of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis Among a Largely Middle-Aged Population Utilizing Ultrasound and Liver Biopsy: A Prospective Study	<i>Gastroenterology</i>	Williams CD/Brooke Army Med Ctr, United States	2011	343
52	Free fatty acids promote hepatic lipotoxicity by stimulating TNF- $\alpha$ expression <i>via</i> a lysosomal pathway	<i>Hepatology</i>	Feldstein AE/Mayo Clin, United States	2004	336
53	<i>In vivo</i> assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease	<i>Hepatology</i>	Wieckowska A/Cleveland Clin Fdn, United States	2006	330
54	Therapeutic effects of restricted diet and exercise in obese patients with fatty liver	<i>J Hepatol</i>	Ueno T/Kurume University School of Medicine, Japan	1997	329
55	Gene expression of tumor necrosis factor $\alpha$ and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients	<i>Hepatology</i>	Crespo J/Hosp Univ Marques Valdecilla, Spain	2001	327
56	Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis	<i>Hepatology</i>	Yamaguchi K/Duke Univ, United States	2007	324
57	Nonalcoholic fatty liver disease: Improvement in liver histological analysis with weight loss	<i>Hepatology</i>	Dixon JB/Monash Univ, Australia	2004	324
58	The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor $\alpha$ in the pathogenesis of non-alcoholic steatohepatitis	<i>Gut</i>	Wigg AJ/Queen Elizabeth Hosp, Australia	2001	324
59	Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity	<i>Proc Natl Acad Sci USA</i>	Fabbrini E/Washington Univ, Greece	2009	323
60	Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: A pilot study	<i>Hepatology</i>	Laurin J/Mayo Clin, United States	1996	317
61	Vitamin E treatment of nonalcoholic steatohepatitis in children: A pilot study	<i>J Pediatr</i>	Lavine JE/Univ Calif San Diego, United States	2000	312
62	A randomized controlled trial of metformin <i>vs</i> vitamin E or prescriptive diet in nonalcoholic fatty liver disease	<i>Am J Gastroenterol</i>	Bugianesi E/Univ Bologna, Italy	2005	309
63	Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: Results of a randomized trial	<i>Hepatology</i>	Lindor KD/Mayo Clin, Canada	2004	305
64	Deletion of NEMO/IKK $\gamma$ in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma	<i>Cancer Cell</i>	Luedde T/Univ Cologne, Belgium	2007	285
65	NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States	<i>Hepatology</i>	Marrero JA/Univ Michigan, United States	2002	283



66	Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis	<i>Am J Gastroenterol</i>	Harrison SA/Univ Texas, United States	2003	281
67	High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: A potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis	<i>Hepatology</i>	Paradis V/Hop Bicetre, France	2001	281
68	Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: A case-control study	<i>Hepatology</i>	Poonawala A/Johns Hopkins Univ, United States	2000	281
69	Insulin resistance-associated hepatic iron overload	<i>Gastroenterology</i>	Mendler MH/Hop Pontchaillou, France	1999	281
70	Free fatty acids induce JNK-dependent hepatocyte lipopoptosis	<i>J Biol Chem</i>	Malhi H/Mayo Clin, United States	2006	280
71	Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis	<i>Hepatology</i>	Musso G/Univ Turin, Italy	2003	279
72	Cytokines and NASH: A pilot study of the effects of lifestyle modification and vitamin E	<i>Hepatology</i>	Kugelmas M/Univ Louisville, United States	2003	275
73	Nonalcoholic fatty liver disease and risk of future cardiovascular events among type 2 diabetic patients	<i>Diabetes</i>	Targher G/Osped Sacro Cuore don G Calabria, Italy	2005	271
74	A lipidomic analysis of nonalcoholic fatty liver disease	<i>Hepatology</i>	Puri P/Virginia Commonwealth Univ, United States	2007	269
75	The Incidence and Risk Factors of Hepatocellular Carcinoma in Patients with Nonalcoholic Steatohepatitis	<i>Hepatology</i>	Ascha MS/Cleveland Clin, United States	2010	268
76	Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients	<i>Diabetes Care</i>	Targher G/Osped Sacro Cuore don Calabria, United Kingdom	2007	268
77	Burden of liver disease in the United States: Summary of a workshop	<i>Hepatology</i>	Kim WR/Mayo Clin, United States	2002	266
78	Plasma Endotoxin Concentrations In Patients With Alcoholic And Nonalcoholic Liver-Disease - Reevaluation With An Improved Chromogenic Assay	<i>J Hepatol</i>	Fukui H/ROBERT BOSCH KRANKENHAUS, Germany	1991	264
79	Histopathology of pediatric nonalcoholic fatty liver disease	<i>Hepatology</i>	Schwinnner JB/Univ Calif San Diego, USA	2005	262
80	A position statement on NAFLD/NASH based on the EASL 2009 special conference	<i>J Hepatol</i>	Ratzl V/Azienda USL Modena, Italy	2010	259
81	Increased intestinal permeability in obese mice: New evidence in the pathogenesis of nonalcoholic steatohepatitis	<i>Am J Physiol-Gastroint Liver Physiol</i>	Brun P/Univ Padua, Italy	2007	258
82	Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease	<i>Hepatology</i>	Villanova N/Alma Mater Studiorum Univ Bologna, Italy	2005	258
83	Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis	<i>Hepatology</i>	Perez-Carreras M/Hosp Univ 12 Octubre, Spain	2003	254
84	Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis	<i>Hepatology</i>	Ratzl V/Hop La Pitie Salpetriere, France	2002	254
85	A pilot study of a thiazolidinedione, troglitazone, in nonalcoholic steatohepatitis	<i>Am J Gastroenterol</i>	Caldwell SH/Univ Virginia, United States	2001	247
86	Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas	<i>J Clin Invest</i>	Horie Y/Akita Univ, Japan	2004	240
87	Randomized Controlled Trial Testing the Effects of Weight Loss on Nonalcoholic Steatohepatitis	<i>Hepatology</i>	Promrat K/Brown Univ, United States	2010	239
88	Insulin resistance in chronic hepatitis C: Association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis	<i>Gastroenterology</i>	Moucari R/Hop Beaujon, France	2008	239
89	Betaine, a promising new agent for patients with nonalcoholic steatohepatitis: Results of a pilot study	<i>Am J Gastroenterol</i>	Abdelmalek MF/Mayo Clin, United States	2001	239
90	Steatosis in chronic hepatitis C: Relative contributions of obesity, diabetes mellitus, and alcohol	<i>Hepatology</i>	Monto A/Univ Calif San Francisco, United States	2002	237
91	Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis	<i>Hepatology</i>	Yokohama S/Dokkyo Univ, Japan	2004	235
92	Hepatic-Effects Of Dietary Weight-Loss In Morbidly Obese Subjects	<i>J Hepatol</i>	Andersen T/Univ Copenhagen, Denmark	1991	236
93	Rosiglitazone for nonalcoholic steatohepatitis: One-year results of the randomized placebo-controlled fatty liver improvement with rosiglitazone therapy trial	<i>Gastroenterology</i>	Ratzl V/Univ Paris, France	2008	234
94	Diagnosis of Fibrosis and Cirrhosis Using Liver Stiffness Measurement in Nonalcoholic Fatty Liver Disease	<i>Hepatology</i>	Wong VWS/Hop Haut Leveque, China	2010	232
95	Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation	<i>Gastroenterology</i>	Weltman MD/Univ Sydney, Australia	1996	230
96	Effect of steatohepatitis associated with irinotecan or oxaliplatin pretreatment on resectability of hepatic colorectal metastases	<i>J Am Coll Surg</i>	Fernandez FG/Washington Univ, United States	2005	229
97	Adiponectin and its receptors in non-alcoholic steatohepatitis	<i>Gut</i>	Kaser S/Univ Innsbruck Hosp, Spain	2005	229
98	Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C	<i>Hepatology</i>	Hui JM/Univ Sydney, Australia	2003	229

99	Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European liver fibrosis panel and exploring simple markers	<i>Hepatology</i>	Guha IN/Guha, United Kingdom	2008	228
100	Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity	<i>J Clin Endocrinol Metab</i>	Bugianesi E/Univ Turin, Italy	2005	227

NAFLD: Nonalcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; TNF: Tumor necrosis factor; ALT: Alanine aminotransferase; PPAR: Peroxisome proliferator activated receptor; HCV: Hepatitis C virus.

**Table 2 Countries of origin of the top 100 articles in nonalcoholic fatty liver disease**

Rank	Nation	TP	FP	SP	CP	RP	TC
1	United States	55	48	45	10	49	26975
2	Italy	20	13	11	9	15	5567
3	Australia	14	10	8	6	9	4767
4	France	9	6	6	3	7	1861
5	United Kingdom	7	5	2	5	2	1826
6	Japan	4	4	4	0	4	1191
7	Spain	3	3	2	1	2	810
8	Sweden	2	2	1	1	1	1074
9	China	2	2	0	2	0	928
10	Canada	2	2	0	2	0	694
11	Germany	2	1	1	1	1	264
12	Finland	1	1	1	0	1	563
13	Greece	1	1	0	1	0	323
14	Belgium	1	1	0	1	0	285
15	Denmark	1	1	1	0	1	236
16	New Zealand	1	0	0	1	1	0
17	Austria	1	0	0	1	1	0
18	South Africa	1	0	0	1	0	0

TP: The number of total 100 top-cited articles; FP: The number of first author articles; SP: The number of single-country articles; CP: The number of internationally collaborative articles; RP: The number of corresponding author articles in total 100 top-cited articles; TC: Total citation of first author articles; Rank: According to the order of TP firstly and TC secondly. As for New Zealand Austria and South Africa, the country with more citation of corresponding author articles took precedence.

**Epidemic studies:** Concepts related to epidemiology such as humans, male/men, female/women, middle aged and adolescent make up another high frequency concepts cluster, which might be closely involved in the accelerating incidence of this disease. The morbidity rate of NAFLD has doubled during last 20 years, whereas the morbidity rate of other chronic liver diseases has remained stable or even decreased. Epidemic investigations of NAFLD primarily focus on human genetic and metabolic studies<sup>[14]</sup>. Several epidemiological investigations such as case series, familial and twin studies have widely revealed the function of heritability<sup>[15]</sup>. Noteworthy, in comparison to high-risk population of NAFLD clustering around middle-aged and elderly adults before, younger age trend has gradually shown especially in Asian countries during the last two decades. Following the epidemics of childhood obesity, NAFLD as the most common form of chronic liver disease in adolescents has become a reality<sup>[16]</sup>.

**Medicalization progress:** Medicalization is also a high frequency concepts cluster. Lack of uniformed

**Table 3 Top productive institutions list with top 100 cited articles in nonalcoholic fatty liver disease**

Rank	Institution	TP	FP	SP	CP	RP	TC
1	Mayo Clinic	12	12	8	4	11	5950
2	University of Bologna	9	5	1	8	5	3627
2	University of Turin	9	4	1	8	4	1591
4	The University of Sydney	7	4	1	6	2	1504
5	University of California, San Diego	6	3	0	6	3	1028
6	University of Texas	5	5	1	4	4	3428
7	Saint Louis University	5	3	3	2	3	2771
8	Virginia Commonwealth University	5	4	2	3	2	2181
9	Westmead Hospital	4	2	0	4	3	907
10	Washington University	4	2	0	4	1	552
11	University of Paris	4	1	0	4	1	234
12	University of California, San Francisco	4	1	1	3	0	237
13	National Cancer Institute	4	1	0	4	1	2151
14	MetroHealth Medical Center	4	0	0	4	0	0

TP: The number of total 100 top-cited articles; FP: The number of first author articles; SP: The number of single-country articles; CP: The number of internationally collaborative articles; RP: The number of corresponding author articles in total 100 top-cited articles; TC: Total citation of first author articles; Rank: According to the order of TP firstly and TC secondly. As for National Cancer Institute and MetroHealth Med Ctr, the institute with more corresponding author articles took precedence.

diagnosis regulation and no established therapy remains a hindrance to be broken through in this field. NASH is characterized by hepatocellular damage, lobular necro-inflammation and fibrogenesis. The early diagnosis of advanced fibrosis in NAFLD is therefore crucial<sup>[17,18]</sup>. The liver biopsy remains the most reliable diagnostic method to appropriately evaluate the severity of liver fibrosis. Facing to limitations of this invasive technique in current use, a number of experimental biomarkers have been developed in order to predict the degree of liver fibrosis<sup>[19]</sup>. Moreover, as a promising method for evaluation of patients with NAFLD, nuclear medicine through liver scintigraphy has recently been proposed<sup>[20]</sup>.

Preventing existing comorbidities such as metabolic disorders, cardiovascular or cerebrovascular events are the primary target for NAFLD treatment, while the secondary goal of NAFLD therapy is reversal of hepatic steatosis<sup>[21-23]</sup>. Lifestyle modification such as weight loss and balanced diet remains the main way of management in NAFLD/NASH. In addition, the benefit of nutritional supplementation on disease progression has attracted growing interest<sup>[24]</sup>. Most recent data has evidenced the effects of nutrients and dietary bioactive compounds intake (*i.e.*, long-chain PUFA, Vitamin E,

**Table 4** Most frequent subspecialties with the top 100 cited articles in nonalcoholic fatty liver disease

Rank	Subject categories	No. of articles	Total citation
1	Gastroenterology and Hepatology	71	33290
2	Endocrinology and Metabolism	7	3341
3	General and Internal Medicine	6	3917
4	Research and Experimental Medicine	4	2172
5	Science and Technology	4	1587
6	Biochemistry and Molecular Biology	2	817
7	Physiology	2	809
8	Pediatrics	2	766
9	Genetics and Heredity	1	614
10	Pathology	1	346
11	Cell Biology	1	285
12	Oncology	1	285
13	Surgery	1	229

Remarks: In the situation of equal numbers of articles, the subspecialties with more total citation took precedence.

**Table 5** Journal distribution of top 100 cited articles in nonalcoholic fatty liver disease

Rank	Journal	No. of articles	Total citation	Impact factor (2014)
1	<i>Hepatology</i>	42	18867	11.055
2	<i>Gastroenterology</i>	16	9490	16.716
3	<i>Am J Gastroenterol</i>	5	2685	10.755
3	<i>J Hepatol</i>	5	1437	11.336
5	<i>J Clin Invest</i>	4	2172	13.215
6	<i>Proc Natl Acad Sci USA</i>	3	1188	9.674
7	<i>J Clin Endocrinol Metab</i>	3	1179	3.457
8	<i>Diabetes</i>	2	1343	8.095
9	<i>New Engl J Med</i>	2	1212	55.873
10	<i>J Biol Chem</i>	2	817	4.573
11	<i>Gut</i>	2	553	14.66

Remarks: In the situation of equal numbers of articles, the journals with more total citation took precedence.

Vitamin D, minerals and polyphenols) on the modulation of molecular mechanisms leading to fat accumulation, oxidative stress, inflammation and liver fibrosis in NAFLD patients<sup>[25]</sup>. In the field of pharmaceutical therapies, a wide range of drugs have been applied in clinical trials, including antioxidants, lipid lowering agents, and rennin-angiotensin system blockers<sup>[26-28]</sup>. Up to the present, lifestyle modification is the main clinical recommendation as an initial step. Although promising results have shown that long-term insulin sensitizers such as metformin, rosiglitazone, and thiazolidinediones are effective in NAFLD therapy, there are no approved drugs<sup>[29-31]</sup>.

In conclusion, it is important to acknowledge the top 100 cited articles because they marked with the leading countries, institutions, journals, hotspots, past and current trends in NAFLD field that could provide the foundation for further investigations. Highly related concepts of the top 100 cited papers in NAFLD suggest that pathogenesis mainly related to metabolic syndrome, epidemiology, and medicalization including diagnosis and

**Table 6** High frequency key words in the top 100 cited articles in nonalcoholic fatty liver disease (frequency > 2)

Rank	Key word	Frequency
1	Hepatic steatosis	4
1	Obesity	4
3	Fibrosis	3
4	Metabolic syndrome	2
4	Insulin resistance	2
4	Biopsies	2
4	Intestinal bacteria	2
4	Endotoxin	2

**Table 7** Highly related concepts of the top 100 articles in nonalcoholic fatty liver disease categorized by GoPubMed® search engine

Rank	Highly related concepts	Frequency	Rank	Highly related concepts	Frequency
1	Fatty liver	97	24	Wounds and injuries	15
2	Male	91	25	Aspartate	14
				Aminotransferases	
3	Humans	89	26	Mice	14
4	Female	84	27	Carcinoma,	13
				Hepatocellular	
5	Middle aged	72	28	Tumor necrosis factor-alpha	12
6	Patients	71	29	Multivariate analysis	12
7	Fibrosis	59	30	Prospective studies	12
8	Biopsy	45	31	Follow-up studies	12
9	Liver	45	32	Hepatitis C	11
10	Obesity	42	33	cell killing	11
11	Aged	36	34	cytolysis	11
12	Insulin	35	35	Medicalization	11
13	Serum	32	36	Metabolic syndrome X	10
14	Body mass index	31	37	Fatty acids, nonesterified	10
15	Syndrome	25	38	Aspartic acid	10
16	Risk Factors	24	39	Hypoglycemic agents	10
17	Alanine transaminase	23	40	Homeostasis	10
18	Alanine transaminase activity	19	41	Severity of illness index	10
19	Pathogenesis	19	42	Men	10
20	Prevalence	18	43	Personal autonomy	10
21	Hepatocytes	17	44	Women	10
22	Alanine	16	45	Adolescent	10
23	Triglycerides	15			

treatment are attracting ever-growing attention.

## ACKNOWLEDGMENTS

We would like to thank Professor Yuh-Shan Ho from Asia University and Hui-Min Guo, PhD, from Logistics University of People's Armed Police Force for their comments on drafting and polishing the manuscript.

## COMMENTS

### Background

Due to the increasing prevalence of obesity and metabolic syndrome worldwide,

nonalcoholic fatty liver disease (NAFLD) becomes the leading cause of chronic liver disease. The rapid growth of NAFLD research recently drives top cited articles in the field to be identified and bibliometric analysis to assess the history and current situation, publication distribution of leading countries and institutes as well as the research hotspots of NAFLD.

### Research frontiers

A systematic review in 2015 covered total articles relevant to NAFLD from Science Citation Index-Expanded (SCI-Expanded) showed article amount has appeared to geometric growth in recent decades. However, bibliometric result from total articles is not sufficient to indicate the evolution and direction in NAFLD research. The citation times by other authors has been used as a measurable comparison to evaluate the academic impact of an article in its subject field. To date, there have no top cited articles analysis were carried out in NAFLD field.

### Innovations and breakthroughs

This paper summarized the current findings from the analysis of the top 100 cited articles in NAFLD field. It is the first global look at the history and current situation of NAFLD research to assess the performances of leading countries/territories and institutes and research hotspots of this disease. In terms of the number of published 100 top-cited articles in NAFLD, United States was the most predominant country and Mayo Clin was the most productive institution. Highly related concepts of the top 100 cited papers in NAFLD suggest that pathogenesis (mainly related to metabolic syndrome), epidemiology, and medicalization (including diagnosis and treatment) are attracting ever-growing attention.

### Applications

Top 100 cited articles marked with the leading countries, institutions, journals, hotspots, past and current trends in NAFLD field that could provide the foundation for further investigations. Medical bibliometric analysis on top 100 cited articles is expected to provide a reference for the researchers to get involved in NAFLD area.

### Terminology

The articles involved in bibliometric analysis were collected based on online version of SCI-Expanded from Thomson Reuters. Keywords for bibliography retrieval in database consisted of "nonalcoholic steatohepatitis" and "nonalcoholic fatty liver disease".

### Peer-review

This study retrieved the top 100 cited articles in the field of NAFLD and determined the country of origin, peak of highly-cited articles and international collaborations. The present study is very interesting on a high prevalent chronic liver disease.

## REFERENCES

- 1 **LaBrecque DR**, Abbas Z, Anania F, Ferenci P, Khan AG, Goh KL, Hamid SS, Isakov V, Lizarzabal M, Peñaranda MM, Ramos JF, Sarin S, Stimac D, Thomson AB, Umar M, Krabshuis J, LeMair A. World Gastroenterology Organisation global guidelines: Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2014; **48**: 467-473 [PMID: 24921212 DOI: 10.1097/MCG.000000000000116]
- 2 **Dyson JK**, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to treatment. *Frontline Gastroenterol* 2014; **5**: 277-286 [PMID: 25285192 DOI: 10.1136/flgastro-2013-100404]
- 3 **Murray MR**, Wang T, Schroeder GD, Hsu WK. The 100 most cited spine articles. *Eur Spine J* 2012; **21**: 2059-2069 [PMID: 22526702 DOI: 10.1007/s00586-012-2303-2]
- 4 **Lefaiivre KA**, Shadgan B, O'Brien PJ. 100 most cited articles in orthopaedic surgery. *Clin Orthop Relat Res* 2011; **469**: 1487-1497 [PMID: 20922583 DOI: 10.1007/s11999-010-1604-1]
- 5 **Neuschwander-Tetri BA**. Nonalcoholic steatohepatitis and the metabolic syndrome. *Am J Med Sci* 2005; **330**: 326-335 [PMID: 16355018 DOI: 10.1097/00000441-200512000-00011]
- 6 **Farrell GC**, Wong VW, Chitturi S. NAFLD in Asia--as common and important as in the West. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 307-318 [PMID: 23458891 DOI: 10.1038/nrgastro.2013.34]
- 7 **Zhang TS**, Qin HL, Wang T, Li HT, Li H, Xia SH, Xiang XH. Global publication trends and research hotspots of nonalcoholic fatty liver disease: a bibliometric analysis and systematic review. *Springerplus* 2015; **4**: 776 [PMID: 26697286 DOI: 10.1186/s40064-015-1542-1]
- 8 **Picknett T**, Davis K. The 100 most-cited articles from JMB. *J Mol Biol* 1999; **293**: 171-176 [PMID: 10529345 DOI: 10.1006/jmbi.1999.3148]
- 9 **Baltussen A**, Kindler CH. Citation classics in anesthetic journals. *Anesth Analg* 2004; **98**: 443-451, table of contents [PMID: 14742385 DOI: 10.1213/01.ANE.0000096185.13474.0A]
- 10 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923 [PMID: 12668987 DOI: 10.1053/jhep.2003.50161]
- 11 **Boppidi H**, Daram SR. Nonalcoholic fatty liver disease: hepatic manifestation of obesity and the metabolic syndrome. *Postgrad Med* 2008; **120**: E01-E07 [PMID: 18654060 DOI: 10.3810/pgm.2008.07.1800]
- 12 **Liu Q**, Bengmark S, Qu S. The role of hepatic fat accumulation in pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Lipids Health Dis* 2010; **9**: 42 [PMID: 20426802 DOI: 10.1186/1476-511X-9-42]
- 13 **Wu JW**, Wang SP, Alvarez F, Casavant S, Gauthier N, Abed L, Soni KG, Yang G, Mitchell GA. Deficiency of liver adipose triglyceride lipase in mice causes progressive hepatic steatosis. *Hepatology* 2011; **54**: 122-132 [PMID: 21465509 DOI: 10.1002/hep.24338]
- 14 **Cohen JC**, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science* 2011; **332**: 1519-1523 [PMID: 21700865 DOI: 10.1126/science.1204265]
- 15 **Macaluso FS**, Maida M, Petta S. Genetic background in non-alcoholic fatty liver disease: A comprehensive review. *World J Gastroenterol* 2015; **21**: 11088-11111 [PMID: 26494964 DOI: 10.3748/wjg.v21.i39.11088]
- 16 **Marzuillo P**, Grandone A, Perrone L, Miraglia Del Giudice E. Controversy in the diagnosis of pediatric non-alcoholic fatty liver disease. *World J Gastroenterol* 2015; **21**: 6444-6450 [PMID: 26074683 DOI: 10.3748/wjg.v21.i21.6444]
- 17 **Rinella ME**. Nonalcoholic fatty liver disease: a systematic review. *JAMA* 2015; **313**: 2263-2273 [PMID: 26057287 DOI: 10.1001/jama.2015.5370]
- 18 **Stål P**. Liver fibrosis in non-alcoholic fatty liver disease - diagnostic challenge with prognostic significance. *World J Gastroenterol* 2015; **21**: 11077-11087 [PMID: 26494963 DOI: 10.3748/wjg.v21.i39.11077]
- 19 **Enomoto H**, Bando Y, Nakamura H, Nishiguchi S, Koga M. Liver fibrosis markers of nonalcoholic steatohepatitis. *World J Gastroenterol* 2015; **21**: 7427-7435 [PMID: 26139988 DOI: 10.3748/wjg.v21.i24.7427]
- 20 **Tovo CV**, de Mattos AZ, Coral GP, Branco FS, Suwa E, de Mattos AA. Noninvasive imaging assessment of non-alcoholic fatty liver disease: focus on liver scintigraphy. *World J Gastroenterol* 2015; **21**: 4432-4439 [PMID: 25914452 DOI: 10.3748/wjg.v21.i15.4432]
- 21 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]
- 22 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]



- 23 **Başaranoğlu M**, Örmeci N. Nonalcoholic fatty liver disease: diagnosis, pathogenesis, and management. *Turk J Gastroenterol* 2014; **25**: 127-132 [PMID: 25003670 DOI: 10.5152/tjg.2014.7675]
- 24 **Gupta V**, Mah XJ, Garcia MC, Antonypillai C, van der Poorten D. Oily fish, coffee and walnuts: Dietary treatment for nonalcoholic fatty liver disease. *World J Gastroenterol* 2015; **21**: 10621-10635 [PMID: 26457022 DOI: 10.3748/wjg.v21.i37.10621]
- 25 **Dongiovanni P**, Lanti C, Riso P, Valenti L. Nutritional therapy for nonalcoholic fatty liver disease. *J Nutr Biochem* 2016; **29**: 1-11 [PMID: 26895659 DOI: 10.1016/j.jnutbio.2015.08.024]
- 26 **Della Corte C**, Alisi A, Iorio R, Alterio A, Nobili V. Expert opinion on current therapies for nonalcoholic fatty liver disease. *Expert Opin Pharmacother* 2011; **12**: 1901-1911 [PMID: 21639814 DOI: 10.1517/14656566.2011.587123]
- 27 **Gossard AA**, Lindor KD. Current therapies for nonalcoholic fatty liver disease. *Drugs Today (Barc)* 2011; **47**: 915-922 [PMID: 22348916 DOI: 10.1358/dot.2011.47.12.1688530]
- 28 **Xiao J**, Fai So K, Liong EC, Tipoe GL. Recent advances in the herbal treatment of non-alcoholic Fatty liver disease. *J Tradit Complement Med* 2013; **3**: 88-94 [PMID: 24716162 DOI: 10.4103/2225-4110.110411]
- 29 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in non-alcoholic steatohepatitis. *Lancet* 2001; **358**: 893-894 [PMID: 11567710 DOI: 10.1016/S0140-6736(01)06042-1]
- 30 **Ratzliff V**, Giral P, Jacqueminet S, Charlotte F, Hartemann-Heurtier A, Serfaty L, Podevin P, Lacorte JM, Bernhardt C, Bruckert E, Grimaldi A, Poynard T. Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) Trial. *Gastroenterology* 2008; **135**: 100-110 [PMID: 18503774 DOI: 10.1053/j.gastro.2008.03.078]
- 31 **Tolman KG**, Fonseca V, Tan MH, Dalpiaz A. Narrative review: hepatobiliary disease in type 2 diabetes mellitus. *Ann Intern Med* 2004; **141**: 946-956 [PMID: 15611492 DOI: 10.7326/0003-4819-141-12-200412210-00011]

**P- Reviewer:** Clouston AD, Mendez-Sanchez N, Streba LA

**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 December 8; 8(34): 1489-1540





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*



**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**Stephen DH Malnick, *Rehovot***Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**Kamal E Bani-Hani, *Zarqa***Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**Angela Peltec, *Chishinev***Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**CA Asabamaka Onyekwere, *Lagos***Pakistan**Bikha Ram Devrajani, *Jamshoro***Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**Jacek Zielinski, *Gdansk***Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**Reem Al Olaby, *Doha***Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**Ser Yee Lee, *Singapore***South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**MINIREVIEWS**

- 1489 Role of nitric oxide in liver transplantation: Should it be routinely used?

*Fukazawa K, Lang JD*

**ORIGINAL ARTICLE****Case Control Study**

- 1497 Fractional excretion of sodium in hepatorenal syndrome: Clinical and pathological correlation

*Alsaad AA, Wadei HM*

**Retrospective Study**

- 1502 Resection margin influences the outcome of patients with bilobar colorectal liver metastases

*Di Carlo S, Yeung D, Mills J, Zaitoun A, Cameron I, Gomez D*

- 1511 On-treatment quantitative hepatitis B e antigen predicted response to nucleos(t)ide analogues in chronic hepatitis B

*Gao YH, Meng QH, Zhang ZQ, Zhao P, Shang QH, Yuan Q, Li Y, Deng J, Li T, Liu XE, Zhuang H*

**Observational Study**

- 1521 Seroprevalence of hepatitis B surface antigen in pregnant women attending antenatal clinic in Honiara Solomon Islands, 2015

*Getahun A, Baekalia M, Panda N, Lee A, Puiahi E, Khan S, Tahani D, Manongi D*

**Prospective Study**

- 1529 Prevalence and risk factors of acute-on-chronic liver failure in a single center from Argentina

*Dominguez C, Romero E, Graciano J, Fernandez JL, Viola L*

**CASE REPORT**

- 1535 Major hepatectomy using the glissonean approach in cases of right umbilical portion

*Ome Y, Kawamoto K, Park TB, Ito T*



**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Dr. Andrea Mancuso, MD, Medicina Interna 1, ARNAS Civico, 90100 Palermo, Italy

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

**I-IV** Editorial Board

**EDITORS FOR THIS ISSUE**

**Responsible Assistant Editor:** *Xiang Li*  
**Responsible Electronic Editor:** *Dan Li*  
**Proofing Editor-in-Chief:** *Lian-Sheng Ma*

**Responsible Science Editor:** *Fang-Fang Ji*  
**Proofing Editorial Office Director:** *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL BOARD MEMBERS**  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

**EDITORIAL OFFICE**  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
December 8, 2016

**COPYRIGHT**  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
<http://www.wjgnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esps/>

## Role of nitric oxide in liver transplantation: Should it be routinely used?

Kyota Fukazawa, John D Lang

Kyota Fukazawa, Division of Transplant Anesthesiology, Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA 98195, United States

John D Lang, Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA 98195, United States

Author contributions: Both authors contributed to the manuscript.

Conflict-of-interest statement: No relevant conflicts of interest were declared for each author.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: John D Lang, MD, Associate Professor, Department of Anesthesiology and Pain Medicine, University of Washington, 1959 NE Pacific Street, Seattle, WA 98195, United States. [jlange@uw.edu](mailto:jlange@uw.edu)  
Telephone: +1-206-5432673  
Fax: +1-206-5432958

Received: April 29, 2016  
Peer-review started: May 4, 2016  
First decision: July 4, 2016  
Revised: August 6, 2016  
Accepted: October 17, 2016  
Article in press: October 18, 2016  
Published online: December 8, 2016

### Abstract

Ischemia-reperfusion injury (IRI) continues to be a major contributor to graft dysfunction, thus supporting the

need for therapeutic strategies focused on minimizing organ damage especially with growing numbers of extended criteria grafts being utilized which are more vulnerable to cold and warm ischemia. Nitric oxide (NO $\cdot$ ) is highly reactive gaseous molecule found in air and regarded as a pollutant. Not surprising, it is extremely bioactive, and has been demonstrated to play major roles in vascular homeostasis, neurotransmission, and host defense inflammatory reactions. Under conditions of ischemia, NO $\cdot$  has consistently been demonstrated to enhance microcirculatory vasorelaxation and mitigate pro-inflammatory responses, making it an excellent strategy for patients undergoing organ transplantation. Clinical studies designed to test this hypothesis have yielded very promising results that includes reduced hepatocellular injury and enhanced graft recovery without any identifiable complications. By what means NO $\cdot$  facilitates extra-pulmonary actions is up for debate and speculation. The general premise is that they are NO $\cdot$  containing intermediates in the circulation, that ultimately mediate either direct or indirect effects. A plethora of data exists explaining how NO $\cdot$ -containing intermediate molecules form in the plasma as S-nitrosothiols (*e.g.*, S-nitrosoalbumin), whereas other compelling data suggest nitrite to be a protective mediator. In this article, we discuss the use of inhaled NO $\cdot$  as a way to protect the donor liver graft against IRI in patients undergoing liver transplantation.

**Key words:** Liver; Nitric oxide; Ischemia-reperfusion; Nitrite; Transplantation

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Our manuscript assesses the basic and clinical literature of nitric oxide and liver transplantation and creates a scientific/clinical justification for its routine use.

Fukazawa K, Lang JD. Role of nitric oxide in liver trans-

plantation: Should it be routinely used? *World J Hepatol* 2016; 8(34): 1489-1496 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i34/1489.htm> DOI: <http://dx.doi.org/10.4254/wjgh.v8.i34.1489>

## INTRODUCTION

Liver transplantation has become a viable treatment option for recipients suffering from irreversible liver failure for more than three decades. However, the number of recipients on the waiting list continues to grow due to the major mismatch between organ supply and demand, creating tremendous pressure on for the development of techniques to expand the donor pool. There is about 7000 liver transplants performed annually with a trend that is increasing due to demand. As a result, according to national transplant registry database from the Organ Procurement and Transplant Network, about 1000 potential recipients on the waiting list die annually (Figure 1). Therefore, strategies are actively being sought to increase in donor pool. The transplant community has evaluated an option to relax the standard for donors to include donors with suboptimal quality [more damage from anoxic preservation and ischemia-reperfusion injury (IRI)], including the advanced age donor, prolonged cold and warm ischemia time, and hepatic steatosis. Currently, extended criteria donors make up 5%-10% of all donors and this number is increasing. IRI to the liver remains a significant contributor to graft dysfunction, or primary non-function, resulting in increased intensive care unit and hospital stay, increase financial burden, re-transplantation and, in a worst case scenario, death<sup>[1]</sup>.

Nitric oxide (NO $\cdot$ ) is an important endogenously produced biological mediator affecting vascular function, metabolic function and host defense mechanisms<sup>[2]</sup>. It is produced by macrophages, dendritic cells and plays a critical role in host innate and adaptive immunological processes<sup>[3,4]</sup>. Inhaled NO $\cdot$  has been clinically used to treat pulmonary hypertension due to its vasodilating effect in pulmonary microcirculation without causing any unfavorable systemic hemodynamic changes. More recent evidence has suggested a relative NO $\cdot$  deficiency due to IRI and that the use of preemptive inhaled NO $\cdot$  can attenuate liver IRI during liver transplantation.

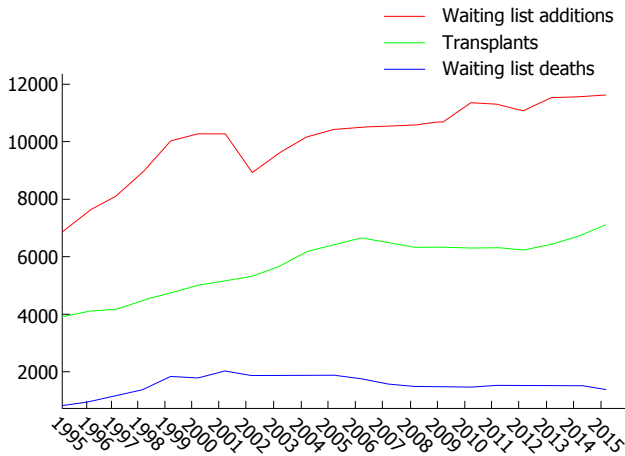
## ENDOGENOUS NO $\cdot$ AND THE LIVER DURING ISCHEMIA-REPERFUSION

Liver graft injury from ischemia-reperfusion is the principal mechanism of liver injury related to procedures involving clamping of hepatic inflow such as hepatectomy and liver transplantation. Cessation of oxygen delivery to sinusoidal microcirculation causes severe ATP depletion which leads to retraction of sinusoidal endothelial cells and Kupffer cells, bleb formation in the microcirculation<sup>[5]</sup>. There is obstruction of sinusoid and

microcirculatory disturbance due to bleb formation as well as accumulation of leukocytes and platelets leads to prolonged ischemia of hepatocytes, so-called, "no-flow phenomenon". In addition, due to the upregulation and generation of inflammatory mediators such as oxygen free radicals, cytokines and chemokines from the Kupffer cells occupying sinusoid there is both a local and systemic inflammatory response after reperfusion<sup>[6]</sup>. Therefore, IRI of the liver is generally believed to cause severe hepatocellular injury as well as extrahepatic organ inflammation and injury that contributes to perioperative morbidity and mortality.

Reductions of NO $\cdot$  during ischemia-reperfusion of liver aggregates liver injury in both animals and humans<sup>[7,8]</sup>. In fact, decreased hepatic production of NO $\cdot$  from endothelial nitric oxide synthase or endothelial nitric oxide synthase (eNOS) (responsible for the constitutive production of NO $\cdot$ ) within 60 min after reperfusion in human liver transplantation contributes to the ischemia-reperfusion graft injury<sup>[8]</sup>. In addition to reduced production, NO $\cdot$  is inactivated from the reactions with reactive oxygen species, such as superoxide radical leading to reduced bioavailability<sup>[9,10]</sup>. As a consequence, reduced NO $\cdot$  bioavailability leads to apoptosis, leukocyte adhesion, increase microcirculatory resistance, and mitochondrial dysfunction<sup>[10]</sup>. Not surprisingly, the restoration of NO $\cdot$  concentrations lessens liver ischemia injury *via* reversing the most of the previously mentioned adverse actions. Additional studies support the finding that eNOS is essential for reduction liver graft injury during liver ischemia-reperfusion. Injury to the liver was decreased in the wild type when compared to mice where eNOS was knocked out. When eNOS expression was exogenously increased or NO $\cdot$  donors enhanced protection was realized<sup>[11,12]</sup>. In addition, established NO $\cdot$  concentrations resulting from inflammation are generally greater due to more robust inducible nitric oxide synthase (iNOS) expression. At the present time the role of iNOS in liver protection is not well known. In a rat liver ischemia-reperfusion model, iNOS enzyme activity was significantly increased in parallel with increased iNOS mRNA expression after reperfusion, which suggests that induction of iNOS has an important role in liver ischemia-reperfusion<sup>[13]</sup>. Counter to this observation, in a porcine ischemia-reperfusion model, is that portal injection of aminoguanidine, a selective iNOS inhibitor, decreased IRI<sup>[14]</sup>. Additionally, when iNOS was knocked out in mice and then exposed to warm liver ischemia-reperfusion, they incurred more injury when compared to wild types. While the injury was greater in the iNOS deficient animals, iNOS mRNA was also undetectable in the wild types. While iNOS is crucial in increasing net NO $\cdot$  concentrations and contributing to liver injury resulting from ischemia-reperfusion, further work is needed.

Additional hepatoprotective studies thought to due to endogenous NO $\cdot$  production have been published. Nitric oxide-mediated protection has been shown to inhibit apoptosis depending on concentration *via* inhibition



**Figure 1** Number of transplants, waiting list additions and waiting list deaths in the United States between 1995 and 2015. Number of waiting list additions and deaths are based on the candidates and candidate who is listed more than one place is counted as one candidate. Data are available from: URL: <https://optn.transplant.hrsa.gov/data/>.

of caspase proteases *via* S-nitrosylation<sup>[15]</sup> (Figure 2). Reduced compartmental concentrations of NO $\cdot$  inhibits apoptosis while increasing concentrations yields toxic reactive nitrogen species such as peroxynitrite or oxygen radicals that have shown to cause apoptosis and necrosis<sup>[16]</sup>. Other proposed mechanisms of how NO $\cdot$  protects include down-regulation of nuclear factor kappa B<sup>[17]</sup>, mitochondrial complex I inhibition that is reversible, and reductions in mitochondrial calcium accumulation<sup>[18]</sup>. Controversy continues in regards to how NO $\cdot$  may mitigate inflammation and injury. In fact, Jaeschke *et al.*<sup>[19]</sup> demonstrated that NOS inhibition did not contribute to hepatic injury at the time of reperfusion. Additionally, the inhibition of NO $\cdot$  did not influence neutrophil function as related to migration or adhesion<sup>[20]</sup>. Nonetheless, most evidence whether lab-based or clinical, demonstrates favorable effects of NO $\cdot$  during liver ischemia-reperfusion. It is difficult to reconcile the results, but no doubt diversity exists in the role of NO $\cdot$  in different cell types, as well as differing cellular compartmental NO $\cdot$  concentrations, timing of administration, and duration of NO $\cdot$  exposure. Also laboratory methods applied may have some role on this conflicting results.

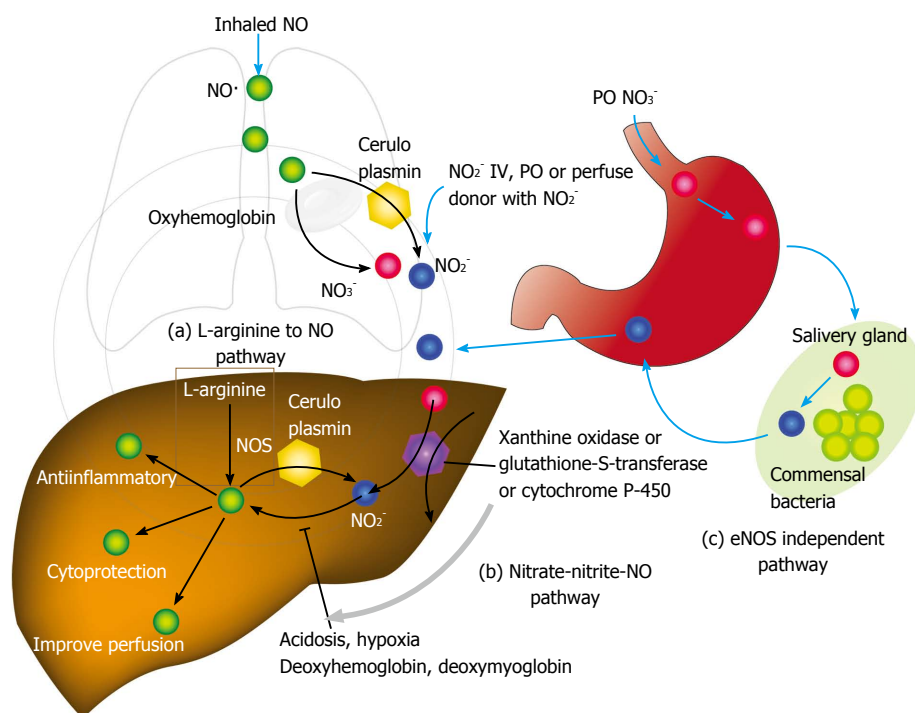
## IMPACT OF EXOGENOUS NO $\cdot$ DELIVERY IN ATTENUATING LIVER IRI

Administration of inhaled NO $\cdot$  has demonstrated efficacy both in animal and human studies<sup>[21-25]</sup>. NO $\cdot$  inhalation decreases pulmonary and systemic vascular resistance with resultant improvements in tissue oxygenation increases in renal blood flow, and glomerular filtration rate<sup>[26-28]</sup>. Moreover, inhaled NO $\cdot$  has been demonstrated to exert extra-pulmonary or peripheral effects to the microvasculature as measured by enhanced perfusion and anti-inflammatory properties during post-reperfusion period<sup>[21,22,29,30]</sup>. Due to this seminal work, administration

of inhaled NO $\cdot$  has undergone more extensive assessment as an anti-inflammatory therapy in humans subjected to predictable ischemia-reperfusion<sup>[23,30-32]</sup>. How extra-pulmonary effects of inhaled NO $\cdot$  remains unclear but generally it is believed in transformation of unstable NO $\cdot$  to relatively stable, NO $\cdot$ -containing intermediate upon entering in the circulation, and then recycled back to NO $\cdot$  at targeted remote location<sup>[31]</sup>. Study with a feline ischemia-reperfusion model suggested the intermediate molecule may be plasma S-nitrosothiols (e.g., S-nitrosoalbumin), while studies in both mice and humans points to nitrite as a possible intermediate<sup>[31,33,34]</sup>. A protective role for nitrite in ischemia-reperfusion is also demonstrated by direct administration of nitrite in murine hepatic ischemia-reperfusion models and together with the demonstration of nitrite conversion to NO $\cdot$  under ischemic location<sup>[33,35]</sup>. NO $\cdot$ -containing molecules in the blood that are labile under biological conditions and can also be formed with the inhalation of NO $\cdot$  (*via* nitrosylation or S-nitrosation reactions). These also includes S-nitrosothiols in the red blood cell, ferrous-nitrosylhemoglobin and C- or N-nitrosamines<sup>[31,36-38]</sup>.

Specifically, inhaled NO $\cdot$  (80 ppm, co-administered with 50% oxygen and 50% nitrous oxide, approximately 5 h) was administered preemptively to healthy patients undergoing lower extremity surgery requiring approximately two hours of tourniquet-induced ischemia and continued until the completion of the surgery<sup>[32]</sup>. Administration of inhaled nitric oxide (80 ppm) reduces the expression of CD11b/CD18, P-selectin, and NF- $\kappa$ B. Also there are associated increase in plasma levels of nitrate/nitrite, and reduced oxidative stress. In this health cohort inhaled NO $\cdot$  administered at 80 ppm significantly reduced inflammation from ischemia-reperfusion of lower extremity. Therefore, under conditions of impaired NO $\cdot$  metabolism, inhaled NO $\cdot$  may be an effective therapy to replenish systemic NO $\cdot$ , thus mitigates injury. A subsequent randomized controlled clinical trial evaluated the effects of preemptive inhaled NO $\cdot$  in recipients ( $n = 20$ ) undergoing liver transplantation<sup>[39]</sup>. Again, inhaled NO $\cdot$  (80 ppm, approximately 4 h) vs placebo was randomly administered to the recipients after patients were anesthetized and stopped upon case completion. Patients who received inhaled NO $\cdot$  significantly demonstrated shorter hospital stay and enhanced recovery of graft function (alanine transaminase and aspartate aminotransferase, prothrombin time and activated partial thromboplastin time) by approximately 2-3 d when compared to the placebo group. The intraoperative transfusion of platelets was reduced by 50% in recipients who received inhaled NO $\cdot$ . As would be expected plasma nitrite levels were significantly increased in inhaled NO $\cdot$  group compare when compared to placebo. Commonly cited untoward side effects such as the formation of critical levels of met hemoglobin, nitrogen dioxide or bleeding complications were not observed. Lang *et al.*<sup>[39]</sup> then compared a placebo group of patients who received 80 ppm of inhaled NO $\cdot$  during the operative phase of liver transplantation. Patients receiving NO $\cdot$  had better allograft function and





**Figure 2 Delivery of nitric oxide to donor liver graft in liver transplantation.** Preemptive treatment with inhaled nitric oxide can attenuate ischemia-reperfusion injury via modulation of a myriad of inflammatory, cellular and vascular mechanisms. IV: Intravenous; NO: Nitric oxide; NO<sub>2</sub><sup>-</sup>: Nitrite; NO<sub>3</sub><sup>-</sup>: Nitrate; eNOS: Endothelial nitric oxide synthase; PO: Per oral.

reduced hepatobiliary complications 9 mo following the initial operation. Also, inhaled NO<sup>•</sup> significantly increased concentrations of serum nitrate, nitrite, and nitrosylhemoglobin. Consistent with previous data, nitrite was hypothesized to be critical to the findings of allograft protection. No adverse metabolic or hematologic effects were observed between groups. Mean costs of inhaled NO<sup>•</sup> was \$1020 per transplantation. Use of inhaled NO<sup>•</sup> has also been tested in models of a non-heart beating donor model and steatotic liver, and demonstrated the injury attenuation and enhanced microcirculatory perfusion<sup>[40,41]</sup>. These studies are in line with other studies demonstrating inhaled NO<sup>•</sup> mitigates injury when utilized prior to predictable IRI. Replenishing NO<sup>•</sup> maybe more important in extended criteria donors which appear even susceptible to ischemia (cold and warm)-reperfusion.

Methemoglobinemia is a well-documented side effect of high dose supplemental inhaled NO<sup>•</sup>. Methemoglobin (MetHb) is rapidly formed by the oxidation of nitrosylhemoglobin, which is a byproduct from the binding of NO<sup>•</sup> to hemoglobin. This has been shown to occur in a dose and time-dependent fashion. MetHb has a fewer hemes to bind oxygen despite that methemoglobin has a higher affinity to oxygen compared to hemoglobin (1500 times higher affinity compared to carbon monoxide)<sup>[42]</sup>, thus diminishing the oxygen carrying capacity of the blood. MetHb level of 10% of total hemoglobin cause clinically apparent cyanosis, and MetHb level of 35% cause headache, weakness, and dyspnea, and MetHb level of more than 70% are fatal. As aforementioned, clinically significant methemoglobinemia

has not been reported when low-dose inhaled NO<sup>•</sup> is used. Only few case reports of methemoglobinemia has been reported when inhaled NO<sup>•</sup> was used in high dose (> 80 ppm)<sup>[43-45]</sup>. However it is worth mentioning that two cases of methemoglobinemia associated with low dose inhaled NO<sup>•</sup> due to delivery failure have been reported<sup>[46]</sup>. In both cases, methemoglobin reductase levels were confirmed to be normal, excluding of heredity methemoglobin reductase deficiency. Authors have speculated that variable (phasic) main flow provided from mechanical ventilator caused periodical accumulation of NO<sup>•</sup> in the inspiratory limb of airway circuit, leading to variable inhaled NO<sup>•</sup> concentration. Incorporated slow-response chemiluminescent analyzer was unable to detect this fluctuation of inhaled NO<sup>•</sup>. This fluctuation of NO<sup>•</sup> was also shown in lung model. Yamaguchi *et al.*<sup>[47]</sup> showed that inhaled NO<sup>•</sup> was more concentrated when it was administered more distally in the inspiratory limb of the circuit as well as administered with lower flow rates. They speculated that delivered NO<sup>•</sup> was diluted by backflow in the NO<sup>•</sup> tubing from the higher pressure in the circuit in the early inspiratory phase of ventilation. This concentrated NO<sup>•</sup> in NO<sup>•</sup> tubing was delivered in the early expiratory phase, leading to fluctuation in NO<sup>•</sup> concentration. Therefore, inhaled NO<sup>•</sup> treatment requires caution during administration and other form of supplementation of NO<sup>•</sup> may be favored in terms of avoiding life-threatening methemoglobinemia.

Other possible complication is the generation of cytotoxic oxidant, "peroxynitrite" by rapidly reacting with superoxide anion<sup>[48]</sup>. Peroxynitrite can induce lipid

peroxidation and inhibit mitochondrial respiration<sup>[49,50]</sup>. Indeed lung damage has been reported after inhaled NO $\cdot$  administration<sup>[51]</sup>. Hydrogen gas discover to have an anti-oxidative effect by scavenging peroxynitrite and other hydroxyl radicals<sup>[52]</sup>. Hydrogen gas has been shown to ameliorate lipopolysaccharide-induced<sup>[53]</sup>, ventilator-associated<sup>[54]</sup>, and hyperoxia-induced acute lung injury<sup>[55]</sup>. Therefore co-administration of hydrogen gas has been investigated to enhance lung protection by NO $\cdot$ .

Underlying mechanisms of how inhaled NO $\cdot$  decreases injury remains speculative. Nitrite, an oxidative product of NO $\cdot$  metabolism, seems to play a protectant role, however<sup>[33,34,56-58]</sup>. Thus, consistent with this line of thinking, sodium nitrite has been shown to alleviate acute injury from ischemia-reperfusion in both murine heart (decreased myocardial infarct size) and liver (decrease apoptosis in hepatocytes)<sup>[33]</sup>. Nitrite-mediated protection seems to involve biochemical pathways that connect ischemia to nitrite reduction to NO $\cdot$  production, therefore exerting cytoprotection by multiple possible mechanisms. In a model of murine liver transplantation, harvested syngeneic liver grafts were perfused with Lactated Ringers, and University of Wisconsin solution, and sodium nitrite supplemented solution during cold preservation period. Several recipients were treated with or without nitrite *via* an intraperitoneal injection. Liver injury demonstrated by enzyme release was significantly mitigated with both nitrite-supplemented solutions. The protective role of nitrite against cold ischemic-induced injury was more robust with longer preservation periods. Cell morphology and architecture was better preserved with grafts treated with nitrite. Hepatocyte cell death/necrosis was significantly reduced in the nitrite supplemented liver grafts. Liver grafts with extended cold preservation times demonstrated both improved tissue histology and liver function after reperfusion when treated with either the nitrite-supplemented preservation solution or in just the nitrite-treated recipients. Surprisingly, combination treatment of both liver graft and recipient did not demonstrate protection. Further clinical studies in the use of inhalation of NO $\cdot$  or injection of NO $\cdot$  donors for extended criteria donor may have a large clinical impact given that there is a surge in use of extended criteria donors to expand donor pool and warrants further investigation.

Other potential route of NO $\cdot$  donor administration is per gastrointestinal tract. In fact, dietary intake of nitrate is major source of NO $\cdot$  donor in mammals<sup>[59]</sup>. Dietary nitrate is abundant in many vegetables and water. Ingested nitrate is absorbed from intestine. One quarter of absorbed nitrate is concentrated in saliva and metabolized to nitrite by commensal bacteria<sup>[60]</sup>. Inorganic nitrite is metabolized to NO $\cdot$  in the presence of gastric acid<sup>[61-63]</sup>. This production pathway of nitric oxide is independent of eNOS (eNOS - independent NO $\cdot$  production) and accounts for majority of nitrite and nitrate in mammalian body<sup>[62,63]</sup>. Absorbed nitrite,

nitrate, or NO $\cdot$  from small intestine is directly delivered to liver through portal vein (Figure 2). Therefore, per oral administration of NO $\cdot$  donor can be a potential route of administration, especially post-transplant period.

Additional drugs that donate NO $\cdot$  have been thoroughly assessed<sup>[64-67]</sup>. Only two types of these drugs feasible to use clinically: Nitrates, and sodium nitroprusside. Nitrates, such as nitroglycerin are widely used to treat patients suffering from coronary ischemia and/or myocardial infarction due to the pronounced venodilatory effect that assists in reducing venous return and myocardial oxygen demand. Several formulations are available commercially including a slow release oral form, an ointment, a transdermal patch, a nebulizer, and lastly intravenous formulations. A major limitation of organic nitrates is tachyphylaxis due to sustained usage. Nitroglycerin releases NO $\cdot$  *via* the enzyme mitochondrial aldehyde dehydrogenase<sup>[68]</sup>. Sodium nitroprusside is another commercially available drug that is an NO $\cdot$  donor. Sodium nitroprusside's release of NO $\cdot$  is complex and but its net effect is to significantly diminish mRNA expression of a few pro-inflammatory mediators that promote hepatic injury<sup>[12]</sup>. Lastly, enhanced eNOS up-regulation confers hepatoprotection during IRI and may allow for another therapeutic option. When agents that increase eNOS expression such as trimetazidine, 5-amino-4-imidazole carboxamide riboside or activated protein C, are added to liver preservation solutions, hepatic allograft protection is afforded<sup>[12]</sup>.

## CONCLUSION

IRI has been well characterized the liver especially as it relates to liver resections and liver transplantation. The contribution of NO $\cdot$  deficiency is a newer finding and may have a central role in the pathogenesis of this injury. Replenishing the liver with NO $\cdot$  *via* either by inhalation, inhaled or intravenous nitrate or *via* other donor drugs represents a pragmatic means of mitigating injury. Clinical studies incorporating inhaled NO $\cdot$  provide solid evidence in mitigating injury from IRI. Inhaled NO $\cdot$  has demonstrated repeated efficacy without any demonstrable metabolic or hematological toxicities. Costs of routine NO $\cdot$  administration during liver transplantation is negligible when the entire spectrum of care is considered. Therefore, NO $\cdot$  has a potential to be a good therapeutic option for organ resuscitation in liver transplantation, especially for the extended criteria (marginal quality) donors, but further investigation is still warranted for routine clinical use.

## REFERENCES

- 1 **de Rougemont O**, Dutkowski P, Clavien PA. Biological modulation of liver ischemia-reperfusion injury. *Curr Opin Organ Transplant* 2010; **15**: 183-189 [PMID: 20125019 DOI: 10.1097/MOT.0b013e3283373ced]
- 2 **Pacher P**, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 2007; **87**: 315-424 [PMID: 17237348 DOI: 10.1152/physrev.00029.2006]
- 3 **Panjwani NN**, Popova L, Srivastava PK. Heat shock proteins

- gp96 and hsp70 activate the release of nitric oxide by APCs. *J Immunol* 2002; **168**: 2997-3003 [PMID: 11884472 DOI: 10.4049/jimmunol.168.6.2997]
- 4 **Chen C**, Lee WH, Zhong L, Liu CP. Regulatory T cells can mediate their function through the stimulation of APCs to produce immunosuppressive nitric oxide. *J Immunol* 2006; **176**: 3449-3460 [PMID: 16517713 DOI: 10.4049/jimmunol.176.6.3449]
  - 5 **Caldwell-Kenkel JC**, Currin RT, Tanaka Y, Thurman RG, Lemasters JJ. Kupffer cell activation and endothelial cell damage after storage of rat livers: effects of reperfusion. *Hepatology* 1991; **13**: 83-95 [PMID: 1988348]
  - 6 **Kupiec-Weglinski JW**, Busuttil RW. Ischemia and reperfusion injury in liver transplantation. *Transplant Proc* 2005; **37**: 1653-1656 [PMID: 15919422 DOI: 10.1016/j.transproceed.2005.03.134]
  - 7 **Köken T**, Inal M. The effect of nitric oxide on ischemia-reperfusion injury in rat liver. *Clin Chim Acta* 1999; **288**: 55-62 [PMID: 10529458 DOI: 10.1016/S0009-8981(99)00138-2]
  - 8 **Varadarajan R**, Golden-Mason L, Young L, McLoughlin P, Nolan N, McEntee G, Traynor O, Geoghegan J, Hegarty JE, O'Farrelly C. Nitric oxide in early ischaemia reperfusion injury during human orthotopic liver transplantation. *Transplantation* 2004; **78**: 250-256 [PMID: 15280686 DOI: 10.1097/01.TP.0000128188.45553.8C]
  - 9 **Ma XL**, Weyrich AS, Lefer DJ, Lefer AM. Diminished basal nitric oxide release after myocardial ischemia and reperfusion promotes neutrophil adherence to coronary endothelium. *Circ Res* 1993; **72**: 403-412 [PMID: 8418991 DOI: 10.1161/01.RES.72.2.403]
  - 10 **Abe Y**, Hines I, Zibari G, Grisham MB. Hepatocellular protection by nitric oxide or nitrite in ischemia and reperfusion injury. *Arch Biochem Biophys* 2009; **484**: 232-237 [PMID: 18940177 DOI: 10.1016/j.abb.2008.10.006]
  - 11 **Duranski MR**, Elrod JW, Calvert JW, Bryan NS, Feelisch M, Lefer DJ. Genetic overexpression of eNOS attenuates hepatic ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2006; **291**: H2980-H2986 [PMID: 16877550 DOI: 10.1152/ajpheart.01173.2005]
  - 12 **Katsumi H**, Nishikawa M, Yamashita F, Hashida M. Prevention of hepatic ischemia/reperfusion injury by prolonged delivery of nitric oxide to the circulating blood in mice. *Transplantation* 2008; **85**: 264-269 [PMID: 18212632 DOI: 10.1097/TP.0b013e31815e902b]
  - 13 **Hur GM**, Ryu YS, Yun HY, Jeon BH, Kim YM, Seok JH, Lee JH. Hepatic ischemia/reperfusion in rats induces iNOS gene transcription by activation of NF-kappaB. *Biochem Biophys Res Commun* 1999; **261**: 917-922 [PMID: 10441525 DOI: 10.1006/bbrc.1999.1143]
  - 14 **Isobe M**, Katsuramaki T, Kimura H, Nagayama M, Matsuno T, Yagihashi A, Hirata K. Role of inducible nitric oxide synthase on hepatic ischemia and reperfusion injury. *Transplant Proc* 2000; **32**: 1650-1652 [PMID: 11119875 DOI: 10.1016/S0041-1345(00)01435-4]
  - 15 **Maejima Y**, Adachi S, Morikawa K, Ito H, Isobe M. Nitric oxide inhibits myocardial apoptosis by preventing caspase-3 activity via S-nitrosylation. *J Mol Cell Cardiol* 2005; **38**: 163-174 [PMID: 15623433 DOI: 10.1016/j.yjmcc.2004.10.012]
  - 16 **Kim PK**, Billiar TR. Give me iNOS or give me death. *Hepatology* 2001; **34**: 436-437 [PMID: 11481632 DOI: 10.1053/jhep.2001.0340436]
  - 17 **Marshall HE**, Hess DT, Stamler JS. S-nitrosylation: physiological regulation of NF-kappaB. *Proc Natl Acad Sci USA* 2004; **101**: 8841-8842 [PMID: 15187230 DOI: 10.1073/pnas.0403034101]
  - 18 **Burwell LS**, Brookes PS. Mitochondria as a target for the cardioprotective effects of nitric oxide in ischemia-reperfusion injury. *Antioxid Redox Signal* 2008; **10**: 579-599 [PMID: 18052718 DOI: 10.1089/ars.2007.1845]
  - 19 **Jaeschke H**, Bautista AP, Spolarics Z, Spitzer JJ. Superoxide generation by Kupffer cells and priming of neutrophils during reperfusion after hepatic ischemia. *Free Radic Res Commun* 1991; **15**: 277-284 [PMID: 1666625 DOI: 10.3109/10715769109105223]
  - 20 **Jaeschke H**, Schini VB, Farhood A. Role of nitric oxide in the oxidant stress during ischemia/reperfusion injury of the liver. *Life Sci* 1992; **50**: 1797-1804 [PMID: 1375973 DOI: 10.1016/0024-3205(92)90064-V]
  - 21 **Fox-Robichaud A**, Payne D, Hasan SU, Ostrovsky L, Fairhead T, Reinhardt P, Kubes P. Inhaled NO as a viable antiadhesive therapy for ischemia/reperfusion injury of distal microvascular beds. *J Clin Invest* 1998; **101**: 2497-2505 [PMID: 9616221 DOI: 10.1172/JCI2736]
  - 22 **Cannon RO**, Schechter AN, Panza JA, Ognibene FP, Pease-Fye ME, Waclawiw MA, Shelhamer JH, Gladwin MT. Effects of inhaled nitric oxide on regional blood flow are consistent with intravascular nitric oxide delivery. *J Clin Invest* 2001; **108**: 279-287 [PMID: 11457881 DOI: 10.1172/JCI200112761]
  - 23 **Hataishi R**, Rodrigues AC, Neilan TG, Morgan JG, Buys E, Shiva S, Tambouret R, Jassal DS, Raher MJ, Furutani E, Ichinose F, Gladwin MT, Rosenzweig A, Zapol WM, Picard MH, Bloch KD, Scherrer-Crosbie M. Inhaled nitric oxide decreases infarction size and improves left ventricular function in a murine model of myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2006; **291**: H379-H384 [PMID: 16443673 DOI: 10.1152/ajpheart.01172.2005]
  - 24 **Kinsella JP**, Cutter GR, Walsh WF, Gerstmann DR, Bose CL, Hart C, Sekar KC, Auten RL, Bhutani VK, Gerdes JS, George TN, Southgate WM, Carriedo H, Couser RJ, Mammel MC, Hall DC, Pappagallo M, Sardesai S, Strain JD, Baier M, Abman SH. Early inhaled nitric oxide therapy in premature newborns with respiratory failure. *N Engl J Med* 2006; **355**: 354-364 [PMID: 16870914 DOI: 10.1056/NEJMoa060442]
  - 25 **Ignarro LJ**, Napoli C. Novel features of nitric oxide, endothelial nitric oxide synthase, and atherosclerosis. *Curr Atheroscler Rep* 2004; **6**: 281-287 [PMID: 15191702 DOI: 10.1007/s11883-004-0059-9]
  - 26 **Frostell C**, Fratacci MD, Wain JC, Jones R, Zapol WM. Inhaled nitric oxide. A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation* 1991; **83**: 2038-2047 [PMID: 2040056 DOI: 10.1161/01.CIR.83.6.2038]
  - 27 **Takahashi Y**, Kobayashi H, Tanaka N, Sato T, Takizawa N, Tomita T. Nitrosyl hemoglobin in blood of normoxic and hypoxic sheep during nitric oxide inhalation. *Am J Physiol* 1998; **274**: H349-H357 [PMID: 9458886]
  - 28 **Troncy E**, Francoeur M, Salazkin I, Yang F, Charbonneau M, Leclerc G, Vinay P, Blaise G. Extra-pulmonary effects of inhaled nitric oxide in swine with and without phenylephrine. *Br J Anaesth* 1997; **79**: 631-640 [PMID: 9422904 DOI: 10.1093/bja/79.5.631]
  - 29 **Kubes P**, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA* 1991; **88**: 4651-4655 [PMID: 1675786 DOI: 10.1073/pnas.88.11.4651]
  - 30 **Gianetti J**, Del Sarto P, Bevilacqua S, Vassalle C, De Filippis R, Kacila M, Farneti PA, Clerico A, Glauber M, Biagini A. Supplemental nitric oxide and its effect on myocardial injury and function in patients undergoing cardiac surgery with extracorporeal circulation. *J Thorac Cardiovasc Surg* 2004; **127**: 44-50 [PMID: 14752411 DOI: 10.1016/j.jtcvs.2002.08.001]
  - 31 **Ng ES**, Jourdain D, McCord JM, Hernandez D, Yasui M, Knight D, Kubes P. Enhanced S-nitroso-albumin formation from inhaled NO during ischemia/reperfusion. *Circulation Res* 2004; **94**: 559-565 [DOI: 10.1161/01.RES.0000117771.63140.D6]
  - 32 **Mathru M**, Huda R, Solanki DR, Hays S, Lang JD. Inhaled nitric oxide attenuates reperfusion inflammatory responses in humans. *Anesthesiology* 2007; **106**: 275-282 [PMID: 17264721]
  - 33 **Duranski MR**, Greer JJ, Dejam A, Jaganmohan S, Hogg N, Langston W, Patel RP, Yet SF, Wang X, Kevil CG, Gladwin MT, Lefer DJ. Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver. *J Clin Invest* 2005; **115**: 1232-1240 [PMID: 15841216 DOI: 10.1172/JCI22493]
  - 34 **Gladwin MT**, Schechter AN, Kim-Shapiro DB, Patel RP, Hogg N, Shiva S, Cannon RO, Kelm M, Wink DA, Espey MG, Oldfield EH, Pluta RM, Freeman BA, Lancaster JR, Feelisch M, Lundberg JO. The emerging biology of the nitrite anion. *Nat Chem Biol* 2005; **1**: 308-314 [PMID: 16408064 DOI: 10.1038/nchembio1105-308]
  - 35 **Li W**, Meng Z, Liu Y, Patel RP, Lang JD. The hepatoprotective effect of sodium nitrite on cold ischemia-reperfusion injury. *J*



- Transplant* 2012; **2012**: 635179 [PMID: 22530108 DOI: 10.1155/2012/635179]
- 36 **Bryan NS**, Rassaf T, Maloney RE, Rodriguez CM, Saijo F, Rodriguez JR, Feelisch M. Cellular targets and mechanisms of nitros(yl)ation: an insight into their nature and kinetics in vivo. *Proc Natl Acad Sci USA* 2004; **101**: 4308-4313 [PMID: 15014175 DOI: 10.1073/pnas.0306706101]
  - 37 **McMahon TJ**, Doctor A. Extrapulmonary effects of inhaled nitric oxide: role of reversible S-nitrosylation of erythrocytic hemoglobin. *Proc Am Thorac Soc* 2006; **3**: 153-160 [PMID: 16565424 DOI: 10.1513/pats.200507-066BG]
  - 38 **Gladwin MT**, Crawford JH, Patel RP. The biochemistry of nitric oxide, nitrite, and hemoglobin: role in blood flow regulation. *Free Radic Biol Med* 2004; **36**: 707-717 [PMID: 14990351 DOI: 10.1016/j.freeradbiomed.2003.11.032]
  - 39 **Lang JD**, Teng X, Chumley P, Crawford JH, Isbell TS, Chacko BK, Liu Y, Jhala N, Crowe DR, Smith AB, Cross RC, Frenette L, Kelley EE, Wilhite DW, Hall CR, Page GP, Fallon MB, Bynon JS, Eckhoff DE, Patel RP. Inhaled NO accelerates restoration of liver function in adults following orthotopic liver transplantation. *J Clin Invest* 2007; **117**: 2583-2591 [PMID: 17717604 DOI: 10.1172/JCI31892]
  - 40 **Srinivasan PK**, Yagi S, Doorschodt B, Nagai K, Afify M, Uemoto S, Tolba R. Impact of venous systemic oxygen persufflation supplemented with nitric oxide gas on cold-stored, warm ischemia-damaged experimental liver grafts. *Liver Transpl* 2012; **18**: 219-225 [PMID: 21987402 DOI: 10.1002/lt.22442]
  - 41 **Nagai K**, Yagi S, Afify M, Bleilevens C, Uemoto S, Tolba RH. Impact of venous-systemic oxygen persufflation with nitric oxide gas on steatotic grafts after partial orthotopic liver transplantation in rats. *Transplantation* 2013; **95**: 78-84 [PMID: 23263502 DOI: 10.1097/TP.0b013e318277e2d1]
  - 42 **Gibson QH**, Roughton FJ. The kinetics and equilibria of the reactions of nitric oxide with sheep haemoglobin. *J Physiol* 1957; **136**: 507-524 [PMID: 13429517 DOI: 10.1113/jphysiol.1957.sp005777]
  - 43 **Nakajima W**, Ishida A, Arai H, Takada G. Methaemoglobinaemia after inhalation of nitric oxide in infant with pulmonary hypertension. *Lancet* 1997; **350**: 1002-1003 [PMID: 9329519 DOI: 10.1016/S0140-6736(05)64067-6]
  - 44 **Heal CA**, Spencer SA. Methaemoglobinaemia with high-dose nitric oxide administration. *Acta Paediatr* 1995; **84**: 1318-1319 [PMID: 8580636 DOI: 10.1111/j.1651-2227.1995.tb13558.x]
  - 45 **Hovenga S**, Koenders ME, van der Werf TS, Moshage H, Zijlstra JG. Methaemoglobinaemia after inhalation of nitric oxide for treatment of hydrochlorothiazide-induced pulmonary oedema. *Lancet* 1996; **348**: 1035-1036 [PMID: 8855888 DOI: 10.1016/S0140-6736(05)64968-9]
  - 46 **Taylor MB**, Christian KG, Patel N, Churchwell KB. Methemoglobinemia: Toxicity of inhaled nitric oxide therapy. *Pediatr Crit Care Med* 2001; **2**: 99-101 [PMID: 12797897 DOI: 10.1097/00130478-200101000-00019]
  - 47 **Yamaguchi N**, Togari H, Suzuki S. During neonatal mechanical ventilatory support, the delivered nitric oxide concentration is affected by the ventilatory setting. *Crit Care Med* 2000; **28**: 1607-1611 [PMID: 10834720 DOI: 10.1097/00003246-200005000-00058]
  - 48 **Koppenol WH**, Moreno JJ, Pryor WA, Ischiropoulos H, Beckman JS. Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. *Chem Res Toxicol* 1992; **5**: 834-842 [PMID: 1336991 DOI: 10.1021/tx00030a017]
  - 49 **Grisham MB**, Jourdain H, Wink DA. Nitric oxide. I. Physiological chemistry of nitric oxide and its metabolites: implications in inflammation. *Am J Physiol* 1999; **276**: G315-G321 [PMID: 9950804]
  - 50 **Liaudet L**, Soriano FG, Szabó C. Biology of nitric oxide signaling. *Crit Care Med* 2000; **28**: N37-N52 [PMID: 10807315 DOI: 10.1097/00003246-200004001-00005]
  - 51 **Hallman M**, Bry K, Turbow R, Waffarn F, Lappalainen U. Pulmonary toxicity associated with nitric oxide in term infants with severe respiratory failure. *J Pediatr* 1998; **132**: 827-829 [PMID: 9602194 DOI: 10.1016/S0022-3476(98)70312-9]
  - 52 **Ohsawa I**, Ishikawa M, Takahashi K, Watanabe M, Nishimaki K, Yamagata K, Katsura K, Katayama Y, Asoh S, Ohta S. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat Med* 2007; **13**: 688-694 [PMID: 17486089 DOI: 10.1038/nm1577]
  - 53 **Xie K**, Yu Y, Zhang Z, Liu W, Pei Y, Xiong L, Hou L, Wang G. Hydrogen gas improves survival rate and organ damage in zymosan-induced generalized inflammation model. *Shock* 2010; **34**: 495-501 [PMID: 20351628 DOI: 10.1097/SHK.0b013e3181def9aa]
  - 54 **Huang CS**, Kawamura T, Peng X, Tochigi N, Shigemura N, Billiar TR, Nakao A, Toyoda Y. Hydrogen inhalation reduced epithelial apoptosis in ventilator-induced lung injury via a mechanism involving nuclear factor-kappa B activation. *Biochem Biophys Res Commun* 2011; **408**: 253-258 [PMID: 21473852 DOI: 10.1016/j.bbrc.2011.04.008]
  - 55 **Sun Q**, Cai J, Liu S, Liu Y, Xu W, Tao H, Sun X. Hydrogen-rich saline provides protection against hyperoxic lung injury. *J Surg Res* 2011; **165**: e43-e49 [PMID: 21067781 DOI: 10.1016/j.jss.2010.09.024]
  - 56 **Gladwin MT**. Nitrite as an intrinsic signaling molecule. *Nat Chem Biol* 2005; **1**: 245-246 [PMID: 16408049 DOI: 10.1038/nchembio1005-245]
  - 57 **Shiva S**, Sack MN, Greer JJ, Duranski M, Ringwood LA, Burwell L, Wang X, MacArthur PH, Shoja A, Raghavachari N, Calvert JW, Brookes PS, Lefer DJ, Gladwin MT. Nitrite augments tolerance to ischemia/reperfusion injury via the modulation of mitochondrial electron transfer. *J Exp Med* 2007; **204**: 2089-2102 [PMID: 17682069 DOI: 10.1084/jem.20070198]
  - 58 **Weitzberg E**, Hezel M, Lundberg JO. Nitrate-nitrite-nitric oxide pathway: implications for anesthesiology and intensive care. *Anesthesiology* 2010; **113**: 1460-1475 [PMID: 21045638 DOI: 10.1097/ALN.0b013e3181fcf3cc]
  - 59 **Lundberg JO**, Weitzberg E, Cole JA, Benjamin N. Nitrate, bacteria and human health. *Nat Rev Microbiol* 2004; **2**: 593-602 [PMID: 15197394 DOI: 10.1038/nrmicro929]
  - 60 **Bryan NS**. Nitrite in nitric oxide biology: cause or consequence? A systems-based review. *Free Radic Biol Med* 2006; **41**: 691-701 [PMID: 16895789 DOI: 10.1016/j.freeradbiomed.2006.05.019]
  - 61 **Benjamin N**, O'Driscoll F, Dougall H, Duncan C, Smith L, Golden M, McKenzie H. Stomach NO synthesis. *Nature* 1994; **368**: 502 [PMID: 8139683 DOI: 10.1038/368502a0]
  - 62 **Rocha BS**, Gago B, Barbosa RM, Lundberg JO, Radi R, Laranjinha J. Intra gastric nitration by dietary nitrite: implications for modulation of protein and lipid signaling. *Free Radic Biol Med* 2012; **52**: 693-698 [PMID: 22154654 DOI: 10.1016/j.freeradbiomed.2011.11.011]
  - 63 **Gago B**, Lundberg JO, Barbosa RM, Laranjinha J. Red wine-dependent reduction of nitrite to nitric oxide in the stomach. *Free Radic Biol Med* 2007; **43**: 1233-1242 [PMID: 17893036 DOI: 10.1016/j.freeradbiomed.2007.06.007]
  - 64 **Aiba M**, Takeyoshi I, Ohwada S, Kawashima Y, Iwanami K, Sunose Y, Yamada T, Tsutsumi H, Matsumoto K, Morishita Y. Novel nitric oxide donor (FK409) ameliorates liver damage during extended liver resection with warm ischemia in dogs. *J Am Coll Surg* 2001; **193**: 264-271 [PMID: 11548796 DOI: 10.1016/S1072-7515(01)01002-X]
  - 65 **Kim YM**, de Vera ME, Watkins SC, Billiar TR. Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor- $\alpha$ -induced apoptosis by inducing heat shock protein 70 expression. *J Biol Chem* 1997; **272**: 1402-1411 [PMID: 8995451 DOI: 10.1074/jbc.272.2.1402]
  - 66 **Nilsson B**, Delbro D, Wallin M, Friman S. Protective effect of nitric oxide and prostaglandin E(2) in ischemia/reperfusion injury of the liver. *Transplant Proc* 2001; **33**: 2518-2520 [PMID: 11406233 DOI: 10.1016/S0041-1345(01)02083-8]
  - 67 **Yang SL**, Lou YJ. Sodium nitroprusside decreased leukotriene C4 generation by inhibiting leukotriene C4 synthase expression and activity in hepatic ischemia-reperfusion injured rats. *Biochem*



*Pharmacol* 2007; **73**: 724-735 [PMID: 17194456 DOI: 10.1016/j.bcp.2006.11.011]

68 **Katsumi H**, Nishikawa M, Yasui H, Yamashita F, Hashida M.

Prevention of ischemia/reperfusion injury by hepatic targeting of nitric oxide in mice. *J Control Release* 2009; **140**: 12-17 [PMID: 19646492 DOI: 10.1016/j.jconrel.2009.07.013]

**P- Reviewer:** Boucek CD, Okumura T, Samhan-Arias AK, Zhao Q

**S- Editor:** Qi Y **L- Editor:** A **E- Editor:** Li D



Case Control Study

## Fractional excretion of sodium in hepatorenal syndrome: Clinical and pathological correlation

Ali A Alsaad, Hani M Wadei

Ali A Alsaad, Department of Internal Medicine, Mayo Clinic, Jacksonville, FL 32224, United States

Hani M Wadei, Department of Transplant, Mayo Clinic, Jacksonville, FL 32224, United States

**Author contributions:** Alsaad AA and Wadei HM contributed to the design of the study, acquisition, analysis and interpretation of the data, writing and reviewing the manuscript, and approved the final version of the manuscript for submission.

**Institutional review board statement:** We obtained approval from the Mayo Clinic Institutional Review Board (IRB) to conduct this study.

**Informed consent statement:** Exempted, demonstrated as minimal risk study.

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.

**Data sharing statement:** Consent was not obtained but the presented data are anonymized and risk of identification is low.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Hani M Wadei, MD, Department of Transplant, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, United States. [wadei.hani@mayo.edu](mailto:wadei.hani@mayo.edu)  
Telephone: +1-904-9536259  
Fax: +1-904-9533220

Received: July 1, 2016

Peer-review started: July 4, 2016

First decision: August 10, 2016

Revised: September 2, 2016

Accepted: October 22, 2016

Article in press: October 24, 2016

Published online: December 8, 2016

### Abstract

#### AIM

To determine the accuracy of fractional excretion of sodium (FeNa) in the diagnosis of hepatorenal syndrome (HRS).

#### METHODS

Eighty-eight liver transplantation candidates with renal dysfunction and/or proteinuria were included in the study sample. The baseline characteristics of the patients were obtained. All the 88 patients underwent iothalamate glomerular filtration rate testing, 24-h urine collection for urinary sodium and protein excretions, random urine for sodium and creatinine testing, and percutaneous kidney biopsy. FeNa was calculated using the equation  $[(\text{urine sodium} \times \text{serum creatinine}) / (\text{serum sodium} \times \text{urine creatinine})] \times 100\%$ . Diuretic use was recorded among the participants. Patients on renal replacement therapy were not included in the original sample.

#### RESULTS

Seventy-seven (87%) of the 88 patients had FeNa < 1%. FeNa < 1% was present in 10/10, 10/12, 11/13, 12/15 and 34/38 in patients with HRS, acute tubular necrosis, membranoproliferative glomerulonephritis, minimal histological findings ( $\leq 30\%$ ) and advanced ( $\geq 30\%$ -40%) interstitial fibrosis and/or glomerulosclerosis, respectively ( $P = 0.4$ ). FeNa < 1% was 100% sensitive and 14% specific in diagnosing HRS. Receiver operating characteristic curve confirmed the poor accuracy of FeNa < 1% in diagnosing HRS (area under the curve = 0.58,  $P = 0.47$ ). Calculated positive predictive value

and negative predictive value for FeNa < 1% in HRS diagnosis were 46% and 100%, respectively. When used as a continuous variable, FeNa did not correlate with kidney biopsy findings ( $P = 0.41$ ).

## CONCLUSION

FeNa < 1% was common in cirrhotic patients with renal dysfunction and it did not differentiate between HRS and other causes of renal pathologies. HRS diagnosis should be avoided in patients with FeNa > 1%.

**Key words:** Fractional excretion of sodium; Hepatorenal syndrome; Renal dysfunction; Liver transplantation; Urinary sodium excretion; Accuracy

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In this retrospective analysis of patients with advanced end-stage liver disease, we describe three main concepts. First, our data indicates that fractional excretion of sodium (FeNa) < 1% is a common finding in this group of patients irrespective of the etiology of their renal dysfunction. Second, our study suggests that FeNa < 1% cannot differentiate hepatorenal syndrome (HRS) from other causes of renal pathology. And third, we statistically measured the performance of FeNa < 1% in patients with HRS using kidney biopsy findings as golden diagnostic standard.

Alsaad AA, Wadei HM. Fractional excretion of sodium in hepatorenal syndrome: Clinical and pathological correlation. *World J Hepatol* 2016; 8(34): 1497-1501 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i34/1497.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i34.1497>

## INTRODUCTION

Kidney dysfunction is common in patients with end-stage liver disease (ESLD). It is estimated that nearly 20% to 25% of patients with ESLD will have some type of kidney dysfunction during their course of disease<sup>[1]</sup>. The spectrum of kidney disease can range from reversible kidney injury like acute kidney injury [whether prerenal azotemia or acute tubular necrosis (ATN)] to permanent and chronic kidney damage (CKD) and fibrosis. CKD could be either from causes unrelated to the liver disease such as diabetes, or related to the liver disease such as hepatitis C virus infection.

Hepatorenal syndrome (HRS) is a form of prerenal azotemia that is unique to patients with liver cirrhosis and ascites that is diagnosed after excluding other causes of renal impairment<sup>[2,3]</sup>. HRS occurs in nearly 8% of patients with ascites and 75% of patients with HRS will require dialysis<sup>[2]</sup>.

Early studies have demonstrated that kidneys procured from HRS patients had normal renal histology and exhibited immediate allograft function after transplantation<sup>[4]</sup>. Patho-

physiologically, patients with HRS experience severe vasoconstriction that involves various vascular beds including the kidneys with subsequent reduction in renal blood flow, glomerular filtration rate, and reciprocal increase in proximal tubular sodium re-absorption<sup>[5]</sup>. Indeed, urinary sodium excretion is low in patients with HRS<sup>[6,7]</sup>. Classic teaching has utilized FeNa cut-off of < 1% or > 1% to differentiate between prerenal (including HRS) and intrinsic renal dysfunctions especially those caused by ATN. While this FeNa cut-off has been used as discriminatory tool in cirrhotic patients presenting with elevated serum creatinine, its accuracy has not been tested in diagnosing HRS against a gold standard such as kidney biopsy. Our program utilizes kidney biopsy in evaluating selected liver transplant candidates with renal dysfunction.

In this study, we sought to determine the accuracy of FeNa < 1% in HRS diagnosis using histological data as a gold standard for comparison.

## MATERIALS AND METHODS

### Settings and participants

After obtaining Mayo Clinic Institutional Review Board approval, we retrospectively reviewed the electronic medical record of 88 patients with ESLD who were undergoing LT evaluation at Mayo Clinic in Jacksonville, Florida. All 88 patients had renal dysfunction defined as an iothalamate glomerular filtration rate (GFR) of less than 40 mL/min per 1.73 m<sup>2</sup> or the presence of proteinuria or hematuria. Patients with fulminant hepatic failure were not included.

All study patients had undergone renal biopsy after a stabilization of platelets count of less than 50000 × 10<sup>6</sup>/L with platelets transfusion, and an international normalized ratio of less than 1.5 by fresh frozen plasma transfusion.

Data for GFR and 24-h urine collection for urinary sodium excretion, protein excretion, random urine sodium, and random creatinine were collected. Patients who underwent renal replacement therapy within the last 6 wk prior to evaluation were not included in the original sample, as their serum and urine electrolyte values will be modulated by dialysis. Diuretic use was recorded.

Kidney biopsy specimens were assessed using light microscopy, immunofluorescence and electron microscopy and were interpreted by an experienced nephropathologist as previously described<sup>[8]</sup>. Patients were grouped according to the primary kidney biopsy diagnosis into five main groups: HRS (normal kidney pathology), ATN, membranoproliferative glomerulonephritis (MPGN), minimal histological changes [defined as 10%-30% interstitial fibrosis (IF) and/or glomerulosclerosis (GS)] and advanced (> 30%) IF and/or GS.

FeNa was calculated using the equation: [(urine sodium × serum creatinine)/(serum sodium × urine creatinine)] × 100.

Patients with fulminant hepatic failure were not included in this analysis.

**Table 1** Baseline characteristics of the 88 liver transplant candidates with renal dysfunction

Variable	
Age	60 ± 7
Male gender	57 (65)
Cause of ESLD	
HCV infection	40 (45)
NASH	13 (15)
Alcoholic cirrhosis	12 (14)
Cryptogenic cirrhosis	10 (11)
Other	13 (15)
MELD score	17.5 ± 5.8
History of diabetes	35 (40)
History of hypertension	40 (45)
Iothalamate GFR mL/min per 1.73 m <sup>2</sup>	28 ± 14
Serum creatinine (mg/dL)	1.9 ± 0.9
Serum Na (mEq/dL)	137 ± 5
24-h urinary protein excretion (mg/d)	87 (0-13625)
24-h urinary Na excretion (mEq/d)	56 (0-238)
24-urine protein > 150 mg/d	35 (40)
Hematuria	40 (45)
Diuretic use	64 (72)
FeNa < 1	77 (87)
Kidney Biopsy	
HRS	10 (11)
ATN	12 (14)
MPGN	13 (15)
Minimal histology	15 (17)
≥ 30%-40% IF/GS	38 (43)

Data presented as number (percent), mean ± SD or median (range). ESLD: End-stage liver disease; HCV: Hepatitis C virus; NASH: Non-alcoholic steatohepatitis; MELD: Model of end stage liver disease; GFR: Glomerular filtration rate; Na: Sodium; FeNa: Fractional excretion of sodium; HRS: Hepatorenal syndrome; ATN: Acute tubular necrosis; MPGN: Membranoproliferative glomerulonephritis; IF: Interstitial fibrosis; GS: Glomerulosclerosis.

### Outcome measures

The primary outcome was to calculate the sensitivity, specificity, positive predictive value (PPV) and (NPV) of FeNa < 1% in diagnosing HRS. Secondary outcome was to measure the correlation between FeNa as a continuous variable and the kidney biopsy diagnosis.

### Statistical analysis

Using SPSS software version 22, (Cary, NC), we analyzed the data of the 88 LT patients. Continuous variables were presented as mean ± SD; categorical variables were presented as number (%). The sensitivity, specificity, PPV and NPV in HRS diagnosis were calculated. A receiver operating characteristic (ROC) curve was constructed to assess the diagnostic accuracy of FeNa < 1% in diagnosing HRS.

## RESULTS

Table 1 summarizes the baseline characteristics of the 88 liver transplantation patients. The mean ± SD age of the cohort was 60 ± 7 years and the majority were of male gender (65%). Seventy two percent of the patients were using at least one diuretic at time of FeNa calculation. Out of the 88 LT candidates, 77 (87%) had

FeNa < 1%. As demonstrated in Table 1, FeNa < 1% was present in 10/10, 10/12, 11/13, 12/15 and 34/38 in patients with HRS, ATN, MPGN, minimal histological changes (10%-30% fibrosis) and advanced (≥ 30%) IF and/or glomerulosclerosis (GS), respectively ( $P = 0.4$ ).

### Primary outcome measure

FeNa < 1% was 100% sensitive and 14% specific in diagnosing HRS. Calculated PPV and NPV for FeNa < 1% in the setting of HRS were 46% and 100%, respectively. Figure 1 represents the result of the ROC curve assessing the performance of FeNa < 1% in HRS diagnosis. As demonstrated in Figure 1, FeNa < 1% showed poor accuracy in diagnosing HRS with an area under the curve (AUC) of 0.58,  $P = 0.47$ . To assess the effect of diuretic use on the performance of FeNa < 1% in HRS diagnosis, we compared urinary sodium indices between patients on diuretics ( $n = 64$ ) and those not on diuretic ( $n = 24$ ) treatment. There was no observed difference in the 24-h urinary sodium excretion, FeNa (as a continuous variable) and number of patients with FeNa < 1% between those using and not using diuretics at time of FeNa calculation ( $P > 0.3$  for all). Also, the sensitivity (100%), specificity (12.5%), PPV (14%) and NPV (100%) of FeNa < 1% in diagnosing HRS did not differ when patients not using diuretics were excluded from the analysis.

### Secondary outcome measure

We then correlated FeNa as continuous variable with the kidney biopsy finding. Although FeNa was lowest in HRS patients it did not differentiate between HRS and other renal pathologies (Figure 2).

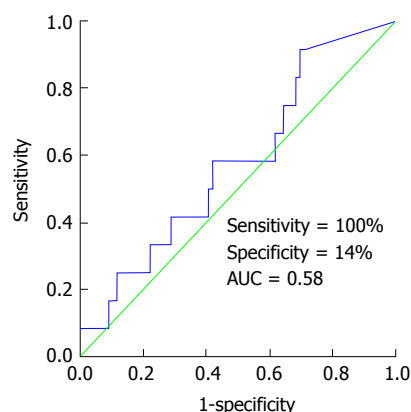
There was also no correlation between the degrees of the IF ( $r = 0.07$ ,  $P = 0.54$ ) or GS ( $r = 0.2$ ,  $P = 0.07$ ) and the 24-h urinary sodium excretion (Figures 3 and 4, respectively). This lack of correlation was not affected by diuretic use (data not shown).

## DISCUSSION

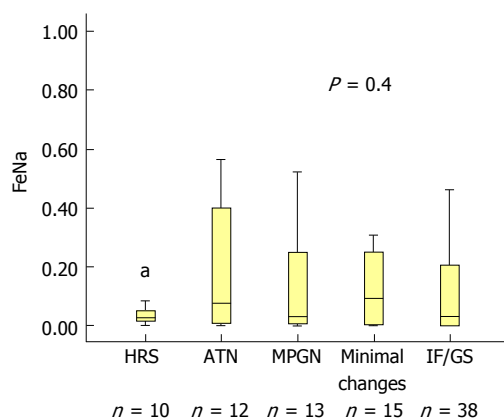
Our study indicates that the majority of ESLD patients with renal dysfunction of unknown etiology or duration have a low 24-h urinary sodium excretion and a FeNa < 1% irrespective of renal pathology on kidney biopsy. Using kidney biopsy findings as a gold standard, we were able to determine the accuracy of FeNa < 1% in diagnosing HRS. Our results defined the sensitivity, specificity, PPV and NPV of FeNa < 1% in HRS as 100%, 14%, 46% and 100%, respectively. These results suggest that a FeNa > 1% excludes HRS diagnosis but a FeNa < 1% is not useful in diagnosing HRS.

Previous studies confirm that cirrhotic patients without renal dysfunction have low urinary sodium excretion rates and increased renal tubular reabsorption due to the activation of various neuro-hormonal mechanism and subsequent increase in renal tubular sodium reabsorption<sup>[5,9]</sup>. In patient with renal dysfunction however, low urinary sodium excretion implies maintained tubular integrity and favors either prerenal azotemia or HRS





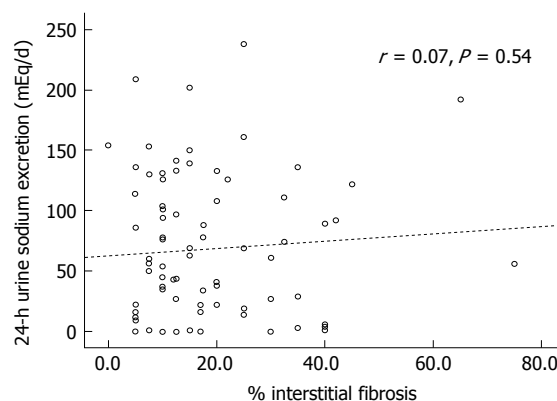
**Figure 1** Receiver operator characteristics curve showing the poor accuracy of fractional excretion of sodium < 1% in diagnosing hepatorenal syndrome with area under the curve of 0.58,  $P = 0.47-0.58$ . AUC: Area under the curve.



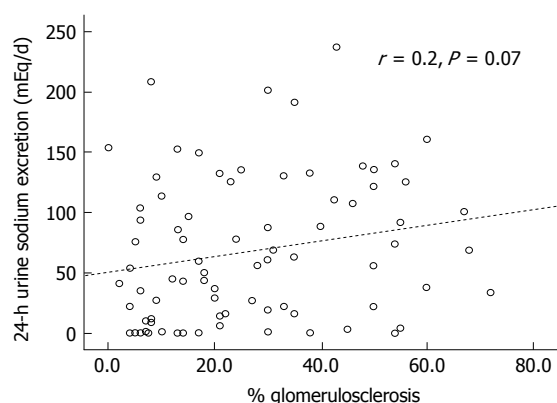
**Figure 2** Correlation between fractional excretion of sodium as a continuous variable and kidney biopsy diagnosis. Although FeNa was lowest in HRS patients, it did not differentiate between HRS and other renal pathologies ( $P = 0.41$ ). FeNa: Fractional excretion of sodium; HRS: Hepatorenal syndrome; ATN: Acute tubular necrosis; MPGN: Membranoproliferative glomerulonephritis; IF: Interstitial fibrosis; GS: Glomerulosclerosis.

diagnosis<sup>[5]</sup>. Results of this study challenge this understanding. Our findings indicated that urinary sodium excretion was similarly low in cirrhotic patients with renal dysfunction due to tubular injury (ATN), glomerular disease (MPGN), irreversible renal damage (advanced IF/GS) or normal renal histology (HRS). There was also no correlation between the degree of IF or GS and the 24-h urinary sodium excretion. These results indicate that a low urinary sodium excretion is present in the majority of cirrhotic patients with renal dysfunction and does not reflect an intact renal tubular integrity but rather reflects the avid renal sodium retention state in these patients irrespective of the cause of renal dysfunction. It is also important to mention that diuretic use did not affect urinary sodium indices or the performance of FeNa < 1% in HRS diagnosis which support the avid sodium retention state in these patients with advanced cirrhosis.

FeNa is more sensitive than urinary sodium concentration in detecting prerenal causes of renal dysfunction as the serum sodium level is factored into the equation.



**Figure 3** Scatter plot depicting the relationship between the percentage of interstitial fibrosis on kidney biopsy and 24-h urine sodium excretion. Correlation was overall poor ( $r = 0.07$ ,  $P = 0.54$ ).



**Figure 4** Scatter plot depicting the relationship between the percentage of glomerulosclerosis on kidney biopsy result and 24-h urine sodium excretion ( $r = 0.2$ ,  $P = 0.07$ ). Correlation was better than the one observed with percentage of interstitial fibrosis but still did not reach statistical significance.

Previously published reports, however, indicated that although FeNa was lowest in patients with HRS, FeNa did not differentiate HRS and other causes of renal dysfunction including ATN<sup>[6,10]</sup>. These studies did not include kidney biopsy evidence of HRS (normal renal histology) and the diagnosis of HRS was made solely on clinical grounds<sup>[10,11]</sup>.

The main strength of the current study is that we used kidney biopsy findings to diagnose HRS, ATN and other renal pathologies. By using histological data, we were able to confirm that FeNa < 1% is present in almost 90% of cirrhotic patients with renal dysfunction. Although FeNa was lowest in HRS and FeNa < 1% was present in 100% of patients with HRS, FeNa did not differentiate between HRS and other causes of acute or chronic renal dysfunction. Of note a previous prospective study demonstrated that FeNa < 1% was present in only 0% to 4% of patients with ATN and no history of liver disease<sup>[12]</sup>. In contrast, in the current study 10 of 12 patients (83%) with biopsy evidence of ATN had a FeNa < 1%. Previous studies also demonstrated that FeNa < 1% had a sensitivity and specificity of 58%-78% and 75%-81% in diagnosing prerenal azotemia, respectively, in subjects without liver disease and varied according to diuretic use<sup>[13]</sup>. The results of the current study indicate

that the sensitivity and specificity of FeNa < 1% in the diagnosis of HRS is much different than in non-cirrhotic patients with prerenal azotemia and that they are not affected by diuretic use. This difference is likely due to the intense renal vasoconstriction manifesting in cirrhotic patients with subsequent increase in renal sodium reabsorption and the diuretic resistant state these patients develop with worsening liver disease.

The performance of FeNa < 1% in diagnosing HRS was overall poor but the test had high sensitivity and high NPV (both 100%), indicating that in patients with negative test results (*i.e.*, FeNa > 1%) HRS diagnosis should be excluded.

The current study is limited by the small number of patients particularly those with normal biopsy findings and HRS diagnosis which could have affected the results. Another important limitation of the study is the lack of detailed information on dietary sodium intake and doses and class of the diuretic medications used. We also measured FeNa at a single time point prior to the kidney biopsy to minimize the selection bias. Future studies should address if serial measurements of FeNa in a given patient will confer similar results.

In conclusion, the current study indicates that FeNa < 1% is common finding in patients with ESLD and renal dysfunction and has a poor accuracy in diagnosing HRS. Our results also indicate that HRS diagnosis should be avoided in patients with FeNa > 1%. Further studies with large number of patients are needed to confirm the findings of this study.

## COMMENTS

### Background

Kidney dysfunction is common in patients with end-stage liver disease (ESLD). It is estimated that nearly 20% to 25% of patients with ESLD will have some type of kidney dysfunction during their course of disease.

### Research frontiers

Classic teaching has utilized fractional excretion of sodium (FeNa) cut-off of < 1% or > 1% to differentiate between prerenal [including hepatorenal syndrome (HRS)] and intrinsic renal dysfunctions especially those caused by acute tubular necrosis. While this FeNa cut-off has been a useful discriminatory tool in cirrhotic patients presenting with elevated serum creatinine, its accuracy has not been tested in diagnosing HRS against a gold standard such as kidney biopsy.

### Innovations and breakthroughs

The authors program utilizes kidney biopsy in evaluating selected liver transplant candidates with renal dysfunction.

### Applications

The authors measured FeNa at a single time point prior to the kidney biopsy to

minimize the selection bias. Future studies should address if serial measurements of FeNa in a given patient will confer similar results.

### Peer-review

In patients with end stage cirrhosis and renal dysfunction (glomerular filtration rate < 40) the estimated FeNa < 1 could not discriminate between HRS and intrarenal kidney injury. The findings are quite relevant, since FeNa is used most often to exclude intrarenal disease in cirrhotic patients.

## REFERENCES

- 1 **Garcia-Tsao G**, Parikh CR, Viola A. Acute kidney injury in cirrhosis. *Hepatology* 2008; **48**: 2064-2077 [PMID: 19003880 DOI: 10.1002/hep.22605]
- 2 **Huschak G**, Kaisers UX, Laudi S. [Hepatorenal syndrome]. *Anaesthesist* 2013; **62**: 571-582 [PMID: 23846211 DOI: 10.1007/s00101-013-2197-3]
- 3 **Mohanty A**, Garcia-Tsao G. Hyponatremia and Hepatorenal Syndrome. *Gastroenterol Hepatol* (NY) 2015; **11**: 220-229 [PMID: 27099594]
- 4 **Iwatsuki S**, Popovtzer MM, Corman JL, Ishikawa M, Putnam CW, Katz FH, Starzl TE. Recovery from "hepatorenal syndrome" after orthotopic liver transplantation. *N Engl J Med* 1973; **289**: 1155-1159 [PMID: 4585359 DOI: 10.1056/NEJM197311292892201]
- 5 **Cárdenas A**, Arroyo V. Mechanisms of water and sodium retention in cirrhosis and the pathogenesis of ascites. *Best Pract Res Clin Endocrinol Metab* 2003; **17**: 607-622 [PMID: 14687592]
- 6 **Bataller R**, Ginès P, Guevara M, Arroyo V. Hepatorenal syndrome. *Semin Liver Dis* 1997; **17**: 233-247 [PMID: 9308128 DOI: 10.1055/s-2007-1007201]
- 7 **Wadei HM**, Mai ML, Ahsan N, Gonwa TA. Hepatorenal syndrome: pathophysiology and management. *Clin J Am Soc Nephrol* 2006; **1**: 1066-1079 [PMID: 17699328 DOI: 10.2215/CJN.01340406]
- 8 **Wadei HM**, Geiger XJ, Cortese C, Mai ML, Kramer DJ, Rosser BG, Keaveny AP, Willingham DL, Ahsan N, Gonwa TA. Kidney allocation to liver transplant candidates with renal failure of undetermined etiology: role of percutaneous renal biopsy. *Am J Transplant* 2008; **8**: 2618-2626 [PMID: 19032225 DOI: 10.1111/j.1600-6143.2008.02426.x]
- 9 **Bichet DG**, Van Putten VJ, Schrier RW. Potential role of increased sympathetic activity in impaired sodium and water excretion in cirrhosis. *N Engl J Med* 1982; **307**: 1552-1557 [PMID: 6755251 DOI: 10.1056/NEJM198212163072504]
- 10 **Belcher JM**, Sanyal AJ, Peixoto AJ, Perazella MA, Lim J, Thiessen-Philbrook H, Ansari N, Coca SG, Garcia-Tsao G, Parikh CR. Kidney biomarkers and differential diagnosis of patients with cirrhosis and acute kidney injury. *Hepatology* 2014; **60**: 622-632 [PMID: 24375576 DOI: 10.1002/hep.26980]
- 11 **Arroyo V**, Fernandez J, Ginès P. Pathogenesis and treatment of hepatorenal syndrome. *Semin Liver Dis* 2008; **28**: 81-95 [PMID: 18293279 DOI: 10.1055/s-2008-1040323]
- 12 **Miller TR**, Anderson RJ, Linas SL, Henrich WL, Berns AS, Gabow PA, Schrier RW. Urinary diagnostic indices in acute renal failure: a prospective study. *Ann Intern Med* 1978; **89**: 47-50 [PMID: 666184]
- 13 **Pépin MN**, Bouchard J, Legault L, Ethier J. Diagnostic performance of fractional excretion of urea and fractional excretion of sodium in the evaluations of patients with acute kidney injury with or without diuretic treatment. *Am J Kidney Dis* 2007; **50**: 566-573 [PMID: 17900456 DOI: 10.1053/j.ajkd.2007.07.001]

P- Reviewer: Chok KSH, Lenz K S- Editor: Qi Y L- Editor: A  
E- Editor: Li D



Retrospective Study

## Resection margin influences the outcome of patients with bilobar colorectal liver metastases

Sara Di Carlo, Derek Yeung, Jamie Mills, Abed Zaitoun, Iain Cameron, Dhanny Gomez

Sara Di Carlo, Department of General Surgery, University of Rome, 00185 Tor Vergata, Italy

Sara Di Carlo, Derek Yeung, Iain Cameron, Dhanny Gomez, Department of Hepatobiliary Surgery and Pancreatic Surgery, Queen's Medical Centre, Nottingham University Hospitals NHS Trust, Nottingham NG5 1PB, United Kingdom

Jamie Mills, Department of Oncology, Queen's Medical Centre, Nottingham University Hospitals NHS Trust, Nottingham NG5 1PB, United Kingdom

Abed Zaitoun, Department of Histo-pathology, Queen's Medical Centre, Nottingham University Hospitals NHS Trust, Nottingham NG5 1PB, United Kingdom

Dhanny Gomez, NIHR Nottingham Digestive Disease Biomedical research Unit, University of Nottingham, Nottingham NG7 2RD, United Kingdom

**Author contributions:** Di Carlo S collected the data and drafted the manuscript; Yeung D collected the data and assisted in drafting of the manuscript; Gomez D analyzed the data, designed and supervised the study; Mills J, Zaitoun A and Cameron I provided analytical oversight and supervision.

**Institutional review board statement:** This study has been registered and approved by the Clinical Audit Department, Nottingham University Hospitals NHS Trust.

**Informed consent statement:** Since this is a retrospective study, individual patient consent was not required, and all local ethical guidelines with respect to retrospective studies in this Trust were adhered to.

**Conflict-of-interest statement:** All the authors have approved the manuscript and there is no conflict of interest to declare.

**Data sharing statement:** The statistical methods of this study were reviewed and performed by Gomez D, who is competent in SPSS statistical software.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external

reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dhanny Gomez, MD, FRCS, Department of Hepatobiliary and Pancreatic Surgery, Queen's Medical Centre, Nottingham University Hospitals NHS Trust, Derby Road, Nottingham NG5 1PB, United Kingdom. [dhanny.gomez@nuh.nhs.uk](mailto:dhanny.gomez@nuh.nhs.uk)  
**Telephone:** +44-115-9249924  
**Fax:** +44-115-8493398

**Received:** July 12, 2016  
**Peer-review started:** July 13, 2016  
**First decision:** August 26, 2016  
**Revised:** September 16, 2016  
**Accepted:** October 22, 2016  
**Article in press:** October 24, 2016  
**Published online:** December 8, 2016

### Abstract

#### AIM

To evaluate the outcome of patients with bilobar colorectal liver metastases (CRLM) and identify clinicopathological variables that influenced survival.

#### METHODS

Patients with bilobar CRLM were identified from a prospectively maintained hepatobiliary database during the study period (January 2010-June 2014). Collated data included demographics, primary tumour treatment, surgical data, histopathology analysis and clinical outcome. Down-staging therapy included Oxaliplatin- or Irinotecan- based regimens, and Cetuximab was also used in patients that were *K-RAS* wild-type. Response

to neo-adjuvant therapy was assessed at the multi-disciplinary team meeting and considered for surgery if all macroscopic CRLM were resectable with a clear margin while preserving sufficient liver parenchyma.

## RESULTS

Of the 136 patients included, thirty-two (23.5%) patients were considered inoperable and referred for palliative chemotherapy, and thirty-four (25%) patients underwent liver resection. Seventy (51.4%) patients underwent down-staging therapy, of which 37 (52.8%) patients responded sufficiently to undergo liver resection. Patients that failed to respond to down-staging therapy ( $n = 33$ , 47.1%) were referred for palliative therapy. There was a significant difference in overall survival between the three groups (surgery *vs* down-staging therapy *vs* inoperable disease,  $P < 0.001$ ). All patients that underwent hepatic resection, including patients that had down-staging therapy, had a significantly better overall survival compared to patients that were inoperable ( $P < 0.001$ ). On univariate analysis, only resection margin significantly influenced disease-free survival ( $P = 0.017$ ). On multi-variate analysis, R0 resection ( $P = 0.030$ ) and female ( $P = 0.036$ ) gender significantly influenced overall survival.

## CONCLUSION

Patients undergoing liver resection with bilobar CRLM have a significantly better survival outcome. R0 resection is associated with improved disease-free and overall survival in this patient group.

**Key words:** Colorectal liver metastases; Chemotherapy; Liver resection

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The management of colorectal liver metastases (CRLM) has evolved over the last decade. More patients are being subjected to potentially curative liver resection following down-staging therapy and the introduction of specialist multi-disciplinary team meetings. The introduction of biological agents has also increased resection rates. The current study analysed patients with bilobar CRLM referred to our centre. Patients that underwent liver resection had a significantly better survival outcome following our multi-disciplinary approach.

Di Carlo S, Yeung D, Mills J, Zaitoun A, Cameron I, Gomez D. Resection margin influences the outcome of patients with bilobar colorectal liver metastases. *World J Hepatol* 2016; 8(34): 1502-1510 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i34/1502.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i34.1502>

## INTRODUCTION

Hepatic resection is the only potentially curative treat-

ment for patients with colorectal liver metastases (CRLM) and the 5-year survival rate is up to 50%<sup>[1,2]</sup>. Patients with extensive, bilateral disease present a surgical challenge in removing all macroscopic disease while preserving sufficient functional liver remnant. Studies have shown that 20%-30% of all patients with CRLM are resectable at the time of diagnosis<sup>[3]</sup>, with bilobar distribution of metastases a major contributing factor for unresectability<sup>[4]</sup>.

More recently, the introduction of biological agents and the improved efficacy of down-staging chemotherapy regimens to treat bilobar CRLM have increased the proportion of patients with initially unresectable disease to subsequently operable disease. In addition, neo-adjuvant chemotherapy can potentially treat systemic disease to lower the risk of distant spread, and allow the identification of patients with biologically aggressive tumours that progress on chemotherapy that would not benefit from liver surgery<sup>[5]</sup>. Down-staging chemotherapy regimens are more toxic than palliative regimens, and hence, it is essential that there is multi-disciplinary team approach in determining the management plan for these patients<sup>[6]</sup>. However, long term outcomes for these patients following down-staging therapy and liver resection are indeterminate.

The aim of this study was to evaluate the outcomes of patients with bilobar CRLM following multi-disciplinary therapy. The secondary aim was to identify clinicopathological variables that influenced disease-free and overall survival in this group of patients.

## MATERIALS AND METHODS

### Patients

Patients with bilobar CRLM were identified from a prospectively maintained hepatobiliary database at Queen's Medical Centre (QMC), Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom during a 4-year period from January 2010 to June 2014. QMC is a tertiary referral center for Nottinghamshire and surrounding regions located in the north of East Midlands, United Kingdom. Pre-operative radiological assessment included a computed tomography (CT) scan of the thorax, abdomen and pelvis and magnetic resonance imaging (MRI) of the liver. Patients with indeterminate lesions, in particular lung nodules, and patients with synchronous presentation underwent a positron emission tomography scan. Synchronous presentation was defined as the presence of liver metastases when colorectal cancer was diagnosed. Prior to any treatment, all patients including patients referred from the surrounding regions were discussed in a specialist multidisciplinary team (MDT) meeting consisting of hepatobiliary surgeons, hepatologists, oncologists, radiologists and pathologists. Patients were selected for liver resection without any prior neo-adjuvant therapy if all macroscopic CRLM were resectable to achieve a clear margin while preserving sufficient liver parenchyma.

Collated data included patient demographics, type of surgical resection, histopathology analysis and clinical



outcome. This study has been registered and approved by the Clinical Audit Department, Nottingham University Hospitals NHS Trust. Since this is a retrospective study, individual patient consent was not required, and all local ethical guidelines with respect to retrospective studies in this Trust were adhered to.

### **Down-staged therapy and adjuvant chemotherapy**

Patients scheduled for preoperative systemic chemotherapy had 3-6 mo of neo-adjuvant treatment. The regimens used were either Oxaliplatin based: Two weekly FOLFOX [5-fluorouracil (FU) 400 mg/m<sup>2</sup> bolus, and 2400 mg/m<sup>2</sup> over 46 h, Leucovorin and Oxaliplatin 85 mg/m<sup>2</sup>] or three weekly CAPOX (Capecitabine 1000 mg/m<sup>2</sup> BD for 14 d and Oxaliplatin 130 mg/m<sup>2</sup>).

However, in patients tested and found to be *K-RAS* wild-type, two weekly FOLFIRI (Irinotecan 180mg/m<sup>2</sup>, 5-FU 400 mg/m<sup>2</sup> bolus, and 2400 mg/m<sup>2</sup> over 46 h) was administered with concurrent Cetuximab (400 mg/m<sup>2</sup> cycle 1, then 250 mg/m<sup>2</sup> cycle 2 onwards).

The response to neo-adjuvant therapy was assessed after 3-6 mo of therapy by CT scan and repeat MRI of the liver if required. Patients were then re-discussed at the MDT and considered for surgery based on absence of new disease, tumour response and extent of disease. Patients deemed to have resectable disease were scheduled for a liver resection, 4-6 wk after their last cycle of chemotherapy. Resectable disease was defined as excision of all macroscopic CRLM to achieve a clear margin while preserving sufficient liver parenchyma based on pre-operative radiological imaging.

Following liver resection, chemotherapy was considered in patients with tumour present at the margin (R1 resection).

### **Surgery**

Liver resection was performed using the Cavi-Pulse Ultrasonic Surgical Aspirator. Intra-operative ultrasound was performed to confirm the findings of pre-operative imaging and to assist in surgical planning. The number of hepatic Couinaud's<sup>[7]</sup> segments resected was determined by the procedure performed as stated in the Brisbane nomenclature<sup>[8]</sup>. Type of surgical procedure was dependent on the resection of all macroscopic disease and achieving a clear resection margin, while preserving sufficient remnant liver. The extent of hepatic resection in this study was classified into two groups; less than hemihepatectomy and hemihepatectomy or more radical resection. Pre-operative PVE was performed if the FRL volume was estimated to be 20% or less of the total liver volume. Liver-first approach was defined when the hepatic resection was performed first prior to colonic or rectal resection<sup>[9,10]</sup>.

In patients where the liver-first approach was adopted, primary tumour resection was usually scheduled 4-8 wk following liver resection, or after completion of chemoradiotherapy for patients with locally advanced rectal cancer. All patients underwent re-staging with a CT scan and MRI to ensure there was no evidence of liver

recurrence or distant metastases. Colorectal resection was performed according to accepted oncological standards, with complete meso-rectal excision for rectal cancers and lymph node dissection for colonic cancers.

### **Histology**

Histopathological data of the resected liver specimen were collated. This included: Tumour size in maximum diameter; tumour number; and status of resection margin. R0 resection was defined as no microscopic evidence of tumour at or within 1 mm of the margin. Lymphatic, peri-neural, biliary and vascular invasion were also determined<sup>[11]</sup>.

### **Follow-up**

Patients were followed up in specialist hepatobiliary clinics. Following initial post-operative review at one month, all patients were examined in the outpatient clinic at 3, 6, 12, 18 and 24 mo and annually thereafter. At each clinical review, carcino-embryonic antigen levels were measured. All patients in this study had a minimum follow-up of 6 mo following hepatic resection for CRLM.

Surveillance imaging included CT scan of the thorax, abdomen and pelvis. Patients underwent 6-monthly CT scan during the first two post-operative years, followed by annual CT scans thereafter. Liver MRI was used to characterise suspicious hepatic lesions demonstrated on CT. Development of symptoms of recurrence at any time-point prompted earlier review than scheduled.

Overall and disease-free survival was recorded, with disease-free survival being defined as the time from primary hepatic resection to the first documented disease recurrence on imaging. Overall survival was defined as the time interval between the date of commencement of neo-adjuvant/induction therapy and the date of death or most recent date of follow-up if the patient was still alive. Following detection of recurrent disease on surveillance imaging, all patients were discussed at the MDT meeting. Patients who had non-resectable disease were referred to the oncologists for consideration of palliative chemotherapy.

### **Statistical analysis**

Categorical data was presented as frequency and percentage. The Kaplan-Meier method was used to assess the actuarial survival and disease-free survival, and presented as median (range). Univariate analysis was performed to assess for a significant difference in clinico-pathological characteristics that influenced disease recurrence and survival. A multivariate analysis was performed by Cox regression (Step-wise forward model) for variables significant on univariate analysis. Statistical analyses were performed using the SPSS for Windows™ version 16.0 (SPSS Inc, Chicago, Ill, United States), and statistical significance was taken at the 5% level. The statistical methods of this study were reviewed and performed by Gomez D, QMC, Nottingham, United Kingdom.

**Table 1 Clinical data of patients with bilobar colorectal liver metastases in this study**

Demographic, clinical and pathological factors	Total (n)
<b>All patients (n = 136)</b>	
<b>All surgery patients (n = 71)</b>	
Demographic factors	
Age > 65 yr	68
Male gender	99
Synchronous presentation	80
Down-staging therapy	70
Oxaliplatin-based regimen	60
Irinotecan-based regimen	10
Addition of biological agent	30
Surgical factors (n = 71)	
Hemi-hepatectomy or more	22
Histo-pathological factor (n = 71)	
Largest tumour size $\geq 5$ cm	11
Number of metastases < 4	44
Lymphatic invasion present	15
Vascular invasion present	28
Peri-neural invasion present	9
Biliary invasion present	25
Resection margin (R0)	40

## RESULTS

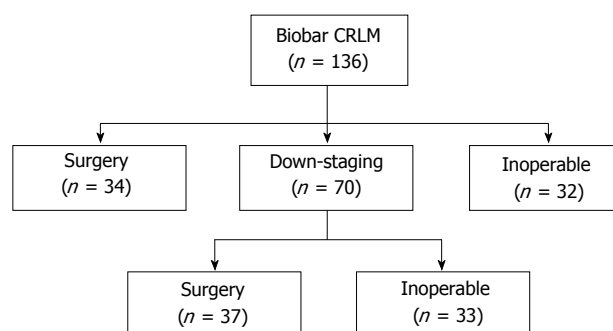
### Patients

During the study period, a total of 136 patients (Table 1) with bilobar CRLM were discussed in the unit's MDT, of which 34 (25.0%) patients underwent surgery with curative intent as their primary treatment (Figure 1). There were 32 (23.5%) patients that had extensive disease and were referred for palliative therapy.

Seventy (51.4%) patients were considered for down-staging therapy, in view to consider liver resection depending on response to therapy. Besides receiving either an Oxaliplatin-based ( $n = 60$ ) or Irinotecan-based ( $n = 10$ ) regimen, 30 (42.8%) patients also had biological agents as part of their down-staging treatment. Within the group of patients that received down-staging therapy, 37 (52.8%) patients had a response to their down-staging therapy and underwent hepatic resection, while the remaining patients [ $n = 33$  (47.2%)] did not undergo surgical resection. These patients did not respond to their down-staging therapy, which included: (1) having new metastases; (2) disease progression; and (3) inability to remove all macroscopic liver disease whilst leaving sufficient remnant liver. This decision was based on MDT review of up to date radiological imaging following down-staging therapy.

### Liver resection

Overall, there were 71 (52.2%) patients that underwent liver resection, of which twenty-two patients had a hemi-hepatectomy or more. The most common surgical procedures performed was multiple non-anatomical resections ( $n = 40$ , 56.3%). Twenty-one patients were female and the median age at the diagnosis was 65 (range: 44-84) years. Seven (9.8%) patients had portal vein embolization prior to liver resection. There were 35

**Figure 1 Outcome of patients with bilobar colorectal liver metastases in this study.** CRLM: Colorectal liver metastases.

patients with synchronous disease, of which 17 patients had a liver-first approach. There was no post-operative mortality.

### Survival outcome

The median overall survival for all patients in this study was 18 (1-48) mo (Figure 2). There was a significant difference in overall survival between the three groups (Surgery vs Down-staging therapy vs Inoperable disease,  $P < 0.001$ ; Figure 3). All patients that underwent hepatic resection, including patients that had down-staging therapy, had a significantly better overall survival compared to patients that were inoperable [24 (6-48) mo vs 17 (1-43) mo;  $P < 0.001$ ; Figure 4]. The disease-free survival for patients that underwent liver resection was 8 (range: 2-36) mo.

### Prognostic factors influencing disease-free and overall survival

With respect to disease-free survival, patients with a clear (R0) resection margin following liver resection had a significantly better disease-free survival compared to patients with a R1 resection ( $P = 0.017$ ; Table 2 and Figure 5).

Patients with a R0 resection ( $P = 0.022$ ; Figure 6) and female gender ( $P = 0.024$ ; Figure 7) has a significantly better overall survival compared to patients with a R1 resection and male gender on univariate analysis. On the multi-variate analysis, both R0 resection and female gender were independent predictors of improved overall survival (Table 3).

## DISCUSSION

With the improvement in chemotherapy agents and the increased efficacy with the addition of biological agents, many centers have reported an increased number of patients being converted from initially unresectable, to resectable disease<sup>[12,13]</sup>. However, although there are an increased number of patients undergoing liver resection with curative intent, some authorities may suggest that these patients are unlikely to be cured<sup>[14]</sup>. Nevertheless, these patients have a better overall survival in comparison to patients treated with palliative systemic

**Table 2** Statistical analysis of prognostic factors with respect to disease-free survival

Demographic, clinical and pathological factors	Survival [median (range) mo]	Uni-variate analysis
Demographic factors		
Age		0.099
< 65 yr ( <i>n</i> = 43)	6 (3-36)	
≥ 65 yr ( <i>n</i> = 28)	12 (2-36)	
Gender		0.343
Male ( <i>n</i> = 50)	6 (3-36)	
Female ( <i>n</i> = 21)	5 (2-36)	
Presentation		0.755
Synchronous ( <i>n</i> = 35)	6 (2-36)	
Metachronous ( <i>n</i> = 36)	6 (3-36)	
Surgical factors		
Less than hemi-hepatectomy ( <i>n</i> = 49)	6 (2-36)	0.760
Hemi-hepatectomy or more ( <i>n</i> = 22)	6 (2-36)	
Histo-pathological factor		
Largest tumour size		0.813
< 5 cm ( <i>n</i> = 60)	6 (2-36)	
≥ 5 cm ( <i>n</i> = 11)	9 (2-36)	
No. of metastases		0.538
< 4 ( <i>n</i> = 44)	7 (2-36)	
> 5 ( <i>n</i> = 27)	6 (3-36)	
Lymphatic invasion		0.256
Positive ( <i>n</i> = 15)	6 (2-24)	
Negative ( <i>n</i> = 56)	6 (2-36)	
Vascular invasion		0.775
Positive ( <i>n</i> = 28)	6 (2-36)	
Negative ( <i>n</i> = 43)	7 (2-36)	
Peri-neural invasion		0.115
Positive ( <i>n</i> = 9)	6 (2-24)	
Negative ( <i>n</i> = 62)	6 (2-36)	
Biliary invasion		0.919
Positive ( <i>n</i> = 25)	6 (2-36)	
Negative ( <i>n</i> = 46)	6 (2-36)	
Resection margin (R0)		0.017
R0 ( <i>n</i> = 40)	8 (2-36)	
R1 ( <i>n</i> = 31)	6 (2-36)	

chemotherapy, with some authors reporting a median survival up to 45 mo<sup>[12,15]</sup>. In the present study, patients with bilobar disease who underwent surgery had a significantly better overall survival compared to patients who failed down-staged chemotherapy and/or treated with palliative chemotherapy.

### Conversion rate

The addition of biological agents to current Oxaliplatin- and Irinotecan- based regimens has led to further improvements in response rates. In a large randomised control trial, Folprecht *et al.*<sup>[16]</sup> showed an increased response rate up to 68% with the addition of Cetuximab. Similarly, Masi *et al.*<sup>[17]</sup> observed that the addition of Bevacizumab to Oxaliplatin- and Irinotecan- based regimens increased the response rate up to 80%. In the present study, the unit's down-staging therapy protocol had a response rate and a conversion of unresectable to resectable disease of more than 50%. These results were consistent with data reported by the groups of Van Cutsem *et al.*<sup>[18]</sup> and Bokemeyer *et al.*<sup>[19]</sup> that observed a conversion rate of approximately 60% following down-staging chemotherapy.

**Table 3** Statistical analysis of prognostic factors with respect to overall survival

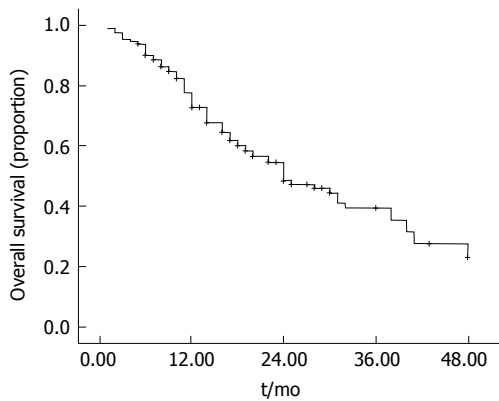
Demographic, clinical and pathological factors	Survival [median (range) mo]	Uni-variate analysis	Multi-variate analysis	Risk ratio (confidence interval)
Demographic factors				
Age		0.173		
< 65 yr ( <i>n</i> = 43)	20 (6-48)			
≥ 65 yr ( <i>n</i> = 28)	27 (7-48)			
Gender		0.024	0.036	3.172 (1.079-9.327)
Male ( <i>n</i> = 50)	19 (6-48)			
Female ( <i>n</i> = 21)	20 (11-48)			
Presentation		0.932		
Synchronous ( <i>n</i> = 35)	23 (6-48)			
Metachronous ( <i>n</i> = 36)	24 (6-48)			
Surgical factors				
Less than hemi-hepatectomy ( <i>n</i> = 49)	22 (6-48)	0.947		
Hemi-hepatectomy or more ( <i>n</i> = 22)	28 (7-48)			
Histo-pathological factor				
Largest tumour size		0.216		
< 5 cm ( <i>n</i> = 60)	24 (6-48)			
≥ 5 cm ( <i>n</i> = 11)	28 (12-48)			
Number of metastases		0.674		
< 4 ( <i>n</i> = 44)	24 (6-48)			
> 5 ( <i>n</i> = 27)	24 (11-48)			
Lymphatic invasion		0.943		
Positive ( <i>n</i> = 15)	24 (11-48)			
Negative ( <i>n</i> = 56)	23 (6-48)			
Vascular invasion		0.367		
Positive ( <i>n</i> = 28)	25 (6-48)			
Negative ( <i>n</i> = 43)	23 (6-48)			
Peri-neural invasion		0.220		
Positive ( <i>n</i> = 9)	12 (11-48)			
Negative ( <i>n</i> = 62)	24 (6-48)			
Biliary invasion		0.608		
Positive ( <i>n</i> = 25)	27 (11-48)			
Negative ( <i>n</i> = 46)	22 (6-48)			
Resection margin (R0)		0.022	0.030	0.403 (0.178-0.917)
R0 ( <i>n</i> = 40)	24 (6-48)			
R1 ( <i>n</i> = 31)	22 (6-48)			

### Survival data

Adam *et al.*<sup>[13]</sup> recently published their long-term survival results following down-sizing chemotherapy and hepatic resection in patients with CRLM and demonstrated that 24 (16%) of 148 patients were alive and disease-free with a minimum of 5-year follow-up. Several studies have shown the improvements in survival after the addition of anti-VEGF/EGFR<sup>[20-22]</sup>. Recently, a number of case series describing 10-year actual survivors after liver resection of CRLM have been published<sup>[23,24]</sup>. The present series demonstrated that hepatic resection for patients with bilobar CRLM had a median disease-free and overall survival of 8 and 24 mo, respectively.

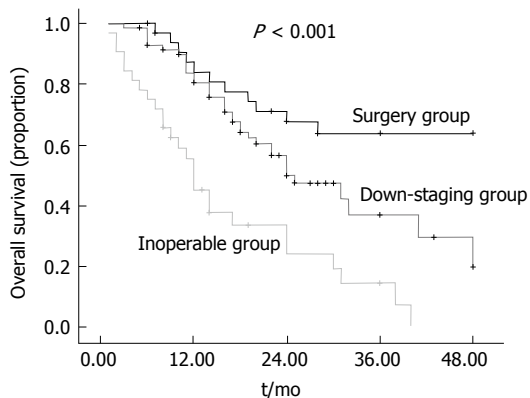
### MDT approach

Definitions of resectable disease have evolved over time, with current consensus suggesting that disease should be considered technically resectable as long as complete macroscopic resection is feasible, whilst maintaining



Numbers at risk					
Patients	0	12	36	48	60
All ( <i>n</i> = 136)	136	97	23	6	0

**Figure 2** Overall survival of patients with bilobar colorectal liver metastases in this study. All patients (*n* = 136): 18 (1-48) mo.

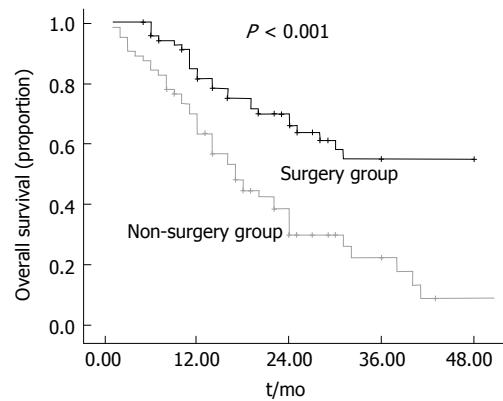


Surgery (*n* = 34): 28 (7-48) mo  
Down-staging therapy (*n* = 70): 18 (3-48) mo  
Inoperable (*n* = 32): 11 (1-40) mo

Numbers at risk					
Patients	0	12	36	48	60
Surgery ( <i>n</i> = 34)	34	32	14	4	0
Down-staging ( <i>n</i> = 70)	70	49	6	2	0
Inoperable ( <i>n</i> = 32)	32	16	3	0	0

**Figure 3** Difference in overall survival in patients that underwent surgery, down-staging therapy followed by surgery or palliative therapy and inoperable patients.

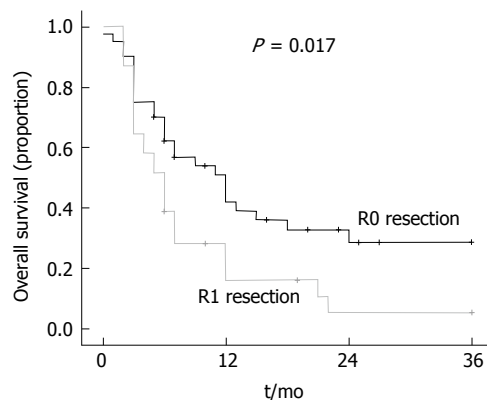
sufficient future liver volume<sup>[25]</sup>. However, there remains concern that not all patients with technically resectable liver-limited metastases benefit from surgery; with approximately half of these patients will develop recurrences within three years of liver resection<sup>[26]</sup>. Therefore, it is crucial that the decision-making process around treatment strategies for metastatic colorectal cancer are made in a MDT environment that consists of specialist hepato-biliary surgeons, radiologists and oncologists that can define optimal patient management on a case by case basis. A recent study demonstrated that almost two-thirds of patients with tumours deemed unresectable by non-specialists were considered potentially resectable



Surgery (*n* = 71): 24 (6-48) mo  
No surgery (*n* = 65): 17 (1-43) mo

Numbers at risk				
Patients	0	12	36	48
Surgery group ( <i>n</i> = 71)	71	66	18	6
Non-surgery group ( <i>n</i> = 65)	65	40	6	0

**Figure 4** Difference in overall survival in patients that underwent surgery following down-staging therapy compared to patients that either failed down-staging therapy or were treated with palliative therapy.



Numbers at risk			
Patients	0	12	36
R0 resection ( <i>n</i> = 40)	40	17	5
R1 resection ( <i>n</i> = 31)	31	7	1

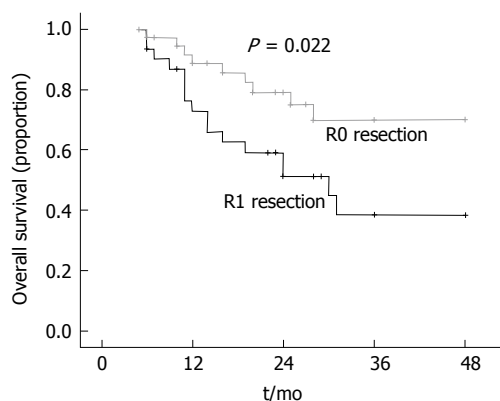
**Figure 5** Difference in disease-free survival in patients with R0 resection compared to patients with R1 resection.

by a panel of specialist hepato-biliary surgeons based on radiological imaging<sup>[27]</sup>.

### Prognostic factors

The role of margin status as a predictor of outcome following resection for CRLM is controversial. Bodingbauer *et al.*<sup>[28]</sup> observed that resection margin and size of margin width did not correlate significantly with survival following resection for CRLM. In a series of 1019 patients, and co-investigators demonstrated that a resection margin > 1 cm was an independent predictor of survival following resection for CRLM<sup>[29]</sup>. Rees *et al.*<sup>[1]</sup> also demonstrated that positive resection margins were an independent predictor of poorer survival. However,





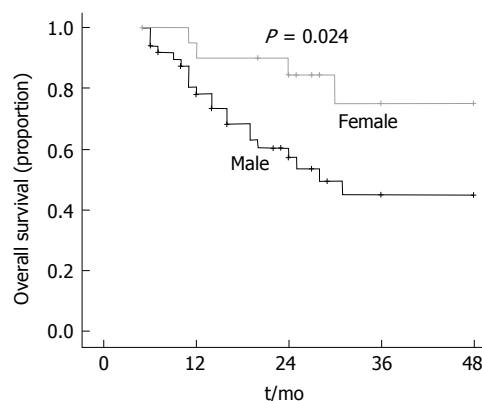
Numbers at risk				
Patients	0	12	36	48
R0 resection (n = 40)	40	34	12	4
R1 resection (n = 31)	31	21	6	2

**Figure 6** Difference in overall survival in patients with R0 resection compared to patients with R1 resection.

Figueras *et al.*<sup>[30]</sup> showed that a margin width < 1 cm in patients who underwent resection for CRLM did not significantly influence recurrent disease in a cohort of 609 patients. Homayounfar *et al.*<sup>[31]</sup> demonstrated that R0 resection in patients with bilobar CRLM have improved survival rates following multi-modal therapy<sup>[32]</sup>. In the present series, a clear resection margin, defined as no microscopic evidence of tumour at or within 1 mm of the margin, was an independent predictor of both disease-free and overall survival. Due to the differences in results observed with respect to resection margin between published studies, it may be that only a selected group of patients undergoing resection for CRLM are influenced by a clear margin. In the present series that focused on patients with bilobar CRLM, that would be considered as having a high tumour burden, benefited from a R0 margin. This could be due to the fact that these patients have an aggressive tumour profile and it is crucial that complete tumour clearance is obtained. Hence, for these patients, “down-sizing” chemotherapy should certainly be considered prior to resection to aid in achieving a clear resection margin. Furthermore, many groups advocate a trial of neo-adjuvant chemotherapy in patients with a high tumour burden, as disease progression on chemotherapy would be a contraindication to surgery<sup>[33]</sup>. Nevertheless, with the increase use of chemotherapy, there is an increase in prevalence of patients undergoing hepatic resection with a background of chemotherapy-related injury, such as steato-hepatitis<sup>[34]</sup> and sinusoidal obstruction syndrome<sup>[35]</sup>. In such cases, the quality, rather than quantity of the remnant liver becomes an important issue to consider prior to extensive resection.

The present study also showed that female gender was an independent prognostic factor for improved overall survival. There are currently no other studies that have reported this finding.

There are limitations in this study. This is a retrospective study, and focused on a group of patients with bilobar liver metastases. These are patients with bad



Numbers at risk				
Patients	0	12	36	48
Male (n = 50)	50	36	11	4
Female (n = 21)	21	20	9	2

**Figure 7** Difference in overall survival in female patients compared to male patients following surgery for colorectal liver metastases.

tumour biology and in most cases, will require down-staging therapy. Nevertheless, although these group of patients have a higher tumour burden; their prognosis can be improved with a MDT approach that focuses on multi-modal therapy.

Patients with bilobar CRLM treated with liver resection as a primary treatment or following down-staging therapy have a better overall survival compared to patients who failed down-staging therapy and/or treated with palliative chemotherapy. Obtaining a clear resection margin in these cases significantly influences outcome. In this group of patients, multi-modal therapy is crucial to achieve a better survival outcome.

## COMMENTS

### Background

Hepatic resection is the only potentially curative treatment for patients with colorectal liver metastases (CRLM) and the 5-year survival rate is up to 50%.

### Research frontiers

The introduction of biological agents and the improved efficacy of down-staging chemotherapy regimens to treat bilobar CRLM have increased the proportion of patients with initially unresectable disease to subsequently operable disease. In addition, neo-adjuvant chemotherapy can potentially treat systemic disease to lower the risk of distant spread, and allow the identification of patients with biologically aggressive tumours that progress on chemotherapy that would not benefit from liver surgery.

### Innovations and breakthroughs

In the present study, patients with bilobar disease who underwent surgery had a significantly better overall survival compared to patients who failed down-staged chemotherapy and/or treated with palliative chemotherapy.

### Applications

Patients with bilobar CRLM treated with liver resection as a primary treatment or following down-staging therapy have a better overall survival compared to patients who failed down-staging therapy and/or treated with palliative chemotherapy.

### Peer-review

This article is interesting but I think epidemiological data are more interesting

than univariate and multivariate analysis, which is the part highlighted by the authors. Structure of the manuscript is correct.

## REFERENCES

- 1 **Rees M**, Tekkis PP, Welsh FK, O'Rourke T, John TG. Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients. *Ann Surg* 2008; **247**: 125-135 [PMID: 18156932 DOI: 10.1097/SLA.0b013e31815aa2e2]
- 2 **Brouquet A**, Abdalla EK, Kopetz S, Garrett CR, Overman MJ, Eng C, Andreou A, Loyer EM, Madoff DC, Curley SA, Vauthey JN. High survival rate after two-stage resection of advanced colorectal liver metastases: response-based selection and complete resection define outcome. *J Clin Oncol* 2011; **29**: 1083-1090 [PMID: 21263087 DOI: 10.1200/JCO.2010.32.6132]
- 3 **Kanas GP**, Taylor A, Primrose JN, Langeberg WJ, Kelsh MA, Mowat FS, Alexander DD, Choti MA, Poston G. Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors. *Clin Epidemiol* 2012; **4**: 283-301 [PMID: 23152705 DOI: 10.2147/CLEP.S34285]
- 4 **Sakamoto Y**, Fujita S, Akasu T, Nara S, Esaki M, Shimada K, Yamamoto S, Moriya Y, Kosuge T. Is surgical resection justified for stage IV colorectal cancer patients having bilobar hepatic metastases?--an analysis of survival of 77 patients undergoing hepatectomy. *J Surg Oncol* 2010; **102**: 784-788 [PMID: 20872814 DOI: 10.1002/jso.21721]
- 5 **Chun YS**, Vauthey JN, Ribero D, Donadon M, Mullen JT, Eng C, Madoff DC, Chang DZ, Ho L, Kopetz S, Wei SH, Curley SA, Abdalla EK. Systemic chemotherapy and two-stage hepatectomy for extensive bilateral colorectal liver metastases: perioperative safety and survival. *J Gastrointest Surg* 2007; **11**: 1498-1504; discussion 1504-1505 [PMID: 17849166 DOI: 10.1007/s11605-007-0272-2]
- 6 **Sutcliffe RP**, Bhattacharya S. Colorectal liver metastases. *Br Med Bull* 2011; **99**: 107-124 [PMID: 21813558 DOI: 10.1093/bmb/ldr034]
- 7 **Couinaud C**. [Liver lobes and segments: notes on the anatomical architecture and surgery of the liver]. *Presse Med* 1954; **62**: 709-712 [PMID: 13177441]
- 8 **Strasberg SM**. Nomenclature of hepatic anatomy and resections: a review of the Brisbane 2000 system. *J Hepatobiliary Pancreat Surg* 2005; **12**: 351-355 [PMID: 16258801 DOI: 10.1007/s00534-005-0999-7]
- 9 **de Rosa A**, Gomez D, Hossaini S, Duke K, Fenwick SW, Brooks A, Poston GJ, Malik HZ, Cameron IC. Stage IV colorectal cancer: outcomes following the liver-first approach. *J Surg Oncol* 2013; **108**: 444-449 [PMID: 24009161 DOI: 10.1002/jso.23429]
- 10 **De Rosa A**, Gomez D, Brooks A, Cameron IC. "Liver-first" approach for synchronous colorectal liver metastases: is this a justifiable approach? *J Hepatobiliary Pancreat Sci* 2013; **20**: 263-270 [PMID: 23325126 DOI: 10.1007/s00534-012-0583-x]
- 11 **Gomez D**, Zaitoun AM, De Rosa A, Hossaini S, Beckingham IJ, Brooks A, Cameron IC. Critical review of the prognostic significance of pathological variables in patients undergoing resection for colorectal liver metastases. *HPB (Oxford)* 2014; **16**: 836-844 [PMID: 24617566 DOI: 10.1111/hpb.12216]
- 12 **Lam VW**, Spiro C, Laurence JM, Johnston E, Hollands MJ, Pleass HC, Richardson AJ. A systematic review of clinical response and survival outcomes of downsizing systemic chemotherapy and rescue liver surgery in patients with initially unresectable colorectal liver metastases. *Ann Surg Oncol* 2012; **19**: 1292-1301 [PMID: 21922338 DOI: 10.1245/s10434-011-2061-0]
- 13 **Adam R**, Wicherts DA, de Haas RJ, Ciaccio O, Lévi F, Paule B, Ducreux M, Azoulay D, Bismuth H, Castaing D. Patients with initially unresectable colorectal liver metastases: is there a possibility of cure? *J Clin Oncol* 2009; **27**: 1829-1835 [PMID: 19273699 DOI: 10.1200/JCO.2008.19.9273]
- 14 **Jones RP**, Jackson R, Dunne DF, Malik HZ, Fenwick SW, Poston GJ, Ghaneh P. Systematic review and meta-analysis of follow-up after hepatectomy for colorectal liver metastases. *Br J Surg* 2012; **99**: 477-486 [PMID: 22261895 DOI: 10.1002/bjs.8667]
- 15 **Jones RP**, Hamann S, Malik HZ, Fenwick SW, Poston GJ, Folprecht G. Defined criteria for resectability improves rates of secondary resection after systemic therapy for liver limited metastatic colorectal cancer. *Eur J Cancer* 2014; **50**: 1590-1601 [PMID: 24661798 DOI: 10.1016/j.ejca.2014.02.024]
- 16 **Folprecht G**, Gruenberger T, Bechstein WO, Raab HR, Lordick F, Hartmann JT, Lang H, Frilling A, Stoecklmaier J, Weitz J, Konopke R, Stroszczynski C, Liersch T, Ockert D, Herrmann T, Goekkurt E, Parisi F, Köhne CH. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. *Lancet Oncol* 2010; **11**: 38-47 [PMID: 19942479 DOI: 10.1016/S1470-2045(09)70330-4]
- 17 **Masi G**, Loupakis F, Salvatore L, Fornaro L, Cremolini C, Cupini S, Ciardo A, Del Monte F, Cortesi E, Amoroso D, Granetto C, Fontanini G, Sensi E, Lupi C, Andreuccetti M, Falcone A. Bevacizumab with FOLFOXIRI (irinotecan, oxaliplatin, fluorouracil, and folinate) as first-line treatment for metastatic colorectal cancer: a phase 2 trial. *Lancet Oncol* 2010; **11**: 845-852 [PMID: 20702138 DOI: 10.1016/S1470-2045(10)70175-3]
- 18 **Van Cutsem E**, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 1408-1417 [PMID: 19339720 DOI: 10.1056/NEJMoa0805019]
- 19 **Bokemeyer C**, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, Donea S, Ludwig H, Schuch G, Stroh C, Loos AH, Zube A, Koralewski P. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009; **27**: 663-671 [PMID: 19114683 DOI: 10.1200/JCO.2008.20.8397]
- 20 **Stintzing S**, Fischer von Weikersthal L, Decker T, Vehlning-Kaiser U, Jäger E, Heintges T, Stoll C, Giessen C, Modest DP, Neumann J, Jung A, Kirchner T, Scheithauer W, Heinemann V. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer-subgroup analysis of patients with KRAS: mutated tumours in the randomised German AIO study KRK-0306. *Ann Oncol* 2012; **23**: 1693-1699 [PMID: 22219013 DOI: 10.1093/annonc/mdr571]
- 21 **Schwartzberg LS**, Rivera F, Karthaus M, Fasola G, Canon JL, Hecht JR, Yu H, Oliner KS, Go WY. PEAK: a randomized, multicenter phase II study of panitumumab plus modified fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) or bevacizumab plus mFOLFOX6 in patients with previously untreated, unresectable, wild-type KRAS exon 2 metastatic colorectal cancer. *J Clin Oncol* 2014; **32**: 2240-2247 [PMID: 24687833 DOI: 10.1200/JCO.2013.53.2473]
- 22 **Gomez D**, De Rosa A, Addison A, Brooks A, Malik HZ, Cameron IC. Cetuximab therapy in the treatment of metastatic colorectal cancer: the future frontier? *Int J Surg* 2013; **11**: 507-513 [PMID: 23660586 DOI: 10.1016/j.ijsu.2013.04.014]
- 23 **Viganò L**, Ferrero A, Lo Tesoriere R, Capussotti L. Liver surgery for colorectal metastases: results after 10 years of follow-up. Long-term survivors, late recurrences, and prognostic role of morbidity. *Ann Surg Oncol* 2008; **15**: 2458-2464 [PMID: 18463927 DOI: 10.1245/s10434-008-9935-9]
- 24 **Pulitanò C**, Castillo F, Aldrighetti L, Bodingbauer M, Parks RW, Ferla G, Wigmore SJ, Garden OJ. What defines 'cure' after liver resection for colorectal metastases? Results after 10 years of follow-up. *HPB (Oxford)* 2010; **12**: 244-249 [PMID: 20590894 DOI: 10.1111/j.1477-2574.2010.00155.x]
- 25 **Adam R**, De Gramont A, Figueras J, Guthrie A, Kokudo N, Kunstlinger F, Loyer E, Poston G, Rougier P, Rubbia-Brandt L, Sobrero A, Tabernero J, Teh C, Van Cutsem E. The oncosurgery approach to managing liver metastases from colorectal cancer: a multidisciplinary international consensus. *Oncologist* 2012; **17**: 1225-1239 [PMID: 22962059 DOI: 10.1634/theoncologist.2012-0121]

- 26 **Jones RP**, Stättner S, Sutton P, Dunne DF, McWhirter D, Fenwick SW, Malik HZ, Poston GJ. Controversies in the oncosurgical management of liver limited stage IV colorectal cancer. *Surg Oncol* 2014; **23**: 53-60 [PMID: 24631118 DOI: 10.1016/j.suronc.2014.02.002]
- 27 **Jones RP**, Vauthey JN, Adam R, Rees M, Berry D, Jackson R, Grimes N, Fenwick SW, Poston GJ, Malik HZ. Effect of specialist decision-making on treatment strategies for colorectal liver metastases. *Br J Surg* 2012; **99**: 1263-1269 [PMID: 22864887 DOI: 10.1002/bjs.8969]
- 28 **Bodingbauer M**, Tamandl D, Schmid K, Plank C, Schima W, Gruenberger T. Size of surgical margin does not influence recurrence rates after curative liver resection for colorectal cancer liver metastases. *Br J Surg* 2007; **94**: 1133-1138 [PMID: 17514637 DOI: 10.1002/bjs.5762]
- 29 **Are C**, Gonen M, Zazzali K, Dematteo RP, Jarnagin WR, Fong Y, Blumgart LH, D'Angelica M. The impact of margins on outcome after hepatic resection for colorectal metastasis. *Ann Surg* 2007; **246**: 295-300 [PMID: 17667509 DOI: 10.1097/SLA.0b013e31811ea962]
- 30 **Figueras J**, Burdio F, Ramos E, Torras J, Llado L, Lopez-Ben S, Codina-Barreras A, Mojal S. Effect of subcentimeter nonpositive resection margin on hepatic recurrence in patients undergoing hepatectomy for colorectal liver metastases. Evidences from 663 liver resections. *Ann Oncol* 2007; **18**: 1190-1195 [PMID: 17434896 DOI: 10.1093/annonc/mdm106]
- 31 **Homayounfar K**, Liersch T, Niessner M, Meller J, Lorf T, Becker H, Ghadimi BM. Multimodal treatment options for bilobar colorectal liver metastases. *Langenbecks Arch Surg* 2010; **395**: 633-641 [PMID: 20213463 DOI: 10.1007/s00423-010-0604-7]
- 32 **Homayounfar K**, Bleckmann A, Conradi LC, Sprenger T, Beissbarth T, Lorf T, Niessner M, Sahlmann CO, Meller J, Becker H, Liersch T, Ghadimi BM. Bilobar spreading of colorectal liver metastases does not significantly affect survival after R0 resection in the era of interdisciplinary multimodal treatment. *Int J Colorectal Dis* 2012; **27**: 1359-1367 [PMID: 22430890 DOI: 10.1007/s00384-012-1455-1]
- 33 **Viganò L**, Capussotti L, Barroso E, Nuzzo G, Laurent C, Ijzermans JN, Gigot JF, Figueras J, Gruenberger T, Mirza DF, Elias D, Poston G, Letoublon C, Isoniemi H, Herrera J, Sousa FC, Pardo F, Lucidi V, Popescu I, Adam R. Progression while receiving preoperative chemotherapy should not be an absolute contraindication to liver resection for colorectal metastases. *Ann Surg Oncol* 2012; **19**: 2786-2796 [PMID: 22622469 DOI: 10.1245/s10434-012-2382-7]
- 34 **Vauthey JN**, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM, Xiong HQ, Eng C, Lauwers GY, Mino-Kenudson M, Risio M, Muratore A, Capussotti L, Curley SA, Abdalla EK. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *J Clin Oncol* 2006; **24**: 2065-2072 [PMID: 16648507 DOI: 10.1200/JCO.2005.05.3074]
- 35 **Rubbia-Brandt L**, Audard V, Sartoretti P, Roth AD, Brezault C, Le Charpentier M, Dousset B, Morel P, Soubrane O, Chaussade S, Mentha G, Terris B. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. *Ann Oncol* 2004; **15**: 460-466 [PMID: 14998849 DOI: 10.1093/annonc/mdh095]

P- Reviewer: Lorenzo D S- Editor: Qi Y L- Editor: A  
E- Editor: Li D



Retrospective Study

## On-treatment quantitative hepatitis B e antigen predicted response to nucleos(t)ide analogues in chronic hepatitis B

Yu-Hua Gao, Qing-Hua Meng, Zhan-Qing Zhang, Ping Zhao, Qing-Hua Shang, Quan Yuan, Yao Li, Juan Deng, Tong Li, Xue-En Liu, Hui Zhuang

Yu-Hua Gao, Yao Li, Juan Deng, Tong Li, Xue-En Liu, Hui Zhuang, Department of Microbiology and Infectious Disease Center, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China

Qing-Hua Meng, Beijing YouAn Hospital, Capital Medical University, Beijing 100069, China

Zhan-Qing Zhang, Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China

Ping Zhao, Department of Hepatology, 302 Military Hospital of China, Beijing 100039, China

Qing-Hua Shang, Department of Hepatology, the No.88 Hospital of the People's Liberation Army, Taian 271000, Shandong Province, China

Quan Yuan, National Institute of Diagnostic and Vaccine Development in Infectious Diseases, School of Public Health, Xiamen University, Xiamen 361000, Fujian Province, China

**Author contributions:** Liu XE and Zhuang H designed the study; Gao YH and Li Y performed the experiments; Meng QH, Zhang ZQ, Zhao P, Shang QH, Yuan Q, Deng J and Li T were involved in samples collection and database establishment; Gao YH analyzed data and wrote the manuscript; Liu XE edit the manuscript for important contents; Zhuang H critically revised the manuscript; all authors have read and approved the final version of the manuscript.

**Supported by** Major Science and Technology Special Project of China Twelfth Five-year Plan, Nos. 2013ZX10002004 and 2012ZX10002003.

**Institutional review board statement:** The study was approved by the Institutional Review Board of Peking University Health Science Center.

**Informed consent statement:** All recruited patients signed written informed consents.

**Conflict-of-interest statement:** The authors declare that they have no competing interests.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Xue-En Liu, MD, Associate Professor, Department of Microbiology and Infectious Disease Center, School of Basic Medical Sciences, Peking University Health Science Center, 38 Xueyuan Road, Haidian District, Beijing 100191, China. [xueenliu@bjmu.edu.cn](mailto:xueenliu@bjmu.edu.cn)  
**Telephone:** +86-10-82802413  
**Fax:** +86-10-82802413

**Received:** August 12, 2016

**Peer-review started:** August 13, 2016

**First decision:** September 13, 2016

**Revised:** September 26, 2016

**Accepted:** October 22, 2016

**Article in press:** October 24, 2016

**Published online:** December 8, 2016

### Abstract

#### AIM

To investigate potential predictors for treatment response to nucleos(t)ide analogues (NAs) in hepatitis B e antigen (HBeAg)-positive chronic hepatitis B (CHB) patients.

#### METHODS

Seventy-six HBeAg-positive CHB patients received 96-wk



NAs optimized therapy (lamivudine and adefovir dipivoxil) were studied retrospectively. Serum hepatitis B surface antigen, HBeAg, hepatitis B core antibody, hepatitis B virus (HBV) DNA and alanine aminotransferase levels were quantitatively measured before and during the treatment at 12 and 24 wk. Stepwise logistic regression analyses were performed to identify predictors for treatment response, and areas under the receiver operating characteristic curves (AUROC) of the independent predictors were calculated.

## RESULTS

Forty-three CHB patients (56.6%) achieved virological response (VR: HBV DNA  $\leq$  300 copies/mL) and 15 patients (19.7%) developed HBeAg seroconversion (SC) after the 96-wk NAs treatment. The HBeAg level (OR = 0.45,  $P$  = 0.003) as well as its declined value (OR = 2.03,  $P$  = 0.024) at 24-wk independently predicted VR, with the AUROC of 0.788 and 0.736, respectively. The combination of HBeAg titer < 1.3 lg PEIU/mL and its decreased value > 1.6 lg PEIU/mL at 24-wk predicted VR with a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of 85%, 100%, 100% and 83%, respectively, and the AUROC increased to 0.923. The HBeAg level (OR = 0.37,  $P$  = 0.013) as well as its declined value (OR = 2.02,  $P$  = 0.012) at 24-wk also independently predicted HBeAg SC, with the AUROC of 0.828 and 0.814, respectively. The HBeAg titer < -0.5 lg PEIU/mL combined with its declined value > 2.2 lg PEIU/mL at 24-wk predicted HBeAg SC with a sensitivity, specificity, PPV, NPV of 88%, 98%, 88% and 98%, respectively, and the AUROC reached 0.928.

## CONCLUSION

The combination of HBeAg level and its declined value at 24-wk may be used as a reference parameter to optimize NAs therapy.

**Key words:** Response predictor; Quantitative detection; Hepatitis B e antigen; Hepatitis B virus DNA; Chronic hepatitis B; Nucleos(t)ide analogues

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Few studies have systematically evaluated quantitative hepatitis B surface antigen, hepatitis B e antigen (HBeAg), hepatitis B core antibody, hepatitis B virus DNA and alanine aminotransferase for predicting treatment response to nucleos(t)ide analogues (NAs) in HBeAg-positive chronic hepatitis B (CHB). In this study, on-treatment HBeAg level as well as its declined value at 24-wk were identified to be the best predictors not only for 96-wk virological response (VR) but also for HBeAg seroconversion (SC). The combination of HBeAg level and its decline at 24-wk strongly predicted 96-wk VR and HBeAg SC with the AUROC of 0.923 and 0.928, respectively. Thus monitoring an early on-treatment HBeAg level and its decline may help to optimize NAs therapy for CHB patients.

Gao YH, Meng QH, Zhang ZQ, Zhao P, Shang QH, Yuan Q, Li Y, Deng J, Li T, Liu XE, Zhuang H. On-treatment quantitative hepatitis B e antigen predicted response to nucleos(t)ide analogues in chronic hepatitis B. *World J Hepatol* 2016; 8(34): 1511-1520 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i34/1511.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i34.1511>

## INTRODUCTION

Hepatitis B virus (HBV) infection is a global public health problem and an estimated 240 million persons are chronically infected worldwide, among which 20%-30% will develop cirrhosis and hepatocellular carcinoma (HCC)-the major complications of chronic hepatitis B (CHB)<sup>[1]</sup>. Antiviral treatment has been proved to be an effective and potent way to reverse the process of liver fibrosis or cirrhosis and decrease the incidence rate of liver complications<sup>[2]</sup>. However, due to the persistence of HBV covalently closed circular DNA (cccDNA) in the nucleus of infected hepatocytes, HBV cannot be completely eradicated by current antiviral drugs, and a long-term treatment are necessary for most patients. It is now clear that sustained viral suppression and hepatitis B e antigen (HBeAg) seroconversion (SC) are two important markers of treatment response for CHB patients receiving antiviral therapy, which is usually associated with a good long-term outcome<sup>[3]</sup>. Generally, after a 1-year course of the current available nucleos(t)ide analogues (NAs) or peginterferon (Peg-IFN) therapy, 7%-76% of patients achieved undetectable serum HBV DNA and 16%-32% developed HBeAg SC for patients with HBeAg-positive CHB<sup>[3]</sup>. Therefore, it is crucial to identify pre-treatment and early on-treatment biomarkers that can effectively predict long-term treatment response and use these biomarkers to choose appropriate antiviral drugs and treatment regimens to optimize therapy and improve efficacy.

Serum HBV DNA is the most widely used virological marker in the management of CHB patients<sup>[2,3]</sup>. A study from Zeuzem *et al*<sup>[4]</sup> reported that both baseline ALT  $\geq$  2  $\times$  upper limit of normal (ULN) (OR = 2.47,  $P$  = 0.0012) and non-detectable serum HBV DNA at treatment week 24 (OR = 2.61,  $P$  < 0.001) were associated with HBeAg SC after 2-year telbivudine (LdT) treatment, and among patients with non-detectable serum HBV DNA at 24-wk as well as favorable pretreatment characteristics [alanine aminotransferase (ALT)  $\geq$  2  $\times$  ULN and HBV DNA < 9 lg copies/mL], 52% obtained HBeAg SC at 2-year of therapy<sup>[4]</sup>. However, the detection of serum HBV DNA is costly and may not always objectively serve as a reliable indicator of sustained response to antiviral therapy<sup>[5]</sup>. Unlike HBV DNA, serum hepatitis B surface antigen (HBsAg), HBeAg and hepatitis B core antibody (anti-HBc) are classical serological markers for HBV infection and are used in clinical diagnosis routinely. The level of HBsAg was identified as an outcome predictor for Peg-IFN therapy among HBeAg-positive CHB patients<sup>[6]</sup>; however,

its predictive value in NAs treatment was inconsistent based on the reported data<sup>[7,8]</sup>. Serum HBeAg level was proposed to be a better outcome predictor for NAs treatment according to recent studies<sup>[8-12]</sup>. However, most of studies applied the semi-quantitative measurement of HBeAg, and some had a limited sample size or a short period of follow-up. Thus the predictive value of the quantitative HBeAg level needs to be further evaluated. In addition, benefiting from a newly developed double-sandwich anti-HBc immunoassay, anti-HBc quantification was identified as a novel biomarker for predicting treatment response<sup>[13]</sup>. Nevertheless, very few studies have systematically evaluated the predictive power of these biomarkers for NAs treatment response.

In the current study, HBeAg-positive CHB patients received 96-wk NAs optimized therapy [lamivudine (LAM) and adefovir dipivoxil (ADV)] were retrospectively investigated. Serum HBsAg, HBeAg, anti-HBc, HBV DNA and ALT were quantitatively tested, and the baseline as well as early on-treatment levels of these parameters were analyzed using logistic regression model to assess their functions in predicting 96-wk virological response (VR) and HBeAg SC.

## MATERIALS AND METHODS

### Patients

We retrospectively analyzed a cohort of HBeAg-positive CHB patients who underwent the 96-wk LAM and ADV optimized therapy between 2011 and 2014 in China. The treatment was continued for CHB patients after week 96, and the data were not available from the patients after 96 wk. The inclusion criteria of patients enrolled for antiviral therapy were briefly summarized as follows: 18-65 years old, HBsAg positive for at least 6 mo, HBeAg positive and hepatitis B e antibody (anti-HBe) negative,  $10^5$  copies/mL  $\leq$  HBV DNA  $\leq$   $10^9$  copies/mL, ALT  $\geq$  2  $\times$  ULN, and no history of antiviral therapy with NAs or interferon within previous six months. The patients were treated with LAM 100 mg/d, and ADV (10 mg/d) was added on when serum HBV DNA > 300 copies/mL at week 24 or a virological breakthrough (> 1 lg increase of serum HBV DNA from nadir or re-detectable after achieving an undetected level) occurred during the 96-wk treatment. Laboratory measurements were done every 12 wk before week 24, and every 24 wk from week 24 to week 96. The main endpoints were VR (defined as HBV DNA  $\leq$  300 copies/mL) and HBeAg SC at 96-wk. A total of 76 patients completed the 96-wk follow-up and finally included in the analyses. The study was approved by the Institutional Review Board of Peking University Health Science Center and conducted in accordance with the ethical standards of the Helsinki Declaration. The informed consents were obtained from recruited patients.

### Laboratory measurements

HBV DNA was quantified by Roche COBAS TaqMan HBV test (Roche Diagnostics, Mannheim, Germany)

with a linear range of 20-10<sup>8</sup> IU/mL (1 IU/mL = 5.82 copies/mL). Serological HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe) were measured by Chemiluminescent Microparticle ImmunoAssay using ARCHITECT i2000SR analyzer (Abbott Diagnostics, North Chicago, IL, United States). HBeAg level was quantified by World Health Organization (WHO) HBeAg reference standard (Paul-Ehrlich-Institute, Germany) also using ARCHITECT i2000SR analyzer<sup>[14]</sup>. Anti-HBc quantification was conducted by using a newly developed double-sandwich immunoassay (Wantai, Beijing, China) validated by WHO anti-HBc standards<sup>[15]</sup>. Biochemical tests (ALT, AST) were detected by the department of laboratory in four hospitals.

### Statistical analysis

Categorical variables were compared using  $\chi^2$  or Fisher's exact tests. Continuous variables were compared using the Student's *t* test or Mann-Whitney test. Stepwise logistic regression analysis was performed to identify independent predictors for VR and HBeAg SC. The predictive value of the independent predictor was further evaluated using areas under the receiver operating characteristic curve (AUROC). The best cut-off value was determined in the condition of the highest Youden index (the sum of sensitivity and specificity minus 1). And the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated at the specified cut-off value. All analysis was done using SPSS 19.0 (SPSS, Chicago, IL, United States). A *P* value < 0.05 was considered as statistically significant.

## RESULTS

### Baseline characteristics

After the 96-wk NAs treatment, 56.6% (43/76) CHB patients achieved VR and 19.7% (15/76) patients developed HBeAg SC. Only 1 patient eliminated HBsAg. The baseline parameters such as age, HBsAg, HBeAg, anti-HBc, HBV DNA and ALT were comparable between patients achieved HBeAg SC and those did not. Compared to patients without VR, those obtained VR had significantly higher baseline ALT level ( $247.95 \pm 150.58$  U/L vs  $169.13 \pm 156.56$  U/L, *P* = 0.029), while HBsAg, HBeAg, anti-HBc, HBV DNA levels were not significantly different between two groups (Table 1).

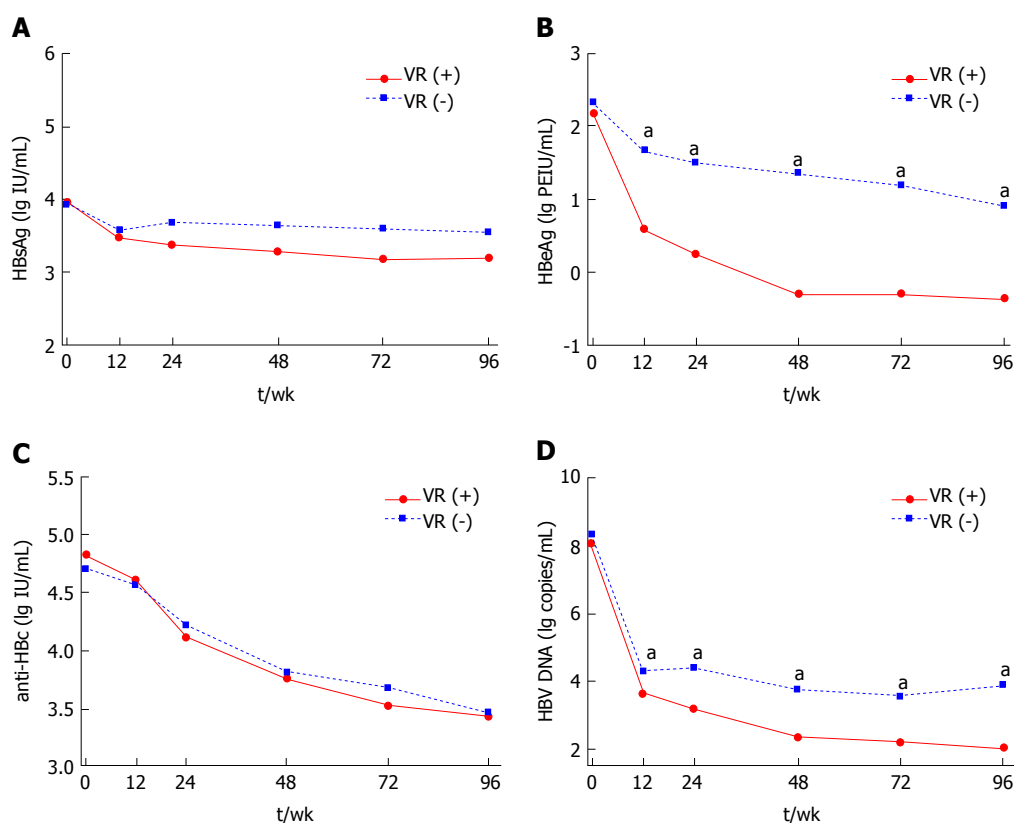
### Dynamic changes in serum levels of HBsAg, HBeAg, anti-HBc and HBV DNA during 96-wk treatment

Serum HBsAg, HBeAg, anti-HBc and HBV DNA levels were all significantly decreased from baseline to week 96 of therapy (HBsAg,  $3.95 \pm 0.83$  lg IU/mL to  $3.36 \pm 0.86$  lg IU/mL; HBeAg,  $2.24 \pm 1.31$  lg PEIU/mL to  $0.20 \pm 1.13$  lg PEIU/mL; anti-HBc,  $4.77 \pm 0.46$  lg IU/mL to  $3.45 \pm 0.65$  lg IU/mL; HBV DNA,  $8.16 \pm 1.34$  lg copies/mL to  $2.85 \pm 1.36$  lg copies/mL; all *P* < 0.001). Significant lower HBeAg and HBV DNA levels were found in patients with VR as compared with patients without VR at every

**Table 1** Baseline clinical characteristics of patients with chronic hepatitis B according to treatment response after 96-wk nucleos(t)ide analogues therapy

Parameters	Overall	VR (+)	VR (-)	P value	SC (+)	SC (-)	P value
n (%)	76	43 (56.6)	33 (43.4)	-	15 (19.7)	61 (80.3)	-
Gender, female/male	20/56	15/28	5/28	0.053	5/10	15/46	0.491
Age, yr	32.63 ± 9.69	32.3 ± 9.83	33.06 ± 9.63	0.738	31.87 ± 11.6	32.82 ± 9.26	0.735
HBsAg, Ig IU/mL	3.95 ± 0.83	3.96 ± 0.68	3.94 ± 1.00	0.881	3.93 ± 0.60	3.96 ± 0.88	0.385
HBeAg, Ig PEIU/mL	2.24 ± 1.31	2.18 ± 1.38	2.32 ± 1.22	0.679	2.36 ± 1.41	2.21 ± 1.29	0.527
anti-HBc, Ig IU/mL	4.77 ± 0.46	4.82 ± 0.43	4.71 ± 0.50	0.311	4.85 ± 0.44	4.75 ± 0.47	0.464
ALT, U/L	213.73 ± 157.17	247.95 ± 150.58	169.13 ± 156.56	0.029	216.49 ± 153.18	213.05 ± 159.38	0.94
ALT strata, ≥/ < 5ULN	31/45	25/18	6/27	< 0.001	9/6	22/39	0.091
HBV DNA, Ig copies/mL	8.16 ± 1.34	8.06 ± 1.45	8.3 ± 1.19	0.608	8.55 ± 0.91	8.07 ± 1.41	0.324
Genotype, C/non-C	53/23	31/12	22/11	0.61	10/5	43/18	0.773

VR (+)/VR (-), with/without virological response at week 96 (virological response: HBV DNA ≤ 300 copies/mL); SC (+)/SC (-), with/without HBeAg seroconversion at week 96. HBsAg: Hepatitis B surface antigen; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; VR: Virological response; SC: HBeAg seroconversion; ULN: Upper limit of normal.



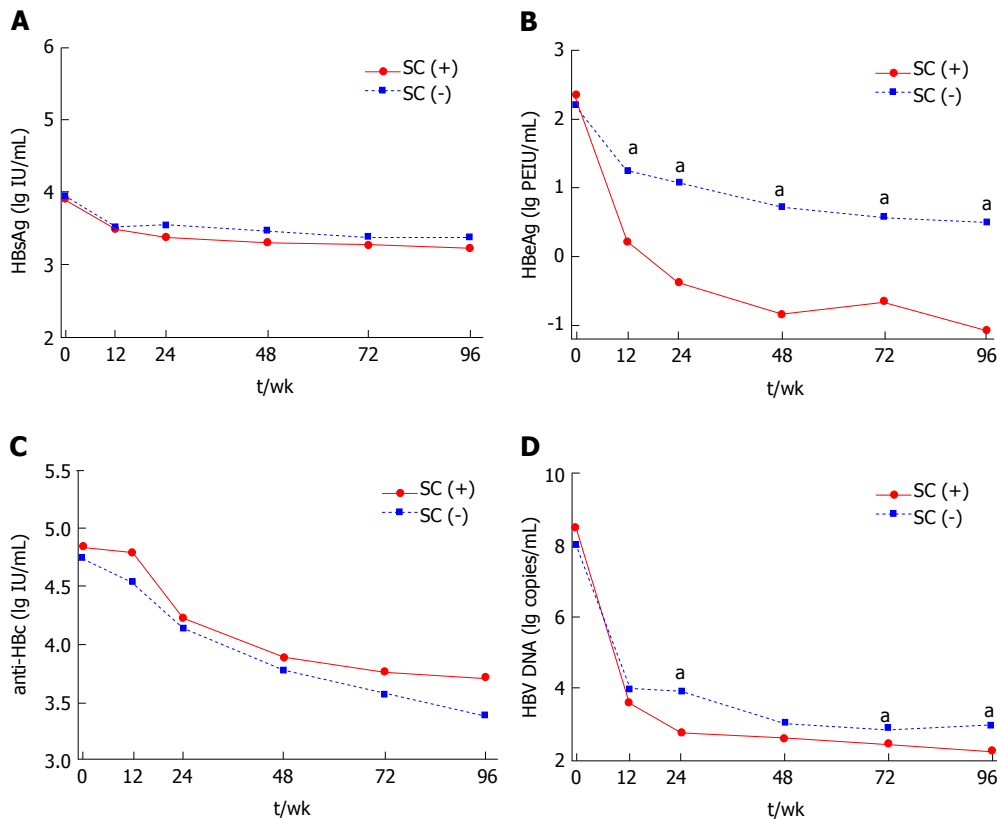
**Figure 1** Dynamic changes of hepatitis B surface antigen (A), hepatitis B e antigen (B), hepatitis B core antibody (C) and hepatitis B virus DNA (D) levels from baseline to 96-wk in chronic hepatitis B patients received nucleos(t)ide analogues therapy stratified by virological response at 96-wk. <sup>a</sup>*P* < 0.05; VR (+): Virological response, HBV DNA ≤ 300 copies/mL; VR (-): Without virological response; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; anti-HBc: hepatitis B core antibody; HBV: Hepatitis B virus; VR: Virological response.

follow-up time-point except baseline (*P* < 0.05), while HBsAg and anti-HBc levels were comparable between two groups (Figure 1). Similarly, HBsAg and anti-HBc levels between patients with and without HBeAg SC were also comparable from baseline to week 96 (Figure 2A and C). However, HBeAg levels were significant lower in patients with HBeAg SC than in patients without HBeAg SC at every follow-up time-point except baseline (*P* < 0.05) (Figure 2B). And a significant difference in HBV DNA levels between patients with and without HBeAg SC

was only observed at week 24, 72 and 96 (*P* < 0.05), as shown in Figure 2D.

#### Baseline and on-treatment parameters associated with 96-wk virological response

At baseline, ALT and ALT ≥ 5 × ULN were associated with VR according to univariate analysis, and multivariate analysis indicated that sex (OR = 3.76, 95%CI: 1.09-13.01, *P* = 0.037) and ALT ≥ 5 × ULN (OR = 7.09, 95%CI: 2.32-21.67, *P* < 0.001) independently predicted VR, re-



**Figure 2** Dynamic changes of hepatitis B surface antigen (A), hepatitis B e antigen (B), hepatitis B core antibody (C) and hepatitis B virus DNA (D) levels from baseline to 96-wk in chronic hepatitis B patients received nucleos(t)ide analogues therapy stratified by hepatitis B e antigen seroconversion at 96-wk. <sup>a</sup> $P < 0.05$ ; SC (+): HBeAg seroconversion; SC (-): Without HBeAg seroconversion; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; anti-HBc: hepatitis B core antibody; HBV: Hepatitis B virus; SC: HBeAg seroconversion.

spectively. At week 12, univariate analysis revealed that HBeAg, HBeAg decline, ALT decline, and HBV DNA were associated with VR, and multivariate analysis identified that HBeAg (OR = 0.62, 95%CI: 0.40-0.95,  $P = 0.03$ ) and HBeAg decline (OR = 2.58, 95%CI: 1.25-5.33,  $P = 0.01$ ) independently predicted VR, respectively. At week 24, HBeAg, HBeAg decline, ALT decline, HBV DNA and HBV DNA decline were associated with VR *via* univariate analysis, and multivariate analysis found that HBeAg (OR = 0.45, 95%CI: 0.27-0.77,  $P = 0.003$ ) and HBeAg decline (OR = 2.03, 95%CI: 1.10-3.74,  $P = 0.024$ ) independently predicted VR, respectively (Table 2).

#### Baseline and on-treatment parameters associated with 96-wk HBeAg seroconversion

At week 12, HBeAg, HBeAg decline, and HBV DNA decline were associated with HBeAg SC through univariate analysis, and HBeAg decline (OR = 2.47, 95%CI: 1.46-4.16,  $P = 0.001$ ) independently predicted HBeAg SC *via* multivariate analysis. At week 24, univariate analysis presented that HBeAg, HBeAg decline, HBV DNA and HBV DNA decline were associated with HBeAg SC, and multivariate analysis identified that HBeAg (OR = 0.37, 95%CI: 0.17-0.81,  $P = 0.013$ ) and HBeAg decline (OR = 2.02, 95%CI: 1.17-3.49,  $P = 0.012$ ) independently predicted HBeAg SC, respectively (Table 2).

Based on the results of above analysis, both of the

HBeAg level and its on-treatment declined value at 12-wk (or 24-wk) as independent predictors were further evaluated using AUROC for predicting 96-wk VR and HBeAg SC.

#### Predictive value of HBeAg titer as well as its declined value at week 12 and 24 for 96-wk virological response

At week 12, the HBeAg titer and its declined value predicted VR with an AUROC of 0.733 (95%CI: 0.617-0.849,  $P = 0.001$ ) and 0.709 (95%CI: 0.590-0.827,  $P = 0.002$ ), respectively, and the best cut-off value for the HBeAg titer and its decline was 0.8 lg PEIU/mL and 0.84 lg PEIU/mL, respectively. Twenty-two patients achieved HBeAg titer < 0.8 lg PEIU/mL as well as the declined value > 0.84 lg PEIU/mL at 12-wk, and among them 91% (20/22) reached VR, whereas only 29% obtained VR among 28 patients without meeting the above two standards. HBeAg titer combined with on-treatment decline at 12-wk predicted VR with an AUROC of 0.812 (95%CI: 0.687-0.936,  $P < 0.001$ ) and the sensitivity, specificity, PPV, NPV was 71%, 91%, 91% and 71%, respectively.

At week 24, the HBeAg titer and its declined value predicted VR with an AUROC of 0.788 (95%CI: 0.683-0.892,  $P < 0.001$ ) and 0.736 (95%CI: 0.620-0.851,  $P < 0.001$ ), respectively, and the best cut-off value for the HBeAg titer and its decline was 1.3 lg PEIU/mL and 1.6 lg PEIU/mL, respectively. All the 22 patients with HBeAg titer < 1.3



**Table 2** Baseline and on-treatment parameters associated with 96-wk virological response and hepatitis B e antigen seroconversion in chronic hepatitis B patients received nucleos(t)ide analogues therapy

Factors	VR				SC			
	Univariate		Multivariate		Univariate		Multivariate	
	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value
Baseline								
Age	0.99 (0.95-1.04)	0.734	-	-	0.99 (0.93-1.05)	0.731	-	-
Sex, female/male	3 (0.96-9.38)	0.059	3.76 (1.09-13.01)	0.037	1.53 (0.45-5.20)	0.493	-	-
HBeAg (lg IU/mL)	1.04 (0.60-1.81)	0.879	-	-	0.96 (0.49-1.88)	0.904	-	-
HBeAg (lg PEIU/mL)	0.92 (0.65-1.31)	0.633	-	-	1.1 (0.70-1.72)	0.682	-	-
Anti-HBc (lg IU/mL)	1.68 (0.62-4.59)	0.308	-	-	1.6 (0.46-5.50)	0.459	-	-
HBV DNA (lg copies/mL)	0.87 (0.62-1.24)	0.451	-	-	1.38 (0.82-2.31)	0.221	-	-
ALT (U/L)	1.004 (1.000-1.007)	0.038	-	-	1 (0.99-1.01)	0.939	-	-
ALT strata, $\geq$ / $<$ 5ULN	6.25 (2.14-18.26)	< 0.001	7.09 (2.32-21.67)	< 0.001	2.66 (0.84-8.46)	0.098	-	-
Genotype, C/non-C	1.29 (0.48-3.46)	0.61	-	-	0.84 (0.25-2.80)	0.773	-	-
Week 12								
HBeAg (lg IU/mL)	0.83 (0.45-1.53)	0.551	-	-	0.95 (0.47-1.92)	0.882	-	-
HBeAg decline (lg IU/mL)	1.43 (0.72-2.84)	0.301	-	-	1.04 (0.46-2.35)	0.919	-	-
HBeAg (lg PEIU/mL)	0.5 (0.33-0.76)	0.001	0.62 (0.40-0.95)	0.03	0.5 (0.30-0.85)	0.011	-	-
HBeAg decline (lg PEIU/mL)	3.04 (1.55-5.98)	0.001	2.58 (1.25-5.33)	0.01	2.47 (1.46-4.16)	< 0.001	2.47 (1.46-4.16)	0.001
Anti-HBc (lg IU/mL)	1.14 (0.49-2.64)	0.764	-	-	2.56 (0.83-7.93)	0.102	-	-
Anti-HBc decline (lg IU/mL)	1.43 (0.45-4.59)	0.546	-	-	0.3 (0.06-1.51)	0.145	-	-
ALT (U/L)	0.99 (0.98-1.01)	0.221	-	-	1 (0.98-1.02)	0.876	-	-
ALT decline (U/L)	1.004 (1.00-1.01)	0.03	-	-	1 (0.99-1.01)	0.918	-	-
HBV DNA (lg copies/mL)	0.57 (0.36-0.90)	0.016	-	-	0.72 (0.42-1.25)	0.249	-	-
HBV DNA decline (lg copies/mL)	1.36 (0.90-2.03)	0.141	-	-	2.11 (1.16-3.84)	0.015	-	-
Week 24								
HBeAg (lg IU/mL)	0.61 (0.31-1.21)	0.155	-	-	0.84 (0.48-1.46)	0.535	-	-
HBeAg decline (lg IU/mL)	1.79 (0.94-3.42)	0.076	-	-	1.21 (0.62-2.37)	0.571	-	-
HBeAg (lg PEIU/mL)	0.39 (0.24-0.64)	< 0.001	0.45 (0.27-0.77)	0.003	0.28 (0.14-0.58)	< 0.001	0.37 (0.17-0.81)	0.013
HBeAg decline (lg PEIU/mL)	2.37 (1.41-3.99)	0.001	2.03 (1.10-3.74)	0.024	2.8 (1.64-4.78)	< 0.001	2.02 (1.17-3.49)	0.012
Anti-HBc (lg IU/mL)	0.73 (0.33-1.63)	0.448	-	-	1.29 (0.48-3.45)	0.608	-	-
Anti-HBc decline (lg IU/mL)	3.11 (0.99-3.79)	0.053	-	-	1.11 (0.31-3.99)	0.874	-	-
ALT (U/L)	0.99 (0.98-1.01)	0.477	-	-	0.99 (0.96-1.02)	0.516	-	-
ALT decline (U/L)	1.004 (1.00-1.01)	0.031	-	-	1 (0.99-1.01)	0.822	-	-
HBV DNA (lg copies/mL)	0.55 (0.37-0.80)	0.002	-	-	0.45 (0.23-0.86)	0.016	-	-
HBV DNA decline (lg copies/mL)	1.39 (1.02-1.88)	0.035	-	-	2.39 (1.39-4.11)	0.002	-	-

HBeAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; Anti-HBc: Hepatitis B core antibody; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; VR: Virological response (HBV DNA  $\leq$  300 copies/mL); SC: HBeAg seroconversion; ULN: Upper limit of normal.

lg PEIU/mL and the declined value  $>$  1.6 lg PEIU/mL at 24-wk achieved VR, whereas only 17% of 23 patients without meeting the above two standards obtained VR. HBeAg titer combined with its decline at 24-wk strongly predicted VR with an AUROC of 0.923 (95%CI: 0.838-1.000,  $P < 0.001$ ) and the sensitivity, specificity, PPV, NPV was 85%, 100%, 100% and 83%, respectively (Table 3).

#### **Predictive value of HBeAg titer as well as its declined value at week 12 and 24 for 96-wk HBeAg seroconversion**

At week 12, the HBeAg declined value predicted HBeAg SC with an AUROC of 0.767 (95%CI: 0.623-0.911,  $P = 0.001$ ), and the best cut-off value for HBeAg decline was 1.8 lg PEIU/mL. The HBeAg declined value  $>$  1.8 lg PEIU/mL at 12-wk predicted HBeAg SC with a sensitivity, specificity, PPV, NPV of 60%, 87%, 53% and 90%, respectively.

At week 24, the HBeAg titer and its declined value predicted HBeAg SC with an AUROC of 0.828 (95%CI: 0.712-0.944,  $P < 0.001$ ) and 0.814 (95%CI: 0.676-0.953,

$P < 0.001$ ), respectively, and the best cut-off value for the HBeAg titer and its decline was -0.5 lg PEIU/mL and 2.2 lg PEIU/mL, respectively. Eight patients achieved HBeAg titer  $<$  -0.5 lg PEIU/mL and the declined value  $>$  2.2 lg PEIU/mL at 24-wk, among them 88% (7/8) achieved HBeAg SC; whereas only 2% of 51 patients who did not meet the above two standards obtained HBeAg SC. HBeAg titer combined with its declined value at 24-wk strongly predicted HBeAg SC with an AUROC of 0.928 (95%CI: 0.791-1.000,  $P < 0.001$ ) and the sensitivity, specificity, PPV, NPV was 88%, 98%, 88% and 98% (Table 4).

## **DISCUSSION**

Achieving a long-term suppression of serum HBV DNA through antiviral therapy is one of important targets of treatment for CHB patients. Several studies tried to investigate the value of an early on-treatment change of HBV markers such as HBeAg in predicting VR to NAs therapy. A study presented that the HBeAg titer decreased by 1 lg PEIU/mL at 12-wk predicted VR

**Table 3** Predictive value of hepatitis B e antigen titer as well as its declined value at week 12 and 24 for virological response after 96-wk nucleos(t)ide analogues therapy

Factors	ROC		Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
	AUROC (95%CI)	P value				
Week 12						
HBeAg < 0.8 lg PEIU/mL	0.733 (0.617-0.849)	0.001	0.63 (0.48-0.78)	0.81 (0.67-0.96)	0.82 (0.68-0.96)	0.62 (0.47-0.77)
HBeAg decline > 0.84 lg PEIU/mL	0.709 (0.590-0.827)	0.002	0.65 (0.50-0.80)	0.75 (0.59-0.91)	0.78 (0.64-0.92)	0.62 (0.46-0.78)
Combined the above	0.812 (0.687-0.936)	< 0.001	0.71 (0.54-0.89)	0.91 (0.78-1.00)	0.91 (0.78-1.00)	0.71 (0.54-0.89)
Week 24						
HBeAg < 1.3 lg PEIU/mL	0.788 (0.683-0.892)	< 0.001	0.88 (0.78-0.98)	0.64 (0.46-0.81)	0.76 (0.63-0.88)	0.81 (0.65-0.97)
HBeAg decline > 1.6 lg PEIU/mL	0.736 (0.620-0.851)	< 0.001	0.55 (0.39-0.70)	0.94 (0.85-1.00)	0.92 (0.81-1.00)	0.62 (0.48-0.76)
Combined the above	0.923 (0.838-1.000)	< 0.001	0.85 (0.70-0.99)	1	1	0.83 (0.66-0.99)

HBeAg: Hepatitis B e antigen; VR: Virological response (HBV DNA  $\leq$  300 copies/mL); AUROC: Area under the receiver operating characteristic curve; PPV: Positive predictive value; NPV: Negative predictive value.

**Table 4** Predictive value of hepatitis B e antigen titer as well as its declined value at week 12 and 24 for hepatitis B e antigen seroconversion after 96-wk nucleos(t)ide analogues therapy

Factors	ROC		Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
	AUROC (95%CI)	P value				
Week 12						
HBeAg decline > 1.8 lg PEIU/mL	0.767 (0.623-0.911)	0.001	0.6 (0.32-0.88)	0.87 (0.78-0.96)	0.53 (0.26-0.79)	0.9 (0.82-0.98)
Week 24						
HBeAg < -0.5 lg PEIU/mL	0.828 (0.712-0.944)	< 0.001	0.67 (0.40-0.94)	0.92 (0.84-0.99)	0.67 (0.40-0.94)	0.92 (0.84-0.99)
HBeAg decline > 2.2 lg PEIU/mL	0.814 (0.676-0.953)	< 0.001	0.73 (0.48-0.99)	0.9 (0.82-0.98)	0.65 (0.39-0.90)	0.93 (0.86-1.00)
Combined the above	0.928 (0.791-1.000)	< 0.001	0.88 (0.58-1.00)	0.98 (0.94-1.00)	0.88 (0.58-1.00)	0.98 (0.94-1.00)

HBeAg: Hepatitis B e antigen; SC: HBeAg seroconversion; AUROC: Area under the receiver operating characteristic curve; PPV: Positive predictive value; NPV: Negative predictive value.

(HBV DNA < 20 IU/mL) after 48-wk entecavir (ETV) therapy with a sensitivity, specificity, PPV, NPV of 67.6%, 87.9%, 86.2% and 70.7%, respectively<sup>[10]</sup>. However, the predictive value of HBeAg titer and its declined value at 12-wk for a long-term treatment response ( $\geq$  2 years) was not reported. In this study, we found that HBeAg titer < 0.8 lg PEIU/mL combined with its declined value > 0.84 lg PEIU/mL at 12-wk predicted VR after 96-wk LAM and ADV optimized therapy with a sensitivity, specificity, PPV, NPV of 71%, 91%, 91% and 71%, respectively, and accompanied with an AUROC of 0.812. In addition, although some studies indicated that the on-treatment HBeAg level or declined value at 24-wk was a predictor for NAs treatment response<sup>[9,12]</sup>, the sample size was small or HBeAg was detected using a semi-quantitative method which cannot accurately quantify HBeAg level, thus limited the validity of the prediction. For example, Zhang *et al.*<sup>[12]</sup> detected serum HBeAg semi-quantitatively and found that the on-treatment declined value of HBeAg (> 65%) at 24-wk was the best predictor for treatment response (HBeAg seroconversion and accompanied by undetectable serum HBV DNA) after 96-wk ETV therapy, and the PPV, NPV, AUROC was 83.3%, 93.6% and 0.885, respectively. However, the finding may have difficulty in applying clinical practice since the absent of accurate quantitative HBeAg levels. In our study, the HBeAg titer (OR = 0.45,  $P$  = 0.003) and its declined value (OR = 2.03,  $P$  = 0.024) at 24-wk were found to predict 96-wk VR with the AUROC of 0.788 and 0.736, respectively.

Moreover, the combination of HBeAg titer < 1.3 lg PEIU/mL and its decrease > 1.6 lg PEIU/mL at 24-wk predicted 96-wk VR with a sensitivity, specificity, PPV, NPV of 85%, 100%, 100% and 83%, respectively, and the AUROC increased to 0.923, which had a better predictive value than the semi-quantitative method reported by Zhang *et al.*<sup>[12]</sup>. With respect to the cost of semi-quantitative and quantitative tests of HBeAg, it is comparable between them. Twenty-two patients in our cohort matched the combination standard and they all achieved 96-wk VR. The results suggested that if patients reached HBeAg titer < 1.3 lg PEIU/mL and declined > 1.6 lg PEIU/mL at 24-wk, there will be a better viral suppression during the continued NAs therapy.

In addition to achieve long-term suppression in serum HBV DNA, HBeAg SC is another important indicator to evaluate the efficacy of antiviral therapy in HBeAg-positive CHB patients. In our study, the declined value of HBeAg at 12-wk (OR = 2.47,  $P$  = 0.001) independently predicted 96-wk HBeAg SC with an AUROC of 0.767, and for the predictive value of HBeAg declined value at 24-wk, an AUROC increased to 0.814. Lee *et al.*<sup>[8]</sup> found that the decline of HBeAg at month 6 was a strongest predictor for HBeAg SC after 2 years of ETV treatment with an AUROC of 0.820 ( $P$  = 0.004), which was similar to our result (AUROC = 0.814). However, Lee's study used the declined value of HBeAg alone to predict treatment response and did not consider combining other indicators. Our data showed that both HBeAg titer as well

as its declined value at 24-wk were independent predictors for 96-wk HBeAg SC, and it strongly predicted HBeAg SC with an AUROC of 0.928 if combining HBeAg titer  $< -0.5$  lg PEIU/mL and declined value  $> 2.2$  lg PEIU/mL at 24-wk. A study from Shin *et al.*<sup>[11]</sup> revealed that HBeAg titer  $< 0.62$  lg PEIU/mL after 48 wk of ETV therapy was a strongest predictor for HBeAg SC at year 3 with an AUROC of 0.86 ( $P < 0.001$ ), which was inferior to the combined prediction validity of HBeAg level and declined value at 24-wk in our study (AUROC = 0.928). Our study presented that 88% (7/8) of patients with HBeAg titer  $< -0.5$  lg PEIU/mL and declined value  $> 2.2$  lg PEIU/mL at 24-wk achieved 96-wk HBeAg SC, whereas only 2% (1/51) of patients without meeting above standards obtained HBeAg SC, thus got a NPV of 98%. Among the 51 patients with unfavorable 24-wk HBeAg titer, 46 patients were added on ADV at 24-wk due to serum HBV DNA  $> 300$  copies/mL. The results suggested that patients with HBeAg titer  $> -0.5$  lg PEIU/mL as well as declined value  $< 2.2$  lg PEIU/mL after 24 wk of LAM therapy will rarely achieve HBeAg SC during the following NAs treatment, even adding on ADV still cannot improve the efficacy. So the patients with unfavorable 24-wk HBeAg titer should be considered to switch to other drugs or use other regimens for a better treatment outcome.

Results of logistic regression analysis in the current study showed that no baseline parameters were associated with 96-wk HBeAg SC, which was consistent with Lee's report<sup>[8]</sup>, who did not find correlations between baseline level of HBeAg (or HBsAg, HBV DNA) and HBeAg SC after 2 years of ETV therapy either. However, other studies showed that pre-treatment serum HBeAg was associated with HBeAg SC during ETV treatment<sup>[10,12]</sup>. The inconsistent results may due to that treatment period was relatively short or HBeAg was detected semi-quantitatively in the latter's studies<sup>[10,12]</sup>. Several recent studies revealed that B lymphocytes played a key role in the regulation of host immune responses to HBV, while anti-HBc was produced and secreted by hepatitis B core antigen-specific B lymphocytes, therefore the serum anti-HBc level may be a surrogate marker for the host immune response to HBV<sup>[16,17]</sup>. Fan *et al.*<sup>[13]</sup> demonstrated that the baseline level of anti-HBc independently predicted HBeAg SC after 2 years of LdT and ADV optimized therapy (OR = 1.99,  $P = 0.001$ ). However, our results presented that baseline anti-HBc was not associated with HBeAg SC or VR after 96-wk LAM and ADV optimized therapy (HBeAg SC, OR = 1.60,  $P = 0.459$ ; VR, OR = 1.68,  $P = 0.308$ ). The discrepancy may due to the little difference of baseline anti-HBc level between patients with and without HBeAg SC (0.10 lg IU/mL) in our study, while the difference in baseline anti-HBc was 0.24 lg IU/mL in Fan's study. Besides, previous studies identified that the elevation of ALT was contributed to T lymphocyte mediated hepatolysis occurred in CHB patients, therefore baseline ALT level may reflect T lymphocyte immune response to HBV which is related to the outcome after antiviral treatment<sup>[18]</sup>. Zeuzem *et al.*<sup>[4]</sup> reported that the

pre-treatment ALT level was associated with VR and baseline ALT  $\geq 2 \times$  ULN could independently predict non-detectable serum HBV DNA after 2 years of LdT treatment (OR = 2.00,  $P = 0.0071$ ). In agreement with these findings, we also found that baseline ALT  $\geq 5 \times$  ULN independently predicted 96-wk VR (OR = 7.09,  $P < 0.001$ ) with the AUROC of 0.700 ( $P = 0.003$ , data not shown), which suggested that patients with a higher pre-treatment ALT level maybe situated in a better immune status and will have an active virological response to NAs treatment.

All the parameters (HBsAg, HBeAg, anti-HBc and HBV DNA) presented continuous descent during the 96-wk treatment. Serum HBV DNA dropped significantly from baseline 8.16 lg copies/mL to 96-wk 2.85 lg copies/mL ( $P < 0.001$ ) and 56.6% (43/76) patients obtained HBV DNA  $\leq 300$  copies/mL in our cohort, showing a similar data to the GLOBE study<sup>[19]</sup>, in which 55.6% HBeAg-positive patients achieved HBV DNA  $< 300$  copies/mL with serum HBV DNA declined by 6.1 lg copies/mL after 2-year LdT treatment. The antiviral effect of NAs agents was reducing viral replication by the inhibition of HBV DNA polymerase, but having limited impacts on the level of HBsAg<sup>[20]</sup>. In our study, HBsAg decreased slowly by 0.59 lg IU/mL after 96-wk therapy. Heathcote *et al.*<sup>[21]</sup> also reported that the mean HBsAg level in HBeAg-positive CHB patients decreased by 0.66 lg IU/mL after 2 years of tenofovir disoproxil (TDF) treatment, which was similar to our data. In addition, anti-HBc level decreased from baseline 4.77 lg IU/mL to 96-wk 3.45 lg IU/mL in our cohort, with an average decrease of 1.32 lg IU/mL. In Fan's report<sup>[13]</sup>, 1.26 lg IU/mL of an average decrease of anti-HBc level was observed in HBeAg-positive CHB patients after 104-wk LdT and ADV optimized therapy (baseline 4.20 lg IU/mL to 104-wk 2.94 lg IU/mL). The average declines in anti-HBc levels were comparable between the two studies, although the baseline anti-HBc level was relatively higher in our study. Concerning the dynamic change of HBeAg, Shin *et al.*<sup>[11]</sup> reported that HBeAg level reduced from baseline 2.23 lg PEIU/mL to 0.96 lg PEIU/mL after 96-wk ETV therapy and 17.1% (14/82) achieved HBeAg SC. In the present study, HBeAg level decreased from baseline 2.24 lg PEIU/mL to 96-wk 0.20 lg PEIU/mL and 19.7% (15/76) obtained HBeAg SC, which presented comparable HBeAg reduction and HBeAg SC rate as compared to Shin's study.

Comparing dynamic changes in HBeAg level between patients with and those without 96-wk VR (or HBeAg SC), our results showed that baseline HBeAg levels were comparable between two groups. Further, HBeAg levels in patients obtained 96-wk VR (or HBeAg SC) were significantly lower than those without VR (or HBeAg SC) from 12-wk to the end of follow-up ( $P < 0.05$ , as shown in Figures 1B and 2B). With respect to dynamic changes of HBV DNA levels, the same trend of decline was observed. The patients achieved 96-wk VR had a significant lower HBV DNA level at every follow-up time-point except baseline when compared with those

without VR ( $P < 0.05$ ), while the significant differences in HBV DNA levels between patients with and without HBeAg SC were only observed at week 24, 72 and 96 (Figures 1D and 2D). These results indicated that HBeAg may have a better predictive value than HBV DNA for treatment response in HBeAg-positive CHB. At the same time, our finding showed that both the HBV DNA level as well as its declined value at 24-wk (or 12-wk) were significantly associated with 96-wk VR and HBeAg SC when performed univariate analysis ( $P < 0.05$ ). However, HBV DNA levels and its declines would not associate with 96-wk VR and HBeAg SC anymore if all parameters including HBeAg levels and its declines were enrolled in multivariate analysis. Other studies also pointed out that HBeAg levels as well as its declines may maintain a better predictive value than HBV DNA to predict NAs treatment response in HBeAg-positive CHB patients<sup>[10,11]</sup>. HBeAg is generated by transcription and translation of HBV cccDNA, and previous studies had reported that serum HBeAg level was significantly correlated with intrahepatic HBV cccDNA ( $r = 0.507$ ,  $P = 0.010$ )<sup>[22]</sup>. Thus, the decline in serum HBeAg level may reflect the reduction of HBV cccDNA and represented a good treatment outcome. In addition, viral persistence and the development of CHB was associated with viral manipulation and evasion of the host's immune system, while HBeAg has been reported to attenuate the host immune response to the nucleocapsid protein and down-regulate the innate and adaptive immune responses<sup>[23,24]</sup>. Therefore, the decline in HBeAg level might weaken this effect and thus the patients may present a better immune control for HBV infection.

There were some limitations in our study. Firstly, LAM and ADV used in the cohort are no longer the first-line antiviral drugs. However, NAs drugs have a similar antiviral mechanism, and HBeAg SC rates in CHB patients are comparable between ETV (or TDF) therapy and an optimized therapy with LAM and ADV. Hence, we propose that the combination parameter of HBeAg level and its declined value at 24-wk might be used as a reference parameter to predict the efficacy of ETV or TDF treatment. Secondly, treatment endpoint evaluated in our study was on-treatment response. For virological response, off-treatment response may be more important than on-treatment response.

To our knowledge, this is the first report that identified the combination of on-treatment quantitative HBeAg level and its decline as the predictor of positive response to long term NAs therapy among HBeAg-positive CHB patients. In particular, the combination of HBeAg titer and its decline at 24-wk strongly predicted 96-wk VR and HBeAg SC with the AUROC of 0.923 and 0.928, respectively. This combination predictor was identified through a retrospective investigation based on the cohort of HBeAg-positive CHB patients received LAM and ADV optimized therapy. We will conduct a prospective study to evaluate and confirm the predictive validity of the combination parameter in the cohort of ETV or TDF therapy in the future, and anticipate that the combination

of on-treatment HBeAg level and its declined value could serve as a reference parameter to guide NAs therapy.

## ACKNOWLEDGMENTS

The authors are grateful to Dr. Shumei Yun, from Missouri Department of Health and Senior Services, Jefferson City, MO, United States for proofreading and editing the manuscript.

## COMMENTS

### Background

The antiviral effect of current available nucleos(t)ide analogues (NAs) or peginterferon drugs are not satisfied. To improve the efficacy, it is crucial to explore the pre-treatment and early on-treatment biomarkers to effectively predict long-term treatment response. However, very few studies have systematically evaluated the predictive power of quantitative hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B core antibody (anti-HBc), hepatitis B virus (HBV) DNA and alanine aminotransferase (ALT) for NAs treatment response.

### Research frontiers

In the current study, serum HBsAg, HBeAg, anti-HBc, HBV DNA and ALT were quantitatively tested during the 96-wk NAs therapy, and the baseline as well as early on-treatment levels of these parameters were comprehensively analyzed to assess their functions in predicting 96-wk virological response (VR) and HBeAg seroconversion (SC).

### Innovations and breakthroughs

This is the first report that the combination parameter of on-treatment quantitative HBeAg level and its declined value was found to predict 96-wk treatment response to lamivudine and adefovir dipivoxil optimized therapy for HBeAg-positive chronic hepatitis B (CHB) patients. In particular, the combination of HBeAg titer and its decline at 24-wk strongly predicted 96-wk VR and HBeAg SC with the AUROC of 0.923 and 0.928, respectively.

### Applications

The combination variable of on-treatment HBeAg level and its declined value may serve as a reference parameter to optimize NAs therapy for HBeAg-positive CHB patients.

### Peer-review

HBV is a major cause of chronic liver disease including chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Reactivation of HBV is closely related to acute exacerbation of HBV carriers which sometimes leads to liver failure. Introduction of NAs dramatically changed the landscape of HBV treatment that is useful weapon to suppress HBV replication. NAs can only suppress HBV replication but not eradicate HBV. Therefore several problems remains including setting of endpoint and adequate cessation of NAs.

## REFERENCES

- 1 WHO Guidelines Approved by the Guidelines Review Committee. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. Geneva: World Health Organization, 2015 [PMID: 26225396]
- 2 Liaw YF, Kao JH, Piratvisuth T, Chan HL, Chien RN, Liu CJ, Gane E, Locarnini S, Lim SG, Han KH, Amarapurkar D, Cooksley G, Jafri W, Mohamed R, Hou JL, Chuang WL, Lesmana LA, Sollano JD, Suh DJ, Omata M. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. *Hepatol Int* 2012; 6: 531-561 [PMID: 26201469 DOI: 10.1007/s12072-012-9365-4]
- 3 European Association For The Study Of The Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; 57: 167-185 [PMID: 22436845]



- DOI: 10.1016/j.jhep.2012.02.010]
- 4 **Zeuzem S**, Gane E, Liaw YF, Lim SG, DiBisceglie A, Buti M, Chutaputti A, Rasenack J, Hou J, O'Brien C, Nguyen TT, Jia J, Poynard T, Belanger B, Bao W, Naoumov NV. Baseline characteristics and early on-treatment response predict the outcomes of 2 years of telbivudine treatment of chronic hepatitis B. *J Hepatol* 2009; **51**: 11-20 [PMID: 19345439 DOI: 10.1016/j.jhep.2008.12.019]
  - 5 **Bowden S**. Serological and molecular diagnosis. *Semin Liver Dis* 2006; **26**: 97-103 [PMID: 16673288 DOI: 10.1055/s-2006-939756]
  - 6 **Sonneveld MJ**, Hansen BE, Piratvisuth T, Jia JD, Zeuzem S, Gane E, Liaw YF, Xie Q, Heathcote EJ, Chan HL, Janssen HL. Response-guided peginterferon therapy in hepatitis B e antigen-positive chronic hepatitis B using serum hepatitis B surface antigen levels. *Hepatology* 2013; **58**: 872-880 [PMID: 23553752 DOI: 10.1002/hep.26436]
  - 7 **Fung J**, Lai CL, Young J, Wong DK, Yuen J, Seto WK, Yuen MF. Quantitative hepatitis B surface antigen levels in patients with chronic hepatitis B after 2 years of entecavir treatment. *Am J Gastroenterol* 2011; **106**: 1766-1773 [PMID: 21826112 DOI: 10.1038/ajg.2011.253]
  - 8 **Lee JM**, Ahn SH, Kim HS, Park H, Chang HY, Kim DY, Hwang SG, Rim KS, Chon CY, Han KH, Park JY. Quantitative hepatitis B surface antigen and hepatitis B e antigen titers in prediction of treatment response to entecavir. *Hepatology* 2011; **53**: 1486-1493 [PMID: 21520167 DOI: 10.1002/hep.24221]
  - 9 **Wang J**, Du LY, Zhu X, Chen EQ, Tang H. The predictive value of early indicators for HBeAg seroconversion in HBeAg-positive chronic hepatitis B patients with Telbivudine treatment for 104 weeks. *Indian J Med Microbiol* 2015; **33** Suppl: 20-25 [PMID: 25657151 DOI: 10.4103/0255-0857.148827]
  - 10 **Kwon JH**, Jang JW, Lee S, Lee J, Chung KW, Lee YS, Choi JY. Pretreatment HBeAg level and an early decrease in HBeAg level predict virologic response to entecavir treatment for HBeAg-positive chronic hepatitis B. *J Viral Hepat* 2012; **19**: e41-e47 [PMID: 22239525 DOI: 10.1111/j.1365-2893.2011.01509.x]
  - 11 **Shin JW**, Jung SW, Park BR, Kim CJ, Eum JB, Kim BG, Jeong ID, Bang SJ, Lee SH, Kim SR, Park NH. Prediction of response to entecavir therapy in patients with HBeAg-positive chronic hepatitis B based on on-treatment HBsAg, HBeAg and HBV DNA levels. *J Viral Hepat* 2012; **19**: 724-731 [PMID: 22967104 DOI: 10.1111/j.1365-2893.2012.01599.x]
  - 12 **Zhang X**, Lin SM, Ye F, Chen TY, Liu M, Chen YR, Zheng SQ, Zhao YR, Zhang SL. An early decrease in serum HBeAg titre is a strong predictor of virological response to entecavir in HBeAg-positive patients. *J Viral Hepat* 2011; **18**: e184-e190 [PMID: 21692931 DOI: 10.1111/j.1365-2893.2010.01423.x]
  - 13 **Fan R**, Sun J, Yuan Q, Xie Q, Bai X, Ning Q, Cheng J, Yu Y, Niu J, Shi G, Wang H, Tan D, Wan M, Chen S, Xu M, Chen X, Tang H, Sheng J, Lu F, Jia J, Zhuang H, Xia N, Hou J. Baseline quantitative hepatitis B core antibody titre alone strongly predicts HBeAg seroconversion across chronic hepatitis B patients treated with peginterferon or nucleos(t)ide analogues. *Gut* 2016; **65**: 313-320 [PMID: 25586058 DOI: 10.1136/gutjnl-2014-308546]
  - 14 **Zhou B**, Liu M, Lv G, Zheng H, Wang Y, Sun J, Hou J. Quantification of hepatitis B surface antigen and E antigen: correlation between Elecsys and architect assays. *J Viral Hepat* 2013; **20**: 422-429 [PMID: 23647959 DOI: 10.1111/jvh.12044]
  - 15 **Li A**, Yuan Q, Huang Z, Fan J, Guo R, Lou B, Zheng Q, Ge S, Chen Y, Su Z, Yeo AE, Chen Y, Zhang J, Xia N. Novel double-antigen sandwich immunoassay for human hepatitis B core antibody. *Clin Vaccine Immunol* 2010; **17**: 464-469 [PMID: 20107008 DOI: 10.1128/CVI.00457-09]
  - 16 **Farci P**, Diaz G, Chen Z, Govindarajan S, Tice A, Agulto L, Pittaluga S, Boon D, Yu C, Engle RE, Haas M, Simon R, Purcell RH, Zamboni F. B cell gene signature with massive intrahepatic production of antibodies to hepatitis B core antigen in hepatitis B virus-associated acute liver failure. *Proc Natl Acad Sci USA* 2010; **107**: 8766-8771 [PMID: 20421498 DOI: 10.1073/pnas.1003854107]
  - 17 **Oliviero B**, Cerino A, Varchetta S, Paudice E, Pai S, Ludovisi S, Zaramella M, Michelone G, Pugnale P, Negro F, Barnaba V, Mondelli MU. Enhanced B-cell differentiation and reduced proliferative capacity in chronic hepatitis C and chronic hepatitis B virus infections. *J Hepatol* 2011; **55**: 53-60 [PMID: 21145853 DOI: 10.1016/j.jhep.2010.10.016]
  - 18 **Chien RN**, Liaw YF, Atkins M. Pretherapy alanine transaminase level as a determinant for hepatitis B e antigen seroconversion during lamivudine therapy in patients with chronic hepatitis B. Asian Hepatitis Lamivudine Trial Group. *Hepatology* 1999; **30**: 770-774 [PMID: 10462384 DOI: 10.1002/hep.510300313]
  - 19 **Liaw YF**, Gane E, Leung N, Zeuzem S, Wang Y, Lai CL, Heathcote EJ, Manns M, Bzowej N, Niu J, Han SH, Hwang SG, Cakaloglu Y, Tong MJ, Papatheodoridis G, Chen Y, Brown NA, Albanis E, Galil K, Naoumov NV. 2-Year GLOBE trial results: telbivudine is superior to lamivudine in patients with chronic hepatitis B. *Gastroenterology* 2009; **136**: 486-495 [PMID: 19027013 DOI: 10.1053/j.gastro.2008.10.026]
  - 20 **Nguyen T**, Thompson AJ, Bowden S, Croagh C, Bell S, Desmond PV, Levy M, Locarnini SA. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. *J Hepatol* 2010; **52**: 508-513 [PMID: 20206400 DOI: 10.1016/j.jhep.2010.01.007]
  - 21 **Heathcote EJ**, Marcellin P, Buti M, Gane E, De Man RA, Krastev Z, Germanidis G, Lee SS, Flisiak R, Kaita K, Manns M, Kotzev I, Tchernev K, Buggisch P, Weilert F, Kuras OO, Shiffman ML, Trinh H, Gurel S, Snow-Lampart A, Borroto-Esoda K, Mondou E, Anderson J, Sorbel J, Rousseau F. Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. *Gastroenterology* 2011; **140**: 132-143 [PMID: 20955704 DOI: 10.1053/j.gastro.2010.10.011]
  - 22 **Tangkijvanich P**, Komolmit P, Mahachai V, Sa-Nguanmoo P, Theamboonlers A, Poovorawan Y. Comparison between quantitative hepatitis B surface antigen, hepatitis B e-antigen and hepatitis B virus DNA levels for predicting virological response to pegylated interferon-alpha-2b therapy in hepatitis B e-antigen-positive chronic hepatitis B. *Hepatol Res* 2010; **40**: 269-277 [PMID: 20070399 DOI: 10.1111/j.1872-034X.2009.00592.x]
  - 23 **Walsh R**, Locarnini S. Hepatitis B precore protein: pathogenic potential and therapeutic promise. *Yonsei Med J* 2012; **53**: 875-885 [PMID: 22869468 DOI: 10.3349/ymj.2012.53.5.875]
  - 24 **Chen MT**, Billaud JN, Sällberg M, Guidotti LG, Chisari FV, Jones J, Hughes J, Milich DR. A function of the hepatitis B virus precore protein is to regulate the immune response to the core antigen. *Proc Natl Acad Sci USA* 2004; **101**: 14913-14918 [PMID: 15469922 DOI: 10.1073/pnas.0406282101]

P- Reviewer: Inoue K S- Editor: Qi Y L- Editor: A  
E- Editor: Li D



Observational Study

# Seroprevalence of hepatitis B surface antigen in pregnant women attending antenatal clinic in Honiara Solomon Islands, 2015

Aneley Getahun, Margaret Baekalia, Nixon Panda, Alice Lee, Elliot Puiahi, Sabiha Khan, Donald Tahani, Doris Manongi

Aneley Getahun, Sabiha Khan, Department of Public Health and Primary Care, College of Medicine Nursing and Health Sciences, Fiji National University, Suva, Fiji Islands

Margaret Baekalia, Department of Health and Social Affairs, Yap State Hospital, Colonia 96943, Federated States of Micronesia

Nixon Panda, School of Nursing and Allied Health Sciences, Solomon Islands National University, Honiara, Solomon Islands

Alice Lee, Concord Repatriation General Hospital, University of Sydney, Concord NSW 2139, Australia

Elliot Puiahi, Donald Tahani, Doris Manongi, National Referral Hospital, Honiara, Solomon Islands

**Author contributions:** Getahun A prepared the proposal, designed the study, drafted and revised the manuscript; Panda N identified the topic and supervised the study implementation; Baekalia M, Puiahi E, Tahani D and Manongi D performed the laboratory tests; Lee A reviewed and edited the manuscript and provided technical support; Khan S design the study, analyzed and interpreted the data; all authors contributed to the write up and the revision of the manuscript.

**Supported by** University Research Publication Committee (URPC), Fiji National University, No. ACT339; and Hepatitis B Free (HBF) Ltd, Australia, No. 25 167 817 389.

**Institutional review board statement:** The study was reviewed and approved by the College Research and Ethics Committee of Fiji National University (FNU) and the National Health Research and Ethics Committee of MHMS, Solomon Islands (HRC14/28).

**Informed consent statement:** All study participants provided written informed consent prior to study enrollment.

**Conflict-of-interest statement:** The study team declared no conflict of interest.

**Data sharing statement:** The de-identified dataset is available from the corresponding author at [aneley.getahun@fnu.ac.fj](mailto:aneley.getahun@fnu.ac.fj).

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Aneley Getahun, MD, MTCM, DTMH, Assistant Professor in Primary Care, Department of Public Health and Primary Care, College of Medicine Nursing and Health Sciences, Fiji National University, Princess Road, Tamavua, Suva, Fiji Islands. [aneley.getahun@fnu.ac.fj](mailto:aneley.getahun@fnu.ac.fj)  
Telephone: +679-9789779  
Fax: +679-3321107

**Received:** June 27, 2016

**Peer-review started:** June 29, 2016

**First decision:** August 5, 2016

**Revised:** September 9, 2016

**Accepted:** October 17, 2016

**Article in press:** October 18, 2016

**Published online:** December 8, 2016

## Abstract

### AIM

To determine the seroprevalence of hepatitis B surface antigen (HBsAg) among pregnant women attending antenatal clinic in Honiara, Solomon Islands.

### METHODS

This descriptive cross-sectional study was carried out in seven area health centers in Honiara. From March to June

2015, identification of eligible pregnant women in each site was conducted using systematic random sampling technique. A total of 243 pregnant women who gave written informed consent were enrolled. Standardized tool was used to record demographics, obstetric history and serology results. HBsAg and hepatitis B e antigen (HBeAg) were tested using point-of-care rapid diagnostic test. All HBsAg positive samples were verified using enzyme-linked immunosorbent assay.

## RESULTS

The mean age of participants was  $26 \pm 6$  years. The overall hepatitis HBsAg prevalence was 13.8% with higher rate (22%) reported in women between 30-34 years of age. Majority of HBsAg positive participants were Melanesians (29 out of 33). None of the pregnant women in the 15-19 years and  $\geq 40$  years tested positive for HBsAg. There was no statistically significant difference in HBsAg prevalence by age, ethnicity, education and residential location. The overall HBeAg seroprevalence was 36.7%. Women between 20-24 years of age had the highest rate of 54.5%. Low level of knowledge about hepatitis B vaccination was reputed. Overall, 54.6% of participants were not aware of their hepatitis B vaccination status and only 65.2% of mothers reported their child had been vaccinated.

## CONCLUSION

Hepatitis B is a disease of public health importance in Solomon Islands and emphasize the need for integrated preventative interventions for its control.

**Key words:** Hepatitis B; Chronic hepatitis; Hepatitis B surface antigen; Hepatitis B e antigen; Seroprevalence; Pregnant women; Solomon Islands

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The objective of this study was to determine the prevalence of chronic hepatitis B infection in a cohort of antenatal women in Honiara. The overall hepatitis HBsAg and hepatitis B e antigen (HBeAg) prevalence was 13.8% and 36.7%, respectively. Our study for the first time reported HBeAg prevalence in pregnant women. Furthermore, the study revealed low level of knowledge about hepatitis B vaccination whereby 54.6% of participants were not aware of their vaccination status. Hepatitis B is a disease of public health importance in Solomon Islands and emphasize the need for efficient delivery of integrated services for its prevention and control.

Getahun A, Baekalia M, Panda N, Lee A, Puiahi E, Khan S, Tahani D, Manongi D. Seroprevalence of hepatitis B surface antigen in pregnant women attending antenatal clinic in Honiara Solomon Islands, 2015. *World J Hepatol* 2016; 8(34): 1521-1528 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i34/1521.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i34.1521>

## INTRODUCTION

Hepatitis B virus (HBV) infection occurs worldwide. It is estimated that over two billion people have been infected with 360 million individuals who remain chronically infected<sup>[1]</sup>. There are around 600000 deaths every year due to the consequences of hepatitis B<sup>[2]</sup>. Hepatitis B is highly endemic in Africa, South-East Asia and the Pacific (excluding Japan, Australia and New Zealand), the Amazon Basin and parts of the Middle East, central Asia and some countries in Eastern Europe. In these areas, about 70% to 90% of the population are exposed to the virus during the first four decades of life, and eight to 20% of people then become chronically infected<sup>[3,4]</sup>. It is estimated that around 45% of the world population live in areas where there are high levels of hepatitis B infection<sup>[4]</sup>.

Hepatitis B virus is a DNA virus classified within the family hepadnaviridae. It is transmitted by percutaneous and permucosal exposure to infected blood and other body fluids, mainly semen and vaginal fluid<sup>[5]</sup>. The predominant mode of transmission varies depending on the endemicity of the disease in a given population. In areas of high endemicity, HBV is mostly acquired in early childhood from mother to child at birth or from person to person in early childhood. Exposure at an earlier age increases likelihood of progress to chronic infection. In low endemic areas, sexual transmission is the predominant route<sup>[1,2]</sup>.

Vaccination is the most cost effective way for the prevention of hepatitis B. The World Health Organization (WHO) recommends the inclusion of hepatitis B vaccine in routine immunization programs in all countries<sup>[1]</sup>. Four doses of vaccines are given, the first one within 24 h after birth followed by three vaccines in infancy. The complete vaccine series induces protective antibody levels in more than 95% of infants, children and young adults. Protection lasts at least 20 years and is likely lifelong<sup>[1]</sup>.

The Solomon Islands is located in the southwest Pacific, stretching about 1700 km from the eastern tip of Papua New Guinea to the northern-most islands of Vanuatu. The Island chain is comprised of nine administrative provinces: Guadalcanal, Malaita, Western, Rennell Bellona, Central, Makara Ulawa, Choiseul, Isabel and Temotu. According to the 2009 census, total population is estimated at 515870 of which 80% live in rural areas<sup>[6]</sup>. In 2013, Solomon Islands was ranked 157 out of 187 countries and territories on the Human Development Index. The country is one of the world's least developed countries and the 2013 Human Development Index was below the average for countries in the low human development group as well as below the average for countries in East Asia and the Pacific region<sup>[7]</sup>.

The current WHO model for Ante Natal Care (ANC) recommend that all pregnant women have at least four ANC assessments by a skilled attendant. WHO also encourages countries to develop national guidelines to

outline the essential minimum packages of ANC based on the local epidemiology and priorities<sup>[8]</sup>. In Solomon Islands, ANC is based on the Manual of Obstetrics and Gynecology for Doctor, Midwives and Nurses in Solomon Islands, 2005<sup>[9]</sup>. This recommends early ANC assessment (after 2-3 missed period) to determine expected date of delivery and identify and treat common conditions such as anemia and syphilis. The first ANC visit includes thorough history taking, physical examination, administering first dose of tetanus toxin, and bloods for hemoglobin, malaria and syphilis and urine for glucose and protein. Antenatal screening for hepatitis B is not part of the routine care.

Hepatitis B infection is hyper endemic in Solomon Islands. A large study conducted in Honiara central hospital in 1994 reported overall Hepatitis B surface antigen (HBsAg) prevalence of 19.6% which ranged from 14.6% in females to 23.4% in males<sup>[10]</sup>. Surveillance reports and prevalence studies in population sub-groups such as pregnant women and blood donors also reported high level of chronic infection, with 14.5%-16% in pregnant women and 25% in blood donors<sup>[11,12]</sup>. HBV C3 and D4 are the two prevalent subgenotypes in Solomon Islands and in the Pacific region<sup>[13-16]</sup>, Utsumi *et al*<sup>[13]</sup>, further reported genotype to be specific with ethnicity where genotype was C were predominant in Melanesians while genotype was D was common among Micronesians. The prevalence of hepatitis B e antigen (HBeAg) was higher among carrier of HBV subtype C compared to carriers of subtype B however it was not statistically significant. In this study there was no statistically significant difference between carriers of the two genotypes in terms of sex, liver function test (AST, serum albumin and total bilirubin) and anti-HBe seroprevalence. Previous study in Solomon Islands reported significantly higher prevalence of HBeAg among carriers of genotype C which could be associated with severe hepatic inflammation and complications<sup>[14]</sup>. However, these studies were not designed to further evaluate the relationship between genotype and clinical progression as they were cross-sectional prevalence studies.

There is paucity of information on the prevalence of complications of HBV infection such as cirrhosis and primary hepatocellular carcinoma in Solomon Islands. Historically, the island reported a high incidence of liver cancer among males<sup>[17]</sup>. According to the 2014 WHO report, liver cancer was the single most common cause of cancer in males<sup>[18]</sup>. Mortality from HBV related complications in Solomon Island remains unknown. Hepatocellular carcinoma is common in neighboring Melanesian islands of Fiji<sup>[19]</sup> and Papua New Guinea<sup>[20]</sup>.

The clinical course of chronic HBV infection generally does not change during pregnancy and chronic infection is not implicated in increased maternal morbidity or mortality<sup>[21,22]</sup>. Most pregnant women with chronic HBV infection are asymptomatic and often detected during routine ANC screening. Pregnancy related complications and perinatal outcomes of chronic HBV are not well elucidated. Some studies reported gestational diabetic,

antepartum hemorrhage, preterm labour and lower Apgar score to be associated with chronic infection<sup>[23,24]</sup>. Recent large scale studies from the United States and China revealed no association between maternal HBV infection and the risk of fetal growth retardation, pregnancy induced hypertension or preeclampsia<sup>[24,25]</sup>. In Solomon Islands, the impact of chronic HBV infection on pregnancy outcomes has not been investigated.

Hepatitis B vaccine was introduced in the national immunization program in 1990-1991 and is recommended for infants at birth, 6, 10 and 14 wk of age<sup>[26]</sup>. In 2009, the coverage of hepatitis B vaccine was 45% at birth and 81% for  $\geq 3$  vaccines<sup>[27]</sup>.

Ongoing transmission at birth and in early childhood is likely to continue to contribute to the significant burden of disease. This early exposure in life increases risk of chronic infection and its complications of liver cirrhosis and its sequelae, liver cancer and early death. There are no recent seroprevalence studies to document the current burden of disease. Therefore, this study aims to contribute to a clearer understanding of the current status of chronic infection in a cohort of antenatal women in Honiara, Solomon Islands.

## MATERIALS AND METHODS

A descriptive, cross-sectional study was carried out in seven area health centers (Kukum, Mataniko, Rove, Vura, Mbokonavera, White River and Mbokona) providing ANC in the catchment areas of Honiara City Council. Ethical clearance was obtained from the College Research and Ethics Committee of Fiji National University and the National Health Research and Ethics Committee of Ministry of Health and Medical Services, Solomon Islands (HRC14/28).

All pregnant women who presented for the first antenatal visit were eligible for the inclusion. Using the one sample population proportion formula, the sample size required was estimated as 239 (based on 16% prevalence of HBV among pregnant women<sup>[12]</sup>, 95%CI, 5% margin error, and 15% non-respondent). Enrolment of eligible pregnant women in each area health center was conducted proportionally based on the monthly average of first ANC bookers using systematic random sampling technique. Potential study participants were invited to participate and those that gave written consent were enrolled. A total of 243 pregnant women were enrolled between March to June 2015. Information was collected using standardized proforma data collection tool which included demographics (age, ethnicity, residential location, education level and occupation), obstetric and medical history as well as HBsAg and HBeAg serology results.

One milliliter of blood that was collected for routine ANC testing was aliquoted and stored at  $-4^{\circ}\text{C}$  in the national referral hospital laboratory. The specimens were thawed back to room temperature for testing according to the manufacturer's instructions<sup>[28]</sup>. All samples were tested for HBsAg with Standard Diagnostics Bioline,



the HBsAg testing kit (30 Tests/kit, Cat. No. 01FK10W, Standard Diagnostics, Inc, South Korea). This is a point of care qualitative immunochromatography testing strip method for the detection of HBsAg. Further testing for HBsAg was performed on all positive sera and 5% of randomly selected nonreactive samples using Murex HBs version 3 enzyme linked immunosorbent assay (ELISA) - horseradish peroxidase conjugated kit which have a specificity of 99.97% and sensitivity of 100% respectively (DiaSorin, S.p.A. United Kingdom branch). Duplicate samples including positive and negative controls were included and the procedure was carried out in accordance to the manufacturer's instructions<sup>[29]</sup> with the washing step done manually. Samples that were positive on both rapid test kits and the ELISA were considered HBsAg positive. From the 33 positive samples for HBsAg, 30 samples were analyzed for HBeAg using the ABON HBV combo test kit (ABON Biopharm, Hangzhou Co., Ltd). The remaining 3 samples were insufficient for further testing. The testing procedure and interpretation was carried out according to the manufacturer's instructions. Positive results were indicated by two red bands; one in the test region and other in the control region. Negative results were indicated by one red band on the control region.

The data was entered into Microsoft Excel spreadsheet and analyzed using Statistical Package for the Social Sciences software version 22. A descriptive analysis was used to determine the demographic, obstetric and medical profile of study participants. The overall HBsAg and HBeAg prevalence was calculated as well as determination by age group, ethnicity and location of residence. Results are presented as proportion, means with standard deviation.  $\chi^2$  test and Fishers' exact tests were used to compare the proportions between hepatitis B seropositive vs sero-negative and demographic variables. Results were considered statistically significant at  $P < 0.05$ .

## RESULTS

A total of 243 pregnant women attending their first antenatal visit were enrolled in the study. Three pregnant women with incomplete information were subsequently excluded from analysis. The data from remaining 240 were used for analysis. The mean age of participants was  $26 \pm 6$  years (range 16 to 45). Majority of participants were Melanesians (91%), Polynesians and Micronesians represented 5.4% and 3.3% respectively. Most pregnant women (62.1%) achieved secondary or tertiary level education, with 7.9% reporting no education. Majority of the pregnant women who took part in the study were unemployed (58.7%). Nearly half of the study participants (46%) were peri-urban dwellers (Table 1).

The average presentation for first ANC visit was in the 6 mo of pregnancy. Most women presented for the first time in their second trimester (58.2%). Majority of women were not aware of their hepatitis B vaccination status (54.6%), with only 4.6% reporting prior vac-

cination. The median number of children was 1 per participant, with most had at least one child (58.3%). Women with children under the age of 5 years (47.9%) were asked about the hepatitis B vaccination of their child/children. Of these, 65.2% of mothers said their child/children had been vaccinated, 27% were uncertain and the remaining 7.8% not having received hepatitis B vaccine.

A total of 33 sera tested positive for HBsAg, with a sero-prevalence of HBsAg among study participants of 13.8%. Highest rate of hepatitis B infection was seen in participants between the ages of 30-34 year (22%). None of the pregnant women in the 15-19 years ( $n = 33$ ) and  $\geq 40$  years ( $n = 2$ ) tested positive for HBsAg. Majority of HBsAg positive participants were Melanesian (29 out of 33). In this study the highest rates of sero-prevalence were reported among Polynesians (23.1%) followed by Melanesians (13.2%). No statistically significant difference in HBsAg prevalence by age group, ethnicity, education level and residential location is seen (Table 2). A total of 44 samples (33 positive and 11 representing 5% of the negative results) were tested with Murex HBs version 3 ELISA for quality assurance. There was 100% concordance in results.

Of the 33 HBsAg positive pregnant women, 30 were tested for HBeAg. The overall prevalence of HBeAg was 36.7%. Higher prevalence was recorded among women between 20-24 years old (54.5%) followed by 25-29 years old (27.3%). All the HBeAg positive women were from Melanesian ethnic group and 54.5% reside in urban areas.

## DISCUSSION

The urgency to address the needs of hepatitis B associated disease and resultant suffering is now being actively addressed with particular attention to those countries with high rates of chronic infection. The hyper prevalence of hepatitis B in the Pacific islands is well accepted but remains poorly defined with gaps in recent data on disease burden. There are complex reasons for this and this study contributes to current understanding in a select cohort of people in Solomon Islands.

We report hepatitis B sero-prevalence rate of 13.8% in this descriptive cross-sectional study of pregnant women attending for their first antenatal visit in seven area health centres in Honiara. Data preceding this dates back to 2008, with comparable rates of 13.7% (41/298) reported amongst a similar antenatal cohort in Honiara, Gizo and Munda<sup>[12]</sup>. Their study determined HBsAg using ELISA (Determine and Serodia). Slightly higher rates of 15.8% were reported amongst women aged 15 to 24 years vs 11.9% in women aged 25-44 years<sup>[12]</sup>. Our study report similar rates of sero-prevalence based on rapid point of care tests in a similar antenatal cohort and hence have similar biases. These studies are both likely to underestimate the burden of disease due to convenience sampling bias in select age in the female population presenting to health care facilities. However,

**Table 1** Demographic characteristics of study participants (*n* = 240)

Demographic profile	<i>n</i> (%)
Age group	
15-19	33 (13.6)
20-24	82 (33.7)
25-29	50 (23.9)
30-34	41 (16.9)
35-39	24 (9.9)
≥ 40	2 (0.8)
Ethnicity	
Melanesian	219 (91.3)
Polynesian	13 (5.4)
Micronesian	8 (3.3)
Education level	
No education	19 (7.9)
Primary	72 (30)
Secondary	127 (52.9)
Tertiary	22 (9.2)
Occupation	
Unemployed	141 (58.8)
Employed (private/government)	77 (32.1)
Student	20 (8.3)
Unknown	2 (0.8)
Residential location	
Peri-urban	111 (46.3)
Urban	104 (43.3)
Rural	25 (10.4)

it does not distract from the high rate of 13.8%. Other previous data are limited to small studies, in select populations. Study on healthy blood donors reported higher prevalence of HBsAg of 19.6% and 22.3%<sup>[10,11]</sup>. Further national random representative sero-surveys are needed to provide a much more accurate assessment of disease burden in Solomon Islands.

We report a trend with increased rates seen with increasing age with peak prevalence of 22% in the 30-34 year group. The increased rates with increasing age may reflect ongoing new infections through sexual contact or other routes including health services. This is proceeded by further decline in the older age group (35-39 years), and no cases seen over the age of 40 years. This decline in the older age group may represent spontaneous sero-conversion over time and nil cases due to small sampling size. No HBsAg positive patients are noted in the youngest cohort (15-19 years). This is likely due to the efforts childhood vaccination program. This draws further attention in the need to ensure high rates of birth dose and vaccination coverage to improve herd immunity and hence overall prevalence over time. Initial effort to assess current vaccination coverage rates as well as addressing the barriers to delivery such as cold chain, birth outside health care facilities and lack of awareness are required.

Amongst the ethnic groups, the highest rates were seen amongst the Polynesian cohort with a prevalence of 23.1% compared with Melanesian and Micronesian (13.2% and 12.5% respectively), lack of statistical significance may be attributed to total numbers recruited for this study, with the largest sampling size from the Polynesian cohort. Similar reports of difference in hepatitis B in

**Table 2** Comparison of hepatitis B surface antigen prevalence by selected socio-demographic variables

	Total	HBsAg positive <i>n</i> (%)	HBsAg negative <i>n</i> (%)	<i>P</i> -value
Age group				-
15-19	33	0	33 (100)	
20-24	82	13 (15.9)	69 (84.1)	
25-29	58	7 (12.1)	51 (87.9)	
30-34	41	9 (22.0)	32 (78.0)	
35-39	24	4 (16.7)	20 (83.3)	
≥ 40	2	0	2 (100)	
Ethnicity				0.513
Melanesian	219	29 (13.2)	190 (86.8)	
Polynesian	13	3 (23.1)	10 (76.9)	
Micronesian	8	1 (12.5)	7 (87.5)	
Education level				0.143
No education	17	5 (26.3)	14 (73.7)	
Primary	77	7 (9.7)	65 (90.3)	
Secondary	127	16 (12.6)	111 (87.4)	
Tertiary	22	5 (22.7)	17 (77.3)	
Occupation				-
Unemployed	141	16 (11.3)	125 (88.7)	
Employed	77	15 (19.5)	62 (80.5)	
Student	20	2 (10.0)	18 (90.0)	
Unknown	2	0	2 (100)	
Residential location				0.112
Urban	104	13 (12.5)	91 (87.5)	
Peri urban	111	13 (11.7)	98 (88.3)	
Rural	25	7 (28.0)	18 (72.0)	

HBsAg: Hepatitis B surface antigen.

ethnic groups are reported from 1994 with highest rates of hepatitis B seen in Micronesians (28.1%), followed by Melanesians (20%) and then Polynesians (8.4%)<sup>[10]</sup>. This ethnic variation is well reported from other parts of the Pacific islands and further understanding of this relevance will assist in the contribution to the understanding of the disease, mode of transmission, disease progress and management strategies. Further work into this is clearly warranted.

Although the prevalence of hepatitis B is noted to be higher in those from rural settings (28%) as compared to those from urban and periurban settings (12.5% and 11.7%), this does not reach statistical significance. There is likely to be a number of factors contributing to this difference including vaccination coverage as well as ongoing risk of horizontal transmission modality and access to health care. Hepatitis B rate is also likely to have geographical variations in different islands. Improved understanding to address this gap is warranted with majority of the population (80.2%) in Solomon Islands living in rural areas where access to clean water and sanitation is not reliable<sup>[7]</sup>. These resource barriers are likely contributors to higher rates of hepatitis B, and ongoing risks of new infection.

Our study for the first time reports the prevalence of HBsAg among pregnant women in Solomon Islands. The overall prevalence of 36.7% is comparable to the rates reported among mothers and the general population. Furusyo *et al.*<sup>[10]</sup>, reported an overall prevalence of 41.3% among 315 HBsAg positive adult patients attending

general outpatients and blood donors. The prevalence did not differ by sex however patients from Melanesian ethnic groups had significantly higher HBeAg seropositive compared to the other two ethnic groups. Another study among mothers of children who received vaccination reported HBeAg prevalence of 40.7%<sup>[30]</sup>. Subsequently in 2001, seroprevalence of 35% was reported among 206 blood donors with chronic hepatitis<sup>[14]</sup>. All studies reported a progressive decline in HBeAg sero-prevalence with increasing age. Wilson *et al*<sup>[31]</sup>, reported high prevalence of HBeAg among pregnant women in the Pacific region which ranged from 48% in Kiribati to 70% in Fiji. HBeAg determines infectivity. High prevalence of HBeAg in pregnant women coupled with low up take of birth dose vaccine in Solomon Island increase the risk for vertical transmission of HBV to their newborns.

Only 4.6% of women screened had received previous hepatitis B vaccination. This low rate represents an opportunity to increase awareness and improve vaccination coverage. More than half the women were not aware of their vaccination status. Despite the routine introduction of childhood vaccination for hepatitis B in 1990-1991, only 65.2% of women with children under the age of 5 were able to report that their child/children had received vaccination. One in three mothers was not aware of their child's vaccination status. This gap in awareness about the vaccination requires attention, with opportunities for education for community as well as health care workers. Birth dose vaccination coverage as well as completion of the three doses remains a significant challenge in resource poor setting and efforts to evaluate this in Solomon islands and address the specific barriers is needed. In particular, challenges include remote settings, lack of cold chain, engagement of health care workers and competing needs of antenatal care, as well as access to vaccines. Solomon Islands have one of the lowest birth dose coverage (45%) in the WHO-Western Pacific Region<sup>[27]</sup>. There are potential solutions to address these gaps that require resource allocation and prioritization with burden compounded by the remote settings. Hence, programs that explore integration into currently systems are should be considered. Even with optimal vaccination delivery, the protective coverage of vaccination is 70% in those born to positive mothers vs 81% to those born to hepatitis B negative mothers<sup>[31]</sup>. Hence, efforts to address this ongoing risk of vertical transmission and its associated high risk of progression to chronic lifelong infection are needed.

The screening tool used for this study was a point of care test (Standards Diagnostics). The 5% of negative samples tested by Murex HBsAg version 3 ELISA method is considered as quality control in the study. According to testing kit evaluation made by independent studies (including WHO), on a number of commercial HBsAg rapid tests, the Murex HBsAg version 3 ELISA is able to detect 0.13-0.21 IU/mL surface antigen concentration with a clinical sensitivity of 100% by one study<sup>[32,33]</sup>. Standard Diagnostics testing kits allows for rapid detection of the HBsAg which is an antigen associated

with hepatitis B. This antigen usually becomes positive very soon after infection and persists if the person is unable to develop protective antibodies, indicating chronic infection if present after 6 mo. These rapid point of care test kits are utilized mostly in resourced limited settings, are cheap, easy to use and interpret and requires less laboratory skills and does not need instruments. On the other hand, ELISA which is the most preferred screening technique with accuracy of 99.9% is time consuming, laborious and needing proficient skills to perform<sup>[34,35]</sup>. Thus rapid HBsAg tests serves as the common testing methods in the Solomon Islands for screening of antenatal mothers, blood donors and patients.

HBV viral load testing remain outside the scope of most of these resource poor settings both in terms of cost of equipment, consumables as well as training for laboratory staff. Additional information is required on the performance characteristics of these rapid tests in terms of their current on field performance and factors that may affect it including cut off viral load, contribution of diversity in hepatitis B variants and the effect of hepatitis B therapy. Other tests that are potentially of use include the use of hepatitis B surface antibody tests and core antibody tests which could add value to the overall understanding of the viral replication status and hence infectivity with potential role in the monitoring of patients who are found to be positive on screening. Further, rapid tests may not be able to detect occult hepatitis B infection, this remaining an area of further study. Although these rapid tests have a clear role, the need for additional laboratory services needs to be considered. Models including the establishment of reference labs could be explored.

The cost effectiveness of routine screening for HBsAg has not been fully investigated. Routine screening of pregnant women for hepatitis B has not been included in the WHO optimum service package for ANC. This could be due to concern over its cost effectiveness and support for mathematical modeling for funding purposes would help clarify. Currently, some PICs countries have included routine screening for Hepatitis B in their ANC package<sup>[36-39]</sup>. In Fiji and French Polynesia, screening is recommended during the first ANC visit and newborns of seropositive mothers are given immunoglobulin as well as birth dose vaccines at birth followed by three subsequent vaccine doses<sup>[36,37]</sup>. Similarly in Vanuatu, universal screening of pregnant women for HBsAg was commenced in Port Vila hospital in 2013<sup>[39]</sup>. In Solomon Islands, introduction of routine screening of pregnant women for HBsAg before childbirth would have two benefits. First, it will enable prompt identification of newborns of positive mothers for the provision of vaccine immediately after birth and subsequent follow up for completion of vaccination. Secondly, in high endemic areas it provides opportunity to immunize HBsAg negative pregnant women (if they have not been immunized) who are at high risk of infection in the community. Identification, screening and vaccination of contacts of positive patients could also be implemented. This targeted immunization coupled with ongoing promotion of current universal

immunization program could provide an opportunity to increase uptake of birth dose and overall hepatitis B vaccine coverage in infants.

Clearly, allocation of resources to allow this both in terms of cost and supporting services related to its delivery is required but need commitment from government. Solomon Islands could provide an example of models of care with an integrated program including awareness, prevention, screening, diagnostics, therapy and management through engagement and education. Specific local needs and resources require attention with focus on efficient delivery of care integrating hepatitis B programs into currently existing program as not to unnecessarily to the existing burden of stretched resources. This could form part of a national strategic plan for the delivery of hepatitis B services in Solomon Islands.

This study found a high rate of HBsAg and HBeAg prevalence among pregnant women in Honiara, with low level of awareness and vaccination uptake among women and their children. The challenges in hepatitis service in Solomon Islands are both unique and similar to those of the many of islands and atolls of the Pacific. Availability and access to care can be addressed by small steps and integration of currently available resources and programs without the need for introduction of separate programs and its associated funding requirements and complex strategies. Rapid point of care tests for screening and diagnosis are also available for further study. The WHO treatment and care guidelines in 2015 provide a framework with direction to further promote momentum on a broader scale to address the multiple facets needed to support hepatitis related service delivery in the resource poor setting<sup>[40]</sup>.

## ACKNOWLEDGMENTS

We would like to express our sincere gratitude to all pregnant women who gave consent and participated in the study, without their participation this study would not have been completed. The research team would also like to thanks the nurses in the seven area health centers and Honiara City Council health division staff and who kindly supported the study.

## COMMENTS

### Background

The Solomon Islands is located in the Southwest Pacific and has total population of 515870. Hepatitis B surface antigen (HBsAg) prevalence of 19.6% was reported among the general population in 1994. Surveillance reports and prevalence studies in population sub-groups such as pregnant women and blood donors also reported high level of chronic infection, with 14.5%-16% in pregnant women and 25% in blood donors. Hepatitis B vaccine was introduced in the national immunization program in 1990-1991 and is recommended for infants at birth, 6, 10 and 14 wk of age. In 2009, the coverage of hepatitis B vaccine was 45% at birth and 81% for  $\geq 3$  vaccines.

### Research frontiers

Hepatitis B infection is hyper endemic in Solomon Islands. Ongoing trans-

mission at birth and in early childhood is likely to continue to contribute to the significant burden of disease. However, there are no recent seroprevalence studies to document the current burden of disease. The research hotspot is to contribute a clearer understanding of the current status of chronic HBV infection in a cohort of antenatal women and provide evidence based information for national HBV prevention and control strategies.

## Innovations and breakthroughs

The hyper prevalence of hepatitis B in Solomon Island is well accepted but remains poorly defined with gaps in recent data on disease burden. This study enrolled 240 pregnant women attending antenatal care in Honiara and found a high rate of HBsAg and hepatitis B e antigen (HBeAg) prevalence with low level of awareness and vaccination uptake among pregnant women and their children respectively.

## Applications

This study found a high rate of HBsAg and HBeAg prevalence among pregnant women which suggests the increased risk of perinatal transmission of HBV. Moreover, the study provided background information on hepatitis B disease burden and described the various challenges and proposed integrated approach for HBV control in Solomon Islands.

## Peer-review

The study design, material-methods are appropriate for the aim. The results are typical for a HBV endemic population but somewhat limited. It would have been more interesting if anti-HBs and anti-HBc total results of the study group were also provided.

## REFERENCES

- 1 **World Health Organization.** Hepatitis B vaccines. *Wkly Epidemiol Rec* 2009; **84**: 405-419 [PMID: 19817017]
- 2 **Goldstein ST,** Zhou F, Hadler SC, Bell BP, Mast EE, Margolis HS. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. *Int J Epidemiol* 2005; **34**: 1329-1339 [PMID: 16249217 DOI: 10.1093/ije/dyi206]
- 3 **Hollinger FB,** Liang TJ. Hepatitis B Virus. In: Knipe DM et al., eds. *Fields Virology*, 4th ed. Philadelphia, Lippincott Williams & Wilkins, 2001: 2971-3036
- 4 **Franco E,** Bagnato B, Marino MG, Meleleo C, Serino L, Zaratti L. Hepatitis B: Epidemiology and prevention in developing countries. *World J Hepatol* 2012; **4**: 74-80 [PMID: 22489259 DOI: 10.4254/wjh.v4.i3.74]
- 5 **Hamborsky J,** Kroger A, Wolfe S, eds. *Epidemiology and prevention of vaccine-preventable diseases*, E-Book: The Pink Book. 13th ed. Washington D.C., Public Health Foundation, 2015: 149-179
- 6 **Solomon Islands Government.** Report on 2009 Population & Housing Census. Statistical bulletin 06/2012. [accessed 2014 Sept 16]. Available from: URL: <http://www.statistics.gov.sb/statistics/demographic-statistics/census>
- 7 **United Nations Development Program.** Human Development Report, Solomon Islands. 2015. [accessed 2014 Oct 8]. Available from: URL: [http://hdr.undp.org/sites/all/themes/hdr\\_theme/country-notes/SLB.pdf](http://hdr.undp.org/sites/all/themes/hdr_theme/country-notes/SLB.pdf)
- 8 **Villar J,** Ba'aqel H, Piaggio G, Lumbiganon P, Miguel Belizán J, Farnot U, Al-Mazrou Y, Carroli G, Pinol A, Donner A, Langer A, Nigenda G, Mugford M, Fox-Rushby J, Hutton G, Bergsjø P, Bakketeig L, Berendes H, Garcia J. WHO antenatal care randomised trial for the evaluation of a new model of routine antenatal care. *Lancet* 2001; **357**: 1551-1564 [PMID: 11377642 DOI: 10.1016/S014-6736(00)04722-X]
- 9 **Ministry of Health and Medical Services.** Manual of Obstetrics and Gynecology for Doctors, Midwives & Nurses in Solomon Islands, 2005
- 10 **Furusyo N,** Hayashi J, Kakuda K, Sawayama Y, Ariyama I, Eddie R, Kashiwagi S. Markedly high seroprevalence of hepatitis B virus infection in comparison to hepatitis C virus and human



- T lymphotropic virus type-1 infections in selected Solomon Islands populations. *Am J Trop Med Hyg* 1999; **61**: 85-91 [PMID: 10432062]
- 11 **Lucas RE**, Faoagali JL. The serological status of Solomon Island blood donors. *Southeast Asian J Trop Med Public Health* 1999; **30**: 542-545 [PMID: 10774666]
- 12 **Ministry of Health and Medical Services of Solomon Islands and Secretariat of the Pacific Community**. Second Generation Surveillance of Antenatal Women and Youth Solomon Islands. 2008. [accessed 2014 Sept 16]. Available from: URL: [http://www.spc.int/hiv1/en/downloads/doc\\_download/250-solomon-islands-sgs-report-2008](http://www.spc.int/hiv1/en/downloads/doc_download/250-solomon-islands-sgs-report-2008)
- 13 **Utsumi T**, Yano Y, Truong BX, Tanaka Y, Mizokami M, Seo Y, Kasuga M, Kawabata M, Hayashi Y. Molecular epidemiological study of hepatitis B virus infection in two different ethnic populations from the Solomon Islands. *J Med Virol* 2007; **79**: 229-235 [PMID: 17245721 DOI: 10.1002/jmv.20791]
- 14 **Furusyo N**, Kubo N, Nakashima H, Kashiwagi K, Hayashi J. Relationship of genotype rather than race to hepatitis B virus pathogenicity: a study of Japanese and Solomon Islanders. *Am J Trop Med Hyg* 2004; **70**: 571-575 [PMID: 15155994]
- 15 **Norder H**, Couroucé AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, Robertson BH, Locarnini S, Magnus LO. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* 2004; **47**: 289-309 [PMID: 15564741 DOI: 10.1159/000080872]
- 16 **Croagh CM**, Desmond PV, Bell SJ. Genotypes and viral variants in chronic hepatitis B: A review of epidemiology and clinical relevance. *World J Hepatol* 2015; **7**: 289-303 [PMID: 25848459 DOI: 10.4254/wjh.v7.i3.289]
- 17 **Taylor R**, Parker M, Ansford A, Davison A. Cancer in Solomon Islands 1970-82. *P N G Med J* 1983; **26**: 102-110 [PMID: 6593952]
- 18 **World Health Organization**. Cancer Country Profiles, Solomon Island. 2014. [accessed 2016 Aug 10]. Available from: URL: [http://www.who.int/cancer/country-profiles/slb\\_en.pdf](http://www.who.int/cancer/country-profiles/slb_en.pdf)
- 19 **Foliaki S**, Best D, Akau'ola S, Cheng S, Borman B, Pearce N. Cancer incidence in four pacific countries: Tonga, Fiji Islands, Cook Islands and Niue. *Pac Health Dialog* 2011; **17**: 21-32 [PMID: 23008968]
- 20 **Intenzo CM**, Park CH, Kim SM. Rapid resolution of pulmonary embolism by tissue plasminogen activator. *Clin Nucl Med* 1989; **14**: 801-802 [PMID: 2513157 DOI: 10.1111/jgh.12684]
- 21 **Cheung KW**, Seto MT, Wong SF. Towards complete eradication of hepatitis B infection from perinatal transmission: review of the mechanisms of in utero infection and the use of antiviral treatment during pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2013; **169**: 17-23 [PMID: 23465469 DOI: 10.1016/j.ejogrb.2013.02.001]
- 22 **Cunningham F**, Leveno KJ, Bloom SL, Spong CY, Dashe JS, Hoffman BL, Casey BM, Sheffield JS. Hepatic, Biliary, and Pancreatic Disorders. In: Cunningham F, Leveno KJ, Bloom SL, Spong CY, Dashe JS, Hoffman BL, Casey BM, Sheffield JS, eds. *Williams Obstetrics*, 24th ed. United States of America: McGraw-Hill Education, 2014: 1089-1091. [accessed 2016 Sept 29]. Available from: <http://accessmedicine.mhmedical.com/content.aspx?bookid=1057&Sectionid=59789200>
- 23 **Tse KY**, Ho LF, Lao T. The impact of maternal HBsAg carrier status on pregnancy outcomes: a case-control study. *J Hepatol* 2005; **43**: 771-775 [PMID: 16139923 DOI: 10.1016/j.jhep.2005.05.023]
- 24 **Reddick KL**, Jhaveri R, Gandhi M, James AH, Swamy GK. Pregnancy outcomes associated with viral hepatitis. *J Viral Hepat* 2011; **18**: e394-e398 [PMID: 21692952 DOI: 10.1111/j.1365-2893.2011.01436.x]
- 25 **Huang X**, Tan H, Li X, Zhou S, Wen SW, Luo M. Maternal Chronic HBV Infection Would Not Increase the Risk of Pregnancy-Induced Hypertension - Results from Pregnancy Cohort in Liuyang Rural China. *2014 PLoS One* 2014; **12**: e1142- 1148 [DOI: 10.1371/journal.pone.0114248]
- 26 **World Health Organization**. Western Pacific Regional Plan for Hepatitis B Control through Immunization. 2007. [accessed 2014 Sept 16]. Available from: URL: [http://www.wpro.who.int/immunization/documents/docs/POA\\_HepB.pdf](http://www.wpro.who.int/immunization/documents/docs/POA_HepB.pdf)
- 27 **World Health Organization**. Progress towards meeting the 2012 hepatitis B control milestone: WHO Western Pacific Region, 2011. *Wkly Epidemiol Rec* 2011; **86**: 180-188 [PMID: 21608201]
- 28 **Bioline SD**. HBsAg instruction for use. [accessed 2015 Nov 18]. Available from: URL: [http://www.standardia.com/en/home/product/Rapid\\_Diagnostic\\_Test/HBsAg.html](http://www.standardia.com/en/home/product/Rapid_Diagnostic_Test/HBsAg.html)
- 29 **Murex HBsAg ELISA test kit**. [accessed 2016 Jul 21]. Available from: URL: [http://www.bio-group.in/Products/Diasorin/IFuk\\_en\\_9F80-05\\_01-Murex\\_HBsAg.pdf](http://www.bio-group.in/Products/Diasorin/IFuk_en_9F80-05_01-Murex_HBsAg.pdf)
- 30 **Milne A**, Rodgers E, Hopkirk N. Hepatitis B vaccination of babies in Melanesia. *Lancet* 1995; **346**: 318 [PMID: 7630277 DOI: 10.1016/S0140-6736(95)92209-1]
- 31 **Wilson N**, Ruff TA, Rana BJ, Leydon J, Locarnini S. The effectiveness of the infant hepatitis B immunisation program in Fiji, Kiribati, Tonga and Vanuatu. *Vaccine* 2000; **18**: 3059-3066 [PMID: 10825610 DOI: 10.1016/S0264-410X(00)00080-3]
- 32 **Scheiblaue H**, El-Nageh M, Diaz S, Nick S, Zeichhardt H, Grunert HP, Prince A. Performance evaluation of 70 hepatitis B virus (HBV) surface antigen (HBsAg) assays from around the world by a geographically diverse panel with an array of HBV genotypes and HBsAg subtypes. *Vox Sang* 2010; **98**: 403-414 [PMID: 20412171 DOI: 10.1111/j.1423-0410.2009.01272]
- 33 **World Health Organization Prequalification of Diagnostics Programme**. Public report on product: Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3. 2014. [accessed 2016 Jul 21]. Available from: URL: [http://www.who.int/diagnostics\\_laboratory/evaluations/141010\\_public\\_report\\_diasorin\\_murex\\_hbsag\\_v1.pdf](http://www.who.int/diagnostics_laboratory/evaluations/141010_public_report_diasorin_murex_hbsag_v1.pdf)
- 34 **Khan JK**, Lone DS, Hameed A, Munim R, Bhatti M, Khattak AA, Usman M, Nadeem MF, Satti HS, Munir M. Evaluation of the performance of two rapid immunochromatographic tests for detection of hepatitis B surface antigen and anti HCV antibodies using ELISA tested samples. *Sp Ed Ann* 2010; **16**: 84-87 [DOI: 10.21649/akemu.v16i1.SI.166]
- 35 **Maity S**, Nandi S, Biswas S, Sadhukhan SK, Saha MK. Performance and diagnostic usefulness of commercially available enzyme linked immunosorbent assay and rapid kits for detection of HIV, HBV and HCV in India. *Viral J* 2012; **9**: 290 [PMID: 23181517 DOI: 10.1186/1743-422X-9-290]
- 36 **Ministry of Health and Medical Services, Government of Fiji**. Obstetrics and Gynecology, Clinical Practice Guidelines, Version 2.1, 2015
- 37 **Patel MK**, Le Calvez E, Wannemuehler K, Ségalin JM. Hepatitis B Vaccination Coverage and Prevalence of Hepatitis B Surface Antigen Among Children in French Polynesia, 2014. *Am J Trop Med Hyg* 2016; **94**: 1370-1375 [PMID: 27001757 DOI: 10.4269/ajtmh.15-0903]
- 38 **Aung M**, Sowter M, Kenealy T. Hepatitis B screening: outcomes and management of pregnant women and infants in the Cook Islands. *Pac J Reprod Health* 2015; **1**: 74-80 [DOI: 10.18313/pjrh.2015.912]
- 39 **Natuman S**, Natuman W, Harrison G, Harry T, Jenney A. Antenatal serology in Vanuatu: The need for hepatitis B screening. Proceedings of the 2nd Pacific Islands Health Research Symposium, 2014 Aug 28-29 Suva, Fiji. Fiji National University, 2014: 36
- 40 **World Health Organization**. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection, 2015 [PMID: 26225396]

P- Reviewer: García-Elorriaga G, Panduro A, Sayiner AA

S- Editor: Ji FF L- Editor: A E- Editor: Li D



Prospective Study

## Prevalence and risk factors of acute-on-chronic liver failure in a single center from Argentina

Cristian Dominguez, Eugenia Romero, Jorgelina Graciano, Jose Luis Fernandez, Luis Viola

Cristian Dominguez, Eugenia Romero, Jorgelina Graciano, Jose Luis Fernandez, Luis Viola, Division of Gastroenterology, Sanatorio Guemes, Buenos Aires C1425EUG, Argentina

Cristian Dominguez, Jose Luis Fernandez, Luis Viola, Centro Integral de Gastroenterología, Buenos Aires C1425EUG, Argentina

**Author contributions:** Dominguez C and Fernandez JL designed the study; Dominguez C, Romero E and Graciano J performed the research; Dominguez C and Fernandez JL analyzed the data; Dominguez C and Fernandez JL wrote the paper; Viola L revised the manuscript for final submission.

**Institutional review board statement:** The study was reviewed and approved by the Guemes Sanatorio institutional review board (BSAS, Argentina).

**Clinical trial registration statement:** Our study is not a clinical trial.

**Informed consent statement:** The protocol was approved by our institutional review board and patients gave the usual written informed consent for hospitalization. No additional procedures other than those indicated by the physicians, based on routine practice and international standards, were performed. Considering this fact, our institutional reviewers considered that another special consent was not required.

**Conflict-of-interest statement:** The authors declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

**Data sharing statement:** There is no additional data sharing available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Cristian Dominguez, MD, Centro Integral de Gastroenterología, Ecuador 1481 PB, Capital Federal, Buenos Aires C1425EUG, Argentina. [cristian.dom@hotmail.com](mailto:cristian.dom@hotmail.com)  
 Telephone: +54-11-48250065

Received: July 12, 2016

Peer-review started: July 14, 2016

First decision: August 22, 2016

Revised: September 20, 2016

Accepted: October 17, 2016

Article in press: October 18, 2016

Published online: December 8, 2016

### Abstract

#### AIM

To study the prevalence, characteristics, risk factors and mortality at 28 d of acute-on-chronic liver failure (ACLF).

#### METHODS

A total of 100 cirrhotic patients admitted to our hospital for more than one day were included during the period between June 2013 and December 2015. We used the European Association for the Study of the Liver-Chronic Liver Failure-Consortium diagnostic criteria for ACLF, considering it as the acute decompensation of cirrhosis associated with the presence of one or more organ failure. For the diagnosis of organic failure the Chronic Liver Failure-Sequential Organ Failure Assessment score was used. Our population was divided into patients with and without ACLF. Clinical characteristics, presence of precipitating events, potential risk factors for developing ACLF and causes of mortality were analyzed. Mortality at 28 d was evaluated.

#### RESULTS

Twenty-nine patients (29%) developed ACLF criteria. Alcoholism, detected in 58 patients (58%), was the

major etiological agent of cirrhosis. Bacterial infections were recognized as a precipitating event in 41.3% of cases and gastrointestinal bleeding in 27.5%. No precipitating event was identifiable in 27.5% of patients with ACLF. Comparing patients with and without ACLF, statistically significant risk factors were: Child Pugh score  $10.2 \pm 2.1$  vs  $8.4 \pm 1.6$  ( $P < 0.0001$ ), MELD score  $20.7 \pm 8.5$  vs  $12.3 \pm 4$  ( $P < 0.0001$ ), presence of ascites 27 (93%) vs 43 (60.5%) ( $P = 0.001$ ), leukocytosis  $15300 \pm 8033$  per cubic millimeter vs  $10770 \pm 5601$  per cubic millimeter ( $P < 0.0001$ ), and high plasma levels of C reactive protein values  $50.9 \pm 46.4$  mg/L vs  $28.6 \pm 23.4$  mg/L ( $P < 0.0019$ ). Mortality rate was 62% (18 patients) vs 5.6% (4 patients), respectively ( $P < 0.0001$ ).

## CONCLUSION

We observed that the ACLF is a frequent entity in this group of patients and has a significantly higher mortality rate.

**Key words:** Acute-on-chronic liver failure; Acute liver decompensation; Cirrhosis; Ascites; Mortality

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Acute-on-chronic liver failure (ACLF) is an increasingly recognized entity that is gaining acceptance in recent times. It is characterized by an acute impairment of an underlying chronic liver disease with high short-term mortality, produced by the development of organic failures and associated with precipitating event. However, little is known about the development and progression of this syndrome. Guided by the European Association for the Study of the Liver-Chronic Liver Failure-Consortium diagnostic criteria and the CANONIC study, we could establish that the prevalence of ACLF in our center was 29%, and that Child Pugh advanced stage, MELD score, presence of ascites and inflammation parameters were significant risk factors for ACLF.

Dominguez C, Romero E, Graciano J, Fernandez JL, Viola L. Prevalence and risk factors of acute-on-chronic liver failure in a single center from Argentina. *World J Hepatol* 2016; 8(34): 1529-1534 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i34/1529.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i34.1529>

## INTRODUCTION

Acute-on-chronic liver failure (ACLF) is an increasingly recognized entity that includes the acute deterioration of a chronic liver disease, usually associated with a precipitating event, the development of one or more organ failure and high short-term mortality.

The term ACLF was initially coined in 1995<sup>[1]</sup>. There are more than thirteen different definitions up to date. Until worldwide diagnostic criteria are accepted, two consensual

definitions are commonly used<sup>[2]</sup>. The first, belonging to the Asian Pacific Association for the Study of the Liver, considers that the ACLF is an "acute hepatic insult manifesting as jaundice and coagulopathy, complicated within four weeks by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease"<sup>[3]</sup>. According to the second definition, developed in a joint symposium of the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases, ACLF is an "acute deterioration of pre-existing chronic liver disease, usually related to a precipitating event, and associated with increased mortality at three months due to multi-system organ failure"<sup>[4]</sup>.

Recently, an European consortium exclusively dedicated to the study of liver failure in patients with chronic liver disease (EASL-CLIF-Consortium) conducted the CANONIC study with the aim to define the ACLF and be able to identify those cirrhotic patients with a high risk of short-term mortality. Based on the analysis of 1343 cirrhotic patients, the EASL-CLIF-Consortium proposed as diagnostic criteria the acute decompensation of the liver disease (defined by the development of ascites, encephalopathy, gastrointestinal bleeding or bacterial infection) associated with the presence of one or more organ failure. The organ failure was defined by the Chronic Liver Failure-Sequential Organ Failure Assessment (CLIF-SOFA) score (Table 1) and a mortality at 28 d higher than 15%<sup>[5,6]</sup>.

Acute decompensation of cirrhosis is the leading cause of hospitalization in cirrhotic patients<sup>[7]</sup>. In many of these patients complications develop in the absence of organic failure, but in others they are associated with impaired function of kidneys, liver or other organs. The last group of patients, falling within the definition of ACLF, are those with a high risk of short term mortality.

The CANONIC study showed that ACLF is an extremely relevant and very common syndrome, with a prevalence of around 30%, differing from a mere acute decompensation by the presence of organ failure, the mortality rate 15 times higher, the clinical characteristics, the association with precipitating events and the parameters of systemic inflammation<sup>[8-10]</sup>.

Due to the lack of a worldwide accepted definition and diagnostic criteria, many aspects of this syndrome, such as prevalence, natural history, precipitating factors, clinical features and pathophysiological mechanisms remain unknown<sup>[11,12]</sup>.

The aims of our study were to determine the prevalence of ACLF in the cirrhotic patients of our institution using the diagnostic criteria established by the CANONIC study, to describe the clinical characteristics of ACLF, to assess the risk factors for developing ACLF, and to evaluate the mortality at 28 d, comparing the cases with and without ACLF.

## MATERIALS AND METHODS

In this prospective observational study we analyzed

**Table 1** Chronic liver failure-sequential organ failure assessment score

Organ/system	0	1	2	3	4
Liver (bilirubin, mg/dL)	< 1.2	≥ 1.2 to ≤ 2	≥ 2 to < 6	≥ 6 to < 12	≥ 12
Kidney (creatinine, mg/dL)	< 1.2	≥ 1.2 to < 2	≥ 2 to < 3.5	≥ 3.5 to < 5 or dialysis	≥ 5 or dialysis
Cerebral (HE grade)	No HE	I	II	III	IV
Coagulation (RIN, platelet count)	< 1.1	≥ 1.1 to < 1.25	≥ 1.25 to < 1.5	≥ 1.5 to < 2.5	≥ 2.5 or platelet count ≤ 20000 per cubic millimeter
Circulation (mean arterial pressure, mmHg), inotropic drugs (μg/kg per minute)	≥ 70	< 70	Dopamine ≤ 5 or dobutamine or terlipressin	Dopamine > 5 or E ≤ 0.1 or NE ≤ 0.1	Dopamine > 15 or E > 0.1 or NE > 0.1
Lungs (SpO <sub>2</sub> /FiO <sub>2</sub> )	> 512	> 357 a ≤ 512	> 214 a ≤ 357	> 89 to ≤ 214	≤ 89

The text in bold indicates the diagnostic criteria for organ failure. HE: Hepatic encephalopathy; E: Epinephrine; NE: Norepinephrine; FiO<sub>2</sub>: Fraction of inspired oxygen; SpO<sub>2</sub>: Pulse oximetric saturation.

patients with cirrhosis, diagnosed by a previous liver biopsy or by indirect signs (clinical examination, laboratory, imaging and endoscopy), who were hospitalized for more than one day in the Sanatorio Güemes, which is one of the biggest high complexity medical centers in Argentina, located in Buenos Aires City, with a capacity of 480 beds.

The protocol was approved by our institutional review board and patients gave the usual written informed consent for hospitalization, no additional procedures other than those indicated by the physicians, based on routine practice and international standards, were performed. Considering this fact, our institutional reviewers considered that another special consent was not required.

Patients were recruited between June 2013 and December 2015. Data were obtained from medical records, including previous episodes of decompensation (ascites, encephalopathy, spontaneous bacterial peritonitis, esophageal varices, variceal bleeding or hepatocellular carcinoma), physical examination, laboratory analysis, presence of potential precipitating factors (infections, active alcohol intake, gastrointestinal bleeding), and etiology of cirrhosis.

For the diagnosis of organic failure the CLIF-SOFA score was used (Table 1). Our population was divided into patients with and without ACLF. Within the group with ACLF the type and number of affected organs were analyzed and divided in 3 grades. ACLF grade 1 included patients with single kidney failure; patients with single failure of the liver, coagulation, circulation, or respiration who had a serum creatinine level ranging from 1.5 to 1.9 mg/dL and/or mild to moderate hepatic encephalopathy; and patients with single cerebral failure, who had a serum creatinine level ranging from 1.5 to 1.9 mg/dL. ACLF grade 2 included patients with failure of two organs and ACLF grade 3 included patients with failure of three or more organs.

After discharge, the mortality at 28 d was evaluated by monitoring on an outpatient basis or by telephone calls when patients did not attend the visit.

Clinical characteristics of each group, presence of precipitating events, potential risk factors for developing ACLF and causes of mortality were analyzed. Within the analyzed clinical parameters, the West-Haven scale for

encephalopathy grades was used<sup>[13]</sup>; ascites was classified in mild (mild ascites only detectable by ultrasound), moderate (moderate ascites evident by moderate symmetrical distension of abdomen) and severe (large or gross ascites with marked abdominal distension)<sup>[14]</sup>; circulation dysfunction implied arterial hypotension (mean arterial pressure below 70 mmHg) or requirement of inotropic drugs; and respiratory failure implied the need for mechanical ventilation.

Laboratory data included a complete blood analysis allowing the calculation of MELD and Child-Pugh scores. Inflammation parameters were evaluated by white blood cell count and C-reactive protein (CRP).

Both the clinical parameters and the laboratory results were recorded when patients were enrolled, when they showed some intercurrent or organic decompensation, and at discharge or previously to death.

### Statistical analysis

For statistical analysis, the  $\chi^2$  test or the Fisher test were used for dichotomous variables as appropriate. For continuous variables the Student *t* test was used. For risk factors, the OR with their respective 95%CI were calculated as association measures.

## RESULTS

A total of 100 patients were included, of which 67 were male (67%) and 33 female (33%). The mean age was  $60 \pm 11$  years and mean Child-Pugh score was  $9 \pm 1.9$ . Regarding to the etiology of cirrhosis, alcohol was found in 58 patients (58%), followed by hepatitis C infection and cryptogenic disease (Table 2).

The total of patients who fulfilled criteria for ACLF was 29 (29%), 10 of them (34.4%) were grade 1, 5 (17.3%) grade 2 and 14 (48.3%) grade 3 (Table 3). Seventeen patients (59%) had criteria for ACLF at admission to the hospital and 12 (41%) developed it during hospitalization, with an average time of presentation of 10 d. Renal failure was the prevalent organ failure for ACLF grade 1. For ACLF grade 2, coagulation failure was the prevalent finding followed by renal and respiratory failure. For ACLF grade 3, the prevalence of all organ failures was high with a significant impact in the circulatory and respiratory



**Table 2** Cirrhosis etiology

Etiology	<i>n</i> (%)
Alcohol	58 (58)
Alcohol + hepatitis C virus	5 (5)
Hepatitis C virus	13 (13)
Nonalcoholic steatohepatitis	4 (4)
Cryptogenic	12 (12)
Autoimmune hepatitis	4 (4)
Primary biliary cirrhosis	1 (1)
Primary biliary cirrhosis + autoimmune hepatitis	1 (1)
Hepatitis B virus + alcohol	1 (1)
Hemochromatosis	1 (1)

**Table 3** Prevalence of acute on chronic liver failure *n* (%)

ACLF	Grade 1	Grade 2	Grade 3
Patients	10 (34.4)	5 (17.3)	14 (48.3)
Mortality	3 (30)	2 (40)	13 (92)

ACLF: Acute-on-chronic liver failure.

system (Table 4).

Analyzing the possible precipitating factors in patients with ACLF, an infectious cause was recognized in 12 (41.3%), being pneumonia the main source of infection, and gastrointestinal bleeding in 8 (27.5%). One patient (3.4%) developed ACLF after a renal failure secondary to acute diarrhea. There was not an evident precipitating factor in 8 cases (27.5%) (Table 5). In the group of patients without ACLF, we observed the following clinical events: Gastrointestinal bleeding in 27 patients (38%), bacterial infections in 20 (29%), other causes such as constipation in 5 (7%) and no event in 19 (26%).

When patients with and without ACLF were compared, we observed, respectively: Male 23 (79%) vs 44 (62%) [ $P = 0.11$ , OR = 2.36 (95%CI: 0.78-7.43)], age  $60 \pm 11$  years vs  $60 \pm 11$  years ( $P = 1.00$ ), active alcohol intake in the last 3 mo 9 (31%) vs 22 (31%) [ $P = 1$ , OR = 1.00 (95%CI: 0.23-2.79)], Child Pugh  $10.2 \pm 2.1$  vs  $8.4 \pm 1.6$  ( $P < 0.0001$ ), MELD score  $20.7 \pm 8.5$  vs  $12.3 \pm 4$  ( $P < 0.0001$ ), previous episodes of ascites 18 (62%) vs 29 (41%) [ $P = 0.07$ , OR = 2.37 (95%CI: 0.89-6.33)], previous episodes of encephalopathy 9 (31%) vs 10 (14%) [ $P = 0.08$ , OR = 2.74 (95%CI: 0.87-8.69)], presence of esophageal varices 18 (62%) vs 37 (52%) [ $P = 0.38$ , OR = 1.5 (95%CI: 0.57-3.99)], prior variceal hemorrhage 4 (13.7%) vs 10 (14%) [ $P = 1.00$ , OR = 0.97 (95%CI: 0.23-3.84)], presence of ascites during hospitalization 27 (93%) vs 43 (60.5%) [ $P = 0.001$ , OR = 8.79 (95%CI: 1.80-8.10)], white blood cell count  $15300 \pm 8.033$  per cubic millimeter vs  $10770 \pm 5.601$  per cubic millimeter ( $P < 0.0001$ ), natremia  $133.3 \pm 6.9$  mEq/L vs  $135.1 \pm 5.3$  mEq/L ( $P = 0.16$ ), and CRP values  $50.9 \pm 46.4$  mg/L vs  $28.6 \pm 23.4$  mg/L ( $P < 0.0019$ ) (Table 6).

Twenty patients were hospitalized in the intensive care unit, 14 received mechanical ventilation and none had artificial liver support because it is not available

**Table 4** Type and number of organ failure *n* (%)

Organs failure	ACLF 1	ACLF 2	ACLF 3
Renal	7 (70)	2 (40)	10 (71)
Cerebral	1 (10)	1 (20)	12 (85)
Coagulation	1 (10)	3 (60)	8 (57)
Liver	1 (10)	1 (20)	2 (14)
Circulatory	0 (0)	1 (20)	14 (100)
Respiratory	1 (10)	2 (40)	14 (100)

ACLF: Acute-on-chronic liver failure.

**Table 5** Precipitating events of acute-on-chronic liver failure

Potential precipitating events of ACLF	<i>n</i> (%)
Bacterial infection	12 (41.3)
Gastrointestinal hemorrhage	8 (27.5)
Renal failure secondary to acute diarrhea	1 (3.4)
No precipitating event	8 (27.5)

ACLF: Acute-on-chronic liver failure.

at our center. ACLF resolved or improved in 11 patients (38%) during hospitalization: 7 patients (70%) in grade 1, 3 (60%) in grade 2 and only 1 (7%) in grade 3. In the group of ACLF, 18 patients (62%) died, due to septic shock 10, type 1 hepatorenal syndrome 3, shock without focus 3, upper gastrointestinal bleeding 1 and bronchoaspiration 1. The mortality was 30% in ACLF grade 1, 40% in grade 2 and 92% in grade 3. In the group without ACLF, 4 patients (5.6%) died, due to infection 3 and cardiac failure 1.

## DISCUSSION

ACLF is a syndrome different from traditional decompensated cirrhosis, not only because of the presence of organ failure and high mortality rate but also because of the alcoholic etiology of cirrhosis, the prevalence of some specific triggers such as bacterial infection and the higher level of systemic inflammation<sup>[15,16]</sup>. To recognize ACLF allows to identify those patients at high risk for death due to organ failure and the CANONIC study provided much more precise diagnostic criteria<sup>[4,5,15]</sup>. So, we followed these criteria in our center and we found a prevalence of 29%, similar to the 30.9% found in the CANONIC study<sup>[5,10]</sup>. It is interesting to point out that cirrhotic patients may develop ACLF during their stay in the hospital, with an incidence of 14.4%. This figure is quite higher than the 10.8% observed in the CANONIC study<sup>[5]</sup>.

It is noteworthy that 65.8% of our patients who developed ACLF had more than one organ involved (grades 2 and 3). This finding differs from the results of the CANONIC study showing that 64.3% of patients had only one organ involvement<sup>[5]</sup>. A possible explanation for this discrepancy may be that our patients had advanced stages of cirrhosis (Child-Pugh C 72%) and high prevalence of alcoholism as etiology of the cirrhosis (58% vs 48.6% in the CANONIC study). An advanced disease

**Table 6** Comparative results between groups with and without acute on chronic liver failure *n* (%)

	ACLF	No ACLF	<i>P</i> value	OR	95%CI
Age (yr $\pm$ SD)	60 $\pm$ 11	60 $\pm$ 11	1.00		
Male	23 (79)	44 (62)	0.11	2.3	0.78-7.43
Child Pugh (score $\pm$ DS)	10.2 $\pm$ 2.1	8.4 $\pm$ 1.6	< 0.0001		
MELD (score $\pm$ DS)	20.7 $\pm$ 8.5	12.3 $\pm$ 4	< 0.0001		
Active alcoholism	9 (31)	22 (31)	1.00	1	0.3-2.8
Prior ascites	18 (62)	29 (41)	0.07	2.3	0.9-6.3
Prior encephalopathy, <i>n</i> (%)	9 (31)	10 (14)	0.08	2.74	0.9-8.7
Esophageal varices	18 (62)	37 (52)	0.38	1.5	0.5-4
Ascites	27 (93)	43 (60.5)	0.001	8.8	1.8-58.1
Variceal hemorrhage	4 (13.7)	10 (14)	1	0.97	0.2-3.8
White cell count ( <i>n</i> /mm <sup>3</sup> $\pm$ SD)	15,300 $\pm$ 10,770	8,033 $\pm$ 5,601	< 0.0001		
Serum sodium (mEq/L $\pm$ SD)	133.3 $\pm$ 6.9	135.1 $\pm$ 5.3	0.16		
CRP (mg/L $\pm$ SD)	50.9 $\pm$ 46.4	28.6 $\pm$ 23.4	0.002		
Mortality	18 (62)	4 (5.6)	< 0.0001		

OR: Odds ratio; 95%CI: Confidence interval 95%; SD: Standard deviation; CRP: C-reactive protein; ACLF: Acute-on-chronic liver failure.

may have been the trigger of irreversible pro- and anti-inflammatory mechanisms<sup>[10,17,18]</sup>. The commonest organ failure was the kidney failure (66%)<sup>[19,20]</sup>. The prevalence of circulatory and respiratory failure was high (51% and 58%) but significant only in patients with ACLF grade 3.

As expected by previous references, bacterial infections primarily and gastrointestinal bleeding secondly were the main precipitating events<sup>[5,21]</sup>. It is important to note that in 27.5% of cases we did not identify an evident precipitating factor to explain ACLF in 27.5% of cases, a fact that was previously observed by other authors<sup>[5]</sup>.

We found that Child-Pugh score, MELD score, presence of ascites, elevated leukocyte count and high CRP values parameters were significant risk factors for the development of ACLF. Although Child-Pugh and MELD scores were not considered as risk factors, the statistical significance of ascites, kidney dysfunction, hepatic encephalopathy, bilirubin, serum creatinine and international normalized ratio in the CANONIC study allows us to infer that our findings agree with these observations. The role of leukocyte count and CRP as inflammatory parameters were also emphasized by these authors<sup>[5,9]</sup>.

As it was previously observed, mortality was significantly higher in our patients with ACLF. Mortality in our patients with ACLF grade 1 was higher when compared with the figures reported by Gustot *et al.*<sup>[22]</sup> (30% vs 6% to 18%), but it was similar in patients with ACLF grade 2 and 3 (40% to 92% vs 42% to 92%). As it was also observed by these authors, mortality increased significantly when three or more organs were involved<sup>[5,10,22]</sup>.

The main strength of our investigation is the prospective design that allowed a rigorous collection of data and its main weakness is that it was performed in a single center with a limited number of patients. Despite this limitation, we can draw several conclusions from our results. ACLF is a syndrome that occurs with high frequency in cirrhotic patients hospitalized for decompensated liver disease, reaching a prevalence of 29%

in our centre. As noted in the literature, ACLF is a very dynamic syndrome. It resolved or improved in 38% of our patients, a figure lower than the 49% observed by Gustot *et al.*<sup>[22]</sup>. Patients may enter the hospital with ACLF but they may also develop it during their stay, there are risk factors that may predict its development and mortality significantly increases when it occurs. Consequently, it is important to recognize this entity, to be aware of its development, to correct the precipitating factors and perhaps to install a more aggressive therapy, in order to reduce the high mortality<sup>[15,23-25]</sup>. To overcome the limitations of our study and to achieve a better knowledge of the epidemiology and clinical characteristics of ACLF in our country, it would be desirable to transfer our bounded experience to a multicenter prolonged study.

## COMMENTS

### Background

Acute-on-chronic liver failure (ACLF) is an increasingly recognized entity that includes the acute deterioration of a chronic liver disease, usually associated with a precipitating event, the development of one or more organ failure and high short-term mortality. However, little is known about the development and progression of this syndrome. This study aimed to determine the prevalence of ACLF and describe the characteristics of this syndrome; assess the risk factors and analyze the mortality at 28 d.

### Research frontiers

Until the development of the CANONIC study there was no established definition of ACLF and the published definition were based only on expert opinions. In this study using the CANONIC diagnostic criteria, the authors describe the clinical characteristics, the prevalence and natural history of ACLF in cirrhotic patients of the authors' institution.

### Innovations and breakthroughs

As suggested in the literature, the authors observed that the ACLF is a frequent entity in this group of patients and has a significantly higher mortality rate.

### Applications

As ACLF is a frequent syndrome, it is important to recognize this entity, to be aware of its development and to install supportive measures in order to reduce the high mortality.

# Terminology

Acute-on-chronic liver failure: Acute deterioration of cirrhosis associated with organ/s failure and short term mortality.

# Peer-review

The paper is well written and includes information about a relevant topic.

# REFERENCES

- 1 Ohnishi H, Sugihara J, Moriwaki H, Muto Y. [Acute-on-chronic liver failure]. *Ryoikibetsu Shokogun Shirizu* 1995; (7): 217-219 [PMID: 8749457]
- 2 Singh H, Pai C. Defining acute-on-chronic liver failure: East, West or middle ground? *World J Hepatol* 2015; 7: 2571-2577 [PMID: 26557949 DOI: 10.4254/wjh.v7.i25.2571]
- 3 Sarin SK, Kumar A, Almeida JA, Chawla YK, Fan ST, Garg H, de Silva HJ, Hamid SS, Jalan R, Komolmit P, Lau GK, Liu Q, Madan K, Mohamed R, Ning Q, Rahman S, Rastogi A, Riordan SM, Sakhuja P, Samuel D, Shah S, Sharma BC, Sharma P, Takikawa Y, Thapa BR, Wai CT, Yuen MF. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). *Hepatol Int* 2009; 3: 269-282 [PMID: 19669378 DOI: 10.1007/s12072-008-9106-x]
- 4 Jalan R, Gines P, Olson JC, Mookerjee RP, Moreau R, Garcia-Tsao G, Arroyo V, Kamath PS. Acute-on chronic liver failure. *J Hepatol* 2012; 57: 1336-1348 [PMID: 22750750 DOI: 10.1016/j.jhep.2012.06.026]
- 5 Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, Durand F, Gustot T, Saliba F, Domenicali M, Gerbes A, Wendon J, Alessandria C, Laleman W, Zeuzem S, Trebicka J, Bernardi M, Arroyo V. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; 144: 1426-1137 [PMID: 23474284 DOI: 10.1053/j.gastro.2013.02.042]
- 6 Younossi ZM, Henry L, Stepanova M. A new comorbidity model for predicting mortality in patients with cirrhosis: does it work? *Gastroenterology* 2014; 146: 19-24 [PMID: 24287302 DOI: 10.1053/j.gastro.2013.11.026]
- 7 Ginès P, Cárdenas A, Arroyo V, Rodés J. Management of cirrhosis and ascites. *N Engl J Med* 2004; 350: 1646-1654 [PMID: 15084697 DOI: 10.1056/NEJMr035021]
- 8 Kim TY, Kim DJ. Acute-on-chronic liver failure. *Clin Mol Hepatol* 2013; 19: 349-359 [PMID: 24459638 DOI: 10.3350/cmh.2013.19.4.349]
- 9 Arroyo V, Moreau R, Jalan R, Gines P. Acute-on-chronic liver failure: a new syndrome that will re-classify cirrhosis. *J Hepatol* 2015 (Suppl 1); 62: S131-S143 [PMID: 25920082 DOI: 10.1016/j.jhep.2014.11.045]
- 10 Blasco-Algora S, Masegoza-Ataz J, Gutierrez-Garcia ML, Alonso-Lopez S, Fernandez-Rodriguez CM. Acute-on-chronic liver failure: pathogenesis, prognostic factors and management. *World J Gastroenterol* 2015; 21: 12125-12140 [PMID: 26576097 DOI: 10.3748/wjg.v21.i42.12125]
- 11 Jalan R, Yurdaydin C, Bajaj JS, Acharya SK, Arroyo V, Lin HC, Gines P, Kim WR, Kamath PS. Toward an improved definition of acute-on-chronic liver failure. *Gastroenterology* 2014; 147: 4-10 [PMID: 24853409 DOI: 10.1053/j.gastro.2014.05.005]
- 12 Jalan R, Stdlbauer V, Sean S, Cheshire L, Chang YM, Mookerjee RP. Role of predisposition, injury, response and organ failure in the prognosis of patients with acute-on-chronic liver failure: a prospective cohort study. *Crit Care* 2012; 16: R227 [PMID: 23186071 DOI: 10.1186/cc11882]
- 13 Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy--definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002; 35: 716-721 [PMID: 11870389 DOI: 10.1053/jhep.2002.31250]
- 14 European Association for the Study of the Liver. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; 53: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]
- 15 Moreau R, Arroyo V. Acute-on-chronic liver failure: a new clinical entity. *Clin Gastroenterol Hepatol* 2015; 13: 836-841 [PMID: 24583872 DOI: 10.1016/j.cgh.2014.02.027]
- 16 Olson JC, Kamath PS. Acute-on-chronic liver failure: concept, natural history, and prognosis. *Curr Opin Crit Care* 2011; 17: 165-169 [PMID: 21326095 DOI: 10.1097/MCC.0b013e328344b42d]
- 17 Moreau R, Jalan R, Arroyo V. Acute-on-Chronic Liver Failure: Recent Concepts. *J Clin Exp Hepatol* 2015; 5: 81-85 [PMID: 25941435 DOI: 10.1016/j.jceh.2014.09.00]
- 18 Sen S, Williams R, Jalan R. The pathophysiological basis of acute-on-chronic liver failure. *Liver* 2002; 22 Suppl 2: 5-13 [PMID: 12220296 DOI: 10.1002/lt.20236]
- 19 Cardenas A, Gines P. Acute-on-chronic liver failure: the kidneys. *Curr Opin Crit Care* 2011; 17: 184-189 [PMID: 21311322 DOI: 10.1097/MCC.0b013e328344b3da]
- 20 Martin-Llahi M, Guevara M, Torre A, Fagundes C, Restuccia T, Gilabert R, Sola E, Pereira G, Marinelli M, Pavesi M, Fernandez J, Rodes J, Arroyo V, Gines P. Prognostic importance of the cause of renal failure in patients with cirrhosis. *Gastroenterology* 2011; 140: 88-96 [PMID: 20682324 DOI: 10.1053/j.gastro.2010.07.04]
- 21 Marciano S, Mauro E, Carena A, Gadano A. Falla hepática aguda sobre crónica. *Actualizaciones en Hepatología* 2013; 5: 17-24
- 22 Gustot T, Fernandez J, Garcia E, Morando F, Caraceni P, Alessandria C, Laleman W, Trebicka J, Elkrief L, Hopf C, Solis-Munoz P, Saliba F, Zeuzem S, Albillos A, Bente D, Montero-Alvarez JL, Chivas MT, Concepción M, Córdoba J, McCormick A, Stauber R, Vogel W, de Gottardi A, Welzel TM, Domenicali M, Rizzo A, Wendon J, Deulofeu C, Angeli P, Durand F, Pavesi M, Gerbes A, Jalan R, Moreau R, Ginés P, Bernardi M, Arroyo V. Clinical Course of acute-on-chronic liver failure syndrome and effects on prognosis. *Hepatology* 2015; 62: 243-252 [PMID: 25877702 DOI: 10.1002/hep.27849]
- 23 Laleman W, Verbeke L, Meersseman P, Wauters J, van Pelt J, Cassiman D, Wilmer A, Verslype C, Nevens F. Acute-on-chronic liver failure: current concepts on definition, pathogenesis, clinical manifestations and potential therapeutic interventions. *Expert Rev Gastroenterol Hepatol* 2011; 5: 523-537; quiz 537 [PMID: 21780899 DOI: 10.1586/egh.11.47]
- 24 Ginès P, Fernández J, Durand F, Saliba F. Management of critically-ill cirrhotic patients. *J Hepatol* 2012; 56 Suppl 1: S13-S24 [PMID: 22300462 DOI: 10.1016/S0168-8278(12)60003-8]
- 25 Gustot T, Durand F, Lebre D, Vincent JL, Moreau R. Severe sepsis in cirrhosis. *Hepatology* 2009; 50: 2022-2033 [PMID: 1985876 DOI: 10.1002/hep.23264]

P- Reviewer: Bossen L, Lenz K S- Editor: Qi Y L- Editor: A  
E- Editor: Li D



## Major hepatectomy using the glissonean approach in cases of right umbilical portion

Yusuke Ome, Kazuyuki Kawamoto, Tae Bum Park, Tadashi Ito

Yusuke Ome, Kazuyuki Kawamoto, Tae Bum Park, Tadashi Ito, Department of Surgery, Kurashiki Central Hospital, Kurashiki, Okayama 710-8602, Japan

Published online: December 8, 2016

**Author contributions:** Ome Y clinically managed the patients, performed the operations, gathered the clinical data, designed the report and wrote the paper; Kawamoto K, Park TB and Ito T supervised the clinical practices and helped draft and revise the manuscript.

**Institutional review board statement:** The Kurashiki Central Hospital Institutional Review Board does not require approval for case reports. Ethics approval was not necessary for this case report.

**Informed consent statement:** Written informed consent was obtained from the patients.

**Conflict-of-interest statement:** None of the authors have conflicts of interest to declare.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Yusuke Ome, MD, Department of Surgery, Kurashiki Central Hospital, 1-1-1 Miwa, Kurashiki City, Okayama 710-8602, Japan. [yo14408@kchnet.or.jp](mailto:yo14408@kchnet.or.jp)  
 Telephone: +81-86-4220210  
 Fax: +81-86-4213424

Received: June 21, 2016  
 Peer-review started: June 24, 2016  
 First decision: August 11, 2016  
 Revised: September 11, 2016  
 Accepted: October 25, 2016  
 Article in press: October 27, 2016

### Abstract

Right umbilical portion (RUP) is a rare congenital anomaly associated with anomalous ramifications of the hepatic vessels and biliary system. As such, major hepatectomy requires a careful approach. We describe the usefulness of the Glissonean approach in two patients with vessel anomalies, such as RUP. The first patient underwent a right anterior sectionectomy for intrahepatic cholangiocarcinoma. We encircled several Glissonean pedicles that entered the right anterior section along the right side of the RUP. We temporarily clamped each pedicle, confirmed the demarcation area, and finally cut them. The operation was performed safely and was successful. The second patient underwent a left trisectionectomy for perihilar cholangiocarcinoma. We secured the right posterior Glissonean pedicle. The vessels in the pedicle were preserved, and the other vessels and contents were resected. Identifying the vessels for preservation facilitated the safe lymphadenectomy and dissection of the vessels to be resected. We successfully performed the operation.

**Key words:** Right anterior sectionectomy; Right umbilical portion; Glissonean approach; Left trisectionectomy; Glissonean pedicle; Cholangiocarcinoma; Hepatocellular carcinoma

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Right umbilical portion (RUP) is a rare congenital anomaly, and its presence is associated with anomalous ramifications of the hepatic artery, portal vein, and biliary system. Major Hepatectomies for patients with this anomaly are complicated and require a careful approach. The Glissonean approach is acknowledged as a successful technique. The targeted Glissonean pedicle to



be resected or preserved is easily identified by clamping; thus, the Glissonean approach can be used in various situations of hepatic resection. This report describes the usefulness of the Glissonean technique, especially in cases with an anomaly, such as RUP.

Ome Y, Kawamoto K, Park TB, Ito T. Major hepatectomy using the glissonean approach in cases of right umbilical portion. *World J Hepatol* 2016; 8(34): 1535-1540 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i34/1535.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i34.1535>

## INTRODUCTION

Right umbilical portion (RUP) is a rare congenital anomaly, and its reported incidence ranges from 0.2% to 1.2%<sup>[1-6]</sup>. The presence of RUP is associated with anomalous ramifications of the hepatic artery, portal vein, and biliary system. During anatomical liver resection, only the vessels feeding the area intended for resection should be resected, whereas the other vessels should be preserved. Consequently, major hepatectomies for cases with RUP are complicated and require a careful approach and attention to the anomalous branching of those vessels. Only a few hepatectomy cases with RUP have been reported in the English literature. Here, we report two successful cases with RUP who safely underwent anatomical hepatectomy. We also describe the usefulness of the Glissonean approach.

## CASE REPORT

### Case 1

A 70-year-old man with hepatitis C presented with a liver tumour. He had a past medical history of distal gastrectomy for gastric ulcer, Graves' disease, and diabetes mellitus. Laboratory tests showed normal levels of carcinoembryonic antigen (CEA), CA19-9 and alpha-fetoprotein (AFP) but elevated PIVKA-II at 808 mAU/mL. The indocyanine green retention rate at 15 min was 12.9% and the Child-Pugh score was 5 points, Grade A. He was diagnosed with intrahepatic cholangiocarcinoma or combined hepatocellular and cholangiocarcinoma located in segment 8. A computed tomography (CT) scan also revealed that his gallbladder was attached to the left side of the liver; RUP was noted (Figure 1).

The patient underwent right anterior sectionectomy (Figure 2). Laparotomy showed that the gallbladder was attached to the round ligament. After the mobilization of the right lobe, the gallbladder was resected. Then, the right anterior Glissonean pedicles, which ramified along the right side of the RUP, were extrahepatically separated and encircled with tape. We temporarily clamped each pedicle and confirmed the demarcation area and blood flow *via* ultrasonography. The demarcation area was the same as the three-dimensional image visualization *via* preoperative simulation. The liver parenchyma was

transected along the demarcation line using the Pringle manoeuvre. We finally ligated and cut the encircled right anterior Glissonean pedicles. The operation succeeded without injuring any of the vessels intended for preservation. The operation required 244 min, and the estimated blood loss was 776 mL.

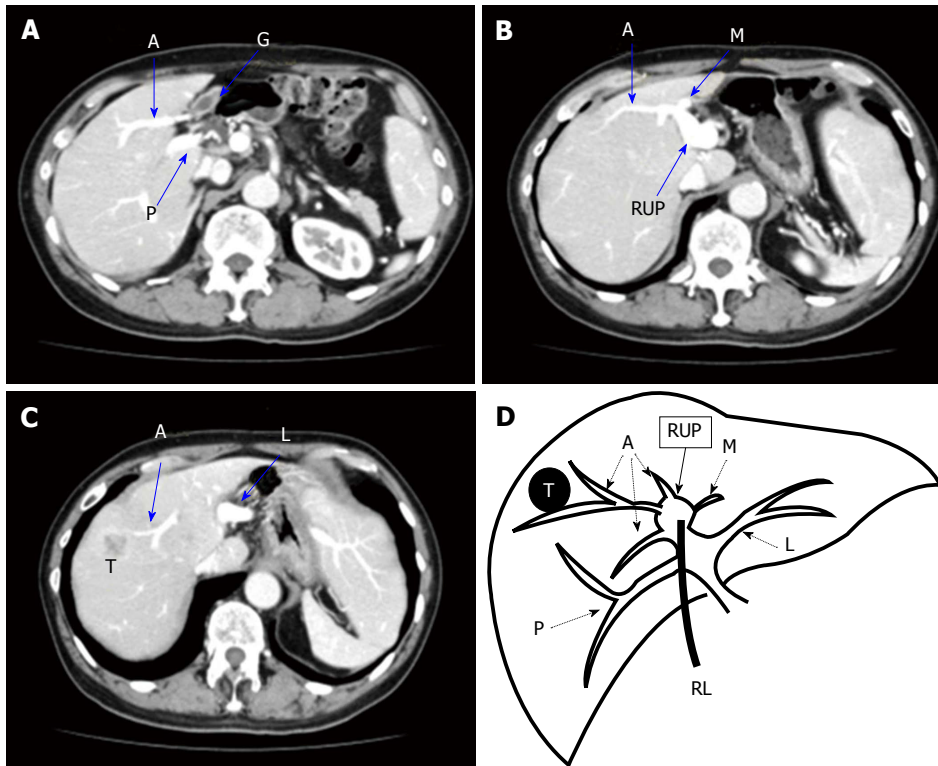
Macroscopic findings showed an irregular mass, 25 mm in size. A histological examination revealed that the tumour was a poorly differentiated intrahepatic cholangiocarcinoma that invaded the intrahepatic portal vein. The patient was diagnosed as stage II (T2N0M0). All of the surgical margins were negative. He recovered uneventfully and was discharged on postoperative day 6.

### Case 2

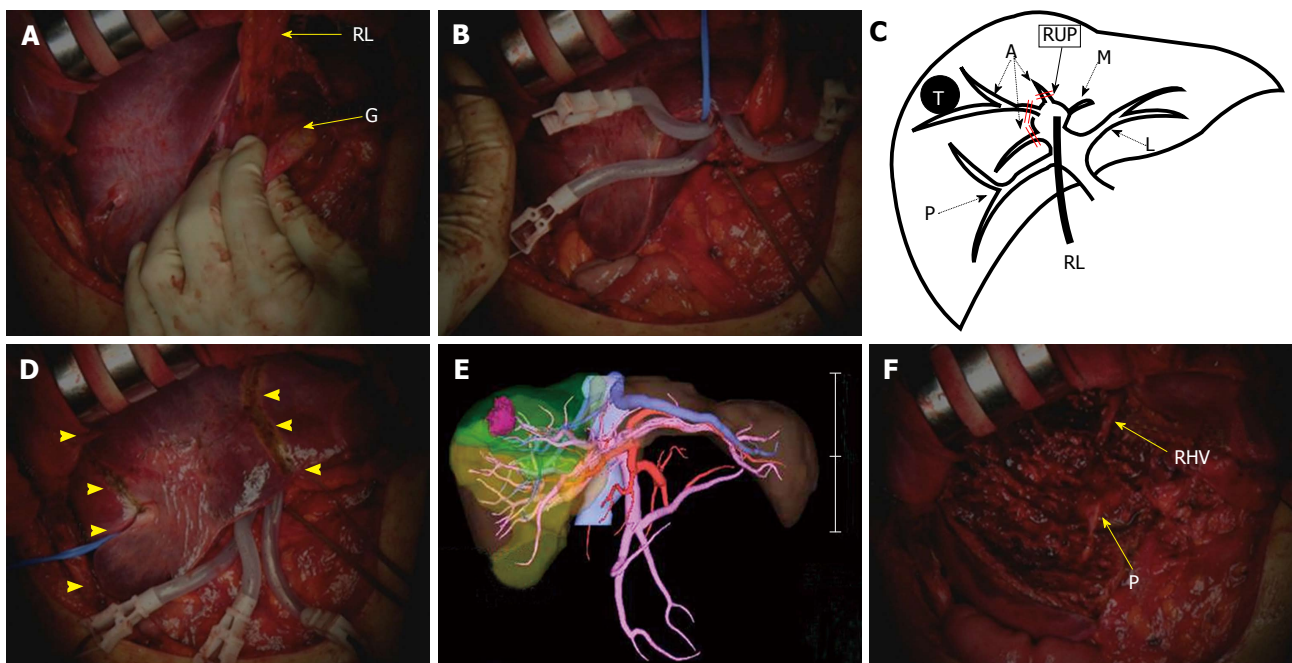
A 70-year-old woman presented with general fatigue and intrahepatic bile duct dilatation. Tumour markers, such as AFP, PIVKA-II and CEA, were normal, but CA19-9 was elevated at 843.6 U/mL. Other laboratory tests showed elevated ALP at 601 IU/L, elevated  $\gamma$ -GTP at 318 IU/L, and impaired serum albumin at 3.3 g/dL. Bilirubin was normal. The indocyanine green retention rate at 15 min was 4.6% and the Child-Pugh score was 6 points, Grade A. She was diagnosed with perihilar cholangiocarcinoma and RUP *via* ultrasound, CT and magnetic resonance cholangiopancreatography (Figure 3). The tumour involved the confluence of the left lateral, left medial, and right anterior hepatic ducts; the right posterior branch was intact.

The patient underwent left trisectionectomy with extrahepatic bile duct resection (Figure 4). First, Kocher's manoeuvre and lymphadenectomy around the pancreas head were performed. The distal common bile duct was transected at the level of the pancreas. Then, we performed lymphadenectomy in the hepatoduodenal ligament. The gallbladder was dissected and we secured and encircled the right lateral Glissonean pedicle with tape. The portal vein, the hepatic artery, and the hilar plate were separated from the other structures just proximal to the secured Glissonean pedicle. The vessels entering the pedicle were preserved and the other vessels and contents were resected. In the preoperative simulation, only one right posterior branch of the hepatic artery was identified. During the operation, however, two arteries were found entering the right posterior section. We preserved the vessels that nourished the right posterior section and resected the root of the left hepatic artery, the right anterior hepatic artery, and the common trunk of the left lateral portal vein and RUP; Next, the demarcation area was confirmed. The left side of the liver was fully mobilized, and the liver parenchyma was transected along the demarcation line; Finally, we cut the right posterior hepatic duct, and the specimen was removed. Hepaticojejunostomy to the right posterior bile duct and jejunojejunostomy were conducted, and the operation was successfully completed. The operative time was 697 min, and the estimated blood loss was 716 mL.

A histological examination showed moderately differen-



**Figure 1 Case 1 enhanced computed tomography.** A: Computed tomography shows the left-sided gallbladder and RUP; B: The right anterior and medial segmental portal branches ramify from the RUP after its trifurcation as well as the right posterior and left lateral branch; C: A 25-mm sized tumour peripherally enhanced in the arterial phase was detected in segment 8; D: Diagram of the intrahepatic portal vein branching and the location of the tumour. A: Right anterior portal vein; P: Right posterior portal vein; G: Gallbladder; M: Left medial portal vein; RUP: Right umbilical portion; L: Left lateral portal vein; T: Tumour; RL: Round ligament.

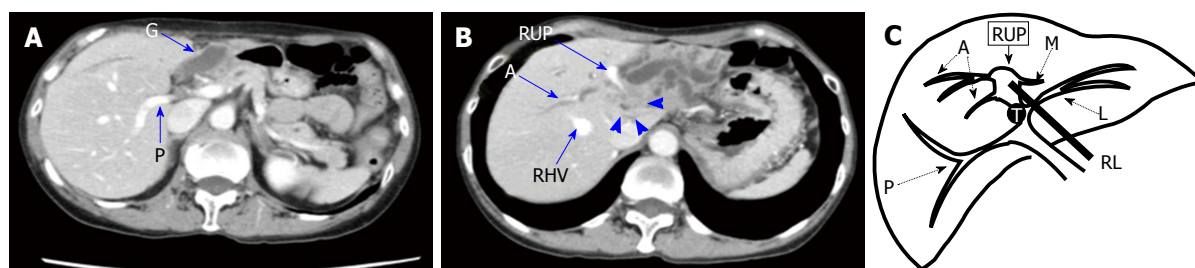


**Figure 2 Case 1 operative findings.** A: The gallbladder was attached to the round ligament; B: Three ramifications of the right anterior Glissonean pedicles were separated and clamped; C: Diagram of the clamped Glissonean pedicles (double line); D and E: The demarcation area (arrow head) was identified as in the preoperative simulation; F: The accomplishment of a right anterior sectionectomy. RL: Round ligament; G: Gallbladder; A: Right anterior branch of the Glissonean pedicle; P: Right posterior branch of the Glissonean pedicle; M: Left medial branch of the Glissonean pedicle; RUP: Right umbilical portion; L: Left lateral branch of the Glissonean pedicle; T: Tumour; RHV: Right hepatic vein.

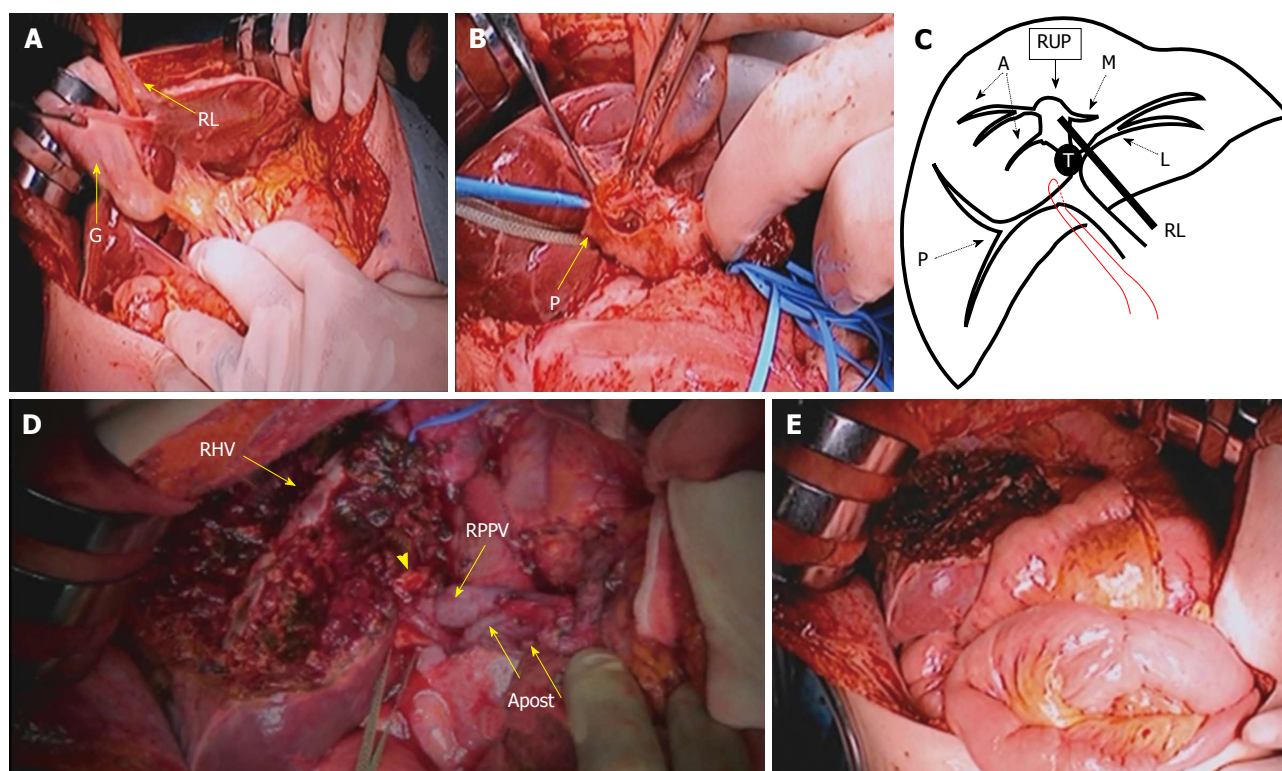
tiated cholangiocarcinoma, 30 mm in size that was invading the hepatic duct and the portal vein. Two lymph node

metastases were revealed. The patient was diagnosed as stage II B (T3N1M0). All of the surgical margins were





**Figure 3 Case 2 enhanced computed tomography.** A and B: CT shows the right posterior portal branch to be solely bifurcated, and the right anterior and medial segmental portal branches ramify from the RUP; B: A 25-mm sized mass (arrow head) is adjacent to the RUP. The RUP is almost occluded, and the intrahepatic distal bile duct is dilated (B); C: Diagram of the intrahepatic portal vein branching and the location of the tumour. RL: Round ligament; A: Right anterior branch of the Glissonean pedicle; P: Right posterior branch of the Glissonean pedicle; M: Left medial branch of the Glissonean pedicle; RUP: Right umbilical portion; L: Left lateral branch of the Glissonean pedicle; T: Tumour; RHV: Right hepatic vein.



**Figure 4 Case 2 operative findings.** A: The gallbladder was attached to the round ligament; B: The right posterior Glissonean pedicle was encircled, and the vessels entering the right posterior Glissonean pedicle were identified; C: Diagram of securing the right posterior branch of the Glissonean pedicle; D: The accomplishment of left trisectionectomy; E: Hepaticojejunostomy was performed. RL: Round ligament; G: Gallbladder; A: Right anterior branch of the Glissonean pedicle; P: Right posterior branch of the Glissonean pedicle; M: Left medial branch of the Glissonean pedicle; RUP: Right umbilical portion; L: Left lateral branch of the Glissonean pedicle; T: Tumour; RHV: Right hepatic vein; RPPV: Right posterior portal vein; Apost: Right posterior hepatic artery; Arrow-head: Stump of the right posterior bile duct.

negative. The postoperative course was uneventful and this patient was discharged on postoperative day 13.

## DISCUSSION

RUP, previously known as a left-sided gallbladder, is a rare congenital anomaly. However, we occasionally encounter it in our daily medical procedures (*e.g.*, cholecystectomy). RUP is an anatomical anomaly in which the umbilical portion exists between the right anterior and left medial section. The right-sided round ligament adheres to the RUP. Other theories exist regarding liver segmentation with RUP. One is that segment 4 is absent<sup>[5]</sup>. Another

is that the right side of the RUP is comparable with the dorsal segment of the right anterior section and the left side of the RUP with the ventral segment of the right anterior section<sup>[7]</sup>. In this report, we defined RUP as the umbilical portion that exists between the right anterior and left medial section. Nagai *et al.*<sup>[1]</sup> reviewed the literature concerning this anomaly and classified the type of portal branching according to bifurcation type and trifurcation type. Nineteen cases with RUP have undergone hepatectomy in the English-language literature<sup>[1,3,6,8-15]</sup> (Table 1). RUP is associated with anomalous ramifications of the hepatic artery, portal vein, and biliary system; thus, surgery for cases with

**Table 1** The reported patients with right umbilical portion who underwent hepatectomy in the English-language literature

Ref.	Age (yr)	Sex	Disease	Surgical procedure	Type of intrahepatic portal venous branching
Uesaka <i>et al</i> <sup>[8]</sup>	53	Male	Liver metastasis of bile duct cancer	Right hepatectomy	Trifurcation type
Idu <i>et al</i> <sup>[9]</sup>	Unknown	Male	Perihilar cholangiocarcinoma	Left hepatectomy	Unknown
Nagai <i>et al</i> <sup>[11]</sup>	67	Male	Bile duct cancer	Right anterior sectionectomy, segmentectomy 1 and pancreatoduodenectomy	Trifurcation type
Nagai <i>et al</i> <sup>[11]</sup>	67	Male	Hepatocellular carcinoma	Segmentectomy 8, and partial resection of segment 1	Trifurcation type
Asonuma <i>et al</i> <sup>[3]</sup>	48	Male	Living donor	Left lateral sectionectomy	Unknown
Asonuma <i>et al</i> <sup>[3]</sup>	29	Male	Living donor	Left lateral sectionectomy	Unknown
Asonuma <i>et al</i> <sup>[3]</sup>	35	Female	Living donor	Left lateral sectionectomy	Bifurcation type
Kaneoka <i>et al</i> <sup>[10]</sup>	53	Male	Perihilar cholangiocellular carcinoma	Left hepatectomy and segmentectomy 1 with extrahepatic bile duct resection	Trifurcation type
Kaneoka <i>et al</i> <sup>[10]</sup>	61	Male	Extrahepatic bile duct cholangiocarcinoma	Left hepatectomy, segmentectomy 1, and pylorus-preserving pancreaticoduodenectomy	Trifurcation type
Tashiro <i>et al</i> <sup>[11]</sup>	53	Male	Hepatocellular carcinoma	Partial hepatectomy	Trifurcation type
Hwang <i>et al</i> <sup>[12]</sup>	18	Male	Living donor	Right hepatectomy	Bifurcation type
Hwang <i>et al</i> <sup>[12]</sup>	24	Unknown	Living donor	Right posterior sectionectomy	Trifurcation type
Hwang <i>et al</i> <sup>[12]</sup>	39	Unknown	Living donor	Left hepatectomy leaving S4a	Bifurcation type
Hsu <i>et al</i> <sup>[6]</sup>	Unknown	Unknown	Hepatocellular carcinoma	Right hepatectomy	Trifurcation type
Hsu <i>et al</i> <sup>[6]</sup>	Unknown	Unknown	Hepatocellular carcinoma	Partial resection of left lateral section	Trifurcation type
Hsu <i>et al</i> <sup>[6]</sup>	Unknown	Unknown	Hepatocellular carcinoma	Left lateral sectionectomy	Bifurcation type
Abe <i>et al</i> <sup>[13]</sup>	70	Female	Liver metastasis of uterine cervical cancer	Right hepatectomy with extrahepatic bile duct resection	Bifurcation type
Sakaguchi <i>et al</i> <sup>[14]</sup>	76	Male	Liver metastasis of rectal cancer	Right posterior sectionectomy and partial resection of segment 1 and right anterior section	Trifurcation type
Almodhaiberi <i>et al</i> <sup>[15]</sup>	67	Male	Perihilar cholangiocarcinoma	Extended left lateral sectionectomy and segmentectomy 1 with extrahepatic bile duct resection	Trifurcation type
Case 1	70	Male	Intrahepatic cholangiocarcinoma	Right anterior sectionectomy	Trifurcation type
Case 2	70	Female	Perihilar cholangiocarcinoma	Left trisectionectomy with extrahepatic bile duct resection	Trifurcation type

RUP requires careful procedures, especially with regard to hepatic resection. Previous reports described the importance of the thorough preoperative and intraoperative recognition of the various anomalies associated with RUP to prevent operative accidents.

CT and three-dimensional imaging have been developed, and preoperative simulation is of great help. We must preoperatively evaluate and recognize the anatomy precisely in cases with this anomaly. However, some vessels go unrecognized during the preoperative survey but can be encountered during the procedure, as was observed in case 2. Thus, paying special attention during the operation is important.

The Glissonean approach is acknowledged as a potentially successful technique for liver surgery, and it is widely performed for liver resection. The ramification pattern of the hepatic artery, portal vein and bile duct in the hepatoduodenal ligament often varies across patients. However, the Glissonean pedicle peripheral to the hilar plate, which is wrapped by connective tissue and contains the hepatic artery, portal vein, and bile duct, enters its proper area and never contains branches that nourish other areas. Consequently, the Glissonean pedicle transection peripheral to the extrahepatic hilar plate is a safe and sure method that enables the cutting of the intended vessels without damaging the vessels to

be preserved. Secondary and tertiary branches of the Glissonean pedicle peripheral to the hilar plate can usually be approached and transected extrahepatically. When the targeted Glissonean pedicle is transiently and selectively clamped, we can recognize the area to be resected. Surgeons do not have to consider any variations in the hepatoduodenal ligament. The Glissonean approach is a successful method, especially in cases with anomalous ramifications of the hepatic artery, portal vein and biliary system. The Glissonean pedicle to be resected was separated in case 1, whereas that to be preserved was encircled in case 2. The Glissonean approach can be used in various situations of hepatic resection and it contributes to a safe and secure liver surgery.

In conclusion, we successfully performed two major hepatectomies using the Glissonean approach in cases with RUP. The Glissonean approach is a useful method and contributes to a safe procedure for cases with an anomalous anatomy such as RUP.

## COMMENTS

### Case characteristics

A 70-year-old man with hepatitis C presented with a liver tumour without any symptoms; a 70-year-old woman presented with general fatigue and intrahepatic bile duct dilatation.



## Clinical diagnosis

Intrahepatic cholangiocarcinoma or combined hepatocellular and cholangiocarcinoma of the right umbilical portion (RUP); perihilar cholangiocarcinoma of the RUP.

## Differential diagnosis

Metastatic liver tumour; intrahepatic cholangiocarcinoma and inflammatory biliary stenosis.

## Laboratory diagnosis

The level of tumour marker PIVKA-II was elevated at 808 mAU/mL; other tumour markers were normal; the level of tumour marker CA19-9 was elevated at 843.6 U/mL; other tumour markers were normal.

## Imaging diagnosis

A computed tomography (CT) scan showed RUP and a 25-mm sized tumour peripherally enhanced in the arterial phase in segment 8; a CT scan showed RUP and a 25-mm sized tumour in the left side of the perihilar region, which caused dilatation of intrahepatic distal bile duct and almost occluded the RUP.

## Pathological diagnosis

A pathological examination showed a poorly differentiated intrahepatic cholangiocarcinoma invading the intrahepatic portal vein; the pathological findings revealed a moderately differentiated cholangiocarcinoma invading RUP.

## Treatment

The patient was treated with right anterior sectionectomy; the patient was treated with left trisectionectomy.

## Related reports

Only nineteen cases of hepatectomy among patients with RUP have been reported in the English-language literature.

## Term explanation

RUP is a congenital anomaly in which the umbilical portion exists between the right anterior section and left medial section.

## Experiences and lessons

This report emphasizes that the Glissonean approach is useful, especially in cases with anomalous ramifications of the hepatic artery, portal vein and biliary system such as RUP. This procedure contributes to a safe and secure liver surgery.

## Peer-review

This paper is the first report about major hepatectomy using the Glissonean approach in cases with RUP, and demonstrates the safety and usefulness of the Glissonean approach for hepatectomy in cases with anomalies such as RUP, and this report is very important guidance for surgeons who perform major hepatectomy for cases with RUP.

## REFERENCES

- 1 Nagai M, Kubota K, Kawasaki S, Takayama T, Bandai Y M. Are left-sided gallbladders really located on the left side? *Ann Surg* 1997; **225**: 274-280 [PMID: 9060583 DOI: 10.1097/00000658-199703000-00006]
- 2 Maetani Y, Itoh K, Kojima N, Tabuchi T, Shibata T, Asonuma

- K, Tanaka K, Konishi J. Portal vein anomaly associated with deviation of the ligamentum teres to the right and malposition of the gallbladder. *Radiology* 1998; **207**: 723-728 [PMID: 9609896 DOI: 10.1148/radiology.207.3.9609896]
- 3 Asonuma K, Shapiro AM, Inomata Y, Uryuhara K, Uemoto S, Tanaka K. Living related liver transplantation from donors with the left-sided gallbladder/portal vein anomaly. *Transplantation* 1999; **68**: 1610-1612 [PMID: 10589965 DOI: 10.1097/00007890-199911270-00031]
- 4 Baba Y, Hokotate H, Nishi H, Inoue H, Nakajo M. Intrahepatic portal venous variations: demonstration by helical CT during arterial portography. *J Comput Assist Tomogr* 2000; **24**: 802-808 [PMID: 11045706 DOI: 10.1097/00004728-200009000-00024]
- 5 Savier E, Taboury J, Lucidarme O, Kitajima K, Cadi M, Vaillant JC, Hannoun L. Fusion of the planes of the liver: an anatomic entity merging the midplane and the left intersectional plane. *J Am Coll Surg* 2005; **200**: 711-719 [PMID: 15848361 DOI: 10.1016/j.jamcollsurg.2004.12.017]
- 6 Hsu SL, Chen TY, Huang TL, Sun CK, Concejero AM, Tsang LL, Cheng YF. Left-sided gallbladder: its clinical significance and imaging presentations. *World J Gastroenterol* 2007; **13**: 6404-6409 [PMID: 18081230]
- 7 Gupta R, Miyazaki A, Cho A, Ryu M. Portal vein branching pattern in anomalous right-sided round ligament. *Abdom Imaging* 2010; **35**: 332-336 [PMID: 19396389 DOI: 10.1007/s00261-009-9520-0]
- 8 Uesaka K, Yasui K, Morimoto T, Torii A, Kodera Y, Hirai T, Yamamura Y, Kato T, Kito T. Left-sided gallbladder with intrahepatic portal venous anomalies. *J Hep Bil Pancr Surg* 1995; **2**: 425-430 [DOI: 10.1007/BF02349262]
- 9 Idu M, Jakimowicz J, Iuppa A, Cuschieri A. Hepatobiliary anatomy in patients with transposition of the gallbladder: implications for safe laparoscopic cholecystectomy. *Br J Surg* 1996; **83**: 1442-1443 [PMID: 8944467 DOI: 10.1002/bjs.1800831037]
- 10 Kaneoka Y, Yamaguchi A, Isogai M, Harada T. Hepatectomy for cholangiocarcinoma complicated with right umbilical portion: anomalous configuration of the intrahepatic biliary tree. *J Hepatobiliary Pancreat Surg* 2000; **7**: 321-326 [PMID: 10982634 DOI: 10.1007/s005340000070321.534]
- 11 Tashiro H, Itamoto T, Nakahara H, Ohdan H, Kobayashi T, Asahara T. Resection of hepatocellular carcinoma in a patient with congenital anomaly of the portal system. *Dig Surg* 2003; **20**: 163-165 [PMID: 12686785 DOI: 10.1159/000069385]
- 12 Hwang S, Lee SG, Park KM, Lee YJ, Ahn CS, Kim KH, Moon DB, Ha TY, Cho SH, Oh KB. Hepatectomy of living donors with a left-sided gallbladder and multiple combined anomalies for adult-to-adult living donor liver transplantation. *Liver Transpl* 2004; **10**: 141-146 [PMID: 14755792 DOI: 10.1002/lt.20007]
- 13 Abe T, Kajiyama K, Harimoto N, Gion T, Shirabe K, Nagaie T. Resection of metastatic liver cancer in a patient with a left-sided gallbladder and intrahepatic portal vein and bile duct anomalies: A case report. *Int J Surg Case Rep* 2012; **3**: 147-150 [PMID: 22365920 DOI: 10.1016/j.ijscr.2012.01.003]
- 14 Sakaguchi T, Suzuki S, Morita Y, Oishi K, Suzuki A, Fukumoto K, Inaba K, Takehara Y, Baba S, Nakamura S, Konno H. Hepatectomy for metastatic liver tumors complicated with right umbilical portion. *Hepatogastroenterology* 2011; **58**: 984-987 [PMID: 21830428]
- 15 Almodhaiberi H, Hwang S, Cho YJ, Kwon Y, Jung BH, Kim MH. Customized left-sided hepatectomy and bile duct resection for perihilar cholangiocarcinoma in a patient with left-sided gallbladder and multiple combined anomalies. *Korean J Hepatobiliary Pancreat Surg* 2015; **19**: 30-34 [PMID: 26155274 DOI: 10.14701/kjhbbs.2015.19.1.30]

P- Reviewer: Bramhall S, Lau WYJ, Qin JM S- Editor: Qiu S  
L- Editor: A E- Editor: Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 December 18; 8(35): 1541-1592





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*



**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vyslouzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*

**MINIREVIEWS**

- 1541 How to assess the efficacy or failure of targeted therapy: Deciding when to stop sorafenib in hepatocellular carcinoma

*Raoul JL, Adhoute X, Gilabert M, Edeline J*

**ORIGINAL ARTICLE****Basic Study**

- 1547 Primary liver injury and delayed resolution of liver stiffness after alcohol detoxification in heavy drinkers with the *PNPLA3* variant I148M

*Rausch V, Peccerella T, Lackner C, Yagmur E, Seitz HK, Longerich T, Mueller S*

**Retrospective Cohort Study**

- 1557 Hepatitis C eradication with sofosbuvir leads to significant metabolic changes

*Morales AL, Junga Z, Singla MB, Sjogren M, Torres D*

**Retrospective Study**

- 1564 Is cirrhosis associated with lower odds of ischemic stroke: A nationwide analysis?

*Goyal A, Chatterjee K, Shah N, Singh S*

**Prospective Study**

- 1569 Immune function biomarker QuantiFERON-monitor is associated with infection risk in cirrhotic patients

*Sood S, Yu L, Visvanathan K, Angus PW, Gow PJ, Testro AG*

**SYSTEMATIC REVIEWS**

- 1576 Intrahepatic pancreatic pseudocyst: A review of the world literature

*Demeusy A, Hosseini M, Sill AM, Cunningham SC*

**META-ANALYSIS**

- 1584 *PNPLA3* polymorphism increases risk for and severity of chronic hepatitis C liver disease

*Salameh H, Masadeh M, Al Hanayneh M, Petros V, Maslonka M, Nanda A, Singal AK*



**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Ahmed O Kaseb, MD, Associate Professor, Department of Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/ABSTRACTING**

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Department of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL BOARD MEMBERS**  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

**EDITORIAL OFFICE**  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
December 18, 2016

**COPYRIGHT**  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
<http://www.wjgnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esps/>

## How to assess the efficacy or failure of targeted therapy: Deciding when to stop sorafenib in hepatocellular carcinoma

Jean-Luc Raoul, Xavier Adhoute, Marine Gilabert, Julien Edeline

Jean-Luc Raoul, Marine Gilabert, Department of Medical Oncology, Paoli-Calmettes Institute, 13273 Marseille, France

Xavier Adhoute, Department of Hepatology, Hopital Saint-Joseph, 13008 Marseille, France

Julien Edeline, Department of Medical Oncology, Centre E Marquis, Bd de la Bataille Frandres-Dunkerque, 35043 Rennes Cedex, France

**Author contributions:** Raoul JL, Adhoute X, Gilabert M and Edeline J wrote the paper and approved its content.

**Conflict-of-interest statement:** Raoul JL has received consultancy fees from Bayer, Taiho, and BTG; Adhoute X has received consultancy fees from Bayer; Gilabert M and Edeline J have no potential conflicts of interest to disclose.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Jean-Luc Raoul, Professor, Department of Medical Oncology, Paoli-Calmettes Institute, BP 156, 13273 Marseille, France. [raouljl@ipc.unicancer.fr](mailto:raouljl@ipc.unicancer.fr)  
Telephone: +33-4-91223679  
Fax: +33-4-91223670

Received: March 15, 2016

Peer-review started: March 18, 2016

First decision: April 18, 2016

Revised: September 20, 2016

Accepted: November 1, 2016

Article in press: November 2, 2016

Published online: December 18, 2016

### Abstract

Sorafenib is thus far the only systemic treatment for hepatocellular carcinoma (HCC) based on the results of two randomized controlled trials performed in Western and in Eastern countries, despite a poor response rate (from 2% to 3.3%) following conventional evaluation criteria. It is now recognized that the criteria (European Association of the Study of the Liver criteria, modified response evaluation criteria in solid tumors) based on contrast enhanced techniques (computed tomography scan, magnetic resonance imaging) aimed to assess the evolution of the viable part of the tumor (hypervascularized on arterial phase) are of major interest to determine the efficacy of sorafenib and of most antiangiogenic drugs in patients with HCC. The role of alpha-fetoprotein serum levels remains unclear. In 2016, in accordance with the SHARP and the Asia-Pacific trials, sorafenib must be stopped when tolerance is poor despite dose adaptation or in cases of radiological and symptomatic progression. This approach will be different in cases of available second-line therapy trials. Some recent data (in renal cell carcinoma) revealed that despite progression in patients who received sorafenib, this drug can still decrease tumor progression compared to drug cessation. Then, before deciding to continue sorafenib post-progression or shift to another drug, knowing other parameters of post-progression survival (Child-Pugh class, Barcelona Clinic Liver Cancer, alpha-fetoprotein, post-progression patterns in particular, the development of extrahepatic metastases and of portal vein thrombosis) will be of major importance.

**Key words:** Tumor evaluation; Response evaluation criteria in solid tumors; Sorafenib; Hepatocellular

carcinoma; Modified response evaluation criteria in solid tumors

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The response rate of sorafenib in hepatocellular carcinoma is low using standard parameters and is better assessed using new criteria based on tumor vascularization (European Association of the Study of the Liver criteria, modified response evaluation criteria in solid tumors). In case of minor progression, if sorafenib is well tolerated, knowing the predictors of post-progression survival will be of value in deciding whether to continue or stop sorafenib.

Raoul JL, Adhoute X, Gilabert M, Edeline J. How to assess the efficacy or failure of targeted therapy: Deciding when to stop sorafenib in hepatocellular carcinoma. *World J Hepatol* 2016; 8(35): 1541-1546 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i35/1541.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i35.1541>

## INTRODUCTION

In the majority of solid tumors, assessing the efficacy or failure of a systemic treatment is based on the tumor size, which is measured either bidimensionally using the World Health Organization criteria (WHO) criteria or unidimensionally using response evaluation criteria in solid tumors (RECIST). The response rate is used as a surrogate marker of drug efficacy in clinical trials, and in clinical practice, the evolution of tumor size is a major parameter to decide whether to stop or continue treatment. In a palliative setting, a treatment is continued as long as the disease is controlled (stable disease or response) and the regimen is tolerated. This approach is less simple with targeted therapies. In gastrointestinal stromal tumors, the efficacy of imatinib was associated with modifications in tumor content and not always with a decrease in tumor size. That finding leads researchers to propose new response criteria<sup>[1]</sup> not only based on tumor size but also based on combining size and density shown on computed tomography (CT) scans. The efficacy of bevacizumab that is associated with chemotherapy is also underestimated under the standard criteria. In a large series, Chun *et al*<sup>[2]</sup> demonstrated that CT scan-based morphologic criteria correlated better with the histological response than the response by RECIST in patients with liver metastases of colorectal cancer treated with bevacizumab-containing chemotherapy. In hepatocellular carcinoma (HCC), despite a low and disappointing response rate (2%) using conventional criteria in a phase II trial<sup>[3]</sup>, sorafenib is thus far the only systemic treatment<sup>[4,5]</sup> that has been demonstrated to improve overall survival. In this era of great expectations regarding new drugs, we would like

to briefly review these response evaluation criteria used in patients with HCC and the determination of when to continue or stop sorafenib treatment.

## CONVENTIONAL CRITERIA FOR EVALUATING TUMOR RESPONSE: WHO AND RECIST

The WHO criteria for defining a response to treatment are based on bidimensionally measured lesions (*i.e.*, the product of the greatest tumor diameter and the greatest perpendicular distance summed over all measured tumors). The RECIST guidelines were published in 2000, with the major change being that the RECIST 1.0 uses unidimensional measurements of the sum of the longest diameters of the tumors. All unmeasurable lesions are considered to be "non-target" lesions, and lymph nodes are not distinguished from extranodal lesions. Progression is defined by an increase of at least 20% of the sum of the longest diameter and the appearance of new lesions or the progression of a non-target lesion. In 14 studies, the application of the WHO criteria and RECIST to the same patients with a large range of cancers has shown similar results<sup>[6]</sup>. A few years later, the RECIST 1.1 criteria were published<sup>[7]</sup>, which better defined the minimal target size and reduced the number of allowed target lesions to 2 per organ and to a total of 5. It was also stated that a lymph node was considered as a target only if the short axis was larger than 15 mm. Ascites, pleural effusion, and lymph nodes from 10 mm to 14 mm on the short axis were considered as non-measurable lesions. Progression of non-target lesions was, by definition, considered to be a sign of disease progression. In a comparison of RECIST 1.0 with RECIST 1.1 in patients with lung cancer treated by erlotinib, the latter group demonstrated a slightly better performance<sup>[8]</sup>.

However, all these criteria were subject to failure in HCC. Ascites or pleural effusions are usually related to the underlying liver cirrhosis, lymph nodes are frequent and may be large in the case of viral hepatitis, and the appearance of non/malignant small liver nodules is common. Moreover, most non-surgical treatments target tumor vascularization (chemo-embolization, radio-embolization, antiangiogenic drugs), and efficacy might be poorly reflected by size only.

## NEW CRITERIA SPECIFICALLY DEDICATED TO HCC

Thus, new, more appropriate criteria were required to assess treatment efficacy in patients with HCC. European Association of the Study of the Liver (EASL) criteria were introduced during the EASL conference in Barcelona in 2000. They were based on bidimensional WHO criteria and the targeting of viable tumors, which were defined as those that showed contrast material-enhancing areas in the arterial phase of a dynamic CT

scan<sup>[9]</sup>. These criteria were later adapted to RECIST<sup>[10]</sup>; in addition to this new definition of target lesions, non-target lesions were revisited, and new hepatic nodules were considered as evidence of progression only if they had typical imaging and a longest diameter of at least 10 mm. Cytopathological confirmation of the neoplastic nature of any effusion that appeared or worsened was required. These new parameters, named modified RECIST (mRECIST), were considered to be a better tool for assessing HCC tumors<sup>[11]</sup>. Several Japanese authors proposed response evaluation criteria in cancer of the liver (RECICL), based on the bidirectional measurement of tumors showing arterial enhancement and considering non-hypervascularized tumors<sup>[12]</sup>. In a series of 156 patients receiving sorafenib for more than 30 d, response rates and the evaluation of overall survival by mRECIST and RECICL were similar. Recently, mRECIST was prospectively validated<sup>[13]</sup> in a phase 3 study (brivanib in second-line treatment). In this study comparing 395 patients who progressed after sorafenib was administered or were intolerant (brivanib to placebo; 2:1 ratio), tumor assessment every 6 wk by contrast-enhanced CT or magnetic resonance imaging was performed by a central review using mRECIST. A partial response was achieved in 8% of patients who received brivanib and 2% of patients who received placebo; the median overall survival was 16.4 mo for mRECIST responders and 8.3 mo for non-responders, and mRECIST evaluation had a prognostic value in multiparametric analysis.

Another way to evaluate tumor vascularization is contrast-enhanced ultrasound. In a short series of 19 patients (16 who received sorafenib and 3 who received sunitinib), this technique seemed effective at distinguishing progressors from non-progressors at 1 mo<sup>[14]</sup>. In a prospective series of 37 patients treated with sorafenib and explored by contrast-enhanced ultrasound before treatment and on days 7, 14 and 28, Sugimoto *et al.*<sup>[15]</sup> found that this technique was not only predictive of tumor response (tumor vascularization) but also of major adverse events (liver parenchyma vascularization). Additional data are still necessary to validate these results.

The impact of alpha-fetoprotein (AFP) evaluation is unclear. In a series of 72 patients who had an elevated baseline AFP and were treated with different antiangiogenic drugs (thalidomide, bevacizumab), a decline of > 20% from the baseline AFP level within the first 4 wk (early AFP response) was associated with a higher response rate and a longer PFS and OS<sup>[16]</sup>. In contrast, in patients who received brivanib<sup>[17]</sup>, a longer survival rate was not associated with either an early AFP response (*i.e.*, a decrease by more than 20% from baseline within the first 4 week) or an AFP response (*i.e.*, an AFP decrease by more than 50% from baseline). In a Japanese retrospective study<sup>[18]</sup>, the best way to assess prognosis was a combination of mRECIST and AFP ratio (AFP under treatment/AFP before treatment), but this ratio (< or > 1) was only associated with survival at 8 wk.

## COMPARISON OF THESE RESPONSE EVALUATION CRITERIA IN HCC CASES

After transarterial chemoembolization (TACE) and percutaneous ablation in 55 patients, Forner *et al.*<sup>[19]</sup> demonstrated that RECIST missed all complete responses (including patients treated by curative options) and underestimated the extent of tissue necrosis. The authors concluded that RECIST should not be used and that dynamic imaging techniques and evaluations must become the standard for assessing treatment efficacy. In a series of 143 patients with HCC who underwent TACE, a comparison of various response criteria showed that volumetric functional imaging is better correlated with outcome than other parameters and that AFP serum levels<sup>[20]</sup> and new 3D-imaging approaches are of great value in differentiating the responders from the non-responders to TACE<sup>[21]</sup> and can be used early to predict outcome after initial TACE. Shim *et al.*<sup>[22]</sup> compared WHO, RECIST, EASL and mRECIST in a cohort of 332 patients with intermediate HCC treated by TACE. They concluded that the enhancement models (EASL guidelines and mRECIST) were the best independent predictors of overall survival after chemoembolization. Similarly, the same results were found in an English series of 83 patients<sup>[23]</sup>. Thus, measuring the viable part of the tumor seems to be the best option after loco-regional treatment of HCC.

In the seminal SHARP<sup>[4]</sup> and AP<sup>[5]</sup> trials, the response rates using RECIST were 2% and 3.3% for patients who received sorafenib and 1% and 1.3% for those who received placebo, respectively; however, the overall survival analysis was clearly in favor of sorafenib, showing a discrepancy between the response rate by RECIST and outcome, with sorafenib efficacy being related to an increase in the time to progression. Many retrospective series have analyzed tumor responses using different criteria for patients receiving sorafenib. Their common features were that the evaluation of the viable part of the tumor based on arterial enhancement provided better results than the usual parameters and showed a real response rate and, thus, should be used for assessing treatment efficacy. Edeline *et al.*<sup>[24]</sup>, in a series of 53 patients, determined that 1 out of 10 patients considered as PD by RECIST was scored as SD using mRECIST. Forty-two patients evaluated as stable by RECIST were reassessed as complete response in 1 case, partial response in 10 cases, SD in 29 cases and PD in 2 cases using mRECIST. Then, the objective response rate of 1.9% by RECIST increased to 22.6% with mRECIST. The mRECIST result was associated with outcome, as those initially considered as SD by RECIST but as responders ( $n = 11$ ), stable ( $n = 29$ ) or progressive ( $n = 2$ ) by mRECIST had different median overall survival rates of 17.1, 9.7 and 3.7 mo, respectively. However, there was no difference between these two criteria regarding the median time to progression. Another retrospective study<sup>[25]</sup> compared RECIST 1.1 with vascularization-based criteria



**Table 1** Parameters of post-progression survival for patients receiving sorafenib

Performance status
Child-Pugh class
BCLC class
CLIP score
Macroscopic venous invasion
AFP serum level
TTP on sorafenib
Pattern of progression

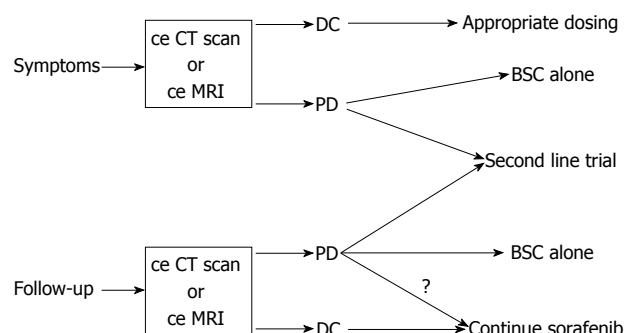
BCLC: Barcelona Clinic Liver Cancer staging classification; CLIP: Cancer of the Liver Italian Program; AFP: Alpha-fetoprotein; TTP: Time to progression.

(Choi criteria, EASL criteria, and mRECIST). The response rates were 3%, 51%, 28% and 28%, respectively, in a cohort of 64 patients treated using sorafenib. The tumor response following RECIST 1.1 did not correlate well with the overall survival rate, whereas other criteria were more appropriate to identify responders with longer survival rates. In two phase II trials (101 patients) evaluating brivanib, an independent review compared the outcomes between the WHO criteria and mRECIST<sup>[17]</sup>. The response rates were higher with mRECIST vs WHO in both cohorts, and PD assessed by mRECIST, was associated with a poorer overall survival rate than when assessed using the WHO criteria.

Thus, these vascularization-based criteria are better than size-only criteria to categorize responders. However, the essential problem exists: How do we define when sorafenib treatment is no longer effective? Progression can be related to an increase in tumor size (or of its viable part) and also to the appearance of new liver nodules (considering vascularization, size, and evolution), effusion and ascites (cytology required), and lymph nodes (size and vascularization). These parameters are listed in a recent paper from the BCLC<sup>[26]</sup>. However, is progression a strict criterion to stop sorafenib treatment?

## IN 2016, WHEN TO STOP SORAFENIB?

In the SHARP trial, treatment was continued until both radiological and symptomatic progression or unacceptable toxicity occurred. In our experience, many patients seem to clinically benefit from the drug despite progression; in clinical practice, progression is not always a clear indication to stop sorafenib, particularly if there is no second-line trial available. In patients with poor prognostic factors at progression (worsening of performance status or of Child-Pugh status), cessation of the drug is recommended. In contrast, if the patients are candidates for second-line therapies, then inclusion is the best option if available. In other cases, we can postulate that sorafenib may retain some efficacy in certain instances despite tumor progression and that cessation of the drug might lead to an acceleration of tumor growth. In metastatic renal cell cancer, some data



**Figure 1** Proposed algorithm for deciding to continue or stop sorafenib in patients with hepatocellular carcinoma. ce CT scan: Contrast-enhanced computed tomography scan; ce MRI: Contrast-enhanced magnetic resonance imaging; DC: Disease controlled; PD: Progressive disease; BSC: Best supportive care.

show that, at progression, the tumor growth rate is lower than before initiation of the treatment using sorafenib and lower than will be observed after cessation of the drug. More interestingly, in renal cell carcinoma, this persistent activity beyond progression with an apparent flare-up effect after drug discontinuation of the drug was observed only with sorafenib and not with everolimus<sup>[27]</sup>. Then, even after progression, this treatment can participate in slowing down the disease. However, continuing sorafenib treatment after progression can be of interest only for patients who have a reasonable life expectancy and an excellent tolerance of the drug. Analysis of post-progression survival (Table 1) showed that, in addition to performance status, Child-Pugh score, and macrovascular invasion at progression, some other parameters are valuable. These include AFP, time to progression (correlation between time to progression using sorafenib and post-progression survival)<sup>[28]</sup>, and pattern of progression<sup>[29]</sup>. Post-progression survival is significantly worse for patients who developed new extrahepatic lesions compared to patients with intra- or extra-hepatic growth or new intrahepatic lesions. These data in a Spanish cohort were later confirmed in Asian patients<sup>[30,31]</sup>. Thus, continuing sorafenib is a possibility if second-line trials are unavailable or if the patient cannot be included. This is particularly relevant for patients who had mild intrahepatic progression, who had a good PS with no worsening in BCLC or the Child-Pugh scores, and who had progressed very slowly (Figure 1).

## CONCLUSION

Contrast-enhanced imaging techniques using mRECIST criteria are the best objective approach to appreciate the efficacy of vascularization targeting agents, particularly sorafenib. The value of AFP serum levels is not clear and not sufficient to impact therapeutic decisions. The enrollment of progressing patients in second-line trials is the best option. If this is not possible, then sorafenib must be discontinued if patients have poor prognostic factors or poor tolerance. In contrast, if patients do not have worsening PS or Child-Pugh classification or

if macrovascular invasion occurs, then sorafenib can be pursued; however, we must consider the important prognostic values of the progression pattern.

## REFERENCES

- 1 **Choi H.** Critical issues in response evaluation on computed tomography: lessons from the gastrointestinal stromal tumor model. *Curr Oncol Rep* 2005; **7**: 307-311 [PMID: 15946591 DOI: 10.1007/s11912-005-0055-4]
- 2 **Chun YS,** Vauthey JN, Boonsirikamchai P, Maru DM, Kopetz S, Palavecino M, Curley SA, Abdalla EK, Kaur H, Charnsangavej C, Loyer EM. Association of computed tomography morphologic criteria with pathologic response and survival in patients treated with bevacizumab for colorectal liver metastases. *JAMA* 2009; **302**: 2338-2344 [PMID: 19952320 DOI: 10.1001/jama.2009.1755]
- 3 **Abou-Alfa GK,** Schwartz L, Ricci S, Amadori D, Santoro A, Figer A, De Greve J, Douillard JY, Lathia C, Schwartz B, Taylor I, Moscovici M, Saltz LB. Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2006; **24**: 4293-4300 [PMID: 16908937 DOI: 10.1200/JCO.2005.01.3441]
- 4 **Llovet JM,** Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]
- 5 **Cheng AL,** Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]
- 6 **Therasse P,** Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**: 205-216 [PMID: 10655437 DOI: 10.1093/jnci/92.3.205]
- 7 **Eisenhauer EA,** Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; **45**: 228-247 [PMID: 19097774 DOI: 10.1016/j.ejca.2008.10.026]
- 8 **Nishino M,** Jackman DM, Hatabu H, Yeap BY, Cioffredi LA, Yap JT, Jänne PA, Johnson BE, Van den Abbeele AD. New Response Evaluation Criteria in Solid Tumors (RECIST) guidelines for advanced non-small cell lung cancer: comparison with original RECIST and impact on assessment of tumor response to targeted therapy. *AJR Am J Roentgenol* 2010; **195**: W221-W228 [PMID: 20729419 DOI: 10.2214/AJR.09.3928]
- 9 **Bruix J,** Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607 DOI: 10.1016/S0168-8278(01)00130-1]
- 10 **Llovet JM,** Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, Schwartz M, Lotze M, Talwalkar J, Gores GJ. Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 698-711 [PMID: 18477802 DOI: 10.1093/jnci/djn134]
- 11 **Lencioni R,** Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: 20175033 DOI: 10.1055/s-0030-1247132]
- 12 **Arizumi T,** Ueshima K, Takeda H, Osaki Y, Takita M, Inoue T, Kitai S, Yada N, Hagiwara S, Minami Y, Sakurai T, Nishida N, Kudo M. Comparison of systems for assessment of post-therapeutic response to sorafenib for hepatocellular carcinoma. *J Gastroenterol* 2014; **49**: 1578-1587 [PMID: 24499826 DOI: 10.1007/s00535-014-0936-0]
- 13 **Lencioni R,** Park JW, Torres F. Objective response by mRECIST predicts survival in hepatocellular carcinoma: a multivariate, time-dependent analysis from the phase 3 BRISK-PS study. *ILCA 2015*
- 14 **Frampas E,** Lassau N, Zappa M, Vullierme MP, Koscielny S, Vilgrain V. Advanced Hepatocellular Carcinoma: early evaluation of response to targeted therapy and prognostic value of Perfusion CT and Dynamic Contrast Enhanced-Ultrasound. Preliminary results. *Eur J Radiol* 2013; **82**: e205-e211 [PMID: 23273822 DOI: 10.1016/j.ejrad.2012.12.004]
- 15 **Sugimoto K,** Moriyasu F, Saito K, Rognin N, Kamiyama N, Furuichi Y, Imai Y. Hepatocellular carcinoma treated with sorafenib: early detection of treatment response and major adverse events by contrast-enhanced US. *Liver Int* 2013; **33**: 605-615 [PMID: 23305331 DOI: 10.1111/liv.12098]
- 16 **Shao YY,** Lin ZZ, Hsu C, Shen YC, Hsu CH, Cheng AL. Early alpha-fetoprotein response predicts treatment efficacy of antiangiogenic systemic therapy in patients with advanced hepatocellular carcinoma. *Cancer* 2010; **116**: 4590-4596 [PMID: 20572033 DOI: 10.1002/cncr.25257]
- 17 **Raoul JL,** Park JW, Kang YK, Finn RS, Kim JS, Yeo W, Polite BN, Chao Y, Walters I, Baudelet C, Lencioni R. Using Modified RECIST and Alpha-Fetoprotein Levels to Assess Treatment Benefit in Hepatocellular Carcinoma. *Liver Cancer* 2014; **3**: 439-450 [PMID: 26280005 DOI: 10.1159/000343872]
- 18 **Kawaoka T,** Aikata H, Murakami E, Nakahara T, Naeshiro N, Tanaka M, Honda Y, Miyaki D, Nagaoki Y, Takaki S, Hiramatsu A, Waki K, Takahashi S, Chayama K. Evaluation of the mRECIST and  $\alpha$ -fetoprotein ratio for stratification of the prognosis of advanced-hepatocellular-carcinoma patients treated with sorafenib. *Oncology* 2012; **83**: 192-200 [PMID: 22890083 DOI: 10.1159/000341347]
- 19 **Forner A,** Ayuso C, Varela M, Rimola J, Hessheimer AJ, de Lope CR, Reig M, Bianchi L, Llovet JM, Bruix J. Evaluation of tumor response after locoregional therapies in hepatocellular carcinoma: are response evaluation criteria in solid tumors reliable? *Cancer* 2009; **115**: 616-623 [PMID: 19117042 DOI: 10.1002/cncr.24050]
- 20 **Bonekamp S,** Halappa VG, Geschwind JF, Li Z, Corona-Villalobos CP, Reyes D, Bhagat N, Cosgrove DP, Pawlik TM, Mezey E, Eng J, Kamel IR. Unresectable hepatocellular carcinoma: MR imaging after intraarterial therapy. Part II. Response stratification using volumetric functional criteria after intraarterial therapy. *Radiology* 2013; **268**: 431-439 [PMID: 23616632 DOI: 10.1148/radiol.13121637]
- 21 **Tacher V,** Lin M, Duran R, Yarmohammadi H, Lee H, Chapiro J, Chao M, Wang Z, Frangakis C, Sohn JH, Maltenfort MG, Pawlik T, Geschwind JF. Comparison of Existing Response Criteria in Patients with Hepatocellular Carcinoma Treated with Transarterial Chemoembolization Using a 3D Quantitative Approach. *Radiology* 2016; **278**: 275-284 [PMID: 26131913 DOI: 10.1148/radiol.2015.142951]
- 22 **Shim JH,** Lee HC, Kim SO, Shin YM, Kim KM, Lim YS, Suh DJ. Which response criteria best help predict survival of patients with hepatocellular carcinoma following chemoembolization? A validation study of old and new models. *Radiology* 2012; **262**: 708-718 [PMID: 22187634 DOI: 10.1148/radiol.11110282]
- 23 **Gillmore R,** Stuart S, Kirkwood A, Hameeduddin A, Woodward N, Burroughs AK, Meyer T. EASL and mRECIST responses are independent prognostic factors for survival in hepatocellular cancer patients treated with transarterial embolization. *J Hepatol* 2011; **55**: 1309-1316 [PMID: 21703196 DOI: 10.1016/j.jhep.2011.03.007]
- 24 **Edeline J,** Boucher E, Rolland Y, Vaulon E, Pracht M, Perrin C, Le Roux C, Raoul JL. Comparison of tumor response by Response Evaluation Criteria in Solid Tumors (RECIST) and modified RECIST in patients treated with sorafenib for hepatocellular

- carcinoma. *Cancer* 2012; **118**: 147-156 [PMID: 21713764 DOI: 10.1002/cncr.26255]
- 25 **Ronot M**, Bouattour M, Wassermann J, Bruno O, Dreyer C, Larroque B, Castera L, Vilgrain V, Belghiti J, Raymond E, Faivre S. Alternative Response Criteria (Choi, European association for the study of the liver, and modified Response Evaluation Criteria in Solid Tumors [RECIST]) Versus RECIST 1.1 in patients with advanced hepatocellular carcinoma treated with sorafenib. *Oncologist* 2014; **19**: 394-402 [PMID: 24652387 DOI: 10.1634/theoncologist.2013-0114]
- 26 **Reig M**, Darnell A, Forner A, Rimola J, Ayuso C, Bruix J. Systemic therapy for hepatocellular carcinoma: the issue of treatment stage migration and registration of progression using the BCLC-refined RECIST. *Semin Liver Dis* 2014; **34**: 444-455 [PMID: 25369306 DOI: 10.1055/s-0034-1394143]
- 27 **Ferté C**, Koscielny S, Albiges L, Rocher L, Soria JC, Iacovelli R, Lorient Y, Fizazi K, Escudier B. Tumor growth rate provides useful information to evaluate sorafenib and everolimus treatment in metastatic renal cell carcinoma patients: an integrated analysis of the TARGET and RECORD phase 3 trial data. *Eur Urol* 2014; **65**: 713-720 [PMID: 23993162 DOI: 10.1016/j.eururo.2013.08.010]
- 28 **Shao YY**, Wu CH, Lu LC, Chan SY, Ma YY, Yen FC, Hsu CH, Cheng AL. Prognosis of patients with advanced hepatocellular carcinoma who failed first-line systemic therapy. *J Hepatol* 2014; **60**: 313-318 [PMID: 24036008 DOI: 10.1016/j.jhep.2013.08.027]
- 29 **Reig M**, Rimola J, Torres F, Darnell A, Rodriguez-Lope C, Forner A, Llarch N, Ríos J, Ayuso C, Bruix J. Postprogression survival of patients with advanced hepatocellular carcinoma: rationale for second-line trial design. *Hepatology* 2013; **58**: 2023-2031 [PMID: 23787822 DOI: 10.1002/hep.26586]
- 30 **Lee IC**, Chen YT, Chao Y, Huo TI, Li CP, Su CW, Lin HC, Lee FY, Huang YH. Determinants of survival after sorafenib failure in patients with BCLC-C hepatocellular carcinoma in real-world practice. *Medicine (Baltimore)* 2015; **94**: e688 [PMID: 25860213 DOI: 10.1097/MD.0000000000000688]
- 31 **Ogasawara S**, Chiba T, Ooka Y, Suzuki E, Kanogawa N, Saito T, Motoyama T, Tawada A, Kanai F, Yokosuka O. Post-progression survival in patients with advanced hepatocellular carcinoma resistant to sorafenib. *Invest New Drugs* 2016; **34**: 255-260 [PMID: 26769245 DOI: 10.1007/s10637-016-0323-1]

**P- Reviewer:** Bayraktar Y, Guo RP, Gwak GY    **S- Editor:** Gong ZM  
**L- Editor:** A    **E- Editor:** Li D



Basic Study

# Primary liver injury and delayed resolution of liver stiffness after alcohol detoxification in heavy drinkers with the *PNPLA3* variant I148M

Vanessa Rausch, Teresa Peccerella, Carolin Lackner, Eray Yagmur, Helmut-Karl Seitz, Thomas Longerich, Sebastian Mueller

Vanessa Rausch, Teresa Peccerella, Helmut-Karl Seitz, Sebastian Mueller, Salem Medical Center and Center for Alcohol Research, University of Heidelberg, 69120 Heidelberg, Germany

Carolin Lackner, Institute for Pathology, Medical University Graz, 8036 Graz, Austria

Eray Yagmur, Laboratory Diagnostics Center, RWTH-University Hospital Aachen, Aachen and Medical Care Center, Dr. Stein and colleagues, 41169 Mönchengladbach, Germany

Thomas Longerich, Institute of Pathology, RWTH-University Hospital Aachen, 52074 Aachen, Germany

**Author contributions:** Rausch V and Mueller S contributed to the conception and design of the study, analyzed and interpreted the data and wrote the manuscript; Rausch V, Peccerella T, Lackner C, Yagmur E and Longerich T contributed to the acquisition and analysis of data; Lackner C, Seitz HK and Longerich T critically revised the manuscript and approved the final version.

**Supported by** The German Research Foundation (DFG, RA-2677/1-1); and the Dietmar Hopp-Foundation, No. 2301196.

**Institutional review board statement:** The study was approved by the Ethical Committee of the University of Heidelberg.

**Conflict-of-interest statement:** The authors declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

**Data sharing statement:** Patients gave informed consent before inclusion in the study; the presented data are anonymized and the risk of identification is very low.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this

work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Sebastian Mueller, MD, PhD, Salem Medical Center and Center for Alcohol Research, University of Heidelberg, Zeppelinstraße 11-33, 69120 Heidelberg, Germany. [sebastian.mueller@urz.uni-heidelberg.de](mailto:sebastian.mueller@urz.uni-heidelberg.de)  
**Telephone:** +49-6221-483210  
**Fax:** +49-6221-483494

**Received:** March 22, 2016

**Peer-review started:** March 23, 2016

**First decision:** July 4, 2016

**Revised:** August 22, 2016

**Accepted:** September 13, 2016

**Article in press:** September 18, 2016

**Published online:** December 18, 2016

## Abstract

### AIM

To investigate the influence of *PNPLA3* genotype in heavy drinkers on serum markers and liver stiffness (LS) during alcohol withdrawal and its association with histology.

### METHODS

Caucasian heavy drinkers ( $n = 521$ ) with a mean alcohol consumption of 192.1 g/d (median alcohol consumption: 169.0 g/d; 95%CI: 179.0-203.3) were enrolled at the Salem Medical Center, University of Heidelberg. LS was measured by transient elastography (Fibroscan, Echosens SA, Paris, France). LS and serum markers were prospectively studied in these patients with all stages



of alcoholic liver disease (steatosis, steatohepatitis, fibrosis) prior and after alcohol detoxification with a mean observation interval of  $6.2 \pm 3.2$  d. A liver biopsy with histological analysis including the Kleiner score was obtained in 80 patients.

## RESULTS

The *PNPLA3* rs738409 genotype distribution for CC, CG and GG was 39.2%, 52.6% and 8.2%. GG genotype primarily correlated with histological steatohepatitis ( $r = 0.404$ ,  $P < 0.005$ ), ballooning ( $r = 0.319$ ,  $P < 0.005$ ) and less with steatosis ( $r = 0.264$ ,  $P < 0.05$ ). Mean LS was lowest in CC carriers (13.1 kPa) as compared to CG and GG carriers (17.6 and 17.2 kPa). Notably, LS primarily correlated with fibrosis stage ( $r = 0.828$ ,  $P < 0.005$ ), ballooning ( $r = 0.516$ ,  $P < 0.005$ ), steatohepatitis ( $r = 0.319$ ,  $P < 0.005$ ) but not with steatosis. After alcohol withdrawal, LS did not change in CC carriers, significantly decreased in CG-carriers from 17.6 to 12.7 kPa but to a lesser extent in GG carriers from 17.6 to 14.5 kPa. This was due to prolonged resolution of inflammation with significantly elevated aspartate transaminase levels after alcohol withdrawal in GG carriers. Non-invasive fibrosis assessment by LS in all patients showed a significantly higher F0 rate as compared to the biopsy cohort (47% vs 6%) with 3.8% more CC carriers while 3.7% less were seen in the F4 cirrhosis group. Thus, about 20% of patients with alcoholic liver cirrhosis would be attributable to *PNPLA3* G variants. The OR to develop cirrhosis corrected for age, gender and body mass index was 1.295 (95%CI: 0.787-2.131) for CG + GG carriers.

## CONCLUSION

In heavy drinkers, *PNPLA3* GG primarily correlates with ballooning/steatohepatitis but not steatosis resulting in a delayed inflammation-associated resolution of LS. Consequently, sustained ballooning-associated LS elevation seems to be a potential risk factor for fibrosis progression in *PNPLA3* GG carriers.

**Key words:** Liver stiffness; Alcoholic liver disease; Adiponutrin; *PNPLA3*; Transient elastography; Alcohol withdrawal; Inflammation

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The role of the *PNPLA3* rs738409 variant (CG and GG) on histology and liver stiffness in response to alcohol detoxification was studied in a large monocentric cohort of heavy drinkers with various stages of ALD. About 20% of our patients with alcoholic liver cirrhosis were attributable to *PNPLA3* G variants with an OR to develop cirrhosis of 1.295. Our data further show that *PNPLA3* GG carriers primarily develop ballooning and not steatosis causing a delayed resolution of liver stiffness after alcohol withdrawal. We suggest that the delayed ballooning-associated stiffness elevation may contribute to fibrosis progression (see also the sinusoidal pressure hypothesis).

Rausch V, Peccerella T, Lackner C, Yagmur E, Seitz HK, Longerich T, Mueller S. Primary liver injury and delayed resolution of liver stiffness after alcohol detoxification in heavy drinkers with the *PNPLA3* variant I148M. *World J Hepatol* 2016; 8(35): 1547-1556 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i35/1547.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i35.1547>

## INTRODUCTION

Alcoholic liver disease (ALD) is the most common chronic liver disease in the Western world<sup>[1]</sup>. ALD encompasses a broad spectrum of disorders ranging from simple steatosis to severe forms of liver injury, including alcoholic steatohepatitis, fibrosis and cirrhosis. Although the majority (80%-90%) of heavy drinkers with an alcohol consumption > 80 g/d develop steatosis, only 35% show signs of inflammation and about 8%-20% progress to cirrhosis<sup>[2]</sup>. The underlying molecular mechanisms of disease progression, especially why some patients rapidly progress to severe liver disease, are still poorly understood. In addition, it remains unclear whether steatosis necessarily precedes steatohepatitis or is a coinciding bystander. The role of environmental factors that affect disease progression such as drinking habits and comorbidities has been known for many years<sup>[3]</sup>. However, twin studies, the enhanced sensitivity of female drinkers and the fact that only a minority of patients progress to cirrhosis despite heavy drinking clearly suggest a genetic pre-disposition<sup>[4,5]</sup>.

Recent studies in multiethnic populations with non-alcoholic fatty liver disease (NAFLD) and ALD have demonstrated that the single-nucleotide polymorphism, the rs738409 variant, that encodes for an isoleucine to methionine substitution at position 148 (I148M) in the patatin-like phospholipase-3 (*PNPLA3*/*Adiponutrin*) gene is a strong disease modifier by influencing steatosis, liver enzymes and fibrosis progression<sup>[6-12]</sup>. So far, the function of *PNPLA3* and the effect of the amino acid substitution remain controversial. *PNPLA3* is closely related to *PNPLA2*/*ATGL*, the major hormone-sensitive lipase of adipose tissue, sharing 56% amino acid identity in the patatin-like domain<sup>[13,14]</sup>. *PNPLA3* is expressed in adipocytes, hepatocytes and hepatic stellate cells<sup>[15-18]</sup>. Despite many efforts, the physiologic role of *PNPLA3* and its direct action in the liver is still incompletely understood and it remains unclear whether the I148M substitution in *PNPLA3* directly causes steatosis, lipotoxicity, or both.

*PNPLA3* GG carriers not only more rapidly progress toward fibrosis but also show elevated liver stiffness (LS)<sup>[19]</sup>. Non-invasive measurement of LS by ultrasound-based elastographic techniques such as transient elastography (TE) are increasingly used to screen for liver fibrosis<sup>[9,20-25]</sup>. However, various conditions have been shown to increase LS in the absence of fibrosis including inflammation and liver damage<sup>[26-28]</sup>, congestion<sup>[29]</sup>, cholestasis<sup>[30]</sup>, arterial pressure<sup>[31]</sup> food intake<sup>[32,33]</sup> or amyloidosis<sup>[34,35]</sup>. For these reasons, we here study in

**Table 1** Patient characteristics before and after alcohol withdrawal

Parameters	Before withdrawal ( <i>n</i> = 521)	After withdrawal ( <i>n</i> = 370)
Demographic characteristics		
Male (%)	72.1	
Age (yr)	50.2 ± 11.3	
Risk factors		
BMI (kg/m <sup>2</sup> )	25.2 ± 4.6	
H/W ratio	1.0 ± 0.1	
Alcohol consumption (g/d)	192.1 ± 139.7	
Duration (yr)	19.9 ± 13.3	
Smoker (%)	70.9	
Diabetes (%)	10.0	
Coronary heart disease (%)	5.1	
RR (%)	34.5	
Ascites (%)	9.0	
F0 (%)	47.4	
F1-2 (%)	17.1	
F3 (%)	10.8	
F4 (%)	24.7	
Noninvasive parameters		
Hepatic steatosis (0-3, US)	1.9 ± 0.9	
Liver stiffness (kPa)	15.8 ± 21.1	12.6 ± 18.1
Laboratory parameters		
AST (U/L)	101 ± 108	54 ± 48
ALT (U/L)	70 ± 79	52 ± 46
GGT (U/L)	398 ± 577	268 ± 360
AP (U/L)	109 ± 76	88 ± 55
Bilirubin (mg/dL)	1.3 ± 2.8	0.9 ± 2.3
Albumin (g/dL)	5.0 ± 6.0	
INR	1.2 ± 3.4	1.2 ± 5.1
Urea (mg/dL)	22.6 ± 16.6	23.7 ± 12.5
Creatinine (mg/dL)	0.7 ± 0.3	0.8 ± 0.2
Hemoglobin (g/dL)	14.2 ± 2.2	13.8 ± 1.8
Platelets (/nL)	209 ± 87	215 ± 82
Glucose (mg/dL)	109.1 ± 36.4	
HbA1C (%)	5.6 ± 1.0	
Triglycerides (mg/dL)	195.7 ± 206.6	
Cholesterol (mg/dL)	215.9 ± 58.2	
HDL cholesterol (mg/dL)	72.3 ± 36.9	
LDL cholesterol (mg/dL)	112.6 ± 45.6	
Lipase (U/L)	63.6 ± 164.8	60.7 ± 56.3
Ferritin (ng/mL)	580.6 ± 650.5	
CRP (mg/dL)	6.1 ± 15.9	7.1 ± 12.5

Data are presented as mean ± SD or in %. BMI: Body mass index; H/W ratio: Hip to waist ratio; RR: Hypertension; F: Fibrosis stage; AST: Aspartate transaminase; ALT: Alanine transaminase; GGT: Gamma-glutamyl-transpeptidase; AP: Alkaline phosphatase; INR: International normalized ratio (Prothrombin); HDL: High-density lipoprotein; LDL: Low-density lipoprotein; CRP: C-reactive protein; US: Ultrasound.

detail the impact of *PNPLA3* I148M substitution on LS and histology in a large population of heavy drinkers primarily admitted to the hospital for alcohol withdrawal. We further analyze the impact of alcohol withdrawal on LS depending on *PNPLA3* status. Our data further suggest that the sustained and drinking-associated LS elevation in *PNPLA3* GG carriers is most likely associated with ballooning and seems to contribute to fibrosis progression.

## MATERIALS AND METHODS

### Patients

Caucasian heavy drinkers (*n* = 521, 148 females/369

males, age range 22-87 years) with a mean alcohol consumption of 192.1 g/d (median alcohol consumption: 169.0; 95%CI: 179.0-203.3) were enrolled at the Department of Gastroenterology, Salem Medical Center in Heidelberg. Patients presented primarily for alcohol detoxification with a mean duration of chronic alcohol consumption of 19.9 years. Patient's characteristics are given in Table 1, a more refined *PNPLA3* genotype-associated data presentation is shown in Table 2. All patients underwent careful clinical examination, standard laboratory routine (venous blood sampling), abdominal ultrasound and liver stiffness measurement. Inclusion criteria were daily alcohol consumption > 60/80 g/d, age > 18 years, and successful assessment of LS. Other causes of liver diseases (exclusion criteria) were ruled out serologically in all patients by screening for AMA, ANA, HCV and HBV. The study protocol was reviewed and approved by the local Ethics Committee of the University of Heidelberg and all patients gave written informed consent prior to inclusion. Laboratory parameters, TE were performed both at day of admission and release with a mean observation interval of 6.2 ± 3.2 d.

### Liver histology and immunostainings

Eighty patients (15.4%) underwent liver biopsy using the Menghini technique (mean biopsy lengths 15.6 mm). Specimens were fixed in formalin and embedded in paraffin. Two experienced pathologists (TL and CL) blinded to the patient's data analyzed all liver biopsies independently. For histological analysis, 4 µm sections were dewaxed and stained with hematoxylin and eosin, Chromotrop-Anilinblue and Sirius-Red using standard procedures. Histological semiquantitative scoring of macro- and microvesicular steatosis, lobular inflammation, hepatocellular ballooning, Mallory-Denk bodies and apoptosis as well as fibrosis staging was performed exactly as described by Kleiner *et al.*<sup>[36]</sup>. In addition, fibrosis was also assessed using the semiquantitative method of Chevallier *et al.*<sup>[37]</sup> and collagen content was quantified by computer-assisted image analysis of Sirius-Red stained sections (morphometry). The histological diagnosis of steatohepatitis was based on the minimal criteria of steatosis (any degree), lobular inflammation and ballooning<sup>[38]</sup>.

### TE and non-invasive fibrosis assessment in ALD patients

LS was measured by TE (Fibroscan, Echosens SA, Paris, France) using the M<sup>[39]</sup> or XL probe<sup>[40,41]</sup>. TE was performed by physicians with at least 12 mo of experience in abdominal ultrasound and transient elastography on the right lobe of the liver in intercostal position according to established protocols<sup>[25]</sup>. Fibrosis stages were determined using the recently established aspartate transaminase (AST)-adapted cut-off values<sup>[42]</sup>. In patients with two measurements prior and after alcohol detoxification, the second measurements were used with less pronounced steatohepatitis and transaminase elevation, since such conditions correlate better with histology<sup>[9,25]</sup>. In addition, liver size, spleen size, ascites formation and semiquan-

**Table 2** Characteristics of alcoholic liver disease sub-cohorts (*n* = 521) based on genotype distribution of rs738409 polymorphism

Parameters	<i>PNPLA3</i> CC ( <i>n</i> = 204)	<i>PNPLA3</i> CG ( <i>n</i> = 274)	<i>PNPLA3</i> GG ( <i>n</i> = 43)	<i>PNPLA3</i> CG + GG ( <i>n</i> = 317)
Demographic characteristics				
Patients (%)	39.2	52.6	8.2	60.8
Age (yr)	49.5 ± 11.0	50.7 ± 11.8	50.1 ± 9.7	50.7 ± 11.5
Risk factors				
BMI (kg/m <sup>2</sup> )	25.4 ± 4.9	25.1 ± 4.5	25.6 ± 3.9	25.2 ± 4.4
H/W ratio	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
Alcohol consumption (g/d)	194.0 ± 136.1	190.8 ± 146.2	181.2 ± 116.1	189.4 ± 142.0
Duration (yr)	18.3 ± 13.3	20.9 ± 13.1	17.2 ± 14.2	20.4 ± 13.3
Smoker (1 = yes)	0.7 ± 0.4	0.7 ± 0.5	0.6 ± 0.5	0.7 ± 0.5
Diabetes (1 = yes)	0.1 ± 0.3	0.1 ± 0.3	0.0 ± 0.2	0.1 ± 0.3
Coronary heart disease (1 = yes)	0.1 ± 0.2	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.3
Noninvasive parameters				
Hepatic steatosis (0-3, US)	1.8 ± 0.9	2.0 ± 0.8	1.9 ± 0.8	2.0 ± 0.8
Liver stiffness (kPa)	13.1 ± 17.7	17.6 ± 23.0 <sup>a</sup>	17.2 ± 22.2	17.5 ± 22.9 <sup>a</sup>
Laboratory parameter				
AST (U/L) before detox	95.2 ± 100.8	102.8 ± 111.4	113.1 ± 116.8	104.0 ± 111.9
AST (U/L) after detox	47.8 ± 32.9	52.6 ± 46.0	82.8 ± 89.5 <sup>a</sup>	56.2 ± 53.5
ALT (U/L) before detox	66.0 ± 59.4	71.9 ± 93.0	76.4 ± 60.4	72.5 ± 89.2
ALT (U/L) after detox	47.5 ± 35.9	52.4 ± 50.9	67.7 ± 55.0 <sup>a</sup>	54.2 ± 51.5
GGT (U/L) before detox	406.3 ± 572.2	365.9 ± 516.1	537.7 ± 869.6	389.6 ± 578.9
GGT (U/L) after detox	254.8 ± 290.9	261.7 ± 347.3	389.7 ± 671.6	276.9 ± 399.6
AP (U/L) before detox	105.5 ± 76.2	111.6 ± 75.8	112.7 ± 72.6	111.8 ± 75.3
AP (U/L) after detox	83.3 ± 45.1	90.5 ± 59.2	97.5 ± 68.4	91.3 ± 60.2
Bilirubin (mg/dL)	1.2 ± 2.8	1.4 ± 3.0	0.9 ± 1.1	1.3 ± 2.8
Albumin (g/dL)	4.7 ± 4.7	5.3 ± 7.2	4.5 ± 0.5	5.2 ± 6.7
INR	1.4 ± 5.4	1.0 ± 0.4	0.9 ± 0.2	1.0 ± 0.4
Urea	20.6 ± 10.8	24.6 ± 20.2 <sup>a</sup>	20.1 ± 9.9	24.0 ± 19.2 <sup>a</sup>
Creatinine	0.7 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	0.7 ± 0.3
Hemoglobin (g/dL)	14.2 ± 1.8	14.2 ± 2.5	14.6 ± 2.0	14.2 ± 2.4
Platelets (/nL)	216.7 ± 92.7	201.1 ± 80.0 <sup>a</sup>	224.2 ± 91.4	204.5 ± 82.0
Glucose (mg/dL)	112.0 ± 46.2	107.7 ± 28.5	110.7 ± 34.6	108.1 ± 29.3
HbA1C (%)	5.6 ± 1.1	5.6 ± 0.8	5.8 ± 1.3	5.6 ± 0.9
Triglycerides (mg/dL)	190.6 ± 202.2	192.0 ± 205.8	240.9 ± 230.4	198.7 ± 209.6
Cholesterol (mg/dL)	219.9 ± 55.0	213.1 ± 61.1	222.9 ± 53.4	214.4 ± 60.1
HDL cholesterol (mg/dL)	73.2 ± 35.9	71.4 ± 37.6	75.6 ± 37.3	71.9 ± 37.5
LDL cholesterol (mg/dL)	113.5 ± 46.3	112.4 ± 45.5	118.0 ± 44.7	113.0 ± 45.3
Lipase (U/L)	48.5 ± 45.9	75.9 ± 216.5	45.3 ± 26.0	72.0 ± 202.7
Ferritin (ng/mL)	546.1 ± 611.6	599.6 ± 668.3	685.2 ± 708.2	610.8 ± 673.1
CRP (mg/dL)	4.7 ± 11.1	7.1 ± 18.9	6.0 ± 12.0	7.0 ± 18.1

Data are presented as mean ± SD or in %; significant paired T tests (<sup>a</sup>*P* < 0.05) with CC. BMI: Body mass index; H/W ratio: Hip to waist ratio; AST: Aspartate transaminase; ALT: Alanine transaminase; GGT: Gamma-glutamyl-transpeptidase; AP: Alkaline phosphatase; INR: International normalized ratio (Prothrombin); HDL: High-density lipoprotein; LDL: Low-density lipoprotein; CRP: C-reactive protein; US: Ultrasound; *PNPLA3*: Adiponutrin.

titative liver steatosis (0-3) were assessed by abdominal ultrasound.

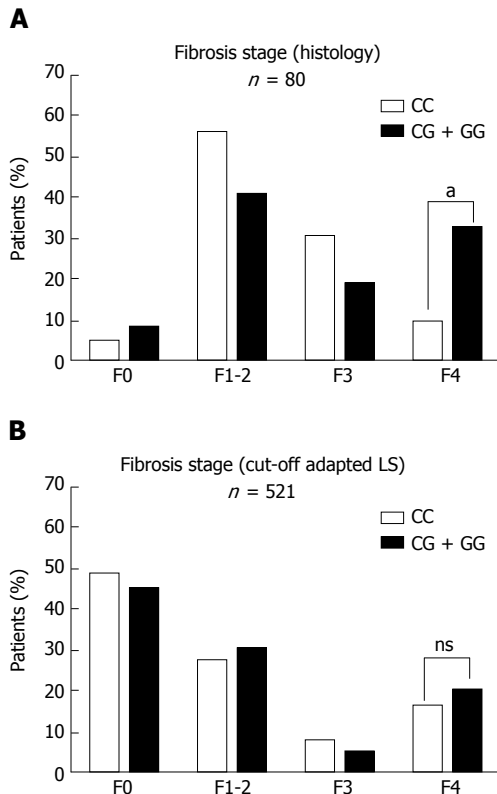
### ***PNPLA3* genotyping**

Genomic DNA was isolated from EDTA anti-coagulated blood using standard protocols. The *PNPLA3* coding SNP I148M was genotyped using tetra-primer ARMS polymerase chain reaction (PCR) technique on the GeneAmp PCR System 2400 (Applied Bioscience) using standard protocol. Primers were designed using Batch Primer 3 software<sup>[43]</sup>, synthesized by Eurofins MWG Operon (Ebersberg, Germany) and are available upon request. PCR reactions were performed in a total volume of 25 µL, containing approximately 30-50 ng of template DNA, 1 × PCR buffer, 2.5 mmol/L MgCl<sub>2</sub>, 0.2 mmol/L dNTPs, 2 nmol/L of outer primer and 20 nmol/L inner allele-specific primers and 1U of Taq polymerase (Roche, Penzberg, Germany). Post-PCR allelic discrimination was

carried out using horizontal non-denaturing polyacrylamide gel (10%) electrophoresis followed by ethidium bromide staining and visualization on a UV transilluminator. To ensure genotyping quality, we included negative controls and DNA samples with known *PNPLA3* genotypes as internal controls.

### **Statistical analysis**

We used descriptive statistics to compute equally distributed data, including means, standard deviations and frequencies. Not normally distributed data were log transformed before statistical analysis. Comparisons of the genotype distribution of CC, GG and combined CG and GG were performed and the Spearman correlation or  $\chi^2$  test for non-parametric variables (regression coefficient *r*, *P*) was used to determine the associations between laboratory findings, LS, histological scores and the genotypes. To determine whether there are significant



**Figure 1** Distribution of fibrosis stages using (A) histology (Kleiner fibrosis score F0-4) or (B) non-invasive liver stiffness measurement (aspartate transaminase-adapted cut-off values). <sup>a</sup> $P < 0.05$ . ns: Not significantly; LS: Liver stiffness.

differences between the variants (CC, CG, GG or CG combined with GG) we used a two-sample Student's *t*-test when the data were normally distributed. Binary logistic regression analysis was calculated to proof possible effects of genotype, gender, age and body mass index (BMI) on the outcome of AST-adapted cut-off values for fibrosis staging. Statistical calculations were performed with SPSS (version 21.0, IBM, SPSS) or SAS (version 9.4, SAS) software and two-sided *P* values  $< 0.05$  were considered statistically significant. Statistical methods of this study were reviewed by Thomas Bruckner from Institute of Medical Biometry and Informatics, University of Heidelberg, Heidelberg, Germany.

## RESULTS

### *PNPLA3* rs738409 GG carrier show more cirrhosis

The *PNPLA3* rs738409 genotype distribution in our cohort of 521 ALD patients was 39.2%, 52.6% and 8.2% ( $n = 204$ , 274 and 43) for CC, CG and GG (Table 2). Notably, fibrosis distribution differed markedly in the non-invasively ( $n = 521$ ) vs histologically ( $n = 80$ ) assessed cohorts (Figure 1), histologically characterized patients showed only a small fraction of F0 stages (6%,  $n = 5$ ). In contrast, the F0 fraction was much higher in the non-invasively assessed cohort by LS (47%,  $n = 245$ , Figure 1B). In both approaches, CG + GG carriers had more F4 cirrhosis as compared to CC carriers as shown in Figure

**Table 3** Risk factors associated with F4 cirrhosis

Factor	OR	95%CI	P value
<i>PNPLA3</i> G (CG + GG)	1.295	0.787-2.131	$> 0.05$
Gender	0.855	0.496-1.475	$> 0.05$
Age	1.040	1.017-1.064	$< 0.001$
BMI	1.037	0.983-1.093	$> 0.05$

BMI: Body mass index; OR: Odds ratio; *PNPLA3*: Adiponutrin.

1A (9.3% vs 32.4%) and 1B (16.3% vs 20.0%). CC carriers represented 42.1% of the F0 cohort but 35.5% of the F4 cohort. In other words, about 3.8% more CC carriers had F0 while they were 3.7% less frequent in the non-invasively assessed F4 cohort. Both cohorts did not differ significantly with respect to age and mean drinking duration (approximately 20 years). Linear regression analysis corrected for age, gender and BMI calculated an OR of 1.295 (95%CI: 0.787-2.131) for CG + GG carriers to develop F4 cirrhosis (Table 3). Taken together, our study indicates a *PNPLA3*-attributable effect on fibrosis stage. Notably and as could be expected, the non-invasively characterized cohort had a much larger proportion of non-fibrotic patients.

### *PNPLA3* rs738409 GG carriers have no pronounced metabolic phenotype

Since *PNPLA3* rs738409 SNP has been primarily identified in NAFLD patients, we next characterized typical features of the NAFLD phenotype. No significant differences were observed between CC, CG and GG carriers with regard to BMI (25.4 vs 25.1 vs 25.6), HbA1c (5.6% vs 5.6% vs 5.8%), and serum fasting glucose concentrations (112 mg/dL vs 108 mg/dL vs 111 mg/dL). This was also the case with regard to coronary heart disease, type II diabetes, smoking habits (assessed by pack years) and arterial hypertension (Table 2 and data not shown). Likewise, no significant differences were observed between levels of high-density lipoprotein and low-density lipoprotein cholesterol and triglycerides (TG) although TG levels were notably higher in GG carriers (Table 2). In summary, in this large cohort of heavy drinkers, GG is associated with advanced fibrosis in the absence of a typical NAFLD-associated metabolic phenotype.

### Ballooning/steatohepatitis is the predominant histological feature of *PNPLA3* rs738409 GG carrier

To learn more about histological association with the *PNPLA3* carrier status, we assessed steatosis, inflammation and fibrosis using the Kleiner and the semiquantitative Chevallier score. Interestingly, GG genotype primarily correlated with steatohepatitis ( $r = 0.404$ ,  $P < 0.005$ ), ballooning ( $r = 0.319$ ,  $P < 0.005$ ), less with steatosis ( $r = 0.264$ ,  $P < 0.05$ ) but not significantly with fibrosis (Table 4). In line with this, CC genotype correlated negatively with ballooning ( $r = -0.221$ ,  $P < 0.05$ ). These data were mirrored in the direct comparison of the genotypes. More fibrosis and ballooning was



**Table 4** Spearman rank correlation of *PNPLA3* carrier status and liver stiffness with histological parameters

Parameter (n = 80)	<i>PNPLA3</i> CC (n = 43)	<i>PNPLA3</i> CG (n = 29)	<i>PNPLA3</i> GG (n = 8)	Liver stiffness (kPa)
Steatohepatitis (score 0-2)	-0.163	-0.099	0.404 <sup>b</sup>	0.391 <sup>b</sup>
Microgranulomas (score 0-1)	-0.095	-0.139	0.357 <sup>b</sup>	0.387 <sup>b</sup>
Ballooning (score 0-2)	-0.221 <sup>a</sup>	0.020	0.319 <sup>b</sup>	0.516 <sup>b</sup>
Glycogenated nuclei (score 0-1)	-0.124	-0.080	0.316 <sup>b</sup>	0.335 <sup>b</sup>
Steatosis (score 0-3)	-0.125	-0.045	0.264 <sup>a</sup>	0.096
Lobular inflammation (score 0-3)	-0.142	-0.003	0.227 <sup>a</sup>	0.420 <sup>b</sup>
Megamitochondria (score 0-1)	-0.121	-0.005	0.198	0.278 <sup>b</sup>
Large lipogranulomas (score 0-1)	0.134	-0.238 <sup>a</sup>	0.145	0.144
Acidophil bodies (score 0-1)	-0.016	-0.072	0.133	0.285 <sup>b</sup>
Pericellular fibrosis (score 0-3)	-0.224	0.141	0.131	0.567 <sup>b</sup>
Chevallier fibrosis score (SSS)	-0.189	0.112	0.131	0.828 <sup>b</sup>
Ballooning k8/18 stain (score 0-2)	-0.537 <sup>b</sup>	0.490 <sup>b</sup>	0.089	0.692 <sup>b</sup>
Kleiner fibrosis score (score 0-4)	-0.163	0.148	0.035	0.745 <sup>b</sup>
Mallory Denk Bodies (score 0-1)	-0.121	0.110	0.026	0.530 <sup>b</sup>
Apoptosis M30 stain (score 0-3)	-0.039	0.031	0.014	0.490 <sup>b</sup>
Pigmented macrophages (score 0-1)	0.003	0.012	-0.022	-0.009
Portal inflammation (score 0-1)	-0.027	0.099	-0.106	0.427 <sup>b</sup>
Liver stiffness (kPa)	-0.045	0.017	0.037	1.000

Liver stiffness primarily correlates with fibrosis and liver damage but not significant with steatosis. In contrast, GG carrier status is tightly associated with liver injury and weakly with steatosis. <sup>a</sup>*P* < 0.05 vs <sup>b</sup>*P* < 0.01. *PNPLA3*: Adiponutrin.

present in the CG + GG carriers (Supplemental Table 1). Interestingly, neither a significant association was found with serum markers of liver damage (data not shown), with signs of liver cirrhosis in the ultrasound and with LS. Taken together, liver injury such as ballooning and steatohepatitis are the primary histological features associated with GG genotype in heavy drinkers while fibrosis and steatosis are less pronounced.

#### **LS is predominantly associated with fibrosis and ballooning/steatohepatitis but not steatosis**

Since previous studies indicated a higher LS in carriers of the *PNPLA3* risk allele (CG + GG) in various liver diseases and LS is increasingly used to screen for liver fibrosis, we next carefully analyzed the correlation of LS with histological subscores and the *PNPLA3* status (Table 4). As expected, LS showed a very tight and significant association with fibrosis stage ( $r = 0.828$ ,  $P < 0.005$ ) but also with ballooning ( $r = 0.692$ ,  $P < 0.005$ ) and steatohepatitis ( $r = 0.391$ ,  $P < 0.005$ ). Notably, no correlation was observed with steatosis ( $r = 0.096$ , ns). In addition, no significant correlation was seen between LS and *PNPLA3* genotype. Taken together, in a cohort of heavy drinkers, LS is correlated with fibrosis, liver injury and inflammation but not with steatosis and the *PNPLA3* status.

#### **Elevated LS in *PNPLA3* rs738409 GG carriers and a delayed resolution after alcohol withdrawal**

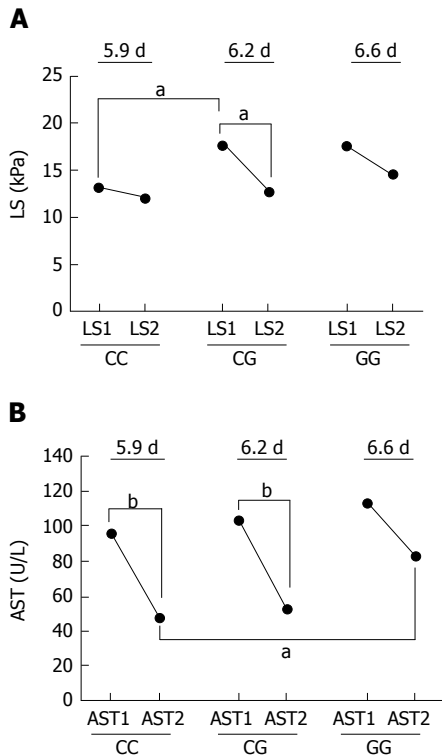
Mean LS was lowest in CC carriers (13.1 kPa) and significantly higher in CG carriers (17.6 kPa, Figure 2). LS was likewise elevated in GG carriers (17.2 kPa) without reaching statistical significance due to the limited number of patients (8.2%). Interestingly, almost no change was observed in CC carrier after alcohol withdrawal (12.0 kPa, LS2). In contrast, LS significantly decreased in CG

carriers to comparable 12.7 kPa after withdrawal from alcohol. Despite a longer observation interval of 6.6 d, LS decreased slower in GG and remained higher (14.5 kPa). This was most likely due to sustained inflammation/ballooning as reflected by elevated AST levels, which were significantly higher after alcohol withdrawal (Figure 2B, Table 2). In summary, GG-associated liver damage results in a reversible, inflammation-associated increase of liver stiffness. In addition, GG carriers show a slower resolution of liver damage and LS after withdrawal from alcohol.

## **DISCUSSION**

We here show in a large monocenter cohort of histologically and non-invasively characterized heavy Caucasian drinkers that the SNP rs738409 in *PNPLA3* (CG and GG) is primarily associated with ballooning/steatohepatitis but less with steatosis. Importantly and as seen previously, G carriers (CG + GG) had higher initial LS values as compared to CC carriers. Notably and in some contrast to the genotype analysis, LS was primarily correlated with fibrosis stage, ballooning/steatohepatitis but not at all with steatosis. GG carriers showed a slower resolution of liver damage and LS after withdrawal from alcohol. Since AST levels were significantly elevated in GG carriers after withdrawal from alcohol, we attributed this to delayed resolution of inflammation/ballooning.

Several findings of this study are unexpected and shed new light on the function of *PNPLA3* and its link to inflammation and fibrosis development. First of all, we see clear differences of the fibrosis distribution between the biopsy and non-invasively characterized cohorts. While only 6% showed no fibrosis (F0) in the biopsy cohort this number increased drastically to 47% in the non-invasive cohort. These numbers are especially



**Figure 2** *PNPLA3* carrier status and its effect on liver stiffness (A) and aspartate transaminase (B) levels prior and after alcohol detoxification (1 and 2). Mean observation of detoxification periods in days are indicated for each genotype. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ . AST: Aspartate transaminase; ALT: Alanine transaminase; LS: Liver stiffness.

impressive with regard to the high negative predictive values of transient elastography<sup>[24,25]</sup>. We believe that these findings clearly indicate an often underestimated selection bias of biopsies in ALD study cohorts. Obviously, significantly less patients with no or mild liver disease are asked or motivated to undergo liver biopsy, whereas more severe patients are willing to agree with the invasive procedure. We believe that this observation is a strong argument to enforce well non-invasively characterized ALD cohorts in future studies.

Second, another interesting finding of the non-invasively characterized cohort is the almost symmetric, mirror-like distribution of CC vs G (CG + GG) carriers in the F0 and F4 population over almost 20 years of alcohol consumption. Circa 4% less CC carriers were seen in the cirrhosis group, an equating circa 4% more CC carriers were observed in the F0 group. Thus, about 20% of patients with alcoholic liver cirrhosis would be attributable to *PNPLA3*-G variants. The odds ratio to develop F4 cirrhosis was 1.3 for our cohort, which corresponds to earlier reports<sup>[12,44]</sup>. Notably and in line with previous reports<sup>[45]</sup>, the genotype distribution did not follow the Hardy-Weinberg equilibrium which could point to phenotype (GG)-related increased mortality, *e.g.*, due to complications of cirrhosis such as primary liver cancer (HCC)<sup>[46]</sup>.

Third, the histological findings are intriguing and partly surprising. Up to date, our study presents the most detailed histological analysis with respect to *PNPLA3*

carrier status and ALD since previous GWAS studies had primarily relied on retrospective samples with laboratory tests such as transaminases and diagnosis of steatosis by ultrasound<sup>[10,12]</sup>. Our data clearly show that signs of liver injury such as steatohepatitis or ballooning are the major and predominant features of GG carriers. In contrast, other widely discussed findings such as steatosis or fibrosis are less pronounced. Our study suggests that rather ballooning and not steatosis is the key feature of the *PNPLA3* GG phenotype in heavy drinkers that later develop ALD. Whether steatosis is either just a consequence of apoptotic liver damage or a bystander needs to be further clarified.

Fourth, special novel insights are seen with the detailed analysis of LS prior and after alcohol withdrawal. It is especially surprising that *PNPLA3* status and LS are differentially associated with histology. These data may also serve as explanation for the rather weak effect of the *PNPLA3* status on LS and less pronounced results in the past<sup>[19]</sup>. Thus, LS is highly associated with fibrosis stage (Kleiner and Chevallier) ( $r = 0.79$ ) and with steatohepatitis/ballooning ( $r = 0.4$ - $0.7$ ) but not at all with steatosis ( $r = 0.09$ ). In contrast, the GG status primarily correlates with liver injury (ballooning, steatohepatitis) ( $r = 0.3$ - $0.5$ ) and weaker with steatosis ( $r = 0.26$ ). Moreover, a striking feature of the protective CC status is the fast resolution of transaminase levels after alcohol detoxification without notable changes of LS. We can only speculate why CC carriers do not respond with a significant LS decrease after alcohol withdrawal despite an almost normalization of liver transaminases. One explanation could be that only 30% of ALD patients with elevated transaminase levels show a change of LS after alcohol withdrawal<sup>[42]</sup>. In other words, liver injury as assessed by elevated AST levels not necessarily increases LS in all patients. It rather suggests that ballooning as predominant histological finding of GG carriers may not necessarily cause an increase of transaminase levels. Indeed, ballooning was not significantly associated with elevated AST levels. We therefore believe that GG carriers not only have higher inflammation but also seem to have a slower resolution of liver damage/ballooning. One possible explanation could be that *PNPLA3* directly affects pressure-mediated LS elevation according to the recently introduced pressure hypothesis of cirrhosis that also encompasses mechano-signaling<sup>[24]</sup>. In line with this the co-presence of steatosis in GG carriers could lower LS since steatosis and LS seem not to associate directly (tissue softening of fat).

One of the limitations of our study is the fact that the exact time point of stopping drinking cannot always be determined with absolute correctness nor the adherence to abstaining from alcohol. In addition, the individual response of both laboratory parameters and LS to alcohol withdrawal may also vary considerably. Nevertheless, we strongly feel that the delayed resolution of alcohol-induced inflammation and LS in GG carriers could contribute to fibrosis progression in drinkers who typically show a pulsatile exposure to alcohol and in line with the

recently proposed sinusoidal pressure hypothesis<sup>[47]</sup>. Consequently, GG carriers could have a longer overall exposure to liver inflammation and elevated LS finally resulting in fibrosis progression.

Taken together, liver damage (inflammation/ballooning) with increased LS appears to be the primary event in GG carriers in response to heavy alcohol consumption, which resolves after alcohol withdrawal. Interestingly, GG carriers require a longer period of medical care in the hospital for alcohol detoxification showing advanced liver fibrosis and pointing toward more severe alcohol-related health problems. However, as demonstrated by our non-invasive fibrosis assessment of the whole study population, *PNPLA3* carrier status accounts only for circa 20% of alcoholic cirrhosis corresponding to about 4% of our overall study cohort and suggesting additional other, hitherto not recognized pro-fibrogenic factors. On a final note, we would like to emphasize the importance of non-invasive characterization of ALD study cohorts in the light of potential study bias of solely biopsy-based designs.

## COMMENTS

### Background

Polymorphisms of *PNPLA3* gene (Adiponutrin) have been identified as important genetic progression factor both of nonalcoholic fatty liver disease and alcoholic liver disease (ALD), the most common liver diseases worldwide. However, *PNPLA3* function and its molecular role in liver fibrosis are still unsettled.

### Research frontiers

Several studies in different populations have confirmed the association of a *PNPLA3* polymorphism with chronic liver disorders ranging from steatosis, inflammation to fibrosis progression and even hepatocellular carcinoma. It has also been shown that *PNPLA3* I148M elevates liver stiffness, an increasingly used non-invasive parameter to screen for liver cirrhosis.

### Innovations and breakthroughs

This is the first study, which investigated in detail the impact of *PNPLA3* I148M status, first, on detailed histological subscores in heavy drinkers, and, second, on liver stiffness and other laboratory parameters in response to alcohol withdrawal.

### Applications

In heavy drinkers, *PNPLA3* GG primarily correlates with ballooning/steatohepatitis but not steatosis resulting in a delayed inflammation-associated resolution of liver stiffness (LS). Consequently, sustained ballooning-associated LS elevation seems to be a potential risk factor for fibrosis progression in *PNPLA3* GG carriers. Significantly more patients without fibrosis (F0) were seen in the non-invasively characterized cohort as compared to the liver biopsy cohort (47% vs 6%) underlining the potential bias of biopsy-based studies.

### Terminology

ALD is the most common chronic liver disease in the Western world. ALD encompasses a broad spectrum of disorders ranging from simple steatosis to severe forms of liver injury, including alcoholic steatohepatitis, fibrosis and cirrhosis. It has been shown, that the SNP rs738409 in *PNPLA3* encoding for an isoleucine to methionine substitution at position 148 (I148M) is a strong liver disease modifier responsible for disease progression.

### Peer-review

Rausch *et al* analyzed the influence of *PNPLA3* genotype in heavy drinkers on serum markers and LS during all stages of alcoholic liver disease (steatosis, steatohepatitis and fibrosis) prior and after alcohol detoxification. This is a study of great interest that can help the researchers in evolving in this field.

## REFERENCES

- Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* 2011; **141**: 1572-1585 [PMID: 21920463 DOI: 10.1053/j.gastro.2011.09.002]
- Seitz HK, Mueller S. Alcoholic liver disease. In: Dancygier H, editor *Clinical Hepatology: Principles and Practice of Hepatobiliary Diseases*. Heidelberg, Dordrecht, Londong, New York: Springer, 2009: 1111-1152
- O'Shea RS, Dasarthy S, McCullough AJ. Alcoholic liver disease. *Hepatology* 2010; **51**: 307-328 [PMID: 20034030 DOI: 10.1002/hep.23258]
- Reed T, Page WF, Viken RJ, Christian JC. Genetic predisposition to organ-specific endpoints of alcoholism. *Alcohol Clin Exp Res* 1996; **20**: 1528-1533 [PMID: 8986199 DOI: 10.1111/j.1530-0277.1996.tb01695.x]
- Hrubec Z, Omenn GS. Evidence of genetic predisposition to alcoholic cirrhosis and psychosis: twin concordances for alcoholism and its biological end points by zygosity among male veterans. *Alcohol Clin Exp Res* 1981; **5**: 207-215 [PMID: 7018299 DOI: 10.1111/j.1530-0277.1981.tb04890.x]
- Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]
- Sookoian S, Castaño GO, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. *J Lipid Res* 2009; **50**: 2111-2116 [PMID: 19738004 DOI: 10.1194/jlr.P900013-JLR200]
- Romeo S, Huang-Doran I, Baroni MG, Kotronen A. Unravelling the pathogenesis of fatty liver disease: patatin-like phospholipase domain-containing 3 protein. *Curr Opin Lipidol* 2010; **21**: 247-252 [PMID: 20480550 DOI: 10.1097/MOL.0b013e328338ca61]
- Mueller S, Millonig G, Sarovska L, Friedrich S, Reimann FM, Pritsch M, Eisele S, Stickel F, Longerich T, Schirmacher P, Seitz HK. Increased liver stiffness in alcoholic liver disease: differentiating fibrosis from steatohepatitis. *World J Gastroenterol* 2010; **16**: 966-972 [PMID: 20180235 DOI: 10.3748/wjg.v16.i8.966]
- Stickel F, Buch S, Lau K, Meyer zu Schwabedissen H, Berg T, Ridinger M, Rietschel M, Schafmayer C, Braun F, Hinrichsen H, Günther R, Arlt A, Seeger M, Mueller S, Seitz HK, Soyka M, Lerch M, Lammert F, Sarrazin C, Kubitz R, Häussinger D, Hellerbrand C, Bröring D, Schreiber S, Kiefer F, Spanagel R, Mann K, Datz C, Krawczak M, Wodarz N, Völzke H, Hampe J. Genetic variation in the *PNPLA3* gene is associated with alcoholic liver injury in caucasians. *Hepatology* 2011; **53**: 86-95 [PMID: 21254164 DOI: 10.1002/hep.24017]
- Trepo E, Franchimont D, Moreno C. Association of *PNPLA3* (rs738409 C>G) with liver damage in liver diseases: one step closer to personalized medicine? *Pers Med* 2011; **8**: 595-597 [DOI: 10.2217/pme.11.66]
- Stickel F, Hampe J, Trépo E, Datz C, Romeo S. *PNPLA3* genetic variation in alcoholic steatosis and liver disease progression. *Hepatobiliary Surg Nutr* 2015; **4**: 152-160 [PMID: 26151055 DOI: 10.3978/j.issn.2304-3881.2014.11.04]
- Wilson PA, Gardner SD, Lambie NM, Commans SA, Crowther DJ. Characterization of the human patatin-like phospholipase family. *J Lipid Res* 2006; **47**: 1940-1949 [PMID: 16799181 DOI: 10.1194/jlr.M600185-JLR200]
- Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science* 2004; **306**: 1383-1386 [PMID: 15550674 DOI: 10.1126/science.1100747]
- Huang Y, He S, Li JZ, Seo YK, Osborne TF, Cohen JC, Hobbs HH. A feed-forward loop amplifies nutritional regulation of *PNPLA3*. *Proc Natl Acad Sci USA* 2010; **107**: 7892-7897 [PMID: 20385813 DOI: 10.1073/pnas.1003585107]

- 16 **Lake AC**, Sun Y, Li JL, Kim JE, Johnson JW, Li D, Revett T, Shih HH, Liu W, Paulsen JE, Gimeno RE. Expression, regulation, and triglyceride hydrolase activity of Adiponutrin family members. *J Lipid Res* 2005; **46**: 2477-2487 [PMID: 16150821 DOI: 10.1194/jlr.M500290-JLR200]
- 17 **He S**, McPhaul C, Li JZ, Garuti R, Kinch L, Grishin NV, Cohen JC, Hobbs HH. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J Biol Chem* 2010; **285**: 6706-6715 [PMID: 20034933 DOI: 10.1074/jbc.M109.064501]
- 18 **Pirazzi C**, Valenti L, Motta BM, Pingitore P, Hedfalk K, Mancina RM, Burza MA, Indiveri C, Ferro Y, Montalcini T, Maglio C, Dongiovanni P, Fargion S, Rametta R, Pujia A, Andersson L, Ghosal S, Levin M, Wiklund O, Iacovino M, Borén J, Romeo S. PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. *Hum Mol Genet* 2014; **23**: 4077-4085 [PMID: 24670599 DOI: 10.1093/hmg/ddu121]
- 19 **Krawczyk M**, Grünhage F, Lammert F. Identification of combined genetic determinants of liver stiffness within the SREBP1c-PNPLA3 pathway. *Int J Mol Sci* 2013; **14**: 21153-21166 [PMID: 24152445 DOI: 10.3390/ijms141021153]
- 20 **Castéra L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350 [PMID: 15685546]
- 21 **Ganne-Carrié N**, Ziol M, de Lédinghen V, Douvin C, Marcellin P, Castera L, Dhumeaux D, Trinchet JC, Beaugrand M. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. *Hepatology* 2006; **44**: 1511-1517 [PMID: 17133503 DOI: 10.1002/hep.21420]
- 22 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077 DOI: 10.1053/j.gastro.2008.01.034]
- 23 **Castera L**, Pinzani M. Biopsy and non-invasive methods for the diagnosis of liver fibrosis: does it take two to tango? *Gut* 2010; **59**: 861-866 [PMID: 20581229 DOI: 10.1136/gut.2010.214650]
- 24 **Mueller S**, Sandrin L. Liver stiffness: a novel parameter for the diagnosis of liver disease. *Hepat Med* 2010; **2**: 49-67 [PMID: 24367208]
- 25 **Mueller S**, Seitz HK, Rausch V. Non-invasive diagnosis of alcoholic liver disease. *World J Gastroenterol* 2014; **20**: 14626-14641 [PMID: 25356026 DOI: 10.3748/wjg.v20.i40.14626]
- 26 **Sagir A**, Erhardt A, Schmitt M, Häussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. *Hepatology* 2008; **47**: 592-595 [PMID: 18098325 DOI: 10.1002/hep.22056]
- 27 **Arena U**, Vizzutti F, Corti G, Ambu S, Stasi C, Bresci S, Moscarella S, Boddi V, Petrarca A, Laffi G, Marra F, Pinzani M. Acute viral hepatitis increases liver stiffness values measured by transient elastography. *Hepatology* 2008; **47**: 380-384 [PMID: 18095306 DOI: 10.1002/hep.22007]
- 28 **Dechène A**, Sowa JP, Gieseler RK, Jochum C, Bechmann LP, El Fouly A, Schlattjan M, Saner F, Baba HA, Paul A, Dries V, Odenthal M, Gerken G, Friedman SL, Canbay A. Acute liver failure is associated with elevated liver stiffness and hepatic stellate cell activation. *Hepatology* 2010; **52**: 1008-1016 [PMID: 20684020 DOI: 10.1002/hep.23754]
- 29 **Millonig G**, Friedrich S, Adolf S, Fonouni H, Golriz M, Mehrabi A, Stiefel P, Pöschl G, Büchler MW, Seitz HK, Mueller S. Liver stiffness is directly influenced by central venous pressure. *J Hepatol* 2010; **52**: 206-210 [PMID: 20022130 DOI: 10.1016/j.jhep.2009.11.018]
- 30 **Millonig G**, Reimann FM, Friedrich S, Fonouni H, Mehrabi A, Büchler MW, Seitz HK, Mueller S. Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis. *Hepatology* 2008; **48**: 1718-1723 [PMID: 18836992 DOI: 10.1002/hep.22577]
- 31 **Piecha F**, Peccerella T, Bruckner T, Seitz HK, Rausch V, Mueller S. Arterial pressure suffices to increase liver stiffness. *Am J Physiol Gastrointest Liver Physiol* 2016; **311**: G945-G953 [PMID: 27288426 DOI: 10.1152/ajpgi.00399.2015]
- 32 **Mederacke I**, Wursthorn K, Kirschner J, Rifai K, Manns MP, Wedemeyer H, Bahr MJ. Food intake increases liver stiffness in patients with chronic or resolved hepatitis C virus infection. *Liver Int* 2009; **29**: 1500-1506 [PMID: 19732330 DOI: 10.1111/j.1478-3231.2009.02100.x]
- 33 **Hines CD**, Lindstrom MJ, Varma AK, Reeder SB. Effects of postprandial state and mesenteric blood flow on the repeatability of MR elastography in asymptomatic subjects. *J Magn Reson Imaging* 2011; **33**: 239-244 [PMID: 21182146 DOI: 10.1002/jmri.22354]
- 34 **Lanzi A**, Gianstefani A, Mirarchi MG, Pini P, Conti F, Bolondi L. Liver AL amyloidosis as a possible cause of high liver stiffness values. *Eur J Gastroenterol Hepatol* 2010; **22**: 895-897 [PMID: 19701091 DOI: 10.1097/MEG.0b013e3283309d5b]
- 35 **Bastard C**, Bosisio MR, Chabert M, Kalopissis AD, Mahrouf-Yorgov M, Gilgenkrantz H, Mueller S, Sandrin L. Transient micro-elastography: A novel non-invasive approach to measure liver stiffness in mice. *World J Gastroenterol* 2011; **17**: 968-975 [PMID: 21448348 DOI: 10.3748/wjg.v17.i8.968]
- 36 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]
- 37 **Chevallier M**, Guerret S, Chossegros P, Gerard F, Grimaud JA. A histological semiquantitative scoring system for evaluation of hepatic fibrosis in needle liver biopsy specimens: comparison with morphometric studies. *Hepatology* 1994; **20**: 349-355 [PMID: 8045495 DOI: 10.1002/hep.1840200213]
- 38 **Yip WW**, Burt AD. Alcoholic liver disease. *Semin Diagn Pathol* 2006; **23**: 149-160 [PMID: 17355088 DOI: 10.1053/j.semmp.2006.11.002]
- 39 **Sandrin L**, Fournier C, Miette V, Millonig G, Mueller S. Fibroscan in hepatology: a clinically-validated tool using vibration-controlled transient elastography. Proceedings of the Ultrasonics Symposium (IUS), 2009 IEEE International; 2009: 1431-1434 [DOI: 10.1109/ultsym.2009.5441658]
- 40 **Durango E**, Dietrich C, Seitz HK, Kunz CU, Pomier-Layrargues GT, Duarte-Rojo A, Beaton M, Elkhatab M, Myers RP, Mueller S. Direct comparison of the FibroScan XL and M probes for assessment of liver fibrosis in obese and nonobese patients. *Hepat Med* 2013; **5**: 43-52 [PMID: 24696623 DOI: 10.2147/HMER.S45234]
- 41 **Kohlhaas A**, Durango E, Millonig G, Bastard C, Sandrin L, Golriz M, Mehrabi A, Büchler MW, Seitz HK, Mueller S. Transient elastography with the XL probe rapidly identifies patients with nonhepatic ascites. *Hepat Med* 2012; **4**: 11-18 [PMID: 24367229 DOI: 10.2147/HMER.S30256]
- 42 **Mueller S**, Englert S, Seitz HK, Badea RI, Erhardt A, Bozaari B, Beaugrand M, Lupşor-Platon M. Inflammation-adapted liver stiffness values for improved fibrosis staging in patients with hepatitis C virus and alcoholic liver disease. *Liver Int* 2015; **35**: 2514-2521 [PMID: 26121926 DOI: 10.1111/liv.12904]
- 43 **You FM**, Huo N, Gu YQ, Luo MC, Ma Y, Hane D, Lazo GR, Dvorak J, Anderson OD. BatchPrimer3: a high throughput web application for PCR and sequencing primer design. *BMC Bioinformatics* 2008; **9**: 253 [PMID: 18510760 DOI: 10.1186/1471-2105-9-253]
- 44 **Singal AG**, Manjunath H, Yopp AC, Beg MS, Marrero JA, Gopal P, Waljee AK. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 325-334 [PMID: 24445574 DOI: 10.1038/ajg.2013.476]
- 45 **Guyot E**, Sutton A, Rufat P, Laguillier C, Mansouri A, Moreau R, Ganne-Carrié N, Beaugrand M, Charnaux N, Trinchet JC, Nahon P. PNPLA3 rs738409, hepatocellular carcinoma occurrence and risk model prediction in patients with cirrhosis. *J Hepatol* 2013; **58**:



- 312-318 [PMID: 23069476 DOI: 10.1016/j.jhep.2012.09.036]
- 46 **Salameh H**, Raff E, Erwin A, Seth D, Nischalke HD, Falletti E, Burza MA, Leathert J, Romeo S, Molinaro A, Corradini SG, Toniutto P, Spengler U, Daly A, Day CP, Kuo YF, Singal AK. *PNPLA3* Gene Polymorphism Is Associated With Predisposition to
- and Severity of Alcoholic Liver Disease. *Am J Gastroenterol* 2015; **110**: 846-856 [PMID: 25964223 DOI: 10.1038/ajg.2015.137]
- 47 **Mueller S**. Does pressure cause cirrhosis? The sinusoidal pressure hypothesis and role of arterialization. *World J Gastroenterol* 2016; In press

**P- Reviewer:** Shih TT, Stasi C, van Erpecum K    **S- Editor:** Gong ZM  
**L- Editor:** A    **E- Editor:** Li D



## Retrospective Cohort Study

# Hepatitis C eradication with sofosbuvir leads to significant metabolic changes

Amilcar L Morales, Zachary Junga, Manish B Singla, Maria Sjogren, Dawn Torres

Amilcar L Morales, Hepatology Service, San Antonio Military Medical Center, San Antonio, TX 78234, United States

Amilcar L Morales, Zachary Junga, Manish B Singla, Maria Sjogren, Dawn Torres, Gastroenterology Service, Walter Reed National Military Medical Center, Bethesda, MD 20889, United States

Zachary Junga, Department of Internal Medicine, Walter Reed National Military Medical Center, Bethesda, MD 20889, United States

**Author contributions:** Morales AL designed the study; Morales AL and Junga Z collected and analyzed the data, and drafted the manuscript; Singla MB performed statistical analysis and manuscript review; Sjogren M and Torres D revised the manuscript for important intellectual content, and help with development of the manuscript and study design; all authors have read and approved the final version to be published.

**Institutional review board statement:** The study protocol was reviewed and approved by the Walter Reed National Military Medical Center Institutional Review Board.

**Informed consent statement:** Informed consent was not provided by patients. The Walter Reed National Military Medical Center Institutional Review Board provided a waiver of informed consent authorizing the use of de-identified patient data for research purpose.

**Conflict-of-interest statement:** No potential conflicts of interest to report.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at [amilcar.l.moralescardona.mil@mail.mil](mailto:amilcar.l.moralescardona.mil@mail.mil).

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and

the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

**Correspondence to:** Amilcar L Morales, MD, Transplant Hepatology Staff, Hepatology Service, San Antonio Military Medical Center, 3551 Roger Brooke Dr, Fort Sam Houston, San Antonio, TX 78234, United States. [amcardona2002@yahoo.com](mailto:amcardona2002@yahoo.com)  
 Telephone: +1-210-8729581

Received: August 15, 2016

Peer-review started: August 23, 2016

First decision: September 6, 2016

Revised: September 26, 2016

Accepted: October 22, 2016

Article in press: October 24, 2016

Published online: December 18, 2016

## Abstract

### AIM

To assess the effect of sofosbuvir (SOF) based regimens on glycemic and lipid control.

### METHODS

This is a retrospective analysis of hepatitis C virus (HCV)-infected patients treated and cured with a SOF regimen [SOF/ribavirin/interferon, SOF/simeprevir, or SOF/ledipasvir (LDV) ± ribavirin] from January 2014 to March 2015. Patients with hemoglobin A1C (HbA1C) and lipid panels within six months before and six months after therapy were identified and included in our study. Due to the known hemolytic effect of ribavirin, HbA1C was obtained a minimum of three months post-treatment for the patients treated with a ribavirin regimen. Medical history, demographics, HCV genotype, pre-therapy RNA, and liver biopsies were included in our analysis. The patients who started a new medication or had an adjustment of baseline medical management for hyper-

lipidemia or diabetes mellitus (DM) were excluded from our analysis.

## RESULTS

Two hundred and thirty-four patients were reviewed, of which 60 patients met inclusion criteria. Sixty-three point three percent were male, 26.7% were Caucasian, 41.7% were African American and 91.7% were infected with hepatitis C genotype 1. Mean age was  $60.6 \pm 6.7$  years. Thirty-nine patients had HbA1C checked before and after treatment, of which 22 had the diagnosis of DM type 2. HbA1C significantly decreased with treatment of HCV (pretreatment  $6.66\% \pm 0.95\%$  vs post-treatment  $6.14\% \pm 0.65\%$ ,  $P < 0.005$ ). Those treated with SOF/LDV had a lower HbA1C response than those treated with other regimens ( $0.26\% \pm 0.53\%$  vs  $0.71\% \pm 0.83\%$ ,  $P = 0.070$ ). Fifty-two patients had pre- and post-treatment lipid panels; there was a significant increase in low-density lipoprotein (LDL) and total cholesterol (TC) after treatment (LDL:  $99.5 \pm 28.9$  mg/dL vs  $128.3 \pm 34.9$  mg/dL,  $P < 0.001$ ; TC:  $171.6 \pm 32.5$  mg/dL vs  $199.7 \pm 40.0$  mg/dL,  $P < 0.001$ ). Pre-treatment body-mass index (BMI) did not differ from post-treatment BMI ( $P = 0.684$ ).

## CONCLUSION

Eradication of HCV with a SOF regimen resulted in a significant drop in HbA1C and an increase in LDL and TC post therapy.

**Key words:** Hepatitis C; Sofosbuvir; Hyperlipidemia; Hemoglobin A1c; Low-density lipoprotein

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In our retrospective study, we evaluated the changes in glucose and lipid metabolism in a group of hepatitis C patients treated and cured with a sofosbuvir-containing regimen. We used hemoglobin A1c (HbA1c) and lipid panels to assess those two parameters. Six months post eradication, we found a statistically significant drop in HbA1c and an increase in low-density lipoprotein and total cholesterol. The use of HbA1c, although not perfect, is easy to understand and is frequently used by primary care doctors as a tool to assess glucose control.

Morales AL, Junga Z, Singla MB, Sjogren M, Torres D. Hepatitis C eradication with sofosbuvir leads to significant metabolic changes. *World J Hepatol* 2016; 8(35): 1557-1563 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i35/1557.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i35.1557>

## INTRODUCTION

Hepatitis C virus (HCV) is a leading cause of chronic liver disease, with a prevalence of infection in the United States of approximately 1.6%<sup>[1]</sup>. It remains the leading cause of death from liver disease and is the leading

indication for liver transplantation in the United States despite recent medical advances in HCV therapy. HCV can cause major alterations in insulin resistance (IR) and lipid homeostasis<sup>[2-4]</sup>. HCV infection has been associated with the development of diabetes mellitus type 2<sup>[5,6]</sup> as well as a 3.5-fold increase in the prevalence of glucose alterations in non-diabetics<sup>[7]</sup>. The pathogenesis between HCV and IR seems to be multifactorial, with cytokine upregulation and direct interactions between viral particles and insulin signaling pathways<sup>[8-12]</sup>. In the traditional pegylated interferon (PegIFN) based regimens, IR was associated with decreased sustained virological response (SVR)<sup>[13]</sup>; multiple studies have shown an association between viral suppression or clearance and improvement of IR<sup>[14-17]</sup>.

Along with IR, steatosis is also very common in patients infected with HCV<sup>[18]</sup>. The exact mechanism has not been fully elucidated, but host lipid alterations seem to play a major role. The virus utilizes very low-density lipoproteins (LDLs) to infect hepatocytes and several other lipid secretory mechanisms to perpetuate replication<sup>[4]</sup>. Several proteins, including Seipin and the HCV core protein, have been shown to alter the production of free fatty acids, as well as the proper excretion of lipids, increasing steatosis in the host<sup>[19-21]</sup>. Hypocholesterolemia is another finding that seems to be closely related to HCV replication mechanisms. After successful treatment with interferon-based therapy, it has been shown that hypocholesterolemia was resolved, with significant increases in LDL, triglycerides, and cholesterol<sup>[22,23]</sup>.

The era of direct-acting antiviral (DAA) agents has increased SVR rates to over 90%, with dramatically improved side effect profiles<sup>[24-30]</sup>. IR and lipid alterations do not seem to affect treatment outcomes, and there is limited data on the effects of DAA therapy on metabolic and lipid profiles. A recent study evaluating the effects of sofosbuvir (SOF) and ribavirin (RBV) therapy in a mostly non-diabetic population demonstrated fluctuations in LDL levels throughout treatment, with elevations in LDL in patients achieving SVR as well as a small decrease in hemoglobin A1C (HbA1C) levels ( $5.58\% \pm 0.08\%$  to  $5.45\% \pm 0.91\%$ ;  $P = 0.0046$ )<sup>[31]</sup>. Additional data regarding metabolic alterations after therapy with the new DAA is scarce. In this retrospective study, the effects of HCV eradication on glucose and lipid metabolism in patients treated with SOF-based regimens at WRNMMC from 2014 to 2015 were assessed.

## MATERIALS AND METHODS

### Eligibility criteria

Patients aged 18 years or older with confirmed infections with HCV (by RNA) treated and cured at our institution with any combination of a NS5B inhibitor (SOF), NS5A inhibitor [ledipasvir (LDV)], protease inhibitor (Simeprevir), RBV and PegIFN from January 2014 to March 2015 were eligible for the study. Electronic records were reviewed to look for patients with a HbA1C and/or lipid panel drawn

before and after therapy. A total of 234 patient charts were reviewed.

### HbA1C

The HbA1C closest to starting day of HCV therapy (up to six months pre-therapy) and the closest HbA1C post-therapy (up to six months) were included in our analysis. RBV is known to cause hemolysis, with remarkable drops in hemoglobin. Since HbA1C is closely related to red blood cell lifespan and could be altered by RBC destructions and anemia, the HbA1C in this population was selected between three to six months post therapy. Hemoglobin levels were reviewed pre-therapy and post-therapy and added to the analysis.

In patients with the diagnosis of diabetes mellitus type 2, a review of concomitant hypoglycemic medications was performed. All clinic encounters up to a year prior to starting HCV therapy, during therapy, and up to six months post therapy were reviewed, looking for adjustments of medications that could have altered HbA1C values. Those patients who started a medication or had an adjustment of baseline medical management were removed from the analysis. Patients on stable doses of oral hypoglycemic medications or insulin regimens during the study period were included. Attempts to account for diet and exercise regimens were beyond the scope of this analysis.

### Lipids

Lipid panels closest to the starting and end dates of therapy (up to six months pre and post therapy) were included. All samples were drawn during the morning. However, due to the retrospective nature of the analysis, fasting could not be confirmed for all patients. Patients on stable doses of lipid-lowering agents were included, while patients started on new medications or with adjustments during the study period were removed from the analysis.

### Data collection

Basic demographic and clinical information was collected for all patients, including age, gender, race, body-mass index (BMI) pre-and post-HCV therapy, and specific HCV anti-viral therapy used. Pre-therapy aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase, total bilirubin, albumin, total protein, Hepatitis C RNA, Hepatitis C genotype, and liver biopsy staging were also included. Patients with other liver diseases, such as hemochromatosis, Wilson disease, and alcoholic liver disease, were excluded from the analysis. A total of 60 patients were included in the final analysis. The study protocol was approved by the Institutional Review Board of Walter Reed National Military Medical Center.

### Statistical analysis

Data were collated and analyzed using statistical software package IBM SPSS Statistics 21.0 (IBM, Armonk, New York). Continuous data was reported as means  $\pm$  SDs. Paired *t*-test was used to compare variables measured before and after HCV treatment.

Student's *t* test was used to analyze between group comparisons of continuous data including the change in HbA1C, HA1C, LDL, and high density lipoprotein (HDL) over the HCV treatment. A probability value of less than 0.05 was considered statistically significant. The statistical review of the study was performed by a biomedical statistic.

## RESULTS

A total of 234 patients were treated for HCV during the study period. Of these, 60 patients met the inclusion criteria. Their average age was  $60.6 \pm 6.7$  years; 26.7% were Caucasian, 41.7% were African American, and 63.3% were male. Clinical history in the cohort was significant for diabetes (38.3%) and hyperlipidemia (HLD) (33.3%). Patients had a mean viral load of  $4.7 \times 10^6 \pm 7.6 \times 10^6$ ; 50.0% were infected with genotype 1a, and 26.7% were infected with genotype 1b. The mean pre-treatment AST was  $61.0 \pm 49.3$  units/L, ALT  $72.1 \pm 56.2$  units/L, alkaline phosphatase  $102.3 \pm 71.3$  units/L, total bilirubin  $0.8 \pm 1.5$  mg/dL, albumin  $4.2 \pm 0.4$  g/dL, and total protein  $7.4 \pm 0.7$  g/dL.

All patients were treated with a SOF-based regimen. Of the 23 patients with diabetes, 15 were treated with a stable dose of anti-diabetic medications, most commonly metformin. Of the 20 patients with HLD, 12 were taking a statin, and only three had their statins held during therapy (Table 1).

A total of 39 patients had pre- and post-treatment HbA1C measured. Overall, there was a significant drop in HbA1C during treatment (Figure 1). This was not accompanied by a significant decrease in BMI (pre-treatment  $28.86 \pm 5.15$  kg/m<sup>2</sup>, post treatment  $28.48 \pm 4.72$  kg/m<sup>2</sup>,  $P = 0.683$ ). There was no significant difference in HbA1C effect between males and females ( $P = 0.793$ ). There was no significant difference in HbA1C drop between genotype 1a and 1b ( $P = 0.605$ ). Although not statically significant, patients with a history of diabetes tended to have a larger drop in HbA1C than those without diabetes, and Caucasians tended to have a larger drop in HA1C than African Americans. Patients aged 65 and older were less likely to have a drop in their HbA1C with treatment (younger than 65,  $0.68\% \pm 0.75\%$ , 65 and older,  $-0.01\% \pm 0.47\%$ ,  $P = 0.0187$ ). Sixteen of these patients were treated in conjunction with ribavirin; this did not have a significant effect on HbA1C change (drop in HbA1C with ribavirin  $0.44\% \pm 0.76\%$ ; without ribavirin  $0.68\% \pm 0.74\%$ ,  $P = 0.342$ ). Patients with a high viral load ( $> 6000000$  copies) tended to have a larger drop in HbA1C with treatment (high VL  $0.87\% \pm 0.97\%$ , low VL  $0.40\% \pm 0.62\%$ ,  $P = 0.080$ ).

Fifty-two patients had pre- and post-treatment lipid panels measured. Overall, there was a significant increase in LDL and total cholesterol (TC) with minimal change in HDL (pre  $52.8 \pm 18.3$  mg/dL, post  $51.4 \pm 18.5$  mg/dL,  $P = 0.699$ ) and triglycerides (pre  $132.1 \pm 99.7$  mg/dL, post  $129.8 \pm 80.8$  mg/dL,  $P = 0.853$ ) (Figure 2). Patients with a history of HLD did not have



**Table 1 Patient characteristics (n = 60)**

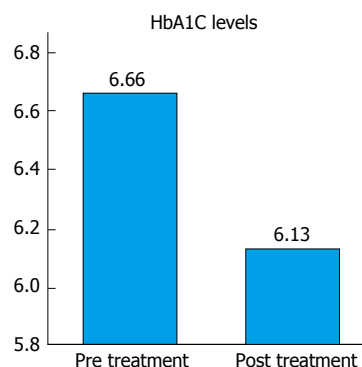
Male (n = 38)	63.3%
Female (n = 22)	36.7%
Race	
Caucasian (n = 16)	26.7%
African American (n = 25)	41.7%
Hispanic (n = 3)	5.0%
Asian (n = 2)	3.3%
Not listed (n = 14)	23.3%
Mean age ± SD	60.6 ± 6.7
Diabetic (n = 23)	38.3%
Hyperlipidemia (n = 20)	33.3%
Hypertension (n = 42)	70.0%
Treatment	
Sofosbuvir/ribavirin/interferon (n = 21)	35.0%
Sofosbuvir/simeprevir (n = 11)	18.3%
Sofosbuvir/ledipasvir (n = 23)	38.3%
Sofosbuvir/ribavirin (n = 4)	8.3%
Sofosbuvir (n = 1)	1.7%
Biopsy stage (n = 49)	
1 (n = 8)	13.3%
2 (n = 21)	35.0%
3 (n = 5)	8.3%
4 (n = 15)	25.0%
Statin use (n = 20)	20.0%
Statin held during tx (n = 3)	5.0%
Mean viral load	4746471 ± 7641768
Mean ALT	72.1 ± 56.2
Genotype	
1a (n = 30)	50.0%
1b (n = 16)	26.7%
1 undistinguished (n = 9)	15.0%
2 (n = 2)	3.3%
3 (n = 3)	5.0%

ALT: Alanine transaminase.

significantly larger increase in LDL than those without a history of hyperlipidemia (HLD  $27.8 \pm 30.7$  mg/dL, non-HLD  $30.4 \pm 44.6$  mg/dL,  $P = 0.810$ ). Patient's age 65 or older did not have a significantly larger increase in LDL than younger patients (65 or older  $22.4 \pm 32.3$  mg/dL, younger  $30.4 \pm 37.1$  mg/dL,  $P = 0.511$ ). Caucasians and African Americans had similar increases in LDL (Caucasians  $35.7 \pm 36.0$  mg/dL, African Americans  $33.4 \pm 35.5$  mg/dL,  $P = 0.847$ ). Those treated with SOF + ledipasvir tended to have a larger increase in LDL than those treated with other regimens (SOF + LED  $36.7 \pm 39.3$  mg/dL, other therapy  $22.1 \pm 33.1$  mg/dL,  $P = 0.157$ ). Treatment regimen including interferon did not affect LDL increase ( $P = 0.755$ ). High VL ( $> 6000000$  copies) prior to treatment did not affect significantly impact the increase in LDL ( $P = 0.221$ ). Patients with hepatitis C genotype 3 ( $n = 3$ ) on average had an increase in LDL of 59 mg/dL (pre 87.3 mg/dL, post 146.3 mg/dL) and an increase in TC of 60 mg/dL (pre 148.7 mg/dL, post 208.7 mg/dL).

## DISCUSSION

The main finding of this retrospective study was a significant decrease in HbA1C up to six months post-HCV eradication. The mechanism responsible for this



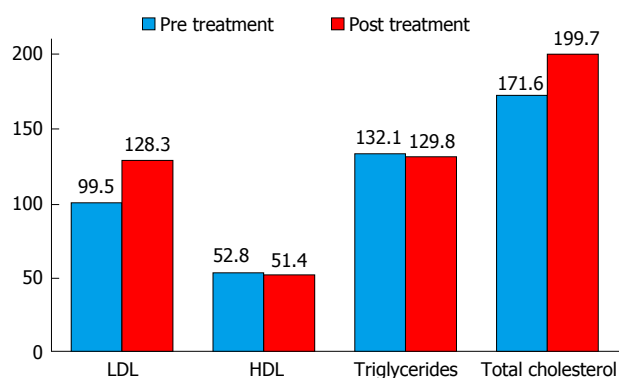
**Figure 1 Effects of hepatitis C eradication on hemoglobin A1C.** Vertical axis represents HbA1C levels in mg/dL. HbA1C significantly decreased after eradication of hepatitis C virus (pretreatment  $6.66 \pm 0.95$  mg/dL vs post-treatment  $6.14 \pm 0.65$  mg/dL,  $P < 0.005$ ). HbA1C: Hemoglobin A1C.

improvement in glycemic control is unknown although likely multifactorial. It is well known that HCV alters glucose metabolism by inducing inflammatory cascades and promoting IR. Defects in pathways important in hepatic gluconeogenesis such as PI3K and AKT phosphorylation have been reported in patients infected with HCV. Insulin receptor substrates 1 and 2 are closely related to the PI3K/AKT pathways; these two receptors are key components in the development of IR in patients infected with HCV. The virus can degrade these two receptors, directly affecting the PI3K/AKT pathways<sup>[32-34]</sup>. Eradication of the virus restores homeostasis of these pathways, leading to an improvement in IR.

In the interferon/RBV era, several studies have demonstrated an improvement of IR with SVR. Early work by Thompson *et al.*<sup>[17]</sup> demonstrated a 10% decrease in IR in genotype 1 patients who achieved SVR, which was supported by the more recent results from Chien *et al.*<sup>[35]</sup> that showed a significant decrease in HOMA-IR at EOT after eradication of the virus with this combination. Similarly, a study by Meissner *et al.*<sup>[31]</sup> demonstrated a small but significant decrease in HbA1c in patients treated with SOF/RBV ( $5.58\% \pm 0.08\%$  to  $5.45\% \pm 0.91\%$ ;  $P = 0.0046$ ). While the majority of these patients were non-diabetic or pre-diabetics, the patients included in this analysis had a significantly higher rate of diabetes, at 56%. When compared to the non-diabetic patients, the diabetics had a greater improvement in HbA1C. Gender, race, HCV genotype, and HCV RNA did not affect HbA1C drop.

In a subgroup analysis, patients treated with the SOF/LDV had a lower drop in HbA1C when compared to SOF/RBV and SOF/SIM groups. One possible explanation is the relationship between the new DAA and its target. The non-structural proteins of the virus NS5A and NS5B are key components in the activation of inflammatory cascades promoting insulin resistance<sup>[34]</sup>. It is plausible that the interaction of the medication or the duration of therapy alters the effects of insulin resistance, although further study is required.

Although this study was not designed to identify the



**Figure 2 Effects of hepatitis C eradication on lipids.** Vertical axis represents lipid levels in mg/dL. LDL and total cholesterol were significantly higher post hepatitis C eradication (LDL:  $99.5 \pm 28.9$  mg/dL vs  $128.3 \pm 34.9$  mg/dL,  $P < 0.001$ ; total cholesterol  $171.6 \pm 32.5$  mg/dL vs  $199.7 \pm 40.0$  mg/dL,  $P < 0.001$ ). No significant changes were noted for HDL and triglycerides. LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

long-term implications of hepatitis C eradication in glucose control, it is possible that these changes could have long-term implications regarding medical management. One of 16 patients on medical therapy for diabetes required a decrease in insulin therapy post viral eradication and another was taken off completely of therapy. The savings from a drop of even 0.5% of HbA1c are significant, and many oral hypoglycemics maximum efficacy is only a 1% improvement in HbA1c. Even more important than potential cost savings are the implications of better glucose control in the development of microvascular and macrovascular disease as small drops in HbA1c can alter the course of these complications. Primary care physician should monitor diabetic patients post HCV eradication to assess if changes in medical management are required and to prevent complications such as hypoglycemia.

The implications of insulin resistance, especially in diabetic patients infected with HCV, are well established. Huang *et al.*<sup>[36]</sup> showed an increased risk of liver disease progression to cirrhosis in HCV-infected patients with diabetes. Hui *et al.*<sup>[3]</sup> demonstrated that insulin resistance was an independent predictor for the degree of fibrosis and fibrosis progression in HCV-infected patients. Everhart *et al.*<sup>[37]</sup> showed that not only hepatic steatosis was associated with liver disease progression, but also the degree of insulin resistance. They suggested that addressing these two issues might modify disease progression<sup>[37]</sup>. Taking into account this information and the results of our study, we should consider adding diabetic HCV-infected patients to the high-risk group that would benefit from priority in treatment.

These results also correlate with previous studies evaluating the effects of HCV eradications and lipids. An increase in TC and LDL post therapy was demonstrated irrespective of anti-viral therapy or genotype. Chronic infection with HCV has been implicated in the development of hypolipidemia<sup>[38,39]</sup>. A reversal of these findings has been reported in patients treated with INF/RBV regimens, as well as SOF/RBV regimens that have

achieved SVR, suggesting this is most likely related to viral clearance rather than a medication effect<sup>[22,23,31]</sup>. The implications of these alterations in cardiovascular and cerebrovascular disease are beyond the scope of this retrospective study but should be further investigated.

The study does have several limitations including its retrospective nature and the small number of patients. Even though all lipids were drawn during the morning time, fasting was unable to be confirmed. Other parameters that could have altered the results, such as dietary changes and exercise, were not available. Medication reconciliation was not directly obtained, but an evaluation of several encounters from the electronic medical record from different providers was performed, looking for adequate medication reconciliation. The length of analysis was also limited to six months post-HCV therapy, so an analysis of the long-term implications of these results cannot be made.

This analysis did strengthen the knowledge pertaining to the metabolic effects of SOF-based regimens and confirmed that eradication of the virus could have extra-hepatic benefits. Even though HOMA-IR is a more direct measurement of IR, HbA1c is a more practical parameter that can be used to assess glucose control, and this study confirmed an improvement in HgA1c with SVR.

In conclusion, this study showed a significant drop in HbA1c up to six months after the eradication of HCV with SOF-based regimens. Future studies are needed to see if this change is sustainable. The effects of virus eradication on lipid panels were also determined, and they confirmed previous analyses that showed an increase in lipid panels, including LDL and TC, with SVR. This study suggests that physicians treating HCV patients should reassess preventive medicine measures after therapy, as the benefits of eradicating HCV may extend beyond eliminating the effects of chronic liver inflammation.

## COMMENTS

### Background

The hepatitis C virus (HCV) is a leading cause of chronic liver disease, with a prevalence of infection in the United States of approximately 1.6%. It is the leading cause of death from liver disease and is the leading indication for liver transplantation in the United States. Chronic hepatitis C infection (CHC) is known to induce systemic changes regarding glucose control and lipid metabolism. Glycemic balance can be affected by direct effect over insulin activation cascades and as a systemic response to inflammatory cytokines. Patients with CHC developed diabetes mellitus earlier than non-infected patients. Lipid metabolism is also affected due to known impaired lipid secretions associated with the infectious mechanism of the virus (possible use of lipid receptor to infect hepatocytes). Steatosis is another major finding in patients infected with CHC.

### Research frontiers

Hepatitis C therapy has changed drastically in the last four years. The authors are now able to achieve cure rates of over 90%, with minimal side effects. The long-term implications of these new agents are still unclear, and previous studies have shown mixed results regarding alterations in glucose and lipid control after eradication. There is limited data regarding the newer anti-viral agents and their effects on metabolic derangements. The study attempts to assess the metabolic changes associated with these new agents.

## Innovations and breakthroughs

Similar studies evaluating metabolic changes associated with hepatitis C eradication have used HOMA-IR as a surrogate of glucose homeostasis. Although this is a very accurate way of assessing glucose changes post hepatitis C eradication, its use on a daily clinic encounter is limited. In the study, the authors used HgA1c as a surrogate for glucose homeostasis. This laboratory test is easy to use and is well known by non-gastroenterology/hepatology providers. This laboratory test is more practical for daily clinic encounters. Previous studies on patients treated and cured with interferon and Ribavirin have shown alterations in lipid homeostasis, similar to the results. As the data on lipid alterations with the new direct antiviral agents is limited, the study adds to the knowledge on non-hepatic effects associated with a sustained virological response.

## Applications

As reported in the study, several patients required adjustments in their hyperglycemic regimens, and one patient was completely taken off medication. The study suggests that shortly after completing hepatitis C therapy, primary care doctors should monitor diabetic patients, under medical management, to assess if changes to their medications are needed. Although the study was not meant to assess long-term effects, changes in HbA1c, as seen in the study, can add benefits in cost savings, as well as prevent microvascular and macrovascular disease. The changes seen in lipid homeostasis are worrying and require further investigation. These patients are still at risk of developing other liver diseases, such as non-alcoholic fatty liver disease. Primary care doctors should implement close monitoring of lipids after hepatitis C eradication, and those who meet the criteria for therapy should be treated accordingly.

## Terminology

NS5A: Non-structural protein 5A. This protein plays a key role in HCV replication. It is one of the main targets for some of the new direct acting antiviral agents; NS5B: Non-structural protein 5B. Involved in Hepatitis C RNA replication. Main target for some of the new anti-viral agents, such as Sofosbuvir; Sustain virological response: Patients with undetectable hepatitis C viral load 12 wk after completing hepatitis C therapy.

## Peer-review

The manuscript is well presented and of interest and the results can contribute to increase the knowledge of this topic.

## REFERENCES

- Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374 [PMID: 19330875 DOI: 10.1002/hep.22759]
- Moucari R, Asselah T, Cazals-Hatem D, Voitto H, Boyer N, Ripault MP, Sobesky R, Martinot-Peignoux M, Maylin S, Nicolas-Chanoine MH, Paradis V, Vidaud M, Valla D, Bedossa P, Marcellin P. Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. *Gastroenterology* 2008; **134**: 416-423 [PMID: 18164296 DOI: 10.1053/j.gastro.2007.11.010]
- Hui JM, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. *Gastroenterology* 2003; **125**: 1695-1704 [PMID: 14724822]
- Negro F. Abnormalities of lipid metabolism in hepatitis C virus infection. *Gut* 2010; **59**: 1279-1287 [PMID: 20660700 DOI: 10.1136/gut.2009.192732]
- Mehta SH, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J, Szklo M, Thomas DL. Hepatitis C virus infection and incident type 2 diabetes. *Hepatology* 2003; **38**: 50-56 [PMID: 12829986 DOI: 10.1053/jhep.2003.50291]
- Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. *Ann Intern Med* 2000; **133**: 592-599 [PMID: 11033586]
- Huang JF, Yu ML, Dai CY, Hsieh MY, Hwang SJ, Hsiao PJ, Lee LP, Lin ZY, Chen SC, Hsieh MY, Wang LY, Shin SJ, Chang WY, Chuang WL. Reappraisal of the characteristics of glucose abnormalities in patients with chronic hepatitis C infection. *Am J Gastroenterol* 2008; **103**: 1933-1940 [PMID: 18637090 DOI: 10.1111/j.1572-0241.2008.01996.x]
- Nelson DR, Lim HL, Marousis CG, Fang JW, Davis GL, Shen L, Urdea MS, Kolberg JA, Lau JY. Activation of tumor necrosis factor-alpha system in chronic hepatitis C virus infection. *Dig Dis Sci* 1997; **42**: 2487-2494 [PMID: 9440625]
- Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, Taniguchi E, Kumemura H, Hanada S, Maeyama M, Baba S, Koga H, Kumashiro R, Ueno T, Ogata H, Yoshimura A, Sata M. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 2004; **165**: 1499-1508 [PMID: 15509521 DOI: 10.1016/S0002-9440(10)63408-6]
- Pazienza V, Clément S, Pugnale P, Conzelman S, Foti M, Mangia A, Negro F. The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms. *Hepatology* 2007; **45**: 1164-1171 [PMID: 17465001 DOI: 10.1002/hep.21634]
- Miyamoto H, Moriishi K, Moriya K, Murata S, Tanaka K, Suzuki T, Miyamura T, Koike K, Matsuura Y. Involvement of the PA28gamma-dependent pathway in insulin resistance induced by hepatitis C virus core protein. *J Virol* 2007; **81**: 1727-1735 [PMID: 17135326 DOI: 10.1128/JVI.01683-06]
- Bernsmeier C, Duong FH, Christen V, Pugnale P, Negro F, Terracciano L, Heim MH. Virus-induced over-expression of protein phosphatase 2A inhibits insulin signalling in chronic hepatitis C. *J Hepatol* 2008; **49**: 429-440 [PMID: 18486982 DOI: 10.1016/j.jhep.2008.04.007]
- Dai CY, Huang JF, Hsieh MY, Hou NJ, Lin ZY, Chen SC, Hsieh MY, Wang LY, Chang WY, Chuang WL, Yu ML. Insulin resistance predicts response to peginterferon-alpha/ribavirin combination therapy in chronic hepatitis C patients. *J Hepatol* 2009; **50**: 712-718 [PMID: 19231011 DOI: 10.1016/j.jhep.2008.12.017]
- Kawaguchi T, Ide T, Taniguchi E, Hirano E, Ito M, Sumie S, Nagao Y, Yanagimoto C, Hanada S, Koga H, Sata M. Clearance of HCV improves insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate 1 and 2. *Am J Gastroenterol* 2007; **102**: 570-576 [PMID: 17223221 DOI: 10.1111/j.1572-0241.2006.01038.x]
- Delgado-Borrego A, Jordan SH, Negre B, Healey D, Lin W, Kamegaya Y, Christofi M, Ludwig DA, Lok AS, Chung RT. Reduction of insulin resistance with effective clearance of hepatitis C infection: results from the HALT-C trial. *Clin Gastroenterol Hepatol* 2010; **8**: 458-462 [PMID: 20156586 DOI: 10.1016/j.cgh.2010.01.022]
- Huang JF, Yu ML, Huang CF, Juo SH, Dai CY, Hsieh MY, Hou NJ, Yeh ML, Hsieh MH, Yang JF, Lin ZY, Chen SC, Shin SJ, Chuang WL. The outcomes of glucose abnormalities in pre-diabetic chronic hepatitis C patients receiving peginterferon plus ribavirin therapy. *Liver Int* 2012; **32**: 962-969 [PMID: 22356575 DOI: 10.1111/j.1478-3231.2012.02771.x]
- Thompson AJ, Patel K, Chuang WL, Lawitz EJ, Rodriguez-Torres M, Rustgi VK, Flisiak R, Pianko S, Diago M, Arora S, Foster GR, Torbenson M, Benhamou Y, Nelson DR, Sulkowski MS, Zeuzem S, Pulkstenis E, Subramanian GM, McHutchison JG. Viral clearance is associated with improved insulin resistance in genotype 1 chronic hepatitis C but not genotype 2/3. *Gut* 2012; **61**: 128-134 [PMID: 21873466 DOI: 10.1136/gut.2010.236158]
- Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; **33**: 1358-1364 [PMID: 11391523 DOI: 10.1053/jhep.2001.24432]
- Perlemuter G, Sabile A, Letteron P, Vona G, Topilco A, Chrétien Y, Koike K, Pessayre D, Chapman J, Barba G, Bréchet C. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model

- of viral-related steatosis. *FASEB J* 2002; **16**: 185-194 [PMID: 11818366 DOI: 10.1096/fj.01-0396com]
- 20 **Syed GH**, Amako Y, Siddiqui A. Hepatitis C virus hijacks host lipid metabolism. *Trends Endocrinol Metab* 2010; **21**: 33-40 [PMID: 19854061 DOI: 10.1016/j.tem.2009.07.005]
  - 21 **Simon TG**, Butt AA. Lipid dysregulation in hepatitis C virus, and impact of statin therapy upon clinical outcomes. *World J Gastroenterol* 2015; **21**: 8293-8303 [PMID: 26217081 DOI: 10.3748/wjg.v21.i27.8293]
  - 22 **Chang ML**, Tsou YK, Hu TH, Lin CH, Lin WR, Sung CM, Chen TH, Cheng ML, Chang KC, Chiu CT, Yeh CT, Pang JH, Shiao MS. Distinct patterns of the lipid alterations between genotype 1 and 2 chronic hepatitis C patients after viral clearance. *PLoS One* 2014; **9**: e104783 [PMID: 25122116 DOI: 10.1371/journal.pone.0104783]
  - 23 **Kuo YH**, Chuang TW, Hung CH, Chen CH, Wang JH, Hu TH, Lu SN, Lee CM. Reversal of hypolipidemia in chronic hepatitis C patients after successful antiviral therapy. *J Formos Med Assoc* 2011; **110**: 363-371 [PMID: 21741004 DOI: 10.1016/S0929-6646(11)60054-5]
  - 24 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]
  - 25 **Lawitz E**, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naïve and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; **383**: 515-523 [PMID: 24209977 DOI: 10.1016/S0140-6736(13)62121-2]
  - 26 **Gane EJ**, Stedman CA, Hyland RH, Ding X, Svarovskaia E, Subramanian GM, Symonds WT, McHutchison JG, Pang PS. Efficacy of nucleotide polymerase inhibitor sofosbuvir plus the NS5A inhibitor ledipasvir or the NS5B non-nucleoside inhibitor GS-9669 against HCV genotype 1 infection. *Gastroenterology* 2014; **146**: 736-743.e1 [PMID: 24262278 DOI: 10.1053/j.gastro.2013.11.007]
  - 27 **Lawitz E**, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, DeJesus E, Pearlman B, Rabinovitz M, Gitlin N, Lim JK, Pockros PJ, Scott JD, Fevery B, Lambrecht T, Ouwerkerk-Mahadevan S, Callewaert K, Symonds WT, Picchio G, Lindsay KL, Beumont M, Jacobson IM. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naïve patients: the COSMOS randomised study. *Lancet* 2014; **384**: 1756-1765 [PMID: 25078309 DOI: 10.1016/S0140-6736(14)61036-9]
  - 28 **Lawitz E**, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **369**: 678-679 [PMID: 23944316 DOI: 10.1056/NEJMc1307641]
  - 29 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
  - 30 **Arase Y**, Suzuki F, Suzuki Y, Akuta N, Kobayashi M, Kawamura Y, Yatsuji H, Sezaki H, Hosaka T, Hirakawa M, Ikeda K, Kumada H. Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. *Hepatology* 2009; **49**: 739-744 [PMID: 19127513 DOI: 10.1002/hep.22703]
  - 31 **Meissner EG**, Lee YJ, Osinusi A, Sims Z, Qin J, Sturdevant D, McHutchison J, Subramanian M, Sampson M, Naggie S, Patel K, Remaley AT, Masur H, Kottlilil S. Effect of sofosbuvir and ribavirin treatment on peripheral and hepatic lipid metabolism in chronic hepatitis C virus, genotype 1-infected patients. *Hepatology* 2015; **61**: 790-801 [PMID: 25203718 DOI: 10.1002/hep.27424]
  - 32 **Adinolfi LE**, Zampino R, Restivo L, Lonardo A, Guerrera B, Marrone A, Nascimbeni F, Florio A, Loria P. Chronic hepatitis C virus infection and atherosclerosis: clinical impact and mechanisms. *World J Gastroenterol* 2014; **20**: 3410-3417 [PMID: 24707124 DOI: 10.3748/wjg.v20.i13.3410]
  - 33 **Romero-Gómez M**, Fernández-Rodríguez CM, Andrade RJ, Diago M, Alonso S, Planas R, Solá R, Pons JA, Salmerón J, Barcena R, Perez R, Carmona I, Durán S. Effect of sustained virological response to treatment on the incidence of abnormal glucose values in chronic hepatitis C. *J Hepatol* 2008; **48**: 721-727 [PMID: 18308416 DOI: 10.1016/j.jhep.2007.11.022]
  - 34 **Ampuero J**, Romero-Gómez M. Assessing cardiovascular risk in hepatitis C: An unmet need. *World J Hepatol* 2015; **7**: 2214-2219 [PMID: 26380047 DOI: 10.4254/wjh.v7.i19.2214]
  - 35 **Chien CH**, Lin CL, Hu CC, Chang JJ, Chien RN. Clearance of Hepatitis C Virus Improves Insulin Resistance During and After Peginterferon and Ribavirin Therapy. *J Interferon Cytokine Res* 2015; **35**: 981-989 [PMID: 26308911 DOI: 10.1089/jir.2014.0200]
  - 36 **Huang YW**, Yang SS, Fu SC, Wang TC, Hsu CK, Chen DS, Hu JT, Kao JH. Increased risk of cirrhosis and its decompensation in chronic hepatitis C patients with new-onset diabetes: a nationwide cohort study. *Hepatology* 2014; **60**: 807-814 [PMID: 24919583 DOI: 10.1002/hep.27212]
  - 37 **Everhart JE**, Lok AS, Kim HY, Morgan TR, Lindsay KL, Chung RT, Bonkovsky HL, Ghany MG. Weight-related effects on disease progression in the hepatitis C antiviral long-term treatment against cirrhosis trial. *Gastroenterology* 2009; **137**: 549-557 [PMID: 19445938 DOI: 10.1053/j.gastro.2009.05.007]
  - 38 **Dai CY**, Chuang WL, Ho CK, Hsieh MY, Huang JF, Lee LP, Hou NJ, Lin ZY, Chen SC, Hsieh MY, Wang LY, Tsai JF, Chang WY, Yu ML. Associations between hepatitis C viremia and low serum triglyceride and cholesterol levels: a community-based study. *J Hepatol* 2008; **49**: 9-16 [PMID: 18486265 DOI: 10.1016/j.jhep.2008.03.016]
  - 39 **Serfaty L**, Andreani T, Giral P, Carbonell N, Chazouillères O, Poupon R. Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. *J Hepatol* 2001; **34**: 428-434 [PMID: 11322205]

**P- Reviewer:** Aghakhani A, Blanco JR, Gutierrez JA, Hwang SG, Rezaee-Zavareh MS **S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D





## Retrospective Study

# Is cirrhosis associated with lower odds of ischemic stroke: A nationwide analysis?

Abhinav Goyal, Kshitij Chatterjee, Nishi Shah, Shailender Singh

Abhinav Goyal, Department of Internal Medicine, Einstein Medical Center, Philadelphia, PA 19141, United States

Kshitij Chatterjee, Nishi Shah, Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205, United States

Shailender Singh, Division of Gastroenterology, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198, United States

**Author contributions:** All the authors contributed to study design, analysis and writing of the manuscript.

**Institutional review board statement:** As this study was conducted using a de-identified commercially available database Institutional Review Board (IRB) approval was not required.

**Informed consent statement:** As this was a retrospective study conducted using a de-identified commercially available database, informed consent was neither feasible nor required.

**Conflict-of-interest statement:** The authors do not have any conflict of interest to disclose. No financial support of any kind was used for this study.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Abhinav Goyal, MD, Department of Internal Medicine, Einstein Medical Center, 5501 Old York Road, Suite 363, Klein Building, Philadelphia, PA 19141, United States. [goyalabh@einstein.edu](mailto:goyalabh@einstein.edu)  
 Telephone: +1-215-4566500  
 Fax: +1-215-4551933

**Received:** August 21, 2016

**Peer-review started:** August 23, 2016

**First decision:** September 28, 2016

**Revised:** October 1, 2016

**Accepted:** November 1, 2016

**Article in press:** November 2, 2016

**Published online:** December 18, 2016

## Abstract

### AIM

To determine the association between cirrhosis and ischemic stroke in a large nationally representative sample.

### METHODS

A retrospective cross-sectional study of all hospitalized patients during 2012 and 2013 in the United States was performed using the National Inpatient Sample database. Hospitalizations with acute stroke, cirrhosis and other risk factors were identified using ICD-9-CM codes.

### RESULTS

There were a total of 72082638 hospitalizations in the United States during the years 2012 and 2013. After excluding hospitalizations with missing demographic variables, that there were a total of 1175210 (1.6%) out of these were for acute ischemic stroke. Cirrhosis was present among 5605 (0.4%) cases of ischemic stroke. Mean age among the cirrhotic and non-cirrhotic groups with ischemic stroke were 66.4 and 70.5 years, respectively. Prevalence of risk factors among the two groups was also calculated. After adjusting for various known risk factors the odds of having an ischemic stroke (OR = 0.28,  $P < 0.001$ ) were 72% lower in cirrhotics compared to non-cirrhotics.

### CONCLUSION

Our study suggests that in a large, nationally representative sample of the United States population, cirrhosis

is associated with a lower likelihood of stroke.

**Key words:** Cirrhosis; Ischemic stroke; Cerebrovascular accident; National Inpatient Sample

© **The Author(s)** 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Our study demonstrates that in a large, nationally representative sample, cirrhosis is associated with a lower likelihood of having an ischemic stroke, after adjusting for known risk factors. Although the odds of having a stroke are lower in cirrhotics, the mortality is significantly higher in them compared to non-cirrhotics.

Goyal A, Chatterjee K, Shah N, Singh S. Is cirrhosis associated with lower odds of ischemic stroke: A nationwide analysis? *World J Hepatol* 2016; 8(35): 1564-1568 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i35/1564.htm> DOI: <http://dx.doi.org/10.4254/wjv.v8.i35.1564>

## INTRODUCTION

Cirrhosis is among the top ten leading causes of death in the United States<sup>[1,2]</sup>. With recent advances in the management of various complications of cirrhosis, it has become one of the most prevalent chronic conditions, that patients live with for a considerable duration of time<sup>[3]</sup>. For instance, there were 633323 patients living with cirrhosis in 2010<sup>[4]</sup>.

Due to altered homeostasis and hemodynamics in cirrhosis it is reasonable to assume that the risk of an ischemic cerebrovascular event [acute ischemic stroke (AIS)] in cirrhotics would be different from that of the general population<sup>[5-8]</sup>. The question whether cirrhosis is associated with a reduced risk of stroke has been a source of controversy for a long time. There have been various studies reporting increased incidence of carotid plaques and atherosclerosis in patients with advanced liver disease, both known risk factors for ischemic stroke<sup>[9-11]</sup>. On the other hand, it is also well known that liver disease causes thrombocytopenia and coagulopathy which should in turn be protective against an ischemic cerebrovascular accident (CVA)<sup>[12]</sup>. Recently, Chen *et al.*<sup>[12]</sup> and Berzigotti *et al.*<sup>[13]</sup> showed that patients with liver cirrhosis may be at a lower risk of experiencing an ischemic CVA<sup>[12-14]</sup>. However, due to predominance of one ethnic group in the former and the relatively small sample size in the latter, the impact of cirrhosis on risk of stroke still remains inconclusive.

We therefore aim to define the impact of cirrhosis and extent of its association with ischemic stroke by using the largest national database for hospitalized patients in the United States.

## MATERIALS AND METHODS

### Data source

The National Inpatient Sample (NIS) formerly known as

Nationwide Inpatient Sample database is an administrative database developed by the Agency of Healthcare Research and Quality for Healthcare Cost and Utilization Project (HCUP). It is the largest all-payer database of hospitalized patients in the United States. NIS is a 20% stratified sample of all discharges from United States community hospitals<sup>[15]</sup>. Thus, manufacturer provided sampling weights were used to produce national estimates. We used NIS databases for the years 2012 and 2013 in this study. The NIS database provides de-identified information regarding the demographic characteristics (age, gender, race), mortality, principal and secondary diagnoses, *etc.*, for each hospitalization. It however does not contain any lab values, imaging or other advanced diagnostic information.

### Study design

This is a retrospective cross-sectional study using a national inpatient database. We used International Classification of Diseases, 9<sup>th</sup> Revision, Clinical Modification (ICD-9-CM) codes 571.2 (Alcoholic cirrhosis of liver), 571.5 (biliary cirrhosis) and 571.6 (cirrhosis without mention of alcohol) to identify the patients with cirrhosis<sup>[3,16]</sup>. ICD-9-CM codes 433.x1, 434.x1, 435 and 436 listed as principal diagnoses were used to identify hospitalizations for acute ischemic cerebrovascular events. These ICD-9-CM codes with modifiers have been previously validated and used to identify AIS in administrative databases with good accuracy<sup>[17-20]</sup>. All the patients with missing age, gender or race information were excluded. The patients with missing age and gender information constituted < 1% of the included population. The hospitalization with missing race were more prevalent, however they were due to non-participation of some states in reporting ethnic information and thus did not result in under-representation of any particular ethnic group. The basic demographic characteristics for different sub-groups have been described in Table 1. We used ICD-9-CM codes to identify the known risk factors for ischemic stroke<sup>[21-25]</sup>. Prevalence of these risk factors was also calculated for different subgroups (Table 1). Since, this study was conducted using a de-identified commercially available database Institutional Review Board approval was not required.

### Statistical analysis

Stata 13.1 (Stata Corp, College Station TX) and SPSS 23.0 (SPSS Inc., Chicago, Ill) were used for statistical analysis. National estimates were produced by using the sampling weights provided by HCUP.  $\chi^2$  test and Independent-samples *t*-test for means were used to determine statistical significance of differences in the prevalence of risk factors and demographic variables among the two groups. Due to the binary nature of the outcome/dependent variable, *i.e.*, presence of ischemic stroke, multivariate logistic regression model was used to assess the association between cirrhosis and ischemic stroke while controlling for known risk factors (as listed in Table 1) of ischemic stroke. Wald's

**Table 1** Demographic characteristics and prevalence of risk factors in patients with ischemic stroke among cirrhotic and non-cirrhotic groups

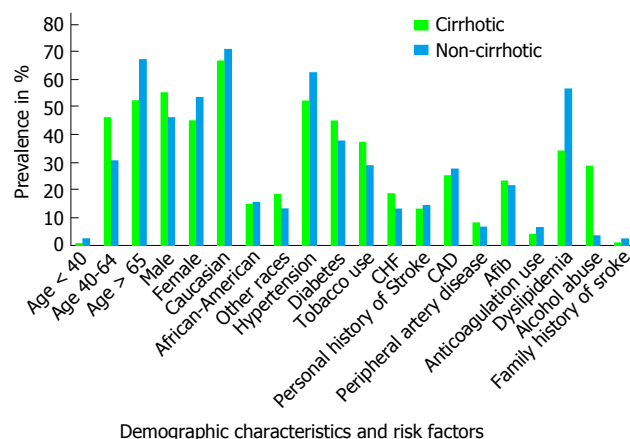
	Cirrhotic	Non-cirrhotic	P value
Mean age (SD)	66.4 (11.9)	70.5 (14.3)	< 0.001
Age categories			
Age < 40	0.8	2.3	< 0.001
Age 40-64	46.6	30.6	
Age > 65	52.6	67.1	
Gender			
Male	55.1	46.5	< 0.001
Female	44.9	53.5	
Race			
Caucasian	66.6	70.8	< 0.001
African-American	14.9	15.9	
Other races	18.5	13.3	
Hospital characteristics			
Teaching status			
Teaching	51.3	48.3	0.13
Non-teaching	48.7	51.7	
Location			
Rural	9.3	10.9	0.04
Urban	90.7	89.1	
Bed size			
Small	10.3	12	0.46
Medium	26.8	26.7	
Large	62.9	61.3	
Risk factors			
Hypertension	52.2	62.5	< 0.001
Diabetes	45.1	37.8	< 0.001
Tobacco use	37.2	28.8	< 0.001
CHF	18.9	13.1	< 0.001
Personal history of stroke	13.1	14.8	< 0.001
CAD	25.3	27.7	< 0.001
Peripheral artery disease	8.7	6.9	< 0.001
Atrial fibrillation	23.8	21.9	0.001
Anticoagulation use	4.4	6.8	< 0.001
Dyslipidemia	34.4	56.5	< 0.001
Alcohol abuse	28.8	3.8	< 0.001
Family history of stroke	1.5	2.3	< 0.001

The numbers in each cell represent percentage of patients with particular characteristics/risk factor in each group. SD: Standard deviation; CAD: Coronary artery disease; CHF: Congestive heart failure.

test was used to determine statistical significance of association between the factors used in the regression model and the outcome, *i.e.*, stroke. *P*-value less than 0.05 was considered statistically significant. The coefficient obtained as a result of the regression model was converted to OR for ease of understanding and is being reported here along with 95%CI. Since the prevalence of our outcome (Stroke) was less than 10%, the OR provides a good estimate of the relative risk<sup>[26]</sup>. The biostatistical methods and tests used in this study were reviewed by a biomedical statistician.

## RESULTS

There were a total of 72082638 hospitalizations in the United States during the year 2012 and 2013. After excluding hospitalizations with missing demographic variables (age, gender, and race), a total of 1175210 (1.6%) hospitalizations were for AIS. Out of these, 5605 (0.4%) were identified to have co-existing cirrhosis of liver. Decompensated cirrhosis which was defined by

**Figure 1** Distribution of demographic characteristics and risk factors in cirrhotic and non-cirrhotic groups with ischemic stroke. CAD: Coronary artery disease; CHF: Congestive heart failure; Afib: Atrial fibrillation.

presence of variceal hemorrhage, ascites, spontaneous bacterial peritonitis or hepatic encephalopathy, constituted 14.3% of the cirrhotic group<sup>[27]</sup>. Mean age among cirrhotic and non-cirrhotic patients were 66.4 (SD = 11.9) and 70.5 (SD = 14.3) years, respectively. Proportion of males among the two groups was 55.1% and 46.5% respectively. The racial distribution was similar among the two groups with 66.6% and 70.8% Caucasians; and 14.9% and 15.9% African-Americans in cirrhotic and non-cirrhotics respectively. The prevalence of some risk factors (Figure 1) for AIS like - diabetes (45.1% vs 37.8%), hypertension (52.2% vs 62.5%), congestive heart failure (18.9% vs 13.1%), smoking (37.2% vs 28.8%), atrial fibrillation (23.8% vs 21.9%), alcohol use (28.8% vs 3.8%) and peripheral vascular disease (8.7% vs 6.9%), were all higher among cirrhotics compared to non-cirrhotics (Table 1). Whereas, others like Coronary atherosclerosis (25.3% vs 27.7%), previous history of stroke (13.1% vs 14.8%), hypercholesterolemia (34.4% vs 56.5%) were found to be higher among the non-cirrhotic group (Table 1). Anticoagulation use, which is considered to be associated with a lower risk of ischemic stroke was more prevalent among the non-cirrhotics at 6.8% compared to 4.4% in cirrhotics.

The overall *P*-value of the logistical regression model was statistically significant at < 0.001. The odds of having an AIS for patients with cirrhosis were 72% lower than patients without cirrhosis (OR = 0.28, 95%CI: 0.26 to 0.29) and was statistically significant with a *P*-value < 0.001. The all cause in-hospital mortality among the cirrhotic group (5%) with AIS was significantly higher than non-cirrhotic group (3.3%) (*P* < 0.001). Even after adjusting (using logistic regression) for various co-morbidities using Charlson comorbidity index (modified to exclude liver disease)<sup>[28,29]</sup>, patient demographics, and hospital characteristics; the mortality remained higher among cirrhotics with stroke (OR = 1.6, 95%CI: 1.22-2.10, *P* = 0.001).

## DISCUSSION

The impact of cirrhosis on stroke has been controversial

for a long time. Our study demonstrates that the odds of having an AIS for cirrhotics are significantly lower (72%) than non-cirrhotics. This is consistent with some of the other smaller non-United States based studies done previously. The magnitude of association however, is different<sup>[12,13]</sup>. The study by Chen *et al.*<sup>[12]</sup> showed that risk of having an AIS was lower in non-alcoholic cirrhosis. But, it was conducted in Taiwan, and has limited generalizability due to predominance of only one kind of ethnic population. Since, ethnicity is itself an independent risk factor of AIS, our results have a more generalized applicability. Our study also had a much larger sample size and adjusted for the most number of risk factors of stroke in any study so far. The reduced likelihood of AIS in patients with cirrhosis represents a very important clinical finding. It may aid a clinician in determining the optimal management of often complicated cirrhotic patients with co-morbidities, that put them at a higher than usual risk of AIS, such as atrial fibrillation. The mechanism of this “protective effect of cirrhosis” is unclear but could be related to the underlying coagulopathy, thrombocytopenia or the altered hemodynamic flow patterns<sup>[12,13]</sup>. This study demonstrates the need for a prospective study to further explore this “protective effect of cirrhosis” on AIS.

Our study is to date the largest of its kind, and likely represents the true association between cirrhosis and strokes after adjusting for several known risk factors. Despite patients with cirrhosis being less likely to have a stroke, the mortality was significantly higher in them compared to non-cirrhotics. This is likely due to complications arising from the cirrhosis which may interfere with the usual management of stroke, for example, the coagulopathy due to cirrhosis may pose problems for planned interventions, if needed.

Our study findings, although important, need to be interpreted in light of some limitations. Firstly, NIS being an administrative database is not free from coding errors, especially related to liver diseases and acute strokes. However, we have used either the previously validated or commonly used codes for cirrhosis and AIS which have been shown to have good accuracy<sup>[3,30-32]</sup>. Secondly, the OR provides a close estimate of Relative Risk due to relatively low prevalence of Stroke in our population, but its not a replacement for the true relative risk which can only be obtained from a prospective cohort study.

Our study demonstrates that in a large, nationally representative sample, cirrhosis is associated with a lower likelihood of having an ischemic stroke, after adjusting for known risk factors. Although the odds of having a stroke are lower in cirrhotics, the mortality is significantly higher in them compared to non-cirrhotics. Prospective studies are needed to establish the causal relationship and better define this association in future.

## COMMENTS

### Background

Cirrhosis is one of the leading causes of morbidity and mortality in the United States. Cirrhotic patients usually suffer from coagulopathy while simultaneously being at an increased risk of deep venous thrombosis. These problems

along with the usually encountered thrombocytopenia imply that the risk of an ischemic cerebrovascular accident (CVA) in a cirrhotic would be different from that of general population. This impact of cirrhosis on the risk of ischemic cerebrovascular events (ischemic stroke) has been controversial.

### Research frontiers

The relationship between cirrhosis and ischemic CVA has not been studied in detail.

### Innovations and breakthroughs

The study is the first study with such a large sample size that controls for so many known risk factors of stroke to explore the true relationship between ischemic stroke and cirrhosis.

### Applications

The reduced likelihood of acute ischemic stroke (AIS) in patients with cirrhosis represents a very important clinical finding. It may aid a clinician in determining the optimal management of often complicated cirrhotic patients with co-morbidities, that put them at a higher than usual risk of AIS, such as atrial fibrillation (Afib).

### Terminology

Charlson comorbidity index is a tool to adjust for the impact of co-morbidities developed for use with administrative databases utilizing ICD-9 codes.

### Peer-review

This paper is described in detail, which, as valuable information, could help the readers that have better understand the first-hand knowledge of this topic to start novel studies.

## REFERENCES

- 1 **Vong S**, Bell BP. Chronic liver disease mortality in the United States, 1990-1998. *Hepatology* 2004; **39**: 476-483 [PMID: 14768001 DOI: 10.1002/hep.20049]
- 2 **Murray CJL**, Atkinson C, Bhalla K, Birbeck G, Burstein R, Chou D, Dellavalle R, Danaei G, Ezzati M, Fahimi A, Flaxman D, Foreman, Gabriel S, Gakidou E, Kassebaum N, Khatibzadeh S, Lim S, Lipshultz SE, London S, Lopez, MacIntyre MF, Mokdad AH, Moran A, Moran AE, Mozaffarian D, Murphy T, Naghavi M, Pope C, Roberts T, Salomon J, Schwebel DC, Shahrzaz S, Sleet DA, Murray, Abraham J, Ali MK, Atkinson C, Bartels DH, Bhalla K, Birbeck G, Burstein R, Chen H, Criqui MH, Dahodwala, Jarlais, Ding EL, Dorsey ER, Ebel BE, Ezzati M, Fahimi, Flaxman S, Flaxman AD, Gonzalez-Medina D, Grant B, Hagan H, Hoffman H, Kassebaum N, Khatibzadeh S, Leasher JL, Lin J, Lipshultz SE, Lozano R, Lu Y, Mallinger L, McDermott MM, Michal R, Miller TR, Mokdad AA, Mokdad AH, Mozaffarian D, Naghavi M, Narayan KMV, Omer SB, Pelizzari PM, Phillips D, Ranganathan D, Rivara FP, Roberts T, Sampson U, Sanman E, Sapkota A, Schwebel DC, Sharaz S, Shivakoti R, Singh GM, Singh D, Tavakkoli M, Towbin JA, Wilkinson JD, Zabetian A, Murray, Abraham J, Ali MK, Alvarado M, Atkinson C, Baddour LM, Benjamin EJ, Bhalla K, Birbeck G, Bolliger I, Burstein R, Carnahan E, Chou D, Chugh SS, Cohen A, Colson KE, Cooper LT, Couser W, Criqui MH, Dabhadkar KC, Dellavalle RP, Jarlais, Dicker D, Dorsey ER, Duber H, Ebel BE, Engell RE, Ezzati M, Felson DT, Finucane MM, Flaxman S, Flaxman AD, Fleming T, Foreman, Forouzanfar MH, Freedman G, Freeman MK, Gakidou E, Gillum RF, Gonzalez-Medina D, Gosselin R, Gutierrez HR, Hagan H, Havmoeller R, Hoffman H, Jacobsen KH, James SL, Jasrasaria R, Jayarman S, Johns N, Kassebaum N, Khatibzadeh S, Lan Q, Leasher JL, Lim S, Lipshultz SE, London S, Lopez, Lozano R, Lu Y, Mallinger L, Meltzer M, Mensah GA, Michaud C, Miller TR, Mock C, Moffitt TE, Mokdad AA, Mokdad AH, Moran A, Naghavi M, Narayan KMV, Nelson RG, Olives C, Omer SB, Ortblad K, Ostro B, Pelizzari PM, Phillips D, Raju M, Razavi H, Ritz B, Roberts T, Sacco RL, Salomon J, Sampson U, Schwebel DC, Shahrzaz S, Shibuya K, Silberberg D, Singh JA, Steenland



- K, Taylor JA, Thurston GD, Vavilala MS, Vos T, Wagner GR, Weinstock MA, Weisskopf MG, Wulf S, Murray. The state of US health, 1990-2010: burden of diseases, injuries, and risk factors. *JAMA* 2013; **310**: 591-608 [PMID: 23842577 DOI: 10.1001/jama.2013.13805]
- 3 **Fc I**, States U. Exam 2: Decreasing Mortality Among Patients Hospitalized With Cirrhosis in the United States From 2002 Through 2010. *Gastroenterology* 2015; **148**: e15-16 [DOI: 10.1053/j.gastro.2015.03.018]
- 4 **Scaglione S**, Kliethermes S, Cao G, Shoham D, Durazo R, Luke A, Volk ML. The Epidemiology of Cirrhosis in the United States: A Population-based Study. *J Clin Gastroenterol* 2015; **49**: 690-696 [PMID: 25291348 DOI: 10.1097/MCG.0000000000000208]
- 5 **Froekjaer VG**, Strauss GI, Mehlsen J, Knudsen GM, Rasmussen V, Larsen FS. Autonomic dysfunction and impaired cerebral autoregulation in cirrhosis. *Clin Auton Res* 2006; **16**: 208-216 [PMID: 16572350 DOI: 10.1007/s10286-006-0337-4]
- 6 **Lagi A**, La Villa G, Barletta G, Cencetti S, Bacalli S, Cipriani M, Foschi M, Lazzeri C, Del Bene R, Gentilini P, Laffi G. Cerebral autoregulation in patients with cirrhosis and ascites. A transcranial Doppler study. *J Hepatol* 1997; **27**: 114-120 [PMID: 9252083]
- 7 **Strauss GI**, Hansen BA, Herzog T, Larsen FS. Cerebral autoregulation in patients with end-stage liver disease. *Eur J Gastroenterol Hepatol* 2000; **12**: 767-771 [PMID: 10929904]
- 8 **Larsen FS**, Olsen KS, Ejlersen E, Hansen BA, Paulson OB, Knudsen GM. Cerebral blood flow autoregulation and transcranial Doppler sonography in patients with cirrhosis. *Hepatology* 1995; **22**: 730-736 [PMID: 7657276]
- 9 **O'Leary DH**, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med* 1999; **340**: 14-22 [PMID: 9878640]
- 10 **Targher G**, Bertolini L, Padovani R, Rodella S, Zoppini G, Zenari L, Cigolini M, Falezza G, Arcaro G. Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. *Diabetes Care* 2006; **29**: 1325-1330 [PMID: 16732016 DOI: 10.2337/dc06-0135]
- 11 **Nahandi MZ**, Khoshbaten M, Ramazanadeh E, Abbaszadeh L, Javadrashid R, Shirazi KM, Gholami N. Effect of non-alcoholic fatty liver disease on carotid artery intima-media thickness as a risk factor for atherosclerosis. *Gastroenterol Hepatol Bed Bench* 2014; **7**: 55-62 [PMID: 25436098]
- 12 **Chen YH**, Chen KY, Lin HC. Non-alcoholic cirrhosis and the risk of stroke: a 5-year follow-up study. *Liver Int* 2011; **31**: 354-360 [PMID: 20860634 DOI: 10.1111/j.1478-3231.2010.02350.x]
- 13 **Berzigotti A**, Bonfiglioli A, Muscari A, Bianchi G, Libassi S, Bernardi M, Zoli M. Reduced prevalence of ischemic events and abnormal supraortic flow patterns in patients with liver cirrhosis. *Liver Int* 2005; **25**: 331-336 [PMID: 15780058]
- 14 **Lee HJ**, Hinrichs CR. Is coagulopathic liver disease a factor in spontaneous cerebral hemorrhage? *J Comput Assist Tomogr* 2002; **26**: 69-72 [PMID: 11801906 DOI: 10.1097/00004728-200201000-00010]
- 15 HCUP-US NIS Overview [Internet]. 2015. [accessed 2016 Apr 23]. Available from: URL: <http://www.hcup-us.ahrq.gov/nisoverview.jsp>
- 16 **Kramer JR**, Davila JA, Miller ED, Richardson P, Giordano TP, El-Serag HB. The validity of viral hepatitis and chronic liver disease diagnoses in Veterans Affairs administrative databases. *Aliment Pharmacol Ther* 2008; **27**: 274-282 [PMID: 17996017 DOI: 10.1111/j.1365-2036.2007.03572.x]
- 17 **Kokotailo RA**, Hill MD. Coding of stroke and stroke risk factors using international classification of diseases, revisions 9 and 10. *Stroke* 2005; **36**: 1776-1781 [PMID: 16020772 DOI: 10.1161/01.STR.0000174293.17959.a1]
- 18 **Roumie CL**, Mitchel E, Gideon PS, Varas-Lorenzo C, Castellsague J, Griffin MR. Validation of ICD-9 codes with a high positive predictive value for incident strokes resulting in hospitalization using Medicaid health data. *Pharmacoepidemiol Drug Saf* 2008; **17**: 20-26 [PMID: 17979142 DOI: 10.1002/pds.1518]
- 19 **Moradiya Y**, Crystal H, Valsamis H, Levine SR. Thrombolytic utilization for ischemic stroke in US hospitals with neurology residency program. *Neurology* 2013; **81**: 1986-1995 [PMID: 24186911 DOI: 10.1212/01.wnl.0000436946.08647.b5]
- 20 **Goldstein LB**. Accuracy of ICD-9-CM coding for the identification of patients with acute ischemic stroke: effect of modifier codes. *Stroke* 1998; **29**: 1602-1604 [PMID: 9707200 DOI: 10.1161/01.STR.29.8.1602]
- 21 **Teunissen LL**, Rinkel GJ, Algra A, van Gijn J. Risk factors for subarachnoid hemorrhage: a systematic review. *Stroke* 1996; **27**: 544-549 [PMID: 8610327]
- 22 **Juvela S**, Hillbom M, Palomäki H. Risk factors for spontaneous intracerebral hemorrhage. *Stroke* 1995; **26**: 1558-1564 [PMID: 7660398 DOI: 10.1161/01.STR.26.9.1558]
- 23 **Martin-Schild S**, Albright KC, Halleve H, Barreto AD, Philip M, Misra V, Grotta JC, Savitz SI. Intracerebral hemorrhage in cocaine users. *Stroke* 2010; **41**: 680-684 [PMID: 20185779 DOI: 10.1161/STROKEAHA.109.573147]
- 24 **Sacco RL**, Benjamin EJ, Broderick JP, Dyken M, Easton JD, Feinberg WM, Goldstein LB, Gorelick PB, Howard G, Kittner SJ, Manolio TA, Whisnant JP, Wolf PA. American Heart Association Prevention Conference. IV. Prevention and Rehabilitation of Stroke. Risk factors. *Stroke* 1997; **28**: 1507-1517 [PMID: 9227708 DOI: 10.1161/01.STR.28.7.1507]
- 25 **McEvoy AW**, Kitchen ND, Thomas DG. Lesson of the week: intracerebral haemorrhage in young adults: the emerging importance of drug misuse. *BMJ* 2000; **320**: 1322-1324 [PMID: 10807629 DOI: 10.1136/bmj.320.7245.1322]
- 26 **Viera AJ**. Odds ratios and risk ratios: what's the difference and why does it matter? *South Med J* 2008; **101**: 730-734 [PMID: 18580722 DOI: 10.1097/SMJ.0b013e31817a7ee4]
- 27 **Schuppan D**, Afdhal NH. Liver cirrhosis. *Lancet* 2008; **371**: 838-851 [PMID: 18328931 DOI: 10.1016/S0140-6736(08)60383-9]
- 28 **Bajaj JS**, Ananthakrishnan AN, Hafeezullah M, Zadornova Y, Dye A, McGinley EL, Saeian K, Heuman D, Sanyal AJ, Hoffmann RG. Clostridium difficile is associated with poor outcomes in patients with cirrhosis: A national and tertiary center perspective. *Am J Gastroenterol* 2010; **105**: 106-113 [PMID: 19844204 DOI: 10.1038/ajg.2009.615]
- 29 **Deyo RA**, Cherkin DC, Ciol MA. Adapting a clinical comorbidity index for use with ICD-9-CM administrative databases. *J Clin Epidemiol* 1992; **45**: 613-619 [PMID: 1607900 DOI: 10.1016/0895-4356(92)90133-8]
- 30 **Lo Re V**, Lim JK, Goetz MB, Tate J, Bathulapalli H, Klein MB, Rimland D, Rodriguez-Barradas MC, Butt AA, Gibert CL, Brown ST, Kidwai F, Brandt C, Dorey-Stein Z, Reddy KR, Justice AC. Validity of diagnostic codes and liver-related laboratory abnormalities to identify hepatic decompensation events in the Veterans Aging Cohort Study. *Pharmacoepidemiol Drug Saf* 2011; **20**: 689-699 [PMID: 21626605 DOI: 10.1002/pds.2148]
- 31 **Lo Re V**, Haynes K, Goldberg D, Forde KA, Carbonari DM, Leidl KB, Hennessy S, Reddy KR, Pawloski PA, Daniel GW, Cheetham TC, Iyer A, Coughlin KO, Toh S, Boudreau DM, Selvam N, Cooper WO, Selvan MS, VanWormer JJ, Avigan MI, Houstoun M, Zornberg GL, Racoonin JA, Shoaibi A. Validity of diagnostic codes to identify cases of severe acute liver injury in the US Food and Drug Administration's Mini-Sentinel Distributed Database. *Pharmacoepidemiol Drug Saf* 2013; **22**: 861-872 [PMID: 23801638 DOI: 10.1002/pds.3470]
- 32 **Singla A**, Hart JL, Li Y, Tseng JF, Shah SA. Hospitalization for complications of cirrhosis: does volume matter? *J Gastrointest Surg* 2011; **15**: 330-335 [PMID: 21108014 DOI: 10.1007/s11605-010-1398-1]

P- Reviewer: Gong ZJ, Kai K S- Editor: Ji FF L- Editor: A  
E- Editor: Li D



Prospective Study

## Immune function biomarker QuantiFERON-monitor is associated with infection risk in cirrhotic patients

Siddharth Sood, Lijia Yu, Kumar Visvanathan, Peter William Angus, Paul John Gow, Adam Gareth Testro

Siddharth Sood, Department of Gastroenterology and Hepatology, University of Melbourne, Royal Melbourne Hospital, Parkville, VIC 3050, Australia

Siddharth Sood, Peter William Angus, Paul John Gow, Adam Gareth Testro, Liver Transplant Unit Victoria, University of Melbourne, Austin Health, Heidelberg, VIC 3084, Australia

Lijia Yu, Kumar Visvanathan, Innate Immune Laboratory, University of Melbourne, St Vincent's Hospital, Fitzroy, VIC 3065, Australia

**Author contributions:** Sood S and Testro AG designed the research, recruited participants, performed the research and wrote the paper; Yu L and Visvanathan K performed the research; Angus PW and Gow PJ analysed the data and helped write the paper.

**Institutional review board statement:** Approval was granted from the Austin Human Research Ethics Committee prior to this study being undertaken.

**Clinical trial registration statement:** As an observational study, this trial was not prospectively registered.

**Informed consent statement:** All study participants, or their legal guardian, provided informed consent prior to study enrolment.

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Siddharth Sood, MBBS, BMedSci,

PhD, Head of Hepatology, Department of Gastroenterology and Hepatology, University of Melbourne, Royal Melbourne Hospital, Royal Parade, Parkville, VIC 3050, Australia. [siddharth.ood@mh.org.au](mailto:siddharth.ood@mh.org.au)  
Telephone: +61-3-93427470  
Fax: +61-3-93427848

Received: July 12, 2016

Peer-review started: July 14, 2016

First decision: September 7, 2016

Revised: October 6, 2016

Accepted: October 22, 2016

Article in press: October 24, 2016

Published online: December 18, 2016

### Abstract

#### AIM

To investigate whether a novel immune function biomarker QuantiFERON-Monitor (QFM) can identify cirrhotic patients at greatest risk of infection.

#### METHODS

Adult cirrhotic patients on the liver transplant waiting list were recruited for this observational cohort study from a tertiary liver transplant referral unit. The immune function biomarker, QFM was performed using the same method as the widely available Quantiferon-gold assay, and measures output in interferon gamma in IU/mL after dual stimulation of the innate and adaptive immune systems. Ninety-one cirrhotic patients were recruited, with 47 (52%) transplanted on the day of their QFM. The remaining 44 (48%) were monitored for infections until transplant, death, or census date of 1<sup>st</sup> February 2014.

#### RESULTS

Cirrhotic patients express a median QFM significantly lower than healthy controls (94.5 IU/mL vs 423 IU/mL), demonstrating that they are severely immunosuppressed.

Several factors including model for end stage liver disease, presence of hepatocellular carcinoma, bilirubin, international normalized ratio and haemoglobin were associated with QFM on univariate analysis. Disease aetiology did not appear to impact QFM. On multivariate analysis, only Child-Pugh score and urea were significantly associated with a patient's immune function as objectively measured by QFM. In the 44 patients who were not transplanted immediately after their blood test and could be monitored for subsequent infection risk, 13 (29.5%) experienced a pre-transplant infection a median 20 d (range 2-182) post-test. QFM < 214 IU/mL was associated with HR = 4.1 ( $P = 0.01$ ) for infection. A very low QFM < 30 IU/mL was significantly associated ( $P = 0.003$ ) with death in three patients who died while awaiting transplantation (HR = 56.6).

### CONCLUSION

QFM is lower in cirrhotics, allowing objective determinations of an individual's unique level of immune dysfunction. Low QFM was associated with increased susceptibility to infection.

**Key words:** Infection; Biomarker; Immune dysfunction; Immune function; Immunosuppression; Liver; Immune system; Cirrhosis; Mortality

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** QuantiFERON-Monitor (QFM) is a net immune function biomarker that measures interferon- $\gamma$  after stimulation of the innate and adaptive immune systems and is based on a readily available pathology platform. Measuring QFM in cirrhotic patients provides an objective marker of their immune dysfunction, which has otherwise been difficult to quantify. Low QFM is significantly associated with the risk of pre-transplant infection, and very low QFM may be associated with increased risk of mortality.

Sood S, Yu L, Visvanathan K, Angus PW, Gow PJ, Testro AG. Immune function biomarker QuantiFERON-monitor is associated with infection risk in cirrhotic patients. *World J Hepatol* 2016; 8(35): 1569-1575 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i35/1569.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i35.1569>

### INTRODUCTION

QuantiFERON-Monitor (QFM, Qiagen Ltd, United States) was developed as an immune function monitoring tool in the post-transplant setting and provides a general biomarker of immune function based on stimulation of both the innate and adaptive immune systems<sup>[1]</sup>. It was developed based on the same diagnostic platform as the widely available QuantiFERON-Gold assay (QFN-gold, Qiagen Ltd, United States) and requires minimal

laboratory processing. A high QFM result suggests a robust immune response, whilst a low result implies impaired immunity. Initial pilot data showed low QFM compared with age-sex matched controls not just in patients on immunosuppression post-transplant, but also in cirrhotic patients on the waiting list prior to transplant<sup>[1]</sup>.

Patients with decompensated cirrhosis have inherently impaired immune responses, with bacterial infections occurring in 20%-60% of patients hospitalized for cirrhosis<sup>[2]</sup> and responsible for up to 25% of deaths in patients with liver disease<sup>[3]</sup>. The immune dysfunction in cirrhosis involves impairments of both quantity and quality of many immune cells that have been individually studied but are not always appreciated in clinical care.

In this study we present data that represents the first well described clinical cohort of patients to be evaluated with the QFM assay. We describe their immune function and investigate whether low QFM is associated with infection risk in this prospective cohort of pre-transplant cirrhotic patients.

### MATERIALS AND METHODS

We performed a prospective observational cohort study on 91 patients with cirrhosis awaiting liver transplantation at a single centre. Patients were recruited between November 2011 to December 2013 and followed until the census date of 1<sup>st</sup> February 2014. Approximately half the patients had blood taken immediately prior to their transplant surgery, while the remainder had a period of time in between their blood test and transplantation, death or the census date.

The QFM assay was performed on 1 mL of whole blood. As per manufacturer's guidelines, blood was stimulated with the QFM immune ligands anti-CD3 and R848 in the form of a single lyophilized ball within 8 h of being taken. Stimulated blood was incubated overnight at 37 °C. Following incubation, the blood underwent centrifugation and plasma harvested. An enzyme-linked immunosorbent assay (ELISA) was performed by a separate investigator who was blinded to clinical data. Clinicians caring for the cirrhotic patients were blinded from the QFM assay results. QFM output was measured as interferon- $\gamma$  (IFN- $\gamma$ ) production measured as IU/mL, in a process similar to that applied to perform a QFN-gold assay: Samples were brought to room temperature and given 60 min to equilibrate. The lyophilized IFN- $\gamma$  standard was reconstituted with deionized water. This was gently mixed to minimize frothing and ensure complete solubilisation. Dilutions were prepared to validate the standard curve.

The lyophilized conjugate was reconstituted with 0.3 mL of deionized water and mixed gently to minimize frothing and ensure complete solubilisation. Further dilutions were performed with addition of Green Diluent. Fifty microliters of prepared conjugate was added to each ELISA well, after which 50  $\mu$ L of each sample were added. Plates were covered and mixed using a microplate shaker for 1 min and then incubated at room

**Table 1** Baseline characteristics of cirrhotic patients

	Demographics	Median QFM (95%CI) IU/mL
Age (median, yr)	54 (20-72)	94.5 (37.3-158)
Male	62 (68.1%)	124.5 (37.3-223)
Female	29 (31.9%)	73.9 (7.50-158)
Child-Pugh score		
A	7	381 (12.9-1234)
B	29	224 (94.4-506)
C	55	37.3 (19.5-128)
MELD score		
0-10	10	319 (12.9-904)
11-20	42	155.5 (94.5-240)
21-30	34	30.0 (9.16-157)
≥ 30	5	8.81 (0.63-47.6)
Primary aetiology of cirrhosis, n (%)		
HCV	39 (42.9)	130 (47.6-223)
PSC	10 (11.0)	61.6 (1.19-279)
ETOH	10 (11.0)	113.3 (8.81-385)
NASH	9 (9.89)	20.3 (6.20-375)
AIH	5 (5.49)	37.3 (0.04-137)
PBC	4 (4.40)	93.0 (24.1-168)
HBV	3 (3.30)	904 (799-1132)
Retransplant	3 (3.30)	163 (2.06-318)
Other	8 (8.79)	6.59 (0.07-774)
HCC	31 (34.1)	194 (87.9-425)
No HCC	61 (65.9)	73.9 (28.0-154)

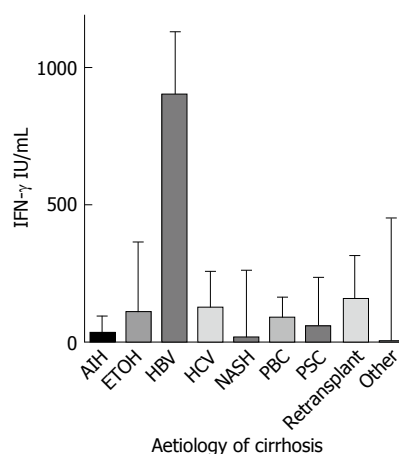
QFM: QuantiFERON-Monitor; HCV: Hepatitis C virus; PSC: Primary sclerosing cholangitis; ETOH: Alcohol; NASH: Non-alcoholic steatohepatitis; AIH: Autoimmune hepatitis; PBC: Primary biliary cirrhosis; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma.

temperature for 120 min  $\pm$  5 min.

Wells were then washed with 400  $\mu$ L of working strength wash buffer for at least 6 cycles in a microplate washer. Plates were tapped, while facing down on absorbent, low-lint towels to remove residual wash buffer. One hundred microliters of enzyme substrate solution was then added to each well, and plates covered with a lid. These were mixed using a microplate shaker, and then incubated at room temperature for a further 30 min.

Following this further incubation, 50  $\mu$ L of enzyme stopping solution was added to each well and mixed thoroughly with the microplate shaker. The optical density was then measured within 5 min of stopping the reaction using a microplate reader fitted with a 450 nm filter, as well as a 620 nm-650 nm reference filter. The optical density values were used to calculate the output result of IFN- $\gamma$  in IU/mL. Low QFM was suggestive of an immunosuppressed state.

Basic clinical data was collected from participants who were recruited as part of a post-transplant research trial. Collected data included age, gender, disease aetiology and blood biochemistry. Patients were also evaluated for commonly used scoring systems for the severity of liver disease, the Child-Pugh Score and model for end stage liver disease (MELD) score. Patients were monitored prospectively for infection occurring after their QFM sample and up to either liver transplant, infection, death



**Figure 1** Median QuantiFERON ( $\pm$  IQR) by aetiology of liver disease. IFN- $\gamma$ : Interferon- $\gamma$ ; AIH: Autoimmune hepatitis; ETOH: Alcohol; HBV: Hepatitis B virus; HCV: Hepatitis C virus; NASH: Non-alcoholic steatohepatitis; PBC: Primary biliary cirrhosis; PSC: Primary sclerosing cholangitis.

or the census date. Infections were per pre-defined criteria of "probable" or "definite" infection adjusted from The International Sepsis Forum Consensus Conference on Definitions of Infection in the Intensive Care Unit<sup>[4]</sup>. All patients were admitted to hospital for intravenous antimicrobial treatment.

Logistic regression, Mann-Whitney *U* test and Kaplan-Meier survival curves were analyzed with GraphPad Prism 6.0 for Mac (IBM, United States). All variables that showed potential predictive capacity of 15% ( $P < 0.15$ ) were entered into a multivariate logistic regression mode using STATA/SE version 12.0 for Mac (Statacorp, United States). *P* values under 0.05 were considered significant. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the appropriate institutional review committee. All patients provided written informed consent.

## RESULTS

Ninety-one cirrhotic patients were recruited (Table 1). The majority ( $n = 62$ , 68.1%) were male. The mean age was 51 years (median 54, range 20-72 years). The most common aetiology of liver disease was hepatitis C virus infection (HCV, 43%), followed by Primary Sclerosing Cholangitis (PSC, 11%), alcoholic liver disease (ETOH, 11%), and non-alcoholic steatohepatitis (NASH, 10%) (Figure 1). ETOH was a significant co-factor in 14/24 (58.3%) of patients with HCV. QFM level did not vary significantly by aetiology ( $P = 0.08$ ).

The mean QFM in cirrhotics was 214.3 IU/mL, median 94.5 IU/mL compared to a historical cohort of healthy controls (mean 555.2 IU/mL, median 423 IU/mL)<sup>[11]</sup>. There was no significant difference between QFM in males and females ( $P = 0.11$ ). Of the patient group as a whole, the median MELD was 20 and Child-Pugh Score was 10. Hepatocellular carcinoma (HCC) was present in



**Table 2** Univariate analysis of QuantiFERON-Monitor in cirrhotic patients

	Coefficient	P value	95%CI
MELD score	-17.3	< 0.001	-25.3:-9.29
Child-Pugh score	-65.6	< 0.001	-91.1:-40.2
Alcohol	-69.3	0.285	-197.4:58.7
HCC	193.9	0.002	71.6:316.1
Age	3.18	0.252	-2.30:8.66
WCC	-9.4	0.351	-29.2:10.5
Neutrophils	-19.0	0.137	-44.1:6.17
HCV	-44.4	0.476	-168.0:79.1
Male	70.1	0.288	-60.1:200.4
Bilirubin	-0.59	0.001	-0.95:-0.24
Urea	-14.5	0.023	-26.9:-2.00
Creatinine	0.17	0.702	-0.71:1.06
Haemoglobin	5.04	< 0.001	2.48:7.59
Platelets	0.59	0.164	-0.24:1.42
Albumin	9.50	0.055	-0.21:19.2
INR	-230.6	< 0.001	-342:-119

HCC: Hepatocellular carcinoma; WCC: White cell count; HCV: Hepatitis C virus; MELD: Model for end stage liver disease; INR: International normalized ratio.

31 patients (34.1%) and associated with a lower median MELD compared with non-HCC patients (15 vs 20,  $P < 0.0001$ ). Accordingly, HCC patients who expressed a more robust immune response with a median QFM more than double that of non-HCC patients (194 IU/mL vs 73.9 IU/mL,  $P = 0.03$ ).

Several other factors were associated with QFM on univariate analysis. Along with presence of HCC, haemoglobin level was positively associated with QFM. Alternatively, an inverse association was found with advancing MELD score, Child-Pugh score, urea and international normalized ratio (Table 2). On a multivariate regression model, only Child-Pugh score and urea were independently associated with QFM levels in cirrhotic patients (Table 3).

### Predicting pre-transplant infection

Of the 91 cirrhotic patients, approximately half ( $n = 47$ , 51.6%) were transplanted on the day of their QFM measurement. The remaining 44 (48.4%) had the QFM assay performed a median 46 d (range 2-591) prior to the date of censor. This sub-group were further investigated for rates of infection prior to transplantation. Most were receiving antibiotic prophylaxis (34/44, 77.3%).

At the census date, 33 patients (75%) had been transplanted, 3 patients had died (6.8%) and 8 (18.2%) were still awaiting transplantation. Advanced MELD ( $r^2 = 0.27$ ,  $P = 0.002$ ) and Child-Pugh score ( $r^2 = 0.15$ ,  $P = 0.03$ ) were associated with shorter time to transplant, while QFM was not ( $r^2 = 0.01$ ,  $P = 0.64$ ).

Thirteen of 44 patients (29.5%) experienced a pre-transplant infection at a median of 20 d (range 2-182) after their pre-transplant blood test. Three patients had spontaneous bacterial peritonitis (SBP), 4 pneumonia, 3 bacteraemia, 1 fungaemia, 1 urinary tract infection and 1 cholangitis. Most patients ( $n = 9$ , 69%) who experienced

**Table 3** Multivariate regression analysis

	Coefficient	P value	95%CI
Child-Pugh score	-51.9	0.013	-92.6:-11.3
MELD score	16.0	0.131	-4.88:36.9
HCC	62.9	0.366	-74.8:201
Bilirubin	-0.39	0.131	-0.91:0.120
Urea	-14.3	0.046	-28.3:-0.261
Haemoglobin	1.77	0.250	-1.27:4.82
Albumin	7.15	0.141	-2.43:16.7
INR	-114	0.168	-278:49.3

HCC: Hepatocellular carcinoma; MELD: Model for end stage liver disease; INR: International normalized ratio.

an infection had Child-Pugh C cirrhosis but Child-Pugh score was not associated with risk of infection (Figure 2A,  $P = 0.2$ ), whereas MELD score ( $\geq 20$ ) was (Figure 2B; HR = 4.7,  $P = 0.01$ ). Urea above the laboratory reference range of 6.7 mmol/L was not associated with infection risk ( $P = 0.15$ ).

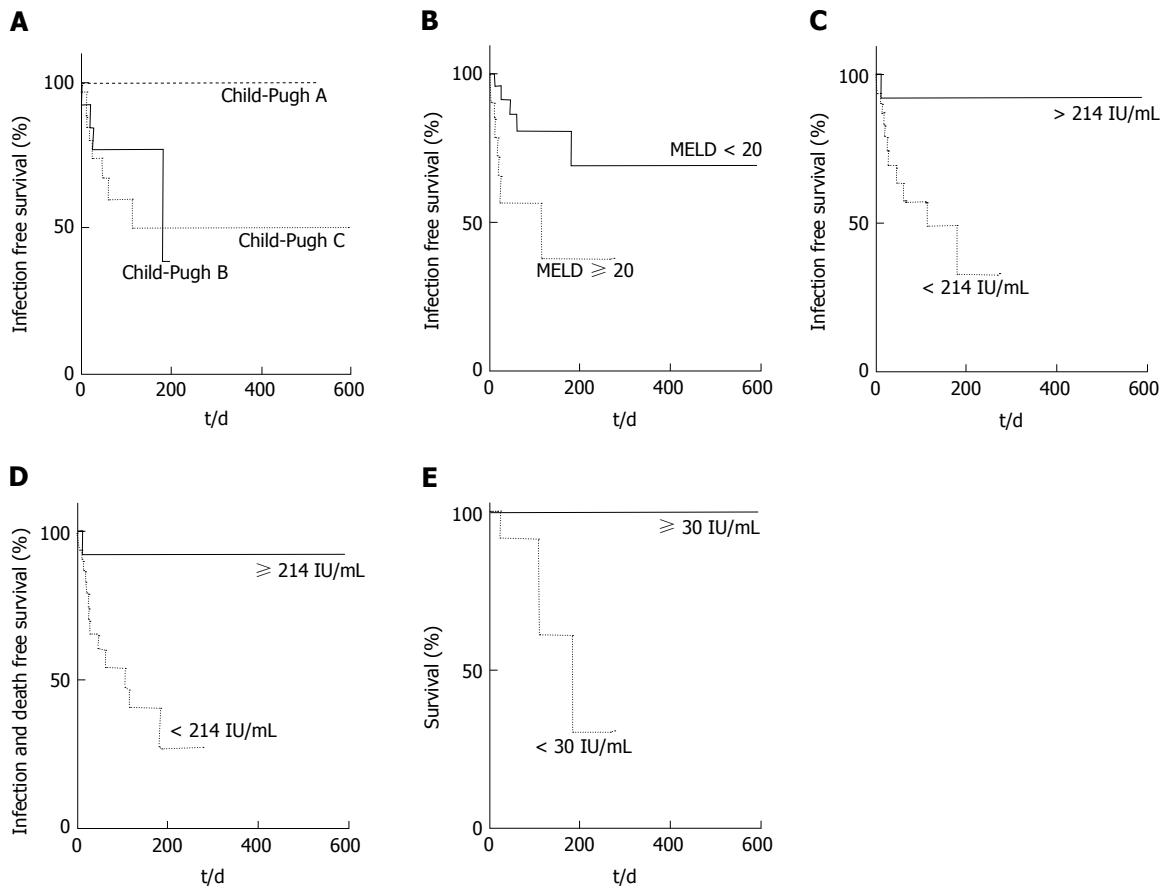
A QFM under the cohort mean of 214 IU/mL was significantly associated with infection pre-transplant (HR = 4.1, Figure 2C,  $P = 0.01$ ) and the combined outcome of infection or death on the waiting list (Figure 2D, HR = 4.4,  $P = 0.006$ ).

Three patients died in this cohort while awaiting transplantation, two from bleeding (one intracranial, one variceal) and one from sepsis and multiorgan failure. The median MELD of these patients was 24 and Child-Pugh score of 12. Patients who died pre-transplant had a significantly lower QFM (AUROC 0.88,  $P = 0.03$ ), and on survival analysis, a very low QFM ( $< 30$  IU/mL) was most associated with a HR of 56.6 for death (Figure 2E,  $P = 0.003$ ).

## DISCUSSION

Infections are implicated in up to 25% of deaths of patients with cirrhosis<sup>[3]</sup>, and are the second leading cause of death in patients with end-stage liver disease awaiting liver transplantation<sup>[5,6]</sup>. Immune dysfunction in cirrhosis is likely multifactorial, with impaired function identified in neutrophils<sup>[7-10]</sup>, monocytes<sup>[11]</sup> and lymphocytes<sup>[12]</sup>. Many of which also show impaired numbers, partly as a result of portal hypertension and splenic sequestration. Advanced cirrhosis is also associated with deficiencies in both structure and function of the reticuloendothelial system<sup>[13,14]</sup>, complement production<sup>[15]</sup>, and a chronic immune activation that appears to result in a systemic immune paralysis<sup>[16-20]</sup>. Although each individual aspect of immune deficiency has been studied in isolation, estimating a patient's overall level of immune function has been unattainable.

QFM was designed as a net immune function biomarker to manage immunosuppression in the post-transplant setting. Unlike other immune function assays that are predominantly confined to research settings, it has potential clinical utility as it is based on QFN-gold, an assay already in widespread use, and requires



**Figure 2** Pretransplant survival based on very low QuantiFERON. A: Infection free survival by Child-Pugh; B: Infection free survival by MELD; C: Infection free survival by QFM; D: Infection and mortality free survival by QFM; E: Pretransplant survival based on very low QFM. QFM: QuantiFERON-Monitor; MELD: Model for end stage liver disease.

only minimal laboratory processing. QFM incorporates both an innate and adaptive stimulant which offers an objective, albeit non-specific overview of a patient's individual immune response. A perhaps not unexpected finding of the original pilot study was that low QFM was identified in patients awaiting, and not only after liver transplantation<sup>[1]</sup>. In this study, we confirm and are able to quantify the immunosuppressed status of cirrhotic patients, with a median QFM of 94.5 IU/mL less than 25% that of healthy controls (423 IU/mL)<sup>[1]</sup>. Most importantly, we not only demonstrate a low QFM in cirrhotic patients (indicative of inherent immunosuppression), but that the most severe immune dysfunction is associated with heightened infection risk. Low QFM in cirrhotic patients had a HR of 4.1 for pre-transplant infection risk. A simple blood test that could highlight a patient's individual risk of subsequent infection would be of value to treating clinicians.

There are some limitations when performing a study in a transplant-waitlisted population. Firstly, with the sickest patients (based on MELD) often receiving priority organ selection, there was risk of patients with greatest risk of infection (and lowest QFM) being transplanted earlier. This risks a type II error, which potentially underestimates the clinical value of the assay in predicting infections; Secondly, we may have underestimated the infection

rate as diagnosing infections in patients with cirrhosis can be difficult, and empirical antibiotics are often used on presentation to hospital with conditions such as variceal bleeding or hepatic encephalopathy; and thirdly, since this data represents the first clinical cohort of non-transplant recipients evaluated with the QFM assay, readily defined set-points for low and very low QFM have not previously been evaluated or described.

Conversely, studying a transplant wait-listed population does offer some advantages since transplant listed patients are more unwell and at greatest susceptibility to infections (reducing the potential sample size and necessary follow-up period). They are closely monitored, with all events being reported to the transplant centre even if occurring at peripheral hospitals, thus allowing all clinical events to be documented.

Early identification and treatment of infections is essential in the management of cirrhotic patients, particularly given the morbidity and mortality often attributed to infections in this vulnerable population. However, infections can be difficult to distinguish from other non-infectious causes of systemic inflammatory response syndrome and symptoms of liver deterioration<sup>[21]</sup>. Serum biomarkers are therefore being examined, although currently available tests such as C-reactive protein, ferritin and white blood cells lack specificity<sup>[21]</sup>.

To prevent infections, some cirrhotic patients are offered antibiotic prophylaxis, although mainly they have been used to prevent episodes of SBP. SBP has a recurrence rate of 70% within 12 mo<sup>[22]</sup>, and secondary prophylaxis is a part of internationally accepted guidelines<sup>[23]</sup>. Primary antibiotic prophylaxis has also been recommended in patients with low protein in ascitic fluid as there is an understanding that it improves incidence of infections and short-term survival<sup>[24,25]</sup>. However, adherence to these guidelines is low, and in part may be due to fears over antimicrobial resistance and reduced effectiveness over time<sup>[26,27]</sup>. An objective immune function biomarker that could highlight patients with the most severe immune-deficiency could enable the use of more targeted antibiotic prophylaxis to those most at risk of all infections, and not just SBP.

There was no significant difference in QFM based on aetiology of the underlying liver disease. Patients with hepatocellular carcinoma as their primary indication for transplant were often not as unwell as other cirrhotic patients, and therefore had significantly higher QFM results on univariate analysis. This was verified in multivariate analysis where HCC was not independently associated with high QFM. Only Child-Pugh score and urea were individually identified on a logistic multivariate regression model as being associated with low QFM. This suggests that commonly used disease scoring systems such as MELD or biochemical investigations such as white cell count are not truly indicative of a patient's underlying level of immune dysfunction and further highlights the possible value of an objective immune function biomarker. The significance of urea with QFM is interesting and would need further study. It could be a surrogate marker of renal function which is known to impact mortality in cirrhosis, although interestingly creatinine had no relationship to QFM. An alternate hypothesis may be that the urea level reflects an increased catabolic state associated with nutritional deficiencies that may impact immune function.

A very low QFM was significantly associated with pre-transplant mortality in this cohort. Although two patients subsequently died of bleeding rather than sepsis, this may highlight the severely immunosuppressed state of patients with critical illness, and offer QFM as an alternate overall prognostic marker. However, despite reaching statistical significance, it is difficult with low numbers to make any firm conclusions regarding QFM and mortality risk. Further studies in a non-transplant wait-listed cirrhotic population would be needed to further explore and confirm this association.

In conclusion, patients with cirrhosis are at high risk of infection, but quantification of immune dysfunction has been difficult in clinical practice. Immune functional assays are often isolated to one small component of immunity, associated with significant laboratory processing or confined to limited situations and medical research. This study describes the first clinical assessment of the QFM immune function assay in patients not receiving

immunosuppressant medications. We show that patients with cirrhosis are not only significantly immunosuppressed, but that a low level of QFM (suggestive of significant immune dysfunction) is associated with a four-fold increased risk for infection. The ability to employ a clinical assay that can objectively provide a biomarker of an individual's innate and adaptive immune function offers obvious benefits to patient care, even outside the transplantation setting. This study also serves as a proof of concept that immune function monitoring may be available and have clinical utility in other fields of medicine where patients are either inherently or iatrogenically immunosuppressed.

## COMMENTS

### Background

Patients with cirrhosis are known to be immunosuppressed and infections are a significant cause of morbidity and mortality. Predicting patients at greatest risk of infection is difficult. The QuantiFERON-Monitor assay (QFM) is a novel immune function biomarker designed to assess immune function in a transplant setting. In this first study to employ QFM in a non-transplant setting, the authors aim to identify whether QFM can objectively measure a patient's immune dysfunction and whether this correlates with the risk of infection.

### Research frontiers

Identifying cirrhotic patients at greatest risk of infection is difficult. Usual biochemical measures such as C-reactive protein and white cell count are not associated with infection in cirrhotic patients.

### Innovations and breakthroughs

QFM is a novel immune function biomarker that provides an objective measure of immune function. In particular, it benefits from measuring interferon gamma production after stimulation of both arms of the immune system (innate and adaptive). In this study, the assay is shown to objectively measure immune dysfunction in cirrhotic patients, and that the patients with lower values had the greatest risk of infection.

### Applications

QFM may have utility in measuring the level of immune function in patients with cirrhosis. This could then identify patients at greatest risk of infection, and who may benefit from either earlier transplantation or antibiotic prophylaxis. Furthermore, the assay may be useful in other medical conditions where patients are either inherently or iatrogenically immunosuppressed.

### Peer-review

This is an questionable topic. First of all, the material method section should be described in a detailed manner. More aspects should be enlightened. Moreover, discussion part should be enlarged properly and more recent studies should be mentioned and more recent studies should be added to references part.

## REFERENCES

- 1 Sood S, Cundall D, Yu L, Miyamasu M, Boyle JS, Ong SY, Gow PJ, Jones RM, Angus PW, Visvanathan K, Testro AG. A novel biomarker of immune function and initial experience in a transplant population. *Transplantation* 2014; **97**: e50-e51 [PMID: 24732902 DOI: 10.1097/TP.0000000000000078]
- 2 Garcia-Tsao G. Spontaneous bacterial peritonitis: a historical perspective. *J Hepatol* 2004; **41**: 522-527 [PMID: 15464231 DOI: 10.1016/j.jhep.2004.09.001]
- 3 Wyke RJ. Problems of bacterial infection in patients with liver disease. *Gut* 1987; **28**: 623-641 [PMID: 3297941 DOI: 10.1136/gut.28.5.623]

- 4 **Calandra T**, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. *Crit Care Med* 2005; **33**: 1538-1548 [PMID: 16003060 DOI: 10.1097/01.CCM.0000168253.91200.83]
- 5 **Fernández J**, Gustot T. Management of bacterial infections in cirrhosis. *J Hepatol* 2012; **56** Suppl 1: S1- S12 [PMID: 22300459 DOI: 10.1016/S0168-8278(12)60002-6]
- 6 **Arvaniti V**, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, Burroughs AK. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* 2010; **139**: 1246-1256 [PMID: 20558165 DOI: 10.1053/j.gastro.2010.06.019]
- 7 **Rajkovic IA**, Williams R. Abnormalities of neutrophil phagocytosis, intracellular killing and metabolic activity in alcoholic cirrhosis and hepatitis. *Hepatology* 1986; **6**: 252-262 [PMID: 3007318 DOI: 10.1002/hep.1840060217]
- 8 **Fiuza C**, Salcedo M, Clemente G, Tellado JM. In vivo neutrophil dysfunction in cirrhotic patients with advanced liver disease. *J Infect Dis* 2000; **182**: 526-533 [PMID: 10915084 DOI: 10.1086/315742]
- 9 **Tritto G**, Bechlis Z, Stadlbauer V, Davies N, Francés R, Shah N, Mookerjee RP, Such J, Jalan R. Evidence of neutrophil functional defect despite inflammation in stable cirrhosis. *J Hepatol* 2011; **55**: 574-581 [PMID: 21236309 DOI: 10.1016/j.jhep.2010.11.034]
- 10 **Ono Y**, Watanabe T, Matsumoto K, Ito T, Kunii O, Goldstein E. Opsonophagocytic dysfunction in patients with liver cirrhosis and low responses to tumor necrosis factor-alpha and lipopolysaccharide in patients' blood. *J Infect Chemother* 2004; **10**: 200-207 [PMID: 15365859 DOI: 10.1007/s10156-004-0321-7]
- 11 **Lin CY**, Tsai IF, Ho YP, Huang CT, Lin YC, Lin CJ, Tseng SC, Lin WP, Chen WT, Sheen IS. Endotoxemia contributes to the immune paralysis in patients with cirrhosis. *J Hepatol* 2007; **46**: 816-826 [PMID: 17328986 DOI: 10.1016/j.jhep.2006.12.018]
- 12 **Doi H**, Iyer TK, Carpenter E, Li H, Chang KM, Vonderheide RH, Kaplan DE. Dysfunctional B-cell activation in cirrhosis resulting from hepatitis C infection associated with disappearance of CD27-positive B-cell population. *Hepatology* 2012; **55**: 709-719 [PMID: 21932384 DOI: 10.1002/hep.24689]
- 13 **Jenne CN**, Kubes P. Immune surveillance by the liver. *Nat Immunol* 2013; **14**: 996-1006 [PMID: 24048121 DOI: 10.1038/ni.2691]
- 14 **Bolognesi M**, Merkel C, Bianco S, Angeli P, Sacerdoti D, Amodio P, Gatta A. Clinical significance of the evaluation of hepatic reticuloendothelial removal capacity in patients with cirrhosis. *Hepatology* 1994; **19**: 628-634 [PMID: 8119687 DOI: 10.1002/hep.1840190313]
- 15 **Homann C**, Varming K, Høgåsen K, Mollnes TE, Graudal N, Thomsen AC, Garred P. Acquired C3 deficiency in patients with alcoholic cirrhosis predisposes to infection and increased mortality. *Gut* 1997; **40**: 544-549 [PMID: 9176087 DOI: 10.1136/gut.40.4.544]
- 16 **Albillos A**, de la Hera A, González M, Moya JL, Calleja JL, Monserrat J, Ruiz-del-Arbol L, Alvarez-Mon M. Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. *Hepatology* 2003; **37**: 208-217 [PMID: 12500206 DOI: 10.1053/jhep.2003.50038]
- 17 **Guarner C**, Soriano G, Tomas A, Bulbena O, Novella MT, Balanzo J, Vilardell F, Mourelle M, Moncada S. Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxemia. *Hepatology* 1993; **18**: 1139-1143 [PMID: 8225220 DOI: 10.1002/hep.1840180520]
- 18 **Campillo B**, Bories PN, Benvenuti C, Dupeyron C. Serum and urinary nitrate levels in liver cirrhosis: endotoxemia, renal function and hyperdynamic circulation. *J Hepatol* 1996; **25**: 707-714 [PMID: 8938549 DOI: 10.1016/S0168-8278(96)80242-X]
- 19 **Lin RS**, Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, Hsu WC, Huang CC, Wang SS, Lo KJ. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. *J Hepatol* 1995; **22**: 165-172 [PMID: 7790704 DOI: 10.1016/0168-8278(95)80424-2]
- 20 **Testro AG**, Visvanathan K. Toll-like receptors and their role in gastrointestinal disease. *J Gastroenterol Hepatol* 2009; **24**: 943-954 [PMID: 19638078 DOI: 10.1111/j.1440-1746.2009.05854.x]
- 21 **Bunchorntavakul C**, Chamroonkul N, Chavalitthamrong D. Bacterial infections in cirrhosis: A critical review and practical guidance. *World J Hepatol* 2016; **8**: 307-321 [PMID: 26962397 DOI: 10.4254/wjh.v8.i6.307]
- 22 **Titó L**, Rimola A, Ginès P, Llach J, Arroyo V, Rodés J. Recurrence of spontaneous bacterial peritonitis in cirrhosis: frequency and predictive factors. *Hepatology* 1988; **8**: 27-31 [PMID: 3257456 DOI: 10.1002/hep.1840080107]
- 23 **Runyon BA**. Management of Adult Patients with Ascites Due to Cirrhosis: Update 2012. AASLD Practice Guideline: AASLD, 2012
- 24 **Fernández J**, Navasa M, Planas R, Montoliu S, Monfort D, Soriano G, Vila C, Pardo A, Quintero E, Vargas V, Such J, Ginès P, Arroyo V. Primary prophylaxis of spontaneous bacterial peritonitis delays hepatorenal syndrome and improves survival in cirrhosis. *Gastroenterology* 2007; **133**: 818-824 [PMID: 17854593 DOI: 10.1053/j.gastro.2007.06.065]
- 25 **Saab S**, Hernandez JC, Chi AC, Tong MJ. Oral antibiotic prophylaxis reduces spontaneous bacterial peritonitis occurrence and improves short-term survival in cirrhosis: a meta-analysis. *Am J Gastroenterol* 2009; **104**: 993-1001; quiz 1002 [PMID: 19277033 DOI: 10.1038/ajg.2009.3]
- 26 **Ngamruengphong S**, Nugent K, Rakvit A, Parupudi S. Potential preventability of spontaneous bacterial peritonitis. *Dig Dis Sci* 2011; **56**: 2728-2734 [PMID: 21394460 DOI: 10.1007/s10620-011-1647-5]
- 27 **Bruns T**, Zimmermann HW, Stallmach A. Risk factors and outcome of bacterial infections in cirrhosis. *World J Gastroenterol* 2014; **20**: 2542-2554 [PMID: 24627590 DOI: 10.3748/wjg.v20.i10.2542]

P- Reviewer: Tanoglu A S- Editor: Qi Y L- Editor: A  
E- Editor: Li D





## Intrahepatic pancreatic pseudocyst: A review of the world literature

Andrew Demeusy, Motahar Hosseini, Anne M Sill, Steven C Cunningham

Andrew Demeusy, Motahar Hosseini, Anne M Sill, Steven C Cunningham, Department of Surgery, Saint Agnes Hospital, Baltimore, MD 21229, United States

**Author contributions:** Demeusy A and Cunningham SC designed and performed the research; Sill AM contributed analytic tools; Demeusy A, Sill AM and Cunningham SC analyzed the data; Demeusy A, Hosseini M and Cunningham SC wrote the paper; all authors drafted the article or revised it critically for important intellectual content, and approved the current version.

**Conflict-of-interest statement:** The authors declare no conflicts of interest regarding this study.

**Data sharing statement:** There are no additional data available for this study.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Noncommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Steven C Cunningham, MD, FACS, Director of Pancreatic and Hepatobiliary Surgery, Director of Research, Department of Surgery, Saint Agnes Hospital, 900 Caton Avenue, MB 207, Baltimore, MD 21229, United States. [steven.cunningham@stagnes.org](mailto:steven.cunningham@stagnes.org)  
Telephone: +1-410-3688815  
Fax: +1-410-7190094

Received: June 25, 2016  
Peer-review started: June 28, 2016  
First decision: September 7, 2016  
Revised: September 26, 2016  
Accepted: November 1, 2016  
Article in press: November 2, 2016  
Published online: December 18, 2016

### Abstract

#### AIM

To investigate and summarize the literature regarding the diagnosis and management of intrahepatic pancreatic pseudocysts (IHPP).

#### METHODS

A literature search was performed using PubMed (MEDLINE) and Google Scholar databases, followed by a manual review of reference lists to ensure that no articles were missed. All articles, case reports, systematic reviews, letters to editors, and abstracts were analyzed and tabulated. Bivariate analyses were performed, with significance accepted at  $P < 0.05$ . Articles included were primarily in the English language, and articles in other languages were reviewed with native speakers or, if none available, were translated with electronic software when possible.

#### RESULTS

We found 41 published articles describing 54 cases since the 1970s, with a fairly steady rate of publication. Patients were predominantly male, with a mean age of 49 years. In 42% of published cases, the IHPP was the only reported pseudocyst, but 58% also had concurrent pseudocysts in other extrapancreatic locations. Average IHPP size was 9.5 cm and they occurred most commonly (48%) in the left hemiliver. Nearly every reported case was managed with an intervention, most with a single intervention, but some required up to three interventions. Percutaneous treatment with either simple aspiration or with an indwelling drain were the most common interventions, frequently performed along with stenting of the pancreatic duct. The size of the IHPP correlated significantly with both the duration of treatment ( $P = 0.006$ ) and with the number of interventions required ( $P = 0.031$ ). The duration of therapy also correlated with the initial white blood cell (WBC) count ( $P = 0.048$ ).

**CONCLUSION**

Diagnosis of IHPP is difficult and often missed. Initial size and WBC are predictive of the treatment required. With appropriate intervention, most patients achieve resolution.

**Key words:** Pseudocyst; Intrahepatic; Percutaneous; Pancreatic; Drainage

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Intrahepatic pancreatic pseudocysts (IHPPs) are rare and the pathophysiology is not entirely clear, but they likely result from proteolytic pancreatic fluid tracking from the pancreas into the surrounding tissue. This fluid may then migrate along planes such as the hepatogastric or hepatoduodenal ligaments, to penetrate the hepatic parenchyma. The initial size of the IHPP and the initial white blood cell are predictive of the number of treatments required and the overall duration of treatment required. Percutaneous approaches have been successful and result in good clinical outcomes.

Demeusy A, Hosseini M, Sill AM, Cunningham SC. Intrahepatic pancreatic pseudocyst: A review of the world literature. *World J Hepatol* 2016; 8(35): 1576-1583 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i35/1576.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i35.1576>

**INTRODUCTION**

A pancreatic pseudocyst is an abnormal collection of pancreatic fluid generally due to pancreatitis, exists for at least 4 wk, have a well-defined wall, and contain essentially no solid material<sup>[1,2]</sup>. They are more commonly seen in patients with alcohol-associated pancreatitis (20%) than with gallstone pancreatitis (6.6%)<sup>[3]</sup>. Although most commonly immediately peripancreatic or intrapancreatic, they can occur in truly extrapancreatic locations throughout the peritoneal cavity as well as the mediastinum<sup>[4,5]</sup>.

Extrapancreatic pseudocysts are relatively uncommon, estimated to occur in up to 22% of patients with pancreatic pseudocysts<sup>[5]</sup>. The location depends on where the pancreatic enzymes are released and the path they travel. One of the least common locations for truly extrapancreatic pseudocysts is within the liver<sup>[4,5]</sup>. Here we describe such a case of an intrahepatic pancreatic pseudocyst (IHPP), and exhaustively review, and analyze, the world literature on IHPP.

A 56-year-old male with a history of acute alcoholic pancreatitis presented with intermittent chronic abdominal pain. Magnetic resonance imaging revealed a 1.3-cm lesion in the body of the pancreas consistent with a small pancreatic pseudocyst. Computed tomography (CT) 4 mo later revealed a new, 18-cm-long, bilobed fluid collection, wrapped about the hepatoduodenal ligament,

not only communicating with the original fluid collection but also insinuating itself deeply into the hepatic parenchyma (Figures 1A), with evidence of communication to the erstwhile intrapancreatic pseudocyst (Figure 1B). Given worsening right upper quadrant abdominal pain, fever, chills, anorexia and significant weight loss, and an unknown age of the new IHPP, percutaneous transhepatic drainage was performed of the more superficial, inferior lobe (Figure 2, fluid was high-amylase and culture-negative), as well as endoscopic pancreatic sphincterotomy, and pancreatic-duct stenting. Follow-up CT one week later revealed a significant reduction in the size of both lobes of the pseudocyst. Three weeks later, however, he developed worsening abdominal fullness, pain and fevers. Repeat CT showed the superficial, inferior lobe to be well drained with the pigtail in place (Figure 3A), but the deeper superior collection was found to be larger containing a small bubble of gas (Figure 3B), with the connecting bridge collapsed. The drain was therefore repositioned into this deeper lobe (Figure 4, culture-positive). Following this procedure, the patient improved clinically and was discharged on 4 more weeks of IV antibiotics. Two weeks later he required aspiration of a small liver abscess (low-amylase, culture-positive), although his pseudocysts remained collapsed. At this point the drain was removed. Interval imaging one month and three months (Figure 5) later revealed no residual fluid collections and he remains drain-free, off antibiotics, gaining weight, and productive at work.

**MATERIALS AND METHODS**

A PubMed and Google Scholar search using key words "pseudocyst", "pancreatic", and "intrahepatic" followed by extensive cross-reference review revealed 41 published articles on patients with IHPP. All articles, case reports, systematic reviews which also added a case, letters to editors, and abstracts were analyzed and the data tabulated for comprehensive review and statistical analysis. Bivariate analyses were performed in Statistical Package for the Social Sciences (IBM Corporation, New York, NY, United States). Statistical review of the study was performed by a biomedical statistician.

Articles included were primarily in the English language, but also included French, German, Portuguese, Czech, Korean, and Japanese. Foreign-language articles were reviewed with native speakers or, if native speakers were not available, then the articles were translated with electronic software when possible.

**RESULTS****Prevalence and patient characteristics**

We identified 41 articles containing 54 cases of IHPP in the literature, the earliest identified case being published in 1974 (Table 1). These are primarily single case reports and mini case series but included two relatively thorough review articles which reviewed 26 cases<sup>[6]</sup> and 23 cases<sup>[7]</sup>. Two of the cases were notable in that the IHPP formation

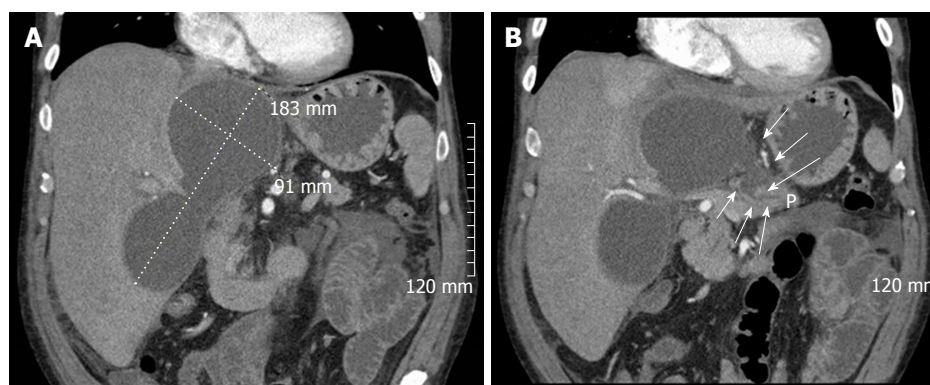


Figure 1 Abdominal computed tomography images showing bilobed intrahepatic pancreatic pseudocysts (A), including connection to main pancreatic duct (B, arrows).



Figure 2 Abdominal computed tomography image showing percutaneous transhepatic drainage of the more superficial, inferior lobe.

was thought to be secondary to ectopic pancreatic tissue and not an inflammatory pancreatic process<sup>[8,9]</sup>. In many of the cases (42%), the IHPP was the only reported pseudocyst, but a significant number also had other concurrent pseudocysts, the most common of which were intra- or peripancreatic pseudocysts (71%).

### Diagnosis

Diagnosis of an IHPP can be difficult as it is uncommon and it is not often part of the initial differential of a patient presenting with abdominal pain. Furthermore, if the presentation is delayed, imaging may reveal the IHPP but without inflammatory changes of the pancreas. Abdominal pain was the primary complaint in 91% of cases, but physical exam was generally nonspecific. Only 17% ( $n = 9$ ) of patients were noted to have a palpable abdominal mass or hepatomegaly, and 15% ( $n = 8$ ) had peritoneal signs. Initial diagnosis was often *via* CT (53%) or ultrasound (US) (33%) but nearly every patient in our database (91% of cases where imaging is mentioned) did get a CT scan at some point in the diagnostic or therapeutic process, and CT is generally considered to be the imaging modality of choice for these patients currently. Prior to the widespread availability of the CT scan, however, a significant workup was often done to identify the etiology of a patient's presentation

and in some cases would include a gastrointestinal transit studies, endoscopy, venogram, arteriogram, or exploratory laparotomy where the lesions were finally identified<sup>[10-12]</sup>. Endoscopy has been used effectively in several cases, not only including initial diagnosis<sup>[13]</sup>, but also with therapeutic intervention<sup>[14,15]</sup>, as discussed further below.

The diagnosis of an IHPP was often delayed with the lesions often initially being mistaken for intrahepatic biliary dilatation, hemangioma, hepatic cyst, pyogenic liver abscess, amebic abscess, biloma, malignancy, echinococcal cyst, or even peritoneal tuberculosis<sup>[10,13,16-19]</sup>. Although IHPP lesions may be clinically suspected in a patient based on the presentation and radiological imaging, definitive diagnosis was rarely made until analysis of the cystic fluid was performed demonstrating a high amylase content<sup>[6,7,17,20,21]</sup>.

### Management

Despite advancements in, and the increasing availability of, imaging modalities, especially the CT scan, the number of reported cases and the type of management techniques have not evolved significantly. There are no widely accepted management guidelines for IHPPs and therefore clinicians have tailored the treatment to the individual patient based on judgment, taking into account many factors, such as underlying etiology, location of the pseudocyst, concomitant lesions, and other patient comorbidities.

Most patients reviewed were symptomatic (91% of reported cases) and required either transcutaneous or surgical intervention. Prior to the development of advanced radiological imaging, more patients underwent a laparotomy and open drainage<sup>[10,12,22]</sup>.

In recent years, however, several less invasive methods have been used to manage IHPPs. Unlike the more commonplace peripancreatic or intrapancreatic pseudocysts, for IHPPs the most common method was percutaneous aspiration or drainage (Table 2) which provided a definitive diagnosis, and was usually well tolerated with minimal complications in these patients<sup>[6,7,23]</sup>. Simple needle aspiration alone with either US or CT

Table 1 Published cases

Ref.	Year	Language	Clinical features
Gautier-Benoit <i>et al</i> <sup>[12]</sup>	1974	French	Abdominal pain, weight loss
Cécile <i>et al</i> <sup>[10]</sup>	1974	French	Same patient as published by Gautier
Quevedo <i>et al</i> <sup>[16]</sup>	1975	Portuguese	Unknown location, died prior to intervention
Siegelman <i>et al</i> <sup>[4]</sup>	1980	English	Edematous pancreas, IHPP aspirated
Epstein <i>et al</i> <sup>[21]</sup>	1982	English	2 patients. Abdominal pain, distension, vomiting, diarrhea, chest pain, ascites
Hospitel <i>et al</i> <sup>[18]</sup>	1983	French	
Atienza <i>et al</i> <sup>[38]</sup>	1987	French	Abdominal pain, jaundice, palpable liver
Roche <i>et al</i> <sup>[11]</sup>	1987	French	Weight loss, hepatomegaly, splenomegaly
Shimayama <i>et al</i> <sup>[39]</sup>	1988	Japanese	Abdominal pain, febrile
Lantink <i>et al</i> <sup>[22]</sup>	1989	English	Abdominal pain
Schaefer <i>et al</i> <sup>[8]</sup>	1989	German	Abdominal pain, anorexia, DVT/PE
Okuda <i>et al</i> <sup>[34]</sup>	1991	English	2 patients, abdominal pain, anorexia, guarding; 1 resolved spontaneously
Slim <i>et al</i> <sup>[40]</sup>	1992	French	
Aiza <i>et al</i> <sup>[37]</sup>	1993	English	Right epigastric pain
Hamm <i>et al</i> <sup>[5]</sup>	1993	German	Abd pain, fever, weight loss
Králík <i>et al</i> <sup>[9]</sup>	1993	Czech	8 patients
Wang <i>et al</i> <sup>[27]</sup>	1993	English	Abdominal pain, pruritis, dark urine, light stools
Scappaticci <i>et al</i> <sup>[35]</sup>	1995	English	Abdominal pain, weight loss
Bayo Poleo <i>et al</i> <sup>[41]</sup>	1997	Spanish	Abdominal pain, blood per rectum
Lederman <i>et al</i> <sup>[23]</sup>	1997	French	Epigstric pain and tenderness, peritonitis
Mehler <i>et al</i> <sup>[30]</sup>	1998	French	Abdominal pain, palpable liver
Mofredj <i>et al</i> <sup>[6]</sup>	2000	English	3 patients, abdominal pain, vomiting, diarrhea, jaundice, guarding
Sugiyama <i>et al</i> <sup>[42]</sup>	2000	Japanese	
Shibaski <i>et al</i> <sup>[33]</sup>	2002	English	Abdominal pain, tenderness, guarding, diarrhea
Bong <i>et al</i> <sup>[43]</sup>	2003	Korean	Abdominal pain
Ancel <i>et al</i> <sup>[44]</sup>	2005	French	Abdominal pain
Balzan <i>et al</i> <sup>[29]</sup>	2005	English	Abdominal pain, cystic dystrophy of duodenal wall
Bhasin <i>et al</i> <sup>[25]</sup>	2005	English	Abdominal pain
Gamanagatti <i>et al</i> <sup>[20]</sup>	2006	English	Abdominal pain, rigid abdomen
Les <i>et al</i> <sup>[17]</sup>	2006	English	Vomiting, melena, tachycardia
Casado <i>et al</i> <sup>[26]</sup>	2007	English	Abdominal pain, nausea
Yi <i>et al</i> <sup>[45]</sup>	2008	Korean	Abdominal pain
Al-Ani <i>et al</i> <sup>[19]</sup>	2009	English	Epigastric pain, fever, diaphoresis, guarding, palpable abdominal mass
Atia <i>et al</i> <sup>[36]</sup>	2009	English	
Chahal <i>et al</i> <sup>[13]</sup>	2009	English	Abdominal pain, nausea, vomiting, hepatomegaly
Guesmi <i>et al</i> <sup>[7]</sup>	2009	English	Abdominal pain
Bhasin <i>et al</i> <sup>[24]</sup>	2010	English	Abdominal pain, vomiting, weight loss
Kibria <i>et al</i> <sup>[14]</sup>	2010	English	2 patients, abdominal pain
Baydar <i>et al</i> <sup>[15]</sup>	2013	English	Abdominal pain
Devangan <i>et al</i> <sup>[28]</sup>	2015	English	Abdominal pain, nausea, vomiting, jaundice
Martínez-Sanz <i>et al</i> <sup>[46]</sup>	2015	English	Abdominal pain, weight loss, anorexia, palpable epigastric mass
Current case	2016	English	Abdominal pain

DVT: Deep-vein thrombosis; PE: Pulmonary embolism; IHPP: Intrahepatic pancreatic pseudocysts.

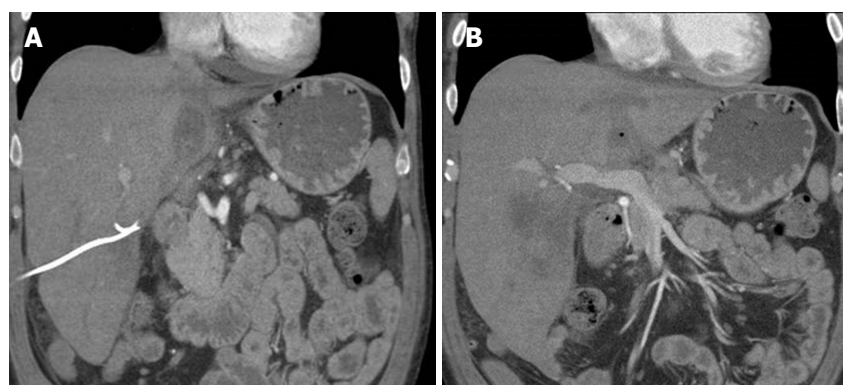


Figure 3 Abdominal computed tomography images showing the superficial, inferior lobe to be well drained with the pigtail in place (A), but the deeper superior collection containing a small bubble of gas (B).

guidance was performed as often as drainage (Table 2). While it aided in the definitive diagnosis by providing an

amylase value of the fluid, it was often not completely therapeutic, with 38% of the aspiration-only cases in



**Table 2 Summary of cases (*n* = 54)**

Mean age (range)	Gender (%)	No. of IHPP (% of cases)	Size (range)	Location (% , <i>n</i> )	No. of interventions (% , <i>n</i> ) <sup>1</sup>	Intervention (% , <i>n</i> ) <sup>2</sup>	Infection (% , <i>n</i> ) <sup>3</sup>
49 (15-76) yr	Male (80%)	1 (67)	9.5 (3-18) cm	Right lobe (11%, 6)	0 (9%, 4)	Operative (25%, 15)	Culture positive
		2 (15)		Left lobe (48%, 26)	1 (60%, 27)	Simple aspiration (28%, 17)	(16%, 5)
	Female (20%)	3 (13)		Right and left lobes (17%, 9)	2 (24%, 11)	Percutaneous drainage (28%, 17)	Culture negative
		4 (4)		Unavailable (24%, 13)	3 (7%, 3)	Endoscopic (8%, 5)	(84%, 27)

<sup>1</sup>Excludes three cases lacking mention of an intervention, and two cases with non-IHPP interventions; <sup>2</sup>Accounts for total number of interventions performed on patient population; some patients underwent several interventions. Does not include those patients who underwent nasopancreatic drainage (5%, 3) or medical intervention (5%, 3); <sup>3</sup>Excludes 15 reports which did not make mention of culture status. IHPP: Intrahepatic pancreatic pseudocysts.



**Figure 4** Abdominal computed tomography image showing drain was repositioned into the deeper lobe seen in Figure 3B.



**Figure 5** Abdominal computed tomography image showing resolution of the intrahepatic pancreatic pseudocysts at 3 mo following the initial intervention.

the literature requiring additional interventions.

In addition to either percutaneous drainage or aspiration, there were several other approaches or adjunctive procedures which have been utilized to manage an IHPP. Although most cases are managed percutaneously or operatively, there is an increasing experience with endoscopic approaches. These have included endoscopic retrograde pancreatography (ERCP) with pancreatic duct stenting, endoscopic transpapillary nasopancreatic drainage, pancreatic duct balloon dilatation, and ERCP-guided aspiration (Table 2)<sup>[13-15,24,25]</sup>. Bhasin *et al.*<sup>[24,25]</sup> for example, reviewed 11 patients with atypically located pseudocysts, treated with ERCP and transpapillary nasopancreatic drainage. Placement of a nasopancreatic drain across the disruption was successful in 10 of the 11 patients (90.9%), with resolution of the extrapancreatic pseudocysts in 4-8 wk, with a follow-up period of 3-70 mo.

Operative interventions on patients with IHPPs have been generally reserved for those refractory to, or inappropriate for, nonoperative treatment, such as cases of diagnostic uncertainty<sup>[26]</sup>, rupture<sup>[22]</sup>, or severe infection<sup>[27]</sup>. All 15 operative interventions (Table 2) to manage these IHPPs were open operations and included partial resection with drainage of the cavity into a Roux limb<sup>[8,9,22]</sup> and complete resection/excision of the lesion<sup>[26,28]</sup>. In 10 reports the operation was the first intervention, in 4 reports it was the second intervention, and in one report it was the third intervention (likely,

see below). The four second-intervention operations followed percutaneous aspiration in two cases<sup>[8,22]</sup> and percutaneous drainage in two cases<sup>[12,29]</sup>. The one third-intervention report<sup>[5]</sup>, however, included 19 extrapancreatic pseudocysts, eight of which were intrahepatic, but it is not clearly reported which if any of those eight IHPP patients underwent which operation. We found no report of postoperative pancreatic fistula development complicating operation.

### Outcomes/complications

Although spontaneous resolution of pseudocysts with conservative (noninterventional) management has been reported, complications in these cases included persistent nausea and vomiting, rupture, fistula tract formation, abscess formation if not sterile, or obstruction of the venous or biliary system due to mass effect.

Outcomes were generally very good for patients presenting with these IHPP, with 45% of patients achieved complete resolution of both the cyst and symptoms. In addition, 21% of patients experienced partial resolution of the cyst but total resolution of their symptoms by the time of the follow-up. In our analysis, we noted a statistically significant correlation between the size of the IHPP and both the duration of treatment ( $P = 0.006$ ) and the number of interventions required ( $P = 0.031$ ).

Infection of these pseudocysts was reported in

16% of the cases (Table 2), but an organism is not always reported and it is usually unknown whether organisms were part of the original process, or later infected the pseudocyst. Many cases were associated with leukocytosis [mean reported white blood cell (WBC) count of 15000] but without correlation to positive cultures on pseudocyst aspiration. Although there is no correlation between infection and final outcome, we did note a statistically significant positive correlation between the initial WBC count and the duration of treatment ( $P = 0.048$ ).

There are four reported deaths in the IHPP literature, three of which had undergone a percutaneous drainage procedure<sup>[7,16,20,30]</sup>. Of note, two of these cases had an infectious component either of the intrahepatic pseudocyst or another concomitant pseudocyst<sup>[20,30]</sup>.

## DISCUSSION

IHPPs frequently present with abdominal pain and are diagnosed with either US or CT imaging. Although the mechanism by which IHPPs develop is not entirely clear, the time to presentation varies tremendously with reports ranging from 6 d to 2 mo<sup>[26,29]</sup>. It is understood, however, that although a collection of pancreatic fluid is not called a "pseudocyst" until it has been present for at least 4 wk, according to the 2012 revision of the Atlanta classification and definitions by international consensus<sup>[1]</sup>, many of the IHPP reports reviewed here predate that nomenclature. Therefore, we have retained the term "pseudocyst" in these cases.

The process of IHPP formation begins of course with an inflammatory or traumatic episode during which pancreatic duct disruption occurs, resulting in the leakage of pancreatic fluid into the surrounding tissue. Then, once the pancreatic proteolytic enzymes are found outside the pancreatic parenchyma, they may migrate along planes (e.g., hepatogastric, hepatoduodenal) or, by digesting tissue, across planes into the hepatic parenchyma. The end result of this is often observable by imaging and on anatomical-pathological findings, evidencing rupture of the main pancreatic duct and active communication with the intrahepatic collection, as shown in several reported cases<sup>[5,21,31-34]</sup>, and in our case (Figure 1). However, communication does not always persist and in these select cases, may actually be more amenable to conservative management or observation.

The most common extrapancreatic location for pancreatic pseudocyst development is within the lesser sac and may be seen alone or along with an IHPP<sup>[4]</sup>. An IHPP may be either subcapsular or intraparenchymal with CT imaging of the former characterized by peripheral location and a biconvex appearance<sup>[20,29]</sup>. They are further characterized by their spatial location in either the right lobe, left lobe, or involving both lobes. It has been hypothesized that the location of the pancreatic inflammation (e.g., head vs tail) is correlated with the tract the fluid takes and eventual location in

the liver of the IHPP with several different paths described<sup>[4,5,13,15,16,19,33-37]</sup>. However, we did not find this to be a statistically significant correlation. The left lobe was by far the most common location for an IHPP (Table 2) with fluid that likely traveled through the hepatogastric ligament.

Although IHPPs may resolve spontaneously, this is uncommon. As in our case, symptoms, or occasionally diagnostic uncertainty, generally require intervention to prevent complications such as infection, fistula, rupture, and mass-effect obstruction of the biliary or portal systems. Our experience certainly echoes that in the literature, *viz.*, that percutaneous or surgical drainage is usually well tolerated and results in resolution of the pseudocyst and improvement in associated symptoms. Treatment of course depends on the location, size, and effects of the pseudocyst, patient stability, and whether or not the lesion remains in persistent communication with the pancreas. In addition to the primary drainage methods to address the IHPP, several adjunctive procedures have been done, some of which were reportedly novel for this indication. Examples include placement of pancreatic duct stent, endoscopic placement of a nasopancreatic drain, or FNA during endoscopy<sup>[13,24,25]</sup>. Recurrence of these pseudocysts has not been described in the literature although is certainly possible, and indeed likely, that there were recurrences, the absence of which may be due to lack of longitudinal follow-up, lack of publication, or the rarity of the condition.

Our case was particularly interesting in that the pseudocyst was very large and bilobed, originating around the hepatoduodenal ligament and extending into the liver. The interval presentation between his pancreatitis flare and initial presentation allowed the pancreas to return to fairly normal appearance. This supports the idea that the hepatoduodenal ligament may be a critical structure in the formation of IHPPs.

In conclusion, although IHPPs are often not included in the differential diagnosis of a patient presenting with an intrahepatic lesion, in the right setting and population of patients, it should be considered as an important differential diagnosis. Analysis of this sparse literature has been instructive in revealing a significant correlation between the size of the IHPP and both the duration of treatment and the number of interventions required. The duration of therapy was also correlated with the initial WBC count. These observations may help with prediction of the clinical course in future cases.

## COMMENTS

### Background

The authors have summarized and analyzed the literature on intrahepatic pancreatic pseudocysts (IHPP), to facilitate an appreciation for this study's relevance and to help understand its significance for the field as a whole.

### Research frontiers

Current important areas in the research field as related this study include the establishment of a registry.

## Innovations and breakthroughs

The key advances in the current study is the recognition that size of the IHPP correlates with both the duration of treatment and the number of interventions required. The duration of therapy was also correlated with the initial white blood cell count.

## Applications

These observations may help with prediction of the clinical course in future cases.

## Peer-review

This is an interesting paper on intrahepatic pseudocyst. Conclusions are interesting. Please elaborate if possible more on the role of endoscopic treatment in such cases.

## REFERENCES

- Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS. Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]
- Sabo A**, Goussous N, Sardana N, Patel S, Cunningham SC. Necrotizing pancreatitis: a review of multidisciplinary management. *JOP* 2015; **16**: 125-135 [PMID: 25791545 DOI: 10.6092/1590-8577/2947]
- Cho JH**, Kim TN, Kim SB. Comparison of clinical course and outcome of acute pancreatitis according to the two main etiologies: alcohol and gallstone. *BMC Gastroenterol* 2015; **15**: 87 [PMID: 26209440 DOI: 10.1186/s12876-015-0323-1]
- Siegelman SS**, Copeland BE, Saba GP, Cameron JL, Sanders RC, Zerhouni EA. CT of fluid collections associated with pancreatitis. *AJR Am J Roentgenol* 1980; **134**: 1121-1132 [PMID: 6770619 DOI: 10.2214/ajr.134.6.1121]
- Hamm B**, Franzen N. [Atypically located pancreatic pseudocysts in the liver, spleen, stomach wall and mediastinum: their CT diagnosis]. *Rofo* 1993; **159**: 522-527 [PMID: 8298111 DOI: 10.1055/s-2008-1032813]
- Mofredj A**, Cadranet JF, Dautreux M, Kazerouni F, Hadj-Nacer K, Deplaix P, Francois G, Danon O, Lukumbo S, Collot G, Levy P, Harry G. Pancreatic pseudocyst located in the liver: a case report and literature review. *J Clin Gastroenterol* 2000; **30**: 81-83 [PMID: 10636217 DOI: 10.1097/00004836-200001000-00016]
- Guesmi F**, Zoghalmi A, Saidi Y, Najeh N, Dziri C. Pancreatic pseudocysts located in the liver: a systematic review of the literature. *Tunis Med* 2009; **87**: 801-804 [PMID: 20209844]
- Schaefer B**, Meyer G, Arnholdt H, Hohlbach G. [Heterotopic pancreatic pseudocyst of the liver]. *Chirurg* 1989; **60**: 556-558 [PMID: 2791745]
- Králík J**, Pesula E. [A pancreatic pseudocyst in the liver]. *Rozhl Chir* 1993; **72**: 91-93 [PMID: 8211403]
- Cécile JP**, Gautier-Benoit G, Luez J, Gaquière A. [False cyst of the pancreas with intrahepatic development]. *J Radiol Electrol Med Nucl* 1974; **55**: 51-54 [PMID: 4364892]
- Roche J**, Frairot A, Volle L, Bory R. [Intrahepatic localization of pancreatic pseudocyst. Treatment by simple puncture under ultrasonography]. *Presse Med* 1987; **16**: 2230 [PMID: 2963322]
- Gautier-Benoit C**, Luez J, Cécile JP. [Pseudocyst of the pancreas with intra-hepatic development]. *Sem Hop* 1974; **50**: 1235-1237 [PMID: 4372705]
- Chahal P**, Baron TH, Topazian MD, Levy MJ. EUS-guided diagnosis and successful endoscopic transpapillary management of an intrahepatic pancreatic pseudocyst masquerading as a metastatic pancreatic adenocarcinoma (with videos). *Gastrointest Endosc* 2009; **70**: 393-396 [PMID: 19394005 DOI: 10.1016/j.gie.2008.10.011]
- Kibria R**, Akram S, Ali SA. Successful endoscopic transpapillary management of intrahepatic pancreatic pseudocyst. *JOP* 2010; **11**: 41-44 [PMID: 20065551]
- Baydar B**, Cantürk F, Alper E, Aslan F, Akpınar Z, Cengiz O, Kandemir A, Ünsal B. Intrahepatic localization of pancreatic pseudocyst: a case report. *Turk J Gastroenterol* 2013; **24**: 447-449 [PMID: 24557971 DOI: 10.4318/tjg.2013.0805]
- Quevedo FC**, Achilles P, Franco MF. [Pancreatic pseudocysts involving the liver and the spleen. Report of 2 cases]. *Rev Hosp Clin Fac Med Sao Paulo* 1975; **30**: 371-374 [PMID: 1188250]
- Les I**, Córdoba J, Vargas V, Guarner L, Boyé R, Pineda V. Pancreatic pseudocyst located in the liver. *Rev Esp Enferm Dig* 2006; **98**: 616-620 [PMID: 17048998 DOI: 10.4321/S1130-01082006000800007]
- Hospitel S**, Guinot B, Teyssou H, Meyblum J, Tessier JP. [Intrahepatic localization of a pancreatic pseudocyst]. *J Radiol* 1983; **64**: 355-358 [PMID: 6876020]
- Al-Ani R**, Ramadan K, Abu-Zidan FM. Intrahepatic pancreatic pseudocyst. *N Z Med J* 2009; **122**: 75-77 [PMID: 19448777]
- Gamanagatti S**, Kandpal H, Mishra V. Acute pancreatitis complicated by intrasplenic and intrahepatic pseudocysts. *Eur J Radiol Extra* 2006; **60**: 29-31 [DOI: 10.1016/j.ejrex.2006.06.008]
- Epstein BM**, Condaris C. Pseudocysts involving the left lobe of the liver. CT demonstration. *Br J Radiol* 1982; **55**: 928-930 [PMID: 7171940 DOI: 10.1259/0007-1285-55-660-928]
- Lantink JA**, Heggelman BG, Geerdink RA. Intrahepatic rupture of a pancreatic pseudocyst: sonographic and CT demonstration. *AJR Am J Roentgenol* 1989; **152**: 1129 [PMID: 2650486 DOI: 10.2214/ajr.152.5.1129]
- Lederman E**, Cajot O, Canva-Delcambre V, Ernst O, Notteghem B, Paris JC. [Pseudocysts of the left liver: uncommon complication of acute pancreatitis]. *Gastroenterol Clin Biol* 1997; **21**: 340-341 [PMID: 9208003]
- Bhasin DK**, Rana SS, Nanda M, Chandail VS, Masoodi I, Kang M, Kalra N, Sinha SK, Nagi B, Singh K. Endoscopic management of pancreatic pseudocysts at atypical locations. *Surg Endosc* 2010; **24**: 1085-1091 [PMID: 19915913 DOI: 10.1007/s00464-009-0732-8]
- Bhasin DK**, Rana SS, Chandail VS, Nanda M, Nadkarni N, Sinha SK, Nagi B. An intra-hepatic pancreatic pseudocyst successfully treated endoscopic transpapillary drainage alone. *JOP* 2005; **6**: 593-597 [PMID: 16286711]
- Casado D**, Sabater L, Calvete J, Mayordomo E, Aparisi L, Sastre J, Lledo S. Multiple intrahepatic pseudocysts in acute pancreatitis. *World J Gastroenterol* 2007; **13**: 4655-4657 [PMID: 17729426 DOI: 10.3748/wjg.v13.i34.4655]
- Wang SJ**, Chen JJ, Changchien CS, Chiou SS, Tai DI, Lee CM, Kuo CH, Chiu KW, Chuah SK. Sequential invasions of pancreatic pseudocysts in pancreatic tail, hepatic left lobe, caudate lobe, and spleen. *Pancreas* 1993; **8**: 133-136 [PMID: 8419901 DOI: 10.1097/00006676-199301000-00024]
- Devangan M**, Sonkar SK, Sharma S. A rare case of pancreatic pseudocyst involving liver and spleen. *Int J Med Sci Res Pract* 2015; **2**: 150-152
- Balzan S**, Kianmanesh R, Farges O, Sauvanet A, O'toole D, Levy P, Ruszniewski P, Ogata S, Belghiti J. Right intrahepatic pseudocyst following acute pancreatitis: an unusual location after acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2005; **12**: 135-137 [PMID: 15868077 DOI: 10.1007/s00534-004-0929-0]
- Mehler CI**, Soyer P, Kardache M, Pelage JP, Boudiaf M, Panis Y, Abitbol M, Hamzi L, Rymer R. [Computed tomography of intrahepatic pancreatic pseudocysts]. *J Radiol* 1998; **79**: 751-755 [PMID: 9757305]
- Goyal S**, Raju R, Yadav S. Pancreatic pseudocyst of gastrohepatic ligament: a case report and review of management. *JOP* 2012; **13**: 439-442 [PMID: 22797402]
- Gould L**, Khademi M, Guarnaccia M, Patel NK. Pancreatic pseudocyst simulating an intrahepatic mass. *Am J Gastroenterol* 1979; **72**: 75-78 [PMID: 463852]
- Shibasaki M**, Bandai Y, Ukai T. Pancreatic pseudocyst extending into the liver via the hepatoduodenal ligament: a case report. *Hepatogastroenterology* 2002; **49**: 1719-1721 [PMID: 12397775]

- 34 **Okuda K**, Sugita S, Tsukada E, Sakuma Y, Ohkubo K. Pancreatic pseudocyst in the left hepatic lobe: a report of two cases. *Hepatology* 1991; **13**: 359-363 [PMID: 1995443 DOI: 10.1002/hep.1840130225]
- 35 **Scappaticci F**, Markowitz SK. Intrahepatic pseudocyst complicating acute pancreatitis: imaging findings. *AJR Am J Roentgenol* 1995; **165**: 873-874 [PMID: 7676984 DOI: 10.2214/ajr.165.4.7676984]
- 36 **Atia A**, Kalra S, Rogers M, Murthy R, Borthwick TR, Smalligan RD. A wayward cyst. *JOP* 2009; **10**: 421-424 [PMID: 19581748]
- 37 **Aiza I**, Barkin JS, Casillas VJ, Molina EG. Pancreatic pseudocysts involving both hepatic lobes. *Am J Gastroenterol* 1993; **88**: 1450-1452 [PMID: 8362849]
- 38 **Atienza P**, Couturier D, Grandjouan S, Guerre J, Bettan L, Chapuis Y, Vasile N. [Intrahepatic collections of fluid of pancreatic origin. A case]. *Presse Med* 1987; **16**: 1195-1198 [PMID: 2955363]
- 39 **Shimayama T**, Katsuki T, Kosai S, Yogi Y. [A case of pancreatic pseudocyst intruded into the right lobe of the liver]. *Nihon Shokakibyo Gakkai Zasshi* 1988; **85**: 1708-1711 [PMID: 3246761]
- 40 **Slim K**, Hendaoui L, Larabi B. [Multiple intrahepatic pseudocysts in acute pancreatitis]. *Gastroenterol Clin Biol* 1992; **16**: 902 [PMID: 1483564]
- 41 **Bayo Poleo R**, Zaheri M, Córdoba López A. [Bleeding intrahepatic cyst in a patient with chronic pancreatitis]. *Gastroenterol Hepatol* 1997; **20**: 46-47 [PMID: 9072201]
- 42 **Sugiyama H**, Sasaki M, Asano T, Kawai H, Kato T, Moriwaki H, Kuroiwa M. [A case of pancreatic pseudocyst intruded into the left lobe of the liver]. *Nihon Shokakibyo Gakkai Zasshi* 2000; **97**: 605-611 [PMID: 10846418]
- 43 **Bong HK**, Kim JK, Lee SY, Lee JS, Lee MS, Kim JH, Cho SW, Shim CS. A case of chronic pancreatitis complicated by intrahepatic pseudocyst. *Korean J Gastroenterol* 1993; **25**: 1375-1380
- 44 **Ancl D**, Lefebvre M, Peyrin-Biroulet L, Chone L, Sido A, Regent D, Bigard MA. [Pancreatic pseudocysts of the right hepatic lobe during acute biliary pancreatitis]. *Gastroenterol Clin Biol* 2005; **29**: 743-745 [PMID: 16142012 DOI: 10.1016/S0399-8320(05)82166-9]
- 45 **Yi CY**, Na GJ, Baek HC, Kim JH, Bae SH, Kim DH, Je IS, Kwon BP. [A case of intrahepatic pseudocyst complicating acute pancreatitis]. *Korean J Gastroenterol* 2008; **51**: 56-59 [PMID: 18349565]
- 46 **Martínez-Sanz N**, González-Valverde FM, Vicente-Ruiz M, Pastor-Pérez P, Ruiz-Marín M, Albarracín-Marín-Blázquez A. Intrahepatic pancreatic pseudocyst: a case report. *Rev Esp Enferm Dig* 2015; **107**: 249-250 [PMID: 25824934]

**P- Reviewer:** Furihata M, Somani P **S- Editor:** Gong ZM

**L- Editor:** A **E- Editor:** Li D





## PNPLA3 polymorphism increases risk for and severity of chronic hepatitis C liver disease

Habeeb Salameh, Maen Masadeh, Muhannad Al Hanayneh, Vincent Petros, Matthew Maslonka, Arjun Nanda, Ashwani K Singal

Habeeb Salameh, Muhannad Al Hanayneh, Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Texas Medical Branch, Galveston, TX 77555, United States

Maen Masadeh, Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Iowa, Iowa City, IA 52242, United States

Vincent Petros, Matthew Maslonka, Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX 77555, United States

Arjun Nanda, Ashwani K Singal, Division of Gastroenterology and Hepatology, University of Alabama, Birmingham, AL 35294, United States

**Author contributions:** Salameh H study design, data collection and interpretation, drafting and editing manuscript; Masadeh M literature review, data extraction and study quality assessment; Al Hanayneh M and Maslonka M literature review, data extraction and study quality assessment; Petros V literature review, drafting and editing the manuscript; Nanda A drafting and editing the manuscript; Singal AK study design, data analysis and interpretation, and manuscript editing; all the authors approved the final version of the manuscript.

**Conflict-of-interest statement:** The authors deny any conflict of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Habeeb Salameh, MD, CMQ, Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555, United States. [habeeb.salameh@yahoo.com](mailto:habeeb.salameh@yahoo.com)  
**Telephone:** +1-409-7721501  
**Fax:** +1-409-7724789

**Received:** June 28, 2016

**Peer-review started:** June 28, 2016

**First decision:** August 10, 2016

**Revised:** September 9, 2016

**Accepted:** October 17, 2016

**Article in press:** October 18, 2016

**Published online:** December 18, 2016

### Abstract

#### AIM

To examine the association of *PNPLA3* polymorphisms in chronic hepatitis C patients and development of liver disease spectrum.

#### METHODS

Literature was searched systematically from PubMed/MEDLINE, EMBASE, and Cochrane search engines for full-length articles written in English that examined *PNPLA3* polymorphism in chronic hepatitis C (CHC) patients. Studies evaluating the association of *PNPLA3* polymorphism spectrum (fatty liver, steatohepatitis, cirrhosis, and hepatocellular carcinoma) of CHC were included. Pooled data are reported as OR with 95%CI. Our study endpoint was the risk of the entire liver disease spectrum including: Steatosis/fatty liver, cirrhosis, and hepatocellular carcinoma in CHC patients with *PNPLA3* polymorphisms.

#### RESULTS

Of 380 studies identified, a total of 53 studies were included for full-text review. Nineteen on chronic he-

patitis C were eligible for analysis. Pooled ORs for rs738409 GG compared to CC and CG among patients with fatty liver was 2.214 (95%CI: 1.719-2.853). ORs among advanced fibrosis/cirrhosis were 1.762 (95%CI: 1.258-2.468). Similar odds ratios among hepatocellular carcinoma patients were 2.002 (95%CI: 1.519-2.639). Pooled ORs for rs738409 GG and CG compared to CC among patients with fatty liver were 1.750 (95%CI: 1.542-1.986). Pooled ORs for advanced fibrosis/cirrhosis patients were 1.613 (95%CI: 1.211-2.147). All analyses were homogenous and without publication bias except one. The associations were maintained after adjusting for publication bias and heterogeneity.

## CONCLUSION

*PNPLA3* polymorphisms have strong association with increased risk and severity of the liver disease spectrum in CHC patients.

**Key words:** *PNPLA3* polymorphism; Cirrhosis; rs738409; Hepatitis C virus; Hepatocellular carcinoma

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** *PNPLA3* polymorphisms (rs738409 CG and GG) are associated with increased risk of steatosis, advanced fibrosis, cirrhosis, and hepatocellular carcinoma in chronic hepatitis C patients.

Salameh H, Masadeh M, Al Hanayneh M, Petros V, Maslonka M, Nanda A, Singal AK. *PNPLA3* polymorphism increases risk for and severity of chronic hepatitis C liver disease. *World J Hepatol* 2016; 8(35): 1584-1592 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i35/1584.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i35.1584>

## INTRODUCTION

Hepatitis C virus (HCV) infection is one of the most important causes of chronic liver disease in the United States<sup>[1]</sup>. About 27% of cases of cirrhosis and 25% of hepatocellular carcinoma (HCC) worldwide are secondary to HCV infection<sup>[2]</sup>. Multiple genetic factors identified within the past few years have been shown to be associated with the predisposition to chronic liver disease and the progression to cirrhosis and HCC<sup>[3,4]</sup>. The single nucleotide polymorphism (SNP) rs738409 C>G (isoleucine to methionine substitution at position 148, I148M) in the *PNPLA3* gene has been strongly linked to progression of liver disease in multiple studies, and this association was confirmed in meta-analyses of the spectrum of alcoholic liver disease (ALD)<sup>[5]</sup> as well as non-alcoholic fatty liver disease (NAFLD)<sup>[6-9]</sup>.

The frequency of hepatic steatosis varies with ethnicity where it was reported as 45%, 33% and 24% in Hispanics, Whites and Blacks respectively<sup>[10]</sup>. At the

same time the frequencies of the *PNPLA3* rs738409[G] allele were 0.49, 0.23, and 0.17 in Hispanics, European Americans and African Americans<sup>[11]</sup>. In addition, the prevalence of the GG genotype in different races in fact correlates with the rate of NAFLD in each respective population, with nearly half of all Hispanics possessing the allele, who in turn are also most likely to have NAFLD. The same is true of the inverse, with less than one quarter of African Americans having the *PNPLA3* rs738409[G] allele, and they are least likely to develop NAFLD compared to Hispanics and Caucasians<sup>[10,11]</sup>.

Given that the association between *PNPLA3* polymorphism and liver disease spectrum in chronic hepatitis C (CHC) patients has not been consistent, especially for HCC<sup>[12,13]</sup> and cirrhosis<sup>[14,15]</sup>, we performed this meta-analysis to further examine the association of *PNPLA3* polymorphisms with the predisposition to the entire spectrum of liver disease among patients with CHC.

## MATERIALS AND METHODS

### Literature search

Utilizing the Meta-analysis of Observational Studies in Epidemiology guidelines, literature was searched from PubMed/Medline, Embase, and Cochrane search engines for full-length articles written in English that examined *PNPLA3* polymorphism in CHC patients<sup>[16]</sup>. The initial medical subject headings search terms were: "Hepatitis C, Chronic" and "adiponutrin, human". The search was then expanded using the terms: "rs738409" and "patatin-like phospholipase domain-containing 3 protein". All databases were searched from their inception date through March 2015. Meeting abstracts from major gastroenterology conferences over the past 3 years were also searched to identify studies that were potentially overlooked in our database search. Articles were selected for full text review based on title and abstract.

### Study selection

Three independent investigators (Masadeh M, Al Hanayneh M and Maslonka M) manually search the retrieved publications to ensure all appropriate articles were discovered and included. Two authors (HS and AKS) reviewed articles in question for possible inclusion. The following inclusion criteria were set for inclusion in this meta-analysis: (1) studies published as full-length articles which reported association of the *PNPLA3* variant (rs738409 C>G) among CHC patients; and (2) studies which analyzed patients with other liver diseases and reported separate data on *PNPLA3* polymorphisms for CHC.

The following exclusion criteria were set: (1) studies without available gene frequency data for analysis; and (2) studies including subjects with other liver diseases without separate data on CHC patients.

### Definitions

HCV infection was diagnosed with both positive serum

anti-HCV antibodies and serum HCV ribonucleic acid (RNA). The disease spectrum was defined as the following: steatosis = fatty liver (FL) on imaging without evidence of cirrhosis or HCC; advanced fibrosis and cirrhosis = biopsy-proven bridging fibrosis, or clinical evaluation supported by hematological, biochemical, and radiologic imaging findings; and HCC = diagnostic imaging findings on triple phase magnetic resonance imaging or computed tomography, or using histological confirmation from liver tissue. Healthy controls were defined as subjects without liver disease and without HCV infection.

### Data extraction

After determining eligibility for inclusion, two reviewers (Masadeh M and Al Hanayneh M) independently extracted data for (1) study characteristics: Author and year of publication, and study design (population based or not, using controls or not); (2) study population: Liver disease spectrum and sample size; (3) frequencies of *PNPLA3* polymorphism genotypes (rs738409 CC, CG, and GG); and (4) OR: For association of *PNPLA3* polymorphism and the spectrum of liver disease and for severity of liver disease. Any discrepancies amongst the reviewers were resolved by jointly reviewing the study in question. Among studies comparing diseased population with healthy controls, similar data were also extracted on healthy controls.

### Endpoints and outcomes

Our study endpoint was the risk of the entire liver disease spectrum including: Steatosis/fatty liver, cirrhosis, and HCC in CHC patients with *PNPLA3* polymorphisms.

### Quality assessment

The quality of included studies was assessed independently by three authors (Masadeh M, Al Hanayneh M and Maslonka M) using the Newcastle-Ottawa Quality Assessment Scale for case-control studies<sup>[17]</sup>. This scale has one instrument for assessing case-control studies and another one for cohort studies. Each of these instruments includes measures of quality in selection, comparability, and exposure domains. While one point is granted for each of the areas measured within the selection and exposure domains, a maximum of two points can be assigned within the comparability domain with highest possible total score of nine. Previous studies have reported that a score of seven or greater denotes a high-quality study<sup>[18]</sup>. Any discrepancies between the three coauthors were addressed by a joint reevaluation of the original article.

### Statistical analysis

The strength of the association between rs738409 and CHC liver disease spectrum prevalence was expressed by OR and their corresponding 95%CI. The Random effects model was used for analyzing pooled data for all the analyses<sup>[19]</sup>. Heterogeneity was measured using  $I^2$  statistics for inter-study variance, with the  $\chi^2$  test

used for statistical analysis. Heterogeneity was defined with  $I^2 \geq 50\%$  or  $\chi^2 P < 0.10$ <sup>[20]</sup>. At least two studies are needed to examine and report heterogeneity. To examine the heterogeneous data and source of heterogeneity, sensitivity analyses were performed in a stepwise fashion for (1) study quality; and (2) excluding studies with the highest and lowest OR. Publication bias was assessed using Egger regression and the Begg-Mazumdar rank correlation tests<sup>[21-23]</sup>. Egger test is a regression method checking for association between effect sizes and standard error and uses actual effect size for each study<sup>[23]</sup>. Begg-Mazumdar is a rank correlation test examining the potential association between effect estimates (taken as a rank and not exact effect size) and sampling variance (or standard error)<sup>[22]</sup>. At least three studies are needed for examining and reporting publication bias. For analyses with publication bias, the analyses were repeated either by performing sensitivity analysis or using the Duval and Tweedie Trim and Fill method, a nonparametric (rank-based) data augmentation technique<sup>[24]</sup>. The method can be used to estimate the number of studies missing from a meta-analysis resulting in a skew of the data due to the suppression of the most extreme results on one side of the funnel plot. The method then amplifies the observed data so that the funnel plot is more symmetric and re-computes the summary estimate based on the comprehensive data. The method should not be regarded as a way of yielding a more "valid" estimate of the overall effect or outcome, but as a means of examining the sensitivity of the results to one particular selection mechanism<sup>[25]</sup>. All statistical analyses were performed using R (Foundation for Statistical Computing) utilizing the metaphor package, or Comprehensive Meta-analysis (Biostat, Englewood, NJ). Singal AK from University of Alabama, Birmingham, reviewed the statistical methods of this study.

## RESULTS

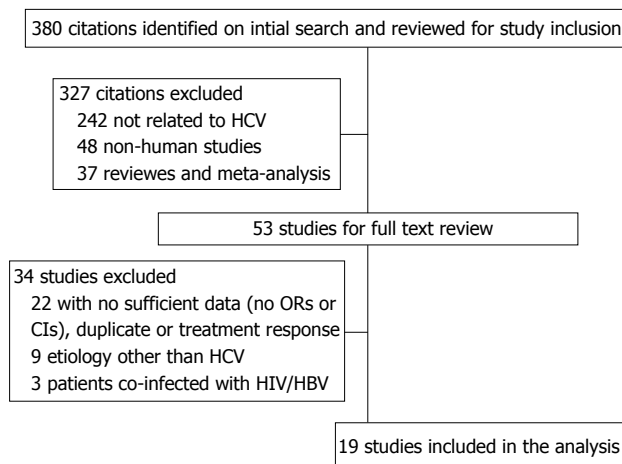
### Baseline study characteristics

A total of 380 citations were retrieved on initial search. After reviewing article titles and abstracts, a total of 53 studies were included for full-text review (Figure 1). Of these, twenty articles were excluded because they did not have sufficient data for our analysis. Nine studies were excluded for including subjects with liver disease not caused by HCV<sup>[11,26-33]</sup>, and three studies were excluded for including subjects co-infected with human immunodeficiency virus and/or hepatitis B virus infection<sup>[34-36]</sup>. One duplicate study<sup>[37]</sup> and one study that looked at treatment response<sup>[38]</sup> were excluded. Nineteen studies evaluating 9093 patients (57.6% males, mean body mass index 25.1 kg/m<sup>2</sup>) on association of *PNPLA3* polymorphisms in CHC<sup>[12-15,39-53]</sup> were included for the analysis. Data on study design, ethnicity and genotype frequency are summarized in Table 1.

**Table 1** Baseline characteristics of patients from studies included in the analysis

Ref.	Study design	Controls (n)	Cases								
			n	Ethnicity	M%	Mean age	Mean BMI	rs 738409 genotype count (CC:CG:GG) <sup>4</sup>			
								FL	Hepatitis	Cirrhosis	HCC
Cai <i>et al</i> <sup>[39]</sup> (2011)	R	-	626	C	61.8	44.7	23.7	62:28 <sup>1</sup>	-	-	-
Valenti <i>et al</i> <sup>[40]</sup> (2011)	R	179	819	C + NA	56.4	57.4	24.8	269:219:73	-	119:172:229	17:21:12
Trépo <i>et al</i> <sup>[41]</sup> (2011)	R	-	537	C	63	49.4	25.5	136:106:31	-	108:85:23	-
Corradini <i>et al</i> <sup>[42]</sup> (2011)	P <sup>6</sup>	-	221	C	63	58	-	-	-	-	-
Nischalke <i>et al</i> <sup>[13]</sup> (2011)	P	190	162	C	57	56	28.4	-	-	45:31:05	40:33:08
Valenti <i>et al</i> <sup>[43]</sup> (2012)	P	-	567	NS	-	-	-	-	-	-	-
Valenti <i>et al</i> <sup>[15]</sup> (2012)	P <sup>6</sup>	-	602	NS	51	51	25.1	364:42 <sup>2</sup>	-	158:21 <sup>2</sup>	-
Guyot <i>et al</i> <sup>[12]</sup> (2013)	P	-	253	NS	54.2	56.7	27.3	-	-	140:75:38	54:26:13
Ezzikouri <i>et al</i> <sup>[44]</sup> (2013)	P	132	230	NA	45.2	63.63	-	-	47:71:11	-	43:35:23
Stättermayer <i>et al</i> <sup>[45]</sup> (2014)	R	-	478	NS	65.7	44.9	25.6	190:23 <sup>2</sup>	-	101:57 <sup>2</sup>	-
Ampuero <i>et al</i> <sup>[46]</sup> (2014)	P <sup>6</sup>	-	474	M	64.8	43.4	25.7	94:126 <sup>3</sup>	-	-	-
Sato <i>et al</i> <sup>[47]</sup> (2014)	R	-	358	A	55.9	69.76	-	41:20 <sup>2</sup>	-	112:37 <sup>2</sup>	100:176:82
Yasui <i>et al</i> <sup>[48]</sup> (2014)	P <sup>6</sup>	-	276	A	40.6	58.2	23	23:75:39	45:66:38	20:31:21	-
Petta <i>et al</i> <sup>[14]</sup> (2015)	P	-	434	C	53.9	51.7	-	-	40:35:12	71:36:13	-
Nakaoka <i>et al</i> <sup>[49]</sup> (2015 )	P	-	231	A	44.6	62.9	22.5	-	-	90:27 <sup>2</sup>	12:22:14
Tamaki <i>et al</i> <sup>[50]</sup> (2015)	R	-	176	A	39.8	56.5	22.9	-	-	52:87:37	-
Huang <i>et al</i> <sup>[51]</sup> (2015)	R	-	1018	A	56.6	51.8	24.9	175:205:75	-	-	-
Petta <i>et al</i> <sup>[52]</sup> (2016)	P <sup>6</sup>	-	694	C	53	54	26.5	151:151:45 <sup>5</sup>	-	-	-
Ali <i>et al</i> <sup>[53]</sup> (2016)	P	-	937	M	70.1	49.5	-	-	-	172:212 <sup>3</sup>	-
Summary		501	9093		57.6	52.7	25.1				

<sup>1</sup>C allele: G allele; <sup>2</sup>CC + CG: GG; <sup>3</sup>CC: CG + GG; <sup>4</sup>Genotype counts were reported as ratios (CC wild genotype: CG heterozygote genotype: GG homozygote genotype) unless indicated by star(s); <sup>5</sup>Calculated from percentages in the original article; <sup>6</sup>Population based studies. A: Asian; BMI: Body mass index; C: Caucasian; FL: Fatty liver; HCC: Hepatocellular carcinoma; M: Mixed (Caucasians and non-Caucasians<sup>[46]</sup> and White/Black/Hispanic<sup>[53]</sup>); P: Prospective; R: Retrospective; M%: Males percentage; N: Number of cases; NA: North African; NS: Not specified in the original manuscripts (although all 4 studies included European referral centers only).



**Figure 1** Attrition on literature search and study inclusion. HCV: Hepatitis C virus; HBV: Hepatitis B virus; HIV: Human immunodeficiency virus.

### Study quality assessment

Based on the Newcastle-Ottawa Scale, nine studies were of “high quality” with a score of seven or more, and the remaining ten studies had a score of six or below (Table 2).

### Association between *PNPLA3* polymorphism and liver disease spectrum (GG vs CG and CC analysis)

**Association of *PNPLA3* polymorphisms with FL in CHC patients:** Among six studies on 3310 patients, the pooled OR for rs738409 GG genotype compared to CC and CG genotypes in CHC was 2.214 (95%CI:

1.719-2.853) (Figure 2A). The data was homogeneous ( $I^2 = 9.4\%$ ,  $P = 0.36$ ) and without publication bias as assessed by Egger test ( $P = 0.08$ ) and Begg-Mazumdar test ( $P = 0.14$ ).

### Association of *PNPLA3* polymorphisms with advanced fibrosis and cirrhosis in CHC patients:

Among seven studies on 3377 patients, the pooled OR for rs738409 GG genotype compared to CC and CG genotypes in CHC was 1.762 (CI: 1.258-2.469) (Figure 2B). The data was heterogeneous ( $I^2 = 65.9\%$ ,  $P = 0.081$ ), with evidence of publication bias as assessed by Begg-Mazumdar test ( $P = 0.036$ ) and tendency for publication bias as assessed by Egger test ( $P = 0.059$ ). Sensitivity analysis after excluding studies with lowest<sup>[14]</sup> and highest<sup>[49]</sup> OR revealed similar effect size: 1.82 (95%CI: 1.41-2.34) with  $I^2 = 18.8\%$ ,  $P = 0.30$ . Additionally, when Dual and Tweedie trim and fall test was used to assess publication bias, 3 studies were trimmed with no change in effect size (OR = 1.39, 95%CI: 1.01-1.92).

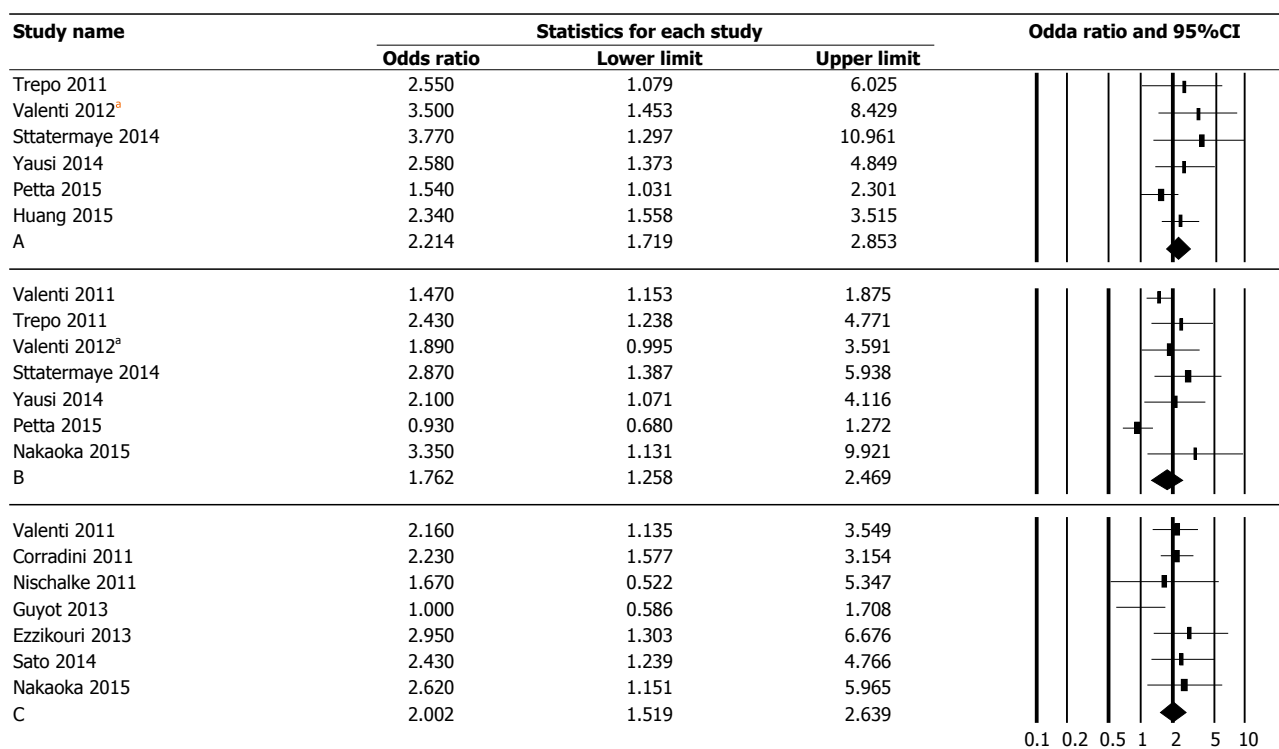
### Association of *PNPLA3* polymorphisms and HCC in CHC patients:

Among seven studies on 2274 patients, the pooled OR for rs738409 GG genotype compared to CC and CG genotypes in CHC was 2.002 (95%CI: 1.519-2.639) (Figure 2C). The data was homogenous ( $I^2 = 30\%$ ,  $P = 0.19$ ), without publication bias as assessed by Egger test ( $P = 0.91$ ) and Begg-Mazumdar test ( $P = 0.99$ ).



**Table 2** Newcastle - Ottawa Scale on quality score of the included studies

Ref.	Selection				Comparability		Exposure		Total
	Independent validation	Case representation	Controls selection	Controls definition	Case and control design/analysis	Ascertainment of exposure	Same method of ascertainment	Same response rate	
Cai <i>et al</i> <sup>[39]</sup> (2011)	1	1			2	1	1	1	7
Valenti <i>et al</i> <sup>[40]</sup> (2011)	1	1	1	1	2	1	1	1	9
Trépo <i>et al</i> <sup>[41]</sup> (2011)	1	1			2	1			5
Corradini <i>et al</i> <sup>[42]</sup> (2011)	1	1			2	1			5
Nischalke <i>et al</i> <sup>[13]</sup> (2011)	1	1	1	1	2	1	1	1	9
Valenti <i>et al</i> <sup>[43]</sup> (2012)	1	1			2	1	1		6
Valenti <i>et al</i> <sup>[15]</sup> (2012)	1	1			2	1	1		6
Guyot <i>et al</i> <sup>[12]</sup> (2013)	1	1			2	1			5
Ezzikouri <i>et al</i> <sup>[44]</sup> (2013)	1	1	1	1	2	1	1	1	9
Stättermayer <i>et al</i> <sup>[45]</sup> (2014)	1	1			2	1			5
Ampuero <i>et al</i> <sup>[46]</sup> (2014)	1	1			2	1	1	1	7
Sato <i>et al</i> <sup>[47]</sup> (2014)	1	1			2	1			5
Yasui <i>et al</i> <sup>[48]</sup> (2014)	1	1			2	1			5
Petta <i>et al</i> <sup>[14]</sup> (2015)	1	1			2	1			5
Nakaoka <i>et al</i> <sup>[49]</sup> (2015)	1	1			2	1			5
Tamaki <i>et al</i> <sup>[50]</sup> (2015)	1	1			2	1	1	1	7
Huang <i>et al</i> <sup>[51]</sup> (2015)	1	1			2	1	1	1	7
Petta <i>et al</i> <sup>[52]</sup> (2016)	1	1			2	1	1	1	7
Ali <i>et al</i> <sup>[53]</sup> (2016)	1	1			2	1	1	1	7



**Figure 2** Forest plots for analysis of chronic hepatitis C studies on the association of *PNPLA3* polymorphisms GG vs CG and CC with fatty liver in (A), cirrhosis in (B), and hepatocellular carcinoma in (C). The effect size is reported as odds ratio with 95%CI. The bottom line in "the statistics for each study" heading is the pooled effect size analyzed using the random effects model. OR greater than 1 denotes risk for the respective outcome or positive association, and OR less than 1 indicates a protective effect or negative association. The 95%CI not crossing 1 indicates a significant association. Valenti 2012<sup>a</sup> in panel (A) refers to reference<sup>[43]</sup>, and in panel (B) refers to reference<sup>[14]</sup>.

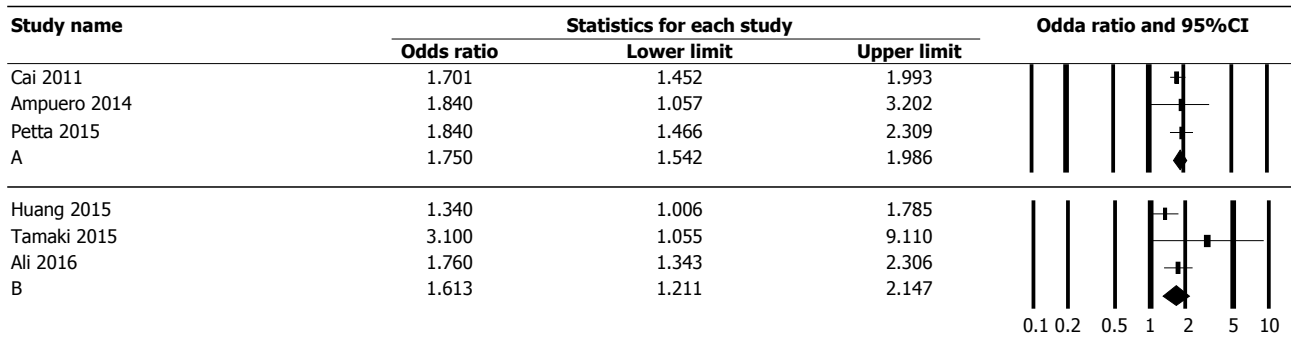
### Association between *PNPLA3* polymorphism and liver disease spectrum (GG and CG vs CC analysis)

**Association of *PNPLA3* polymorphisms with FL in CHC patients:** Among three studies on 1794 patients, the pooled OR for rs738409 GG and CG genotypes compared to CC genotype in CHC was 1.750 (95%CI: 1.542-1.986) (Figure 3A). The data was homogeneous

( $I^2 = 0.0\%$ ,  $P = 0.84$ ), without publication bias as assessed by Egger test ( $P = 0.57$ ) and Begg-Mazumdar test ( $P = 0.99$ ).

### Association of *PNPLA3* polymorphisms with advanced fibrosis, and cirrhosis in CHC patients:

Among three studies on 2131 patients, the pooled OR



**Figure 3** Forest plots for analysis of chronic hepatitis C studies on the association of *PNPLA3* polymorphisms GG and CG vs CC with fatty liver in (A), and cirrhosis in (B). The effect size is reported as odds ratio with 95%CI. The bottom line in the "statistics for each study" heading is the pooled effect size analyzed using the random effects model. OR greater than 1 denotes risk for the respective outcome or positive association, and OR less than 1 indicates a protective effect or negative association. The 95%CI not crossing 1 indicates a significant association.

for rs738409 GG and CG genotypes compared to CC genotype in CHC was 1.613 (95%CI: 1.211-2.147) (Figure 3B). The data was homogeneous ( $I^2 = 41\%$ ,  $P = 0.18$ ), without publication bias as assessed by Egger test ( $P = 0.056$ ) and Begg-Mazumdar test ( $P = 0.99$ ).

## DISCUSSION

We have previously described that *PNPLA3* polymorphism is a modifier in the natural history of ALD<sup>[5]</sup> and NAFLD<sup>[6-8]</sup>. In this meta-analysis, we found a clear association between *PNPLA3* polymorphisms and the entire spectrum (steatosis/fatty liver, cirrhosis, and HCC) of liver disease in CHC patients.

It was previously reported that *PNPLA3* polymorphisms were an independent predictor of more rapid fibrosis progression in patients with chronic hepatitis C<sup>[50]</sup>. The mechanism whereby rs738409 influences the development of fatty liver likely involves a decreased ability of the 148M *PNPLA3* variant to regulate hepatic lipid metabolism<sup>[54]</sup>. It is not known whether the rs738409 SNP influences the steatogenic effect of HCV and the progression of CHC. However, if steatosis causes fibrosis progression in CHC, then it may be assumed the rs738409 SNP should also be associated with advanced fibrosis and HCC<sup>[40]</sup>.

Like any other meta-analysis, our study had to face the possibility of publication bias. In order to minimize this possibility, and the subsequent overestimation of the true effect size due to negative study identification failure<sup>[55]</sup>, we combined searches from PubMed/Medline, Embase and Cochrane with manual searches. Although we used procedures in agreement with current guidelines, we cannot formally rule out the possibility that we overlooked studies that were not accessible<sup>[55]</sup>. Another limitation of this meta-analysis is the inclusion of case-control studies in which the potential for biases (e.g., selection and reporting) is higher when compared to randomized trials, and they are more inherent to confounding factors. In contrary to the previous meta-analysis on *PNPLA3* polymorphisms in alcoholic and non-alcoholic liver diseases that compared different genotypes<sup>[5-8]</sup>, our current analysis used the recessive

model when comparing GG genotype vs CC and CG genotypes, and the dominant model when comparing GG and CG genotypes related to the CC genotype. Finally, no pooled data were provided on steatohepatitis in chronic HCV patients as only one study had reported such an association<sup>[14]</sup>, while the studies by Ezzikouri *et al.*<sup>[44]</sup> and Yasui *et al.*<sup>[48]</sup> either did not have biopsies performed or reported "necroinflammatory changes". Lack of standard definition amongst these studies prevented pooling them together.

The *PNPLA3* GG genotype was negatively associated with sustained virological response and early viral kinetics in patients receiving peginterferon and ribavirin<sup>[15]</sup>. Also, in patients with chronic hepatitis C who failed to achieve sustained virologic response following interferon-based therapy, IL28B and *PNPLA3* were independent predictors of rapid fibrosis progression<sup>[50]</sup>. Tamaki *et al.*<sup>[50]</sup> developed a fibrosis progression-score by combining IL28B and *PNPLA3* genotypes and ALT values, which stratified patients into low, intermediate, and high-risk groups for fibrosis progression. However, this fibrosis progression score needs external validation. In the era of direct-acting antiviral therapy, the question that remains unanswered is whether or not *PNPLA3* polymorphisms identify high-risk CHC patients that are responsive to new treatment regimens. In summary, this meta-analysis provides strong evidence for the association of *PNPLA3* polymorphisms and the spectrum of liver disease in patients with CHC, beginning with fatty liver disease and extending as far as cirrhosis and even HCC in patients with CHC. Further studies on treatment response are needed in this group of patients who carry a higher risk for more rapidly progressive liver disease.

## COMMENTS

### Background

Hepatitis C virus (HCV) infection is one of the most important causes of chronic liver disease in the United States. About 27% of cases of cirrhosis and 25% of hepatocellular carcinoma (HCC) worldwide are secondary to HCV infection.

### Research frontiers

Given that the association between *PNPLA3* polymorphism and liver disease

spectrum in chronic hepatitis C (CHC) patients has not been consistent, especially for HCC and cirrhosis, the authors performed this meta-analysis to further examine the association of *PNPLA3* polymorphisms with the predisposition to the entire spectrum of liver disease among patients with CHC.

### Innovations and breakthroughs

In this meta-analysis, they found a clear association between *PNPLA3* polymorphisms and the entire spectrum (steatosis/fatty liver, cirrhosis, and HCC) of liver disease in CHC patients.

### Peer-review

This manuscript is very well designed; the authors did a great effort in selecting the articles to be included in the meta-analysis with a proper quality scoring of selected articles.

## REFERENCES

- 1 **Younossi ZM**, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, Srishord M. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin Gastroenterol Hepatol* 2011; **9**: 524-530.e1; quiz e60 [PMID: 21440669 DOI: 10.1016/j.cgh.2011.03.020]
- 2 **Alter MJ**. Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007; **13**: 2436-2441 [PMID: 17552026 DOI: 10.3748/wjg.v13.i17.2436]
- 3 **Labib HA**, Ahmed HS, Shalaby SM, Wahab EA, Hamed EF. Genetic polymorphism of IL-23R influences susceptibility to HCV-related hepatocellular carcinoma. *Cell Immunol* 2015; **294**: 21-24 [PMID: 25666505 DOI: 10.1016/j.cellimm.2015.01.012]
- 4 **Singal AG**, Manjunath H, Yopp AC, Beg MS, Marrero JA, Gopal P, Waljee AK. The effect of *PNPLA3* on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 325-334 [PMID: 24445574 DOI: 10.1038/ajg.2013.476]
- 5 **Salameh H**, Raff E, Erwin A, Seth D, Nischalke HD, Falletti E, Burza MA, Leathert J, Romeo S, Molinaro A, Corradini SG, Toniutto P, Spengler U, Daly A, Day CP, Kuo YF, Singal AK. *PNPLA3* Gene Polymorphism Is Associated With Predisposition to and Severity of Alcoholic Liver Disease. *Am J Gastroenterol* 2015; **110**: 846-856 [PMID: 25964223 DOI: 10.1038/ajg.2015.137]
- 6 **Sookoian S**, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (*PNPLA3*) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 1883-1894 [PMID: 21381068 DOI: 10.1002/hep.24283]
- 7 **Xu R**, Tao A, Zhang S, Deng Y, Chen G. Association between patatin-like phospholipase domain containing 3 gene (*PNPLA3*) polymorphisms and nonalcoholic fatty liver disease: a HuGE review and meta-analysis. *Sci Rep* 2015; **5**: 9284 [PMID: 25791171 DOI: 10.1038/srep09284]
- 8 **Zhang L**, You W, Zhang H, Peng R, Zhu Q, Yao A, Li X, Zhou Y, Wang X, Pu L, Wu J. *PNPLA3* polymorphisms (rs738409) and non-alcoholic fatty liver disease risk and related phenotypes: a meta-analysis. *J Gastroenterol Hepatol* 2015; **30**: 821-829 [PMID: 25641744 DOI: 10.1111/jgh.12889]
- 9 **Salameh H**, Al Hanayneh M, Masadeh M, Nasseemuddin M, Matin T, Erwin A, Singal A. *PNPLA3* as a Genetic Determinant of Risk for and Severity of Non-alcoholic Fatty Liver Disease Spectrum. *J Clin Trans Hepatol* 2016; **4**: 175-191 [PMID: 27777887 DOI: 10.14218/JCTH.2016.00009]
- 10 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]
- 11 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]
- 12 **Guyot E**, Sutton A, Rufat P, Laguillier C, Mansouri A, Moreau R, Ganne-Carrié N, Beaugrand M, Charnaux N, Trinchet JC, Nahon P. *PNPLA3* rs738409, hepatocellular carcinoma occurrence and risk model prediction in patients with cirrhosis. *J Hepatol* 2013; **58**: 312-318 [PMID: 23069476 DOI: 10.1016/j.jhep.2012.09.036]
- 13 **Nischalke HD**, Berger C, Luda C, Berg T, Müller T, Grünhage F, Lammert F, Coenen M, Krämer B, Körner C, Vidovic N, Oldenburg J, Nattermann J, Sauerbruch T, Spengler U. The *PNPLA3* rs738409 148M/M genotype is a risk factor for liver cancer in alcoholic cirrhosis but shows no or weak association in hepatitis C cirrhosis. *PLoS One* 2011; **6**: e27087 [PMID: 22087248 DOI: 10.1371/journal.pone.0027087]
- 14 **Petta S**, Vanni E, Bugianesi E, Rosso C, Cabibi D, Cammà C, Di Marco V, Eslam M, Grimaudo S, Macaluso FS, McLeod D, Pipitone RM, Abate ML, Smedile A, George J, Craxi A. *PNPLA3* rs738409 1748M is associated with steatohepatitis in 434 non-obese subjects with hepatitis C. *Aliment Pharmacol Ther* 2015; **41**: 939-948 [PMID: 25801076 DOI: 10.1111/apt.13169]
- 15 **Valenti L**, Aghemo A, Stättermayer AF, Maggioni P, De Nicola S, Motta BM, Rumi MG, Dongiovanni P, Ferenci P, Colombo M, Fargion S. Implications of *PNPLA3* polymorphism in chronic hepatitis C patients receiving peginterferon plus ribavirin. *Aliment Pharmacol Ther* 2012; **35**: 1434-1442 [PMID: 22530607 DOI: 10.1111/j.1365-2036.2012.05109.x]
- 16 **Stroup DF**, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008-2012 [PMID: 10789670]
- 17 **Wells GA**, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available from: URL: [http://www.ohri.ca/programs/clinical\\_epidemiology/oxford.asp](http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp)
- 18 **Ungaro R**, Bernstein CN, Gearry R, Hviid A, Kolho KL, Kronman MP, Shaw S, Van Kruiningen H, Colombel JF, Atreja A. Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 1728-1738 [PMID: 25223575 DOI: 10.1038/ajg.2014.246]
- 19 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188 [PMID: 3802833]
- 20 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]
- 21 **Deeks JJ**, Altman DG, Bradburn MJ. Statistical methods for examining heterogeneity and combining results from several studies in meta-analysis. In: Egger M DSG, Altman DG, eds, editor Systematic Reviews in Health Care: Meta-Analysis in Context. 2nd edition ed. London: BMJ Books, 2005: 285-312
- 22 **Begg CB**, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; **50**: 1088-1101 [PMID: 7786990 DOI: 10.2307/2533446]
- 23 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563 DOI: 10.1136/bmj.315.7109.629]
- 24 **Duval S**, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000; **56**: 455-463 [PMID: 10877304 DOI: 10.1111/j.0006-341X.2000.00455.x]
- 25 Trim and Fill Analysis for 'rma.uni' Objects. Available from: URL: [http://handbook.cochrane.org/chapter\\_10/10\\_4\\_4\\_2\\_trim\\_and\\_fill.htm](http://handbook.cochrane.org/chapter_10/10_4_4_2_trim_and_fill.htm)
- 26 **Hamza S**, Petit JM, Masson D, Jooste V, Binquet C, Sgro C, Guieu B, Bronowicki JP, Thieffin G, Di Martino V, Doffoel M, Barraud H, Richou C, Jouve JL, Raab JJ, Bouvier AM, Cottet V, Verges B, Minello A, Bonithon Kopp C, Hillon P. *PNPLA3* rs738409 GG homozygote status is associated with increased risk of hepatocellular carcinoma in cirrhotic patients. *J Hepatol* 2012; S281-S282 [DOI: 10.1016/S0168-8278(12)60725-9]

- 27 **Way M**, McQuillin A, Gurling HMD, Morgan MY. The PNPLA3 I148M mutation significantly increases the risk of developing alcohol-related cirrhosis in alcohol-dependent individuals. *J Hepatol* 2013; **58**: S563-S564 [DOI: 10.1016/S0168-8278(13)61403-8]
- 28 **Dutta AK**. Genetic factors affecting susceptibility to alcoholic liver disease in an Indian population. *Ann Hepatol* 2013; **12**: 901-907 [PMID: 24114820]
- 29 **Nguyen-Khac E**, Houchi H, Dreher M-L, Herpe Y-E, Naassila M. Is PNPLA3 polymorphism involved in severe acute alcoholic hepatitis. *Hepatology* 2011; 976A
- 30 **Bhatt SP**, Nigam P, Misra A, Guleria R, Pandey RM, Pasha MA. Genetic variation in the patatin-like phospholipase domain-containing protein-3 (PNPLA-3) gene in Asian Indians with nonalcoholic fatty liver disease. *Metab Syndr Relat Disord* 2013; **11**: 329-335 [PMID: 23734760 DOI: 10.1089/met.2012.0064]
- 31 **Burza MA**, Pirazzi C, Maglio C, Sjöholm K, Mancina RM, Svensson PA, Jacobson P, Adiels M, Baroni MG, Borén J, Ginanni Corradini S, Montalcini T, Sjöström L, Carlsson LM, Romeo S. PNPLA3 I148M (rs738409) genetic variant is associated with hepatocellular carcinoma in obese individuals. *Dig Liver Dis* 2012; **44**: 1037-1041 [PMID: 22704398 DOI: 10.1016/j.dld.2012.05.006]
- 32 **Kottrönen A**, Johansson LE, Johansson LM, Roos C, Westerbacka J, Hamsten A, Bergholm R, Arkkila P, Arola J, Kiviluoto T, Fisher RM, Ehrenborg E, Orho-Melander M, Ridderstråle M, Groop L, Yki-Järvinen H. A common variant in PNPLA3, which encodes adiponutrin, is associated with liver fat content in humans. *Diabetologia* 2009; **52**: 1056-1060 [PMID: 19224197 DOI: 10.1007/s00125-009-1285-z]
- 33 **Santoro N**, Kursawe R, D'Adamo E, Dykas DJ, Zhang CK, Bale AE, Calì AM, Narayan D, Shaw MM, Pierpont B, Savoye M, Lartaud D, Eldrich S, Cushman SW, Zhao H, Shulman GI, Caprio S. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is associated with fatty liver disease in obese children and adolescents. *Hepatology* 2010; **52**: 1281-1290 [PMID: 20803499 DOI: 10.1002/hep.23832]
- 34 **Zampino R**, Pisaturo MA, Cirillo G, Marrone A, Macera M, Rinaldi L, Stanzione M, Durante-Mangoni E, Gentile I, Sagnelli E, Signoriello G, Miraglia Del Giudice E, Adinolfi LE, Coppola N. Hepatocellular carcinoma in chronic HBV-HCV co-infection is correlated to fibrosis and disease duration. *Ann Hepatol* 2015; **14**: 75-82 [PMID: 25536644]
- 35 **Morse CG**, McLaughlin M, Matthews L, Proschan M, Thomas F, Gharib AM, Abu-Asab M, Orenstein A, Engle RE, Hu X, Lempicki R, Hadigan C, Kleiner DE, Heller T, Kovacs JA. Nonalcoholic Steatohepatitis and Hepatic Fibrosis in HIV-1-Monoinfected Adults With Elevated Aminotransferase Levels on Antiretroviral Therapy. *Clin Infect Dis* 2015; **60**: 1569-1578 [PMID: 25681381 DOI: 10.1093/cid/civ101]
- 36 **Mandorfer M**, Payer BA, Schwabl P, Steiner S, Ferlitsch A, Aichelburg MC, Stättermayer AF, Ferenci P, Obermayer-Pietsch B, Grabmeier-Pfistershammer K, Trauner M, Peck-Radosavljevic M, Reiberger T. Revisiting liver disease progression in HIV/HCV-coinfected patients: the influence of vitamin D, insulin resistance, immune status, IL28B and PNPLA3. *Liver Int* 2015; **35**: 876-885 [PMID: 24905495 DOI: 10.1111/liv.12615]
- 37 **Huang CF**, Dai CY, Yeh ML, Huang CI, Tai CM, Hsieh MH, Liang PC, Lin YH, Hsieh MY, Yang HL, Huang JF, Lin ZY, Chen SC, Yu ML, Chuang WL. Association of diabetes and PNPLA3 genetic variants with disease severity of patients with chronic hepatitis C virus infection. *J Hepatol* 2015; **62**: 512-518 [PMID: 25457210 DOI: 10.1016/j.jhep.2014.10.011]
- 38 **Wong GL**, Chan HL, Tse CH, Chan PO, Cheng JC, Cheng JS, Lau SH, Lee EK, Ma JM, Chan AW, Choi PC, Wong VW. Impact of IL28B and PNPLA3 polymorphisms on treatment outcomes in patients infected with genotype 6 hepatitis C virus. *J Gastroenterol Hepatol* 2015; **30**: 1040-1048 [PMID: 25639146 DOI: 10.1111/jgh.12890]
- 39 **Cai T**, Dufour JF, Muellhaupt B, Gerlach T, Heim M, Moradpour D, Cerny A, Malinverni R, Kaddai V, Bochud M, Negro F, Bochud PY. Viral genotype-specific role of PNPLA3, PPARG, MTP, and IL28B in hepatitis C virus-associated steatosis. *J Hepatol* 2011; **55**: 529-535 [PMID: 21236304 DOI: 10.1016/j.jhep.2010.12.020]
- 40 **Valenti L**, Rumi M, Galmozzi E, Aghemo A, Del Menico B, De Nicola S, Dongiovanni P, Maggioni M, Fracanzani AL, Rametta R, Colombo M, Fargion S. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. *Hepatology* 2011; **53**: 791-799 [PMID: 21319195 DOI: 10.1002/hep.24123]
- 41 **Trépo E**, Pradat P, Potthoff A, Momozawa Y, Quertinmont E, Gustot T, Lemmers A, Berthillon P, Amininejad L, Chevallier M, Schlué J, Kreipe H, Devière J, Manns M, Trépo C, Sninsky J, Wedemeyer H, Franchimont D, Moreno C. Impact of patatin-like phospholipase-3 (rs738409 C& gt; G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. *Hepatology* 2011; **54**: 60-69 [PMID: 21488075 DOI: 10.1002/hep.24350]
- 42 **Corradini SG**, Burza MA, Molinaro A, Romeo S. Patatin-like phospholipase domain containing 3 sequence variant and hepatocellular carcinoma. *Hepatology* 2011; **53**: 1776; author reply 1777 [PMID: 21351112 DOI: 10.1002/hep.24244]
- 43 **Valenti L**, Aghemo A, Stättermayer AF. Interaction between IL28B and PNPLA3 genotypes in the pathogenesis of steatosis in chronic hepatitis C non genotype-3 patients. *J Hepatol* 2012; **56**: 1209-1210; author reply 1210-1212 [PMID: 22230871 DOI: 10.1016/j.jhep.2011.10.024]
- 44 **Ezzikouri S**, Alaoui R, Tazi S, Nadir S, Elmdaghri N, Pineau P, Benjelloun S. The adiponutrin I148M variant is a risk factor for HCV-associated liver cancer in North-African patients. *Infect Genet Evol* 2014; **21**: 179-183 [PMID: 24269995 DOI: 10.1016/j.meegid.2013.11.005]
- 45 **Stättermayer AF**, Rutter K, Beinhardt S, Wrba F, Scherzer TM, Strasser M, Hofer H, Steindl-Munda P, Trauner M, Ferenci P. Role of FDF1T polymorphism for fibrosis progression in patients with chronic hepatitis C. *Liver Int* 2014; **34**: 388-395 [PMID: 23870067 DOI: 10.1111/liv.12269]
- 46 **Ampuero J**, Del Campo JA, Rojas L, García-Lozano JR, Solá R, Andrade R, Pons JA, Navarro JM, Calleja JL, Buti M, González-Escribano MF, Forns X, Diago M, García-Samaniego J, Romero-Gómez M. PNPLA3 rs738409 causes steatosis according to viral & amp; IL28B genotypes in hepatitis C. *Ann Hepatol* 2014; **13**: 356-363 [PMID: 24927606]
- 47 **Sato M**, Kato N, Tateishi R, Muroyama R, Kowatari N, Li W, Goto K, Otsuka M, Shiina S, Yoshida H, Omata M, Koike K. Impact of PNPLA3 polymorphisms on the development of hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Hepatol Res* 2014; **44**: E137-E144 [PMID: 24125181 DOI: 10.1111/hepr.12258]
- 48 **Yasui K**, Kawaguchi T, Shima T, Mitsuyoshi H, Seki K, Sendo R, Mizuno M, Itoh Y, Matsuda F, Okanoue T. Effect of PNPLA3 rs738409 variant (I148 M) on hepatic steatosis, necroinflammation, and fibrosis in Japanese patients with chronic hepatitis C. *J Gastroenterol* 2015; **50**: 887-893 [PMID: 25543233 DOI: 10.1007/s00535-014-1018-z]
- 49 **Nakaoka K**, Hashimoto S, Kawabe N, Nitta Y, Murao M, Nakano T, Shimazaki H, Kan T, Takagawa Y, Ohki M, Kurashita T, Takamura T, Nishikawa T, Ichino N, Osakabe K, Yoshioka K. PNPLA3 I148M associations with liver carcinogenesis in Japanese chronic hepatitis C patients. *Springerplus* 2015; **4**: 83 [PMID: 25713769 DOI: 10.1186/s40064-015-0870-5]
- 50 **Tamaki N**, Kurosaki M, Higuchi M, Takada H, Nakakuki N, Yasui Y, Suzuki S, Tsuchiya K, Nakanishi H, Itakura J, Takahashi Y, Ogawa S, Tanaka Y, Asahina Y, Izumi N. Genetic Polymorphisms of IL28B and PNPLA3 Are Predictive for HCV Related Rapid Fibrosis Progression and Identify Patients Who Require Urgent Antiviral Treatment with New Regimens. *PLoS One* 2015; **10**: e0137351 [PMID: 26352693 DOI: 10.1371/journal.pone.0137351]
- 51 **Huang CF**, Chen JJ, Yeh ML, Huang CI, Hsieh MY, Yang HL, Dai CY, Huang JF, Lin ZY, Chen SC, Chuang WL, Chen YL, Yu ML. PNPLA3 genetic variants determine hepatic steatosis in non-obese chronic hepatitis C patients. *Sci Rep* 2015; **5**: 11901 [PMID: 26139292 DOI: 10.1038/srep11901]



- 52 **Petta S**, Maida M, Grimaudo S, Pipitone RM, Macaluso FS, Cabibi D, Cammà C, Di Marco V, Sferrazza S, Craxi A. TM6SF2 rs58542926 is not associated with steatosis and fibrosis in large cohort of patients with genotype 1 chronic hepatitis C. *Liver Int* 2016; **36**: 198-204 [PMID: 26259026 DOI: 10.1111/liv.12918]
- 53 **Ali M**, Yopp A, Gopal P, Beg MS, Zhu H, Lee W, Singal AG. A Variant in PNPLA3 Associated With Fibrosis Progression but not Hepatocellular Carcinoma in Patients With Hepatitis C Virus Infection. *Clin Gastroenterol Hepatol* 2016; **14**: 295-300 [PMID: 26305067 DOI: 10.1016/j.cgh.2015.08.018]
- 54 **He S**, McPhaul C, Li JZ, Garuti R, Kinch L, Grishin NV, Cohen JC, Hobbs HH. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J Biol Chem* 2010; **285**: 6706-6715 [PMID: 20034933 DOI: 10.1074/jbc.M109.064501]
- 55 **Thornton A**, Lee P. Publication bias in meta-analysis: its causes and consequences. *J Clin Epidemiol* 2000; **53**: 207-216 [PMID: 10729693 DOI: 10.1016/S0895-4356(99)00161-4]

**P- Reviewer:** Kohla MAS, Pekgoz M, Yang SS **S- Editor:** Qi Y  
**L- Editor:** A **E- Editor:** Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



# World Journal of *Hepatology*

*World J Hepatol* 2016 December 28; 8(36): 1593-1644





## Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

### EDITORS-IN-CHIEF

Clara Balsano, *Rome*  
Wan-Long Chuang, *Kaohsiung*

### ASSOCIATE EDITOR

Thomas Bock, *Berlin*  
Silvia Fargion, *Milan*  
Ze-Guang Han, *Shanghai*  
Lionel Hebbard, *Westmead*  
Pietro Invernizzi, *Rozzano*  
Valerio Nobili, *Rome*  
Alessandro Vitale, *Padova*

### GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*  
Tai-An Chiang, *Tainan*  
Chi-Tan Hu, *Hualien*  
Sen-Yung Hsieh, *Taoyuan*  
Wenya Huang, *Tainan*  
Liang-Yi Hung, *Tainan*  
Jih RU Hwu, *Hsinchu*  
Jing-Yi Lee, *Taipei*  
Mei-Hsuan Lee, *Taipei*  
Chih-Wen Lin, *Kaohsiung*  
Chun-Che Lin, *Taichung*  
Wan-Yu Lin, *Taichung*  
Tai-Long Pan, *Tao-Yuan*  
Suh-Ching Yang, *Taipei*  
Chun-Yan Yeung, *Taipei*

### MEMBERS OF THE EDITORIAL BOARD



**Algeria**

Samir Rouabhia, *Batna*



**Argentina**

Fernando O Bessone, *Rosario*  
Maria C Carrillo, *Rosario*  
Melisa M Dirchwolf, *Buenos Aires*  
Bernardo Frider, *Buenos Aires*  
Jorge Quarleri, *Buenos Aires*  
Adriana M Torres, *Rosario*



**Armenia**

Narina Sargsyants, *Yerevan*



**Australia**

Mark D Gorrell, *Sydney*



**Austria**

Harald Hofer, *Vienna*  
Gustav Paumgartner, *Vienna*  
Matthias Pinter, *Vienna*  
Thomas Reiberger, *Vienna*



**Bangladesh**

Shahinul Alam, *Dhaka*  
Mamun Al Mahtab, *Dhaka*



**Belgium**

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*  
Luisa Vonghia, *Antwerp*



**Botswana**

Francesca Cainelli, *Gaborone*  
Sandro Vento, *Gaborone*



**Brazil**

Edson Abdala, *Sao Paulo*  
Ilka FSF Boin, *Campinas*  
Niels OS Camara, *Sao Paulo*  
Ana Carolina FN Cardoso, *Rio de Janeiro*  
Roberto J Carvalho-Filho, *Sao Paulo*  
Julio CU Coelho, *Curitiba*  
Flavio Henrique Ferreira Galvao, *Sao Paulo*  
Janaina L Narciso-Schiavon, *Florianopolis*  
Sílvia HC Sales-Peres, *Bauru*  
Leonardo L Schiavon, *Florianópolis*  
Luciana D Silva, *Belo Horizonte*  
Vanessa Souza-Mello, *Rio de Janeiro*  
Jaques Waisberg, *Santo André*



**Bulgaria**

Mariana P Penkova-Radicheva, *Stara Zagora*  
Marieta Simonova, *Sofia*



**Canada**

Runjan Chetty, *Toronto*  
Michele Molinari, *Halifax*  
Giada Sebastiani, *Montreal*



**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*  
 En-Qiang Chen, *Chengdu*  
 Gong-Ying Chen, *Hangzhou*  
 Jin-lian Chen, *Shanghai*  
 Jun Chen, *Changsha*  
 Alfred Cheng, *Hong Kong*  
 Chun-Ping Cui, *Beijing*  
 Shuang-Suo Dang, *Xi'an*  
 Ming-Xing Ding, *Jinhua*  
 Zhi-Jun Duang, *Dalian*  
 He-Bin Fan, *Wuhan*  
 Xiao-Ming Fan, *Shanghai*  
 James Yan Yue Fung, *Hong Kong*  
 Yi Gao, *Guangzhou*  
 Zuo-Jiong Gong, *Wuhan*  
 Zhi-Yong Guo, *Guangzhou*  
 Shao-Liang Han, *Wenzhou*  
 Tao Han, *Tianjin*  
 Jin-Yang He, *Guangzhou*  
 Ming-Liang He, *Hong Kong*  
 Can-Hua Huang, *Chengdu*  
 Bo Jin, *Beijing*  
 Shan Jin, *Hohhot*  
 Hui-Qing Jiang, *Shijiazhuang*  
 Wan-Yee Joseph Lau, *Hong Kong*  
 Guo-Lin Li, *Changsha*  
 Jin-Jun Li, *Shanghai*  
 Qiang Li, *Jinan*  
 Sheng Li, *Jinan*  
 Zong-Fang Li, *Xi'an*  
 Xu Li, *Guangzhou*  
 Xue-Song Liang, *Shanghai*  
 En-Qi Liu, *Xi'an*  
 Pei Liu, *Shenyang*  
 Zhong-Hui Liu, *Changchun*  
 Guang-Hua Luo, *Changzhou*  
 Yi Lv, *Xi'an*  
 Guang-Dong Pan, *Liuzhou*  
 Wen-Sheng Pan, *Hangzhou*  
 Jian-Min Qin, *Shanghai*  
 Wai-Kay Seto, *Hong Kong*  
 Hong Shen, *Changsha*  
 Xiao Su, *Shanghai*  
 Li-Ping Sun, *Beijing*  
 Wei-Hao Sun, *Nanjing*  
 Xue-Ying Sun, *Harbin*  
 Hua Tang, *Tianjin*  
 Ling Tian, *Shanghai*  
 Eric Tse, *Hong Kong*  
 Guo-Ying Wang, *Changzhou*  
 Yue Wang, *Beijing*  
 Shu-Qiang Wang, *Chengdu*  
 Mary MY Wayne, *Hong Kong*  
 Hong-Shan Wei, *Beijing*  
 Danny Ka-Ho Wong, *Hong Kong*  
 Grace Lai-Hung Wong, *Hong Kong*  
 Bang-Fu Wu, *Dongguan*  
 Xiong-Zhi Wu, *Tianjin*  
 Chun-Fang Xu, *Suzhou*  
 Rui-An Xu, *Quanzhou*  
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*  
 Shi-Ying Xuan, *Qingdao*  
 Ming-Xian Yan, *Jinan*  
 Lv-Nan Yan, *Chengdu*  
 Jin Yang, *Hangzhou*  
 Ji-Hong Yao, *Dalian*  
 Winnie Yeo, *Hong Kong*  
 Zheng Zeng, *Beijing*  
 Qi Zhang, *Hangzhou*  
 Shi-Jun Zhang, *Guangzhou*  
 Xiao-Lan Zhang, *Shijiazhuang*  
 Xiao-Yong Zhang, *Guangzhou*  
 Yong Zhang, *Xi'an*  
 Hong-Chuan Zhao, *Hefei*  
 Ming-Hua Zheng, *Wenzhou*  
 Yu-Bao Zheng, *Guangzhou*  
 Ren-Qian Zhong, *Shanghai*  
 Fan Zhu, *Wuhan*  
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vysloulzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*  
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*  
 NGB G Bader EL Din, *Cairo*  
 Hatem Elalfy, *Mansoura*  
 Mahmoud M El-Bendary, *Mansoura*  
 Mona El SH El-Raziky, *Cairo*  
 Mohammad El-Sayed, *Cairo*  
 Yasser M Fouad, *Minia*  
 Mohamed AA Metwally, *Benha*  
 Hany Shehab, *Cairo*  
 Mostafa M Sira, *Shebin El-koom*  
 Ashraf Taye, *Minia*  
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*  
 Sophie Conchon, *Nantes*  
 Daniel J Felmlee, *Strasbourg*  
 Herve Lerat, *Creteil*  
 Dominique Salmon, *Paris*  
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*  
 Enrico N De Toni, *Munich*  
 Oliver Ebert, *Muenchen*  
 Rolf Gebhardt, *Leipzig*  
 Janine V Hartl, *Regensburg*  
 Sebastian Hinz, *Kiel*  
 Benjamin Juntermanns, *Essen*  
 Roland Kaufmann, *Jena*  
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*  
 Benjamin Maasoumy, *Hannover*  
 Jochen Mattner, *Erlangen*  
 Nadja M Meindl-Beinker, *Mannheim*  
 Ulf P Neumann, *Aachen*  
 Margarete Odenthal, *Cologne*  
 Yoshiaki Sunami, *Munich*  
 Christoph Roderburg, *Aachen*  
 Frank Tacke, *Aachen*  
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*  
 George N Dalekos, *Larissa*  
 Ioanna K Delladetsima, *Athens*  
 Nikolaos K Gatselis, *Larissa*  
 Stavros Gourgiotis, *Athens*  
 Christos G Savopoulos, *Thessaloniki*  
 Tania Siahaidou, *Athens*  
 Emmanouil Sinakos, *Thessaloniki*  
 Nikolaos G Symeonidi, *Thessaloniki*  
 Konstantinos C Thomopoulos, *Larissa*  
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*  
 Peter L Lakatos, *Budapest*  
 Maria Papp, *Debrecen*  
 Ferenc Sipos, *Budapest*  
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*  
 Girish M Bhopale, *Pune*  
 Sibnarayan Datta, *Tezpur*  
 Nutan D Desai, *Mumbai*  
 Sorabh Kapoor, *Mumbai*  
 Jaswinder S Maras, *New Delhi*  
 Nabeen C Nayak, *New Delhi*  
 C Ganesh Pai, *Manipal*  
 Amit Pal, *Chandigarh*  
 K Rajeshwari, *New Delhi*  
 Anup Ramachandran, *Vellore*  
 D Nageshwar Reddy, *Hyderabad*  
 Shivaram P Singh, *Cuttack*  
 Ajith TA, *Thrissur*  
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*  
 Cosmas RA Lesmana, *Jakarta*  
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*  
 Sedigheh Kafi-Abad, *Tehran*  
 Iradj Maleki, *Sari*  
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*  
 Alfonso W Avolio, *Rome*  
 Francesco Bellanti, *Foggia*  
 Marcello Bianchini, *Modena*  
 Guglielmo Borgia, *Naples*  
 Mauro Borzio, *Milano*  
 Enrico Brunetti, *Pavia*  
 Valeria Cento, *Roma*  
 Beatrice Conti, *Rome*  
 Francesco D'Amico, *Padova*  
 Samuele De Minicis, *Fermo*  
 Fabrizio De Ponti, *Bologna*  
 Giovan Giuseppe Di Costanzo, *Napoli*  
 Luca Fabris, *Padova*  
 Giovanna Ferraioli, *Pavia*  
 Matteo Garcovich, *Rome*  
 Edoardo G Giannini, *Genova*  
 Rossano Girometti, *Udine*  
 Alessandro Granito, *Bologna*  
 Alberto Grassi, *Rimini*  
 Alessandro Grasso, *Savona*  
 Francesca Guerrieri, *Rome*  
 Quirino Lai, *Aquila*  
 Andrea Lisotti, *Bologna*  
 Marcello F Maida, *Palermo*  
 Lucia Malaguarnera, *Catania*  
 Andrea Mancuso, *Palermo*  
 Luca Maroni, *Ancona*  
 Francesco Marotta, *Milano*  
 Pierluigi Marzuillo, *Naples*  
 Sara Montagnese, *Padova*  
 Giuseppe Nigri, *Rome*  
 Claudia Piccoli, *Foggia*  
 Camillo Porta, *Pavia*  
 Chiara Raggi, *Rozzano (MI)*  
 Maria Rendina, *Bari*  
 Maria Ripoli, *San Giovanni Rotondo*  
 Kryssia I Rodriguez-Castro, *Padua*  
 Raffaella Romeo, *Milan*  
 Amedeo Sciarra, *Milano*  
 Antonio Solinas, *Sassari*  
 Aurelio Sonzogni, *Bergamo*  
 Giovanni Squadrito, *Messina*  
 Salvatore Sutti, *Novara*  
 Valentina Svicher, *Rome*  
 Luca Toti, *Rome*  
 Elvira Verduci, *Milan*  
 Umberto Vespasiani-Gentilucci, *Rome*  
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*  
 Nabil AS Eid, *Takatsuki*  
 Kenichi Ikejima, *Tokyo*  
 Shoji Ikuo, *Kobe*  
 Yoshihiro Ikura, *Takatsuki*  
 Shinichi Ikuta, *Nishinomiya*  
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*  
 Takanobu Kato, *Tokyo*  
 Saiho Ko, *Nara*  
 Haruki Komatsu, *Sakura*  
 Masanori Matsuda, *Chuo-city*  
 Yasunobu Matsuda, *Niigata*  
 Yoshifumi Nakayama, *Kitakyushu*  
 Taichiro Nishikawa, *Kyoto*  
 Satoshi Oeda, *Saga*  
 Kenji Okumura, *Urayasu*  
 Michitaka Ozaki, *Sapporo*  
 Takahiro Sato, *Sapporo*  
 Junichi Shindoh, *Tokyo*  
 Ryo Sudo, *Yokohama*  
 Atsushi Suetsugu, *Gifu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Reiji Sugita, *Sendai*  
 Koichi Takaguchi, *Takamatsu*  
 Shinji Takai, *Takatsuki*  
 Akinobu Takaki, *Okayama*  
 Yasuhiro Tanaka, *Nagoya*  
 Takuji Tanaka, *Gifu City*  
 Atsunori Tsuchiya, *Niigata*  
 Koichi Watashi, *Tokyo*  
 Hiroshi Yagi, *Tokyo*  
 Taro Yamashita, *Kanazawa*  
 Shuhei Yoshida, *Chiba*  
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*  
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*  
 María de F Higuera-de la Tijera, *Mexico City*  
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*  
 Frank G Schaap, *Maastricht*  
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*  
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*  
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*  
 Liana Gheorghe, *Bucharest*  
 George S Gherlan, *Bucharest*  
 Romeo G Mihaila, *Sibiu*  
 Bogdan Procopet, *Cluj-Napoca*  
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*  
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*  
 Ihab MH Mahmoud, *Riyadh*  
 Ibrahim Masoodi, *Riyadh*  
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*  
 Jeong Heo, *Busan*  
 Dae-Won Jun, *Seoul*  
 Bum-Joon Kim, *Seoul*  
 Do Young Kim, *Seoul*  
 Ji Won Kim, *Seoul*  
 Moon Young Kim, *Wonu*  
 Mi-Kyung Lee, *Suncheon*  
 Kwan-Kyu Park, *Daegu*  
 Young Nyun Park, *Seoul*  
 Jae-Hong Ryoo, *Seoul*  
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*  
 Javier Ampuero, *Sevilla*  
 Jaime Arias, *Madrid*  
 Andres Cardenas, *Barcelona*  
 Agustin Castiella, *Mendaro*  
 Israel Fernandez-Pineda, *Sevilla*  
 Rocio Gallego-Duran, *Sevilla*  
 Rita Garcia-Martinez, *Barcelona*  
 José M González-Navajas, *Alicante*  
 Juan C Laguna, *Barcelona*  
 Elba Llop, *Madrid*  
 Laura Ochoa-Callejero, *La Rioja*  
 Albert Pares, *Barcelona*  
 Sonia Ramos, *Madrid*  
 Francisco Rodriguez-Frias, *Córdoba*  
 Manuel L Rodriguez-Peralvarez, *Córdoba*  
 Marta R Romero, *Salamanca*  
 Carlos J Romero, *Madrid*  
 Maria Trapero-Marugan, *Madrid*



#### **Sri Lanka**

Niranga M Devanarayana, *Ragama*



#### **Sudan**

Hatim MY Mudawi, *Khartoum*



#### **Sweden**

Evangelos Kalaitzakis, *Lund*



#### **Switzerland**

Christoph A Maurer, *Liestal*



#### **Thailand**

Taned Chitapanarux, *Chiang mai*  
 Temduang Limpai boon, *Khon Kaen*  
 Sith Phongkitkarun, *Bangkok*  
 Yong Poovorawan, *Bangkok*



#### **Turkey**

Osman Abbasoglu, *Ankara*  
 Mesut Akarsu, *Izmir*  
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*  
 Yasemin H Balaban, *Istanbul*  
 Bulent Baran, *Van*  
 Mehmet Celikbilek, *Yozgat*  
 Levent Doganay, *Istanbul*  
 Fatih Eren, *Istanbul*  
 Abdurrahman Kadayifci, *Gaziantep*  
 Ahmet Karaman, *Kayseri*  
 Muhsin Kaya, *Diyarbakir*  
 Ozgur Kemik, *Van*  
 Serdar Moralioglu, *Uskudar*  
 A Melih Ozel, *Gebze - Kocaeli*  
 Seren Ozenirler, *Ankara*  
 Ali Sazci, *Kocaeli*  
 Goktug Sirin, *Kocaeli*  
 Mustafa Sunbul, *Samsun*  
 Nazan Tuna, *Sakarya*  
 Ozlem Yonem, *Sivas*



#### **Ukraine**

Rostyslav V Bubnov, *Kyiv*  
 Nazarii K Kobylak, *Kyiv*  
 Igor N Skrypnyk, *Poltava*



#### **United Kingdom**

Safa Al-Shamma, *Bournemouth*  
 Jayantha Arnold, *Southall*  
 Marco Carbone, *Cambridge*  
 Rajeev Desai, *Birmingham*  
 Ashwin Dhanda, *Bristol*  
 Matthew Hoare, *Cambridge*  
 Stefan G Hubscher, *Birmingham*  
 Nikolaos Karidis, *London*  
 Lemonica J Koumbi, *London*  
 Patricia Lalor, *Birmingham*  
 Ji-Liang Li, *Oxford*  
 Evaggelia Liaskou, *Birmingham*  
 Rodrigo Liberal, *London*  
 Wei-Yu Lu, *Edinburgh*  
 Richie G Madden, *Truro*  
 Christian P Selinger, *Leeds*  
 Esther Una Cidon, *Bournemouth*  
 Feng Wu, *Oxford*



#### **United States**

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*  
 Mohammed Sawkat Anwer, *North Grafton*  
 Kalyan Ram Bhamidimarri, *Miami*  
 Brian B Borg, *Jackson*  
 Ronald W Busuttil, *Los Angeles*  
 Andres F Carrion, *Miami*  
 Saurabh Chatterjee, *Columbia*  
 Disaya Chavalitdhamrong, *Gainesville*  
 Mark J Czaja, *Bronx*  
 Jonathan M Fenkel, *Philadelphia*  
 Catherine Frenette, *La Jolla*  
 Lorenzo Gallon, *Chicago*  
 Kalpana Ghoshal, *Columbus*  
 Hie-Won L Hann, *Philadelphia*  
 Shuang-Teng He, *Kansas City*  
 Wendong Huang, *Duarte*  
 Rachel Hudacko, *Suffern*  
 Lu-Yu Hwang, *Houston*  
 Ijaz S Jamall, *Sacramento*  
 Neil L Julie, *Bethesda*  
 Hetal Karsan, *Atlanta*  
 Ahmed O Kaseb, *Houston*  
 Zeid Kayali, *Pasadena*  
 Timothy R Koch, *Washington*  
 Gursimran S Kochhar, *Cleveland*  
 Steven J Kovacs, *East Hanover*  
 Mary C Kuhns, *Abbott Park*  
 Jiang Liu, *Silver Spring*  
 Li Ma, *Stanford*  
 Francisco Igor Macedo, *Southfield*  
 Sandeep Mukherjee, *Omaha*  
 Natalia A Osna, *Omaha*  
 Jen-Jung Pan, *Houston*  
 Christine Pocha, *Minneapolis*  
 Yury Popov, *Boston*  
 Davide Povero, *La Jolla*  
 Phillip Ruiz, *Miami*  
 Takao Sakai, *Cleveland*  
 Nicola Santoro, *New Haven*  
 Eva Schmelzer, *Pittsburgh*  
 Zhongjie Shi, *Philadelphia*  
 Nathan J Shores, *New Orleans*  
 Siddharth Singh, *Rochester*  
 Shailendra Singh, *Pittsburgh*  
 Veysel Tahan, *Iowa City*  
 Mehlika Toy, *Boston*  
 Hani M Wadei, *Jacksonville*  
 Gulam Waris, *North Chicago*  
 Ruliang Xu, *New York*  
 Jun Xu, *Los Angeles*  
 Matthew M Yeh, *Seattle*  
 Xuchen Zhang, *West Haven*  
 Lixin Zhu, *Buffalo*  
 Sasa Zivkovic, *Pittsburgh*



## Contents

Three issues per month Volume 8 Number 36 December 28, 2016

### REVIEW

- 1593 Biliary atresia: Where do we stand now?

*Govindarajan KK*

### MINIREVIEWS

- 1602 Restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension

*Garbuzenko DV, Arefyev NO, Belov DV*

### ORIGINAL ARTICLE

#### Basic Study

- 1610 Fractionation of gamma-glutamyltransferase in patients with nonalcoholic fatty liver disease and alcoholic liver disease

*Sueyoshi S, Sawai S, Satoh M, Seimiya M, Sogawa K, Fukumura A, Tsutsumi M, Nomura F*

#### Retrospective Cohort Study

- 1617 Spontaneous bacterial peritonitis prevalence in pre-transplant patients and its effect on survival and graft loss post-transplant

*Shah NL, Intagliata NM, Henry ZH, Argo CK, Northup PG*

#### Observational Study

- 1623 Prevalence of significant liver disease in human immunodeficiency virus-infected patients exposed to Didanosine: A cross sectional study

*Logan S, Rodger A, Maynard-Smith L, O'Beirne J, Fernandez T, Ferro F, Smith C, Bhagani S*

- 1629 Enzyme pattern of biliary colic: A counterintuitive picture

*Resnick E, Shteingart S, Melamud B, Bdolah-Abram T, Zalut T, Reuben A, Lurie Y*

### SYSTEMATIC REVIEWS

- 1637 Isolated bilateral Tapia's syndrome after liver transplantation: A case report and review of the literature

*Bilbao I, Dopazo C, Caralt M, Castells L, Pando E, Gantxegi A, Charco R*



## Contents

*World Journal of Hepatology*  
Volume 8 Number 36 December 28, 2016

### ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Mohammed Joutei Hassani Tahiri, MD, PhD, Associate Professor, Department of Gastroenterology and Hepatology, Ibn Rochd University Hospital Centre Casablanca, Casablanca 20170, Morocco

### AIM AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

### INDEXING/ABSTRACTING

*World Journal of Hepatology* is now indexed in PubMed, PubMed Central, and Scopus.

### FLYLEAF

#### I-IV Editorial Board

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Dan Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL  
*World Journal of Hepatology*

ISSN  
ISSN 1948-5182 (online)

LAUNCH DATE  
October 31, 2009

FREQUENCY  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

EDITORS-IN-CHIEF  
**Clara Balsano, PhD, Professor**, Department of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL BOARD MEMBERS  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

EDITORIAL OFFICE  
Xiu-Xia Song, Director  
Fang-Fang Ji, Vice Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLISHER  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

PUBLICATION DATE  
December 28, 2016

COPYRIGHT  
© 2016 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS  
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION  
<http://www.wjgnet.com/esps/>

## Biliary atresia: Where do we stand now?

Krishna Kumar Govindarajan

Krishna Kumar Govindarajan, Department of Pediatric Surgery, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry 605006, India

**Author contributions:** The author solely contributed to this manuscript.

**Conflict-of-interest statement:** None.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Krishna Kumar Govindarajan, Department of Pediatric Surgery, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry Road, Puducherry 605006, India. [sasisang@rediffmail.com](mailto:sasisang@rediffmail.com)  
 Telephone: +91-413-2297328  
 Fax: +91-413-2297325

Received: April 3, 2016  
 Peer-review started: April 7, 2016  
 First decision: June 7, 2016  
 Revised: September 9, 2016  
 Accepted: November 1, 2016  
 Article in press: November 2, 2016  
 Published online: December 28, 2016

### Abstract

The pathway from clinical suspicion to establishing the diagnosis of biliary atresia in a child with jaundice is a daunting task. However, investigations available help to point towards the correct diagnosis in reasonable time frame. Imaging by Sonography has identified several parameters which can be of utility in the diagnostic

work up. Comparison of Sonography with imaging by Nuclear medicine can bring out the significant differences and also help in appropriate imaging. The battery of Biochemical tests, available currently, enable better understanding of the line-up of investigations in a given child with neonatal cholestasis. Management protocols enable standardized care with optimal outcome. The place of surgical management in biliary atresia is undisputed, although Kasai procedure and primary liver transplantation have been pitted against each other. This article functions as a platform to bring forth the various dimensions of biliary atresia.

**Key words:** Biliary atresia; Neonatal cholestasis; Kasai procedure; Neonatal jaundice; Hyperbilirubinemia

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The etiology of biliary atresia is intriguing with a myriad of diagnostics available to work up a child with neonatal jaundice. This article attempts to review the pathogenesis, evaluation, management and outcome for current update of biliary atresia.

Govindarajan KK. Biliary atresia: Where do we stand now? *World J Hepatol* 2016; 8(36): 1593-1601 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i36/1593.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i36.1593>

### INTRODUCTION

Biliary atresia is the commonest surgical cause for neonatal cholestasis, although the diagnosis is one of exclusion of the various causes of neonatal cholestasis which require non-surgical management. Incidence of neonatal cholestasis is noted to be 1 in 2500 newborn children<sup>[1]</sup>. Among the group of children with neonatal cholestasis, about 34%-42% have been noted to have Biliary atresia<sup>[2,3]</sup>. The actual incidence is around 1 in

8000-18000 live births<sup>[4]</sup>. The etio-pathogenesis is not entirely convincing to point towards a particular offending agent, inspite of several studies describing the causal association of infective or autoimmune origin. The investigations for the establishment of the diagnosis are elaborate, and require extensive workup. As timely surgical intervention is essential, an appropriate and prompt work up is required.

## ETIOLOGY

For the purpose of better understanding, biliary atresia is categorized into 2 forms, the perinatal or the acquired form and the embryonic or the congenital form. The embryonic form is the less common variant of the two (20%), with a link to syndromic association such as Biliary atresia Splenic Malformation (BASM - polysplenia, intestinal malrotation, preduodenal portal vein, absent inferior vena cava, aberrant hepatic artery, abdominal heterotaxia), known to be due to gene mutations controlling the bile duct development. The commoner perinatal form (80%) is supposed to be the end result of viral trigger and complex interactions between innate and adaptive immune responses<sup>[5]</sup>.

The complete deletion of inversin gene in mice was shown to produce laterality defects in the abdominal organs along with malformations of the hepatobiliary system, similar to that of the fetal form<sup>[6]</sup>. However, the role of the inversin gene in humans is unlikely in the fetal form of Biliary atresia, as established by Schön *et al*<sup>[7]</sup>.

Several viral agents such as human papilloma virus, cytomegalo virus, respiratory syncytial virus, reovirus, rotavirus<sup>[8-12]</sup>, epstein barr virus, herpes virus, hepatitis B virus<sup>[13-15]</sup> have been implicated in the past, but none have been consistently and convincingly shown to be associated with the pathogenesis of Biliary atresia in humans.

The cystic biliary atresia is believed to be an exclusive subtype, based on the following observations: (1) jaundice noticed at birth; (2) diagnosed antenatally by the identification of a cystic lesion at the porta on sonography; and (3) intra-operatively a cystic lesion seen, not communicating with intrahepatic ductal system or duodenum.

Reports on cystic type biliary atresia declare that the entity has a better outcome<sup>[16]</sup>.

## PATHOGENESIS

To explain the pathogenesis of Biliary atresia, the concept of an initial viral infection damaging the biliary duct, followed by exaggerated autoimmune directed inflammation of biliary ducts and secondary biliary cirrhosis as a result of progressive ductal injury and obstruction has been mooted<sup>[17,18]</sup>.

Interestingly, studies have noted the ability of Rota virus to target cholangiocytes and cause tissue specific inflammation and pathogenic effects in mouse models. The theory of viral mediated damage and progressive

obliterative inflammation of bile ductules has been put forward, on the basis of this murine model. The virus is found to be tropic to cholangiocytes, leaving behind gamma interferon producing CD4 and 8 lymphocytes which target the hepatobiliary system, culminating in fibrosis of the injured ductal elements, bearing the striking resemblance to Biliary atresia<sup>[19]</sup>.

Furthermore, it was concluded that the gamma interferon triggered the inflammatory changes responsible for progressive bile duct obstruction and obliteration<sup>[19]</sup>. It is believed that DNA hypomethylation changes in CD4 lymphocytes leads onto uncontrolled gamma interferon expression<sup>[20]</sup>. Gamma interferon through release from T lymphocytes, has been projected as the pivotal player, orchestrating the sequence of events, specifically the later occurrence of intraductal inflammation, ductal fibrosis and loss of epithelial integrity. However, the initial response of neutrophilic inflammation to the provoking viral agent was not altered, lending to the surmise that the gamma interferon is responsible for the ultimate damage and loss of extrahepatic bile ducts<sup>[21]</sup>. It is noteworthy that, in their attempt to achieve viral clearance, the CD8 lymphocytes secondarily cause ductular damage resulting in the experimental type of Biliary atresia<sup>[19]</sup>. Alpha2 beta1 integrin has been identified to be the medium of interaction responsible for predisposition of the cholangiocytes to Rhesus Rota virus infection<sup>[22]</sup>.

Regulatory T lymphocyte defects in the presence of viral infection, has also found to be contributory to the unchecked bile ductal inflammation and destruction<sup>[23]</sup>.

## ANIMAL MODELS

The use of intrahepatic injection of chemicals like carbon tetrachloride, ethanol, formalin have been found to simulate inflammation similar to biliary atresia in adult rat<sup>[24,25]</sup>. Other animals like lamb fetus has also been studied<sup>[26]</sup>. Attempted *in vivo* replication of biliary atresia includes bile duct excision or ligation. Sea Lamprey as a model has been propagated with the advantage of seamless progress into biliary atresia without the need for intervention with injection of chemicals or surgical bile duct ligation<sup>[27]</sup>.

## CLINICAL PRESENTATION

The consistent passage of clay coloured stools, dark coloured urine, icterus at about 2 wk of age in a neonate should prompt the complete work up for cholestasis, especially biliary atresia.

## INVESTIGATIONS

Simple macroscopic examination showing clay coloured acholic stool raises a strong suspicion of biliary atresia. When the stools are not acholic, additional features such as fecal fat and consistency can provide more information. Soil like consistency of stool with massive fat droplets on Sudan III stain is a finding which has

high sensitivity, although not specific for biliary atresia detection<sup>[28]</sup>.

### Blood biochemistry

Gamma glutamyl transpeptidase (GGT) has been found to be an important parameter in the differential diagnosis of neonatal cholestasis. Children with Biliary atresia consistently had higher GGT levels than those without Biliary atresia (902.7 mmol/L vs 263.2 mmol/L)<sup>[29]</sup>. Tang *et al*<sup>[30]</sup> demonstrated that an elevated GGT more than 300 IU/L had a specificity of 98% and sensitivity of 38% to differentiate biliary atresia from Neonatal Hepatitis. In addition, the association between GGT and Alanine transferase ALT (GGT/ALT ratio more than 2) was put forth as a useful adjunct in the differential diagnosis of biliary atresia<sup>[30]</sup>.

It is to be noted that, more relevance is placed on the correlation of GGT with age, than an absolute GGT value. To elaborate further, GGT is best diagnostic when evaluating cholestasis in children aged less than 120 d. Among infants aged 31-60 d, GGT levels more than 268 IU/L had a sensitivity of 80.5% and specificity of 75.6%, respectively, with an accuracy of 79.1% in the diagnostic evaluation of Biliary atresia. Recommended cut-off values of GGT for various age groups include 303 IU/L for age 61-90 d, 298 IU/L for age 91-120 d, 252 IU/L for age more than 121 d<sup>[31]</sup>. Another study brought out the optimal threshold for GGT for various ages, 150 IU/L for age less than 4 wk, 250 IU/L for age between 4-8 wk and 300 IU/L for age more than 8 wk<sup>[32]</sup>.

On the contrary, alkaline phosphatase levels were noted to be higher in those children without biliary atresia<sup>[31]</sup>.

The Apolipoprotein E has been found to be useful in the diagnostic workup as the serum levels have been consistently elevated in biliary atresia<sup>[33]</sup>. Rafeey *et al*<sup>[34]</sup> in a recent study showed Apolipoprotein E to have positive predictive value of 71% and negative predictive value of 67% in differentiating biliary atresia from other neonatal cholestatic disorders, indicating that its utility as a stand-alone diagnostic test is limited. Similar results have been seen with procalcitonin, which is an inflammatory marker, synthesized in the liver. Hence it could be used possibly in combination with other tests to improve the diagnostic accuracy<sup>[34]</sup>.

Recently, microRNA assay has been pointed to be a novel method of quick diagnosis of biliary atresia. Injury to liver tissue in biliary atresia is supposed to release certain microRNAs, which are non-coding RNAs regulating target genes. High levels of these micro RNAs are found in the intrahepatic bile ducts confirming the source of release and their specificity. The study by Zahm *et al*<sup>[35]</sup> has established the high levels of serum miR220b/429 in Biliary atresia patients in comparison to other cholestatic disorders, implying the potential and promising utility of these in aiding in the early diagnosis.

### Imaging

Sonography has distinct advantages of being non-

invasive, repeatable, less expensive, readily available bedside and non-ionising, although limited by operator dependancy. Hence, this is used as the initial screening modality in the work up of neonatal cholestasis.

The usefulness of sonography, as an initial diagnostic tool is well brought out in several studies. Presence of a triangular cord sign which is the visualization of the fibrotic cord in the portal hilum is one of the hallmarks of sonographic imaging with a positive predictive value of 95%<sup>[36]</sup>. Triangular or tubular structure with echogenic density cranial to portal vein bifurcation at the liver hilum is indicative of triangular cord sign<sup>[37]</sup>. Gall bladder (GB) morphology is looked into as the primary diagnostic factor on sonography. If the GB morphology is normal on sonography, the next step of measuring the triangular cord thickness is undertaken, which if more than 3.4 mm, the sonographic diagnosis of Biliary atresia is very likely<sup>[38]</sup>.

In addition, the GB contractility, size and dimension, regular mucosal contour all go together in the diagnostic imaging. Findings pertaining to GB on sonography can be absent/non visualized GB, irregular contour of GB, small shrunken GB, non contractile GB despite 4 h of fasting, cystic structure replacing GB and absent echogenic mucosal lining of GB. The liver echotexture signifying the presence of cirrhosis is another finding useful on sonography for prognostication<sup>[39]</sup>. At a cut off GB length of 1.5 cm, high index of suspicion for biliary atresia to be kept while evaluating a baby with neonatal cholestasis<sup>[40]</sup>. In the early stage of the disease, the triangular cord sign may be not prominent, leading to missed diagnosis. Triangular cord sign combined with GB length can act as twin hallmarks in the sonographic diagnosis of biliary atresia. In the setting of periportal inflammation or cirrhosis, sonographic diagnosis may be difficult as triangular cord sign may not be apparent. Utility of the GB ghost triad, including GB length less than 1.9 cm, irregular contour of GB and lack of smooth, regular mucosal echogenicity of GB may be helpful in the above scenario. With an accuracy of 97%, it appears to be an invaluable diagnostic feature on sonography<sup>[41]</sup>.

As adjuncts to the above sonographic parameters, the right hepatic artery diameter more than 1.5 mm and ratio of the right hepatic artery to that of the portal vein more than 0.45 were of use in the sonographic evaluation<sup>[42]</sup>.

The visualisation of hepatic subcapsular flow due to hepatic arteriopathy and fibrosis in biliary atresia is another sonographic feature on colour Doppler study<sup>[43]</sup>. El-Guindi *et al*<sup>[44]</sup> in a recent study reported the superiority of demonstration of hepatic subcapsular flow over the other sonographic parameters such as triangular cord sign, GB contractility, GB size and dimensions of hepatic artery. Even when the sonographic hallmark of triangular cord sign could not be satisfactorily demonstrated, presence of hepatic sub capsular flow can be of significant value in sonographic examination<sup>[45]</sup>.

The measurement of liver span below the costal margin by sonography can help in the workup, as consistently "small" livers are seen in non-biliary atresia



children<sup>[29]</sup>.

Using a special transducer sonography probe, it is now possible to measure liver fibrosis, based on the technique of transient elastography. Consequently, prognostication of the state of the advanced liver disease can be predicted in a non-invasive manner. It is predicted to be useful as a follow up tool, without the need to resort to performing a liver biopsy. However, the sensitivity of this test in identifying early stages of liver fibrosis is limited<sup>[46]</sup>.

It is recommended that a confident demonstration of the triangular cord sign can route the algorithm towards operative cholangiography, rather than subjecting to liver biopsy, in view of the accuracy of the sonographic sign<sup>[29]</sup>.

### **Antenatal diagnosis**

The presence of a cystic structure at the porta hepatis without intrahepatic biliary ductular dilatation goes towards the diagnosis of biliary atresia, in the antenatal period. This is also known to be associated with additional anomalies<sup>[47,48]</sup>.

### **Sonography vs scintigraphy**

Compared to nuclear scintigraphy, sonography has better discriminatory value in the differential diagnosis. This is evidenced by the higher specificity of the triangular cord sign (95.8%) against scintigraphy (72.9%). Also, the positive predictive value of the triangular cord sign scoring twice higher than scintigraphy (77.8% vs 38.1%) puts sonography ahead, in the correct detection of biliary atresia<sup>[49]</sup>.

### **Magnetic resonance cholangiopancreatography**

The utility of Magnetic resonance cholangiopancreatography (MRCP) has not been encouraging in view of the cost, varying results and the need for immobilisation. Negative and positive predictive value have been reported as 91%-100%, 75%-96% respectively<sup>[50,51]</sup>. The requirement of sedation, preferably general anesthesia is a significant concern in addition to long image acquisition time. In a recent study, the image acquisition time using three dimensional MRCP has been reported to be around 180 s. The sensitivity 99.08% and negative predictive value 96.88% were high but the specificity 36.05% and positive predictive value 65.19% were low<sup>[52]</sup>.

### **Nuclear scintigraphy**

Nuclear scintigraphy is non-invasive, simple and is supposed to have practical utility in view of the logical assumption of the functional ability of liver to take up the tagged agent and subsequent excretion in the intestine, enabling visualization of gut activity. Studies caution regarding excessive reliance of scintigraphy, as it may contribute to misdiagnosis in infants with jaundice<sup>[29]</sup>. However, in the background of elevated bilirubin levels and likely deranged liver function, ability to take up the agent

may be compromised. To overcome this, cholegogues such as Ursodeoxy cholic acid, Phenobarbitone, Phenytoin are used as pre-treatment agents, to ensure adequate "priming"<sup>[53]</sup>.

The value of delayed or 24 h imaging has been pointed out to decrease the false positive results as nearly 50% of the bowel visualization was seen in the delayed image<sup>[54]</sup>. As an adjunct, SPECT has been put forth in dealing with poor bowel visualization. More studies are required before concluding in favor of its usage<sup>[55]</sup>. However, arguments against, have discouraged the same citing the poor image resolution with consequent difficult interpretation. Excretion is expected to be less due to reduced uptake primarily, given the background of deranged liver function and high bilirubin levels, competing with the tagged agent effectively to decrease the uptake. Furthermore, it has been proposed that this would be time consuming and lead to more delay in the work up<sup>[56]</sup>.

A recent meta-analysis places the scintigraphy in the correct perspective, at a low specificity of 70.4%, although pooled sensitivity was high at 98.7%. This would mean that almost every case of biliary atresia gets detected, but when the scintigraphy shows no excretion, it does not necessarily diagnose biliary atresia amongst the other causes of neonatal cholestasis<sup>[53]</sup>.

### **Histology**

Liver biopsy is considered as gold standard in the diagnosis of biliary atresia, with an accuracy of 88.2%-96.9%<sup>[57,58]</sup>. To cope with the delayed referrals and the negative laparotomy rate, histology of liver biopsy is proposed as the best alternative. Also, histology has a definitive role, where the various imaging modalities may not be able to suggest the suitable diagnosis, especially in younger neonatal cholestatic children. Among the several findings in histology, ductular proliferation, bile plugs in the ducts and the ductules and portal fibrosis were found to be statistically significant in the diagnostic workup of biliary atresia. On further multivariate analysis, the ductular proliferation emerged as the sole parameter of paramount importance. Of note, age was not found to be a factor in altering the diagnostic histological features in biliary atresia. Multinucleate giant cell formation and myeloid metaplasia were noted to be seen more commonly in neonatal hepatitis<sup>[58]</sup>. Utility of the liver biopsy in the work up of neonatal cholestasis has been recommended as a guideline<sup>[59]</sup>.

Histology can also prognosticate in addition to providing a diagnosis, by cirrhosis assessment and ductal plate malformation. Ductal plate malformation which refers to presence of fetal type intrahepatic duct, is identified to be a poor prognostic factor as it is known to be associated with poor bile flow after Portoenterostomy<sup>[60]</sup>.

Ductal diameter less than 100 microns was a feature identified with children requiring liver transplantation<sup>[61]</sup>. Whereas, when ductal size was more than 150 microns, in combination with a columnar lined epithelium, it was predictive of good prognosis after surgical mana-

gement<sup>[62]</sup>.

Fibrosis as an independent prognostic marker in histological evaluation is established by various studies<sup>[63-65]</sup>. Also, it has been utilized to predict the long term outcome in post-operative biliary atresia patients<sup>[66]</sup>.

Ductopenia and secondary biliary cirrhosis were consistently found to be late histological features<sup>[67]</sup>.

### Endoscopy

Use of endoscopy in biliary atresia is mainly for dealing with the sequelae of portal hypertension and varices. However, endoscopy can be of use in aiding diagnostic workup, in addition to duodenal intubation for bile detection. Sampling of Duodenal contents to improve the accuracy of scintigraphy, as gamma camera may not pick up minimal activity, is a step towards improvisation by means of non-imaging method<sup>[68]</sup>.

### Scoring system

Based on the variable nature of the diagnostic tests and their overlapping tendency, it would be best to rely on a combination of investigations with correlation to the clinical condition, to reach a prompt and confident diagnosis in the individual child with neonatal cholestasis. Most investigations by themselves do not point to a clear cut differentiation between biliary atresia and other causes of neonatal cholestasis. Hence this has led to a strategy of mix and match of modalities to evolve a meaningful scoring system to attempt to objectively categorize the children with biliary atresia from the group of Neonatal cholestasis. The proposal of El-Guindi *et al*<sup>[69]</sup> consists of a twelve-point scoring system, according to clinical, laboratory, ultrasonographic, and histopathological parameters, with a reported accuracy of 98.3% in pin pointing biliary atresia. Strikingly, scintigraphy was not included in their scoring, referring to its low specificity and time lost to prime the patient. Confining to histology, Chen *et al*<sup>[70]</sup> have evolved a 8-feature (liver fibrosis, portal ductal proliferation, bile plugs in portal ductules, cholestasis, hepatocellular changes inflammatory cells infiltration in portal region, extramedullary hematopoiesis, and ductal plate malformation), 21-point (0 to 21) scoring system declaring an accuracy of 91.9% in correctly identifying biliary atresia<sup>[70]</sup>.

## OPERATIVE MANAGEMENT

Surgery is the main stay of treatment in biliary atresia to effectively establish bile drainage and jaundice clearance. Left untreated, there is an incessant progression towards Biliary cirrhosis, end stage liver failure and death by 3 years of age<sup>[4]</sup>. The hallmark of biliary atresia is the difficulty in prediction of the natural course and outcome, given that it should not be considered a single disease entity with a predictable natural history and stereotypical response to surgery<sup>[71]</sup>.

Kasai portoenterostomy relies on the realization that the microscopic structures in the porta hepatis will act as micro-conduits of bile as an internal biliary fistula is

created with a segment of bowel. Use of gall bladder, appendix has been tried earlier as conduits instead of the bowel segment, but none were successful like the bowel. In view of higher revision rates, other conduits except bowel have been abandoned<sup>[72]</sup>.

The extended Kasai procedure attempts at utilising more anastomotic area for achieving effective bile drainage by extending the dissection into the Rex recess (the space between segments III and IV under the liver bridge) and around the bifurcation of the right vascular pedicle of portal hilum<sup>[73,74]</sup>.

Laparoscopic portoenterostomy has not been shown to have better outcome than the open portoenterostomy<sup>[75]</sup>. Although proponents have defended the minimal access approach with the claim that the risk for damage to small bile ductules around the porta hepatis is minimal, due to avoidance of deep suturing and extensive dissection<sup>[76]</sup>. The advantage of minimal adhesions after laparoscopic intervention, enabling future liver transplantation has also been negated<sup>[77]</sup>. Hence, the open portoenterostomy continues to be the gold standard for biliary atresia<sup>[78]</sup>.

The recommendation to perform per op cholangiography directly without a liver biopsy where clinical suspicion is high, reflects the equivocal state of the liver biopsy<sup>[3]</sup>.

### Post-operative management

The role of corticosteroids is hotly debated and controversial, as there is no conclusive evidence in terms of long term improved outcome<sup>[79]</sup>. However, there does seem to be a positive impact of improved clearance of jaundice when steroids are used for a short course in the post-operative period. Thus the lack of translation of beneficial effect with usage of steroids has generally discouraged its prescription in the long term management, although there is a strong link between continuing inflammation, altered immunity and ongoing fibrosis in biliary atresia after Kasai procedure<sup>[80]</sup>. Unlike steroids, Urso deoxycholic acid does play a positive and significant role in the bile flow and finds a place in the post-operative protocol of Biliary atresia management<sup>[81]</sup>.

## OUTCOME

Lower degree of biliary fibrosis, bile ductular proliferation, absence of ductal plate malformation, large ducts more than 150 µm and younger age were found to be associated with better long term outcome<sup>[66]</sup>.

The cystic dilatation of the intrahepatic biliary system on sonography following Kasai during long term follow-up, is considered as a poor prognostic feature lowering the survival rate with native liver<sup>[82]</sup>.

The children with BASM tend to have a poorer prognosis<sup>[83,84]</sup>. Younger age at Kasai was linked with better outcome in those with the cystic type biliary atresia and BASM. Whereas younger age at surgery was not a determining factor in isolated biliary atresia<sup>[83]</sup>.

The long term survival with native liver is significantly lower, establishing the dictum that liver transplant is the

ultimate recipe for biliary atresia management. Adult outcome studies in Biliary atresia patients quote the survival with native liver at 20% in the adults 20 years post Kasai and 10% among those who are 30 years post Kasai<sup>[85,86]</sup>.

Centralisation of services, such that biliary atresia surgery is managed at select centres, has been shown to remarkably increase surgical outcome and overall survival. Standardisation of protocolised management with uniform pre operative work up, surgical technique, post operative management and follow-up seem to be the cohesive factors towards achieving a better outcome. To quote the Finnish study, jaundice clearance rate improved from 27% to 75% and overall survival from 64% to 92% with all the above measures<sup>[87]</sup>.

Kasai portoenterostomy effectively acts as a bridging procedure, enabling retention of native liver in about a quarter of patients and maintaining the rest till an organ is available for transplant in the long term<sup>[88]</sup>. The importance of surveillance is underlined by the fact that majority of the patients (58.3%) after Kasai procedure develop features of chronic liver disease such as Cirrhosis and Portal hypertension<sup>[89]</sup>.

Early neonatal screening with stool charts has a beneficial effect as evidenced by the fact that 5 year survival with native liver increased from 27.3% to 64.3%<sup>[90]</sup>.

Nutritional management for optimal outcome would include feeding regime with a medium chain triglyceride formula. Also, the follow-up of these children should monitor the regular vitamin supplementation of fat soluble vitamins. However, the question of nutritional resuscitation is relevant from the point of view of those awaiting liver transplant<sup>[4]</sup>.

## SCREENING

Various screening methods other than stool charts have been studied, but none are effective as a simple, cost effective and useful tool in screening general population. Serum bile acid, direct bilirubin, Apo C II/CIII proteins, urine sulfated bile acid, fecal bilirubin and fat<sup>[91-95]</sup> were some of the biomarkers used in the literature for screening of Biliary atresia.

## CONCLUSION

Biliary atresia is a multifactorial disorder with varied outcome depending upon the time of surgical treatment and histology. Strict adherence to protocols in the form of investigations would lead to seamless progression from diagnosis to management. Post operative management with appropriate medications is required to ensure an optimal outcome. Long term follow-up is essential as the native liver can fail over a period of time requiring the need for liver transplantation. Although advances regarding understanding of progressive inflammation after portoenterostomy have been made, translation into significant treatment has not evolved yet.

## REFERENCES

- Poddar U**, Thapa BR, Das A, Bhattacharya A, Rao KL, Singh K. Neonatal cholestasis: differentiation of biliary atresia from neonatal hepatitis in a developing country. *Acta Paediatr* 2009; **98**: 1260-1264 [PMID: 19469771 DOI: 10.1111/j.1651-2227.2009.01338.x]
- Indian Academy of Pediatrics. Pediatric Gastroenterology Subspecialty Chapter**. Consensus report on neonatal cholestasis syndrome. *Pediatric Gastroenterology Subspecialty Chapter of Indian Academy of Pediatrics. Indian Pediatr* 2000; **37**: 845-851 [PMID: 10951633]
- Shah I**, Bhatnagar S, Dhabe H. Clinical and biochemical factors associated with biliary atresia. *Trop Gastroenterol* 2012; **33**: 214-217 [PMID: 23600053]
- Sokol RJ**, Mack C, Narkewicz MR, Karrer FM. Pathogenesis and outcome of biliary atresia: current concepts. *J Pediatr Gastroenterol Nutr* 2003; **37**: 4-21 [PMID: 12827000]
- Mack CL**, Feldman AG, Sokol RJ. Clues to the etiology of bile duct injury in biliary atresia. *Semin Liver Dis* 2012; **32**: 307-316 [PMID: 23397531 DOI: 10.1055/s-0032-1329899]
- Mazziotti MV**, Willis LK, Heuckeroth RO, LaRegina MC, Swanson PE, Overbeek PA, Perlmuter DH. Anomalous development of the hepatobiliary system in the Inv mouse. *Hepatology* 1999; **30**: 372-378 [PMID: 10421642 DOI: 10.1002/hep.510300223]
- Schön P**, Tsuchiya K, Lenoir D, Mochizuki T, Guichard C, Takai S, Maiti AK, Nihei H, Weil J, Yokoyama T, Bouvagnet P. Identification, genomic organization, chromosomal mapping and mutation analysis of the human INV gene, the ortholog of a murine gene implicated in left-right axis development and biliary atresia. *Hum Genet* 2002; **110**: 157-165 [PMID: 11935322 DOI: 10.1007/s00439-001-0655-5]
- Drut R**, Drut RM, Gómez MA, Cueto Rúa E, Lojo MM. Presence of human papillomavirus in extrahepatic biliary atresia. *J Pediatr Gastroenterol Nutr* 1998; **27**: 530-535 [PMID: 9822318]
- Fischler B**, Ehrnst A, Forsgren M, Orvell C, Nemeth A. The viral association of neonatal cholestasis in Sweden: a possible link between cytomegalovirus infection and extrahepatic biliary atresia. *J Pediatr Gastroenterol Nutr* 1998; **27**: 57-64 [PMID: 9669727]
- Nadal D**, Wunderli W, Meurmann O, Briner J, Hirsig J. Isolation of respiratory syncytial virus from liver tissue and extrahepatic biliary atresia material. *Scand J Infect Dis* 1990; **22**: 91-93 [PMID: 2320967]
- Morecki R**, Glaser JH, Cho S, Balistreri WF, Horwitz MS. Biliary atresia and reovirus type 3 infection. *N Engl J Med* 1982; **307**: 481-484 [PMID: 6285193 DOI: 10.1056/NEJM198208193070806]
- Riepenhoff-Talty M**, Gouvea V, Evans MJ, Svensson L, Hoffenberg E, Sokol RJ, Uhnou I, Greenberg SJ, Schäkel K, Zhaori G, Fitzgerald J, Chong S, el-Yousef M, Nemeth A, Brown M, Piccoli D, Hyams J, Ruffin D, Rossi T. Detection of group C rotavirus in infants with extrahepatic biliary atresia. *J Infect Dis* 1996; **174**: 8-15 [PMID: 8656017]
- Mahjoub F**, Shahsiah R, Ardalan FA, Iravanloo G, Sani MN, Zarei A, Monajemzadeh M, Farahmand F, Mamishi S. Detection of Epstein Barr virus by chromogenic in situ hybridization in cases of extra-hepatic biliary atresia. *Diagn Pathol* 2008; **3**: 19 [PMID: 18442403 DOI: 10.1186/1746-1596-3-19]
- Domati-Saad R**, Dawson DB, Margraf LR, Finegold MJ, Weinberg AG, Rogers BB. Cytomegalovirus and human herpesvirus 6, but not human papillomavirus, are present in neonatal giant cell hepatitis and extrahepatic biliary atresia. *Pediatr Dev Pathol* 2000; **3**: 367-373 [PMID: 10890252]
- Landing BH**. Considerations of the pathogenesis of neonatal hepatitis, biliary atresia and choledochal cyst--the concept of infantile obstructive cholangiopathy. *Prog Pediatr Surg* 1974; **6**: 113-139 [PMID: 4856850]
- Caponcelli E**, Knisely AS, Davenport M. Cystic biliary atresia: an etiologic and prognostic subgroup. *J Pediatr Surg* 2008; **43**: 1619-1624 [PMID: 18778995 DOI: 10.1016/j.jpedsurg.2007.12.058]
- Mack CL**, Sokol RJ. Unraveling the pathogenesis and etiology of biliary atresia. *Pediatr Res* 2005; **57**: 87R-94R [PMID: 15817506]



- DOI: 10.1203/01.PDR.0000159569.57354.47]
- 18 **Asai A**, Miethke A, Bezerra JA. Pathogenesis of biliary atresia: defining biology to understand clinical phenotypes. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 342-352 [PMID: 26008129 DOI: 10.1038/nrgastro.2015.74]
  - 19 **Shivakumar P**, Sabla G, Mohanty S, McNeal M, Ward R, Stringer K, Caldwell C, Choungnet C, Bezerra JA. Effector role of neonatal hepatic CD8+ lymphocytes in epithelial injury and autoimmunity in experimental biliary atresia. *Gastroenterology* 2007; **133**: 268-277 [PMID: 17631148 DOI: 10.1053/j.gastro.2007.04.031]
  - 20 **Dong R**, Zhao R, Zheng S. Changes in epigenetic regulation of CD4+ T lymphocytes in biliary atresia. *Pediatr Res* 2011; **70**: 555-559 [PMID: 21857377 DOI: 10.1203/PDR.0b013e318232a949]
  - 21 **Shivakumar P**, Campbell KM, Sabla GE, Miethke A, Tiao G, McNeal MM, Ward RL, Bezerra JA. Obstruction of extrahepatic bile ducts by lymphocytes is regulated by IFN-gamma in experimental biliary atresia. *J Clin Invest* 2004; **114**: 322-329 [PMID: 15286798 DOI: 10.1172/JCI21153]
  - 22 **Jafri M**, Donnelly B, Allen S, Bondoc A, McNeal M, Rennert PD, Weinreb PH, Ward R, Tiao G. Cholangiocyte expression of alpha2beta1-integrin confers susceptibility to rotavirus-induced experimental biliary atresia. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G16-G26 [PMID: 18436621 DOI: 10.1152/ajpgi.00442.2007]
  - 23 **Brindley SM**, Lanham AM, Karrer FM, Tucker RM, Fontenot AP, Mack CL. Cytomegalovirus-specific T-cell reactivity in biliary atresia at the time of diagnosis is associated with deficits in regulatory T cells. *Hepatology* 2012; **55**: 1130-1138 [PMID: 22105891 DOI: 10.1002/hep.24807]
  - 24 **Tatekawa Y**, Nakada A, Nakamura T. Intrahepatic biliary ablation with pure ethanol: an experimental model of biliary atresia. *Surg Today* 2013; **43**: 661-669 [PMID: 23073846 DOI: 10.1007/s00595-012-0379-2]
  - 25 **Dumont M**, D'Hont C, Moreau A, Mbape H, Feldmann G, Erlinger S. Retrograde injections of formaldehyde into the biliary tree induce alterations of biliary epithelial function in rats. *Hepatology* 1996; **24**: 1217-1223 [PMID: 8903401 DOI: 10.1053/jhep.1996.v24.pm0008903401]
  - 26 **Spitz L**. Ligation of the common bile duct in the fetal lamb: an experimental model for the study of biliary atresia. *Pediatr Res* 1980; **14**: 740-748 [PMID: 7383750 DOI: 10.1203/00006450-198005000-00007]
  - 27 **Chung-Davidson YW**, Yeh CY, Li W. The Sea Lamprey as an Etiological Model for Biliary Atresia. *Biomed Res Int* 2015; **2015**: 832943 [PMID: 26101777 DOI: 10.1155/2015/832943]
  - 28 **Okajima K**, Nagaya K, Azuma H, Suzuki T. Biliary atresia and stool: its consistency and fat content, another potentially useful clinical information. *Eur J Gastroenterol Hepatol* 2016; **28**: 118 [PMID: 26594917 DOI: 10.1097/MEG.0000000000000504]
  - 29 **Sun S**, Chen G, Zheng S, Xiao X, Xu M, Yu H, Dong R. Analysis of clinical parameters that contribute to the misdiagnosis of biliary atresia. *J Pediatr Surg* 2013; **48**: 1490-1494 [PMID: 23895960 DOI: 10.1016/j.jpedsurg.2013.02.034]
  - 30 **Tang KS**, Huang LT, Huang YH, Lai CY, Wu CH, Wang SM, Hwang KP, Huang FC, Tiao MM. Gamma-glutamyl transferase in the diagnosis of biliary atresia. *Acta Paediatr Taiwan* 2007; **48**: 196-200 [PMID: 18265540]
  - 31 **Chen X**, Dong R, Shen Z, Yan W, Zheng S. Value of Gamma-Glutamyl Transpeptidase for Diagnosis of Biliary Atresia by Correlation With Age. *J Pediatr Gastroenterol Nutr* 2016; **63**: 370-373 [PMID: 26963938 DOI: 10.1097/MPG.00000000000001168]
  - 32 **Rendón-Macías ME**, Villasis-Keever MA, Castañeda-Muciño G, Sandoval-Mex AM. Improvement in accuracy of gamma-glutamyl transferase for differential diagnosis of biliary atresia by correlation with age. *Turk J Pediatr* 2010; **50**: 253-259 [PMID: 18773671]
  - 33 **Wang H**, Malone JP, Gilmore PE, Davis AE, Magee JC, Townsend RR, Heuckeroth RO. Serum markers may distinguish biliary atresia from other forms of neonatal cholestasis. *J Pediatr Gastroenterol Nutr* 2010; **50**: 411-416 [PMID: 20216099 DOI: 10.1097/MPG.0b013e3181cb42ee]
  - 34 **Rafeey M**, Saboktakin L, Shoa Hassani J, Farahmand F, Aslanabadi S, Ghorbani-Haghjou A, Poorebrahim S. Diagnostic value of procalcitonin and apo-e in extrahepatic biliary atresia. *Iran J Pediatr* 2014; **24**: 623-629 [PMID: 25793072]
  - 35 **Zahm AM**, Hand NJ, Boateng LA, Friedman JR. Circulating microRNA is a biomarker of biliary atresia. *J Pediatr Gastroenterol Nutr* 2012; **55**: 366-369 [PMID: 22732895 DOI: 10.1097/MPG.0b013e318264e648]
  - 36 **Park WH**, Choi SO, Lee HJ. Technical innovation for noninvasive and early diagnosis of biliary atresia: the ultrasonographic "triangular cord" sign. *J Hepatobiliary Pancreat Surg* 2001; **8**: 337-341 [PMID: 11521178 DOI: 10.1007/s0053410080337]
  - 37 **Sun Y**, Zheng S, Qian Q. Ultrasonographic evaluation in the differential diagnosis of biliary atresia and infantile hepatitis syndrome. *Pediatr Surg Int* 2011; **27**: 675-679 [PMID: 21207229 DOI: 10.1007/s00383-010-2814-z]
  - 38 **Lee SM**, Cheon JE, Choi YH, Kim WS, Cho HH, Kim IO, You SK. Ultrasonographic Diagnosis of Biliary Atresia Based on a Decision-Making Tree Model. *Korean J Radiol* 2015; **16**: 1364-1372 [PMID: 26576128 DOI: 10.3348/kjr.2015.16.6.1364]
  - 39 **Li SX**, Zhang Y, Sun M, Shi B, Xu ZY, Huang Y, Mao ZQ. Ultrasonic diagnosis of biliary atresia: a retrospective analysis of 20 patients. *World J Gastroenterol* 2008; **14**: 3579-3582 [PMID: 18567090]
  - 40 **Tan Kendrick AP**, Phua KB, Ooi BC, Subramaniam R, Tan CE, Goh AS. Making the diagnosis of biliary atresia using the triangular cord sign and gallbladder length. *Pediatr Radiol* 2000; **30**: 69-73 [PMID: 10663514 DOI: 10.1007/s002470050017]
  - 41 **Tan Kendrick AP**, Phua KB, Ooi BC, Tan CE. Biliary atresia: making the diagnosis by the gallbladder ghost triad. *Pediatr Radiol* 2003; **33**: 311-315 [PMID: 12695863 DOI: 10.1007/s00247-003-0867-z]
  - 42 **Kim WS**, Cheon JE, Youn BJ, Yoo SY, Kim WY, Kim IO, Yeon KM, Seo JK, Park KW. Hepatic arterial diameter measured with US: adjunct for US diagnosis of biliary atresia. *Radiology* 2007; **245**: 549-555 [PMID: 17890351 DOI: 10.1148/radiol.2452061093]
  - 43 **Lee MS**, Kim MJ, Lee MJ, Yoon CS, Han SJ, Oh JT, Park YN. Biliary atresia: color doppler US findings in neonates and infants. *Radiology* 2009; **252**: 282-289 [PMID: 19561262 DOI: 10.1148/radiol.2522080923]
  - 44 **El-Guindi MA**, Sira MM, Konsowa HA, El-Abd OL, Salem TA. Value of hepatic subcapsular flow by color Doppler ultrasonography in the diagnosis of biliary atresia. *J Gastroenterol Hepatol* 2013; **28**: 867-872 [PMID: 23425046 DOI: 10.1111/jgh.12151]
  - 45 **Ramesh RL**, Murthy GV, Jadhav V, Ravindra S. Hepatic subcapsular flow: An early marker in diagnosing biliary atresia. *Indian J Radiol Imaging* 2015; **25**: 196-197 [PMID: 25969645 DOI: 10.4103/0971-3026.155875]
  - 46 **Shin NY**, Kim MJ, Lee MJ, Han SJ, Koh H, Namgung R, Park YN. Transient elastography and sonography for prediction of liver fibrosis in infants with biliary atresia. *J Ultrasound Med* 2014; **33**: 853-864 [PMID: 24764341 DOI: 10.7863/ultra.33.5.853]
  - 47 **Hinds R**, Davenport M, Mieli-Vergani G, Hadzić N. Antenatal presentation of biliary atresia. *J Pediatr* 2004; **144**: 43-46 [PMID: 14722517 DOI: 10.1016/j.jpeds.2003.09.027]
  - 48 **Casaccia G**, Bilancioni E, Nahom A, Trucchi A, Aite L, Marcellini M, Bagolan P. Cystic anomalies of biliary tree in the fetus: is it possible to make a more specific prenatal diagnosis? *J Pediatr Surg* 2002; **37**: 1191-1194 [PMID: 12149700]
  - 49 **Imanieh MH**, Dehghani SM, Bagheri MH, Emad V, Haghighat M, Zahmatkeshan M, Forutan HR, Rasekhi AR, Gheisari F. Triangular cord sign in detection of biliary atresia: is it a valuable sign? *Dig Dis Sci* 2010; **55**: 172-175 [PMID: 19229615 DOI: 10.1007/s10620-009-0718-3]
  - 50 **Han SJ**, Kim MJ, Han A, Chung KS, Yoon CS, Kim D, Hwang EH. Magnetic resonance cholangiography for the diagnosis of biliary atresia. *J Pediatr Surg* 2002; **37**: 599-604 [PMID: 11912518]
  - 51 **Norton KI**, Glass RB, Kogan D, Lee JS, Emre S, Shneider BL.



- MR cholangiography in the evaluation of neonatal cholestasis: initial results. *Radiology* 2002; **222**: 687-691 [PMID: 11867786 DOI: 10.1148/radiol.2223010969]
- 52 **Liu B**, Cai J, Xu Y, Peng X, Zheng H, Huang K, Yang J. Three-dimensional magnetic resonance cholangiopancreatography for the diagnosis of biliary atresia in infants and neonates. *PLoS One* 2014; **9**: e88268 [PMID: 24505457 DOI: 10.1371/journal.pone.0088268]
  - 53 **Kianifar HR**, Tehranian S, Shojaei P, Adinehpour Z, Sadeghi R, Kakhki VR, Keshtgar AS. Accuracy of hepatobiliary scintigraphy for differentiation of neonatal hepatitis from biliary atresia: systematic review and meta-analysis of the literature. *Pediatr Radiol* 2013; **43**: 905-919 [PMID: 23519699 DOI: 10.1007/s00247-013-2623-3]
  - 54 **Stipsanelli K**, Koutsikos J, Papantoniou V, Arka A, Palestidis C, Tsiouris S, Manolaki A, Zerva C. Hepatobiliary scintigraphy and gamma-GT levels in the differential diagnosis of extrahepatic biliary atresia. *Q J Nucl Med Mol Imaging* 2007; **51**: 74-81 [PMID: 17220819]
  - 55 **Yang JG**, Ma DQ, Peng Y, Song L, Li CL. Comparison of different diagnostic methods for differentiating biliary atresia from idiopathic neonatal hepatitis. *Clin Imaging* 2009; **33**: 439-446 [PMID: 19857804 DOI: 10.1016/j.clinimag.2009.01.003]
  - 56 **Guan YX**, Chen Q, Wan SH, Huang JS, Yang XQ, Pan LJ, Zhang QI, Zhang Q, Ou YJ, Peng XW, Liu SZ, Chen QI, Lou J. Effect of different time phases of radionuclide hepatobiliary scintigraphy on the differential diagnosis of congenital biliary atresia. *Genet Mol Res* 2015; **14**: 3862-3868 [PMID: 25966156 DOI: 10.4238/2015.April.22.15]
  - 57 **Dehghani SM**, Haghighat M, Imanieh MH, Geramizadeh B. Comparison of different diagnostic methods in infants with Cholestasis. *World J Gastroenterol* 2006; **12**: 5893-5896 [PMID: 17007060]
  - 58 **Rastogi A**, Krishnani N, Yachha SK, Khanna V, Poddar U, Lal R. Histopathological features and accuracy for diagnosing biliary atresia by prelaparotomy liver biopsy in developing countries. *J Gastroenterol Hepatol* 2009; **24**: 97-102 [PMID: 19196397 DOI: 10.1111/j.1440-1746.2008.05737.x]
  - 59 **Moyer V**, Freese DK, Whittington PF, Olson AD, Brewer F, Colletti RB, Heyman MB. Guideline for the evaluation of cholestatic jaundice in infants: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2004; **39**: 115-128 [PMID: 15269615]
  - 60 **Shimadera S**, Iwai N, Deguchi E, Kimura O, Ono S, Fumino S, Higuchi K. Significance of ductal plate malformation in the postoperative clinical course of biliary atresia. *J Pediatr Surg* 2008; **43**: 304-307 [PMID: 18280279 DOI: 10.1016/j.jpedsurg.2007.10.023]
  - 61 **Baerg J**, Zuppan C, Klooster M. Biliary atresia--a fifteen-year review of clinical and pathologic factors associated with liver transplantation. *J Pediatr Surg* 2004; **39**: 800-803 [PMID: 15185199]
  - 62 **Gautier M**, Eliot N. Extrahepatic biliary atresia. Morphological study of 98 biliary remnants. *Arch Pathol Lab Med* 1981; **105**: 397-402 [PMID: 6894845]
  - 63 **Kang N**, Davenport M, Driver M, Howard ER. Hepatic histology and the development of esophageal varices in biliary atresia. *J Pediatr Surg* 1993; **28**: 63-66 [PMID: 8429476]
  - 64 **Wildhaber BE**, Coran AG, Drongowski RA, Hirschl RB, Geiger JD, Lelli JL, Teitelbaum DH. The Kasai portoenterostomy for biliary atresia: A review of a 27-year experience with 81 patients. *J Pediatr Surg* 2003; **38**: 1480-1485 [PMID: 14577071]
  - 65 **Arii R**, Koga H, Arakawa A, Miyahara K, Lane GJ, Okazaki T, Urao M, Yamataka A. How valuable is ductal plate malformation as a predictor of clinical course in postoperative biliary atresia patients? *Pediatr Surg Int* 2011; **27**: 275-277 [PMID: 21069347 DOI: 10.1007/s00383-010-2793-0]
  - 66 **Mukhopadhyay SG**, Roy P, Chatterjee U, Datta C, Banerjee M, Banerjee S, Basu AK, Ganguli M. A histopathological study of liver and biliary remnants in the long-term survivors (> 10 years) of cases of biliary atresia. *Indian J Pathol Microbiol* 2014; **57**: 380-385 [PMID: 25118727 DOI: 10.4103/0377-4929.138722]
  - 67 **Kahn E**. Biliary atresia revisited. *Pediatr Dev Pathol* 2004; **7**: 109-124 [PMID: 14994122 DOI: 10.1007/s10024-003-0307-y]
  - 68 **Liu SX**, Huang ZH. The value of radionuclide hepatobiliary scintigraphy in combination with determination of bilirubin from duodenal drainage in differential diagnosis of infantile persistent jaundice. *Front Med China* 2010; **4**: 342-345 [PMID: 21191842 DOI: 10.1007/s11684-010-0099-1]
  - 69 **El-Guindi MA**, Sira MM, Sira AM, Salem TA, El-Abd OL, Konsowa HA, El-Azab DS, Allam AA. Design and validation of a diagnostic score for biliary atresia. *J Hepatol* 2014; **61**: 116-123 [PMID: 24657403 DOI: 10.1016/j.jhep.2014.03.016]
  - 70 **Chen G**, Xue P, Zheng S, Chen L, Ma Y. A pathological scoring system in the diagnosis and judgment of prognosis of biliary atresia. *J Pediatr Surg* 2015; **50**: 2119-2123 [PMID: 26577909 DOI: 10.1016/j.jpedsurg.2015.08.041]
  - 71 **Davenport M**, Grieve A. Maximizing Kasai portoenterostomy in the treatment of biliary atresia: medical and surgical options. *S Afr Med J* 2012; **102**: 865-867 [PMID: 23116745]
  - 72 **Zhao R**, Li H, Shen C, Zheng S, Xiao X. Hepatic portocholecystostomy (HPC) is ineffective in the treatment of biliary atresia with patent distal extrahepatic bile ducts. *J Invest Surg* 2011; **24**: 53-58 [PMID: 21345004 DOI: 10.3109/08941939.2010.530737]
  - 73 **Endo M**, Katsumata K, Yokoyama J, Morikawa Y, Ikawa H, Kamagata S, Nakano M, Nirasawa Y, Ueno S. Extended dissection of the portahepatis and creation of an intussuscepted ileocolic conduit for biliary atresia. *J Pediatr Surg* 1983; **18**: 784-793 [PMID: 6663407]
  - 74 **Kobayashi H**, Yamataka A, Urao M, Okazaki T, Yanai T, Koga H, Lane GJ, Miyano T. Innovative modification of the hepatic portoenterostomy. Our experience of treating biliary atresia. *J Pediatr Surg* 2006; **41**: e19-e22 [PMID: 16677870 DOI: 10.1016/j.jpedsurg.2005.12.056]
  - 75 **Sun X**, Diao M, Wu X, Cheng W, Ye M, Li L. A prospective study comparing laparoscopic and conventional Kasai portoenterostomy in children with biliary atresia. *J Pediatr Surg* 2016; **51**: 374-378 [PMID: 26589186 DOI: 10.1016/j.jpedsurg.2015.10.045]
  - 76 **Yamataka A**. Laparoscopic Kasai portoenterostomy for biliary atresia. *J Hepatobiliary Pancreat Sci* 2013; **20**: 481-486 [PMID: 23572285 DOI: 10.1007/s00534-013-0607-1]
  - 77 **Oetzmann von Sochaczewski C**, Petersen C, Ure BM, Osthaus A, Schubert KP, Becker T, Lehner F, Kuebler JF. Laparoscopic versus conventional Kasai portoenterostomy does not facilitate subsequent liver transplantation in infants with biliary atresia. *J Laparoendosc Adv Surg Tech A* 2012; **22**: 408-411 [PMID: 22577810 DOI: 10.1089/lap.2012.0077]
  - 78 **Lishuang M**, Zhen C, Guoliang Q, Zhen Z, Chen W, Long L, Shuli L. Laparoscopic portoenterostomy versus open portoenterostomy for the treatment of biliary atresia: a systematic review and meta-analysis of comparative studies. *Pediatr Surg Int* 2015; **31**: 261-269 [PMID: 25627699 DOI: 10.1007/s00383-015-3662-7]
  - 79 **Sarkhy A**, Schreiber RA, Milner RA, Barker CC. Does adjuvant steroid therapy post-Kasai portoenterostomy improve outcome of biliary atresia? Systematic review and meta-analysis. *Can J Gastroenterol* 2011; **25**: 440-444 [PMID: 21912769]
  - 80 **Bezerra JA**, Spino C, Magee JC, Shneider BL, Rosenthal P, Wang KS, Erlichman J, Haber B, Hertel PM, Karpen SJ, Kerkar N, Loomes KM, Molleston JP, Murray KF, Romero R, Schwarz KB, Shepherd R, Suchy FJ, Turmellet YP, Whittington PF, Moore J, Sherker AH, Robuck PR, Sokol RJ. Use of corticosteroids after hepatopuertoenterostomy for bile drainage in infants with biliary atresia: the START randomized clinical trial. *JAMA* 2014; **311**: 1750-1759 [PMID: 24794368 DOI: 10.1001/jama.2014.2623]
  - 81 **Willot S**, Uhlen S, Michaud L, Briand G, Bonnevalle M, Sfeir R, Gottrand F. Effect of ursodeoxycholic acid on liver function in children after successful surgery for biliary atresia. *Pediatrics* 2008; **122**: e1236-e1241 [PMID: 19029197 DOI: 10.1542/peds.2008-0986]
  - 82 **Shimadera S**, Iwai N, Deguchi E, Kimura O, Ono S, Furukawa T, Fumino S. Predicting factors on the occurrence of cystic dilatation

- of intrahepatic biliary system in biliary atresia. *Pediatr Surg Int* 2010; **26**: 611-614 [PMID: 20428877 DOI: 10.1007/s00383-010-2601-x]
- 83 **Davenport M**, Caponcelli E, Livesey E, Hadzic N, Howard E. Surgical outcome in biliary atresia: etiology affects the influence of age at surgery. *Ann Surg* 2008; **247**: 694-698 [PMID: 18362634 DOI: 10.1097/SLA.0b013e3181638627]
  - 84 **Shneider BL**, Brown MB, Haber B, Whittington PF, Schwarz K, Squires R, Bezerra J, Shepherd R, Rosenthal P, Hoofnagle JH, Sokol RJ. A multicenter study of the outcome of biliary atresia in the United States, 1997 to 2000. *J Pediatr* 2006; **148**: 467-474 [PMID: 16647406 DOI: 10.1016/j.jpeds.2005.12.054]
  - 85 **Lykavieris P**, Chardot C, Sokhn M, Gauthier F, Valayer J, Bernard O. Outcome in adulthood of biliary atresia: a study of 63 patients who survived for over 20 years with their native liver. *Hepatology* 2005; **41**: 366-371 [PMID: 15660386 DOI: 10.1002/hep.20547]
  - 86 **Howard ER**, MacLean G, Nio M, Donaldson N, Singer J, Ohi R. Survival patterns in biliary atresia and comparison of quality of life of long-term survivors in Japan and England. *J Pediatr Surg* 2001; **36**: 892-897 [PMID: 11381420 DOI: 10.1053/jpsu.2001.23965]
  - 87 **Lampela H**, Ritvanen A, Kosola S, Koivusalo A, Rintala R, Jalanko H, Pakarinen M. National centralization of biliary atresia care to an assigned multidisciplinary team provides high-quality outcomes. *Scand J Gastroenterol* 2012; **47**: 99-107 [PMID: 22171974 DOI: 10.3109/00365521.2011.627446]
  - 88 **Karrer FM**, Price MR, Bensard DD, Sokol RJ, Narkewicz MR, Smith DJ, Lilly JR. Long-term results with the Kasai operation for biliary atresia. *Arch Surg* 1996; **131**: 493-496 [PMID: 8624194]
  - 89 **Lee S**, Park H, Moon SB, Jung SM, Kim JM, Kwon CH, Kim SJ, Joh JW, Seo JM, Lee SK. Long-term results of biliary atresia in the era of liver transplantation. *Pediatr Surg Int* 2013; **29**: 1297-1301 [PMID: 23948814 DOI: 10.1007/s00383-013-3366-9]
  - 90 **Lien TH**, Chang MH, Wu JF, Chen HL, Lee HC, Chen AC, Tiao MM, Wu TC, Yang YJ, Lin CC, Lai MW, Hsu HY, Ni YH. Effects of the infant stool color card screening program on 5-year outcome of biliary atresia in Taiwan. *Hepatology* 2011; **53**: 202-208 [PMID: 21140377 DOI: 10.1002/hep.24023]
  - 91 **Matsui A**, Kasano Y, Yamauchi Y, Momoya T, Shimada T, Ishikawa T, Abukawa D, Kimura A, Adachi K, Tazuke Y. Direct enzymatic assay of urinary sulfated bile acids to replace serum bilirubin testing for selective screening of neonatal cholestasis. *J Pediatr* 1996; **129**: 306-308 [PMID: 8765633]
  - 92 **Akiyama T**, Yamauchi Y. Use of near infrared reflectance spectroscopy in the screening for biliary atresia. *J Pediatr Surg* 1994; **29**: 645-647 [PMID: 8035274]
  - 93 **Mushtaq I**, Logan S, Morris M, Johnson AW, Wade AM, Kelly D, Clayton PT. Screening of newborn infants for cholestatic hepatobiliary disease with tandem mass spectrometry. *BMJ* 1999; **319**: 471-477 [PMID: 10454398]
  - 94 **Mowat AP**, Davidson LL, Dick MC. Earlier identification of biliary atresia and hepatobiliary disease: selective screening in the third week of life. *Arch Dis Child* 1995; **72**: 90-92 [PMID: 7717750]
  - 95 **Song Z**, Dong R, Fan Y, Zheng S. Identification of serum protein biomarkers in biliary atresia by mass spectrometry and enzyme-linked immunosorbent assay. *J Pediatr Gastroenterol Nutr* 2012; **55**: 370-375 [PMID: 22569524 DOI: 10.1097/MPG.0b013e31825bb01a]

**P- Reviewer:** Liu HY, Santetti D   **S- Editor:** Gong ZM   **L- Editor:** A  
**E- Editor:** Li D



## Restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension

Dmitry Victorovich Garbuzenko, Nikolay Olegovich Arefyev, Dmitry Vladimirovich Belov

Dmitry Victorovich Garbuzenko, Nikolay Olegovich Arefyev, Department of Faculty Surgery, South Ural State Medical University, 454092 Chelyabinsk, Russia

Dmitry Vladimirovich Belov, Department of Hospital Surgery, South Ural State Medical University, 454092 Chelyabinsk, Russia

**Author contributions:** Garbuzenko DV contributed to the conception and design, acquisition, analysis and interpretation of data, drafting the article, final approval of the version; all authors wrote this manuscript.

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dmitry Victorovich Garbuzenko, MD, PhD, Professor, Department of Faculty Surgery, South Ural State Medical University, Box 12317, 454092 Chelyabinsk, Russia. [garb@inbox.ru](mailto:garb@inbox.ru)  
 Telephone: +8-909-7459826  
 Fax: +8-351-2687772

Received: August 18, 2016  
 Peer-review started: August 19, 2016  
 First decision: September 13, 2016  
 Revised: September 23, 2016  
 Accepted: November 1, 2016  
 Article in press: November 2, 2016  
 Published online: December 28, 2016

### Abstract

In recent years, defined progress has been made in

understanding the mechanisms of hemodynamic disturbances occurring in liver cirrhosis, which are based on portal hypertension. In addition to pathophysiological disorders related to endothelial dysfunction, it was revealed: There is the restructuring of the vasculature, which includes vascular remodeling and angiogenesis. In spite of the fact that these changes are the compensatory-adaptive response to the deteriorating conditions of blood circulation, taken together, they contribute to the development and progression of portal hypertension causing severe complications such as bleeding from esophageal varices. Disruption of systemic and organ hemodynamics and the formation of portosystemic collaterals in portal hypertension commence with neovascularization and splanchnic vasodilation due to the hypoxia of the small intestine mucosa. In this regard, the goal of comprehensive treatment may be to influence on the chemokines, proinflammatory cytokines, and angiogenic factors (vascular endothelial growth factor, placental growth factor, platelet-derived growth factor and others) that lead to the development of these disorders. This review is to describe the mechanisms of restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension. Development of pathogenetic methods, which allow correcting portal hypertension, will improve the efficiency of conservative therapy aimed at prevention and treatment of its inherent complications.

**Key words:** Portal hypertension; Vascular remodeling; Angiogenesis; Pathogenesis; Liver cirrhosis

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The purpose of the review is to describe the mechanisms of restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension. In addition to pathophysiological disorders related to endothelial dysfunction, it was revealed: There is the restructuring of the vasculature, which includes vascular remodeling and angiogenesis. In spite of the fact that these changes are the compensatory-

adaptive response to the deteriorating conditions of blood circulation, taken together, they contribute to the development and progression of portal hypertension causing severe complications such as bleeding from esophageal varices.

Garbuzenko DV, Arefyev NO, Belov DV. Restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension. *World J Hepatol* 2016; 8(36): 1602-1609 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i36/1602.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i36.1602>

## INTRODUCTION

The majority of severe complications of liver cirrhosis are directly related to its characteristic hemodynamic disturbances, and portal hypertension is at their core<sup>[1]</sup>. Due to various anatomical and functional factors, the increase in hepatic vascular resistance causes the development of portal hypertension. The synthesis of extracellular matrix components leads to serious changes in the liver cytoarchitectonics. Accompanying this process, hypoxia with the participation of vascular endothelial growth factor (VEGF) induce pathological angiogenesis. In addition, sinusoidal endothelial dysfunction in liver cirrhosis disturbs the balance between vasoconstrictors and vasodilators produced by the endothelium. There are endothelin-1 (ET-1) and nitrogen oxide (NO), the most studied at present<sup>[2]</sup>.

In spite of the formation of portosystemic shunts, the subsequent development of hyperdynamic circulatory syndrome contributes to the progression of portal hypertension. Appearing in this case circulatory disorders are caused by endothelial dysfunction and restructuring of the vascular bed that includes vascular remodeling and angiogenesis<sup>[3]</sup> (Figure 1).

The purpose of the review is to describe the mechanisms of restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension.

## CURRENT CONCEPT OF VASCULAR REMODELING

The term "remodeling" started to be used in the 1980s of the last century, mainly in cardiology. In the strict interpretation, it means the process of reorganization of the existing structure, during which it joins a new material or it is entirely changed. Vascular remodeling is an adaptive response to long-term hemodynamic disturbances. This process includes several stages<sup>[4]</sup>: (1) perception of signals about modified conditions of blood circulation and circulating humoral factors; (2) signal transmission within a cell and between adjacent cells; (3) synthesis, activation, or release of substances affecting cell growth, death, migration, or extracellular matrix

construction; and (4) structural changes of the vascular wall (both cellular and extracellular components), its mechanics, and function.

Endothelial cells are the main sensors perceiving changes in blood flow and the impact of various humoral factors. They are constantly activated by mechanical stimuli, such as shear stress and intravascular pressure, which are transformed into intracellular and extracellular chemical signals within endothelial cells. These changes occur in the early stage of the mechanotransduction process<sup>[5]</sup>. It involves a variety of physiological elements including ion channels, molecules of cell-matrix and cell-cell interactions [integrins, platelet-endothelial cell adhesion molecule-1 (PECAM-1) or CD31, adherent compounds], tyrosine kinase receptors, caveolae, G protein-coupled receptors and G-proteins, glycocalyx, endothelial cell cytoskeletal components, and others. In response to mechanotransduction, there are formation and secretion of biologically active substances produced by the endothelium. They regulate the development of extracellular matrix, proliferation, migration, and organization of endothelial and smooth muscle cells, as well as sensitivity to growth factors - the key events in the vascular remodeling. Currently, the most studied ones are the vasodilators NO and prostacyclin (PGI<sub>2</sub>), and the vasoconstrictor ET-1<sup>[6]</sup>.

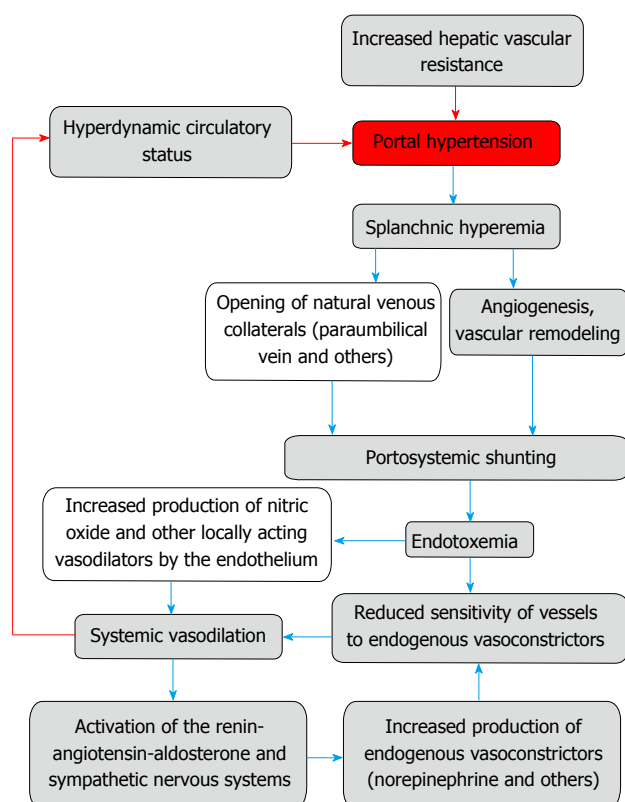
Structural changes of blood vessels consist in eutrophic, hypertrophic, and hypotrophic remodeling. In inward eutrophic remodeling, outer and lumen diameters are reduced, and media cross-sectional area is unaltered. Inward hypertrophic remodeling is characterized by an increase in media/lumen ratio owing to media cross-sectional area increasing. Outward hypotrophic remodeling refers to an increase in lumen diameter and a decrease in cross-sectional area<sup>[7]</sup>.

Distensibility is an important mechanical characteristic of the blood vessels defined as the percentage of change of their volume for each 1 mmHg change in intraluminal pressure. It depends primarily on the stiffness of the vascular wall, which mainly consists of collagen, elastin, and smooth muscles. It has been shown on the experimental model of spontaneously hypertensive rats that reduced distensibility of major arteries is related to increased quantity and changing structure of elastin, whereas its reduction in mesenteric resistance vessels mainly results from the modification of the elastin structure and possibly collagen accumulation<sup>[8]</sup>.

## MOLECULAR MECHANISMS OF THE ANGIOGENIC PROCESS

Angiogenesis is the complicated physiological process of creating new blood vessels. It occurs *via* activation of endothelial cells, expression of proteases in them, extracellular matrix destruction, proliferation, migration of endothelial cells, and creation of the highly permeable primary vascular structures, which is reconstructed into the three-dimensional vascular network after stabilization





**Figure 1** Potential mechanisms of the pathogenesis of portal hypertension in liver cirrhosis.

and “maturation” by attracting pericytes and smooth muscle cells<sup>[9]</sup>.

The major inducer of angiogenesis in both pathological and physiological conditions is hypoxia. The cells react to lack of oxygen by several mechanisms including accumulation of hypoxia-inducible factors (HIFs). They are activated in the physiologically important sites of regulation of the oxygen pathways to provide quick and adequate responses to hypoxic stress and activate genes that regulate the angiogenesis process, vaso-motor control, energy metabolism, erythropoiesis, and apoptosis<sup>[10]</sup>.

The HIFs family include three  $\alpha$ -subunits, which are connected with a  $\beta$ -subunit (HIF-1 $\beta$ ). HIF-1 $\alpha$  is ubiquitously expressed, whereas HIF-2 $\alpha$  is found in a more limited set of cell types, particularly in vascular ECs, type II pneumocytes, hepatocytes, and macrophages. The role of HIF-3 $\alpha$  is less well understood in hypoxic responses<sup>[11]</sup>.

HIFs are considered to be the major transcriptional regulators of genes involved in response to the lack of oxygen, whereas miRNAs regulate gene expression post-transcriptionally<sup>[12]</sup>.

The most investigated angiogenic growth factors are the family of VEGF. It consists of five homologs: VEGF-A, B, C, D and placental growth factor (PlGF). All VEGFs binds to various related receptors: VEGFR-1, VEGFR-2, VEGFR-3. Only the first two of them are responsible for transmission of angiogenic signals. What is more, the binding of VEGF-A to VEGFR-2 and the increase of

vascular permeability under the influence of nitric oxide are the mechanisms triggering vasculogenesis and angiogenesis<sup>[13]</sup>.

Members of the fibroblast growth factor (FGF) family are also capable of stimulating angiogenesis. Cellular response to the impact of FGFs develops *via* specific binding to FGF-receptors (FGFR), which have internal tyrosine kinase activity. Dimerization of FGFR is a precondition for activation and phosphorylation of signaling molecules occurring with the participation of heparin-binding proteins. The process stimulates cell differentiation, proliferation, migration, and destruction of the extracellular matrix. It is important to note that while members of the VEGF family are involved mostly in the capillary formation, the FGF mainly involved in arteriogenesis<sup>[14]</sup>.

Although the platelet-derived growth factor (PDGF) influence on angiogenesis is not so explicit in contrast with VEGF and FGF, studies *in vivo* have shown that it is capable of causing the formation of blood vessels and regulating their tone<sup>[15]</sup>.

Tie-2 (Tek), a tyrosine kinase receptor expressed by endothelial cells, and its ligands, the angiopoietins (Ang), play an important role in the coordination of the angiogenesis process. Angiopoietin-1 (Ang-1) promotes stabilization of vessels *via* inhibiting endothelial cells apoptosis and stimulating its formation. In contrast, Ang-2, an antagonist of Ang-1, results in destabilization of vessels by converting the endothelial cells to the proliferative phenotype from the stable state. However, Ang-2 is also able to stimulate angiogenesis with the participation of VEGF<sup>[16]</sup>.

Integrin  $\alpha$  v  $\beta$  3 ( $\alpha$ v $\beta$ 3) and  $\alpha$  v  $\beta$  5 ( $\alpha$ v $\beta$ 5) were regarded as positive regulators of angiogenesis for a long time. Nevertheless, the recent studies have shown their inhibitory role in this process<sup>[17]</sup>.

Vascular endothelial (VE)-cadherin, an endothelial-specific adhesion molecule, promotes cell-cell junctions during neovascularization and manages the transport of molecules through the endothelial lining<sup>[18]</sup>.

Thrombospondin-1, an antiangiogenic protein, has a direct impact on migration and apoptosis of endothelial cells. Furthermore, it prevents the release of VEGF from the extracellular matrix by suppressing the activation of matrix metalloproteinases (MMPs)<sup>[19]</sup>. Endostatin, a fragment of the C-terminal part of the collagen XVIII  $\alpha$ 1-chain, and angiostatin, a plasminogen degradation product, are also inhibitors of angiogenesis<sup>[20]</sup>.

The first step in the formation of new blood vessels is vasodilatation. Ang-2 and VEGF affect the formation of endothelial fenestrae. Increased vascular permeability causes extravasation of plasma proteins, which will further serve as a scaffold for migrating endothelial cells. Meanwhile, integrins provide them information about the presence of the angiogenic sites. The next step is the destruction of the basement membrane and the extracellular matrix by activated MMPs. This induces the subsequent migration and proliferation of endothelial cells with the participation of angiogenic growth factors such

as VEGF, FGF, and epidermal growth factor (EGF). One of the regulators of this process is a transmembrane protein ESDN - endothelial and smooth muscle cell-derived neuropilin-like protein<sup>[21]</sup>.

Endothelial progenitor cells differentiate into endothelial cells. VE-cadherin and integrins coordinate their binding, and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), FGF, and PDGF create conditions for the formation of new capillaries. Endothelial cells form the new basement membrane and the extracellular matrix with the participation of surrounding pericytes. Ang-1 provides final vascular stabilization<sup>[9]</sup>.

## MECHANISM OF PORTAL-SYSTEMIC COLLATERALS FORMATION

In the early stages of the development of portal hypertension, a moderate increase in the portal pressure leads to a redistribution of blood flow toward the muscle layer of the small intestine. The appearance of mucosal hypoxia causes a significant increase in NAD(P)H oxidase activity, the main source of reactive oxygen species (ROS) in the mucous membrane, and also leads to increased production of VEGF and NO by arterioles, contributing splanchnic vasodilation<sup>[22]</sup>. In addition, multiple signaling pathways are stimulated, such as mitogen-activated protein kinases, tyrosine kinases, and transcription factors that are involved in VEGF-induced neovascularization<sup>[23]</sup>. It was shown that overexpression of Kruppel-like factor 2 in duodenal tissue with the assistance of microRNAs causes hemodynamic stimuli integration and VEGF-driven angiogenesis in patients with liver cirrhosis<sup>[24]</sup>. Besides the wall of the small intestine<sup>[25]</sup>, the elevated levels of VEGF, VEGFR-2, and CD31 (PECAM-1) is observed in the mesentery<sup>[26]</sup>.

These pathophysiological disturbances may be the initial step in the development of portosystemic collateral circulation in portal hypertension<sup>[27]</sup>. Monocytes adhere to the surface of activated endothelial cells and produce growth factors and proteases, such as urokinase plasminogen activator and MMPs, promoting the division and migration of smooth muscle cells. Proinflammatory cytokines [macrophage chemotactic protein-1, granulocyte-macrophage colony-stimulating factor, transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1), TNF- $\alpha$ ] also promote the growth of blood vessels, as well as growth factors such as PIGF, which stimulates the growth of endothelial and smooth muscle cells, FGF - through upregulated expression of PDGF receptor, and VEGF by reaction with Ang-1. At the same time, anti-inflammatory cytokines (e.g., interleukin-10) inhibit the process<sup>[28]</sup>.

It was shown in animal model of prehepatic portal hypertension induced by partial portal vein ligation, that the blockade of VEGFR-2 with anti-VEGFR-2 monoclonal antibody for 5-7 d and inhibition of VEGF/VEGFR-2 signaling using autophosphorylation inhibitor VEGFR-2 for 5 d after the operation resulted in 50% reduction of portosystemic collateral vessel formation<sup>[29,30]</sup>. Blockade

of NAD(P)H also contributed to this owing to the reduced splanchnic expression of VEGF, VEGFR-2 and CD31<sup>[31]</sup>.

It should be noted that the emerging shunts are very dynamic vascular bed because of the expression of various receptor types on the surface of the endothelial lining, for example,  $\alpha$  and  $\beta$  adrenoreceptors, 5-HT<sub>2</sub> receptors. Furthermore, vasoactive substances such as NO, ET-1, prostaglandins can affect their tonus<sup>[32]</sup>. In particular, it was noted that excessive discharge of blood through portosystemic collaterals because of postprandial splanchnic hyperemia promotes their dilation due to shear stress, which in turn induces the overproduction of NO by endothelial cells<sup>[33]</sup>. Although natural portosystemic anastomoses are found in all patients with portal hypertension, they acquire the highest clinical significance in the development of gastroesophageal varices, because their rupture leads to life-threatening bleeding. The determining factor in their formation is the type of hepatofugal blood flow, and a gastroesophageal drainage path is the most important in this situation. The left gastric vein plays the main role in this path. It drains blood from both surfaces of the stomach, ascends from right to left along the lesser curvature into the lesser omentum, to the esophageal opening of the diaphragm, where it receives esophageal veins. It then turns backward and passes from left to right behind the omental bursa and drains into the portal vein. Anastomoses between the left and right gastric veins and the left and short gastric veins, respectively indicated by terms "coronary vein" and "posterior gastric vein", have clinical significance only in portal hypertension, because they are involved in the formation of esophageal and related with them paraesophageal varices<sup>[34]</sup>.

Immunohistochemical studies, which was conducted in patients with portal hypertension, revealed the existence of the pronounced expression of PDGF, basic FGF-2, EGF and TGF- $\alpha$  in the wall of the coronary vein of the stomach. This fact shows that the increase in pressure in this vein activates smooth muscle cells and induces the release of growth factors that stimulate their proliferation, differentiation, and migration, as well as contribute to the disruption of the metabolism of collagen and elastin fibers. Phenotypic changes of smooth muscle cells is a response to chronic mechanical stimuli. They are lead to thickening of the venous wall and reduce its elasticity<sup>[35]</sup>.

## VASCULAR STRUCTURE OF THE LOWER ESOPHAGUS IN CLINICAL PORTAL HYPERTENSION

The venous system of the distal portion of the esophagus includes intraepithelial, subepithelial superficial, deep submucosal and adventitial veins. The largest varices are generally localized 2-3 cm above and 2 cm below the cardia, mainly in the lamina propria of the mucous membrane. They have two types of vascular structure: Palisading type and bar type. The palisading type has

dilated intraepithelial channels and numerous small superficial collateral veins. The bar type has triply dilated subepithelial superficial veins and deep submucosal veins which erode the epithelium<sup>[36]</sup>. Structural changes in the veins of the distal portion of the esophagus in portal hypertension are characterized by thickening of the medial layer because of hyperplasia of elastic and collagen fibers. Elastic fibers become fragmented and sharply tortuous directly in the varicose veins of the esophagus in the background of increasing sclerosis of the vascular wall<sup>[37]</sup>.

Four distinct intramural vascular zones of the gastroesophageal junction were defined as follows: Gastric zone, palisade zone, perforating zone, and truncal zone. Portacaval shunts in this area are formed because of increased pressure in the portal venous system<sup>[38]</sup>.

### **Gastric zone**

The longitudinal veins of the gastric area are located in the submucosa and the lamina propria of the proximal portion of the stomach. They are more abundant near the esophagus, have a small diameter, and form a group of several longitudinal vessels. The veins merge in the submucosa of the distal part of the gastric zone and form large tortuous trunks draining blood into the portal vein system.

### **Palisade zone**

The palisade zone is an extension of the gastric zone. It begins in the projection of the gastroesophageal junction and ends 2-3 cm above it. Veins in that zone are located randomly, close to each other, and are arranged longitudinally and in parallel as a palisade.

Numerous anastomoses are identified between vessels of both gastric and palisade zones. They are localized in the submucosa of the gastroesophageal junction, penetrate the muscularis mucosa, and pass into the lamina propria mainly in a longitudinal direction.

Veins of a proximal portion of the palisade zone simultaneously converge at one point and, perforating the muscularis mucosa, pass into the submucosa again as four or five big trunks. There are arched transverse anastomoses between them. Veins perforating the muscular layer of the esophagus were not detected in this zone.

### **Perforating zone**

Veins of the perforating zone, which is located 3-5 cm above the gastroesophageal junction, are not so homogeneous and constant. Vessels form five polygonal networks in the lamina propria of the esophageal mucosa (as a continuation of the veins of the palisade zone) and perforate the muscular layer, communicating with adventitial veins located on the outer esophageal surface. They were referred to as (treble clef) veins because of their similarity with music symbols.

The perforating zone is the "critical area" for variceal rupture in portal hypertension. This is due to increased

resistance to blood flow in this anatomical area, as well as increased fragility and superficial location of perforating veins<sup>[39]</sup>.

### **Truncal zone**

The truncal zone is a region from 8 to 10 cm in length with the bottom edge 5 cm above the gastroesophageal junction. Large longitudinal venous trunks, discovered here in the lamina propria, constitute a continuation of the polygonal vascular networks of the perforating zone. They have a small diameter in the proximal portion. Between them, there are several transversely oriented anastomoses. Perforating veins, locating randomly along the zone, pass from the submucosa of the esophagus to its outer surface and communicate with adventitial veins.

In physiological terms, palisade zone is the most important part of the vascular structure of the gastroesophageal junction. Veins are located there mainly in the lamina propria. Their superficial location decreases venous blood flow resistance to a minimum, which would otherwise arise in the high-pressure zone in the area of the lower esophageal sphincter.

A large number of small caliber vessels in the palisade zone with a longitudinal stroke and parallel to each other perfectly adapted to the physiological pressure variations that leads to a bi-directional flow during breathing. When the venous outflow is carried out in the caudal direction, the gastric zone collects and drains the blood into the portal vein system.

Deep submucosal veins are enlarged because of the blood outflow in the cranial direction in portal hypertension. They drain the blood into the enlarged adventitial veins (periesophageal collateral veins) through the numerous veins perforating the esophageal smooth muscle layer in the perforating zone. Adventitial veins, in turn, communicate with paraesophageal collateral veins, which are located in the posterior mediastinum. The blood flows from them usually into the azygos vein<sup>[40]</sup>, which structural changes in response to increased blood flow are characterized by focal destruction, hyperplasia and chaotic arrangement of elastic fibers<sup>[37]</sup>.

---

## **THE SYSTEMIC AND SPLANCHNIC ADAPTIVE RESPONSE OF VASCULAR BED TO HEMODYNAMIC DISTURBANCES IN PORTAL HYPERTENSION**

---

The development of portosystemic collateral circulation is a compensatory mechanism, which purpose is decompression of increased portal pressure. However, this does not happen. Conversely, there is a hyperdynamic circulatory state accompanied by increased cardiac output, decreased peripheral vascular resistance, and the opening of arteriovenous communications, which exacerbates portal hypertension. The cause of these disorders may be the flow of vasodilator substances (e.g., glucagon, endocannabinoid, atrial natriuretic

peptide, bacterial endotoxin) through the network of portosystemic shunts, as well as increased production of topical vasodilators by endothelium, such as NO, carbon monoxide, PGI<sub>2</sub>, endothelium-derived hyperpolarizing factor, adrenomedullin, hydrogen sulfide. Furthermore, in spite of increased circulating levels of endogenous vasoconstrictors (noradrenaline, ET-1, angiotensin II), vascular sensitivity to them is significantly reduced<sup>[41]</sup>.

### **Abdominal aorta**

Adaptive response of the abdominal aorta to shear stress, induced by the blood flow in the conditions of the hyperdynamic circulation, may be associated with oxidative stress. Production of ROS, such as superoxide and hydrogen peroxide, which are toxic metabolic products of the cells, leads to non-specific damage of nucleic acids, proteins, lipids, and its other components. ROS regulate vascular tone, endothelial cells sensitivity to oxygen, their growth, proliferation, and apoptosis. Furthermore, they promote the expression of inducible genes by transcription factors, including NF- $\kappa$ B. These genes contribute to the synthesis of chemokines, chemokine receptors, proinflammatory cytokines, and adhesion molecules, inducing an inflammatory response. Potential sources of ROS are various enzyme systems: NAD(P)H oxidase, xanthine oxidase, enzymes of arachidonic acid metabolism (cyclooxygenase and lipoxygenase), and the mitochondrial respiratory chain<sup>[42]</sup>.

Increased levels of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 in the aorta, as a result of oxidative stress, plays an important role in the induction of immune-mediated systemic vascular process in portal hypertension. Particularly, TNF- $\alpha$  induces activation and translocation of NF- $\kappa$ B to the nucleus with activation of NF- $\kappa$ B-dependent target genes. The subsequent increase in expression of connective tissue growth factor may enhance the synthesis of extracellular matrix proteins, particularly, collagen I type, whereas the decrease of the level of MMP-2/TIMP-2 complex (tissue inhibitor of metalloproteinase-2) will contribute to reducing the degradation of extracellular matrix proteins. These processes lead to significant histological changes in the aorta. Its wall thickness decreases, as well as the ratio of medial layer thickness to lumen diameter. Elastic fibers lose their ordered arrangement, and well-marked collagen fibers become more narrow and separated because of the increase of the extracellular matrix in the interstitium of media with a significant decrease in the number of smooth muscle cells<sup>[43,44]</sup>.

The left gastric artery is the first branch of the celiac artery. It is assumed that the hemodynamics in the left gastric artery in portal hypertension may act as the initiator of variceal formation, showing close linkage with variceal recurrence<sup>[45]</sup>.

### **Mesenteric resistance arteries**

Similar infringements also occur in mesenteric resistance arteries. The mechanical stimuli, generated by shear stress, activate endothelial cells and induce hyperproduction of NO and prostaglandins, causing vasodila-

tion<sup>[46]</sup>. The significantly reduced isometric stiffness of blood vessels and their increased elongation may cause structural changes in the internal elastic membrane and increase fenestrations in it<sup>[47]</sup>. This contributes to excessive NO-mediated vascular permeability and angiogenic processes in the mesentery of the small intestine because of the high VEGF and eNOS expression in microvessels located there<sup>[48]</sup>.

### **Portal vein and hepatic artery**

Splanchnic hyperemia leads to increased portal venous inflow. The portal vein becomes dilated under the influence of shear stress. Its intima and media are thickened due to the high content of collagen fibers here, hypertrophy, and hyperplasia of smooth muscle cells, which significantly reduce the vascular wall elasticity<sup>[49]</sup>. At the same time, portal blood flow, supplying the liver, decreases because of collateral circulation, and so-called hepatic arterial buffer response maintains hepatic perfusion constancy. This phenomenon, first described by Lautt<sup>[50]</sup>, was identified in physiological conditions and in various pathological conditions. In liver cirrhosis, it is caused by intrahepatic hypoxia. Oxidative stress contributes to hepatic artery remodeling, which is accompanied by its dilation, decreased elasticity and thinning of the wall, as well as increased expression of adenosine and NO<sup>[51]</sup>. This reduces hepatic arterial vascular resistance and allows to maintain oxygen supply to the liver, providing protection of the organ structure and function<sup>[52]</sup>.

### **Splenic artery and vein**

Significant histopathological changes also occur in the blood vessels of the spleen. Damaged splenic artery intima becomes thicker, and smooth muscle cells grow into it. The internal elastic lamina is stratified, that is accompanied by the destruction of both included in its structure and localized in media elastic fibers.

Smooth muscle cells, randomly located in media, have a different size and morphology, and the content of separating them collagen fibers, as well as the extracellular matrix, increases significantly, causing the "collagenization" of the vascular wall, thickening, and rigidity<sup>[53]</sup>. The splenic vein expanding and its intima and media thickening is due to high content of collagen fibers, hypertrophy, and hyperplasia of smooth muscle cells<sup>[54]</sup>. These pathologic changes in the blood vessels of the spleen lead to a significant reduction of their flexibility.

## **CONCLUSION**

In recent years, defined progress has been made in understanding the mechanisms of hemodynamic disturbances occurring in liver cirrhosis, which are based on portal hypertension. In addition to pathophysiological disorders related to endothelial dysfunction, it was revealed: There is the restructuring of the vasculature, which includes vascular remodeling and angiogenesis. In spite of the fact that these changes are the compensatory-adaptive response to the violated conditions



of blood circulation, taken together, they promote the development and progression of portal hypertension causing severe complications such as bleeding from esophageal varices. Disruption of systemic and organ hemodynamics and the formation of portosystemic collaterals in portal hypertension commence with neovascularization and splanchnic vasodilation due to the hypoxia of the small intestine mucosa. In this regard, the goal of comprehensive treatment may be to influence on the chemokines, proinflammatory cytokines, and angiogenic factors (VEGF, PIGF, PDGF and others) that lead to the development of these disorders. Although pathogenetically reasonable methods of correction of portal hypertension are studied mainly at the molecular, cellular level, and in animal experiments, it can be expected that their clinical implementation will improve the efficiency of conservative therapy aimed at prevention and treatment of its inherent complications.

## REFERENCES

- 1 **Garbuzenko DV.** [Multiorganic hemodynamic disorders in hepatic cirrhosis]. *Ter Arkh* 2007; **79**: 73-77 [PMID: 17460973]
- 2 **Garbuzenko DV, Arefyev NO, Belov DV.** Mechanisms of adaptation of the hepatic vasculature to the deteriorating conditions of blood circulation in liver cirrhosis. *World J Hepatol* 2016; **8**: 665-672 [PMID: 27326313 DOI: 10.4254/wjh.v8.i16.665]
- 3 **Fernandez M.** Molecular pathophysiology of portal hypertension. *Hepatology* 2015; **61**: 1406-1415 [PMID: 25092403 DOI: 10.1002/hep.27343]
- 4 **Gibbons GH, Dzau VJ.** The emerging concept of vascular remodeling. *N Engl J Med* 1994; **330**: 1431-1438 [PMID: 8159199 DOI: 10.1056/NEJM199405193302008]
- 5 **Davies PF, Barbee KA, Volin MV, Robotewskyj A, Chen J, Joseph L, Griem ML, Wernick MN, Jacobs E, Polacek DC, dePaola N, Barakat AI.** Spatial relationships in early signaling events of flow-mediated endothelial mechanotransduction. *Annu Rev Physiol* 1997; **59**: 527-549 [PMID: 9074776 DOI: 10.1146/annurev.physiol.59.1.527]
- 6 **Ngai CY, Yao X.** Vascular responses to shear stress: the involvement of mechanosensors in endothelial cells. *Open Circ Vasc J* 2010; **3**: 85-94 [DOI: 10.2174/1877382601003010085]
- 7 **Mulvany MJ.** Small artery remodelling in hypertension. *Basic Clin Pharmacol Toxicol* 2012; **110**: 49-55 [PMID: 21733124 DOI: 10.1111/j.1742-7843.2011.00758.x]
- 8 **Briones AM, González JM, Somoza B, Giraldo J, Daly CJ, Vila E, González MC, McGrath JC, Arribas SM.** Role of elastin in spontaneously hypertensive rat small mesenteric artery remodelling. *J Physiol* 2003; **552**: 185-195 [PMID: 12844513 DOI: 10.1113/jphysiol.2003.046904]
- 9 **Folkman J.** Angiogenesis: an organizing principle for drug discovery? *Nat Rev Drug Discov* 2007; **6**: 273-286 [PMID: 17396134 DOI: 10.1038/nrd2115]
- 10 **Semenza GL.** O<sub>2</sub>-regulated gene expression: transcriptional control of cardiorespiratory physiology by HIF-1. *J Appl Physiol* (1985) 2004; **96**: 1173-1177; discussion 1170-1172 [PMID: 14766767]
- 11 **Skuli N, Majmudar AJ, Krock BL, Mesquita RC, Mathew LK, Quinn ZL, Runge A, Liu L, Kim MN, Liang J, Schenkel S, Yodh AG, Keith B, Simon MC.** Endothelial HIF-2 $\alpha$  regulates murine pathological angiogenesis and revascularization processes. *J Clin Invest* 2012; **122**: 1427-1443 [PMID: 22426208 DOI: 10.1172/JCI57322]
- 12 **Chen Z, Lai TC, Jan YH, Lin FM, Wang WC, Xiao H, Wang YT, Sun W, Cui X, Li YS, Fang T, Zhao H, Padmanabhan C, Sun R, Wang DL, Jin H, Chau GY, Huang HD, Hsiao M, Shyy JY.** Hypoxia-responsive miRNAs target argonaute 1 to promote angiogenesis. *J Clin Invest* 2013; **123**: 1057-1067 [PMID: 23426184 DOI: 10.1172/JCI65344]
- 13 **Ferrara N.** The role of VEGF in the regulation of physiological and pathological angiogenesis. *EXS* 2005; **(94)**: 209-231 [PMID: 15617481 DOI: 10.1007/3-7643-7311-3\_15]
- 14 **Klein S, Roghani M, Rifkin DB.** Fibroblast growth factors as angiogenesis factors: new insights into their mechanism of action. *EXS* 1997; **79**: 159-192 [PMID: 9002232 DOI: 10.1007/978-3-034-8-9006-9\_7]
- 15 **Hellberg C, Ostman A, Heldin CH.** PDGF and vessel maturation. *Recent Results Cancer Res* 2010; **180**: 103-114 [PMID: 20033380 DOI: 10.1007/978-3-540-78281-0\_7]
- 16 **Kim I, Moon SO, Koh KN, Kim H, Uhm CS, Kwak HJ, Kim NG, Koh GY.** Molecular cloning, expression, and characterization of angiopoietin-related protein. angiopoietin-related protein induces endothelial cell sprouting. *J Biol Chem* 1999; **274**: 26523-26528 [PMID: 10473614 DOI: 10.1074/jbc.274.37.26523]
- 17 **Reynolds LE, Wyder L, Lively JC, Taverna D, Robinson SD, Huang X, Sheppard D, Hynes RO, Hodivala-Dilke KM.** Enhanced pathological angiogenesis in mice lacking beta3 integrin or beta3 and beta5 integrins. *Nat Med* 2002; **8**: 27-34 [PMID: 11786903 DOI: 10.1038/nm0102-27]
- 18 **Kevil CG, Payne DK, Mire E, Alexander JS.** Vascular permeability factor/vascular endothelial cell growth factor-mediated permeability occurs through disorganization of endothelial junctional proteins. *J Biol Chem* 1998; **273**: 15099-15103 [PMID: 9614120 DOI: 10.1074/jbc.273.24.15099]
- 19 **Greenaway J, Lawler J, Moorehead R, Bornstein P, Lamarre J, Petrik J.** Thrombospondin-1 inhibits VEGF levels in the ovary directly by binding and internalization via the low density lipoprotein receptor-related protein-1 (LRP-1). *J Cell Physiol* 2007; **210**: 807-818 [PMID: 17154366 DOI: 10.1002/jcp.20904]
- 20 **Eriksson K, Magnusson P, Dixelius J, Claesson-Welsh L, Cross MJ.** Angiostatin and endostatin inhibit endothelial cell migration in response to FGF and VEGF without interfering with specific intracellular signal transduction pathways. *FEBS Lett* 2003; **536**: 19-24 [PMID: 12586331 DOI: 10.1016/S0014-5793(03)00003-6]
- 21 **Nie L, Guo X, Esmailzadeh L, Zhang J, Asadi A, Collinge M, Li X, Kim JD, Woolls M, Jin SW, Dubrac A, Eichmann A, Simons M, Bender JR, Sadeghi MM.** Transmembrane protein ESDN promotes endothelial VEGF signaling and regulates angiogenesis. *J Clin Invest* 2013; **123**: 5082-5097 [PMID: 24177422 DOI: 10.1172/JCI67752]
- 22 **Abraldes JG, Iwakiri Y, Loureiro-Silva M, Haq O, Sessa WC, Groszmann RJ.** Mild increases in portal pressure upregulate vascular endothelial growth factor and endothelial nitric oxide synthase in the intestinal microcirculatory bed, leading to a hyperdynamic state. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G980-G987 [PMID: 16603731 DOI: 10.1152/ajpgi.00336.2005]
- 23 **Angermayr B, Mejias M, Gracia-Sancho J, Garcia-Pagan JC, Bosch J, Fernandez M.** Heme oxygenase attenuates oxidative stress and inflammation, and increases VEGF expression in portal hypertensive rats. *J Hepatol* 2006; **44**: 1033-1039 [PMID: 16458992 DOI: 10.1016/j.jhep.2005.09.021]
- 24 **Kobus K, Kopycinska J, Kozłowska-Wiechowska A, Urasinska E, Kempinska-Podhorodecka A, Haas TL, Milkiewicz P, Milkiewicz M.** Angiogenesis within the duodenum of patients with cirrhosis is modulated by mechanosensitive Kruppel-like factor 2 and microRNA-126. *Liver Int* 2012; **32**: 1222-1232 [PMID: 22574900 DOI: 10.1111/j.1478-3231.2012.02791.x]
- 25 **Huang HC, Haq O, Utsumi T, Sethasine S, Abraldes JG, Groszmann RJ, Iwakiri Y.** Intestinal and plasma VEGF levels in cirrhosis: the role of portal pressure. *J Cell Mol Med* 2012; **16**: 1125-1133 [PMID: 21801303 DOI: 10.1111/j.1582-4934.2011.01399.x]
- 26 **Fernández M, Semela D, Bruix J, Colle I, Pinzani M, Bosch J.** Angiogenesis in liver disease. *J Hepatol* 2009; **50**: 604-620 [PMID: 19157625 DOI: 10.1016/j.jhep.2008.12.011]
- 27 **Gana JC, Serrano CA, Ling SC.** Angiogenesis and portal-systemic collaterals in portal hypertension. *Ann Hepatol* 2016; **15**: 303-313

- [PMID: 27049484 DOI: 10.5604/16652681.1198799]
- 28 **Carmeliet P.** Manipulating angiogenesis in medicine. *J Intern Med* 2004; **255**: 538-561 [PMID: 15078497 DOI: 10.1111/j.1365-2796.2003.01297.x]
  - 29 **Fernandez M,** Vizzutti F, Garcia-Pagan JC, Rodes J, Bosch J. Anti-VEGF receptor-2 monoclonal antibody prevents portal-systemic collateral vessel formation in portal hypertensive mice. *Gastroenterology* 2004; **126**: 886-894 [PMID: 14988842 DOI: 10.1053/j.gastro.2003.12.012]
  - 30 **Fernandez M,** Mejias M, Angermayr B, Garcia-Pagan JC, Rodés J, Bosch J. Inhibition of VEGF receptor-2 decreases the development of hyperdynamic splanchnic circulation and portal-systemic collateral vessels in portal hypertensive rats. *J Hepatol* 2005; **43**: 98-103 [PMID: 15893841 DOI: 10.1016/j.jhep.2005.02.022]
  - 31 **Angermayr B,** Fernandez M, Mejias M, Gracia-Sancho J, Garcia-Pagan JC, Bosch J. NAD(P)H oxidase modulates angiogenesis and the development of portosystemic collaterals and splanchnic hyperaemia in portal hypertensive rats. *Gut* 2007; **56**: 560-564 [PMID: 16854998 DOI: 10.1136/gut.2005.088013]
  - 32 **Chan CC.** Portal-systemic collaterals and angiogenesis. *J Chin Med Assoc* 2009; **72**: 223-224 [PMID: 19467943 DOI: 10.1016/S1726-4901(09)70060-7]
  - 33 **Albillos A,** Bañares R, González M, Catalina MV, Pastor O, Gonzalez R, Ripoll C, Bosch J. The extent of the collateral circulation influences the postprandial increase in portal pressure in patients with cirrhosis. *Gut* 2007; **56**: 259-264 [PMID: 16837532 DOI: 10.1136/gut.2006.095240]
  - 34 **Arora A,** Rajesh S, Meenakshi YS, Sureka B, Bansal K, Sarin SK. Spectrum of hepatofugal collateral pathways in portal hypertension: an illustrated radiological review. *Insights Imaging* 2015; **6**: 559-572 [PMID: 26337049 DOI: 10.1007/s13244-015-0419-8]
  - 35 **Yang Z,** Tian L, Peng L, Qiu F. Immunohistochemical analysis of growth factor expression and localization in gastric coronary vein of cirrhotic patients. *J Tongji Med Univ* 1996; **16**: 229-233 [PMID: 9389088 DOI: 10.1007/BF02888113]
  - 36 **Hashizume M,** Kitano S, Sugimachi K, Sueishi K. Three-dimensional view of the vascular structure of the lower esophagus in clinical portal hypertension. *Hepatology* 1988; **8**: 1482-1487 [PMID: 3192160 DOI: 10.1002/hep.1840080603]
  - 37 **Turmakhanov ST,** Asadulaev ShM, Akhmetkaliev MN. [Morpho-functional Changes of the Azygos Vein and Other Veins of the Gastroesophageal Zone in Portal Hypertension]. *Ann hir gepatol* 2008; **13**: 58-65
  - 38 **Vianna A,** Hayes PC, Moscoso G, Driver M, Portmann B, Westaby D, Williams R. Normal venous circulation of the gastroesophageal junction. A route to understanding varices. *Gastroenterology* 1987; **93**: 876-889 [PMID: 3623028 DOI: 10.1016/0016-5085(87)90453-7]
  - 39 **Noda T.** Angioarchitectural study of esophageal varices. With special reference to variceal rupture. *Virchows Arch A Pathol Anat Histopathol* 1984; **404**: 381-392 [PMID: 6437071 DOI: 10.1007/BF00695222]
  - 40 **Gaba RC,** Couture PM, Lakhoo J. Gastroesophageal Variceal Filling and Drainage Pathways: An Angiographic Description of Afferent and Efferent Venous Anatomic Patterns. *J Clin Imaging Sci* 2015; **5**: 61 [PMID: 26713177 DOI: 10.4103/2156-7514.170730]
  - 41 **Gracia-Sancho J,** Maeso-Díaz R, Bosch J. Pathophysiology and a Rational Basis of Therapy. *Dig Dis* 2015; **33**: 508-514 [PMID: 26159267 DOI: 10.1159/000374099]
  - 42 **Libby P.** Inflammatory mechanisms: the molecular basis of inflammation and disease. *Nutr Rev* 2007; **65**: S140-S146 [PMID: 18240538 DOI: 10.1301/nr.2007.dec.S140-S146]
  - 43 **de Las Heras N,** Aller MA, Martín-Fernández B, Miana M, Ballesteros S, Regadera J, Cachofeiro V, Arias J, Lahera V. A wound-like inflammatory aortic response in chronic portal hypertensive rats. *Mol Immunol* 2012; **51**: 177-187 [PMID: 22463791 DOI: 10.1016/j.molimm.2012.03.016]
  - 44 **Fernández-Varo G,** Ros J, Morales-Ruiz M, Cejudo-Martín P, Arroyo V, Solé M, Rivera F, Rodés J, Jiménez W. Nitric oxide synthase 3-dependent vascular remodeling and circulatory dysfunction in cirrhosis. *Am J Pathol* 2003; **162**: 1985-1993 [PMID: 12759254 DOI: 10.1016/S0002-9440(10)64331-3]
  - 45 **Kiyono S,** Maruyama H, Kondo T, Sekimoto T, Shimada T, Takahashi M, Yokosuka O. Hemodynamic effect of the left gastric artery on esophageal varices in patients with cirrhosis. *J Gastroenterol* 2016; **51**: 900-909 [PMID: 26781661 DOI: 10.1007/s00535-015-1157-x]
  - 46 **Piva A,** Zampieri F, Di Pascoli M, Gatta A, Sacerdoti D, Bolognesi M. Mesenteric arteries responsiveness to acute variations of wall shear stress is impaired in rats with liver cirrhosis. *Scand J Gastroenterol* 2012; **47**: 1003-1013 [PMID: 22774919 DOI: 10.3109/00365521.2012.703231]
  - 47 **Resch M,** Wiest R, Moleda L, Fredersdorf S, Stoelcker B, Schroeder JA, Schölmerich J, Endemann DH. Alterations in mechanical properties of mesenteric resistance arteries in experimental portal hypertension. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G849-G857 [PMID: 19696142 DOI: 10.1152/ajpgi.00084.2009]
  - 48 **Geerts AM,** De Vriese AS, Vanheule E, Van Vlierbergh H, Mortier S, Cheung KJ, Demetter P, Lameire N, De Vos M, Colle I. Increased angiogenesis and permeability in the mesenteric microvasculature of rats with cirrhosis and portal hypertension: an in vivo study. *Liver Int* 2006; **26**: 889-898 [PMID: 16911473 DOI: 10.1111/j.1478-3231.2006.01308.x]
  - 49 **Wen B,** Liang J, Deng X, Chen R, Peng P. Effect of fluid shear stress on portal vein remodeling in a rat model of portal hypertension. *Gastroenterol Res Pract* 2015; **2015**: 545018 [PMID: 25892988 DOI: 10.1155/2015/545018]
  - 50 **Laufer WW.** Mechanism and role of intrinsic regulation of hepatic arterial blood flow: hepatic arterial buffer response. *Am J Physiol* 1985; **249**: G549-G556 [PMID: 3904482]
  - 51 **Moeller M,** Thonig A, Pohl S, Ripoll C, Zipprich A. Hepatic arterial vasodilation is independent of portal hypertension in early stages of cirrhosis. *PLoS One* 2015; **10**: e0121229 [PMID: 25793622 DOI: 10.1371/journal.pone.0121229]
  - 52 **Eipel C,** Abshagen K, Vollmar B. Regulation of hepatic blood flow: the hepatic arterial buffer response revisited. *World J Gastroenterol* 2010; **16**: 6046-6057 [PMID: 21182219 DOI: 10.3748/wjg.v16.i48.6046]
  - 53 **Li T,** Ni JY, Qi YW, Li HY, Zhang T, Yang Z. Splenic vasculopathy in portal hypertension patients. *World J Gastroenterol* 2006; **12**: 2737-2741 [PMID: 16718761 DOI: 10.3748/wjg.v12.i17.2737]
  - 54 **Yang Z,** Zhang L, Li D, Qiu F. Pathological morphology alteration of the splanchnic vascular wall in portal hypertensive patients. *Chin Med J (Engl)* 2002; **115**: 559-562 [PMID: 12133298]

**P- Reviewer:** Dong L, Mortensen C **S- Editor:** Gong ZM

**L- Editor:** A **E- Editor:** Li D



## Basic Study

# Fractionation of gamma-glutamyltransferase in patients with nonalcoholic fatty liver disease and alcoholic liver disease

Shigeo Sueyoshi, Setsu Sawai, Mamoru Satoh, Masanori Seimiya, Kazuyuki Sogawa, Atsushi Fukumura, Mikihiro Tsutsumi, Fumio Nomura

Shigeo Sueyoshi, Setsu Sawai, Masanori Seimiya, Kazuyuki Sogawa, Fumio Nomura, Department of Molecular Diagnosis, Graduate School of Medicine, Chiba University, Chuo-ku, Chiba 260-8670, Japan

Mamoru Satoh, Fumio Nomura, Clinical Proteomics Research Center, Chiba University Hospital, Chuo-ku, Chiba 260-8670, Japan

Atsushi Fukumura, Mikihiro Tsutsumi, Department of Hepatology, Kanazawa Medical University, Kahoku, Ishikawa 920-0293, Japan

**Author contributions:** Sueyoshi S, Sawai S, Satoh M, Seimiya M, Sogawa K, Fukumura A, Tsutsumi M and Nomura F designed research; Sueyoshi S and Satoh M performed research, contributed new analytic tools and analyzed data; Nomura F wrote the paper.

**Institutional review board statement:** All routine liver biopsy specimens and blood samples from the patients were taken after informed consent and ethical permission was obtained for participation in the study.

**Conflict-of-interest statement:** The authors declare that they have no competing interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Fumio Nomura, MD, Professor, Department of Molecular Diagnosis, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. [fnomura@faculty.chiba-u.jp](mailto:fnomura@faculty.chiba-u.jp)

Telephone: +81-43-2262324  
Fax: +81-43-2262324

Received: June 21, 2016  
Peer-review started: June 23, 2016  
First decision: August 10, 2016  
Revised: September 13, 2016  
Accepted: November 1, 2016  
Article in press: November 2, 2016  
Published online: December 28, 2016

## Abstract

### AIM

To assess how serum gamma-glutamyltransferase (GGT) fractions vary in patients with alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD).

### METHODS

Serum samples were obtained from 14 patients with biopsy-proven alcoholic liver diseases and 9 patients with biopsy proven non-alcoholic fatty liver disease. In addition to these biopsy-proven cases, 16 obese (body mass index > 25) patients without any history of alcohol consumption but with a fatty liver on ultrasound examination and with elevated GGT were included for an additional analysis. Serum GGT fractionation was conducted by high-performance gel filtration liquid chromatography and was separated into the four fractions, big-GGT, medium-GGT, small-GGT (s-GGT), and free-GGT (f-GGT).

### RESULTS

The results were expressed as a ratio of each fraction including the total GGT (t-GGT). The s-GGT/t-GGT ratios

were lowest for the control group and highest for the ALD group. The differences between the control and NAFLD groups and also between the NAFLD and ALD groups were statistically significant. In contrast, the f-GGT/t-GGT ratios were highest in the control group and lowest in the ALD group, with the differences being statistically significant. As a result, the s-GGT/f-GGT ratios were markedly increased in the NAFLD group as compared with the control group. The increase of the s-GGT/t-GGT ratios, the decrease of the f-GGT/t-GGT ratios, and the increase of s-GGT/f-GGT ratios as compared with the control group subjects were also found in obese patients with clinically diagnosed fatty change of the liver.

## CONCLUSION

Serum GGT fractionation by high-performance gel filtration liquid chromatography is potentially useful for the differential diagnosis of ALD and NAFLD.

**Key words:** Gamma-glutamyltransferase; f-GGT/t-GGT ratios; Alcoholic liver disease; Non-alcoholic fatty liver disease; Gel filtration liquid chromatography

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The aim of this study was to assess whether fractionation of serum gamma-glutamyltransferase (GGT) into four fractions by high-performance gel filtration chromatography is useful for the differential diagnosis of alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD). In patients with ALD, small-GGT (s-GGT)/total GGT (t-GGT) ratios were significantly higher and free-GGT (f-GGT)/t-GGT ratios were lower than in those in NAFLD. Consequently, there were marked differences in the s-GGT/f-GGT ratio between ALD and NAFLD. These preliminary results indicate that a large-scale study to clarify the diagnostic values of serum GGT fractionation in the differential diagnosis of ALD and NAFLD is warranted.

Sueyoshi S, Sawai S, Satoh M, Seimiya M, Sogawa K, Fukumura A, Tsutsumi M, Nomura F. Fractionation of gamma-glutamyltransferase in patients with nonalcoholic fatty liver disease and alcoholic liver disease. *World J Hepatol* 2016; 8(36): 1610-1616 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i36/1610.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i36.1610>

## INTRODUCTION

Gamma-glutamyltransferase (GGT) [(5-glutamyl)-peptide: Amino acid 5-glutamyltransferase, EC 2.3.2.2] is present in many tissues, including the kidneys, pancreas, and liver<sup>[1]</sup>. GGT in serum is mainly derived from the liver and this enzyme is often used as a marker of hepatobiliary diseases. Although sensitive, GGT elevation is not specific enough for the differential diagnosis of hepatobiliary disorders. GGT is present in serum in multiple forms in molecular complexes that vary in size, charge, and density<sup>[2]</sup>. These forms were evaluated in the past by

electrophoretic methods to enhance the diagnostic value of GGT measurements<sup>[3]</sup>. These methods, however, were not sensitive enough to facilitate the differential diagnosis of liver diseases. To overcome this limitation, Franzini *et al.*<sup>[4]</sup> developed a high-performance liquid chromatography method to quantify four plasma GGT fractions on the basis of molecular size exclusion chromatography, followed by a GGT-specific post-column reaction.

GGT is widely used as a marker of excessive alcohol intake in patients with alcoholic liver disease (ALD)<sup>[5]</sup>. Induction of hepatic microsomal GGT by chronic alcohol consumption may account, at least in part, for GGT elevation in alcoholics<sup>[6]</sup>. In addition, serum GGT levels are often increased in patients with non-alcoholic fatty liver disease (NAFLD)<sup>[7]</sup>.

Distinguishing ALD from NAFLD is difficult because self-reported history of alcohol consumption is unreliable. Detection of patients with high alcohol intake by general practitioners is not necessarily easy<sup>[8,9]</sup>. Accurate diagnosis of NAFLD relies on a liver biopsy; hence, a less-invasive evaluation strategy is desirable<sup>[10]</sup>.

The aim of this preliminary study was to assess how serum GGT fraction patterns, obtained by a high-performance liquid chromatography method, vary in patients with ALD and NAFLD.

## MATERIALS AND METHODS

### Patients and blood sample preparation

Serum samples were obtained from 23 patients with biopsy-proven NAFLD or ALD at the Department of Hepatology, Kanazawa Medical University. Fourteen patients (11 males and 3 females, age  $53.0 \pm 10.6$  years) with biopsy-proven ALD (3 patients with fatty liver, 2 alcoholic fibrosis, 5 alcoholic hepatitis, 3 alcoholic hepatitis, and 3 liver cirrhosis) and 9 patients (6 males and 3 females, age  $57.2 \pm 9.86$  years) with biopsy-proven NAFLD (6 patients with non-alcoholic steatohepatitis and 3 with simple steatosis) were included in the study. In addition to these biopsy-proven cases, 16 obese (body mass index  $> 25$ ) patients (16 males, age  $48.3 \pm 6.97$  years) without any history of alcohol consumption but with a fatty liver on ultrasound examination and with elevated GGT were included for an additional analysis. Subjects suspected to have autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, Wilson's disease and alpha 1 anti-trypsin deficiency were excluded from this study. Serum samples were also obtained from 10 apparently healthy and age-matched subjects for a control group. The clinical data for these 49 patients are presented in Table 1. All samples were frozen by liquid nitrogen and were stored at  $-80^\circ\text{C}$  until analysis. Written informed consent was obtained from all the patients. The ethics committees of each institute approved the protocol.

### GGT fractionation by high-performance gel filtration chromatography

Serum GGT fractionation by high-performance liquid chromatography was conducted on the basis of the



**Table 1** Comparison of the characteristics of the controls and study patients with biopsy proven

	Biopsy-proven ALD ( <i>n</i> = 14)	Biopsy-proven NAFLD ( <i>n</i> = 9)	Clinically diagnosed NAFLD ( <i>n</i> = 16)	Controls ( <i>n</i> = 10)	<i>P</i> value Biopsy-proven ALD <i>vs</i> NAFLD
Age (yr)	53 (45-60)	54 (49-66)	49 (43-54)	51 (39-60)	NS
Gender (male:female)	11:3	6:3	16:0	10:0	NS
AST (U/L)	74 (41-105)	45 (38-95)	32 (24-42)	19 (15-21)	NS
ALT (U/L)	19 (13-22)	35 (27-67)	48 (35-74.5)	18 (14-20)	0.0166
Albumin (g/dL)	4.1 (3.2-4.4)	4.6 (4.1-4.7)	4.4 (4.3-4.6)	4.6 (4.3-4.8)	NS
Total bilirubin (mg/dL)	1 (0.6-3.1)	0.7 (0.6-0.8)	0.8 (0.6-0.9)	0.9 (0.6-1.2)	NS
Triglyceride (mg/dL)	186 (99-284)	184 (101-298)	187 (102-212)	130 (111-139)	NS
HDL-cholesterol (mg/dL)	40 (30-56)	43 (29-52)	42 (39-54)	50 (42-56)	NS
LDL-cholesterol (mg/dL)	75 (43-105)	116 (97-153)	122 (98-157)	113 (101-136)	0.0181
GGT (U/L)	368 (296-421)	94 (62-170)	74 (57-112)	24 (19-42)	0.0018
b/t-GGT ratio	0.1 (0.07-0.13)	0.16 (0.13-0.26)	0.18 (0.14-0.24)	0.12 (0.08-0.15)	NS
m/t-GGT ratio	0.04 (0.02-0.05)	0.04 (0.02-0.05)	0.04 (0.03-0.05)	0.02 (0.02-0.03)	NS
s/t-GGT ratio	0.78 (0.65-0.80)	0.55 (0.49-0.64)	0.54 (0.49-0.60)	0.4 (0.31-0.44)	0.0158
f/t-GGT ratio	0.12 (0.08-0.15)	0.18 (0.15-0.26)	0.23 (0.15-0.29)	0.45 (0.37-0.63)	0.0055
b/s-GGT ratio	0.14 (0.09-0.20)	0.33 (0.21-0.53)	0.35 (0.24-0.51)	0.29 (0.19-0.52)	0.0456
s/f-GGT ratio	6.68 (3.67-10.58)	3.1 (2.18-4.32)	2.09 (1.69-3.98)	0.96 (0.48-1.11)	0.0086

Data are presented as median (25<sup>th</sup>-75<sup>th</sup> percentile). Statistical analysis: Wilcoxon-Mann-Whitney *U* test. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyltransferase; ALD: Alcoholic liver disease; NAFLD: Non-alcoholic fatty liver disease; NS: Not significant; HDL: High density lipoprotein; LDL: Low density lipoprotein; b-GGT: Big-GGT; m-GGT: Medium-GGT; s-GGT: Small-GGT; f-GGT: Free-GGT.

methods described by Franzini *et al.*<sup>[4]</sup>. A 100- $\mu$ L aliquot of serum was injected into a Superose 6 HR 10/300 GL column (diameter 10 mm, length 300-310 mm; GE Healthcare, Parsippany, NJ, United States) attached to a LC-10AD high-performance liquid chromatography system (Shimadzu Co., Kyoto, Japan). Gel filtration chromatography was performed using the isocratic mode with a binary mobile phase composed of 0.1 mol/L sodium phosphate buffer (pH 7.4), containing 0.2 mol/L NaCl, 0.1 mmol/L EDTA, and 5.4 mmol/L Gly-Gly to support the GGT reaction<sup>[11,12]</sup>. The flow rate of the mobile phase was 0.5 mL/min. Total run time was 60 min, and fractions were collected every 30 s. Serum containing high GGT (> 150 U/L) levels was difficult to separate into small-GGT (s-GGT) and free-GGT (f-GGT) fractions. To make an appropriate comparison of elution profiles of GGT fractions, serum samples with high-GGT-level sera were diluted to approximate 30-50 U/L with the mobile phase solvent prior to analysis. All results were expressed as compared with total GGT activity subjected to the high-performance liquid chromatography analysis.

#### Measurement of serum total and fractionated GGT activities

Serum total GGT (t-GGT) activities were determined using an enzymatic assay (Serotec Co. Ltd., Sapporo, Japan) with an autoanalyzer (JCA-2250; JEOL Ltd., Tokyo, Japan). This measurement conformed to the International Federation of Clinical Chemistry reference mode for GGT, implemented at a serum volume of 1.2  $\mu$ L and a reagent volume of 75  $\mu$ L<sup>[4]</sup>. Moreover, GGT activity in each fraction improved in the sensitometer mode, which was implemented at a serum volume of 25  $\mu$ L and a reagent volume of 40  $\mu$ L. The limit of quantitation at a 10% coefficient of variation was 0.102 U/L.

#### Statistical analysis

Total GGT activity and those in each high-performance liquid chromatography fraction in the NAFLD and ALD groups were analyzed using the non-parametric Wilcoxon-Mann-Whitney *U* test. Between-group comparisons of the laboratory data were made with Spearman's rank correlation coefficient. *P* values of < 0.05 were considered significant.

## RESULTS

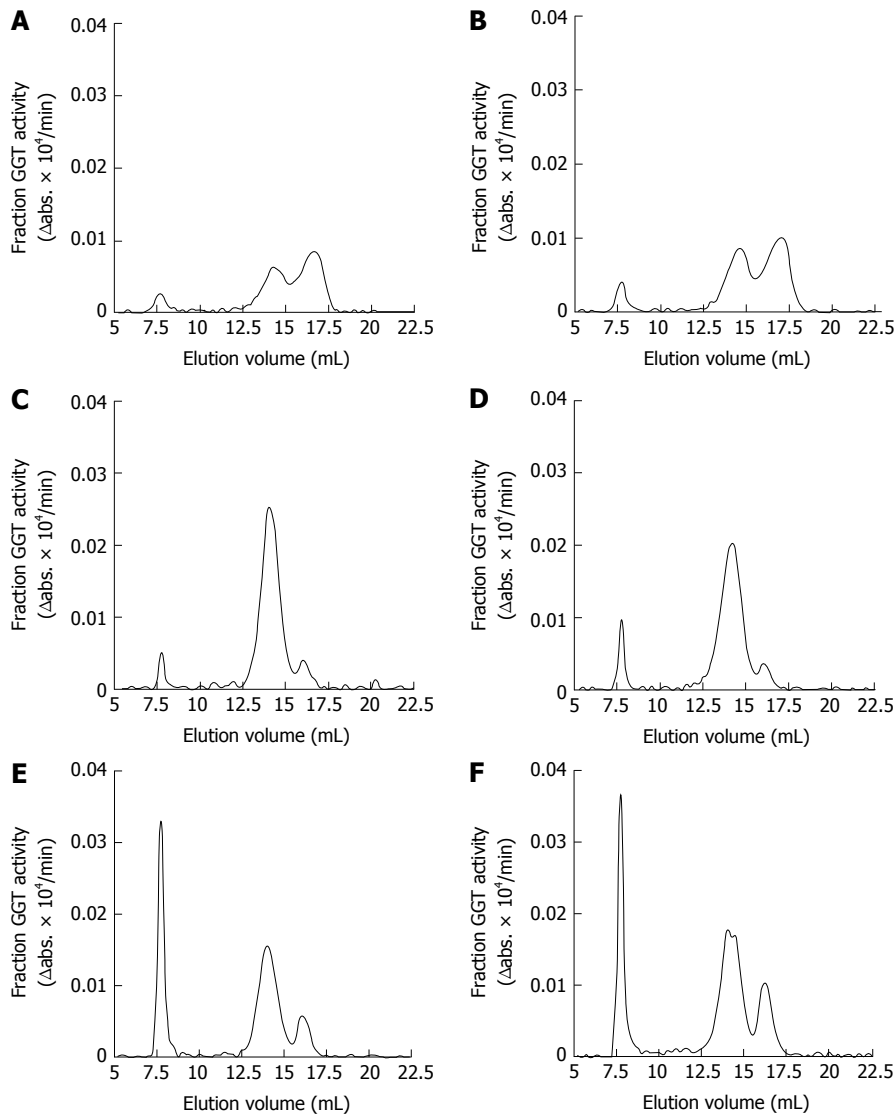
#### Elution pattern of the GGT fractions

Figure 1A-E shows the GGT-specific elution profiles of representative serum samples obtained from the control group (Figure 1A and B), and patients with ALD (Figure 1C and D) and NAFLD (Figure 1E and F). Three distinct peaks and a low one were found by fractionation every 30 s. The area of the peaks was calculated using a blank for the average GGT eluted with elution volumes of 5.00-6.25 mL. Each GGT fraction was calculated by dividing the area of each single peak. As indicated in Figure 2, it was confirmed that the area under the chromatogram curve was proportional to the GGT enzyme activities.

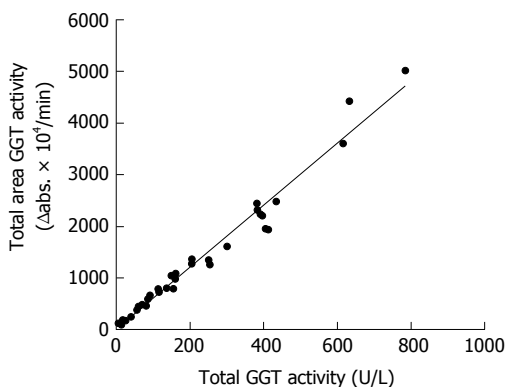
On the basis of the molecular weight calibration curve (data not shown), these four peaks are equivalent to big-GGT (b-GGT) (MW > 2000 kDa, eluted between 6.25-9.50 mL), medium GGT (m-GGT) (MW 940 Da, eluted between 9.5-12.25 mL), s-GGT (MW 140 kDa, eluted between 12.25-15.5 mL) and f-GGT (MW 70 kDa, eluted between 15.5-20 mL), respectively.

#### GGT fraction profiles in four patient groups and the control group

The s-GGT/t-GGT ratios were lowest for the control group

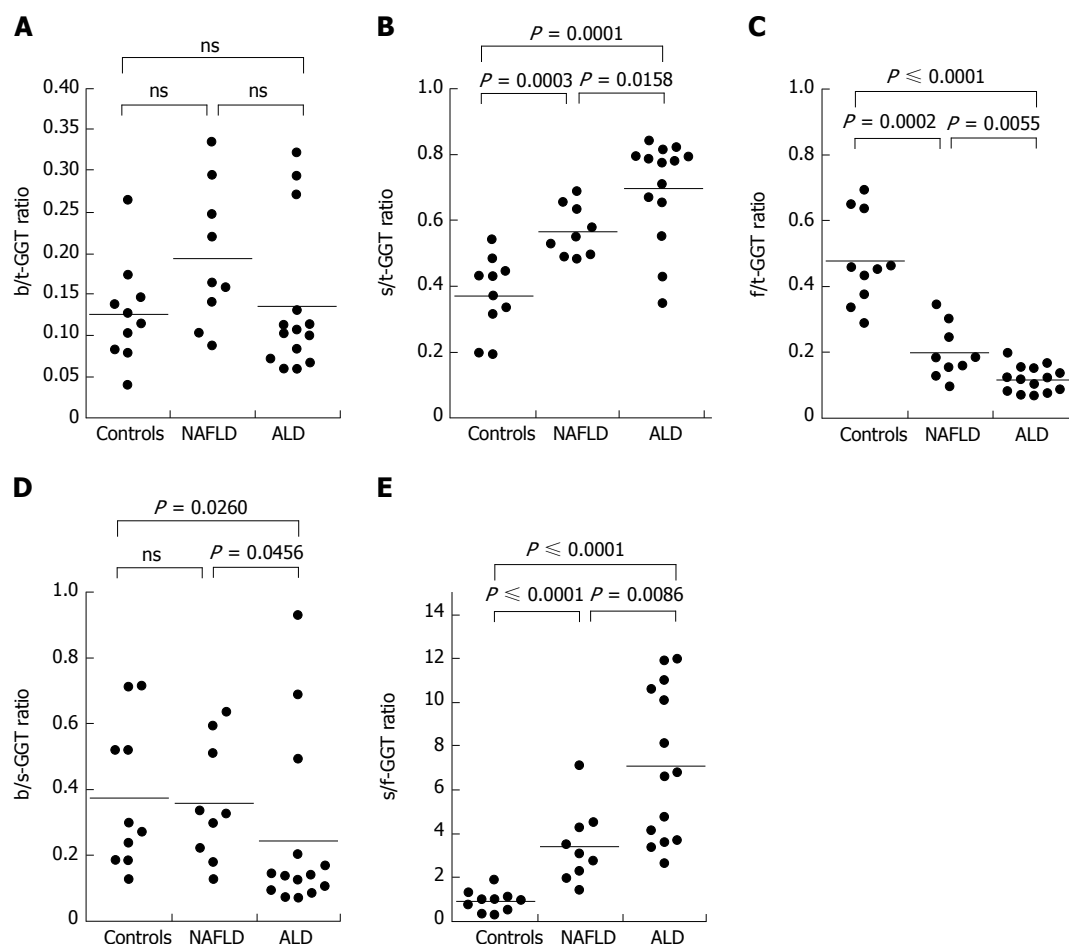


**Figure 1** Gamma-glutamyltransferase-specific elution profiles of representative serum samples (two of each) obtained from the control group subjects (A and B), patients with alcoholic liver disease (C and D), and patients with non-alcoholic fatty liver disease (E and F). On the basis of the molecular weight calibration curve (data not shown), these four peaks are equivalent to big-GGT (MW > 2000 kDa, eluted between 6.25-9.50 mL), medium GGT (MW 940 kDa, eluted between 9.5-12.25 mL), small GGT (MW 140 kDa, eluted between 12.25-15.5 mL) and free-GGT (MW 70 kDa, eluted between 15.5-20 mL), respectively. GGT: Gamma-glutamyltransferase.



**Figure 2** Linear correlation between the total area under the chromatograph curve multiplied by serum dilution factors and total serum gamma-glutamyltransferase activity. Elution volume (between 12-38 mL).  $y = 5.9667x + 15.303$ ;  $r = 0.988$ ;  $P < 0.001$ ;  $n = 49$ . GGT: Gamma-glutamyltransferase.

and highest for the ALD group. The differences between the control and NAFLD groups and also between the NAFLD and ALD groups were statistically significant, as indicated in Figure 3B. In contrast, the f-GGT/t-GGT ratios were highest in the control group and lowest in the ALD group, with the differences being statistically significant (Figure 3C). As a result, the s-GGT/f-GGT ratios were markedly increased in the NAFLD group as compared with the control group (Figure 3E). The increase of the s-GGT/t-GGT ratios, the decrease of the f-GGT/t-GGT ratios, and the increase of s-GGT/f-GGT ratios as compared with the control group subjects were also found in obese patients with clinically diagnosed fatty change of the liver (Figure 4C-E). There was also a positive correlation between b-GGT activity and levels of low-density lipoprotein and apolipoprotein B in the NAFLD group, but not in the ALD



**Figure 3** Serum b-GGT/t-GGT, s-GGT/t-GGT, f-GGT/t-GGT, b-GGT/s-GGT and s-GGT/f-GGT ratios in patients with biopsy-proven non-alcoholic fatty liver disease, biopsy-proven alcoholic liver disease and the controls group. ALD: Alcoholic liver disease; NAFLD: Non-alcoholic fatty liver disease; GGT: Gamma-glutamyltransferase; b-GGT: Big-GGT; t-GGT: Total-GGT; s-GGT: Small-GGT; f-GGT: Free-GGT; ns: No significant differences.

group, in the present study (data not shown).

## DISCUSSION

Franzini *et al*<sup>[4]</sup> described a high-performance gel filtration chromatography method for plasma GGT fraction analysis. This method permitted the quantification of four GGT fractions; b-GGT, m-GGT, s-GGT (likely lipoprotein-bound, molecular masses > 2000, 940 and 140 kDa, respectively) and a f-GGT fraction.

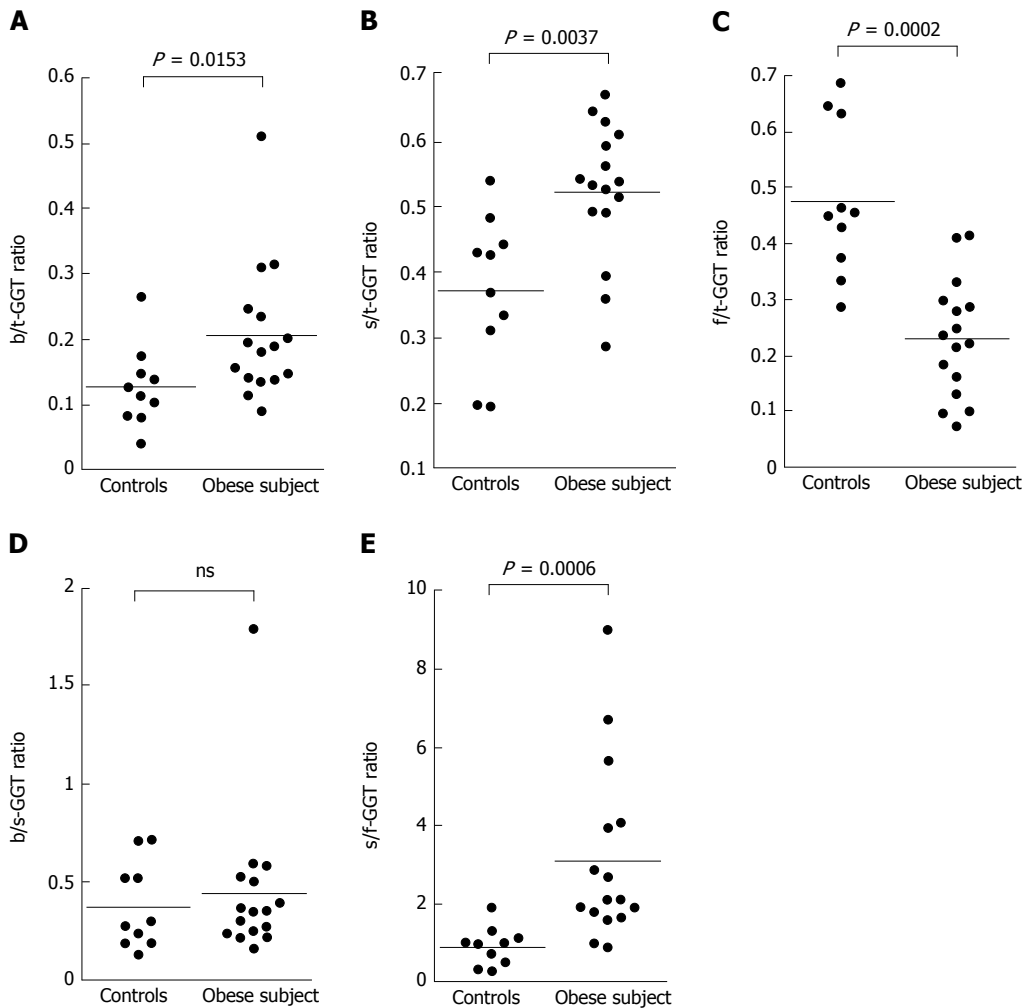
It is common for serum GGT levels to be elevated in patients with ALD<sup>[5]</sup> or obesity-related NAFLD<sup>[7]</sup>. Patients with elevated serum GGT levels who are obese and are also excessive, habitual alcohol drinkers are frequently encountered in clinical practice. It is necessary to have non-invasive measures to assess the relative contribution of overweight and excessive alcohol consumption on GGT elevations. We wondered how serum GGT fraction patterns obtained by the high-performance liquid chromatography method vary in patients with ALD and NAFLD.

The results of this preliminary study indicate that in patients with ALD, s-GGT/t-GGT ratios were significantly increased and f-GGT/t-GGT ratios were lower, compared with those in NAFLD patients. As a result, there was a

marked difference in the s-GGT/f-GGT ratios between patients with ALD and NAFLD. These results indicate that a large-scale study to clarify the diagnostic value of serum GGT fractionation in the differential diagnosis of ALD and NAFLD is warranted.

High-sensitivity GGT fraction patterns of various liver diseases were evaluated by Franzini *et al*<sup>[13-17]</sup>, Elawdi *et al*<sup>[18]</sup>, Fornaciari *et al*<sup>[19]</sup> and Corti *et al*<sup>[20,21]</sup>. They reported that the b-GGT/s-GGT ratio was significantly lower in both alcoholics and abstainers than in the control group, consistent with our study<sup>[13]</sup>. Patients with NAFLD and chronic hepatitis C have different GGT fraction patterns: b-GGT is increased in NAFLD, but not in chronic hepatitis C<sup>[14]</sup>. More recently, GGT fractions were measured in cirrhosis patients, revealing that, irrespective of etiology, s-GGT showed the greatest increase in cirrhotic patients and the b-GGT/s-GGT ratio was even lower than that in patients with chronic hepatitis C<sup>[18]</sup>.

To the best of our knowledge, the present study is the first direct comparison of serum GGT fraction profiles between patients with ALD and NAFLD. However, there are several limitations to the present study. The numbers of the biopsy-proven cases was small. In addition, how serum GGT profiles change with disease progression



**Figure 4** Serum b-GGT/t-GGT, s-GGT/t-GGT, f-GGT/t-GGT, b-GGT/s-GGT, and s-GGT/f-GGT ratios in patients with non-alcoholic fatty liver disease clinically diagnosed by ultrasonography. GGT: Gamma-glutamyltransferase; b-GGT: Big-GGT; t-GGT: Total-GGT; s-GGT: Small-GGT; f-GGT: Free-GGT; ns: No significant differences.

from fatty liver to liver cirrhosis in patients with NAFLD and ALD remains unclear. Also, the diagnostic value of GGT profiles remains to be compared with other markers including cytochrome C<sup>[22]</sup>.

In addition to the well-known alterations in hepatobiliary disorders, GGT is associated with cardiovascular disease (CVD)<sup>[23]</sup>. In a recent review article, the predictive value of GGT for assessing CVD and cancer mortality was described, including assessment at the physiological level of the enzyme activity<sup>[24]</sup>. Taking advantage of the high-performance gel filtration chromatography method for plasma GGT fraction analysis, Franzini *et al*<sup>[15]</sup> demonstrated that CVD risk factors were associated with b-GGT.

In conclusion, the serum GGT fraction patterns in patients with NAFLD are significantly different from those in patients with ALD. In patients with ALD, s-GGT/t-GGT ratios were significantly higher and f-GGT/t-GGT ratios were lower than in those in NAFLD. Consequently, there were marked differences in the s-GGT/f-GGT ratio between ALD and NAFLD. A large-scale study is needed to further evaluate the diagnostic value of serum GGT fractionation in the differential diagnosis of ALD and

NAFLD.

## COMMENTS

### Background

Distinguishing alcoholic liver disease (ALD) from non-alcoholic fatty liver disease (NAFLD) is difficult because self-reported history of alcohol consumption is unreliable. Detection of patients with high alcohol intake by general practitioners is not necessarily easy. Accurate diagnosis of NAFLD relies on a liver biopsy; hence, a less-invasive evaluation strategy is desirable.

### Research frontiers

Gamma-glutamyltransferase (GGT) in serum is mainly derived from the liver and this enzyme is often used as a marker of hepatobiliary diseases. Although sensitive, GGT elevation is not specific enough for the differential diagnosis of hepatobiliary disorders. GGT is present in serum in multiple forms in molecular complexes that vary in size, charge, and density. These methods, however, were not sensitive enough to facilitate the differential diagnosis of liver diseases. To overcome this limitation, Franzini *et al* developed a high-performance liquid chromatography method to quantify four plasma GGT fractions on the basis of molecular size exclusion chromatography, followed by a GGT-specific post-column reaction.

### Innovations and breakthroughs

To the best of our knowledge, the present study is the first direct comparison of



serum GGT fraction profiles between patients with ALD and NAFLD.

## Applications

Although preliminary, determination of serum GGT profiles may serve for differential diagnosis of ALD and NAFLD.

## Terminology

High-performance liquid chromatography (previously called high-pressure liquid chromatography), is a useful analytical tool which is able to separate various compounds based on their size, electrical charge and biochemical affinity.

## Peer-review

The research presents a screening for future investigations about AFLD and NAFLD diagnosis differentiation. It is an interesting approach of ALD and AFLD diagnosis which was not executed in the best possible way.

## REFERENCES

- 1 **Castellano I**, Merlino A.  $\gamma$ -Glutamyltranspeptidases: sequence, structure, biochemical properties, and biotechnological applications. *Cell Mol Life Sci* 2012; **69**: 3381-3394 [PMID: 22527720 DOI: 10.1007/s00018-012-0988-3]
- 2 **Nemesánszky E**, Lott JA. Gamma-glutamyltransferase and its isoenzymes: progress and problems. *Clin Chem* 1985; **31**: 797-803 [PMID: 2859933]
- 3 **Cohen DE**, Carey MC. Rapid (1 hour) high performance gel filtration chromatography resolves coexisting simple micelles, mixed micelles, and vesicles in bile. *J Lipid Res* 1990; **31**: 2103-2112 [PMID: 2086707]
- 4 **Franzini M**, Bramanti E, Ottaviano V, Ghiri E, Scatena F, Barsacchi R, Pompella A, Donato L, Emdin M, Paolicchi A. A high performance gel filtration chromatography method for gamma-glutamyltransferase fraction analysis. *Anal Biochem* 2008; **374**: 1-6 [PMID: 18023410]
- 5 **Rosalki SB**, Rau D. Serum  $\gamma$ -glutamyl transpeptidase activity in alcoholism. *Clin Chim Acta* 1972; **39**: 41-47 [PMID: 5038763]
- 6 **Teschke R**, Rauen J, Neufeld M, Petrides AS, Strohmeyer G. Alcoholic liver disease associated with increased gamma-glutamyltransferase activities in serum and liver. *Adv Exp Med Biol* 1980; **132**: 647-654 [PMID: 6106999]
- 7 **Banderas DZ**, Escobedo J, Gonzalez E, Liceaga MG, Ramirez JC, Castro MG.  $\gamma$ -Glutamyl transferase: a marker of nonalcoholic fatty liver disease in patients with the metabolic syndrome. *Eur J Gastroenterol Hepatol* 2012; **24**: 805-810 [PMID: 22546752 DOI: 10.1097/MEG.0b013e328354044a]
- 8 **Liviero FA**, Acco A. Molecular basis of alcoholic fatty liver disease: From incidence to treatment. *Hepatol Res* 2016; **46**: 111-123 [PMID: 26417962 DOI: 10.1111/hepr.12594]
- 9 **Reid AL**, Webb GR, Hennrikus D, Fahey PP, Sanson-Fisher RW. Detection of patients with high alcohol intake by general practitioners. *Br Med J (Clin Res Ed)* 1986; **293**: 735-737 [PMID: 3094634]
- 10 **Castera L**, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 666-675 [PMID: 24061203 DOI: 10.1038/nrgastro.2013.175]
- 11 **Shaw LM**, Strømme JH, London JL, Theodorsen L. International Federation of Clinical Chemistry, (IFCC), Scientific Committee, Analytical Section. IFCC methods for the measurement of catalytic concentration of enzymes. Part 4. IFCC method for gamma-glutamyltransferase [(gamma-glutamyl)-peptide: amino acid gamma-glutamyltransferase, EC 2.3.2.2]. *J Clin Chem Clin Biochem* 1983; **21**: 633-646 [PMID: 6139407]
- 12 **Schiele F**, Muller J, Colinet E, Siest G. Production and certification of an enzyme reference material for gamma-glutamyltransferase (CRM 319). Part 1: Preparation and characterization. *Clin Chem* 1987; **33**: 1971-1977 [PMID: 2890449]
- 13 **Franzini M**, Fornaciari I, Vico T, Moncini M, Cellesi V, Meini M, Emdin M, Paolicchi A. High-sensitivity  $\gamma$ -glutamyltransferase fraction pattern in alcohol addicts and abstainers. *Drug Alcohol Depend* 2013; **127**: 239-242 [PMID: 22749559 DOI: 10.1016/j.drugalcdep.2012.06.004]
- 14 **Franzini M**, Fornaciari I, Fierabracci V, Elawadi HA, Bolognesi V, Maltinti S, Ricchiuti A, De Bortoli N, Marchi S, Pompella A, Passino C, Emdin M, Paolicchi A. Accuracy of b-GGT fraction for the diagnosis of non-alcoholic fatty liver disease. *Liver Int* 2012; **32**: 629-634 [PMID: 22098947 DOI: 10.1111/j.1478-3231.2011.02673.x]
- 15 **Franzini M**, Paolicchi A, Fornaciari I, Ottaviano V, Fierabracci V, Maltinti M, Ripoli A, Zyw L, Scatena F, Passino C, Pompella A, Emdin M. Cardiovascular risk factors and gamma-glutamyltransferase fractions in healthy individuals. *Clin Chem Lab Med* 2010; **48**: 713-717 [PMID: 20158443 DOI: 10.1515/CCLM.2010.125]
- 16 **Franzini M**, Fornaciari I, Rong J, Larson MG, Passino C, Emdin M, Paolicchi A, Vasan RS. Correlates and reference limits of plasma gamma-glutamyltransferase fractions from the Framingham Heart Study. *Clin Chim Acta* 2013; **417**: 19-25 [PMID: 23247050 DOI: 10.1016/j.cca.2012.12.002]
- 17 **Franzini M**, Fierabracci V, Bolognesi V, Maltinti S, Fornaciari I, Marchi S, Paolicchi A. Plasma gamma-glutamyltransferase (GGT) activity in inflammatory bowel disease: is the clinical laboratory plasma GGT assay sensitive enough for gastroenterology? *Inflamm Bowel Dis* 2013; **19**: E21-E22 [PMID: 22223492 DOI: 10.1002/ibd.22856]
- 18 **Elawdi HA**, Franzini M, Paolicchi A, Emdin M, Fornaciari I, Fierabracci V, De Simone P, Carrai P, Filippini F. Circulating gamma-glutamyltransferase fractions in cirrhosis. *Liver Int* 2014; **34**: e191-e199 [PMID: 24387676 DOI: 10.1111/liv.12455]
- 19 **Fornaciari I**, Fierabracci V, Corti A, Aziz Elawadi H, Lorenzini E, Emdin M, Paolicchi A, Franzini M. Gamma-glutamyltransferase fractions in human plasma and bile: characteristic and biogenesis. *PLoS One* 2014; **9**: e88532 [PMID: 24533101 DOI: 10.1371/journal.pone.0088532]
- 20 **Corti A**, Franzini M, Paolicchi A, Pompella A. Gamma-glutamyltransferase of cancer cells at the crossroads of tumor progression, drug resistance and drug targeting. *Anticancer Res* 2010; **30**: 1169-1181 [PMID: 20530424]
- 21 **Corti A**, Fierabracci V, Caponi L, Paolicchi A, Lorenzini E, Campani D, Belcastro E, Franzini M. Effect of the three-dimensional organization of liver cells on the biogenesis of the  $\gamma$ -glutamyltransferase fraction pattern. *Biomarkers* 2016; **21**: 441-448 [PMID: 27027926 DOI: 10.3109/1354750X.2016.1153719]
- 22 **Tarantino G**, Colao A, Capone D, Conca P, Tarantino M, Grimaldi E, Chianese D, Finelli C, Contaldo F, Scopacasa F, Savastano S. Circulating levels of cytochrome C, gamma-glutamyl transferase, triglycerides and unconjugated bilirubin in overweight/obese patients with non-alcoholic fatty liver disease. *J Biol Regul Homeost Agents* 2011; **25**: 47-56 [PMID: 21382273]
- 23 **Ruttman E**, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H; Vorarlberg Health Monitoring and Promotion Program Study Group. Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation* 2005; **112**: 2130-2137 [PMID: 16186419 DOI: 10.1161/CIRCULATIONAHA.105.552547]
- 24 **Long Y**, Zeng F, Shi J, Tian H, Chen T. Gamma-glutamyltransferase predicts increased risk of mortality: a systematic review and meta-analysis of prospective observational studies. *Free Radic Res* 2014; **48**: 716-728 [PMID: 24684379 DOI: 10.3109/10715762.2014.902055]

P- Reviewer: Dajani A, de Medeiros IC, Liviero FA, Tarantino G

S- Editor: Gong ZM L- Editor: A E- Editor: Li D



Retrospective Cohort Study

# Spontaneous bacterial peritonitis prevalence in pre-transplant patients and its effect on survival and graft loss post-transplant

Neeral L Shah, Nicolas M Intagliata, Zachary H Henry, Curtis K Argo, Patrick G Northup

Neeral L Shah, Nicolas M Intagliata, Zachary H Henry, Curtis K Argo, Patrick G Northup, Division of Gastroenterology and Hepatology, Department of Medicine, University of Virginia, Charlottesville, VA 22908, United States

**Author contributions:** Shah NL and Northup PG and performed designed research; Northup PG analyzed data; Shah NL, Intagliata NM, Henry ZH, Argo CK and Northup PG wrote the paper.

**Institutional review board statement:** Study approved by IRB at University of Virginia.

**Informed consent statement:** A cohort study on a de-identified population did not require consent.

**Conflict-of-interest statement:** We have no conflicts of interest to report.

**Data sharing statement:** No additional data is available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Neeral L Shah, MD, Division of Gastroenterology and Hepatology, Department of Medicine, University of Virginia, Jefferson Park Avenue, PO Box 800708, Charlottesville, VA 22908, United States. [neeral.shah@virginia.edu](mailto:neeral.shah@virginia.edu)  
 Telephone: +1-434-9242626  
 Fax: +1-434-2447586

Received: June 28, 2016  
 Peer-review started: June 30, 2016  
 First decision: August 5, 2016

Revised: October 25, 2016  
 Accepted: November 16, 2016  
 Article in press: November 17, 2016  
 Published online: December 28, 2016

## Abstract

### AIM

To investigate the incidence of spontaneous bacterial peritonitis (SBP) in pre-transplant patients and its effect on post transplant mortality and graft failure.

### METHODS

We conducted a retrospective cohort study of patient records from the organ procurement and transplant network data set. Patients were identified by the presence of SBP pre-transplant. Univariate post-transplant survival models were constructed using the Kaplan-Meier technique and multivariate models were constructed using the Cox proportional hazards model. Variables that affected post-transplant graft survival were identified in the SBP population.

### RESULTS

Forty-seven thousand eight hundred and eighty patient records were included in the analysis for both groups, and 1966 (4.11%) patients were identified in the data set as having pre-transplant SBP. Patients that had pre-transplant SBP had higher rates of graft loss from recurrent hepatitis C virus (HCV) (3.6% vs 2.0%,  $P < 0.0001$ ), infections leading to graft loss (1.9% vs 1.3%,  $P = 0.02$ ), primary non-function (4.3% vs 3.0%,  $P < 0.0001$ ) and chronic rejection (1.1% vs 0.7%,  $P = 0.04$ ). Kaplan-Meier survival analysis showed a statistically significant difference in all-cause survival in patients with a history of SBP vs those without ( $P < 0.0001$ ). Pre-transplant history of SBP was independently predictive

of mortality due to recurrent HCV (HR = 1.11, 95%CI: 1.02-1.21,  $P < 0.017$ ) after liver transplantation.

## CONCLUSION

HCV patients prior to the advent of direct acting anti-viral agents had a higher incidence of pre-transplant SBP than other patients on the liver transplant wait list. SBP history pre-transplant resulted in a higher rate of graft loss due to recurrent HCV infection and chronic rejection.

**Key words:** Spontaneous bacterial peritonitis; Liver transplant; Graft failure; Hepatitis C

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Prevention of spontaneous bacterial peritonitis (SBP) pre-transplant may affect graft outcomes and ultimately patient survival post-transplant. Patients with hepatitis C virus (HCV) in whom therapy is deferred until the time of transplant due to hepatic decompensation, may benefit from expedited treatment if they possess a history of SBP to avoid complications related to HCV recurrence.

Shah NL, Intagliata NM, Henry ZH, Argo CK, Northup PG. Spontaneous bacterial peritonitis prevalence in pre-transplant patients and its effect on survival and graft loss post-transplant. *World J Hepatol* 2016; 8(36): 1617-1622 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i36/1617.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i36.1617>

## INTRODUCTION

According to the Scientific Registry of Transplant Recipients database, since 2005 over 6000 liver transplants have been performed on an annual basis. Hepatitis C virus (HCV) has consistently been the most common indication for liver transplantation and challenging due to the possibility of viral recurrence post-transplant. Recurrent HCV had been a major problem for patients post liver transplantation, but with the new direct acting anti-viral (DAA) therapies, this problem is steadily declining. Even with resolution of this challenge, identifying factors that may accelerate graft loss or damage is still essential.

Originally established for mortality post transjugular intrahepatic portosystemic shunts, the laboratory based Model for End Stage Liver Disease (MELD and MELD-Na) is now the primary measure for liver organ allocation in the United States<sup>[1]</sup>. Since the use of MELD began in 2002, wait list mortality has significantly decreased as patients are prioritized effectively, however patients on the wait list still suffer from complications of end stage liver disease and portal hypertension such as gastrointestinal bleeding, encephalopathy, ascites, renal failure, and infection<sup>[2]</sup>.

Liver disease patients, with decreased levels of com-

plement proteins and decreased opsonization, live in a relatively immunocompromised state and are at high risk of developing bacterial, viral, or fungal infections<sup>[3]</sup>. Ascites reportedly occurs in 7% to 23% of hospitalized end stage liver disease patients<sup>[4]</sup>. An initial episode of spontaneous bacterial peritonitis (SBP) occurs in about 10% of these patients<sup>[5]</sup>. Infection related to SBP can have severe ramifications in the development of renal failure and mortality of patients while on the transplant wait list.

The utility of MELD in organ allocation is well established, but its extrapolation to post-transplant survival or graft outcomes is still unclear. Further, in the post MELD era, limited studies have investigated the role of SBP on post-transplant outcomes<sup>[6]</sup>. Therefore, in order to understand the role of infections on post-transplant outcomes, we aimed to investigate the incidence of SBP in pre-transplant patients and its effect on post transplant mortality and graft failure in the era prior to DAA therapy.

## MATERIALS AND METHODS

We investigated the United States organ procurement and transplant network (OPTN) dataset for liver transplants from February 2002 until November 2009 for liver graft recipients with a reported history of pre-transplant SBP. All patients who eventually underwent liver transplantation were included in the analysis. If the patient did not have a history of SBP or if the question was left blank (or "unknown" was selected) on the Adult Liver Transplant Recipient Registration Worksheet submitted to the United Network for Organ Sharing, the recipient was assumed to not have pre-transplant SBP. The population with a history of SBP was compared to those without the history of SBP for multiple pre- and post-transplant characteristics. Recipient etiology of disease was categorized as HCV, hepatitis B, non-alcoholic steatohepatitis or cryptogenic, alcohol alone, cholestatic liver disease, autoimmune, liver malignancy, or other. Etiologies of graft failure included biliary, *de novo* autoimmune hepatitis, recurrent (non-viral) disease, infection, primary non-function, recurrent viral hepatitis, acute rejection, chronic rejection, and vascular thrombosis. Laboratory (non-exception) MELD scores were used for all recipients.

Demographics, recipient, donor, and surgical characteristics were compared between groups using the  $\chi^2$  test for categorical variables and the Student *t*-test for continuous variables. Univariate post-transplant survival models were constructed using the Kaplan-Meier technique and multivariate models were constructed using the Cox proportional hazards model. Variables known to influence post-transplant survival from previous studies or those variables found to be significant in the univariate analysis to a level of less than 0.20 were included in multivariate models using a whole model (non-stepwise) analysis. Because of the finding of a relationship between SBP and graft failure due to recurrent HCV, a multivariate logistic regression model was developed to determine

**Table 1** Population characteristics

Population characteristic	History of pre-transplant SBP ( <i>n</i> = 1966)	No history of SBP ( <i>n</i> = 45914)	<i>P</i> -value
Recipient age, yr	50.51 (49.96-51.07)	47.97 (47.81-48.13)	< 0.0001
Donor age, yr	38.08 (38.84-39.61)	38.29 (38.12-38.46)	0.165
MELD score at transplant	25.28 (24.83-25.74)	20.34 (20.25-20.44)	< 0.0001
Male	1429 (72.69)	29950 (65.23)	< 0.0001
Ethnicity African American	149 (7.58)	4591 (10.00)	0.027
Etiology of recipient liver disease			< 0.0001
Alcohol alone	318 (16.17)	4621 (10.06)	
Autoimmune	64 (3.26)	1149 (2.50)	
Cholestatic disease	113 (5.75)	3337 (7.27)	
Hepatitis B	69 (3.51)	1014 (2.21)	
Hepatitis C	809 (41.15)	13557 (29.53)	
Liver malignancy	126 (6.41)	6435 (14.02)	
NASH/cryptogenic	175 (8.90)	4214 (9.18)	
Other	292 (14.85)	11587 (25.24)	
Liver retransplantation	145 (7.38)	3711 (8.08)	0.259

Categorical variables are expressed as number and column percent. Continuous variables are expressed as mean and 95% CIs unless otherwise specified. SBP: Spontaneous bacterial peritonitis; MELD: Model for End Stage Liver Disease; NASH: Nonalcoholic steatohepatitis.

those variables independently predictive of recurrent HCV. No data imputation was used. All statistical testing was two sided and the level of type one error deemed to be statistically significant was assumed to be less than or equal to 0.05. All dataset manipulation and analysis was performed using SAS (version 9.2, Cary, NC, United States). Local institutional review board approval was not required for analysis of this de-identified dataset.

## RESULTS

The OPTN data set contained information on 47880 patients transplanted during the study period. The characteristics of the study population are outlined in Table 1. Of this population, 1966 (4.11%) patients were reported to have a history of pre-transplant SBP. Patients with a history of SBP tended to be older (50.5 mean years in the SBP population vs 48.0 in the non-SBP population,  $P < 0.0001$ ), male (72.7% vs 65.2%,  $P < 0.0001$ ), and have a higher MELD score at the time of liver transplantation (25.3 vs 20.3,  $P < 0.0001$ ). The etiology of liver disease was significantly different between those recipients who had pretransplant SBP compared to those that did not. HCV was significantly more prevalent in the SBP population (41.1% vs 29.5%,  $P < 0.0001$ ).

Table 2 shows the distribution and causes of graft failure in the post-transplant time period. While overall graft loss was uncommon, compared to those without a history of SBP, patients that had pre-transplant SBP had higher rates of graft loss from recurrent HCV (3.6% vs 2.0%,  $P < 0.0001$ ), infections leading to graft loss (1.9% vs 1.3%,  $P = 0.02$ ), primary non-function (4.3% vs 3.0%,  $P < 0.0001$ ) and chronic rejection (1.1% vs 0.7%,  $P = 0.04$ ). In regards to all-cause survival, patients having pre-transplant SBP had worse unadjusted one year post-transplant survival (82.8% vs 86.5%,  $P < 0.0001$ ) and this difference widened at two years (76.5% vs 81.6%,  $P < 0.0001$ ). Figure 1 shows the Kaplan-Meier survival analysis. There was a statistically significant difference

in all-cause survival in patients with a history of SBP vs those without ( $P < 0.0001$ ).

In order to account for multiple factors influencing graft loss in this population, we developed a multivariable logistic regression model including factors known to affect survival rates: Age of recipient, age of donor, MELD score, history of previous transplant, and history of HCV. Table 3 shows the results of this analysis. A pre-transplant history of SBP was found to be an independent risk factor for post-transplant graft failure imparting a 57% increased risk of graft failure (OR = 1.57, 95%CI: 1.22-2.02,  $P < 0.001$ ). Table 4 shows the results of a multivariate proportional hazards survival model predicting death due to recurrent HCV. Once again, a pre-transplant history of SBP was independently predictive of mortality due to recurrent HCV (HR = 1.11, 95%CI: 1.02-1.21,  $P < 0.017$ ) after liver transplantation.

## DISCUSSION

We have shown that HCV patients prior to the advent of DAA agents had a higher incidence of pre-transplant SBP than other patients on the liver transplant wait list. Further, SBP history resulted in a higher rate of graft loss due to recurrent HCV infection and chronic rejection. This group also had an 11.2% greater risk of post-transplant mortality. In a multivariate Cox regression model, SBP was found to be an independent risk factor for post-transplant mortality.

Patients with a diagnosis of active HCV infection at the time of transplant have a known predisposition to graft failure due to HCV recurrence. However, the rate of progression to cirrhosis and graft failure is unpredictable. Certain known risk factors such as the donor's age, post-transplant CMV infection, HIV co-infection, the use of T cell depleting therapies, pulsed steroids, and other donor risk factors have all been associated with poorer outcomes<sup>[7]</sup>. SBP has not been studied in this regard. Our results show that a history of pre-transplant SBP may



**Table 2 Cause of graft failure in patients with and without a history of spontaneous bacterial peritonitis**

Cause of graft failure	History of pre-transplant SBP ( <i>n</i> = 1966)	No history of SBP ( <i>n</i> = 45914)	<i>P</i> -value
Biliary	24 (1.22)	423 (0.92)	0.176
<i>De novo</i> autoimmune hepatitis	2 (0.10)	17 (0.04)	0.182
Recurrent viral hepatitis	71 (3.61)	936 (2.04)	< 0.0001
Infection	37 (1.88)	585 (1.27)	0.020
Primary non-function	84 (4.27)	1352 (2.94)	< 0.0001
Recurrent non-viral disease	26 (1.32)	547 (1.19)	0.600
Acute rejection	18 (0.92)	289 (0.63)	0.120
Chronic rejection	21 (1.07)	312 (0.68)	0.042
Vascular thrombosis	44 (2.24)	811 (1.77)	0.122

SBP: Spontaneous bacterial peritonitis.

**Table 3 Multivariate analysis of independent predictors of graft failure due to recurrent viral hepatitis**

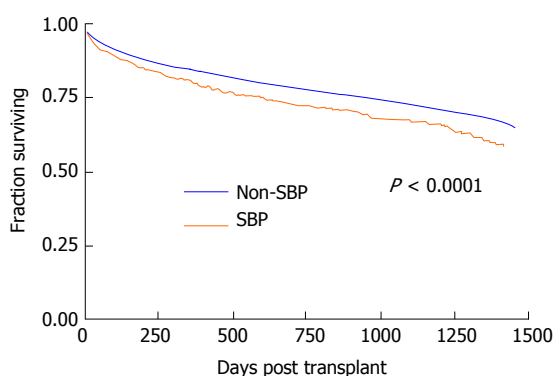
	Odds ratio	95%CI	<i>P</i> -value
History of pre-transplant SBP	1.567	1.218-2.017	< 0.001
Hepatitis C ( <i>vs</i> hepatitis B)	6.777	5.864-7.832	< 0.0001
Previous transplant	2.349	1.923-2.868	< 0.0001
MELD at transplant	0.989	0.982-0.996	0.0014
Age of recipient	0.992	0.986-0.997	0.0041
Age of donor	1.023	1.019-1.027	< 0.0001

SBP: Spontaneous bacterial peritonitis; MELD: Model for End Stage Liver Disease.

**Table 4 Multivariate analysis of independent predictors of all cause survival due to recurrent hepatitis C**

	Hazard ratio	95%CI	<i>P</i> -value
History of pre-transplant SBP	1.112	1.019-1.212	0.017
Hepatitis C	1.176	1.126-1.228	< 0.0001
Previous transplant	1.979	1.860-2.107	< 0.0001
Gender male	0.983	0.941-1.027	0.443
Ethnicity non-African American	0.921	0.866-0.980	0.010
MELD at transplant	1.016	1.014-1.018	< 0.0001
Age of recipient	1.009	1.007-1.010	< 0.0001
Age of donor	1.009	1.008-1.010	< 0.0001

SBP: Spontaneous bacterial peritonitis; MELD: Model for End Stage Liver Disease.

**Figure 1 Kaplan-Meier post-transplant survival analysis of patients with a history of pre-transplant spontaneous bacterial peritonitis.** SBP: Spontaneous bacterial peritonitis.

contribute to worse survival and an increased risk of graft failure. As our study shows, 40% of pre-transplant SBP patients in this cohort suffered from HCV, whereas only 30% of a control, non-SBP pre-transplant group suffered from HCV.

Prior treatment regimens, which included interferon, to decrease HCV levels in pre-transplant candidates possessed their own risks and had shown an increase the rate of bacterial infections in these patients<sup>[8]</sup>. The rate of infection, especially the incidence of SBP in patients not receiving prophylaxis therapy, in Child Pugh B/C patients was higher than matched controls<sup>[8]</sup>. Several etiologies had been proposed, including the neutropenic and granulo-toxic effect of interferon causing an increase susceptibility to bacterial infections. These studies show

an increased risk of infection, including SBP, in HCV patients with poor liver function. With our data showing the deleterious effect of SBP on transplant outcomes, it supports the careful choice of HCV patients that were selected in the past to undergo interferon based therapies pre-transplant and the importance of SBP prophylaxis in this group with proper indications<sup>[8,9]</sup>.

Limited studies from the pre-MELD era did not show that pre-transplant SBP influenced post-transplant outcomes. A study following 100 patients, showed that 32% of patients developed a pre-transplant infection. The infections ranged from SBP (35.6%), blood stream infections (28.9%), cellulitis (13.3%), pneumonia (8.9%), urinary tract infections (6.7%), and other infections (6.7%)<sup>[10]</sup>. After following this group, the study found that patients with pre-transplant infections were less likely to be transplanted from home and required longer hospital stays, but the mortality at 90 and 180 d post-transplant did not differ significantly compared to individuals without pre-transplant infections. As shown by our Kaplan Meier survival curves in Figure 1, the survival of the two groups from our analysis of the OPTN database seems to be similar, but then diverges significantly starting at day 250. Therefore, even though the previously mentioned study did not find differences in survival, we feel that with longer follow-up this difference could have been statistically significant.

Another single center study, reported that patients with a history of SBP had more severe liver disease as measured by MELD and CTP score, but had similar

post-transplant mortality to those without a history of SBP<sup>[11]</sup>. The patients who developed SBP most commonly suffered from liver disease related to HCV or alcohol. The mean follow-up time period for this study was 3 years. While mortality or infection rates were not affected, these patients were more likely to require abdominal surgery 1 year post transplant for hernia repairs, bleeding, and vascular complications<sup>[11]</sup>. This study reinforces our findings of the large burden of SBP on pre-transplant cirrhosis patients and the higher rate of SBP in HCV patients. However, the relatively small sample size and inclusion of patients transplanted in the pre-MELD era may not be as applicable to the general population. Our cohort studies the transplants occurring after 2002, and includes a multi-center analysis with almost 48000 patient records.

There are several limitations to our study. One of the major limitations is the incomplete reporting of SBP in the OPTN database. We believe it is safe to assume that reported instances of SBP are accurate and have a clear documented episode recognized by the listing health care provider, which prompted this designation. However, the low rates of patients with SBP pre-transplant as compared to other studies in the literature, raises the speculation of recall bias from reporting centers on patient's history of SBP. The exact determination of SBP may not be uniform across all centers, and without full accessibility to numbers of SBP episodes, exact cell counts, or ascites fluid analysis we are dependent solely on information from the database. Further, those patients with severe infection due to SBP were likely excluded from transplant listing. Finally, while we may assume that survival statistics and graft loss are accurate in the OPTN database, other post-transplant variables are often incomplete including immunosuppression data and other details regarding HCV recurrence<sup>[12]</sup>. By using a worst case scenario analysis, if we assume that all patients in the OPTN database without data entered for SBP are included in the control group. Any mortality registered in these patients would only reduce the differences between our groups.

It has been shown that HCV patients being treated with interferon based therapies were at an increased risk of developing SBP<sup>[8]</sup>. It is unclear if this association is due to the underlying viral hepatitis disease process or related to the treatment regimen that were used. Regardless, stronger surveillance measures and antibiotic prophylaxis pre-transplant SBP may be necessary to improve post-transplant outcomes. Also, the new issue as we transition fully to DAA regimens is the decision to treat cirrhosis patients pre vs post-transplant<sup>[13,14]</sup>. If patients live in a region with a relatively high average MELD at time of transplant, treating HCV infection pre-transplant may put these patients into MELD "purgatory" - stable MELD that will not increase transplant prioritization, but continued low quality of life<sup>[15]</sup>. Due to the low rates of drug interactions between the DAAs and immunosuppression, some patients at higher MELD are deferred for therapy until after transplant. From these

results and our experience prior to the advent of DAAs, in those HCV patients we deem necessary to treat post-transplant, we should consider prioritizing therapy in those with a history of SBP. Further, we should strive to reduce donor risk factors, minimize pulsed steroids or T cell depleting immunosuppression to prevent post-transplant HCV recurrence and graft failure<sup>[16,17]</sup>. Over time, we predict the number of transplants performed in the United States stemming from HCV infection to decrease, but the decision to treat these patients around the time of transplant and avoiding SBP will continue to be a challenge.

## COMMENTS

### Background

This study examines the effect of spontaneous bacterial peritonitis in cirrhosis patients on the wait list for liver transplantation. Using the patients identified in the organ procurement and transplant network (OPTN)/United Network for Organ Sharing (UNOS) database, in the era prior to direct acting anti-viral agents, we investigated the effect of spontaneous bacterial peritonitis (SBP) on liver transplant graft loss and post-transplant mortality.

### Research frontiers

Future studies focused on the prevention of pre-transplant infections, like SBP, and the effect of direct acting anti-viral (DAA) therapies on graft loss and mortality is a potential area to study. Further, as the treatment paradigm for pre vs post-transplant hepatitis C virus (HCV) therapy changes, it may be worthwhile to examine the change it produces in graft loss from HCV complications.

### Innovations and breakthroughs

From our knowledge, this is the first article to assess the effect of SBP on post transplant outcomes from the OPTN dataset. Further it provides a collective experience of our outcomes from the pre-DAA therapy era which can be used as a basis for future comparison.

### Applications

This article has shown the importance of avoiding SBP infections in the pre-transplant population. Aggressive prophylaxis and treatment of this infection may have long term implications with regards to graft survival and mortality.

### Terminology

SBP: Spontaneous bacterial peritonitis - infection found in the ascites fluid in the peritoneum; DAA: Direct acting anti-viral therapies - the new class of HCV medications which directly inhibits replication; OPTN/UNOS: The national organization responsible for managing organ allocation, obtaining individual center's data on transplantation, and reporting collective outcomes.

### Peer-review

This is a good paper.

## REFERENCES

- 1 **Malinchoc M**, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871 [PMID: 10733541 DOI: 10.1053/he.2000.5852]
- 2 **Kamath PS**, Kim WR. The model for end-stage liver disease (MELD). *Hepatology* 2007; **45**: 797-805 [PMID: 17326206 DOI: 10.1002/hep.21563]
- 3 **Ono Y**, Watanabe T, Matsumoto K, Ito T, Kunii O, Goldstein E. Opsonophagocytic dysfunction in patients with liver cirrhosis and low responses to tumor necrosis factor-alpha and lipopolysaccharide in patients' blood. *J Infect Chemother* 2004; **10**: 200-207 [PMID: 15365859 DOI: 10.1007/s10156-004-0321-7]

- 4 **Runyon BA.** Low-protein-concentration ascitic fluid is predisposed to spontaneous bacterial peritonitis. *Gastroenterology* 1986; **91**: 1343-1346 [PMID: 3770358]
- 5 **Cheruvattath R,** Balan V. Infections in Patients With End-stage Liver Disease. *J Clin Gastroenterol* 2007; **41**: 403-411 [PMID: 17413611 DOI: 10.1097/01.mcg.0000248018.08515.f9]
- 6 **Leong J,** Huprikar S, Schiano T. Outcomes of spontaneous bacterial peritonitis in liver transplant recipients with allograft failure. *Transpl Infect Dis* 2016; **18**: 545-551 [PMID: 27261101 DOI: 10.1111/tid.12565]
- 7 **Watt K,** Veldt B, Charlton M. A practical guide to the management of HCV infection following liver transplantation. *Am J Transplant* 2009; **9**: 1707-1713 [PMID: 19538491 DOI: 10.1111/j.1600-6143.2009.02702.x]
- 8 **Carrión JA,** Martínez-Bauer E, Crespo G, Ramírez S, Pérez-del-Pulgar S, García-Valdecasas JC, Navasa M, Forns X. Antiviral therapy increases the risk of bacterial infections in HCV-infected cirrhotic patients awaiting liver transplantation: A retrospective study. *J Hepatol* 2009; **50**: 719-728 [PMID: 19217183 DOI: 10.1016/j.jhep.2008.11.015]
- 9 **Rimola A,** García-Tsao G, Navasa M, Piddock LJ, Planas R, Bernard B, Inadomi JM. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. International Ascites Club. *J Hepatol* 2000; **32**: 142-153 [PMID: 10673079]
- 10 **Sun HY,** Cacciarelli TV, Singh N. Impact of pretransplant infections on clinical outcomes of liver transplant recipients. *Liver Transpl* 2010; **16**: 222-228 [PMID: 20104499 DOI: 10.1002/lt.21982]
- 11 **Mounzer R,** Malik SM, Nasr J, Madani B, Devera ME, Ahmad J. Spontaneous bacterial peritonitis before liver transplantation does not affect patient survival. *Clin Gastroenterol Hepatol* 2010; **8**: 623-628.e1 [PMID: 20417723 DOI: 10.1016/j.cgh.2010.04.013]
- 12 **Gillespie BW,** Merion RM, Ortiz-Rios E, Tong L, Shaked A, Brown RS, Ojo AO, Hayashi PH, Berg CL, Abecassis MM, Ashworth AS, Fries CE, Hong JC, Trotter JF, Everhart JE. Database comparison of the adult-to-adult living donor liver transplantation cohort study (A2ALL) and the SRTR U.S. Transplant Registry. *Am J Transplant* 2010; **10**: 1621-1633 [PMID: 20199501 DOI: 10.1111/j.1600-6143.2010.03039.x]
- 13 **Russo FP,** Zanetto A, Burra P. Timing for treatment of HCV recurrence after liver transplantation: the earlier the better. *Transpl Int* 2016; **29**: 694-697 [PMID: 26713429 DOI: 10.1111/tri.12739]
- 14 **Suraweera D,** Saab EG, Tong MJ, Saab S. Timing of Hepatitis C Antiviral Therapy in Liver Transplant Recipients With Direct-acting Agents. *Exp Clin Transplant* 2016; **14**: 243-251 [PMID: 27221717]
- 15 **Carrion AF,** Khaderi SA, Sussman NL. Model for end-stage liver disease limbo, model for end-stage liver disease purgatory, and the dilemma of treating hepatitis C in patients awaiting liver transplantation. *Liver Transpl* 2016; **22**: 279-280 [PMID: 26663608 DOI: 10.1002/lt.24383]
- 16 **Eyerson GT,** Trotter J, Forman L, Kugelmas M, Halprin A, Fey B, Ray C. Treatment of advanced hepatitis C with a low accelerating dosage regimen of antiviral therapy. *Hepatology* 2005; **42**: 255-262 [PMID: 16025497 DOI: 10.1002/hep.20793]
- 17 **Bhat I,** Mukherjee S. Hepatitis C recurrence after liver transplantation. *Panminerva Med* 2009; **51**: 235-247 [PMID: 20195234]

**P- Reviewer:** El-Shabrawi MHH, Stanciu C **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Li D



Observational Study

# Prevalence of significant liver disease in human immunodeficiency virus-infected patients exposed to Didanosine: A cross sectional study

Sarah Logan, Alison Rodger, Laura Maynard-Smith, James O'Beirne, Thomas Fernandez, Filippo Ferro, Colette Smith, Sanjay Bhagani

Sarah Logan, Alison Rodger, Laura Maynard-Smith, Thomas Fernandez, Filippo Ferro, Sanjay Bhagani, the Ian Charleson Day Centre, Ground Floor, Royal Free London NHS Foundation Trust, London NW3 2PF, United Kingdom

Alison Rodger, Colette Smith, Research Department of Infection and Population Health, University College London, London NW3 2PF, United Kingdom

James O'Beirne, Department of Hepatology, Nambour General Hospital, Sunshine Coast Hospital and Health Service, Nambour, Queensland 4560, Australia

**Author contributions:** Logan S, Rodger A, O'Beirne J, Smith C and Bhagani S contributed to study conception and design; Logan S, Maynard-Smith L, Fernandez T and Ferro F contributed to data acquisition; Logan S, Rodger A, Smith C and Bhagani S analysed and interpreted the data; Logan S and Rodger A wrote the article; O'Beirne J and Bhagani S contributed to editing reviewing; all authors gave final approval of the manuscript.

**Supported by** The British HIV Association Research Award 2009-£7800 in total awarded.

**Institutional review board statement:** The study was reviewed and approved by the NHS/HSC Research Ethics Committee in North West London. The reference number is 10/H0720/54.

**Informed consent statement:** All study participants provided their informed consent in writing prior to study enrollment.

**Conflict-of-interest statement:** None of the authors has any conflict of interest to declare.

**Data sharing statement:** In order to avoid deductive disclosure of confidential information in patents with HIV, individual level patient data will not be available. Please contact the authors directly if you wish to discuss access to the data.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license,

which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Sanjay Bhagani, Consultant in Infectious Diseases and HIV Medicine, the Ian Charleson Day Centre, Ground Floor, Royal Free London NHS Foundation Trust, Rowland Hill Street, London NW3 2PF, United Kingdom. [s.bhagani@nhs.net](mailto:s.bhagani@nhs.net)  
**Telephone:** +44-207-7940500  
**Fax:** +44-207-4726558

**Received:** June 30, 2016  
**Peer-review started:** July 3, 2016  
**First decision:** August 5, 2016  
**Revised:** September 16, 2016  
**Accepted:** November 1, 2016  
**Article in press:** November 2, 2016  
**Published online:** December 28, 2016

## Abstract

### AIM

To identify significant liver disease [including nodular regenerative hyperplasia (NRH)] in asymptomatic Didanosine (DDI) exposed human immunodeficiency virus (HIV) positive patients.

### METHODS

Patients without known liver disease and with > 6 mo previous DDI use had liver stiffness assessed by transient elastography (TE). Those with alanine transaminase (ALT) above upper limit normal and/or TE > 7.65 kPa underwent ultrasound scan (U/S). Patients with: (1) abnormal U/S; or (2) elevated ALT plus TE > 7.65 kPa;



or (3) TE > 9.4 kPa were offered trans-jugular liver biopsy (TJLB) with hepatic venous pressure gradient (HVPG) assessment.

## RESULTS

Ninety-nine patients were recruited, median age 50 years (range 31-70), 81% male and 70% men who have sex with men. Ninety-five percent with VL < 50 copies on antiretroviral therapy with median CD4 count 639 IU/L. Median DDI exposure was 3.4 years (range 0.5-14.6). Eighty-one had a valid TE readings (interquartile range/score ratio < 0.3): 71 (88%) < 7.65 kPa, 6 (7%) 7.65-9.4 kPa and 4 (6%) > 9.4 kPa. Seventeen (17%) met criteria for TJLB, of whom 12 accepted. All had HVPG < 6 mmHg. Commonest histological findings were steatosis ( $n = 6$ ), normal architecture ( $n = 4$ ) and NRH ( $n = 2$ ), giving a prevalence of previously undiagnosed NRH of 2% (95%CI: 0.55%, 7.0%).

## CONCLUSION

A screening strategy based on TE, liver enzymes and U/S scan found a low prevalence of previously undiagnosed NRH in DDI exposed, asymptomatic HIV positive patients. Patients were more likely to have steatosis highlighting the increased risk of multifactorial liver disease in this population.

**Key words:** Nodular regenerative hyperplasia; Human immunodeficiency virus; Steatosis; Non-cirrhotic portal hypertension; Didanosine

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Human immunodeficiency virus positive patients are at increased risk of liver disease. The aetiology is often multifactorial and includes exposure to antiretroviral therapy. We used a simple screening strategy based on transient elastography, liver enzymes and ultrasound scan to identify that 2% of asymptomatic patients exposed to Didanosine in a clinical cohort had undiagnosed nodular regenerative hyperplasia. A further 6% had undiagnosed steatosis. Implementation of a screening strategy enables identification of liver disease and initiation of earlier targeted interventions in this high-risk group.

Logan S, Rodger A, Maynard-Smith L, O'Beirne J, Fernandez T, Ferro F, Smith C, Bhagani S. Prevalence of significant liver disease in human immunodeficiency virus-infected patients exposed to Didanosine: A cross sectional study. *World J Hepatol* 2016; 8(36): 1623-1628 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i36/1623.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i36.1623>

## INTRODUCTION

Nodular regenerative hyperplasia (NRH) - the develop-

ment of micronodules in liver parenchyma without intervening fibrosis<sup>[1]</sup> - has been reported in human immunodeficiency virus (HIV) positive patients who often present late in the course of the condition with complications associated with non-cirrhotic portal hypertension (NCPH). A strong association with NRH is current or previous use of Didanosine (DDI)<sup>[1-6]</sup>. Although DDI is no longer used as first line antiretroviral therapy (ART), many HIV positive patients have significant previous exposure with a reported prevalence of NRH of between 0.5%-35%<sup>[1,2,6]</sup>. This wide range is indicative of the unreliability of screening strategies and the fact that studies largely included individuals diagnosed with NRH or NCPH as a result of liver biopsy for other conditions or as a result of late presentation with complications of portal hypertension<sup>[1-6]</sup>. However prior to this the disease is largely sub clinical and a screening strategy may be useful to identify patients with DDI associated liver disease earlier in the disease process.

Other studies have reported raised transaminases in NRH, but in many cases transaminases are only mildly elevated or not at all this indicating the unreliability of this alone as a screening tool<sup>[1]</sup>. Hepatic transient elastography (TE) is a validated non-invasive tool with good correlation for identifying hepatic and peri-portal fibrosis<sup>[2,7]</sup>. TE is also associated with hepatic venous pressure gradients (HVPG) in cirrhosis<sup>[8]</sup>. The co-relation with NRH is less well delineated, however increased liver stiffness readings have been reported in patients with both NRH and NCPH<sup>[9-14]</sup>, although one study found that liver stiffness readings did not predict the presence of portal hypertension in NRH<sup>[10]</sup>. Many studies also report reduced platelet levels and the presence of splenomegaly in individuals with NRH<sup>[1]</sup>.

Our aim was to develop and implement a screening strategy incorporating 3 separate measures; TE, platelet and alanine transaminase (ALT) levels, with subsequent ultrasound and trans-jugular liver biopsy (TJLB) in those who met criteria to identify DDI related liver disease in HIV positive patients with previous significant DDI exposure, but who were currently asymptomatic.

## MATERIALS AND METHODS

This study is a cross-sectional study in HIV outpatients at The Royal Free London NHS Foundation Trust from 2010 to 2011. Ethical approval was obtained (REC 10 /H0720/54). Study subjects were identified from the HIV clinical database. HIV positive patients currently under active follow-up and previously exposed to DDI therapy for longer than 6 mo were eligible to take part. Exclusions were viral hepatitis co-infection, age < 18 years, a body mass index (BMI) > 40, pregnancy or ascites. Patients were sequentially recruited as they attended for routine clinic follow-up. Statistical review of the study was performed by a biomedical statistician (Dr. Colette Smith).

Patients completed a study specific questionnaire on sociodemographic factors, medical history, lifestyle

including smoking, alcohol [Michigan Alcoholism Screening test (MAST)] and drug use. Clinical data (HIV viral load, CD4 count, whether on/off treatment, date of diagnosis, date of ART start, lipids, liver panel bloods, blood glucose, BMI) were also collected.

Liver TE was measured using FibroScan (FS) (Echosens, Paris). A median stiffness reading was measured using at least ten readings with a valid reading recording 60% accuracy and an interquartile range (IQR) of less than 30% of the median. Subjects were offered further evaluation with ultrasound of liver and spleen with doppler waveforms of the hepatic vasculature if they had either: (1) an ALT level above 19 IU/mL for women and 31 IU/mL for men; or (2) a platelet count (PLT) less than  $120 \times 10^9/L$ ; or (3) TE reading of  $> 7.65$  kPa (IQR  $< 0.3$ ).

Individuals with evidence on ultrasound of splenomegaly or fatty liver or coarse echotexture or abnormalities of hepatic doppler waveforms in conjunction with a raised ALT or TE reading as above were offered a TJLB with HVPG measurements. Patients with a raised ALT or platelets  $< 120 \times 10^9/L$  for whom an elastography score was unobtainable (centripetal obesity) or uninterpretable (less than 10 valid readings or IQR/LSM  $> 30\%$ ) were offered ultrasound and TJLB.

All TJLB procedures were performed in the interventional radiology suite by an experienced operator (O'Beirne J) after a 6-h fast, under local anaesthesia. Biopsies were taken using a 19G Tru-cut type biopsy needle (Quick core; Cook, William Cook Europe, Denmark). Three or 4 passes were performed through the same hepatic vein wall (right or middle) to ensure that sufficient liver tissue was obtained. Wedge hepatic vein pressures (WHVP) were measured using a 5-F Berenstein balloon catheter (Boston Scientific, Natick, MA) using the technique described by Groszmann and Wongcharatrawee<sup>[15]</sup>. Three sets of measurements were taken. WHVP was measured for at least 1 min each time. HVPG was calculated as the mean of the 3 gradients (the difference between WHVP minus free hepatic pressure). Groups were compared using the Mann Whitney *U* test for non-parametric continuous variables and using the  $\chi^2$  test for categorical variables.

## RESULTS

Four hundred and fifty-nine patients exposed to DDI for longer than 6 mo were identified from the clinic database. Eighty-four patients known to have co-infection with hepatitis B or C were excluded. No patients were excluded due to BMI  $> 40$  or presence of ascites. Of the remaining 376 patients, 99 patients were recruited sequentially as they attended HIV clinic during the study time period and response rates in those approached to take part in the study were  $> 95\%$ .

Characteristics of patients recruited ( $n = 99$ ) were compared to those not approached to take part ( $n = 274$ ) to assess potential for recruitment bias. There were no differences in those recruited by sex: 80.8% (80/99 recruited) vs 78.5% (215/274) not recruited were male;  $P = 0.41$ , total length of DDI exposure (mean 4.1

years recruited vs 4.1 years not recruited;  $P = 0.89$ ), most recent median ALT (34  $\mu/L$  recruited vs 35  $\mu/L$  not recruited;  $P = 0.85$ ) or most recent median platelets (210 recruited vs 210 not recruited;  $P = 0.09$ ). However a larger proportion of those of white ethnicity were recruited (77% vs 64%  $P = 0.02$ ) and of those they had a slightly older mean age (50 years vs 48 years;  $P = 0.001$ ).

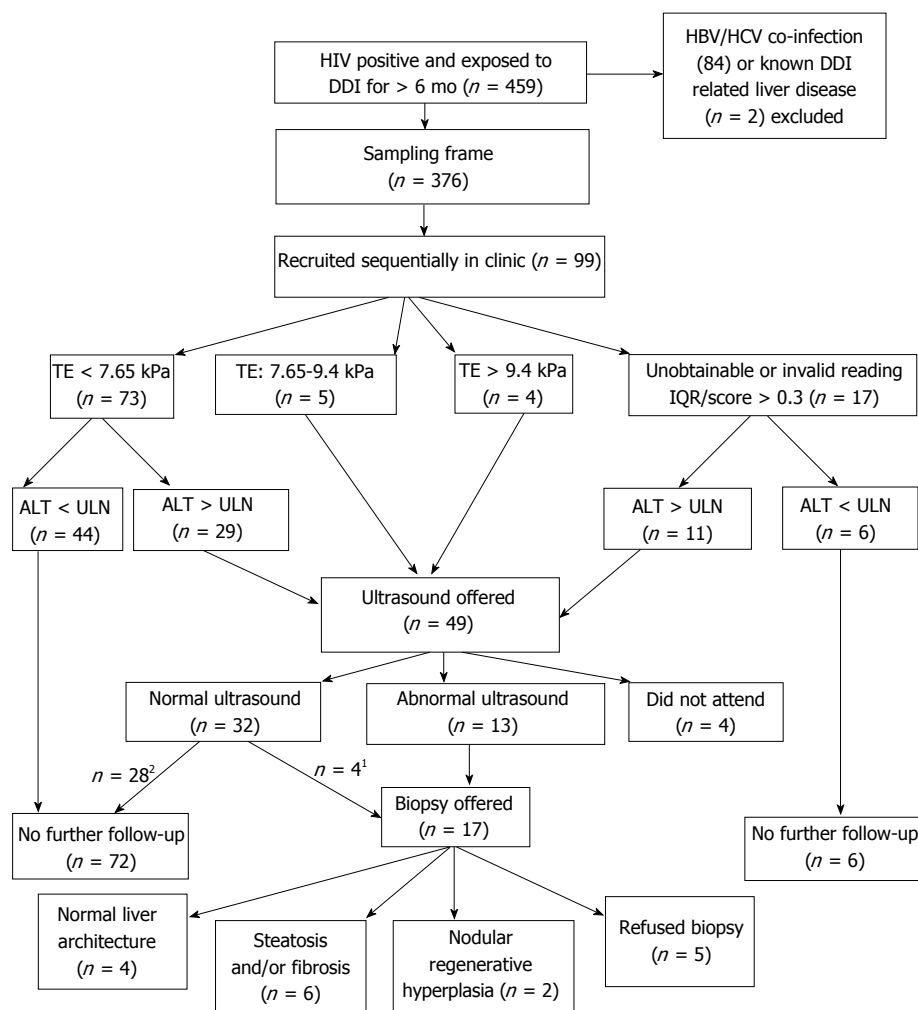
Of the 99 who took part in the study, 75 (75%) were men who have sex with men (MSM). Mean age was 50 years (range: 30 to 70), 76% were White, 19% Black and 5% were of another ethnicity. The majority had well-controlled HIV infection with a median CD4 count of 637  $mm^3$  (IQR: 254 to 1378) and 92% had a suppressed HIV VL at  $< 40$  copies. All were on ART and had been for a median of 15 years (IQR: 13 to 16 years). None were currently on a DDI containing ART regime. Median cumulative time previously on DDI was 43 mo (IQR: 22 mo to 68 mo). Overall, 43 (43%) patients reported never drinking alcohol or consuming less than 2 units monthly. Only 2 patients scored  $> 6$  on the MAST score indicating hazardous drinking.

The screening algorithm is shown in Figure 1. ALT above the upper limit normal (ULN) (19 IU/mL in women and 31 IU/mL in men) was found in 37% ( $n = 7/19$ ) of women and 50% of men ( $n = 40/80$ ). Median ALT in men was 32  $\mu/L$  (IQR 23-44) and 18  $\mu/L$  in women (IQR 15-22). Eight-two (82%) had a valid TE reading (IQR/score ratio  $< 0.3$  and success rate  $> 60\%$ ). Of these, 73 (73%) were  $< 7.65$  kPa, 5 (6%) between 7.65-9.4 kPa and 4 (4%)  $> 9.4$  kPa (Figure 1). Only one subject had platelets  $< 120$  and they were known to have cirrhosis of the liver at study entry.

Ultrasound assessment was offered to 49 patients (49%) based on TE reading and/or ALT result. All those that met the criteria for ultrasound were screened for autoimmune liver disease and thrombophilia with a coagulation screen. Four patients did not attend for ultrasound. The most common abnormality was increased reflectivity indicating fatty filtration in 8 patients (18%). A further 4 patients had a normal liver ultrasound but were offered a TJLB on the basis of their ALT and TE score. In total 17 met criteria for TJLB of whom 12 accepted. The characteristics of these patients are described in Table 1. There were no complications observed from the TJB procedures. In the 5 who did not accept liver biopsy, ultrasound appearances were normal in 2 subjects, indicative of fatty infiltration of the liver in 2 and demonstrated splenomegaly in one. Two had abnormal FS readings  $> 7.76$  kPa.

Overall, the commonest histological finding on liver biopsy was steatosis ( $n = 5$ ) or normal architecture ( $n = 4$ ). All subjects had HVPG  $< 6$  mmHg ( $n = 11$ ) including the 2 patients with previously undiagnosed NRH on biopsy in-keeping with a pre-sinusoidal component. This gives a prevalence of previously undiagnosed NRH in our cohort of 2% (95%CI: 0.55%, 6.8%).

As a sensitivity analyses we applied our study algorithm to two other patients attending the HIV clinic with previously identified DDI related liver disease. One



**Figure 1** Screening algorithm to detect Didanosine related liver disease in human immunodeficiency virus positive patients previously exposed to Didanosine for > 6 mo. <sup>1</sup>ALT > upper limit normal (ULN) or platelets < lower limit normal (LLN) or TE > 7.65 kPa; <sup>2</sup>ALT < ULN and platelets > LLN and TE < 7.65 kPa. TE: Transient elastography; HIV: Human immunodeficiency virus; DDI: Didanosine; ALT: Alanine transaminase; HBV: Hepatitis B virus; HCV: Hepatitis C virus; IQR: Interquartile range.

case had been identified due to complications of portal hypertension and the other from liver biopsy undertaken due to abnormal ultrasound scan (U/S) appearances. Both cases met our study screening criteria to proceed to TJLB indicating they would have been detected using our screening process.

## DISCUSSION

NRH largely presents with complications associated with NCPH after a prolonged asymptomatic period<sup>[1-6,9,12]</sup>. Although the aetiology may be multi-factorial, an overriding association is use of DDI. A recent study also identified an association between single-nucleotide polymorphisms in the 5'-nucleotidase and xanthine oxidase genes and development of NCPH after DDI exposure<sup>[13]</sup> suggesting an element of genetic predisposition *via* the purine metabolism pathway.

Our study is the first to use a screening strategy to identify asymptomatic individuals with a previous DDI exposure but with no known liver disease at study entry. Such a strategy is important to identify liver disease

at an earlier stage so that preventative measures and risk minimisation strategies may be instituted. Using our screening strategy we found 2 cases of previously undiagnosed NRH, but no cases of NCPH. Our strategy was based on TE, platelets and ALT levels, with ultrasound and subsequent TJLB in those who met criteria. We used a combination of methods to improve sensitivity of the screening strategy. Elevated transaminases, low platelets and moderate elevations in TE readings have all been described in known cases of NRH or NCPH. We chose a low TE cut off of 7.65 kPa as one study reported a median FS value of 7.9 kPa in subjects with biopsy confirmed NRH<sup>[10]</sup>. In addition in order to identify individuals with very low-level transaminase elevations we used a ULN cut off of 19 IU/mL in women and 31 IU/mL in men, contrasting with the ULN of 41  $\mu$ L/L used in previous studies<sup>[2]</sup>.

A recent multi-centre cohort of DDI-associated NCPH in HIV-infected adults identified thrombocytopenia, splenomegaly and elevated aminotransferases and alkaline phosphatase as significantly associated with NCPH<sup>[14]</sup>. The authors suggest a screening algorithm for NCPH consisting of DDI exposure or splenomegaly plus either

**Table 1** Characteristics of those with liver disease identified as a result of study screening

Subject number	Gender	Age (yr)	Ethnicity	Prior DDI exposure (mo)	Time since DDI (yr)	BMI (kg/m <sup>2</sup> )	MAST score	ALT (IU/mL)	PLTs (10 <sup>6</sup> /L)	PTT	Alk Phos (U/L)	FS (kPa)	Ultrasound results	Biopsy results	HVPG	Fib 4
Participants who accepted to undergo TJLB as a result of study screening																
1	Male	43	White British	81	7	25.58	0	77	175	14.0	83	10	Splenomegaly	NRH with mild steatosis	5	1.29
2	Male	59	White British	40	6	24.42	0	36	175	14.8	93	8	Normal	NRH	4	2.39
3	Male	48	White British	9	6	29.76	0	32	184	14.3	108	NR	Fatty liver, Splenomegaly	Moderate steatosis	3	1.08
4	Male	52	White British	70	7	24.69	0	50	242	11.7	44	5.6	Fatty liver	Mild steatosis	3	0.79
5	Male	49	White Other	34	4	28.54	1	60	215	14.0	83	12.6	Fatty liver	Moderate steatosis, moderate fibrosis	3	1.38
6	Male	52	White British	21	11	28.67	1	58	257	13.4	43	NR	Fatty liver	Moderate steatosis	Not done	1.02
7	Male	62	White Other	13	13	32.56	2	56	175	11.1	45	NR	Fatty liver	Moderate steatosis	2	2.25
8	Male	50	White British	117	3	31.18	0	125	204	10.7	58	8.7	Fatty liver	Mild fibrosis with mild steatosis	2	1.71
9	Female	56	Black African	24	11	36.96	0	27	208	12.3	67	4.4	Coarse echotexture	Normal	5	1.07
10	Male	57	White British	61	4		0	40	190	13.9	71	NR	Splenomegaly	Architecture Normal	3	1.74
11	Male	51	White Irish	103	4	20.30	0	56	228	10.8	154	4.8	Coarse echotexture	Architecture Normal	3	1.04
12	Male	52	White Other	44	5	21.07	6	24	224	11.4	62	9.1	Normal	Architecture Normal	2	1.16
Participants who refused TJLB offered as a result of study screening																
13	Male	46	White British	74	2	21.46	0	18	177		60	10	Normal	Declined		1.41
14	Male	41	White British	97	3	25.65	0	63	188		101	7.6	Splenomegaly	Declined		1.30
15	Male	47	White British	33	5	24.39	0	34	238		58	7.1	Fatty liver, dampened waveform	Declined		0.99
16	Male	61	White British	110	2	20.75	1	35	246		71	9.2	Normal	Declined		1.39
17	Male	54	White British	28	5	28.93	1	32	333		58	4.3	Fatty liver	Declined		0.67

DDI: Didanosine; BMI: Body mass index; MAST: Michigan Alcoholism Screening test; ALT: Alanine transaminase; PLT: Platelet count; FS: FibroScan; TJLB: Trans-jugular liver biopsy.

a raised serum aminotransferase or thrombocytopenia or raised alkaline phosphatase as a trigger for further assessment. Using this study's algorithm only one third of our cohort would have been offered further investigation and one of the two cases of NRH identified in this study prior to development of NCPH would have been missed. Furthermore, splenomegaly is not uncommon in HIV positive patients<sup>[16]</sup>.

We opted to use TE together with ALT and platelet counts on the basis of ready availability of blood tests and the ease of use of FS in the outpatient ambulatory setting. The most common histological abnormality we found on liver biopsy was steatosis, in association with fibrosis in 2 cases. We are unlikely to have missed cases though cannot exclude NRH in those who declined biopsy, but is unlikely that they had underlying NRH in a greater frequency that that seen in patients who did agree to

undergo biopsy. Whilst DDI-associated NRH and NCPH is a serious condition with potentially life-threatening complications, this prospective study suggests a relatively low prevalence in treated cohorts. A previous study has identified non-alcoholic steatohepatitis (NAFLD) as a significant cause of unexplained serum aminotransferase elevation<sup>[12]</sup> in HIV positive people on ART and we also showed a significant rate of hepatic steatosis in association with hepatic fibrosis in our patients.

In this cross sectional study, we found a low prevalence of previously undiagnosed DDI-associated NRH using a screening strategy that combines TE, serum aminotransferase and platelet measurements followed by an U/S. We did, however, demonstrate a higher prevalence of NAFLD, which requires active management to address risk factors and prevent progression to fibrosis in HIV-positive patients.



## ACKNOWLEDGMENTS

The authors would like to thank all the patients that took part in this observational study.

## COMMENTS

### Background

Human immunodeficiency virus positive patients are at increased risk of liver disease. This is multifactorial and includes co-infection with hepatitis viruses, prescribed and recreational drug use and alcohol. The anti-retroviral drug Didanosine (DDI) has been implicated in the aetiology in some patients, particularly if the type of liver damage is nodular regenerative hyperplasia (NRH) or a patient has non-cirrhotic portal hypertension.

### Research frontiers

The authors used a combination of liver enzyme level (with a lower upper limit of normal) and transient elastography (TE) (which measures the liver stiffness) as an initial screen of patients exposed to DDI. This highly sensitive approach identified 42% who required further investigation with an ultrasound scan and 17% who subsequently were offered a transjugular liver biopsy. The authors, therefore, believe that the prevalence rate of 2% NRH in this DDI exposed asymptomatic cohort is accurate.

### Innovations and breakthroughs

The prevalence study is the first to systematically screen asymptomatic patients exposed to DDI. Other groups have looked at the association between the drug and liver disease but have not screened a large cohort of exposed but asymptomatic patients to establish a prevalence of disease.

### Applications

Use of a simple screening strategy in patients previously exposed to DDI will allow clinicians to identify liver disease which if left undiagnosed may present with the complications of portal hypertension such as variceal bleeding.

### Terminology

TE: A technique combining ultrasound waves and a pressure transducer to assess the stiffness of the liver. This has been validated as a tool to measure liver fibrosis and steatosis; NRH is characterized by small (less than 3 mm) regenerative nodules in the absence of fibrous septa on biopsy. The nodules cause obliteration of the portal veins which leads to portal hypertension.

### Peer-review

It is an interesting study.

## REFERENCES

- Sood A, Castrejón M, Saab S. Human immunodeficiency virus and nodular regenerative hyperplasia of liver: A systematic review. *World J Hepatol* 2014; **6**: 55-63 [PMID: 24653794 DOI: 10.4254/wjh.v6.i1.55]
- Maida I, Núñez M, Ríos MJ, Martín-Carbonero L, Sotgiu G, Toro C, Rivas P, Barreiro P, Mura MS, Babudieri S, García-Samaniego J, González-Lahoz J, Soriano V. Severe liver disease associated with prolonged exposure to antiretroviral drugs. *J Acquir Immune Defic Syndr* 2006; **42**: 177-182 [PMID: 16688096 DOI: 10.1097/01.qai.0000221683.44940.62]
- Mallet V, Blanchard P, Verkarre V, Vallet-Pichard A, Fontaine H, Lascoux-Combe C, Pol S. Nodular regenerative hyperplasia is a new cause of chronic liver disease in HIV-infected patients. *AIDS* 2007; **21**: 187-192 [PMID: 17197809 DOI: 10.1097/QAD.0b013e3280119e47]
- Maida I, García-Gasco P, Sotgiu G, Ríos MJ, Vispo ME, Martín-Carbonero L, Barreiro P, Mura MS, Babudieri S, Albertos S, García-Samaniego J, Soriano V. Antiretroviral-associated portal hypertension: a new clinical condition? Prevalence, predictors and outcome. *Antivir Ther* 2008; **13**: 103-107 [PMID: 18389904]
- Saifee S, Joelson D, Braude J, Shrestha R, Johnson M, Sellers M, Galambos MR, Rubin RA. Noncirrhotic portal hypertension in patients with human immunodeficiency virus-1 infection. *Clin Gastroenterol Hepatol* 2008; **6**: 1167-1169 [PMID: 18639498 DOI: 10.1016/j.cgh.2008.04.023]
- Stebbing J, Wong N, Tan L, Scourfield A, Jiao LR, Shousha S, Grover D, Bower M, Nelson M. The relationship between prolonged antiretroviral therapy and cryptogenic liver disease. *J Acquir Immune Defic Syndr* 2009; **50**: 554-556 [PMID: 19300102]
- Friedrich-Rust M, Ong ME, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077 DOI: 10.1053/j.gastro.2008.01.034]
- Vizzutti F, Arena U, Rega L, Romanelli RG, Colagrande S, Cuofano S, Moscarella S, Belli G, Marra F, Laffi G, Pinzani M. Performance of Doppler ultrasound in the prediction of severe portal hypertension in hepatitis C virus-related chronic liver disease. *Liver Int* 2007; **27**: 1379-1388 [PMID: 18036101 DOI: 10.1111/j.1478-3231.2007.01563.x]
- Scourfield A, Waters L, Holmes P, Panos G, Randell P, Jackson A, Mandalia S, Gazzard B, Nelson M. Non-cirrhotic portal hypertension in HIV-infected individuals. *Int J STD AIDS* 2011; **22**: 324-328 [PMID: 21680667 DOI: 10.1258/ijsa.2010.010396]
- Laharie D, Vergniol J, Bioulac-Sage P, Diris B, Poli J, Foucher J, Couzigou P, Drouillard J, de Ledinghen V. Usefulness of noninvasive tests in nodular regenerative hyperplasia of the liver. *Eur J Gastroenterol Hepatol* 2010; **22**: 487-493 [PMID: 19940782 DOI: 10.1097/MEG.0b013e328334098f]
- Cotte L, Bénet T, Billioud C, Mialhes P, Scoazec JY, Ferry T, Brochier C, Boibieux A, Vanhems P, Chevallier M, Zoulim F. The role of nucleoside and nucleotide analogues in nodular regenerative hyperplasia in HIV-infected patients: a case control study. *J Hepatol* 2011; **54**: 489-496 [PMID: 21056493 DOI: 10.1016/j.jhep.2010.07.030]
- Ingiliz P, Valantin MA, Duvivier C, Medja F, Dominguez S, Charlotte F, Tubiana R, Poynard T, Katlama C, Lombès A, Benhamou Y. Liver damage underlying unexplained transaminase elevation in human immunodeficiency virus-1 mono-infected patients on antiretroviral therapy. *Hepatology* 2009; **49**: 436-442 [PMID: 19085967 DOI: 10.1002/hep.22665]
- Vispo E, Cevik M, Rockstroh JK, Barreiro P, Nelson M, Scourfield A, Boesecke C, Wasmuth JC, Soriano V. Genetic determinants of idiopathic noncirrhotic portal hypertension in HIV-infected patients. *Clin Infect Dis* 2013; **56**: 1117-1122 [PMID: 23315321 DOI: 10.1093/cid/cit001]
- Parikh ND, Martel-Laferrriere V, Kushner T, Childs K, Vachon ML, Dronamraju D, Taylor C, Fiel MI, Schiano T, Nelson M, Agarwal K, Dieterich DT. Clinical factors that predict noncirrhotic portal hypertension in HIV-infected patients: a proposed diagnostic algorithm. *J Infect Dis* 2014; **209**: 734-738 [PMID: 23911709 DOI: 10.1093/infdis/jit412]
- Groszmann RJ, Wongcharatrawee S. The hepatic venous pressure gradient: anything worth doing should be done right. *Hepatology* 2004; **39**: 280-282 [PMID: 14767976 DOI: 10.1002/hep.20062]
- Zambetti EF, Haramati LB, Jenny-Avital ER, Boreczuk AC. Detection and significance of splenomegaly on chest radiographs of HIV-infected outpatients. *Clin Radiol* 1999; **54**: 34-37 [PMID: 9915508 DOI: 10.1016/S0009-9260(99)91237-0]

P- Reviewer: Koubaa M, McQuillan GM S- Editor: Ji FF  
L- Editor: A E- Editor: Li D



Observational Study

## Enzyme pattern of biliary colic: A counterintuitive picture

Elad Resnick, Shimon Shteingart, Bernardo Melamud, Tali Bdolah-Abram, Todd Zalut, Adrian Reuben, Yoav Lurie

Elad Resnick, Department of Interns, Hadassah University Hospital, Kiryat Hadassah, Jerusalem 91120, Israel

Shimon Shteingart, Department of Pathology, Share'e Zedek Medical Center, Jerusalem 91031, Israel

Bernardo Melamud, Digestive Disease Institute, Share'e Zedek Medical Center, Jerusalem 91031, Israel

Tali Bdolah-Abram, Department of Social Medicine, Hebrew University, Hadassah Hospital, Kiryat Hadassah, Jerusalem 91120, Israel

Todd Zalut, Department of Emergency Medicine, Share'e Zedek Medical Center, Jerusalem 91031, Israel

Adrian Reuben, Division of Gastroenterology and Hepatology, Medical University of South Carolina, Charleston, SC 29425-2900, United States

Yoav Lurie, Liver Unit, Digestive Disease Institute, Share'e Zedek Medical Center, Jerusalem 91031, Israel

**Author contributions:** Resnick E, Shteingart S and Lurie Y designed the research; Resnick E, Zalut T, Melamud B and Lurie Y provided the clinical data; Resnick E, Shteingart S, Bdolah-Abram T and Lurie Y analyzed and modeled the data; Resnick E, Shteingart S, Reuben A and Lurie Y wrote the paper.

**Institutional review board statement:** This was an observational chart review and was approved by the Share'e Zedek Medical Center's Helsinki committee (number: p 92/13).

**Informed consent statement:** Not needed, as no patients were contacted.

**Conflict-of-interest statement:** None.

**Data sharing statement:** Technical appendix, statistical code and dataset available from the corresponding author at [yoav@szmc.org.il](mailto:yoav@szmc.org.il). Consent was not obtained but the presented data are anonymized and risk of identification is low.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external

reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Yoav Lurie, MD, Head of Liver Unit, Digestive Disease Institute, Share'e Zedek Medical Center, 12 Shmu'el Bait Street, Jerusalem 91031, Israel. [yoav@szmc.org.il](mailto:yoav@szmc.org.il)  
**Telephone:** +972-2-6555035  
**Fax:** +972-2-6555359

**Received:** June 29, 2016  
**Peer-review started:** July 1, 2016  
**First decision:** September 5, 2016  
**Revised:** October 2, 2016  
**Accepted:** November 1, 2016  
**Article in press:** November 2, 2016  
**Published online:** December 28, 2016

## Abstract

### AIM

To evaluate the diagnostic value of serial biochemical blood tests in the diagnosis of biliary colic.

### METHODS

Files were reviewed of 1039 patients who were admitted to the Share'e Zedek Medical Center emergency department between the years 2012-2013, and received the coding of acute biliary disease. Of these, the first 100 cases were selected that met the following criteria: (1) a diagnosis of biliary colic or symptomatic cholelithiasis; (2) at least two biochemical blood tests performed; and (3) 18 years of age or older. Patients with other acute biliary diseases were excluded. The biochemical profile of the patients was analyzed as were their clinical and radiological findings.

## RESULTS

Three-quarters of the patients were women, whose average age of 37 years was younger than the average of the men, at 50 years. According to their histories, 47% of the patients had previously known cholelithiasis. Pain in either the right upper quadrant or the epigastrium was the presenting symptom in 93% cases. The greatest change in serum biochemical results was seen during the first day of the patients' admissions. Alanine aminotransferase (ALT) showed the highest initial rise above the reference range, followed by aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), bilirubin and alkaline phosphatase (ALKP) - all these increases were statistically significant ( $P < 0.05$ ). AST showed the sharpest decline followed by bilirubin and ALT. GGT and ALKP did not fall. A sharp rise and fall in liver enzymes, especially during the first day, most prominently in AST and ALT, was seen in 70% percent of cases. In 65% of cases trans-abdominal sonography did not give diagnostic findings.

## CONCLUSION

Serial serum liver enzyme measurements are helpful in the initial diagnosis of acute biliary colic.

**Key words:** Biliary colic; Symptomatic cholelithiasis; Gallstones; Liver enzymes; Aspartate aminotransferase; Alanine aminotransferase; Enzyme pattern; Diagnostic tool; Emergency department

© **The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Gallstones are prevalent in affluent countries, more so in women than in men, and their prevalence increases with age. A large proportion of patients presenting to the emergency department with epigastric or right upper quadrant (RUQ) pain present a diagnostic challenge, especially when they belong to the older age group. We found that serial liver and biliary enzyme measurements reveal a characteristic pattern that helps the clinician determine quickly, cheaply and safely that the cause of RUQ/epigastric pain is biliary colic, in 71% of the patients. Serial enzyme testing is a useful adjunct to other diagnostic tools, for the diagnosis of acute upper abdominal pain.

Resnick E, Shteingart S, Melamud B, Bdolah-Abram T, Zalut T, Reuben A, Lurie Y. Enzyme pattern of biliary colic: A counterintuitive picture. *World J Hepatol* 2016; 8(36): 1629-1636 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i36/1629.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i36.1629>

## INTRODUCTION

Gallstones are prevalent in affluent countries, more so in women than in men, and their prevalence increases with age<sup>[1]</sup>. Gallstones usually produce symptoms when they migrate into the cystic duct or common bile duct

(CBD), causing obstruction that increases intraluminal pressure and distends the viscus. The most characteristic symptom of gallstone disease is biliary colic<sup>[2]</sup>, i.e., pain arising from the cystic duct or CBD.

A patient presenting to the emergency department (ED) complaining of epigastric or right upper quadrant (RUQ) pain, with a classic history and physical examination compatible with biliary colic does not present a great diagnostic challenge. A large proportion of patients presenting to the ED with epigastric or RUQ pain do present a diagnostic challenge, especially when they belong to the older age group (in which both a wide array of underlying diseases and polypharmacy are prevalent) which has become more common in Western society over recent decades<sup>[1]</sup>. Unfortunately, "classic" cases are the exception rather than the rule<sup>[3,4]</sup>. The differential diagnosis in an older patient with many co-morbidities includes: An acute coronary syndrome, pericarditis, an aortic dissection, peptic ulcer disease including a perforated ulcer, pulmonary embolism, lower lobe pneumonia, renal colic, pyelonephritis, partial colonic obstruction, diverticulitis, appendicitis, pancreatitis, acute cholecystitis, cholangitis, diabetic ketoacidosis, porphyria and biliary colic<sup>[2]</sup>. The diagnostic pathways are not straightforward, consume time and resources and are subject to pitfalls and misleading findings.

The confirmatory diagnostic test for biliary colic in the ED is the demonstration of gallstones in the cystic duct or CBD by trans-abdominal sonography<sup>[4-6]</sup>. However the demonstration of cholelithiasis alone is not diagnostic<sup>[1]</sup>, and unfortunately, the demonstration of gallstones in the cystic duct or CBD is very difficult even for an experienced radiologist under optimal conditions, namely in a lean, cooperative and fasted patient, when time is not limited. In practice, it is often very difficult to demonstrate gallstones in the cystic duct or CBD<sup>[7]</sup>. Due to these limitations, and in the majority of cases, ultrasonography is usually not diagnostic in the first hours or days of the patient's admission, and a negative ultrasound scan may be misleading<sup>[1,5,7-9]</sup>. A simple, rapid, cheap, non-invasive, reproducible and reliable test is needed to diagnose biliary colic quickly and efficiently.

Laboratory testing of serum "hepatocellular" and cholestatic liver enzymes [namely: Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and alkaline phosphatase (ALKP) and gamma-glutamyl transferase (GGT), respectively] is performed almost routinely in patients presenting with epigastric or RUQ pain, as the clinician searches for diagnostic patterns. For example: A patient with or without pain who has elevated "cholestatic" enzymes, ALKP and GGT, is considered a potentially "surgical" patient who is suffering from a chronic mechanical obstruction of the biliary tract. And indeed, it is rare to find such an obstruction without elevation of these enzymes<sup>[10]</sup>. In this type of obstruction, the hepatocellular enzymes, AST and ALT, will be only mildly elevated. Conversely, a patient with RUQ fullness, malaise, and AST and ALT in the hundreds and even thousands of international units per liter (IU/L), with or

without jaundice, and with only mildly elevated ALKP and GGT is considered a “medical” patient, who is suffering from viral, autoimmune or drug-induced hepatitis. These patterns that have served so well for so many years represent entrenched dogma.

We and others<sup>[11-13]</sup> have also observed another distinct pattern of a sharp (up to 100-fold above the upper limit of the normal reference range), short-lived (usually less than a week) rise in AST and ALT, and only a mild rise in ALKP, bilirubin and GGT, which we think is characteristic of acute “biliary colic”. Our clinical impression is that this pattern has high specificity for the diagnosis of “biliary colic” especially in the first hours of the patient’s admission, which is paradoxically counterintuitive in a patient, who has “surgical” pain with a “medical” enzyme pattern.

Our observations are mentioned (unreferenced) in the two latest editions of the leading textbooks in internal medicine, the leading textbook in gastroenterology as well as in a respected textbook on laboratory tests<sup>[2,8,14,15]</sup>. It is noteworthy that this pattern is not mentioned in the latest versions of two leading textbooks in general surgery<sup>[4,6]</sup>.

Our current study will examine the existence and the utility of this paradoxical “biliary colic” enzyme pattern in our own patient population.

## MATERIALS AND METHODS

### Study design and patients

This was an observational retrospective chart review, which was approved by the Medical Center’s Helsinki committee (number: p 92/13). We reviewed the medical records of 1039 patients, who were admitted to the Share’e Zedek Medical Center ED between December 1<sup>st</sup> 2013 and January 1<sup>st</sup> 2012, and who were assigned the coded diagnosis of “acute biliary disease”. Inclusion criteria were: (1) 18 years of age and older; (2) two or more blood tests including a liver profile; and (3) a diagnosis of biliary colic or symptomatic cholelithiasis. Symptomatic cholelithiasis was also included as it was clear that this term was used interchangeably with biliary colic. It was used to differentiate this entity from acute cholecystitis. Exclusion criteria were: (1) acute cholecystitis; (2) ascending cholangitis; (3) ultrasound-confirmed choledocholithiasis; and (4) sepsis. The search for cases meeting the inclusion and exclusion criteria was closed once the first sequential 100 suitable patients (out of 1039 during 23 mo) were identified. The case acquisition flow chart is shown in Figure 1.

Data were collected from the Medical Center computerized database. Clinical data were derived from the either the ED file (if the patient was not hospitalized) or the hospital chart. Laboratory data were collected from the computerized laboratory records. Laboratory tests were performed using the standard techniques used for all biochemical tests at the hospital. Ultrasound interpretations were retrieved from the diagnostic imaging computerized database in written or dictated format.

### Primary and secondary end points

The primary variables we examined were the serum biochemical laboratory results, including: Bilirubin, ALKP, AST, ALT and GGT, throughout the patient’s stay in the ED or hospital, relating to the particular ED visit with biliary colic.

The secondary variables examined were the location of the patient’s pain, radiation of the pain, whether it was post-prandial, and associated symptoms such as anorexia, nausea, vomiting, fever and chills, and physical examination findings. The interpretation of the radiologic tests performed was also evaluated.

### Statistical analysis

Statistical analysis was performed by Tali Bdolah-Abram from The Hebrew University, Jerusalem, Israel. Paired *t*-tests as well as non-parametric Wilcoxon signed-rank tests were used to assess the differences between pairs of quantitative variables. The one-sample *t*-test was used to test the significance of percent changes between two measurements. Repeated measures ANOVA models were applied to quantitative variables in order to simultaneously test trends over time, the difference between subgroups of patients and the interaction between time and group. The significance of the trends and the interactions were tested using the Greenhouse-Geisser test. The Friedman non-parametric test was used for testing a trend over time for quantitative variables when the data was not normally distributed.

All tests applied were two-tailed, and a *P*-value of 5% or less was considered statistically significant.

## RESULTS

### Demographic characteristics

The basic demographic characteristics of the 100 cases studied are presented in Table 1. The mean age of the patients in our study was 40 years, 76% of cases were women. On average, the women were younger than the men with mean ages of 37.4 years and 50.0 years respectively. In 47% of cases, cholelithiasis was already known from the patient’s history.

### Clinical characteristics

Clinical presentations were extracted from the patients’ files and are reported in Table 2. The clinical variables analyzed were: Location of pain and tenderness, presence of peritoneal irritation, Murphy sign, fever, chills, nausea or vomiting and whether the pain was post-prandial. The location of the pain was noted to be in the RUQ or epigastrium in the large majority of cases (92.8%).

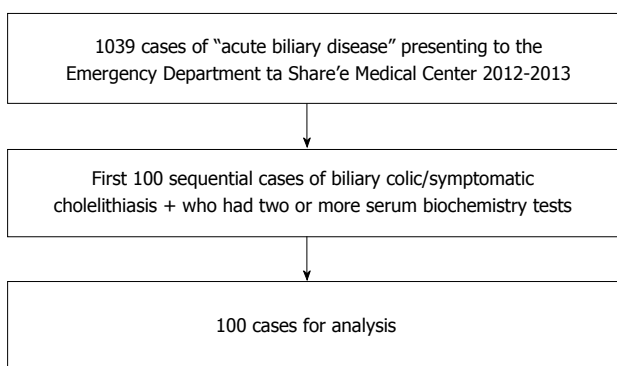
### Analysis of blood biochemistry tests

The primary endpoint of our study was the result of the analysis of the serial blood biochemistry laboratory tests, notably: Bilirubin, AST, ALT, ALKP and GGT. All tests were done according to the standard routine of the hospital laboratory. We retrieved the sequential laboratory results



**Table 1** Demographic characteristics

Age (yr)	
Mean $\pm$ SD	40.5 $\pm$ 19.1
Median	37
Gender (#)	
Men	24
Women	76
Age by gender (yr)	
Men	
Mean $\pm$ SD	50.0 $\pm$ 17.0
Median	49
Women	
Mean $\pm$ SD	37.4 $\pm$ 18.8
Median	31.5
Cholelithiasis known previously (from history)	
Yes	47
No	53



**Figure 1** Flow chart illustrating case selection process. Of 1039 patients who presented to the emergency department (ED) of Share'e Zedek Medical Center during 23 mo (2012-2013) with various forms of acute biliary disease, 100 who were designated in the ED as having Biliary Colic and who fulfilled study criteria, were selected sequentially for analysis of concomitant serum bilirubin and enzyme temporal patterns.

for the 100 cases of interest, and analyzed the results from up to four sequential tests in each patient. Some patients had more than four tests done, but as this subset was small, we limited our analysis to four tests. The goal of the study was to differentiate which enzyme variable was most significantly indicative of the clinical event, namely biliary colic. To facilitate comparison between the four enzyme patterns, we normalized the data as percent changes per hour. For example, a change in AST from 50 to 150 units over a 10 h interval, would calculate to a change of 10 units per hour, representing a 20% per hour increase from 50 units. Figure 2 shows the time intervals between the sequential measurements tests, which allows a visual appreciation of the time course of the changes in enzyme levels. The average time between tests grew longer from the first to the last test, at 15.8, 25.3 and 29.8 h, respectively.

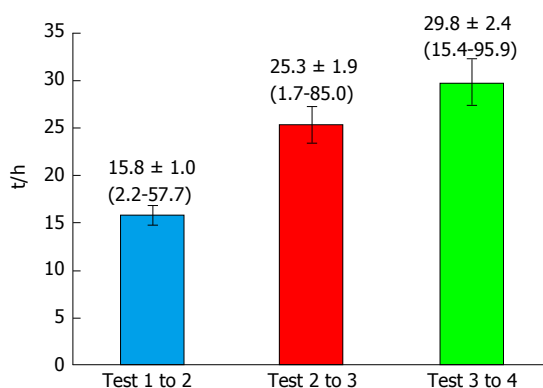
We compared changes in all five primary variables to one another at three different points, *i.e.*, percent changes per hour between tests 1 and 2, 2 and 3, and 3 and 4, as is shown in Figure 3.

It can be readily seen that the largest percent changes in enzyme levels, which were also statistically significant,

**Table 2** Clinical features, *n* (%)

Pain location	
RUQ	41 (42.3)
Epigastric	43 (44.3)
RUQ and/or epigastric + other region	6 (6.2)
Not RUQ or epigastric	7 (7.3)
No data	3
Fever	
Yes	2 (2.1)
No	95 (97.9)
No data	3
Nausea	
Yes	28 (60.9)
No	18 (39.1)
No data	54
Vomiting	
Yes	20 (31.7)
No	43 (68.3)
No data	37
Tenderness to palpation	
RUQ	47 (49.0)
Epigastric	20 (20.8)

RUQ: Right upper quadrant.

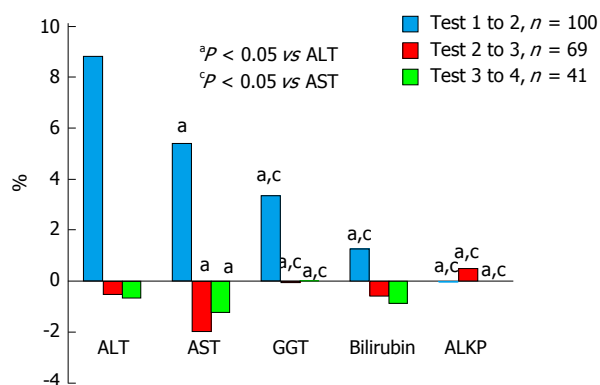


**Figure 2** Time intervals between serological liver tests (hours). Time interval data in hours, are shown as means. Means  $\pm$  SDs, and ranges (*i.e.*, minima to maxima), above the bars.

were seen between the first and second tests, and that ALT changed the most followed by AST, GGT, bilirubin and ALKP. Between tests two and three, the effects were far smaller with AST being the only variable with greater than one percent change. Between tests three and four effect percent changes were of similar magnitude to the changes between test two and three. AST still showed the largest absolute change, but this was smaller than between the previous tests, at 1.2%.

### The enzyme pattern

In order to demonstrate the enzyme pattern we plotted the percent change over time as seen in Figure 4. As this is a retrospective study and not protocol-driven, blood was drawn for testing at different intervals in each case. Therefore, the time between tests is represented as the average time interval between tests. To permit a graphic presentation of the different enzyme patterns, the hourly percent changes are multiplied by the corresponding



**Figure 3 Comparison of relative percent changes (per hour) in bilirubin and liver enzymes, between serial tests.** Percent change per hour is shown for these five variables. <sup>a,c</sup>Denote significant statistical difference from ALT and AST respectively in the same time frame (i.e., ALT between test one and two compared with AST between test one and two). The number of cases in the analysis in the different time frames is shown on the top right. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; ALKP: Alkaline phosphatase.

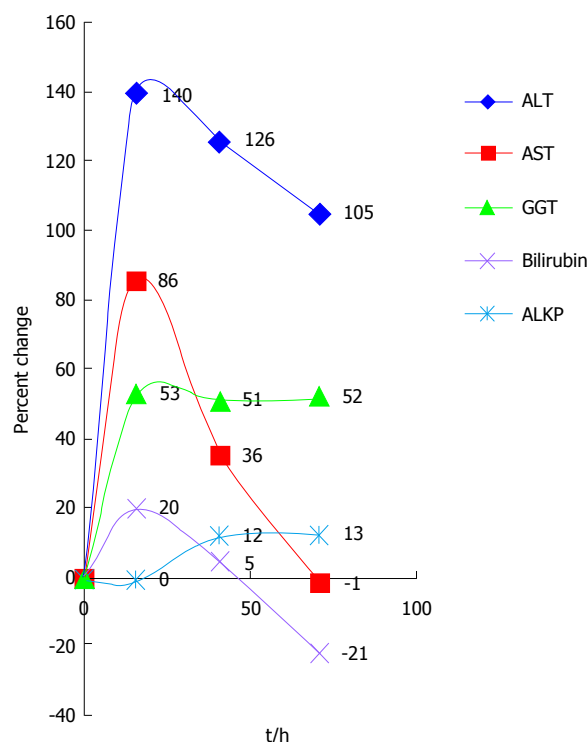
average time intervals.

As shown above, the largest initial change was in ALT, at 140% between the first and second tests. AST rose less dramatically (86%) but showed the most dramatic average fall (50%). GGT rose initially by 53% but then hardly changed at all, while bilirubin and ALKP showed only minor fluctuations.

To determine how many of the 100 patients studied presented with the aforementioned enzyme pattern that is characteristic for biliary colic, we devised two criteria to separate patients into those either positive or negative for the pattern; positivity for either criterion was considered a characteristic pattern.

The first criterion was that a patient had at least doubled the levels of AST or ALT between the first two tests, compared to any subsequent test. The second criterion was that a patient had at least halved the first test results for AST or ALT in any one of the following three tests. Additionally, to be counted positive for the second criterion, only patients who had a first test result of more than double the upper limit of normal (ULN) for AST or ALT were counted. We used more than twice the ULN of normal as a cut-off as this has been used for many years in the field of chronic hepatitis B surveillance and in treatment algorithms (between 1-2 times the ULN is considered minimally raised)<sup>[16,17]</sup>. ULN for AST and ALT in our medical center are 36 and 52 units, respectively.

We found 33 (33%) patients positive for the first criterion (of a doubling in aminotransferases) all of whom showed a rise in ALT and 18 (54.5%) of whom had a rise in AST. Fifty-two (52%) patients were positive for the second criterion (of a significant subsequent fall in enzymes) - all had a fall in AST and 14 (26.9%) of whom also showed a fall in ALT. All told, 71 (71%) patients were positive for either one or the other of the criteria and 14 (57.6%) out of the 71 were positive for both criteria.



**Figure 4 Time trends in percent changes of bilirubin and liver enzymes with serial testing.** Time between tests is represented using the average time interval between tests (as seen in Figure 2). The hourly percent change between each test was multiplied by this interval, to provide a graphic presentation of the enzyme patterns. The intersection of the axes represents the first test and the following points represent each change from the previous result. For example, bilirubin increased by 20% between tests one and two and decreased by 15% between tests two and three; therefore, the next point is 5%. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; ALKP: Alkaline phosphatase.

### Ultrasonography findings

Trans-abdominal ultrasonography was performed in all but one case, a woman who had had sonography performed the previous day, when she was found to have gallbladder stones. The sonography reports given by the hospital radiologists, in either written or dictated form, were reviewed. We divided the findings into two categories, namely (1) highly suggestive or compatible with a cause for biliary colic; and (2) questionable or possibly non-contributory to the diagnosis of biliary colic. The results and the categorization of the different findings are displayed in Table 3. As can be seen, in only a third of the cases were the sonographic findings highly suggestive or compatible with a cause for biliary colic. In other words there was definite, albeit somewhat indirect, evidence of CBD disease. In the remaining 64 cases, whereas there was no evidence of CBD disease, it is conceivable, nonetheless, that gallstones could have migrated from the gallbladder in 62 cases without causing visible CBD injury.

### DISCUSSION

Biliary colic is a common symptom that is defined as

**Table 3** Ultrasound findings

	Frequency
Non contributory	64
Contracted gallbladder	2
Cholelithiasis	58
Contracted gallbladder + cholelithiasis	4
Compatible or highly suggestive	35
Dilated CBD	5
Dilated CBD + filling defect	1
Thickened gallbladder wall	6
Distended gallbladder + cholelithiasis	13
Distended gallbladder	2
Stone in cystic duct	4
Distended gallbladder + pericystic fluid	2
Thickened gallbladder wall + cholelithiasis	2
Total valid	99
Missing	1
Total	100

CBD: Common bile duct.

pain caused by spasm of the cystic duct or CBD, usually caused by intraluminal calculi that have migrated from the gallbladder, or occasionally that form *in situ*<sup>[1]</sup>. Patients diagnosed with biliary colic present with symptoms of RUQ and/or epigastric pain. In some cases, diagnosis is straightforward but in some the differential diagnosis is wide and the evaluation is time and resource-consuming and occasionally potentially harmful.

Trans-abdominal sonography is readily available, but has low sensitivity and a low negative predictive value for choledocholithiasis. Indeed, in our study all but one of the patients were finally diagnosed as having biliary colic due to the passage of a stone through the CBD despite, the fact that choledocholithiasis was not proven directly in 99% of them. Ultrasonic findings, *i.e.*, CBD abnormalities, were compatible or highly suggestive of biliary colic, in only 35% of the patients. But even in this minority, sonography did not clinch the diagnosis, since there seemingly was enough uncertainty to prevent the clinician from discharging the patient or referring him/her for laparoscopic cholecystectomy without additional tests. The same is truer for those 62 patients with typical biliary colic in whom cholelithiasis was present, but there was no visible CBD abnormality. The ED staff in Galveston, Texas, made similar observations on the utility of ultrasound scans, and this led to overuse of computed tomography scanning, especially at night<sup>[18]</sup>.

Jafari *et al.*<sup>[7]</sup> also point out that passage of stones through the CBD can be fast, so that by the time the patient is transported to the radiology department the diagnostic picture - a dilated CBD containing a stone, can be missed. They advocate that ultrasound scans should be performed in the ED, and for CBD diameter to be measured routinely. It is in this situation that the characteristic enzyme elevation and fall pattern comes into its own.

As opposed to these limitations of ultrasound scanning, we found that serial liver and biliary enzyme tests (2-4 tests performed during the first 80 h from admission)

increase and decrease with statistical significance, and reveal a characteristic liver enzyme pattern that helps the ED clinician determine quickly, cheaply and safely, in concert with simultaneous other diagnostic maneuvers, that the cause of RUQ/epigastric pain is biliary colic. Thus, our findings confirm and extend what is already known. We assume that in most of the 29% of patients in whom the characteristic temporal pattern was not seen, the first blood sample was taken too late for the sharp rise and fall to be fully appreciated.

The most statistically significant changes in the variables we examined were seen between the 1<sup>st</sup> and the 2<sup>nd</sup> test. The most prominent effect is seen with ALT followed by AST. However, GGT and ALKP, the classic "obstructive" biliary enzymes, and bilirubin, contribute little, if at all, to the diagnosis. This finding is compatible with our assumption that ALT and AST, the "medical" hepatocellular enzymes, are deranged early in biliary colic, for which the most plausible hypothesis is that high biliary pressures lead to impairment of bile secretion and retention of bile acids with accompanying hepatocyte apoptosis, necrosis or leakage of enzymes<sup>[19]</sup>. The classic rise in the "biliary" enzymes, alkaline phosphatase and GGT, in prolonged obstruction is thought to reflect increased enzyme synthesis rather than cholangiocyte damage and leakage<sup>[20]</sup>. Moreover, the greatest changes in enzyme levels occur early and up to the first 24 hours of the patient's admission, which makes this a diagnostic tool particularly useful in the ED setting.

In our study many patients were seen to have both the rise and the fall of hepatocellular enzymes, unlike the findings in two earlier studies that showed mainly the down-sloping phase of the temporal enzyme pattern<sup>[12,21]</sup>. This can be explained by lesser availability of liver enzyme testing in the late 1980s, when these studies were published. And indeed, in a later study, published in 2010, an enzyme pattern almost identical to ours was found, in which both the ascending and descending phases were detected. This latter study was performed on subjects in whom choledocholithiasis was proven invasively, which, admittedly, is a different and far less common situation<sup>[22]</sup>.

The pattern of a short term (less than a week), sharp rise and sharp (but to a lesser degree) fall in ALT and AST is classically recognized in two clinical scenarios, namely ischemic liver injury - so-called Hypoxic Hepatitis - and acetaminophen intoxication<sup>[23-25]</sup>. It is our opinion that this pattern is also typical of a third clinical scenario - biliary colic due to passage of a biliary stone that causes transient biliary obstruction.

Our impression is that this pattern is not widely appreciated by general clinicians although it is mentioned in several articles and in some leading textbooks<sup>[2,8,12-15,21-22,26]</sup>. One explanation for this pattern's relative anonymity is the dogma that elevated cholestatic enzymes mean biliary obstruction has served so well for prolonged biliary obstruction. The corollary has been assumed, that all biliary obstruction is accompanied by a cholestatic enzyme pattern. This is clearly not the case, as transient

biliary obstruction due to passage of a stone through the CBD causes elevation of ALT and AST - the medical or hepatocellular enzymes.

Limitations of this enzyme temporal analysis include the fact that this is a retrospective study, with its unavoidable selection bias, and that the exact timing of the clinical event, *i.e.*, the passage of the CBD stone, is unknown. Hence, the appropriateness of the time of first blood drawing is also uncertain. A third limitation of this study is that we only included patients who had two or more blood tests performed, which introduces further selection bias of patients who had initial enzyme elevations. Irrespective, the more prominent abnormal enzymes were the hepatocellular. Another bias is that our subjects were already diagnosed as suffering from biliary colic. It remains to be tested, therefore, whether the characteristic "biliary colic" pattern will be useful in the more real life scenario of a patient presenting to the ED with abdominal pains. We hope to be able to resolve this question in a prospective study, in which all patients presenting with abdominal pain will undergo serial liver enzyme measurement at protocol-defined intervals, along with a precise history of the time of onset of the abdominal pain. Finally, although we were careful to exclude cases of cholecystitis, it must still be acknowledged that aminotransferase elevation occurs in active gallbladder inflammation, usually associated with fever and leukocytosis (both absent in our 100 cases) and of slower resolution than seen in our cases. In gallbladder inflammation, the mechanism of aminotransferase rise is usually attributed to cytokine release and other mediators of the Systemic Inflammatory Response Syndrome<sup>[27]</sup>.

### Conclusions and practical implications

Based on a retrospective statistical analysis of 100 patients who were diagnosed as having biliary colic - which is defined as upper abdominal pain due to passage of a gallstone through the cystic duct or CBD, we found that a sharp, short term rise and fall of ALT and AST, typically thought to be indicative of ischemic hepatic injury and acetaminophen intoxication, is also typical of biliary colic. Whereas the current observations are not entirely novel, they are worth emphasizing and bringing to general attention as they do not appear to be widely appreciated. Thus, in the ED, when a patient presents with an appropriate history and physical exam, we recommend adding liver enzymes to the list of blood tests already ordered. If liver enzymes are found to be elevated, we recommend repeating these tests twice or thrice at intervals over the next 24 h. If the pattern that is characteristic of biliary colic is seen, concomitant with the resolution or even only marked improvement of the pain, and no other diagnosis is suspected (*e.g.*, an acute coronary event, pancreatitis, cholecystitis, *etc.*), then the patient can be discharged home or admitted for laparoscopic cholecystectomy according to local practice<sup>[28]</sup>.

## ACKNOWLEDGMENTS

We would like to thank Tzina Lindenberg, director of Share'e Zedek Medical Center's archive, for her support of this project.

## COMMENTS

### Background

Patients complaining of abdominal pain are frequently seen in the emergency department. The differential diagnosis is vast and therefore the clinical workup is resource and time consuming. However, some clinical observations led us to the thought that a few cheap and routine blood tests could greatly aid the clinician in this diagnostic challenge.

### Research frontiers

A few studies, mainly from the 1980's and 1990's described the enzymatic profile of biliary colic and reached. However this very useful profile did not become common knowledge.

### Innovations and breakthroughs

In recent years more patients were tested for liver enzymes as part of the routine workup of acute abdominal pain. Additionally many patients had more than one blood sample taken. This has allowed us to follow the level of liver enzymes and observe a unique pattern.

### Applications

This pattern helps clinicians in the clinical scenario of acute abdominal pain diagnosis. It is cheap, rapid, readily available, accurate and non-invasive.

### Terminology

Biliary colic is a term used to describe pain arising from stones obstructing the cystic duct or the passage of a stone through the common bile duct.

### Peer-review

This manuscript provides the updated evidence to the readers. The topic is an important one and deserves a practical value.

## REFERENCES

- 1 **Duncan CB**, Riall TS. Evidence-based current surgical practice: calculous gallbladder disease. *J Gastrointest Surg* 2012; **16**: 2011-2025 [PMID: 22986769 DOI: 10.1007/s11605-012-2024-1]
- 2 **Longo DL**, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J. Harrison's principles of internal medicine. 18th edition, USA: Mc Graw Hill, 2012: 2615-2628
- 3 **Gunn A**, Keddie N. Some clinical observations on patients with gallstones. *Lancet* 1972; **2**: 239-241 [PMID: 4114503 DOI: 10.1016/S0140-6736(72)91683-2]
- 4 **Brunicaardi F**, Andersen D, Billiar T, Dunn D, Hunter JG, Matthews JB, Pollock RE. Schwartz's Principles of Surgery. 10th edition, USA: McGraw-Hill, 2014: 1309-1340
- 5 **Bar-Meir S**. Gallstones: prevalence, diagnosis and treatment. *Isr Med Assoc J* 2001; **3**: 111-113 [PMID: 11344819]
- 6 **Townsend CM Jr**, Beauchamp RD, Evers BM, Mattox KL. Sabiston Textbook of Surgery. 19th edition, Canada: Elsevier Saunders, 2012: 1476-1514
- 7 **Jafari D**, Cheng AB, Dean AJ. Dynamic changes of common bile duct diameter during an episode of biliary colic, documented by ultrasonography. *Ann Emerg Med* 2013; **62**: 176-179 [PMID: 23489651 DOI: 10.1016/j.annemergmed.2013.01.004]
- 8 **Feldman M**, Friedman LS, Brandt LJ. Sleisenger and Fordtran's Gastrointestinal and Liver Disease. 9th edition, Canada: Elsevier Saunders, 2010: 1089-1120
- 9 **Einstein DM**, Lapin SA, Ralls PW, Halls JM. The insensitivity



- of sonography in the detection of choledocholithiasis. *AJR Am J Roentgenol* 1984; **142**: 725-728 [PMID: 6608231 DOI: 10.2214/ajr.142.4.725]
- 10 **Freitas ML**, Bell RL, Duffy AJ. Choledocholithiasis: evolving standards for diagnosis and management. *World J Gastroenterol* 2006; **12**: 3162-3167 [PMID: 16718834 DOI: 10.3748/wjg.v12.i20.3162]
  - 11 **Isogai M**, Hachisuka K, Yamaguchi A, Nakano S. Etiology and pathogenesis of marked elevation of serum transaminase in patients with acute gallstone disease. *HPB Surg* 1991; **4**: 95-105; discussion 106-107 [PMID: 1931784 DOI: 10.1155/1991/95059]
  - 12 **Halvorsen FA**, Ritland S. [Biliary duct obstruction presenting with laboratory levels indicating liver cell damage]. *Tidsskr Nor Lægeforen* 1989; **109**: 1779-1781 [PMID: 2473542]
  - 13 **Hayat JO**, Loew CJ, Asstress KN, McIntyre AS, Gorard DA. Contrasting liver function test patterns in obstructive jaundice due to biliary strictures [corrected] and stones. *QJM* 2005; **98**: 35-40 [PMID: 15625352 DOI: 10.1093/qjmed/hci004]
  - 14 **Goldman L**, Schafer AI. Goldman's Cecil Medicine. 24th edition, USA: Elsevier Saunders, 2011: 1011-1021
  - 15 **Williamson MA**, Snyder LM. Wallach's Interpretation of Diagnostic Tests. 9th edition, China: Lippincott, Williams & Wilkins, 2011: 38-40
  - 16 **Liaw YF**, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008; **2**: 263-283 [PMID: 19669255 DOI: 10.1007/s12072-008-9080-3]
  - 17 **Liaw YF**, Kao JH, Piratvisuth T, Chan HL, Chien RN, Liu CJ, Gane E, Locarnini S, Lim SG, Han KH, Amarapurkar D, Cooksley G, Jafri W, Mohamed R, Hou JL, Chuang WL, Lesmana LA, Sollano JD, Suh DJ, Omata M. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. *Hepatol Int* 2012; **6**: 531-561 [PMID: 26201469 DOI: 10.1007/s12072-012-9365-4]
  - 18 **Benarroch-Gampel J**, Boyd CA, Sheffield KM, Townsend CM, Riall TS. Overuse of CT in patients with complicated gallstone disease. *J Am Coll Surg* 2011; **213**: 524-530 [PMID: 21862355 DOI: 10.1016/j.jamcollsurg.2011.07.008]
  - 19 **Nathwani RA**, Kumar SR, Reynolds TB, Kaplowitz N. Marked elevation in serum transaminases: an atypical presentation of choledocholithiasis. *Am J Gastroenterol* 2005; **100**: 295-298 [PMID: 15667485 DOI: 10.1111/j.1572-0241.2005.40793.x]
  - 20 **Kaplan MM**, Righetti A. Induction of rat liver alkaline phosphatase: the mechanism of the serum elevation in bile duct obstruction. *J Clin Invest* 1970; **49**: 508-516 [PMID: 5415676 DOI: 10.1172/JCI106260]
  - 21 **Patwardhan RV**, Smith OJ, Farmelant MH. Serum transaminase levels and cholescintigraphic abnormalities in acute biliary tract obstruction. *Arch Intern Med* 1987; **147**: 1249-1253 [PMID: 3300588 DOI: 10.1001/archinte.147.7.1249]
  - 22 **Sharara AI**, Mansour NM, El-Hakam M, Ghaith O, El Halabi M. Duration of pain is correlated with elevation in liver function tests in patients with symptomatic choledocholithiasis. *Clin Gastroenterol Hepatol* 2010; **8**: 1077-1082 [PMID: 20831901 DOI: 10.1016/j.cgh.2010.08.021]
  - 23 **Birrer R**, Takuda Y, Takara T. Hypoxic hepatopathy: pathophysiology and prognosis. *Intern Med* 2007; **46**: 1063-1070 [PMID: 17634701 DOI: 10.2169/internalmedicine.46.0059]
  - 24 **Ebert EC**. Hypoxic liver injury. *Mayo Clin Proc* 2006; **81**: 1232-1236 [PMID: 16970220 DOI: 10.4065/81.9.1232]
  - 25 **Singer AJ**, Carracio TR, Mofenson HC. The temporal profile of increased transaminase levels in patients with acetaminophen-induced liver dysfunction. *Ann Emerg Med* 1995; **26**: 49-53 [PMID: 7793720 DOI: 10.1016/S0196-0644(95)70237-7]
  - 26 **Grau F**, Almela P, Aparisi L, Bautista D, Pascual I, Peña A, Rodrigo JM. Usefulness of alanine and aspartate aminotransferases in the diagnosis of microlithiasis in idiopathic acute pancreatitis. *Int J Pancreatol* 1999; **25**: 107-111 [PMID: 10360223 DOI: 10.1385/IJGC.25.2.107]
  - 27 **Chang CW**, Chang WH, Lin CC, Chu CH, Wang TE, Shih SC. Acute transient hepatocellular injury in cholelithiasis and cholecystitis without evidence of choledocholithiasis. *World J Gastroenterol* 2009; **15**: 3788-3792 [PMID: 19673021 DOI: 10.3748/wjg.15.3788]
  - 28 **Jiang ZY**, Sheng X, Xu CY, Li WW, Chang XX, Sun LY, Yang XB, Yu LF. Gallbladder gallstone disease is associated with newly diagnosed coronary artery atherosclerotic disease: a cross-sectional study. *PLoS One* 2013; **8**: e75400 [PMID: 24058685 DOI: 10.1371/journal.pone.0075400]

P- Reviewer: Liao KF, Shih SC, Zimmer V S- Editor: Ji FF

L- Editor: A E- Editor: Li D



## Isolated bilateral Tapia's syndrome after liver transplantation: A case report and review of the literature

Itxarone Bilbao, Cristina Dopazo, Mireia Caralt, Lluís Castells, Elisabeth Pando, Amaia Gantxegi, Ramón Charco

Itxarone Bilbao, Cristina Dopazo, Mireia Caralt, Lluís Castells, Elisabeth Pando, Amaia Gantxegi, Ramón Charco, Department of Digestive Surgery, Hepatobiliopancreatic Surgery and Liver Transplant Unit, Hospital Universitario Vall d'Hebrón, CIBERehd, Universidad Autónoma de Barcelona, 08035 Barcelona, Spain

**Author contributions:** Bilbao I, Dopazo C, Caralt M and Pando E participated in the liver transplantation surgery; Bilbao I designed the research; Castells L followed the patients; Gantxegi A analyzed the data; Bilbao I and Gantxegi A wrote the paper; Charco R supervised the paper; all authors read and approved the final manuscript.

**Conflict-of-interest statement:** All the authors declare that they have no competing interests.

**Data sharing statement:** The technical appendix and dataset are available from the corresponding author at [ibilbao@vhebron.net](mailto:ibilbao@vhebron.net).

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Itxarone Bilbao, MD, PhD, Department of Digestive Surgery, Hepatobiliopancreatic Surgery and Liver Transplant Unit, Hospital Universitario Vall d'Hebrón, CIBERehd, Universidad Autónoma de Barcelona, Paseo Vall d'Hebrón 119-129, 08035 Barcelona, Spain. [ibilbao@vhebron.net](mailto:ibilbao@vhebron.net)  
Telephone: +34-93-2746113  
Fax: +34-93-2746112

Received: August 19, 2016  
Peer-review started: August 23, 2016  
First decision: September 28, 2016

Revised: October 14, 2016  
Accepted: November 1, 2016  
Article in press: November 2, 2016  
Published online: December 28, 2016

### Abstract

#### AIM

To describe one case of bilateral Tapia's syndrome in a liver transplanted patient and to review the literature.

#### METHODS

We report a case of bilateral Tapia's syndrome in a 50-year-old man with a history of human immunodeficiency virus and hepatitis C virus child. A liver cirrhosis and a bi-nodular hepatocellular carcinoma, who underwent liver transplantation after general anesthesia under orotracheal intubation. Uneventful extubation was performed in the intensive care unit during the following hours. On postoperative day (POD) 3, he required urgent re-laparotomy due to perihepatic hematoma complicated with respiratory gram negative bacilli infection. On POD 13, patient was extubated, but required immediate re-intubation due to severe respiratory failure. At the following day a third weaning failure occurred, requiring the performance of a percutaneous tracheostomy. Five days later, the patient was taken off mechanical ventilation and severe dysphagia, sialorrhea and aphonia revealed. A computerized tomography and a magnetic resonance imaging of the head and neck excluded central nervous injury. A stroboscopy showed bilateral paralysis of vocal cords and tongue and a diagnosis of bilateral Tapia's syndrome was performed. With conservative management, including a prompt establishment of a speech and swallowing rehabilitation program, the patient achieved full recovery within four months after liver transplantation. We carried out MEDLINE search for the term Tapia's syndrome. The inclusion criteria had no restriction by language or year but must provide sufficient

available data to exclude duplicity. We described the clinical evolution of the patients, focusing on author, year of publication, age, sex, preceding problem, history of endotracheal intubation, unilateral or bilateral presentation, diagnostic procedures, type of treatment, follow-up, and outcome.

## RESULTS

Several authors mentioned the existence of around 70 cases, however only 54 fulfilled our inclusion criteria. We found only five published studies of bilateral Tapia's syndrome. However this is the first case reported in the literature in a liver transplanted patient. Most patients were male and young and the majority of cases appeared as a complication of airway manipulation after any type of surgery, closely related to the positioning of the head during the procedure. The diagnosis was founded on a rapid suspicion, a complete head and neck neurological examination and a computed tomography and or a magnetic resonance imaging of the brain and neck to establish the origin of central or peripheral type of Tapia's syndrome and also the nature of the lesion, ischemia, abscess formation, tumor or hemorrhage. Apart from corticosteroids and anti-inflammatory therapy, the key of the treatment was an intensive and multi-disciplinary speech and swallowing rehabilitation. Most studies have emphasized that the recovery is usually completed within four to six months.

## CONCLUSION

Tapia's syndrome is almost always a transient complication after airway manipulation. Although bilateral Tapia's syndrome after general anesthesia is exceptionally rare, this complication should be recognized in patients reporting respiratory obstruction with complete dysphagia and dysarthria after prolonged intubation. Both anesthesiologists and surgeons should be aware of the importance of its preventing measurements, prompt diagnosis and intensive speech and swallowing rehabilitation program.

**Key words:** Liver transplantation; Follow-up; Outcome; Postoperative complications; Bilateral Tapia's syndrome

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Tapia's syndrome is a rare entity characterized by the concomitant extracranial injury of the hypoglossal nerve (XII) and the recurrent laryngeal branch of the vagus nerve (X) at the base of the tongue and the pyriform fossa. Anesthesiologists, surgeons and otorhinolaryngologist should be aware of its presentation at any type of surgery as in the present case, after liver transplantation. The purpose of this study is to present our even rarer presentation of bilateral Tapia's syndrome to the liver transplant community and to review the literature to update the current management and treatment. The most relevant common feature in most cases of bilateral syndrome was orotracheal intubation prolonged for more than 14 d.

Bilbao I, Dopazo C, Caralt M, Castells L, Pando E, Gantxegi A, Charco R. Isolated bilateral Tapia's syndrome after liver transplantation: A case report and review of the literature. *World J Hepatol* 2016; 8(36): 1637-1644 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v8/i36/1637.htm> DOI: <http://dx.doi.org/10.4254/wjh.v8.i36.1637>

## INTRODUCTION

Tapia's syndrome was described for the first time by the Spanish otorhinolaryngologist Antonio García Tapia in 1904<sup>[1]</sup>. It is characterized by the unilateral paralysis of the tongue and the vocal cord caused by extracranial injury to the hypoglossal nerve (XII) and the recurrent laryngeal branch of the vagal nerve (X) at the base of the tongue and the pyriform fossa<sup>[1-6]</sup>. Although the Tapia's syndrome refers to the extracranial lesion of the hypoglossal and recurrent laryngeal nerves, some authors also describe a central type of Tapia's syndrome, referring to those patients with the same symptoms, but whose damage has occurred in the nucleus ambiguus, the nucleus of the hypoglossal nerve, and the pyramidal tract in the central nervous system. We describe one case of bilateral Tapia's syndrome in a liver transplant patient, which is not previously reported in the literature.

## MATERIALS AND METHODS

We report herein a case of bilateral Tapia's syndrome together with a review of the literature. We carried a literature research in the MEDLINE database through the PubMed search service for the term Tapia's syndrome. The inclusion criteria had no restriction by language or year but must provide sufficient available data to exclude duplicity. We described the clinical evolution of the patients, focusing on author, year of publication, age, sex, preceding problem, history of endotracheal intubation, unilateral or bilateral presentation, diagnostic procedures, type of treatment, follow-up, and outcome.

### Case report

A 50-year-old man with a history of human immunodeficiency virus (HIV) and hepatitis C virus positive serology, with class A of Child-Pugh classification liver cirrhosis and a bi-nodular hepatocellular carcinoma underwent liver transplantation after general anesthesia under orotracheal intubation. Body mass index at time of transplantation was 21 kg/m<sup>2</sup>. An 8.0 endotracheal tube was placed. The cuff was inflated with 3 mL of air and verified with a manual manometry to reach a filling pneumotamponade of 20 cm water. Surgery lasted 375 min. The procedure was well tolerated and required a low dose of inotrops (noradrenalin 0.5 mL/h) during surgery. Immunosuppression therapy during induction was based on mycophenolate mophetil and tacrolimus. Patient was transferred to the intensive care unit (ICU) under mechanical ventilation, sedated with remifentanyl. Uneventful weaning was performed during the following

hours. On postoperative day (POD) 3, he required urgent re-laparotomy due to a perihepatic hematoma and was transferred to the ICU under mechanical ventilation, sedated with propofol and remifentanyl. Extubation was postponed due to a respiratory gram negative bacilli infection and agitation after several attempts of decreasing sedation. On POD 13, patient was extubated and required immediate re-intubation after severe respiratory failure. A third weaning failure occurred the following day requiring re-intubation for the third time. Then percutaneous tracheostomy was performed with no events. Five days later, patient was taken off mechanical ventilation progressively and oral diet was started the day after, appearing severe dysphagia and important sialorrhea, being hardly able to swallow a pureed diet. Aphonia was another significant symptom presented at that time. At POD 28 patient was decanulated and persisted with swallowing difficulty, requiring parenteral nutrition. A computerized tomography (CT) of the head and neck and a magnetic resonance imaging (MRI) of the brain and neck were then performed to exclude central nervous injury. Both explorations did not show pathological findings.

At POD 34, patient was transferred to the ward and enteral nutrition was initiated *via* nasogastric tube. He was evaluated by speech and swallow therapists and diagnosis of a bilateral tongue paralysis and aphonia was made. Evaluation by otorhinolaryngologist excluded a recurrent laryngeal nerve injury. Detailed neurological examination revealed bilateral tongue paralysis, severe dysarthria and dysphagia for liquids and solids. A stroboscopy was performed showing bilateral paralysis of vocal cords in addition to the bilateral tongue paralysis. Cervical electromyography was also performed. Bilateral Tapia's syndrome was then diagnosed; a bilateral hypoglossal and laryngeal recurrent nerve neuroapraxia. At three months post-transplant, subjective improvement in aphonia and dysphagia were observed and the patient was discharged with enteral nutrition.

Outpatient neurological follow-up regarding speech and swallow training was performed twice weekly. Satisfactory recovery of his aphonia and dysphagia were observed. At four months post-transplant, videofluoroscopy was performed with no significant findings; however, laryngeal stroboscopy showed severe hypomotility of cricoarthenoideal articulations, cordal atrophy and minimal adduction movements with severe longitudinal hiatus. Despite that, the patient presented no problems during intake, being able to take out the nasogastric feeding tube. At that time, the nasogastric tube was preferred to the percutaneous gastrostomy to avoid invasive procedures in a patient with a complex postoperative.

## RESULTS

In total around 70 cases were initially described in the literature, but only 53 fulfilled the inclusion criteria: To have patients with sufficient available data in the description of cases in order to rule out duplicity. Table

1<sup>[1-2,7-51]</sup> summarizes the 54 cases (including ours) of Tapia's syndrome, focusing on author, year of publication, age, sex, preceding problem, history of endotracheal intubation, unilateral or bilateral presentation, diagnostic procedures, type of treatment, follow-up and outcome.

The majority were young. Only 13 cases were older than 50 years (range 16-95). All cases except 10 were males. Two cases were attributed to a central cause (metastatic hemangiosarcoma in the medulla oblongata<sup>[2]</sup> and infiltration of a large B-cell lymphoma<sup>[14]</sup>), but the remaining 53 patients were peripheral type. Six patients<sup>[8,22,24,36,42,43]</sup>, apart from ours, had a bilateral presentation of the syndrome; four with complete deficit of hypoglossal and recurrent laryngeal nerves and three<sup>[22,24,43]</sup> incomplete with bilateral paralysis of the hypoglossus nerves and unilateral recurrent laryngeal nerve palsy. All the cases, except one<sup>[36]</sup>, followed to a prolonged oro-tracheal intubation for more than 14 d. In the systematic review, we have found two other cases of isolated bilateral hypoglossal paralysis without other nerve involvement after oro-tracheal intubation<sup>[52,53]</sup>.

All, except seven of peripheral cases<sup>[9,15,29,39,40,47,51]</sup>, have been attributed to orotracheal intubation for surgery or respiratory failure. The most frequently involved operations were: Osteoarticular surgery of the shoulder, mandible and cervical spine in 14 cases, otorhinolaryngology surgical procedures in 11 cases, cardiac surgery in 4 cases, thoracic surgery in 2 cases, abdominal surgery in 2 cases, and direct traumatic nerve injury in 2 cases. However, several causes have been described in the literature such as: Vascular (vertebral artery dissection, carotid artery aneurism); metastatic or primary neoplasia (lymphoma, hemangiosarcoma, prostate, pseudotumor of the neck, nasopharyngeal carcinoma, neurilemoma, neurofibroma, etc.); infectious of the neck (bacterial, viral, fungal), etc.

The diagnosis and management of Tapia's syndrome in the majority of cases was based on a complete neurological examination, including laryngeal endoscopy and a head and neck CT or MRI. Some authors have advocated for the use of video-fluoroscopic swallowing and electromyography to confirm the diagnosis and to predict prognosis.

The treatment was supportive in all cases with a prompt establishment of a swallowing rehabilitation program. The administration of intravenous or oral steroids in combinations with B1, B6, B12 vitamins or hyaluronic acid injection has been proposed by many authors in the acute setting. At least 4 patients<sup>[8,17,23]</sup> required percutaneous endoscopic gastrostomy and 2 a naso-gastric tube insertion<sup>[20,42]</sup> to ensure nutritional requirements while the oro-esophageal route was unable to be used. In two cases (Takimoto<sup>[43]</sup> and ours), where bilateral paralyses were discovered, reintubation with subsequent tracheotomy was necessary to prevent respiratory failure.

Recovery was excellent for the majority of non-tumour peripheral cases after a duration of 3 to 6 mo, ranging from 15 d to 3 years. In 9 cases the patients reported only



**Table 1 Cases of Tapia's syndrome reported in the literatura to date (including our case): 54 peripheral type and 2 central type**

Ref.	Age	Sex	Clinical procedure	OTI	Bil	Diagnosis	Treatment	Follow-up	Recovery
Bilbao 2016	50	M	Liver transplantation due to HCV cirrhosis coinfectied with HIV and hepatocellular carcinoma	Yes	Yes	Neurological examination Electromiography Laryngeal endoscopy Head and neck CT and MRI Video fluoroscopic examination	Temporary tracheotomy for airway management Nasogastric tube feeding Speech and swallowing therapy	4 mo	Yes
Cariati <i>et al</i> <sup>[7]</sup> 2016	36	M	Neck abscess drainage	Yes	No	Neurological exam Barium swallow X-ray Swallowing endoscopy	Rehabilitation program	3 mo	Yes
	61	M	Neck abscess drainage	Yes	No	Neurologic exam Airway endoscopy	Rehabilitation program	3 mo	Yes
	42	M	Shoulder fracture reduction	Yes	No	Neurologic exam Airway endoscopy	Rehabilitation program	3 mo	Yes
Coninckx <i>et al</i> <sup>[8]</sup> 2015	64	M	Liver cirrhosis. Pneumonia and respiratory failure	Yes	No	Neurological examination Lumbar puncture Laryngeal endoscopy Head and neck CT and MRI Chest CT	Speech and swallowing therapy Percutaneous endoscopic gastrostomy	22 mo	Yes
	49	M	Myocardial infarction. Percutaneous coronary intervention. Penumonia	Yes	Yes	Neurologic examination Brain CT	Corticosteroid therapy 8 wk Speech and swallowing therapy Percutaneous endoscopic gastrostomy	4 mo	Yes
Yilmaz <i>et al</i> <sup>[9]</sup> 2015	61	M	Bone metastatic prostate cancer	No	No	Neck CT and MRI	-	-	-
Paramalingam <i>et al</i> <sup>[10]</sup> 2015	38	M	Eagle syndrome. Pneumonia	Yes	No	Head and neck CT			
Brandt <i>et al</i> <sup>[11]</sup> 2015	23	M	Otorhinolaryngology surgical procedure	Yes	No	-	-	-	-
	67	-	Arthroscopic intervention of left shoulder	Yes	No	-	-	-	-
Ghorbani <i>et al</i> <sup>[12]</sup> 2014	27	M	Septorhinoplasty	Yes	No	Neurological examination Head and neck MRI	Systemic corticosteroids	6 mo	Yes
Ulusoy <i>et al</i> <sup>[13]</sup> 2014	19	F	Nasoseptal deformity	Yes	No	Neurological examination Head and neck MRI Airway endoscopy	Systemic corticosteroids	6 mo	Yes
Cantalupo <i>et al</i> <sup>[14]</sup> 2014	16	M	Large B-cell Lymphoma	No	No	-	-	-	-
Lo Casto <i>et al</i> <sup>[15]</sup> 2013	42	F	Inflammatory pseudotumor of the neck	No	No	Neurological examination Electromiography Laryngeal endoscopy Head and neck MRI Chest and abdomen CT	Corticosteroid therapy	-	-
Kang <i>et al</i> <sup>[16]</sup> 2013	47	M	Cervical spine surgery	Yes	No	Head and neck CT and MRI	Corticosteroid therapy Speech therapy rehabilitation	8 mo	Partially
Emohare <i>et al</i> <sup>[17]</sup> 2013	17	M	Artrodesis T1-L1	Yes	No	Barium swallow X-ray Head and neck MRI Airway endoscopy	Percutaneous endoscopic gastrostomy Hialuronic acid inyection Rehabilitation program	1 mo	Yes
Varedi <i>et al</i> <sup>[18]</sup> 2013	27	M	Zygomatic complex fracture	Yes	No	Neurological examination Head and neck CT and MRI Laringoscopic examination	Systemic corticosteroids Vitamin B complex Rehabilitation program	9 mo	Yes
Gevorgyan <i>et al</i> <sup>[19]</sup> 2013	48	F	Liposuction 3 yr previously rhinoplasty 25 yr previously	Yes	No	Neurological examination Head and neck CT and MRI Laringoscopic examination	Vocal cord injection Rehabilitation program	3 yr	Partially
Lim <i>et al</i> <sup>[20]</sup> 2013	64	M	Cervical spine surgery	Yes	No	Neurological examination Head and neck CT and MRI Laringoscopic examination Video fluoroscopic examination	Systemic corticosteroids Electrical stimulation therapy Nasogastric tube feeding	3 mo	Yes
Park <i>et al</i> <sup>[21]</sup> 2013	53	M	Posterior cervical spine surgery Posterior cervical spine surgery	Yes	No	Head and neck CT and MRI Laryngeal electromyography	-	6 mo	Yes
	56	M		Yes	No	-	-	2 mo	Yes

Sønnichsen <i>et al</i> <sup>[22]</sup> 2013	-	-	Legionella infection	Yes	Yes	-	-	2 mo	Partially
Nalladuru <i>et al</i> <sup>[23]</sup> 2012	49	M	Cardiac surgery	Yes	No	Neurological examination Head and neck CT and MRI	Systemic corticosteroids Percutaneous endoscopic gastrostomy	2.5 mo	Yes
Turan <i>et al</i> <sup>[24]</sup> 2012	15	M	Acute lymphoblastic leukemia pneumonia	Yes	Yes	Neurological examination Laryngoscopic examination	Systemic corticosteroids	0.5 mo	Partially
Wadelek <i>et al</i> <sup>[25]</sup> 2012	57	M	Arthroscopic shoulder	Yes	No	Neurological examination Head and neck MRI Laryngeal endoscopy	Rehabilitation program	+ 2 mo	Yes
Lykoudis <i>et al</i> <sup>[26]</sup> 2012	32	M	Rhinoplasty	Yes	No	Head and neck CT Laryngeal endoscopy	Oral corticosteroid therapy Speech and swallowing therapy	4 mo	Yes
Park <i>et al</i> <sup>[27]</sup> 2011	42	M	Anterior cervical spine surgery	Yes	No	Neurological examination Electromyography Video fluoroscopic swallowing Laryngeal endoscopy Head and neck MRI	Rehabilitation program	7 mo	Yes
Torres-Morientes <i>et al</i> <sup>[28]</sup> 2011	32	M	Tracheostomy and right thoracostomy	<sup>1</sup>	No	Neurological examination	Speech and swallowing therapy	4 mo	Yes
Al-Sihan <i>et al</i> <sup>[29]</sup> 2011	63	M	Vertebral artery dissection	No	No	-	Clopidogrel for 6 wk Speech and swallowing therapy	-	Partially
Kashyap <i>et al</i> <sup>[30]</sup> 2010	41	M	Mandibular fracture	Yes	No	-	None	16 mo	Partially
Rotondo <i>et al</i> <sup>[31]</sup> 2010	-	-	Cardiac surgery	-	-	-	-	-	-
Boğa <i>et al</i> <sup>[32]</sup> 2010	35	M	Septorhinoplasty	Yes	No	-	Systemic corticosteroids	0.5 mo	Yes
Dursun <i>et al</i> <sup>[33]</sup> 2007	-	-	Hunting rifle-shot	-	-	-	-	-	-
Sotiriou <i>et al</i> <sup>[34]</sup> 2007	-	-	Coronary bypass grafting surgery	Yes	-	-	-	-	-
Tesei <i>et al</i> <sup>[35]</sup> 2006	30	F	Rhinoplasty	Yes	No	Neurological examination Head and neck MRI	Systemic corticosteroids Speech and swallowing therapy	4 mo	Yes
Cinar <i>et al</i> <sup>[36]</sup> 2005	20	M	Open rhinoplasty	Yes	Yes	-	Systemic corticosteroids	1 mo	Yes
Yavuzer <i>et al</i> <sup>[37]</sup> 2004	42	F	Septorhinoplasty	Yes	No	-	Oral corticosteroid therapy	6 mo	Yes
Krasnianski <i>et al</i> <sup>[2]</sup> 2003	77	M	Metastatic hemangiomasarcoma in the medulla oblongata	-	No	-	None	-	-
Boisseau <i>et al</i> <sup>[38]</sup> 2002	42	M	Shoulder surgery	Yes	No	Vertebral and carotid ultrasonography Head and neck CT and MRI	Systemic corticosteroids Speech and swallowing therapy	6 mo	Yes
Johnson <i>et al</i> <sup>[39]</sup> 1999	44	M	Surgical repair of a shoulder injury	No <sup>1</sup>	No	Head and neck CT and MRI	None	2 mo	Partially
Shimohata <i>et al</i> <sup>[40]</sup> 1994	61	F	Aneurism of extracranial internal carotid artery	No	No	Carotid angiography Head and neck CT and MRI	-	-	-
Millán Guevara <i>et al</i> <sup>[41]</sup> 1993	-	-	Viral etiology?	-	-	-	-	-	-
McCleary <i>et al</i> <sup>[42]</sup> 1993	95	F	Fracture of the odontoid process	-	Yes	-	Naso-gastric tube	12 mo	Partially
Takimoto <i>et al</i> <sup>[43]</sup> 1991	18	F	Nasopharyngeal carcinoma radiation	-	Yes	-	Temporary tracheotomy for airway management during pregnancy	4 yr	No
de Freitas <i>et al</i> <sup>[44]</sup> 1991	37	F	Paracoccidioidomycosis fungus in the nasal mucosa	-	-	-	Oral Ketoconazol	2 yr	No
Quattrocchio <i>et al</i> <sup>[45]</sup> 1986	24	M	Neurilemoma of vagus and hypoglossal nerves	-	-	-	-	-	-
Gelmers <i>et al</i> <sup>[46]</sup> 1983	41	M	Thoracotomy	Yes	No	-	-	12 mo	No
Andrioli <i>et al</i> <sup>[47]</sup> 1980	36	M	Thoracotomy	Yes	No	-	-	12 mo	No
	25	M	Neurofibrome of X and XII nerves below the nodose ganglion	No	No	-	Surgery: Resection of the two nerves	-	No
Mayer <i>et al</i> <sup>[48]</sup> 1974	51	M	Hiatus hernia repair. Pneumonia	Yes	No	-	None	0.5 mo	Partially
Ruhrmann <i>et al</i> <sup>[49]</sup> 1963	-	-	Congenital	-	-	-	-	-	-
Babini <i>et al</i> <sup>[50]</sup> 1961	-	-	Obstetrical trauma	-	-	-	-	-	-

Symonds <i>et al</i> <sup>[31]</sup> 1923	35	F	Chronic otitis media	No	No	-	-	2 yr	Partially
Tapia <i>et al</i> <sup>[1]</sup> 1905	-	M	Bullfighter injury behind the angle of the jaw		No				

Interscalene brachial plexus block <sup>1</sup>Tracheostomy. OTI: Orotracheal intubation; BIL: Bilateral; F: Female; M: Male; CT: Computed tomography; MRI: Magnetic resonance imaging; HCV: Hepatitis C virus.

partial recovery.

## DISCUSSION

The case described above, is the first reported case of complete bilateral Tapia's syndrome (paralysis of the tongue muscles and vocal cords because of an extracranial injury of the X and XII cranial nerves) occurring after liver transplantation and oro-tracheal general anaesthesia requiring re-intubation for three times. There are many causes of Tapia's syndrome, including general anaesthesia, fungal infections<sup>[44]</sup>, neoplasms<sup>[2,9,14,15,24,43,45,47]</sup>, vascular<sup>[29,40]</sup> and traumatic problems<sup>[1,33,50]</sup>, being general anaesthesia the main cause. Intubation tube or its cuff and motion of the head during surgery can lead to injury to the pharyngeal wall and its underlying neurovascular structures (X and XII cranial nerves)<sup>[32]</sup>. Excessive dorsiflexion of the head during laryngoscopy, excessive cuff pressure, malposition of the cuff in the larynx rather than the trachea, or extubation while the cuff is still inflated is the most likely cause<sup>[18]</sup>. The tracheal tube and its cuff may press on a localized area just at the crossing of the vagal and hypoglossal nerves, compressing the anterior branch of the inferior laryngeal nerve against the postero-medial part of the thyroid cartilage and this can lead to a recurrent laryngeal paralysis<sup>[6]</sup>. Hypoglossal nerve damage can be caused by a stretching of the nerve against the greater horn of the hyoid bone by an oro-tracheal tube or compression of the posterior part of the laryngoscope or oro-tracheal tube<sup>[35]</sup>. There was no clear mechanism for injury to the hypoglossal and recurrent laryngeal nerves in our patient. Intracranial pathology was unlikely because of negative CT scan and MRI. We postulate that low blood pressure during surgery and post-operatively due to intrabdominal hemorrhage requiring reintervention and the need of several oro-tracheal reintubations (3 times), 2 of them in emergency conditions, in addition to prolonged intubation with probable unnoticed overinflation and malposition of the endotracheal cuff, might have been the source of the bilateral nerve compression. A change in the position of the neck at some point, compression by the endotracheal tube and pressure to the lateral roots of the tongue with the McIntosh blade during intubation could be additional mechanisms. The caquexia of the patient and some degree of lypodistrophy due the HIV coinfection at time of transplant could also play a role. Liver transplantation is usually a long lasting surgical procedure, which could contribute, along with other factors to the development of Tapia's syndrome. This fact should be taken into account by all clinicians involved in the liver transplantation care:

Liver surgeons, anesthetists, intensivists, hepatologists, gastroenterologists, *etc.*

Although most patients were male and young, there is no an explanation to relate the syndrome to sex or age. We believe that this syndrome is more related to anatomical, positional and lasting-time issues than to other characteristics.

The diagnosis is founded on a rapid suspicion, a complete history around the paralysis and a complete head and neck neurological examination. A computed tomography and or a magnetic resonance imaging of the brain and neck is essential to establish the diagnosis of central or peripheral type of Tapia's syndrome and also the nature of the lesion, ischemia, abscess formation, tumor or haemorrhage.

Tapia's syndrome classification and a treatment protocol have been proposed by Aktas and Boğa<sup>[32]</sup>: Grade I /mild type, unilateral cord and tongue paralysis, no uvula distortion, minimal slowdown in speaking, no swelling in tongue and no trouble in swallowing, Corticosteroid treatment is not recommended; Grade II/moderate type, unilateral cord and tongue paralysis, no uvula distortion, mild slowdown in speaking, swelling in tongue, dryness in pharynx, trouble in swallowing, cracked speech and normal feeding and drinking, 15 d of corticosteroid treatment is recommended; Grade III/severe type, unilateral cord and tongue paralysis, significant uvula distortion, significant difficulty in speaking, swelling in tongue, dryness in pharynx, trouble in swallowing and difficulties in feeding and drinking, endovenous corticosteroid is recommended for 1 wk.

To our knowledge, only six cases<sup>[8,22,24,36,42,43]</sup> of isolated bilateral Tapia's syndrome have been reported in the literature and all of them were related to transoral intubation during general anaesthesia. The most relevant common feature was the prolonged oro-tracheal intubation for more than 14 d in all the cases except one<sup>[36]</sup>. Our patient was reintubated three times, two of them as an urgent procedure, and remained ventilated for more than 18 d.

The majority of all reported cases, even unilateral or bilateral, recovered in 4-6 mo and this progressive recovery of function suggests nerve damage of a neuropraxic type, which is typical of compression injury. But there are some reports in the literature regarding its irreversible form<sup>[43,44,46,47]</sup> or partially reversible form<sup>[16,19,22,24,29,30,39,42,48,51]</sup>.

Apart from corticosteroids and anti-inflammatory therapy described above as key of the therapy, other support treatments recommended are speech and swallow therapy and warm air inhalation. Most studies

have emphasized that the recovery is usually completed within 6 mo, but with an intensive and multidisciplinary approach the patients' recovery time could be reduced. In our case, despite no corticosteroids were administered, the recovery was complete four months post-transplant after intensive speech and swallow training.

In conclusion, Tapia's syndrome is mainly a rare complication of airway manipulation. It can occur after any type of surgery under endotracheal general anesthesia. Clinicians should be aware of its preventive strategies, diagnosis, treatment and almost always transient outcomes. Although bilateral Tapia's syndrome after general anaesthesia is exceptionally rare, this complication should be recognized in patients reporting respiratory obstruction with complete dysphagia and dysarthria after extubation. Special attention should be paid to correct positioning of the head during surgery to avoid such problems.

## COMMENTS

### Background

Tapia's syndrome is an extracranial ipsilateral palsy of the recurrent laryngeal and the hypoglossal nerves. It is a very rare complication with few cases reported in the literature. The predisposing factors are most commonly orotracheal intubation for general anesthesia but also other etiologies.

### Research frontiers

This study tries to collect all articles published to date, emphasizing the common aspects of all reported cases.

### Innovations and breakthroughs

The rarity in the presentation of Tapia's syndrome makes its incidence probably underestimated if clinicians are not aware of its symptoms. The publication of this review will help the scientific community to keep in mind Tapia's syndrome and to establish common guidelines for diagnosis, management and treatment.

### Peer-review

This is a very interesting case report and a good literature review about the topic.

## REFERENCES

- 1 **Tapia AG.** Un caso de parálisis del lado derecho de la laringe y de ungue, con parálisis del externo-cleidomastoideo y trapecio del mismo lado. *Siglo Médico* 1905; **52**: 211-213
- 2 **Krasnianski M,** Neudecker S, Schlüter A, Krause U, Winterholler M. Central Tapia's syndrome ("matador's disease") caused by metastatic hemangiosarcoma. *Neurology* 2003; **61**: 868-869 [PMID: 14504349 DOI: 10.1212/01.WNL.0000080370.43712.AA]
- 3 **Chusid JG.** Letter: Tapia syndrome. *JAMA* 1974; **228**: 28 [PMID: 4406142 DOI: 10.1001/jama.1974.03230260022014]
- 4 **Miyazaki M.** [Tapia's syndrome]. *Nihon Rinsho* 1977; **35** Suppl 1: 596-597 [PMID: 612913]
- 5 **Schoenberg BS,** Massey EW. Tapia's syndrome. The erratic evolution of an eponym. *Arch Neurol* 1979; **36**: 257-260 [PMID: 375880 DOI: 10.1001/archneur.1979.00500410035003]
- 6 **Kapoor S.** Tapia's syndrome: a rare complication of airway trauma. *Anesth Analg* 2013; **117**: 1261 [PMID: 24149506 DOI: 10.1213/ANE.0b013e3182a5c717]
- 7 **Cariati P,** Cabello A, Galvez PP, Sanchez Lopez D, Garcia Medina B. Tapia's syndrome: pathogenetic mechanisms, diagnostic management, and proper treatment: a case series. *J Med Case Rep* 2016; **10**: 23 [PMID: 26809980 DOI: 10.1186/s13256-016-0802-1]
- 8 **Coninckx M,** Cardoen S, Hemelsoet D. Tapia's syndrome in the intensive care unit: a rare cause of combined cranial nerve palsy following intubation. *Acta Neurol Belg* 2015; **115**: 533-537 [PMID: 26088745 DOI: 10.1007/s13760-015-0500-6]
- 9 **Yilmaz Z,** Duygulu G, Kiliç S, Terzi R. Tapia's syndrome secondary to metastatic prostate cancer. *Neurol India* 2015; **63**: 782-783 [PMID: 26448244 DOI: 10.4103/0028-3886.166531]
- 10 **Paramalingam S,** Kuok YJ. Eagle Syndrome as a potential cause of Tapia Syndrome. *Med J Aust* 2015; **202**: 491 [PMID: 25971574 DOI: 10.5694/mja14.01227]
- 11 **Brandt L.** [Tapia's syndrome: Rare complication of securing airways]. *Anaesthesist* 2015; **64**: 122-127 [PMID: 25523320 DOI: 10.1007/s00101-014-2397-5]
- 12 **Ghorbani J,** Dabir S, Givhechi G, Najafi M. Co-presentation of Tapia's syndrome and pressure alopecia--A rare event after septorhinoplasty: A case report and literature review. *Acta Anaesthesiol Taiwan* 2014; **52**: 38-40 [PMID: 24999217 DOI: 10.1016/j.aat.2014.02.001]
- 13 **Ulusoy H,** Besir A, Cekic B, Kosucu M, Geze S. Transient unilateral combined paresis of the hypoglossal nerve and lingual nerve following intubation anesthesia. *Braz J Anesthesiol* 2014; **64**: 124-127 [PMID: 24794456 DOI: 10.1016/j.bjane.2012.12.003]
- 14 **Cantalupo G,** Spagnoli C, Cerasti D, Piccolo B, Crisi G, Pisani F. Tapia's syndrome secondary to laterocervical localization of diffuse large cell lymphoma. *Brain Dev* 2014; **36**: 548-550 [PMID: 23958591 DOI: 10.1016/j.braindev.2013.07.008]
- 15 **Lo Casto A,** Spataro R, Purpura P, La Bella V. Unilateral laryngeal and hypoglossal paralysis (Tapia's syndrome) in a patient with an inflammatory pseudotumor of the neck. *Clin Neurol Neurosurg* 2013; **115**: 1499-1501 [PMID: 23265562 DOI: 10.1016/j.clineuro.2012.11.019]
- 16 **Kang JH,** Kim DM, Kim SW. Tapia syndrome after cervical spine surgery. *Korean J Spine* 2013; **10**: 249-251 [PMID: 24891858 DOI: 10.14245/kjs.2013.10.4.249]
- 17 **Emohare O,** Peterson E, Slinkard N, Janus S, Morgan R. Occam paradox? A variation of tapia syndrome and an unreported complication of guidewire-assisted pedicle screw insertion. *Evid Based Spine Care J* 2013; **4**: 132-136 [PMID: 24436711 DOI: 10.1055/s-0033-1357355]
- 18 **Varedi P,** Shirani G, Karimi A, Varedi P, Khiabani K, Bohluli B. Tapia syndrome after repairing a fractured zygomatic complex: a case report and review of the literature. *J Oral Maxillofac Surg* 2013; **71**: 1665-1669 [PMID: 23850042 DOI: 10.1016/j.joms.2013.05.019]
- 19 **Gevorgyan A,** Nedzelski JM. A late recognition of tapia syndrome: a case report and literature review. *Laryngoscope* 2013; **123**: 2423-2427 [PMID: 24078360 DOI: 10.1002/lary.24070]
- 20 **Lim KJ,** Kim MH, Kang MH, Lee HM, Park EY, Kwon KJ, Lee SK, Choi H, Moon HS. Tapia's syndrome following cervical laminoplasty -A case report-. *Korean J Anesthesiol* 2013; **64**: 172-174 [PMID: 23459018 DOI: 10.4097/kjae.2013.64.2.172]
- 21 **Park CK,** Lee DC, Park CJ, Hwang JH. Tapia's Syndrome after Posterior Cervical Spine Surgery under General Anesthesia. *J Korean Neurosurg Soc* 2013; **54**: 423-425 [PMID: 24379951 DOI: 10.3340/jkns.2013.54.5.423]
- 22 **Sønnichsen R,** Lauritsen AO. [Hypoglossus and laryngeal nerves palsy after an intubation for Legionella infection]. *Ugeskr Laeger* 2013; **175**: 2647-2648 [PMID: 24629202]
- 23 **Nalladaru Z,** Wessels A, DuPreez L. Tapia's syndrome--a rare complication following cardiac surgery. *Interact Cardiovasc Thorac Surg* 2012; **14**: 131-132 [PMID: 22108947 DOI: 10.1093/icvts/ivr056]
- 24 **Turan I,** Yildirim ZK, Tan H. Bilateral Tapia syndrome secondary to oropharyngeal intubation. *J Neurosurg Anesthesiol* 2012; **24**: 78 [PMID: 22036876 DOI: 10.1097/ANA.0b013e31823769ef]
- 25 **Wadelek J,** Kolbusz J, Orlicz P, Staniaszek A. Tapia's syndrome after arthroscopic shoulder stabilisation under general anaesthesia and LMA. *Anaesthesiol Intensive Ther* 2012; **44**: 31-34 [PMID: 23801511]
- 26 **Lykoudis EG,** Seretis K. Tapia's syndrome: an unexpected but



- real complication of rhinoplasty: case report and literature review. *Aesthetic Plast Surg* 2012; **36**: 557-559 [PMID: 22179851 DOI: 10.1007/s00266-011-9849-y]
- 27 **Park J**, Ahn R, Weon Y, Yang D. Diagnosing Tapia syndrome using a videofluoroscopic swallowing study and electromyography after anterior cervical spine surgery. *Am J Phys Med Rehabil* 2011; **90**: 948-953 [PMID: 21955952 DOI: 10.1097/PHM.0b013e31823286e0]
  - 28 **Torres-Morientes LM**, Benito-Orejas JI, Landínez-Cepeda GA, Morais-Pérez D. Tapia's syndrome following thoracotomy. *Rev. ORL* 2011; **2**: 16
  - 29 **Al-Sihan M**, Schumacher M, Löhle E. Tapia syndrome caused by a vertebral artery dissection. *Ear Nose Throat J* 2011; **90**: 313-314 [PMID: 21792800]
  - 30 **Kashyap SA**, Patterson AR, Loukota RA, Kelly G. Tapia's syndrome after repair of a fractured mandible. *Br J Oral Maxillofac Surg* 2010; **48**: 53-54 [PMID: 19423205 DOI: 10.1016/j.bjoms.2009.01.021]
  - 31 **Rotondo F**, De Paulis S, Modoni A, Schiavello R. Peripheral Tapia's syndrome after cardiac surgery. *Eur J Anaesthesiol* 2010; **27**: 575-576 [PMID: 19923990 DOI: 10.1097/EJA.0b013e3283340ac3]
  - 32 **Boğa I**, Aktas S. Treatment, classification, and review of Tapia syndrome. *J Craniofac Surg* 2010; **21**: 278-280 [PMID: 20098201 DOI: 10.1097/SCS.0b013e3181c678f0]
  - 33 **Dursun E**, Cincik H, Cekin E. Tapia's Syndrome Followig Hunting Rifle- Shot. *IJHNS* 2007; **1**: 1
  - 34 **Sotiriou K**, Balanika M, Anagnostopoulou S, Gomatou C, Karakitsos D, Saranteas T. Postoperative airway obstruction due to Tapia's syndrome after coronary bypass grafting surgery. *Eur J Anaesthesiol* 2007; **24**: 378-379 [PMID: 17087848 DOI: 10.1017/S0265021506001542]
  - 35 **Tesei F**, Poveda LM, Strali W, Tosi L, Magnani G, Farneti G. Unilateral laryngeal and hypoglossal paralysis (Tapia's syndrome) following rhinoplasty in general anaesthesia: case report and review of the literature. *Acta Otorhinolaryngol Ital* 2006; **26**: 219-221 [PMID: 18236639]
  - 36 **Cinar SO**, Seven H, Cinar U, Turgut S. Isolated bilateral paralysis of the hypoglossal and recurrent laryngeal nerves (Bilateral Tapia's syndrome) after transoral intubation for general anesthesia. *Acta Anaesthesiol Scand* 2005; **49**: 98-99 [PMID: 15675991 DOI: 10.1111/j.1399-6576.2004.00553.x]
  - 37 **Yavuzer R**, Başterzi Y, Özköse Z, Yücel Demir H, Yılmaz M, Ceylan A. Tapia's syndrome following septorhinoplasty. *Aesthetic Plast Surg* 2004; **28**: 208-211 [PMID: 15599532 DOI: 10.1007/s00266-003-3037-7]
  - 38 **Boisseau N**, Rabarjaona H, Grimaud D, Raucoules-Aimé M. Tapia's syndrome following shoulder surgery. *Br J Anaesth* 2002; **88**: 869-870 [PMID: 12173208 DOI: 10.1093/bja/88.6.869]
  - 39 **Johnson TM**, Moore HJ. Cranial nerve X and XII paralysis (Tapia's syndrome) after an interscalene brachial plexus block for a left shoulder Mumford procedure. *Anesthesiology* 1999; **90**: 311-312 [PMID: 9915343 DOI: 10.1097/00000542-199901000-00040]
  - 40 **Shimohata T**, Nakano R, Sato S, Tsuji S. [A patient with aneurysm of extracranial internal carotid artery presenting lower cranial polyneuropathy similar to Tapia's syndrome]. *Rinsho Shinkeigaku* 1994; **34**: 707-711 [PMID: 7955729]
  - 41 **Millán Guevara J**, Royo López J, Pascual Millán LF, Rivas Rodríguez P, Fumanal Senz L, Castellote Armero A. [Idiopathic associated paralysis of the Xth and XIIth cranial nerves]. *An Otorrinolaringol Ibero Am* 1993; **20**: 61-64 [PMID: 8465938]
  - 42 **McCleary AJ**. A fracture of the odontoid process complicated by tenth and twelfth cranial nerve palsies. A case report. *Spine (Phila Pa 1976)* 1993; **18**: 932-935 [PMID: 8316898]
  - 43 **Takimoto T**. Radiographic technique for preoperative diagnosis of plunging ranula. *J Oral Maxillofac Surg* 1991; **49**: 659 [PMID: 2037926]
  - 44 **de Freitas MR**, Nascimento OJ, Chimelli L. Tapia's syndrome caused by Paracoccidioides brasiliensis. *J Neurol Sci* 1991; **103**: 179-181 [PMID: 1880535 DOI: 10.1016/0022-510X(91)90161-Y]
  - 45 **Quattrocchio G**, Giobbe D, Baggione P. Tapia's syndrome caused by a neurilemmoma of vagus and hypoglossal nerves in the neck. *Acta Neurol (Napoli)* 1986; **8**: 535-540 [PMID: 3799257]
  - 46 **Gelmers HJ**. Tapia's syndrome after thoracotomy. *Arch Otolaryngol* 1983; **109**: 622-623 [PMID: 6882274 DOI: 10.1001/archotol.1983.00800230058014]
  - 47 **Andrioli G**, Rigobello L, Mingrino S, Toso V. Tapia's syndrome caused by a neurofibroma of the hypoglossal and vagus nerves: case report. *J Neurosurg* 1980; **52**: 730-732 [PMID: 7373407 DOI: 10.3171/jns.1980.52.5.0730]
  - 48 **Mayer A**, Opran H. Letter: Tapia syndrome. *JAMA* 1974; **227**: 326 [PMID: 4859671 DOI: 10.1001/jama.1974.03230160054024]
  - 49 **Ruhrmann G**. [Congenital right-sided vagus and hypoglossal nerve paralysis (Tapia syndrome) as the cause of congenital stridor]. *Z Kinderheilkd* 1963; **88**: 22-26 [PMID: 13975474]
  - 50 **Babini B**, Scorza P. [Glosso-laryngeal paralysis (Tapia's syndrome) due to obstetrical trauma]. *Clin Pediatr (Bologna)* 1961; **43**: 1006-1012 [PMID: 13863659]
  - 51 **Symonds CP**. Case of Unilateral Affection of Cranial Nerves, 9-12 (Tapia's Syndrome) associated with Chronic Otitis Media. *Proc R Soc Med* 1923; **16**: 53-54 [PMID: 19983080]
  - 52 **Rubio-Nazábal E**, Marey-Lopez J, Lopez-Facal S, Alvarez-Perez P, Martinez-Figueroa A, Rey del Corral P. Isolated bilateral paralysis of the hypoglossal nerve after transoral intubation for general anesthesia. *Anesthesiology* 2002; **96**: 245-247 [PMID: 11753027 DOI: 10.1097/00000542-200201000-00040]
  - 53 **Uña E**, Gandía F, Duque JL. Tongue paralysis after orotracheal intubation in a patient with primary mediastinal tumor: a case report. *Cases J* 2009; **2**: 9301 [PMID: 20062625 DOI: 10.1186/1757-1626-2-9301]

**P- Reviewer:** Coban M, Hilmi I, Marchan-Lopez A, Ramsay MA

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Li D





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

